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German Research Center for Environmental Health

2018 2019 2020

Universität Rostock HelmholtzZentrum münchen
JOINT MASS SPECTROMETRY CENTRE

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Progress Report

Summer Term **2018** – Winter Term **2020**/2021

Joint Mass Spectrometry Centre (JMSC)

JMSC comprehends the research cooperation unit Comprehensive Molecular Analysis – CMA at the Helmholtz Zentrum München and the Chair of Analytical Chemistry – CAC at the University of Rostock

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www.helmholtz-muenchen.de/cma/publications

Foreword

Dear reader,

I am delighted to present the seventh progress report of the research unit *Joint Mass Spectrometry Centre* (JMSC) at the University of Rostock (*"Chair of Analytical Chemistry"* at the *Institute of Chemistry,* CAC) and the *Helmholtz Zentrum München* (research unit *"Comprehensive Molecular Analytics",* CMA) as well as the first progress report of the *Helmholtz International Lab* aeroHEALTH.

The JMSC is operating since 2008 as a cooperative, joint research initiative of the University of Rostock (CAC) and the Helmholtz Zentrum München (CMA), based on a strategic, perpetual cooperation contract between the *Helmholtz Zentrum München* (HMGU) and the *University of Rostock* (UR), which was closed upon my appointment (Berufung) in 2007. In 2018, our network was extended by a strategic cooperation with the University of the Bundeswehr in München (UniBw, Institute for Chemistry and Environmental Engineering, Prof. Dr. T. Adam).

The main scientific interest of JMSC is in the fields of Environmental Health research (research area: *Aerosols and Health*) and of Analytical Chemistry (research area: *Enabling Analytical Technologies*). In the research area *Aerosols and Health,* the nature and principles of adverse biological and toxicological effects of aerosols on lung cells, tissues, animals, and humans are investigated. In the research area *Enabling Analytical Technologies,* new and innovative approaches, based on *e.g.* mass spectrometric-, chromatographic-, laser spectroscopic-, *in-vitro* cell exposure- and bioanalytical-technologies are developed to enable progress in Environmental Health research. JMSC contributed to the successful Program-Oriented Funding (POF) evaluations of the Helmholtz Association at the HMGU and is committed to contribute to the HMGU research program in POF within the HGF research field "Health".

After successful finalization of the "Helmholtz Virtual Institute HICE – Aerosol and Health" project in 2017, which was cofunded by the Helmholtz Association (HGF) and led by the JMSC, the HICE concept was perpetuated and transferred into the base funding scheme of the Helmholtz Zentrum München. In order to reestablish the international cooperative component of the Aerosol and Health research, we (HMGU/CMA) proposed together with the international partner Weizmann Institute of Science (Israel) and the national Partner Forschungszentrum Jülich (FZJ) as well as with the associated partners University of Rostock and University of Eastern Finland to establish a Helmholtz International Lab. After a competitive selection process, the Helmholtz Association (HGF) granted the Helmholtz International Lab aeroHEALTH in 2018 (funding period 2019 – 2024). aeroHEALTH went operational in April 2019, aiming to elucidate the potential health effects of the atmospheric transformation of aerosol emissions (gases, vapors, fine dust particles, and nanoparticles).

In the report at hand, we introduce the JMSC coworkers, actual PhD students, and habilitants. Some historical aspects, facts, highlights as well as selected scientific reports on research projects are provided. Of course, some information on the work in Helmholtz International Lab aeroHEALTH is included. Finally, yet important, the JMSC performance parameters (cooperations, scientific publications, lectures, TV-media coverage, acquired third-party funds, etc.) are listed. Short profiles of all performed and newly acquired third-party projects in the reporting period are given.

Prof. Dr. Ralf Zimmermann, full professor of Analytical Chemistry at the University of Rostock and head of the research unit CMA at the Helmholtz Zentrum München. Head of JMSC and Spokesperson of the Helmholtz International Lab. aeroHEALTH

In the end, I like to thank the rector of the University of Rostock, Prof. Dr. W. Schareck, and the previous scientific CEO of the Helmholtz Zentrum München, Prof. Dr. G. Wess, for the many years of engaged support of the JMSC, HICE, and aeroHEALTH. I am grateful to the president of the Helmholtz association Prof. Wiestler as head of the International Lab selection committee. I thank the current CEO of the HMGU, Prof. Dr. M. Tschöp, for his promise to support the aeroHEALTH International Lab. Furthermore, I thank the administrations at UR and HMGU for making impossible things possible.

The most important factor for JMSC's success are our coworkers. Without the super-engaged work of all JMSC co-workers as well as the PhD-, MSc- and BSc-students, accompanied by the support of the cooperation partners of the JMSC, HICE, and aeroHEALTH, the here presented achievements would not have been possible. I also owe a special acknowledgement to the 10 topic leaders, and in particular, to my deputy JMSC directors, Prof. Dr. T. Adam and Dr. T. Streibel. Finally, I thank Dr. B. Schloter-Hai for compiling the progress report.

I close with the hope, that the following years, after the pandemic and other problems, will be continuing to be similarly successful as the preceding ones.

Ralf Zimmermann
Rostock, 18.12.2020

Health impact of aerosols

Profile of the research focus "Health impacts of aerosols" at the University of Rostock and the Helmholtz Zentrum München within the Joint Mass Spectrometry Centre and the Helmholtz International Lab aeroHEALTH

JMSC – Executive Summary

Motivation: Air pollution by fine dust particles and trace gases (aerosols) massively impacts human health. More than 4 million premature deaths per year are associated with global air pollution (WHO), and the numbers of seriously ill people with typical conditions such as cardiovascular diseases, asthma, COPD, or lung cancer are orders of magnitude higher. This renders air pollution to the most serious cause of environmental illness and death, not only in particularly strongly affected regions such as in Asia. The aging societies in Central Europe are severely affected by air pollution-related health effects as well. Research on the underlying environmental and toxicological issues is very complex (multifactorial problems, see Fig. 1) and requires a multi- and interdisciplinary research approach in which environmental scientists, analytical chemists, physicists, molecular biologists, toxicologists, and other disciplines work closely together. This interdisciplinary approach has been developed and expanded over the past 12 years within the framework of the research focus „Health Effects of Aerosols" of the *Joint Mass Spectrometry Centre* (JMSC), a joint institution of the *University of Rostock* (UR) and the *Helmholtz Zentrum München* (HMGU). The joint research concept of the UR and HMGU was internationalized by two major project-initiatives, which were funded after a competitive selection process by the Helmholtz Association and led by the JMSC. Namely, these are the *Helmholtz Virtual Institute* HICE – Aerosol and Health *(2012 – 2017, with seven national/international partners)* and the *Helmholtz International Lab* aeroHEALTH *(2018 – 2024/27; with the Weizmann Institute in Israel, the Forschungszentrum Jülich and the associated partner University of Eastern Finland)*. The Universität der Bundeswehr München, with its competences in the field of environmental sciences and technology at the Institute of Chemistry and Environmental Engineering (Prof. Adam), has joined the successful JMSC cooperation.

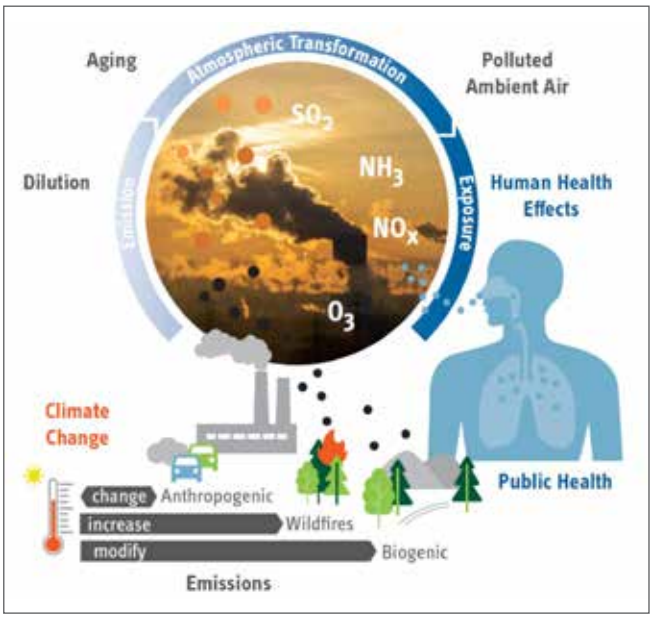

Figure 1: *The study of the complex relationships between emitted pollutants, atmospheric transformation processes, biological effects at the cellular level, and human health are the topic of the research focus between the University of Rostock and the Helmholtz Zentrum München at the JMSC.*

Research approach: The aim of the research activities in the JMSC is a better understanding of the influences of the chemical and physical properties of air pollution (*i.e.* aerosols: particle and gas phase) on the biological effects in cell culture-based human lung models, lung tissues and finally the translation of the results to human health. The results will support evidence-based, effective mitigation and protection measures as well as appropriate legal regulations. In order to achieve this goal, we established two overarching areas within the framework of the cooperation between *UR* and *HMGU*. In the field of *Enabling Analytical Technologies*, new high-technology analytical methods for characterizing aerosols and biological systems are being developed and applied. Through a spin-off company (*Photonion* GmbH), founded with the support of a successfully acquired *Helmholtz Enterprise Funds* (HEF) project-grant, such innovative measurement systems are also made available for scientists from academia and industry worldwide. In the second area, *Aerosols and Health*, in addition to the extensive chemical and physical aerosol characterization and the study of the pollutant transformation and new particle formation in the atmosphere, we investigate, in particular, the toxicology and genotoxicity of aerosols. This includes studying the effects of ultrafine particles (UFP). A key element is the use of so-called Air Liquid Interface (ALI) techniques for direct exposure of human lung cell cultures and

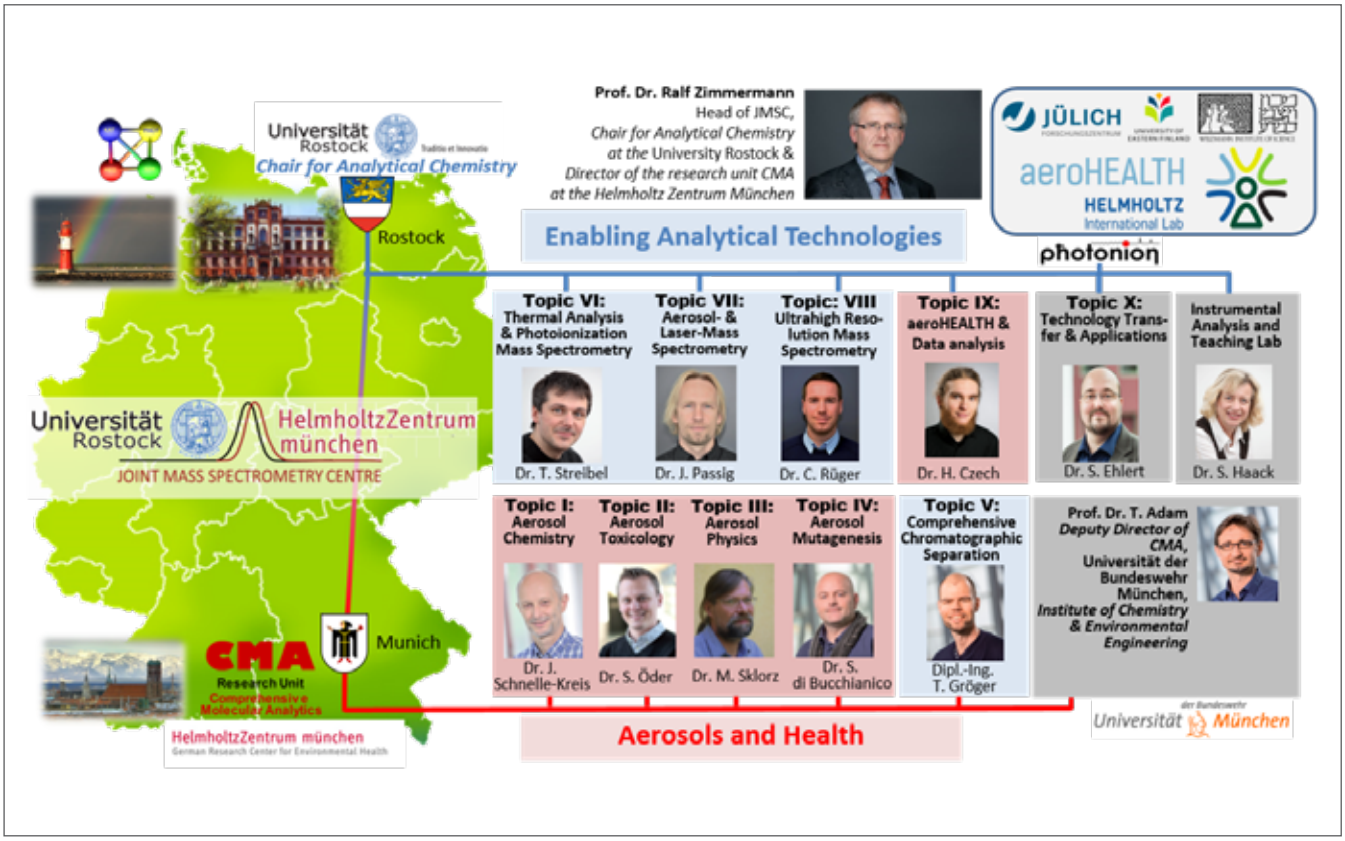

*Figure 2: Structure of the research focus „Health Effects of Fine Dusts and Aerosols" in the **JMSC** of the University of Rostock and the Helmholtz Zentrum München with the 10 scientific sub-areas in the fields of „Aerosols and Health" and „Enabling Analytical Technologies"*

organ models to air pollutants or aerosols. Molecular biological (*e.g.* transcriptome and proteome analyses) and functional (*e.g.* analysis of DNA- or chromosomal-damage) studies are used to investigate the mechanisms of the adverse biological effects. Using modern data processing techniques and AI-based routines, we draw conclusions on harmful components and mixtures in the environmental aerosol and the mechanisms of triggering diseases. Of particular interest are, for example, the assessment of health effects caused by emissions from biomass combustion, wild fires, and the transport sector (ship engines, aircraft turbines, road transport, etc.) as well as the health effects of atmospherically transformed emissions, including the effects of newly formed secondary organic aerosols and the evaluation of mitigation measures.

Structure in the research focus: Within the framework of the contractually agreed cooperation between the HMGU and the UR *in the context of the appointment of Prof. Zimmermann* and the founding of the *Joint Mass Spectrometry Centre,* 10 scientific topics have been established. The topics are organized in two areas, *Enabling Analytical Technologies and Aerosols and Health* (see Figure 2), and are working closely together in a highly interdisciplinary manner. At the University of Rostock, the JMSC research activities are embedded in the Department Life, Light, Matter (LLM); in the LLM research building the JMSC leads the mass spectrometry competence center. The JMSC is contributing to the research program in the Helmholtz Association's research field "Health" in the framework of the Program-Oriented Funding (POF) scheme and leads the HGF-funded Helmholtz International Lab aeroHEALTH (www.aeroHEALTH.eu).

Key facts from JMSC and aeroHEALTH @ University of Rostock and Helmholtz Zentrum München

- Scientific Director: Prof. Dr. Ralf Zimmermann
- Deputy Head: Prof. Dr. Thomas Adam (@Munich) and Dr. Thorsten Streibel (@Rostock)
- Basic funded staff in 2020 (scientists/technical staff): **13/10**
- Third-party funded staff in 2020 (doctoral students and postdocs): **34**
- Publications in peer-reviewed scientific journals (average 2018-2020): **>30** per year
- Third-party funding in 2020 (3-year average): **> € 3 million** per year
- Sponsors: DFG, HGF, BMBF, BMWi, EU, DGUV, Bay. – StMUV, etc. and various industrial companies
- Number of current, approved, or completed third-party funded projects in the period 2018–2020: **> 30**

Gesundheitsauswirkungen von Aerosolen

Steckbrief des Forschungsschwerpunkts „Gesundheitsauswirkungen von Aerosolen“ der Universität Rostock und des Helmholtz Zentrums München im Joint Mass Spectrometry Centre und dem Helmholtz International Lab aeroHEALTH

JMSC – Zusammenfassung

Motivation: Die Luftbelastung hat massive Auswirkungen auf die menschliche Gesundheit. Die weltweiten jährlichen, luftverschmutzungsbedingten vorzeitigen Todesfälle liegen bei über 4 Millionen (WHO), die Zahlen von schwer erkrankten Personen mit typischen Krankheitsbildern wie Herzkreislauferkrankungen, Asthma, COPD oder Lungenkarzinomen sind um Größenordnungen höher. Damit ist die Luftverschmutzung die mit Abstand gravierendste umweltbedingte Erkrankungs- und Todesursache, nicht nur in besonders hochbelasteten Regionen wie z.B. in Asien. Auch die überalterten Gesellschaften in

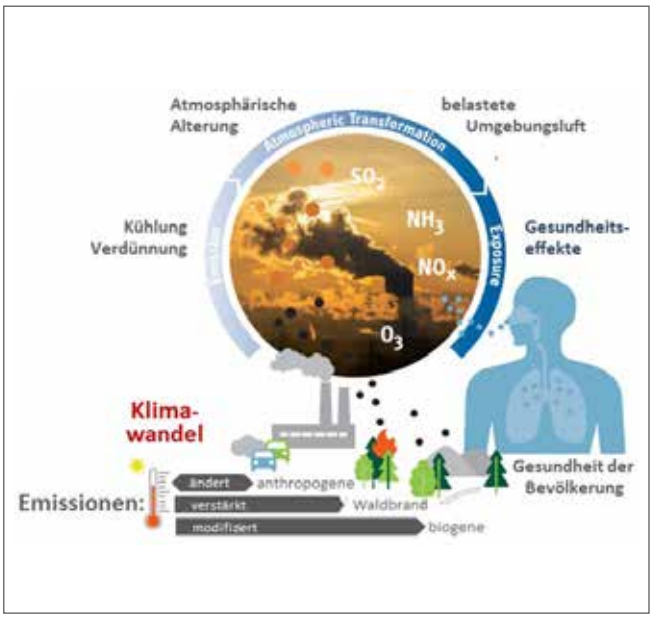

Abbildung 1: *Die Untersuchung der komplexen Zusammenhänge zwischen emittierten Schadstoffen, atmosphärischen Umwandlungsprozessen, biologischen Effekten auf zellulärem Level und der menschlichen Gesundheit sind das Thema des Forschungsschwerpunkts zwischen der Universität Rostock und dem Helmholtz Zentrum München am JMSC.*

Mitteleuropa sind stark von luftverschmutzungsbedingten Gesundheitsauswirkungen betroffen. Die Erforschung der zugrundeliegenden umweltwissenschaftlichen und toxikologischen Fragestellungen ist sehr komplex (multifaktorielle Prozesse, siehe Abbildung 1) und bedarf eines multi- und interdisziplinären Forschungsansatzes, bei dem Umweltwissenschaftler, analytische Chemiker, Physiker, Molekularbiologen, Toxikologen und weitere Disziplinen eng verzahnt zusammenarbeiten. Im Rahmen des hier vorgestellten Forschungsschwerpunkts „Gesundheitsauswirkungen von Aerosolen“ des *Joint Mass Spectrometry Centre* (JMSC), einer gemeinsamen Einrichtung der *Universität Rostock* (UR) und des *Helmholtz Zentrums München* (HMGU), ist dieser interdisziplinäre Ansatz in den letzten 12 Jahren entwickelt und ausgebaut worden. Dabei hat sich das gemeinsame Forschungskonzept der UR und des HMGU über zwei, von der Helmholtz Gemeinschaft nach stringentem Auswahlverfahren ausgewählten und geförderte, vom JMSC geleitete Großprojekte, dem *Helmholtz Virtual Institute* HICE – *Aerosol und Health (2012 – 2017)* und dem *Helmholtz International Lab* aeroHEALTH *(2018 – 2024, mit dem Weizmann Institut in Israel, dem Forschungszentrum Jülich und dem assoziierten Partner University of Eastern Finland)* international vernetzt. Die *Universität der Bunderwehr München,* mit Kompetenzen im Bereich Umweltchemie und Prozessanalytik am Institut für Chemie und Umwelttechnik (Prof. Adam) ist zur erfolgreichen JMSC-Kooperation hinzugestoßen.

Forschungsansatz: Ziel der Forschungsaktivitäten im JMSC ist es, den Einfluss der chemischen und physikalischen Eigenschaften der Luftbelastung (Aerosole: Partikel- und Gasphase) auf die biologischen Wirkungen in zellkulturbasierten menschlichen Lungenmodellen, in Lungengewebe und schließlich auf die menschliche Gesundheit besser zu verstehen. Dadurch können evidenzbasierte, effektive Minderungs- und Schutzmaßnahmen sowie angemessene gesetzliche Regelungen realisiert werden. Zur Umsetzung dieses Zieles wurden im Rahmen der Kooperation zwischen *UR* und *HMGU* zwei übergeordnete Bereiche etabliert. Im Bereich „Instrumentelle Analytische Technologien“ *(Enabling Analytical Technologies)* werden neue analytische Hochtechnologie-Verfahren zu Charakterisierung von Aerosolen und biologischen Systemen entwickelt und zur Anwendung gebracht. Über eine mit Hilfe des *Helmholtz Enterprise Fonds* (HEF) gegründeten Ausgründung (*Photonion* GmbH) werden solche Systeme auch anderen Wissenschaftlern weltweit zur Verfügung gestellt. Im zweiten Bereich, Aerosole und Gesundheit *(Aerosols and Health),* werden neben der umfangreichen chemischen und physikalischen Charakterisierung von Aerosolemissionen und -immissionen und der Erforschung der Transformation und Neubildung von Partikeln und Schadstoffen in der Atmosphäre,

Abbildung 2: *Struktur des Forschungsschwerpunkts „Gesundheitsauswirkungen von Feinstäuben und Aerosolen" im JMSC der Universität Rostock und dem Helmholtz Zentrum München mit den 10 wissenschaftlichen Teilbereichen in den Bereichen „Aerosols and Health" und „Enabling Analytical Technologies"*

vor allem die Toxikologie und Genotoxizität der Aerosole untersucht. Das schließt die Untersuchung der Wirkung von ultrafeinen Partikel (UFP) ein. Ein Kernelement ist der Einsatz von Air Liquid Interface (ALI) Techniken zur direkten Exposition humaner Lungenzellkulturen und Organmodelle mit den Luftschadstoffen bzw. Aerosolen. Durch molekularbiologische (z.B. Transkriptom- und Proteomanalysen) und funktionale (z.B. Analyse von DNA- oder Chromosomen Schäden) Untersuchungen werden die Mechanismen der biologischen Wirkung untersucht. Unter Einsatz moderner Datenverarbeitungsverfahren und KI-basierter Routinen erzielen wir Rückschlüsse auf wirksame Komponenten des Umweltaerosols und ausgelöste Krankheiten. Von besonderem Interesse sind z.B. die Bewertung der Emissionen aus der Biomasseverbrennung, aus Waldbränden und dem Transportsektor (Schiffsmotoren, Flugantriebe, Straßenverkehr etc.) sowie die Gesundheitseffekte atmosphärisch unter Sonnenlichteinwirkung neugebildeter, sekundärer Aerosole und die Evaluierung von Minderungsmaßnahmen.

Struktur im Forschungsschwerpunkt: Im Rahmen der Kooperation des HMGU und der UR, die durch die gemeinsame Berufung von Prof. Zimmermann mit der Gründung des *Joint Mass Spectrometry Centre* etabliert wurde, sind 10 wissenschaftliche Themenbereiche (Themen) in den beiden Bereichen *Enabling Analytical Technologies* und *Aerosols and Health* eingerichtet worden (siehe Abbildung 2), die das Forschungsfeld eng verzahnt und interdisziplinär bearbeiten. An der Universität Rostock ist das JMSC im Department Life, Light, Matter (LLM) die Leitung des Kompetenzzentrums für Massenspektrometrie am LLM-Forschungsbau eingebunden. Das JMSC trägt im Rahmen der Programmorientierten Förderung (POF) zum Forschungsbereich Gesundheit (Health) der Helmholtz Gemeinschaft am HMGU bei und leitet das von der HGF geförderte Helmholtz International Lab. aeroHEALTH (www.aeroHEALTH.eu).

Kennzahlen von JMSC und aeroHEALTH @ Universität Rostock und Helmholtz Zentrum München

- Wissenschaftlicher Leiter: Prof. Dr. Ralf Zimmermann
- Stellvertretende Leiter: Prof. Dr. Thomas Adam (@München) und Dr. Thorsten Streibel (@Rostock)
- Grundfinanzierte Mitarbeiter in 2020 (Wissenschaftler/ Technische Mitarbeiter): **13/10**
- Drittmittelfinanzierte Mitarbeiter in 2020 (Doktoranden und PostDocs): **34**
- Referierte Publikationen in Fachjournalen (Durchschnitt 2018–2020): **> 30** pro Jahr
- Jährliche Drittmitteleinwerbung (Durchschnitt über 3 Jahre): **> 3 Mio. €** pro Jahr
- Fördergeber: DFG, HGF, BMBF, BMWi, EU, DGUV, Bay. – StMUV etc. und diverse Industrieunternehmen
- Zahl laufender, bewilligter oder abgeschlossene Drittmittelprojekte im Zeitraum 2018–2020: **> 30**

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JMSC – A Helmholtz Zentrum München and University of Rostock Cooperation

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JMSC

A Cooperation between
Helmholtz Zentrum München
and the University of Rostock

1 This is JMSC

1.1 The Joint Mass Spectrometry Centre, a permanent scientific and structural cooperation of the University of Rostock and the Helmholtz Zentrum München

In April 2008, the University of Rostock (UR) and the Helmholtz Zentrum München (HMGU,) started a permanent scientific and structural cooperation in the general areas of environmental health research (research topic: **Aerosols and Health**) and analytical instrument development (research topic: **Enabling Analytical Technologies**), as set forth in the cooperation contract signed by the GSF-Forschungszentrum (the predecessor institution of HMGU) and the University of Rostock in December 2007. As a result, a joint research unit of UR and HMGU, the "**Joint Mass Spectrometry Centre**" (JMSC), was established. The JMSC is headed by Prof. Dr. Ralf Zimmermann, who concurrently holds the full professor/chair position at the Chair of Analytical Chemistry (*i.e.* the "Lehrstuhl für Analytische Chemie") of the Institute of Chemistry at the University of Rostock (UR) and directs the independent research unit "Comprehensive Molecular Analytics" (CMA) at HMGU.

In 2012, the UR-HMGU cooperation was further intensified by the successful implementation of the "Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health – Aerosols and Health (HICE)". HICE was established as an international research consortium with eight partner institutions, funded initially for 5 years by the Helmholtz Impulse and Networking Funds of the Helmholtz Association (HGF), and was further supported financially by HMGU and UR. Prof. Dr. Ralf Zimmermann became speaker of HICE. The topic of the HICE project was the integration of molecular biological and toxicological research, engineering and data sciences, environmental and analytical chemistry as well as aerosol physics to create a truly multidisciplinary approach for assessing and understanding the health effects of combustion process-related aerosol emissions (*e.g.* internal combustion engines of all sizes, biomass burning, etc.). A main approach developed within HICE was the online exposure of human lung cell cultures (cell lines, primary cells) at the Air-Liquid-Interface (ALI, *i.e.* method to mimic the situation in the human respiratory tract during exposure) to diluted combustion aerosols followed by a molecular biological multi-omics and toxicological effect analysis. This was accompanied by a truly comprehensive physical-chemical characterization of the exposure aerosols and joint data analysis of the biological, chemical, and physical results. One main outcome was the realization of how important indeed the chemical composition of the airborne particulates is for the observed effects. Furthermore, it was recognized that the gas phase of combustion aerosols contributes more than expected to the observed acute cytotoxicity. Thus already the change of the fuel or the combustion conditions can drastically alter the adverse effects/mechanisms. The pathway of oxidative-stress mediated induction of inflammatory effects was observed. The complex action of the xenobiotic compounds in combustion aerosol includes additive, synergistic as well as antagonistic effects. For example, the results support the hypothesis that acute cytotoxic effects of wood combustion may be reduced by antioxidants in wood smoke (*e.g.* phenols) albeit carcinogenic endpoints should not be influenced. A comprehensive summary of the HICE research can be found in the previous JMSC progress reports: (https://www.helmholtz-muenchen.de/cma/publikation/fortschrittsberichte/fortschrittsberichte-archiv/index.html).

After completion of the HGF funding period of HICE in 2017 and the following outstanding evaluation results of CMA and HICE, the UR, and HMGU as part of the HICE-initiative were permanently transferred by the HMGU management into the HMGU institutional funding (internally referred to as "newHICE") and also put into the program-oriented funding (POF) scheme of HMGU at the HGF. In addition to JMSC (*i.e.* UR and HMGU) also the University of Eastern Finland (UEF) was included in newHICE via an HMGU–funded cooperation contract (*i.e.* the inhaleHICE contract), in particular, to enable joint measurements at the ILMARI-facility of UEF (https://www3.uef.fi/en/web/ilmari). The long-term aims of newHICE are to extend the concept of HICE, namely to investigate the biological and health impacts of other aerosol and fine dust emissions and of photochemically aged aerosols (*i.e.* the ambient air). This includes the development and application of novel analytical techniques, *e.g.* in the field of aerosol mass spectrometry. New concepts for the characterization of complex molecular mixtures in environmental health research are established. Novel sensitive techniques and biological approaches will address the impact of polluted ambient air, photochemically aged aerosol emissions, and bioaerosols (allergy) onto human lung cells and tissues.

1.2 JMSC Research Facilities and Equipment

At the University of Rostock, the JMSC is represented by the Chair of Analytical Chemistry (CAC), which is located in buildings of the University of Rostock in the Dr.-Lorenz-Weg 2 (figure 1) and the competence Centre "Mass Spectrometry" in the new Research Building of the "Life, Light & Matter Department of the Interdisciplinary Faculty (LLM, Albert Einstein Straße 25, figure 2). Currently, 22 scientists (senior scientists and postdoc level), PhD- and MSc- students as well as technical co-workers are working at the Chair of Analytical Chemistry. Four scientists occupy permanent academic positions, while 14 scientific members of CAC have temporary third-party, project-funded employment contracts. Furthermore, there are 3.5 technical staff positions located at CAC (two technicians, one secretary, and a half-time electronic engineer position). From this personnel, three co-workers are permanently delegated from the HMGU to the CAC in the framework of the cooperation contract. A new building for the Institute of Chemistry (extension building, figure 3) is currently under construction next to the LLM Research Building in the Albert Einstein Straße, to which we will move from Dr.-Lorenz-Weg in summer 2021.

The JMSC operates at Rostock several large-scale, high-end analytical instruments. For in-depth analysis of aerosol particles and fuels, **ultra-high resolution mass spectrometry** approaches are used at the CAC (figure 4). For this purpose, among others, a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR MS) system is used. The system is based on a 7T superconducting cryomagnet and has two FT-ICR MS carts (Solarix and APEX III, Bruker GmbH, Germany). The system can be used with MALDI, ESI, and GC-APCI ion sources and interfaces and reaches mass resolutions of more than 500.000. Furthermore, the CAC has an Orbitrap high-resolution mass spectrometer with an ESI ion source as well as an experimental direct low-pressure photoionization Orbitrap mass spectrometer system.

Two **Single Particle time-of-flight aerosol Mass Spectrometer** (SPMS), as well as one Aerodyne Aerosol Mass Spectrometer (AMS), are in use for the online characterization of aerosol particles and method developments (figure 5). The SPMS units can be operated in a newly invented and patented, laser desorption resonance-enhanced multiphoton ionization process which also enables laser desorption-ionization of the same particle. The introduction of novel single-particle characterization methods in aerosol science has the potential to become a game-changer for understanding aerosol processes, sources, and health effects.

High-end laser laboratories are available for the application and development of photoionization mass spectrometry techniques (Nd:YAG laser, tunable OPO and dye laser, CO_2 laser, various excimer lasers, rare-gas excimer light sources). The use of fs-laser radiation promises a steering of desorption and ionization steps in laser mass spectrometry (figure 6).

An **on-line mass spectrometry application laboratory** for online analyses of gases and aerosols and hyphenated analysis systems is equipped with seven photoionization time-of-flight-mass spectrometers (PIMS) and one proton transfer mass spectrometer (figure 7). The systems are applied for on-line analyses during the Aerosol and Health measurement campaigns. For analytical pyrolysis, combustion, and pyrolysis research, a pyrolysis furnace system with two chambers or three thermal balance systems (TG), partially equipped with fast-gas chromatography units can be hyphenated with the PIMS units. A special application is the off-line analysis of aerosol particles collected on filters by a Thermal/Optical Carbon Analyzer (TOCA). The TOCA-system analyses the carbon content (elemental and organic carbon fractions) of aerosols and has been coupled in our laboratory to a photoionization time-of-flight mass spectrometer or an electron ionization quadrupole mass spectrometer. The analysis of the chemical composition of the carbon fractions is helpful for aerosol source apportionment and toxicant profiling. Finally, also liquid chromatography tandem mass spectrometry (LC-MS/MS) and gas chromatography mass spectrometry (GC-MS) systems are available in the laboratories for method development and routine analyses.

With the new **S2 cell and molecular biology laboratory** (figure 8) and the aerosol source and exposure unit, as well as the oxidation flow tube reactor in the new Chemistry extension building (figure 3), from 2021 on new research possibilities will be available at the University of Rostock, urgently anticipated for the fulfillment of the Helmholtz International Lab aeroHEALTH and HMGU POF programs.

Figure 1: *Research Building of the University of Rostock at Dr.-Lorenz-Weg 2 where the Chair of Analytical Chemistry/JMSC currently still has its main research and teaching premises. The main large equipment units are several photoionization mass spectrometers, partially in hyphenation with different separation and pyrolysis devices.*

Figure 2: *Research Building LLM of the Interdisciplinary Faculty of the University of Rostock (since 2016), where the Chair of Analytical Chemistry/JMSC operates the Mass Spectrometry Competence Centre with the high-resolution MS laboratory (FT-ICR MS, Orbitrap MS), a short-pulse (fs) laser mass spectrometry laboratory, an aerosol mass spectrometry laboratory as well as an organic chemical analysis laboratory (LC-MS/MS, GC-MS).*

Figure 3: *The nearly finalized new Chemistry "extension building" of the University of Rostock. The Chair of Analytical Chemistry/ JMSC will move to the new Building in 2021. Several high-end special laboratories, including an S2 biosafety laboratory, an aerosol source, and aerosol aging oxidation flow-tube laboratory were jointly planned by HMGU and UR and are currently realized.*

Figure 4: *High-resolution MS laboratory in the Research Building LLM of the University of Rostock where the Chair of Analytical Chemistry/JMSC operates an FT-ICR MS System.*
Top: The 7 T FT-ICR MS exhibits two carts allowing MALDI, ESI, GC-/TA-APCI, APPI, APLI ionization experiments.
Bottom: An Orbitrap for photoionization of gases and particles is developed in the framework of the AerORBI EU Project.

Figure 5: *Aerosol Mass Spectrometry Lab. A single-particle mass spectrometer with advanced laser ionization technology developed in our group.*

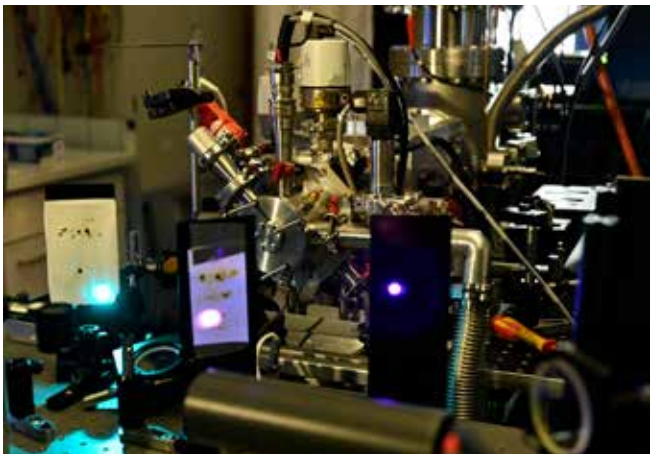


Figure 6: *fs-Laserionization MS Lab. Ultra-short laser pulses of only 35 fs duration are investigated as a novel tool for fragment-free desorption and ionization of particle-bound molecules.*

Figure 7: *On-line gas-phase characterization. Dual on-line photoionization mass spectrometer (PhotoTOF; Photonion GmbH, Schwerin, Germany) for parallel acquisition of single-photon ionization using a VUV-lamp and resonance-enhanced multi-photon ionization (REMPI) mass spectra on a field campaign at the research laboratories of PROBAT-Werke von Gimborn Maschinenfabrik GmbH in Emmerich am Rhein.*

The independent research unit "Comprehensive Molecular Analytics" (CMA) at Helmholtz Zentrum München in Oberschleißheim and Sendling consists of 13 senior or postdoc scientists. 7 scientists are permanently employed at Helmholtz Zentrum München. 9 PhD students are employed on a temporary basis with contracts through third-party funding or are scholarship-holders. The cooperation unit also has 5 technical staff members and two scientists serving as associated scientific consultants. The CMA laboratory rooms are currently dispersed in two separate locations, one on the main campus of the Helmholtz Zentrum München in Oberschleißheim and the other at Gmunder Straße 37 in München Sendling (main premises, see figure 9). At the main campus of HMGU in Building 24 some special laboratories are situated (see figure 10).

The CMA research unit operates two HPLC-MS/MS systems, two HPLC systems equipped with a diode array, and a fluorescence detector. Four instruments for the multidimensional analysis of highly complex samples in bioscience and environmental science are currently available at CMA: one system for multi-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS), one system for multi-dimensional gas chromatography fast quadrupole mass spectrometry (GCxGC-QMS), and two multi-dimensional gas chromatography high-resolution multi reflection time-of-flight mass spectrometer GCxGC-HRTOFMS systems equipped with direct insertion probe (DIP), a coupling to thermal analysis devices and a prototype photoionization setup (cooperation with LECO GmbH, Germany).

Furthermore, four GC-MS systems (partially equipped for thermal desorption of aerosol loaded filters) and an FTIR gas analyzer are available. Physicochemical characterization of particulate matter and aerosol particles is performed by two scanning mobility particle size spectrometers (SMPS), two electric low-pressure impactors (ELPI), an aerodynamic particle size spectrometer (APS), a white light optical particle size spectrometer (WELAS), and diverse particle sampling equipment (several Berner impactors, a rotating-drum impactor, a MOUDI impactor, and several low volume samplers). A major activity of CMA is the toxicological and molecular biological analysis of the effects of air-pollution, particulate matter and nanoparticles on human lung cells and tissues. In addition to the research topic Aerosol Toxicology at CMA/JMSC in 2019 the topic Aerosol Mutagenesis was newly established. Thus the biological/toxicological work field was very much expanded during the establishment of the permanent newHICE funding at CMA. The topics Aerosol Toxicology and Aerosol Mutagenesis are equipped with three automated air liquid interface (ALI) cell exposures stations within total 60 exposure places (see figure 11), facilities for cell culture work (incubators, clean benches, etc.) a fluorescence microscope, and a real-time PCR unit. In figure 12 some views into the biological laboratories at CMA are taken. Furthermore, it operates the mobile S2 biosafety laboratory of CMA (HICE MobiLab, see figure 13).

The JMSC cooperates in the framework of the HICE extension ("newHICE") with the University of Eastern Finland in Kuopio. The joint research activities are regulated by a cooperation contract. At Kuopio, extensive measurement facilities for

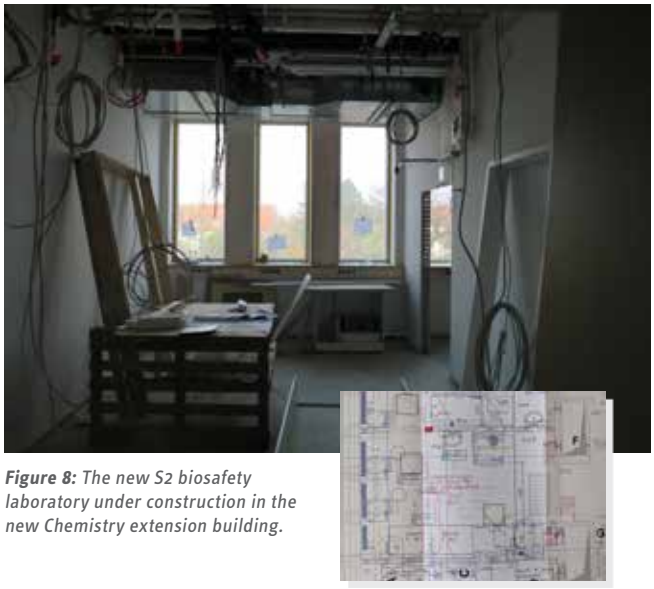


Figure 8: *The new S2 biosafety laboratory under construction in the new Chemistry extension building.*

Figure 9: *The main research premises of CMA/JMSC are located at Gmunder Straße 37 in München Sendling and comprise the whole 2nd floor of the shown building.*

Figure 10: *At the HMGU campus in Oberschleißheim (Building 24) JMSC/CMA operates climate chambers and special aerosol laboratories, partly in conjunction with the HICE MobiLab (see below).*

aerosol research can be used by the consortium. This includes state-of-the-art appliances for wood combustion and diesel generators as well as an aerosol chamber and flow tube reactors for aerosol aging experiments. Further HICE partners have joined the activities via third-party funding (*e.g.* MDC Berlin, University of Luxemburg, Photonion GmbH). Particularly important in this context is the contractually regulated partnership to the Universität der Bundeswehr München (UniBw M, Prof. Dr. Thomas Adam), as described below.

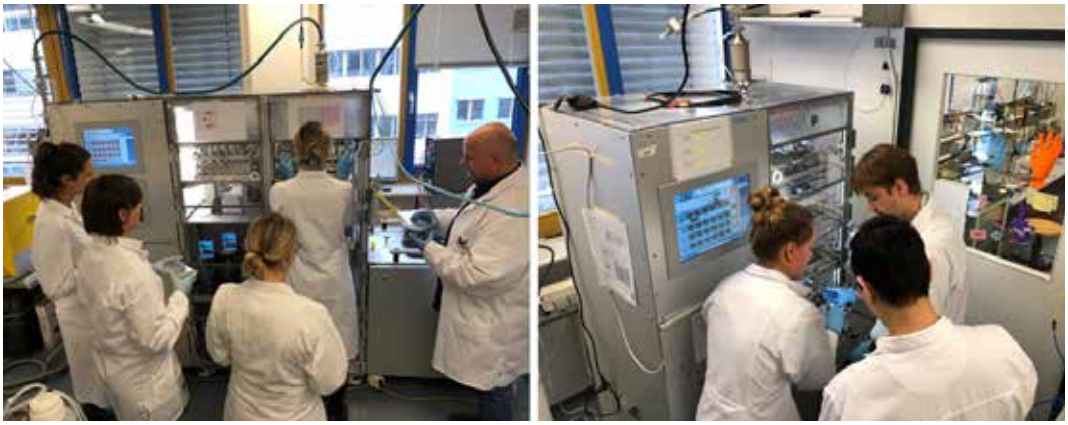


Figure 11: *Bio laboratories at the CMA/JMSC location in Gmunder Straße in München Sendling. Three automated Air-Liquid-Interface (ALI) Exposure-Units are used to expose human lunge cells (cell lines and primary cells) to fine and nanoparticles and gases (aerosols) under realistic conditions to investigate the health effects of air pollution.*

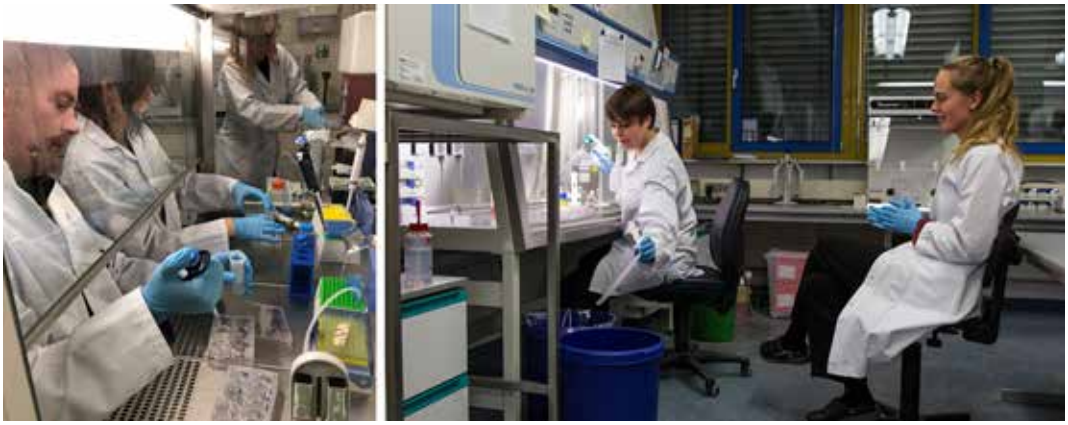


Figure 12: *Bio laboratories at the CMA/JMSC location in Gmunder Straße in München, Sendling, after the exposure cytotoxicity and genotoxic effects are quantified. Omics analyses (proteome, transcriptome) reveal the adverse biological mechanisms. Effect validation with e.g. animal testing is performed with the cooperation partners in Finland (UEF).*

Figure 13: *The HICE MobiLab, a mobile aerosol exposure and biosafety level 2 laboratory based on a 20 ft. shipping container. The HICE MobiLab contains two rooms. Left) HICE MobiLab in use at the University of Eastern Finland during a measurement campaign, the small insert shows the HICE MobiLab in Munich before shipping. Right) Photo of cell extraction and biological analysis work after cell exposures to wood combustion exhaust, taken through the HICE MobiLab window.*

1.3 JMSC in the framework of the research building of the Department Life, Light & Matter at the Interdisciplinary Faculty, University of Rostock

In 2007 the University of Rostock established its 10th Faculty, the Interdisciplinary Faculty (INF). Within the INF, research is organized and focused in four overarching research fields, organized in a Department structure. The Departments of the INF currently are:

- Department Life, Light & Matter (LLM)
- Department Maritime Systems (MTS)
- Department Aging of Individuals and Society (AGIS)
- Department Knowledge – Culture – Transformation (WKT)

By institutionalizing this thematic concentration and cross-subject and discipline cooperation, the university breaks new ground and connects the new structure with a clear vision, intending to bundle and enhance comprehensive expertise related to these topics. The Department Life, Light, and Matter (LLM), currently led by Prof. Dr. K. H. Meiwes-Broer, was established as a focal point between natural and engineering sciences and medicine. In 2008, a proposal for an LLM Research Building to the German Council of Science (state/federal funding scheme) was worked out by a professorial consortium under the direction of Prof. Dr. Meiwes-Broer and with the participation of Prof. Dr. Zimmermann. The proposal was finally granted and in 2015 the new research building of the department LLM (see figure 2) was inaugurated. The LLM research building comprises of project laboratories and five competence centers: Interfaces and Surfaces; Microscopy and Spectroscopy; Calorimetry and Thermal Analysis Methods; Nuclear Magnetic Resonance Spectroscopy; and Mass Spectrometry. This modern building allows scientists from diverse scientific areas to conduct cooperative and cutting-edge research on over 2500 square meters of excellently equipped laboratories. The five competence centers house large-scale equipment and facilities, some of which are unique, such as a high-performance electron microscope successfully funded in 2017. Figure 14 shows a sketch of the current LLM structure, including the competence centers in the LLM research building. The JMSC, led by Prof. Dr. Ralf Zimmermann, is heading the competence center Mass Spectrometry at the LLM research building with the vision to bring together high-performance mass spectrometric instrumentation. Currently, more than ten members of the JMSC, including eight PhD-students, are working in the framework of the competence center Mass Spectrometry in various research projects. Five research laboratory rooms, organized in the **Ultra-High Resolution and Routine Mass Spectrometry Laboratory (HRMS)** and the **Laser and Aerosol Mass Spectrometry Laboratory (LAMS)** are used by the competence center Mass Spectrometry. Unique mass spectrometric platforms are operated, including two single-particle time-of-flight mass spectrometer, and one Aerodyne aerosol mass spectrometer and an fs-laser – mass spectrometry system (LAMS: Topic VI, Dr. Johannes Passig), as well as an ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS), and two Orbitrap high-resolution mass spectrometer (HRMS: Topic VIII, Dr. Christopher P. Rüger). Moreover, routine chromatographic mass spectrometric systems are maintained, such as liquid and gas chromatography. Due to current third-party projects with LLM partners, Photonion and JMSC are jointly using a project laboratory in the LLM research building (Dr. Robert Irsig and Dr. Sven Ehlert).

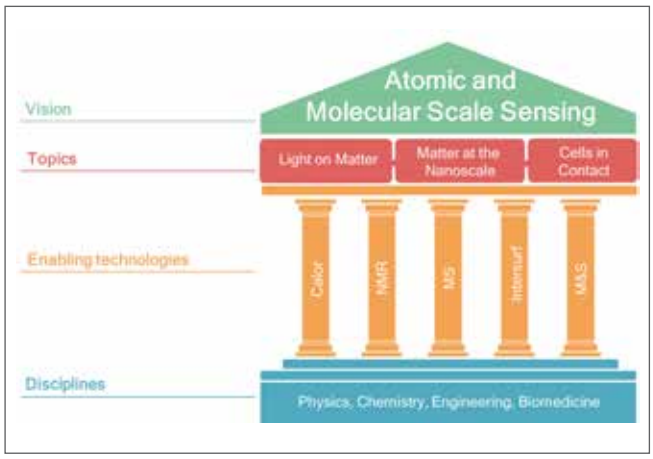


Figure 14: Structure of the Department LLM. The Disciplines and Competence centers (Intersurf: Interfaces and Surfaces, M&S: Microscopy and Spectroscopy, MS: Mass Spectrometry, NMR: Nuclear Magnetic Resonance Spectroscopy, Calor: Calorimetry and Thermal Analysis Methods) support the topics, which reflect the LLM motto: "Atomic and Molecular Scale Sensing".

In the following, the labs of the competence center Mass Spectrometry with several selected cooperative research activities and the unique instrumentation located in the department LLM are presented.

Ultra-High Resolution and Routine Mass Spectrometry Laboratory (HRMS): The JMSC operates an Ultrahigh Resolution Mass spectrometry Lab in the LLM research building. The Ultra-High Resolution and Routine Mass Spectrometry Laboratory is part of the European network of FT-ICR MS laboratories (EU_FT-ICR_MS, see third party project (23)) and is operating two Bruker FT-ICR MS systems equipped with a 7 Tesla superconducting magnet in the laboratories of the LLM research building. The EU_FT-ICR_MS consortium provides foreign researchers access to state-of-the-art mass spectrometric instrumentation via so-called trans-national access. This allows interdisciplinary and

unique investigations together with international researchers fully covered by the project funding and fully in accordance with the scope of the INF. The FT-ICR MS devices are the heart of HRMS and deliver unbeaten mass accuracy (< 1 ppm) and mass resolving power (> 500,000 @ *m/z* 400 achievable) for the chemical description of ultra-complex organic mixtures. Various direct liquid infusion ionization sources are available, such as electrospray (ESI), atmospheric pressure chemical (APCI), and atmospheric pressure photo ionization (APPI). Most importantly and unique for the competence center Mass Spectrometry is the strong focus towards evolved gas analysis coupled to atmospheric pressure ionization mass spectrometry. Besides direct inlet probe and gas chromatography coupled to FT-ICR MS, the only-known thermogravimetric (TG) FT-ICR MS coupling is operated (see Enabling Technologies (08)). This coupling allows the direct investigation of highly viscous and solid sample materials (figure 17a). Information on the composition and structure of the constituents is gained via desorption and intended pyrolysis combined with comprehensive data processing. Selective ionization approaches, *e.g.*, by atmospheric pressure laser ionization at 266 (4th Nd:YAG harmonic) or 157 nm (Fluorine excimer) are performed together with the Laser mass spectrometry lab, allow for further structural insights (see Enabling Technologies (02)). The expertise of the thermal analysis (DIP and TG) mass spectrometric hyphenation is strongly linked to the JMSC Topic VII and the competence center Calorimetry and Thermal Analysis Methods within the department LLM. The expertise in thermal analysis mass spectrometry is further utilized within a recently funded DFG-ANR project (Thermal analysis and ion mobility coupled to high-resolution mass spectrometry for organic aerosol characterization – TIMSAC, see third party project 04). Figure 15 b schematically depicts the instrumental setup foreseen at the University of Rostock. Despite chromatographic and mass spectrometric information, in this international cooperation together with the University of Rouen-Normandy (France), the structural elucidation aspects of ion mobility spectrometry will be further explored. Ion mobility spectrometry gives access to the collision-cross section (CCS) a direct measure for the size and shape of an ion. Computational approaches, so-called *in-silico* prediction models, will be brought together with experimental data for unraveling the isomeric distribution. In particular, for the theoretical modeling, collaborative work together with the competence center Nuclear Magnetic Resonance Spectroscopy and the Institute of Physics is foreseen.

Laser and Aerosol Mass Spectrometry Laboratory (LAMS): In the Laser and Aerosol Mass Spectrometry Laboratory, several gas-phase photoionization and laser desorption/(photo) ionization based ionization approaches for mass spectrometry are developed, investigated, and applied. The investigated samples are gaseous, solid, liquid, or aerosolized (nano-particles). Several JMSC projects, *e.g.*, are using single-particle mass spectrometry (SPMS) or they directly focus on the further development of this approach (see contribution in the sections Selected Research Projects and Topic VI). In this method, individual particles are exposed to laser pulses in order to obtain size and chemical information on a single-particle level. One emphasis of our SPMS research is to control the laser-particle interaction and to exploit resonances between the laser light, the particle, and the evolving plume surrounding the excited particle. Several laser excitation and ionization schemes have been developed in the LLM labs during the last years. Recently, a new laser control scheme was developed to trigger chirped-pulse-amplification femtosecond laser systems to irregularly incoming particles from atmospheric aerosols, a key prerequisite to exploit the potential of fs-lasers for excitation and ionization control for real-world aerosols (see figure 16). All SPMS research is bundled in the aerosol- and laser labs of LAMS in the LLM building. Thus, the JMSC is synergistically working hand-in-hand with the many groups in the LLM using complex optical approaches and ultra-short laser pulses. This work culminates in a joint project within a recently proposed Collaborative Research Centre with the Institute of Physics (DFG-SFB).

In cooperation with research groups of the Department Maritime Systems (MTS) the LAMS is involved in projects to study the gaseous and particulate emission of ships engine (*e.g.* SAARUS (see third party projects (17)) or the EU project ULTRHAS (see third party projects (01))). Another example is the direct on-line mass spectrometry measurement of trace contaminants in water. A new project, AMMOTRACe, funded by MarTERA, an ERA-NET Cofund scheme of the European Commission, draws together European companies and research organizations that develop analytical techniques and instruments to detect historic munitions in marine waters and sediments and conduct munition clearance operations (see third party projects (02)). Together with the German project partners from the Leibniz

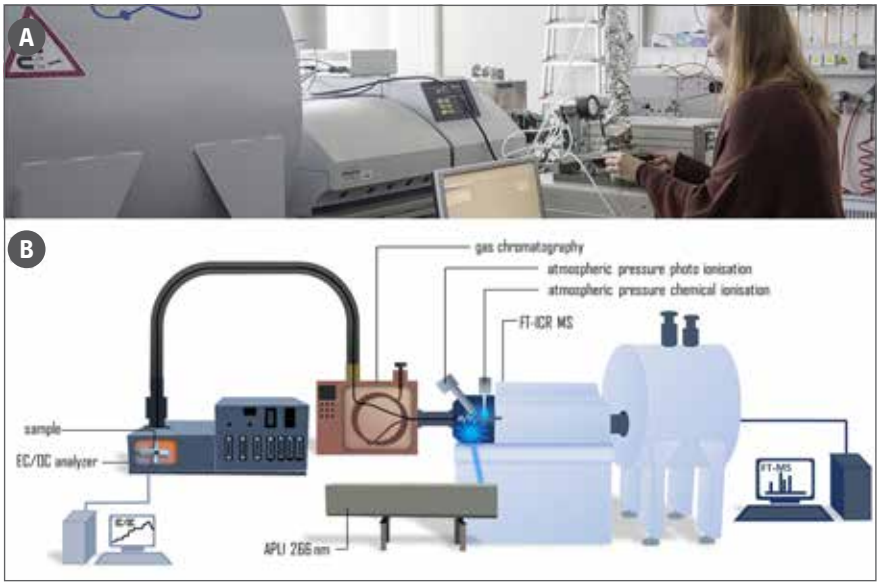


Figure 15: *A) Photographic image of the ultrahigh-resolution mass spectrometry laboratory picturing the work of a PhD-student at the thermogravimetric coupling to FT-ICR MS. B) Schematic setup of the anticipated EGA FT-MS system foreseen in the DFG-ANR TIMSAC project featuring TOCA coupling and gas chromatographic coupling with large volume injector both enabling direct solid sample introduction. Various types of ionization, such as atmospheric pressure chemical, laser, and photoionization can be deployed. Further structural information is gained by ion dissociation, e.g., utilizing the quadrupole collision-induced dissociation (CID) option. Graphic not to scale.*

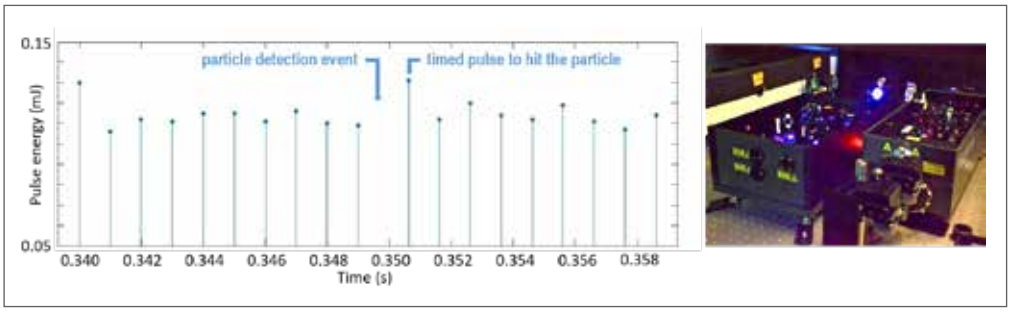


Figure 16: *(left) Single-pulse output energy of the fs-laser system in the LLM (35 fs, max. 6 mJ, 800 nm) during synchronizing an emitted pulse to an individual aerosol particle. The developed electronics take timing control over the entire fs-laser, including the amplifier system. (Right) Detail photographic image of the fs-laser source setup.*

Institute for Baltic Sea Research Warnemünde (IOW, also a member of MTS), the Helmholtz Centre for Ocean Research Kiel (Geomar) and the spin-off company Photonion GmbH, the JMSC develops novel laser mass spectrometry and ion mobility techniques for the trace detection of munitions in seawater, see section third party project AMMOTRACe (third party project (02)) and figure 17. The laser-MS experiments are performed in the LLM laboratories using tunable optical parametric oscillators (OPO) as light sources. By exploring multi-step and multi-color approaches, the optimum photoionization schemes for ultra-sensitive detection of ammunition compounds are developed. These pathways will provide the scientific basis for the realization of both an ultra-sensitive on-ship mass spectrometer with tunable laser sources for water column screening and a simplified and robust underwater system for in-situ localization of munition. The physical developments greatly benefit from the laser expertise of further groups in the LLM (competence center Microscopy and Spectroscopy).

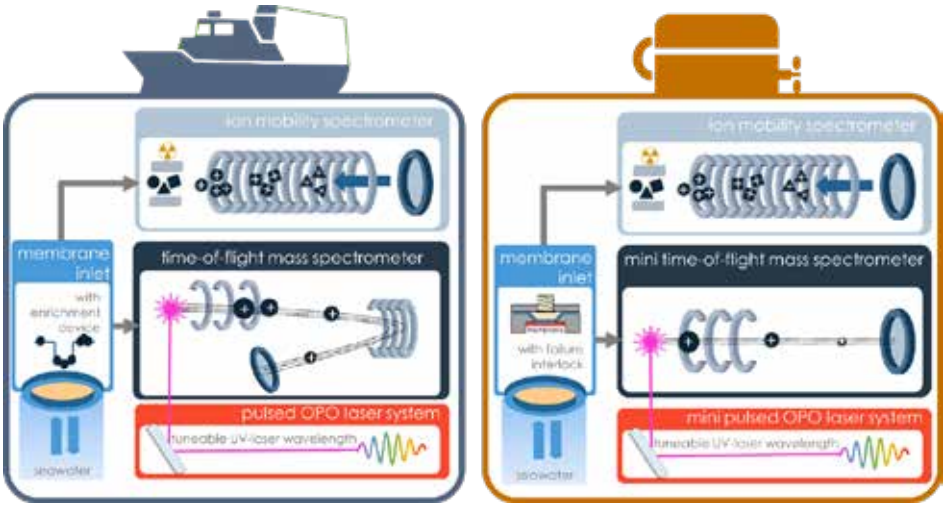
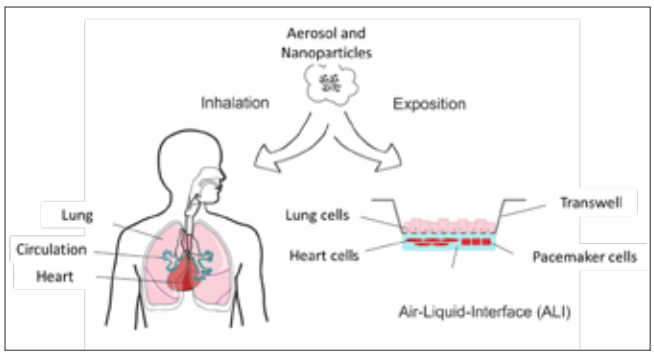


Figure 17: *Schematic setups of the ship-based (left) and remotely operated underwater system (right) for detection of munition sites. The membrane inlet and laser mass spectrometry technology is developed with the partners of the Interdisciplinary Faculty in the LLM building at the University of Rostock.*

In addition to the activities in the Ultra-High Resolution and Routine Mass Spectrometry Laboratory and the Laser and Aerosol Mass Spectrometry Laboratory within the LLM Mass Spectrometry competence center, further activities in the framework of LLM are ongoing or planned. With the anticipated move of JMSC into the new building in Rostock (see figure 3) also the cooperation with biomedical research groups in LLM will be fostered. For example, in the labs of the LLM, the research group for cardiac stem cell therapy at the University of Rostock (Prof. Robert David) and the JMSC are jointly developing a concept for a novel *in-vitro* system to detect the impact of aerosols and nanoparticles on the function of specialized heart cells and the lung epithelium in real-time. The motivation for this joint research is that about one half of the several million deaths caused by ambient air pollution is currently associated with acute cardiovascular effects and ischemic strokes. In order to resemble the real situation in the human body as close as possible, the heart cells will be exposed to the aerosol under realistic conditions in an air-liquid-interface (ALI) system, separated from the air by a monolayer of lung cells (see also various contributions in the section Selected Research Projects referencing the ALI approach). The cell metabolism of this unique design will be monitored in real-time using luminescence methods. The key idea of the approach is the detection of the contraction activity of the heart cells using multi-electrode array systems. This provides a real-time measure of the cell system functionality under realistic aerosol exposure. Subsequent genotoxicity analysis will complement the studies. A special feature facilitated by the large equipment in LLM is the control of particle deposition and mobility in the cell system using laser scanning microscopy in collaboration with the competence center Microscopy and Spectroscopy. Currently, third party funding proposals are under preparation.

Figure 18: *Working scheme of the interdisciplinary collaboration. Systems of human lung and heart cells are exposed to aerosols and nanoparticles under realistic conditions in an air-liquid-interface (ALI) system, modeling the natural exposure and biological effects on the cardiovascular system of the human body.*



1.4 A new JMSC partner: The Universität der Bundeswehr München

The "Institute for Chemistry and Environmental Engineering" at the Faculty for Mechanical Engineering of the Universität der Bundeswehr München (UniBW M) specializes in the chemical analysis of environmental samples. The institute has a broad range of measurement techniques at its disposal to investigate organic and inorganic substances in liquid, solid and gaseous matrices.

Research activities focus on aerosol research, in particular, the chemical and physical characterization of air pollutants and climate-relevant compounds, as well as on the measurement of exhaust gases in combustion processes. One focal point of current and future research lies in examining the exhaust gases of ships, aircraft engines, and road traffic together with testing modern exhaust gas after-treatment systems. For this purpose, the faculty's facilities, *e.g.*, a vehicle chassis dynamometer incl. CVS tunnel, various light-duty engine test benches, an aircraft combustion chamber simulator, and a helicopter gas turbine test bench can be used. Another main emphasis is the research on transport-related emissions from non-combustion processes. These include the abrasion of brakes, tires, drive components, and road surfaces. Research in this field is carried out by using the institute's brake and tire test benches.

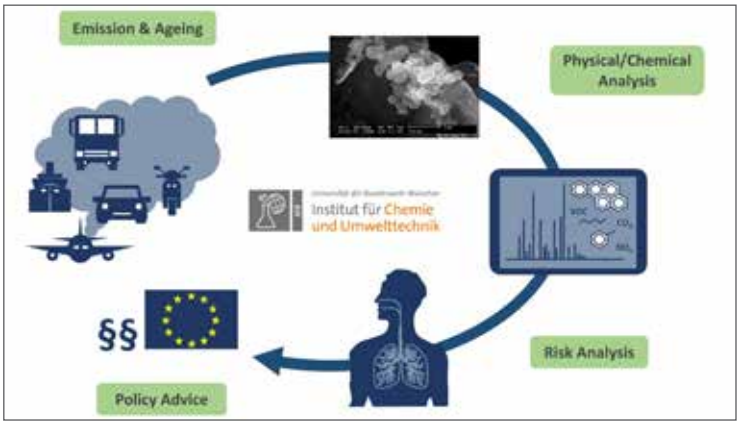


Figure 19: *Research activities at the "Institute for Chemistry and Environmental Engineering" at the Universität der Bundeswehr München.*

The institute is headed by Prof. Dr. rer. nat. Thomas W. Adam – a member of the expert commission PMP (Particle Measurement Program) at UNECE (United Nations Economic Commission For Europe) – which is currently developing a statutory testing method to regulate brake dust in the automotive industry.

Facts file Prof. Dr. Thomas Adam, graduate chemist & aerosol scientist

- since 2017: Full Professor at the Institute for Chemistry and Environmental Engineering at the Universität der Bundeswehr München.
- since 2017: Deputy Director at CMA-HMGU
- 2016: Founding Adviron Environmental Consulting
- 2011 – 2015: AUDI AG Ingolstadt, Emission test center
- 2007 – 2010: European Commission Joint Research Centre Ispra, Transport and Air Quality Unit
- 2002 – 2007: GSF – Forschungszentrum für Umwelt und Gesundheit, now HMGU
- 2001: Alfred Wegener Institute for Polar & Marine Research Bremerhaven

Another field of activity lies in investigating aging processes in the atmosphere and their influence on the composition of urban, rural, and Alpine ambient air.

The chemical/physical tests carried out by the institute are supported by biological analyses (*e.g. in-vitro* cell studies) conducted at JMSC. The goal here is to provide an indication of potential health hazards for humans. For this purpose, the institute has got a modern mobile measurement laboratory for immission and emission analyses. One of the institute's specialist areas lies in the real-time measurement of gaseous organic pollutants in combustion processes. It's a procedure, making it possible to simultaneously detect a large number of hydrocarbons which damage health at trace levels. The procedure enables online process controls on combustion engines, residential heating, power plants, and industrial plants. The institute uses the technique of time-of-flight mass spectrometry coupled with soft photoionization processes, *i.e.* single-photon ionization (SPI) and resonance-enhanced multiphoton ionization (REMPI) to achieve this. The use cases for the work done by the "Institute for Chemistry and Environmental Engineering" are numerous. But to stay ahead of the curve, strategic cross-discipline collaboration will be critical. As a result, in summer 2018, the institute signed a cooperation agreement with the Helmholtz Zentrum München. Through this cooperation, approximately 50 scientists and engineers at the Universität der Bundeswehr München, Helmholtz Zentrum München, and the University of Rostock spanning mechanical and environmental engineering, chemistry, physics, and biology are currently working on the research of gaseous pollutants and particulate matter in emissions and in the atmosphere as well as their impact on human health and the environment.

Current staff at the Institute for Chemistry and Environmental Engineering:

- Prof. Dr. Thomas Adam: Institute Director
- Dr. Sara Padoan: Chemist
- Dr. Mohamed Saraji: Physicist
- Ajit Mudan: Chemist
- Jan Bendl: Environmental Engineer
- NN: Automotive Engineer
- NN: Bundeswehr officer in mechanical engineering
- Günter Obermaier: Technician
- Wolfgang Christ: Technician

All three institutions thus benefit from the integration of classic scientific disciplines with health-related research and engineering disciplines.

1.5 The technology transfer partner: Photonion GmbH

Continuous developments in on-line photoionization mass spectrometry (PIMS) at CMA, UR, and UniBw M have led to a number of patents and third party projects, often carried out with direct industrial support or with other research partners. To satisfy the growing demand in photoionization mass spectrometry instrumentation developed at JMSC and to allow long-term employment of scientific co-workers, a spin-off company of JMSC was founded in 2009, the Photonion GmbH. The founding of the spin-off was supported by a Helmholtz Enterprise Funds (HEF) grant. Photonion is closely connected to scientific research and aims at the further development of innovative trace gas analysis instruments based on the JMSC photoionization and mass spectrometry techniques and expertise. Mass spectrometric measurement devices with both, single-photon ionization (SPI) and resonance-enhanced multi-photon ionization (REMPI) techniques are developed and marketed. Customers are research departments of companies, universities, or research centers. Photonion is also engaged in fundamental and applied research projects together with JMSC and other research partners. These projects are prospectively leading to new products, services and applications. At Photonion currently five scientists are employed, working together with the JMSC scientists in Munich and Rostock in the research areas "Aerosols and Health" and "Enabling Analytical Technologies". In line with the focus of the JMSC on aerosol research, Photonion is concentrating on the development of advanced measurement techniques for aerosol characterization. The classical portfolio of Photonion in photoionization mass spectrometry for gas-phase analysis can be readily applied for aerosol research. A new approach developed at JMSC aims to better characterize organic compounds in the particulate fraction of aerosols. For this, the coupling of a thermo/optical carbon analyzer (TOCA) to photoionization mass spectrometry (PIMS) was realized. This TOCA-PIMS system was developed at JMSC together with the Desert Research Institute (DRI; Reno, USA). In the TOCA system, the organic carbon fractions (OC) are quantified, based on a stepwise desorption of organic material from a quartz fiber filter, loaded with aerosol particulate matter (PM). The hyphenation of TOCA to the Photonion PIMS technology allows the chemical analysis of the different OC-fractions during an OC/EC analysis process. A first system was already ordered by the Chinese Academy of Science Institute. The second measurement technique, which was recently developed in cooperation of JMSC and Photonion, is a new version of single particle mass spectrometry (SPMS). In order to understand the health effects of aerosolized ambient particulate matter it is important to know the internal and external mixing behavior of toxic compounds in the aerosol particle ensemble in order to calculate local doses at the cellular level in the lung. SPMS systems can on-line sample aerosol particles from the air. After size measurement, the particles are laser-vaporized and ionized in a mass spectrometer. Up to now, however, SPMS systems only were able to detect inorganic compounds and a sum fraction representing organic compounds. A molecular speciation of organic compounds in addition to the inorganic characterization has not been possible yet. With a newly developed laser desorption and combined laser-plasma/REMPI-ionization, we enabled to perform the inorganic speciation in parallel to analyze the molecular pattern of aromatic compounds from the same individual aerosol particle. This opens new perspectives in analyzing the distribution of toxic transition metals and Polycyclic Aromatic Hydrocarbons (PAH) on the particles. Current projects with this technique are focusing on emissions of ship engines and the detection of shipping emissions in coastal regions (see third party projects (06)) as well as on detection of aerosolized hazardous compounds (drugs, toxicants, etc. (see third party projects (07)). The first of the new SPMS systems (figure 15) has been ordered by a renowned Taiwanese research university. In current research projects, Photonion is involved also in the detection of ammunition dumped in the Baltic Sea by membrane inlet PIMS (see third party project 02). Furthermore, Photonion is taking part in a project to implement REMPI and SPI photoionization for high resolution Orbitrap mass spectrometry (see third party project 22) and a project aiming to develop an automated aerosol sampling-derivatization-thermal-desorption gas chromatography – mass spectrometry system for organic aerosol monitoring (see third party projects (15)). The current coworkers of Photonion are:

- Dr. Sven Ehlert, Chief Scientific Officer (also leads topic X)
- Dr. Matthias Bente
- Dr. Dumitru Duca
- Dr. Robert Irsig
- Dr. Mohammad Reza Saraji-Bozorgzad

Figure 20: *Photonion Booth at the European Aerosol Conference (EAC 2017) in Zurich, Switzerland. One of the latest Photonion products lines, based on a new Single Particle Mass Spectrometer (SPMS) concept is presented. Using a patented ion source, organic and inorganic chemicals can be on-line analyzed from individual aerosol particles in the size range from 150 nm-1 µm.*

1.6 JMSC Research Areas – Structuring the scientific work

The scientific activities of the JMSC are currently structured in two general research areas, **Aerosols and Health** and **Enabling Analytical Technologies** (see figure 21). The research program is oriented towards the Helmholtz Mission (https://www.helmholtz.de/en/about_us/the_association/mission/) of the national Helmholtz Association of German Research Centers (HGF) and the HGF's Program-Oriented Funding (POF) scheme for Environmental Health at HMGU.

The objective of the first research area, **Aerosols and Health**, is to study the impact of aerosols and air pollution on human health. Environmental factors directly or indirectly relevant to human health and wellbeing are studied. A pronounced activity in this research area is the investigation of the health effects of ambient and emission aerosols. The research area is also directly related to the Helmholtz Virtual Institute HICE – Aerosol and Health or rather its perpetuation (newHICE) in the base funding/POF at the HMGU and the Helmholtz International Lab aeroHEALTH, which is led by CMA.

The second general research area, **Enabling Analytical Technologies**, comprehends the area of development and application of mass spectrometric and chromatographic analytical techniques for the characterization of complex molecular mixtures. The application of the techniques, methods, and approaches developed in this research area is focusing on Aerosol and Health and other Environmental Health research aspects.

The work in the two research areas of JMSC is organized in ten corresponding and interlinked research topics at UR and HMGU, respectively, each represented and led by an experienced scientific topic leader. The organigram is shown in figure 21. Furthermore, the HMGU/UR spin-off company Photonion GmbH and the Universität der Bundeswehr are involved in the research (figure 22). The respective ten topic leaders and the chief scientific officer of Photonion are indicated in the figure. The research topic of ***Aerosol Chemistry*** (Dr. Jürgen Schnelle-Kreis, topic I) performs the chemical characterization of all kinds of aerosols, such as ambient aerosols as well as combustion aerosols and artificially aged aerosols. The research topic ***Aerosol Physics*** (Dr. Martin Sklorz, topic III) is responsible for the physical aerosol characterization, *i.e.* by determining size distributions and working on questions of aerosol dosage in ALI systems and on workplace aerosols. The research topic of ***Aerosol Toxicology*** (Dr. Sebastian Öder, topic II) deals with biological effects of cell culture models, which are exposed to aerosols at the Air-Liquid Interface (ALI). The biological response characterization is performed on different omics-levels as well as by functional assays. In the topic of ***Aerosol Mutagenesis*** (Dr. Sebastiano Di Bucchianico, topic IV) the primary, as well as secondary genotoxic and mutagenic effects of aerosols, are investigated. In addition, biomarkers of exposure and oxidative stress are evaluated utilizing chromatographic and mass spectrometric methods. The Helmholtz International Lab aeroHEALTH is managed in the research topic **aeroHEALTH *& Data Analysis*** (Dr. Hendryk Czech, topic IX). Furthermore, the topic leads the simulation of aerosol aging processes as well as the chemometric data analysis and the integration of physico-chemical and biological data in Aerosol and Health research. The development and application of multidimensional comprehensive chromatographic separation methods, often coupled with high-resolution mass spectrometry is conducted in the topic ***Comprehensive Chromatographic Separation*** (Dipl.-Ing. Thomas Gröger, topic V). The research topic ***Thermal Processes & Photoionization Mass Spectrometry*** (Dr. Thorsten Streibel, topic VI) utilizes hyphenated analytical techniques between thermal methods and mass spectrometry as well as online mass spectrometric monitoring approaches for analysis of thermal processes. In the research area, ***Aerosol and Laser Mass Spectrometry*** (Dr. Johannes Passig, topic VII) single-particle laser mass spectrometry (SPMS) is performed. In addition to applications for, *e.g.*, the detection of toxic ship emissions, new laser ionization schemes are developed, involving among others a femtosecond laser spectroscopy unit. The topic of ***High Resolution Mass Spectrometry*** (Dr. Christopher Rüger, topic VIII) addresses the development and application of ultra-high resolution mass spectrometry utilizing Fourier-Transform Ion Cyclotron Mass Spectrometry (FT-ICR) and Orbitrap Mass Spectrometry. Application in the field of Aerosols and Health is accompanied by petrochemical analyses of *e.g.* ship fuels. The spin-off ***Photonion GmbH*** (Dr. Sven Ehlert, topic X) is involved in many Enabling Analytical Technologies and Aerosols and Health research activities by developing and supplying the required instrumental analytical devices.

In addition to the above-mentioned scientific tasks, Prof. Dr. Zimmermann, Dr. Haack, and Dr. Streibel, as well as the Habilitants Dr. Czech and Dr. Rüger, are involved in teaching student courses on the freshmen-, BSc- and MSc-level in Analytical Chemistry at the University of Rostock. The practical courses are organized by Dr. Sabine Haack (see figure 21) who is supported by a technician and the PhD Students at the Chair of Analytical Chemistry.

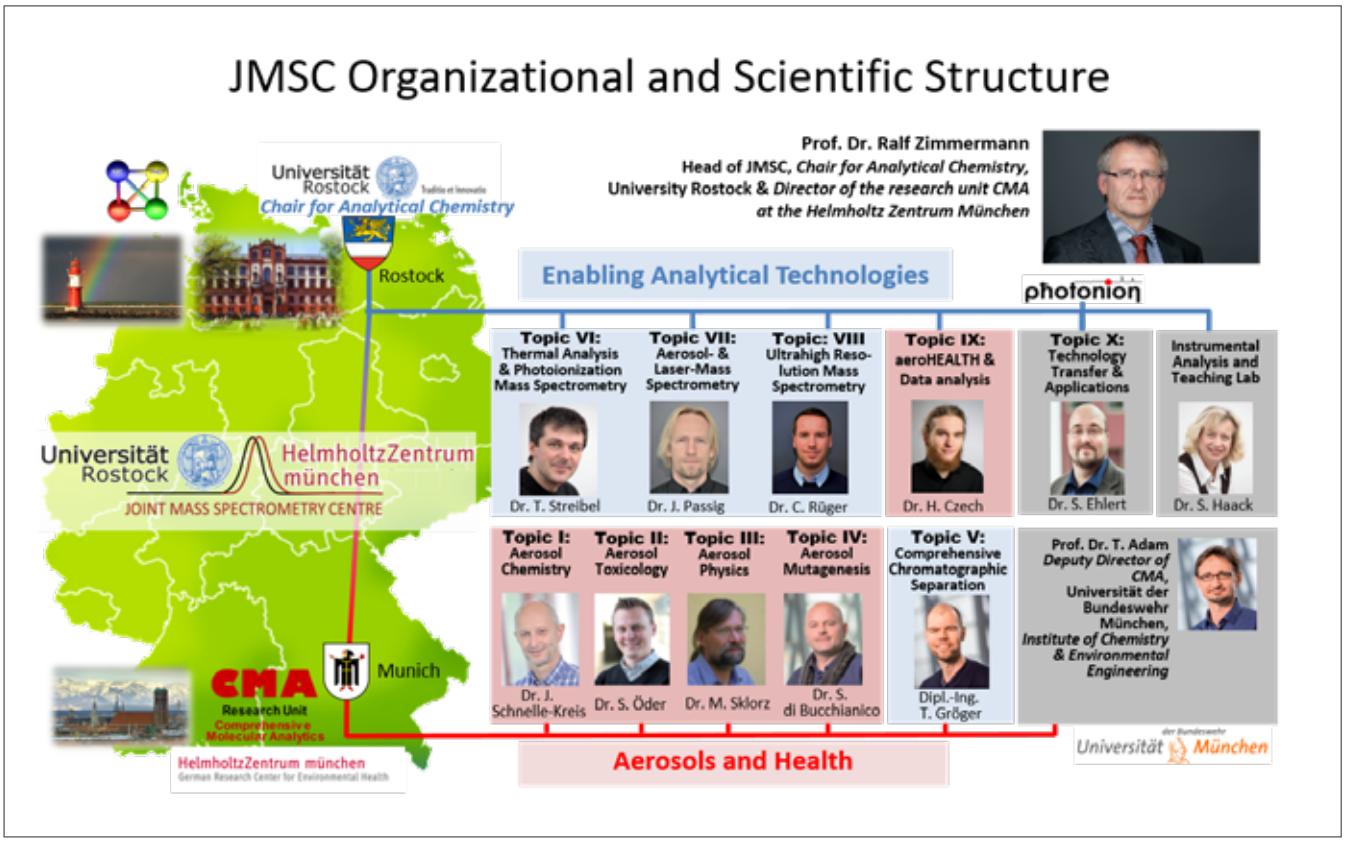


Figure 21: *Organization chart of the JMSC depicting the two general research areas* ***Aerosols and Health*** *and* ***Enabling Analytical Technologies*** *and the ten research topics with the respective topic leaders and their localization in Rostock or Munich.*

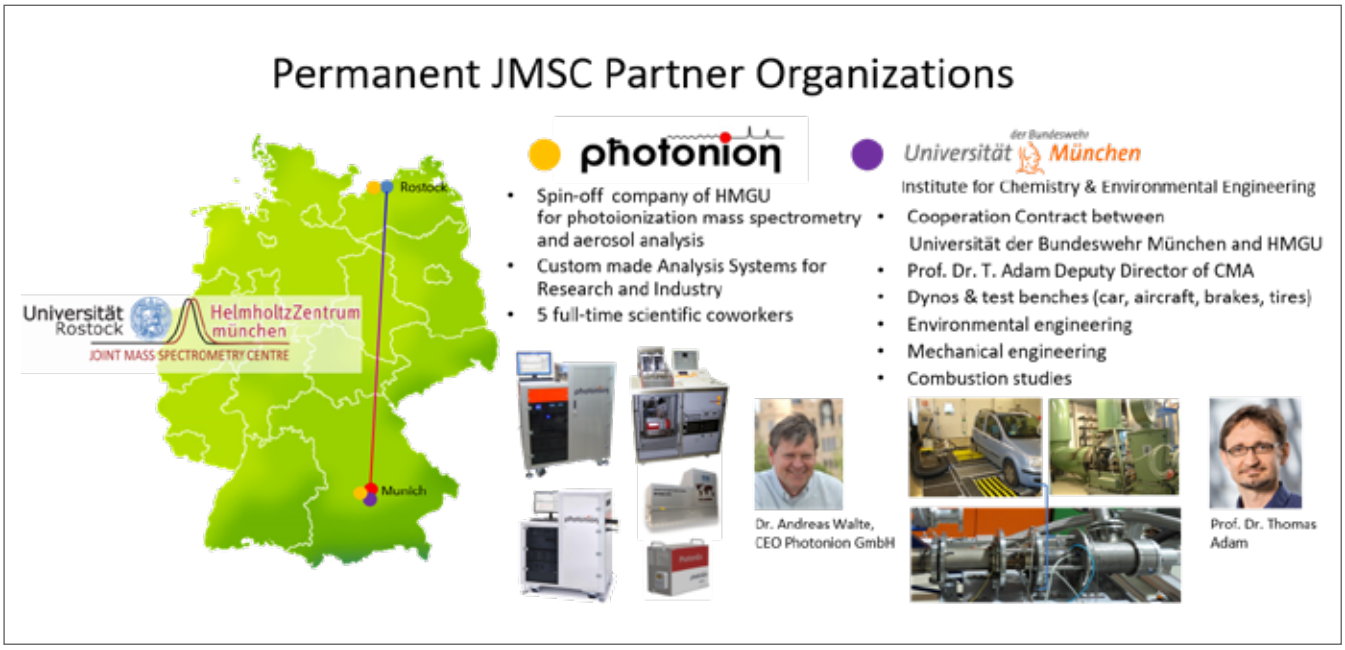


Figure 22: *Permanent partner organizations in JMSC are the JMSC spin-off* ***Photonion GmbH*** *and the* ***Universität der Bundeswehr München.***

1.7 Brief representation of the Scientific Topics

Topic I: Aerosol Chemistry

Topic Leader: Dr. Jürgen Schnelle-Kreis

Air pollution is an important determinant of human health. The effects of particulate matter (PM) on health are particularly well documented. However, the fundamental mechanisms leading to the adverse effects are not fully understood. Moreover, there is no evidence of a safe exposure level or threshold below which no adverse health effects occur.

In close cooperation with national and international research groups, the topic Aerosol Chemistry addresses priority questions that have been identified in the WHO REVIHAAP project. The focus of our investigations is on the role of the chemical fractions or metrics of PM in the formation of negative health effects. Among other things, the importance of smaller particle fractions (*e.g.* ultrafine particles), soot, chemical constituents, or source types (*e.g.* road traffic industry, PM of natural origin, primary or secondary PM) is investigated. In addition, questions related to the temporal and spatial variability of exposure are investigated, among others in cooperation with the Institute of Epidemiology of the HMGU.

The basis for the activities of the Aerosol Chemistry group is always the quantification of the chemical components of the particles and/or the gas phase of the aerosols. For this purpose, we usually use gas chromatography coupled with mass spectrometry. The chemical analytical methods are continuously developed further to be able to address current problems. The focus is on extending the list of target analytes, *e.g.* with specific markers for secondary aerosols, and on the development of increasingly sensitive methods. Sensitive quantitative methods are particularly needed to better characterize the personal exposure of volunteers in epidemiological studies, but also to determine the chemical composition of ultrafine particles in the environment, which only make up a very small part of the mass of ambient PM.

The activities of the topic Aerosol Chemistry can be roughly divided into two areas:

The first areas comprise the chemical characterization of aerosols from anthropogenic and natural sources as well as model aerosols for fundamental aerosol research and aerosol toxicological studies. Starting from fresh emissions from combustion sources (*e.g.* car engines, ship engines, biomass combustion) the focus of the investigation in this area shifts more and more to aged anthropogenic and natural aerosols. In collaboration with national (*e.g.* Forschungszentrum Jülich, Leibnitz Institute for Tropospheric Research) and international working groups (*e.g.* Paul Scherrer Institute, Switzerland, University of Kuopio, Finland) and together with the topic Aerosol Physics we investigate the changes in the chemical composition of aerosols in chamber experiments and flow tube reactor experiments. In close collaboration with the topic Aerosol Physics and the two toxicologically oriented topics (Aerosol Toxicology and Aerosol Mutagenesis), we investigate which properties and constituents of aerosols are involved in the development of diseases on a molecular level.

The second area of the topic deals with the characterization of ambient particulate matter. Numerous international studies in megacities (*e.g.* Beijing, Nanjing, China; Tehran, Iran; Moscow, Russia), small to medium-sized cities (Sernaglia della Battaglia, Italy; Augsburg, Germany), and rural regions (Preila, Lithuania; Pha Din, Vietnam), in which we have participated, investigate the composition, source fraction, and aging processes of environmental aerosols. Since 2012, ultrafine particles in environmental aerosols have increasingly become a focus of our investigations. In a first study, we investigated the composition and source fraction of urban quasi ultrafine particles in a joint project with the Institute of Epidemiology (see research area 1 (04)). The further development of the work on sampling and analysis of UFP, which was started here, is continued in the project Method optimization for chemical analysis of UFP (see third party projects (09) and (10)), which is part of the Bavarian project network „Measurement, characterization, and evaluation of ultrafine particles“.

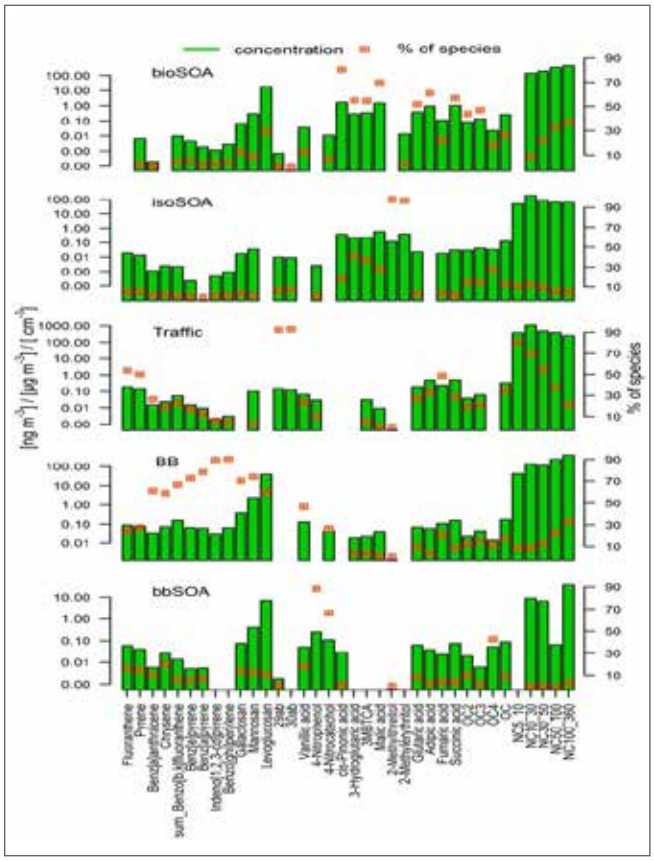


Figure 1: *Source factors contributing to ambient quasi ultrafine particulate matter. The combined PMF analysis of the chemical composition and the size distribution of the particles allows the identification of the main source contributions to the ultrafine particles.*

Topic II: Aerosol Toxicology

Topic Leader: Dr. Sebastian Öder

There is strong evidence for a correlation between air pollution and the ever-increasing amount of people suffering from respiratory, allergic, and cardiovascular diseases. The goal of topic II – Aerosol Toxicology is to understand, what components of air pollution are involved in the development and exacerbation of these diseases on a molecular level and improve the basic knowledge of aerosol toxicology. For our analyses, cells of the human respiratory system are grown in air-liquid interface (ALI) conditions, where they are supplied with nutrients and liquids only from their basolateral side and exposed to air and aerosols on their apical side. Whenever possible, we use an ALI exposure approach (*e.g.* by Vitrocell®, Waldkirch, Germany, figure 1A), to mimic the physiological conditions in the human lung and investigate the effects of environmental aerosols. However, if an experimental situation does not allow the use of the ALI exposures, we collect the particulate phase of the aerosols and use them for submerged toxicological studies. In ALI exposures, complete aerosols (including both, gases and particles) are guided onto the cells that mimic the actual aerosol exposure in the human lungs as realistically as possible and prevent the use of animal tests. The combination of classic toxicological assays with comprehensive multi-omics analyses allows us to obtain an in-depth evaluation of the biological effects of aerosol exposure. The close cooperation with the other topics within JMSC and their expertise in the mutagenic and physicochemical characterization of aerosols enables us to identify substances that are responsible for specific cellular responses and can influence environmental diseases. We want to develop strategies to prevent and improve those diseases by the targeted avoidance of these so far unknown risk factors.

Topic II is participating in several national and international projects using ALI exposure models. The newHICE project is an extension to the Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health (HICE), with the aim of finding out if the negative effects of environmental aerosol in our *in-vitro* models correspond well to the negative effects in a mouse model. Our main cooperation partners for animal studies are the Inhalation Toxicology group at the University of Eastern Finland and the Institute of Lung Biology and Disease of the HMGU (research area 1 (06)). In the Carbon Concrete Composite (C^3) project, we use a combination of human respiratory cells and fibroblasts to account for the more specific fibrotic effects of inhalable fibers. By culturing both cell types on different sides of a semipermeable membrane, only respiratory cells are in direct contact with the fibers. Any effects in the fibroblasts are thus likely to be secondary effects because of cell-cell interactions (research area 1 (03)). The main aim of the Allergy Project of the HMGU is to investigate the adjuvant and protective effects of combustion-derived air pollution as well as biologic aerosols from livestock farms. We expose respiratory cells first to combustion aerosols and then to the biologic aerosols or vice versa. This way, it becomes evident, if either exposure has aggravating or mitigating effects on the other and to what extent (see research area 1 (09)).

For some of our research projects, however, it was not possible to use the direct ALI exposure. In the wind turbine blade recycling-project we examine the toxicity of particles and fibers, which are formed in the thermal recycling process of wind turbine rotor blades at the end of their life cycle. The inhalable dust from this process will be collected in cooperation with topics VI and VII. Exposure experiments, both in submerged conditions and using the droplet settling ALI approach (Vitrocell, Cloud system) are carried out in cooperation with topic III and IV (see also research area 1 (10)). The goal of the SAARUS project *(Abgaswäsche Technologie zur Reduktion umweltschädlicher Schiffsemissionen)* is to determine, whether the use of wet scrubbers to remove sulfur from ship emissions is as efficient as using sulfur-free marine fuel. In the first stage of SAARUS, we collected particulate emissions from the combustion of six marine fuels and characterized their physicochemical properties (topic I and III). The second stage of the project includes exposure experiments with human respiratory cells to investigate the cytotoxic, genotoxic, and inflammatory potential of these particles (see report (11) in research area 1).

Even though our *in-vitro* models and exposure methods are already quite realistic, they cannot address certain negative effects of air pollution, like immunomodulation or effects on the cardiovascular or nervous system. Therefore, the development of more advanced *in-vitro* models, which incorporate also immune cells and cardiomyocytes, is another key research area within topic II. The use of a co-culture of respiratory cells and fibroblasts (figure 1B) is already well established in our laboratory, and we have also demonstrated that a triple culture of respiratory cells, fibroblasts, and immune cells can be used for toxicity testing. Upcoming projects also involve the development of an *in-vitro* cardiopulmonary model (see chapter on department LLM above). Additionally, ambient air exposures using ALI cell cultures need a cell model, which is stable and easy to maintain for long periods of time (up to 120 h). Therefore, the ALIAS-LUNG project *(Entwicklung einer Expositionseinheit zur automatisierten Langzeit-In-Vitro-Zelllexposition zur Analyse schädlicher Effekte der Luftverschmutzung auf Lungenzellen)* is targeted at developing such stable cell models and improving the technical parameters in the Vitrocell-ALI to enable these long-term exposures (third party projects (15)).

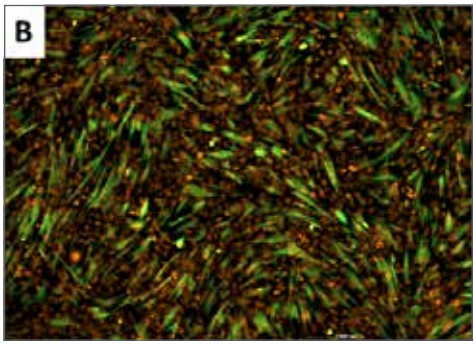


Figure 1:
(A) The Automated ALI Exposure Station (Vitrocell®),
(B) Fluorescence micrograph of a co-culture of A549 alveolar epithelial cells (orange) and MRC-5 lung fibroblasts (green) cultured at ALI conditions.

Topic III: Aerosol Physics

Topic Leader: Dr. Martin Sklorz

Aerosols are mixtures of gaseous components and particles (solid particles or liquid droplets) in the air and their effect on human health is clearly related to the chemical composition of particles and gas-phase (see Topic I, Aerosol Chemistry). Nevertheless, biological responses, as well as atmospheric transportation and inhalation properties of aerosols, depend strongly on the distribution of potentially hazardous substances between gas- and particle-phase and its physical nature (*e.g.* size, shape, or density of particles; vapor pressure, sorption equilibrium, and capacity, etc.).

The Aerosol Physics team advances research of air quality, combustion emission, atmospheric aerosols, aerosol personal exposure, occupational safety, and relevant sampling/measurement techniques. In particular, we focus on the application and method development for physical particle characterization and the investigation of gas-particle partitioning of semi-volatile organic substances. A battery of online instruments (*e.g.* for determination of particle number, size- and mobility, online mass concentration, and optical properties are applied – total organic carbon and selected gaseous components are analyzed by *e.g.* a flame ionization detector and online mass spectrometers, resp.). Sampling on impaction substrates or filters and selected absorber materials enable a further offline in-depth chemical analysis. Furthermore, sophisticated computer models were developed and applied to study particle deposition in the human lungs, animal models, and *in-vitro* incubation systems (ALI = "Air Liquid Interface").

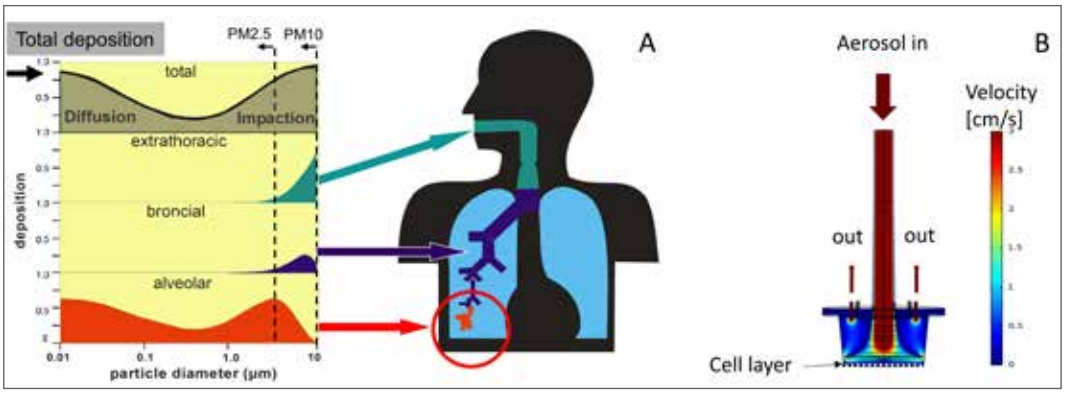


Figure 1: *(A) Particle size dependent lung deposition – model results from the "Hygroscopic Particle Deposition Model" by Ferron et al. (HPLD06 online calculator, see https://www1.helmholtz-muenchen.de/ioec/lung-deposition/hpldb06_d/index3d.php, and report (14) in Research Area 1). (B) Calculated aerosol velocity profile of the "Air Liquid Interface" (ALI) exposure unit.*

In addition, we support the JMSC with laboratory studies using several particle generators and characterize the produced model aerosol in cooperation with the Aerosol Chemistry group. In several extended off-site measurement campaigns (*e.g.* at the University of Eastern Finland or at the Engineering Faculty of the University of Rostock) aerosol diluting and distributing sampling trains were set up for the ALI exposure studies, adapted analyses, and in-depth characterization of aerosol from different emission sources and artificially aged aerosols.

In the framework of two currently granted projects of the Bavarian State Ministry of the Environment and Consumer Protection (see report (09) and (10) in third party projects), ultra-fine particles (UFP, having a diameter smaller than 100m) will be studied. Soot model particles will be generated with standardized combustion instruments, suited sampling procedures evaluated and optimized and effects of UFP towards human lung cells will be investigated by *in-vivo* cell exposures.

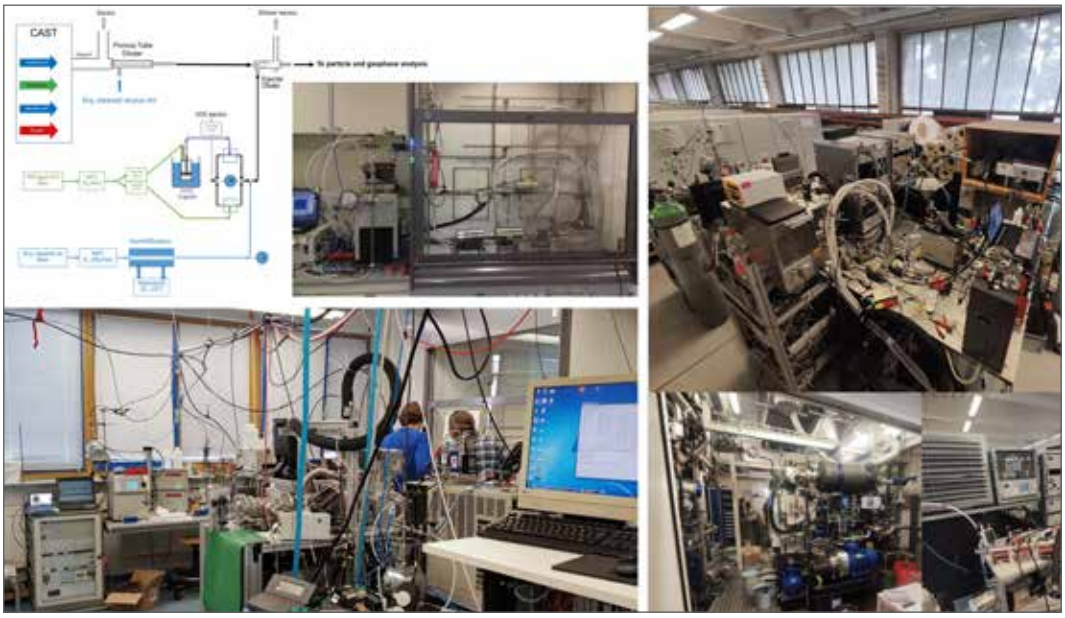


Figure 2: *Some impressions of laboratory-scale aerosol generation and characterization for workplace exposure investigation (see report (20) in third party projects), during the first aeroHEALTH campaign (see reports aeroHEALTH 4 to 8) in our lab and as well as an off-site measuring campaign for the investigation of ship diesel emissions from various heavy fuel oils at the University of Rostock (see report (11) in Research Area 1 and (17) in third party projects).*

Topic IV: Aerosol Mutagenesis

Topic Leader: Dr. Sebastiano Di Bucchianico

A new research topic was introduced in 2019 to the CMA research area 1, Aerosol and Health, with a focus on the mutagenic potential of aerosols. Air pollution affects human health, both in the short and long-term exposure, resulting in persisting inflammation that may have a number of associated consequences including oxidative burst. Deriving reactive oxygen and nitrogen species may interact with nucleic acids producing DNA or chromosomal rearrangements that can increase the spontaneous mutation rate. However, not only DNA instabilities act as direct pro-mutagenic lesions, but oxidation products may also lead to secondary genotoxicity and pro-mutagenic effects. Primary genotoxic actions can be elicited also by particle uptake, and/or aerosol gas phase, and subsequent direct or indirect interactions with DNA in the absence of inflammatory responses. Conversely, secondary genotoxic mechanisms imply DNA damage resulting from oxidative DNA insults generated during inflammatory processes, which play a central role in several lung diseases.

In this scientific context, the objective of our research aims to identify and characterize the connection between exposure to aerosols and mutagenesis and lung diseases. We are also engaged in studies into genotoxic and mutagenic effects of nanomaterials, both in terms of respirable nanoparticles or fibers and in terms of engineered nanomaterials for nanomedicine. We work with both submerged and Air Liquid Interface (ALI) respiratory models, linking OECD recognized testing methods with refined cell culture model systems to increase our insight into the genotoxic, epigenotoxic, as well as the mutagenic potential of aerosols and nanomaterials possibly affecting human health. The analysis and integration of transcriptome and proteome data are used to provide broad-scale insights into molecular mechanisms of aerosols-induced effects to identify lung diseases associated genes and biomarkers.

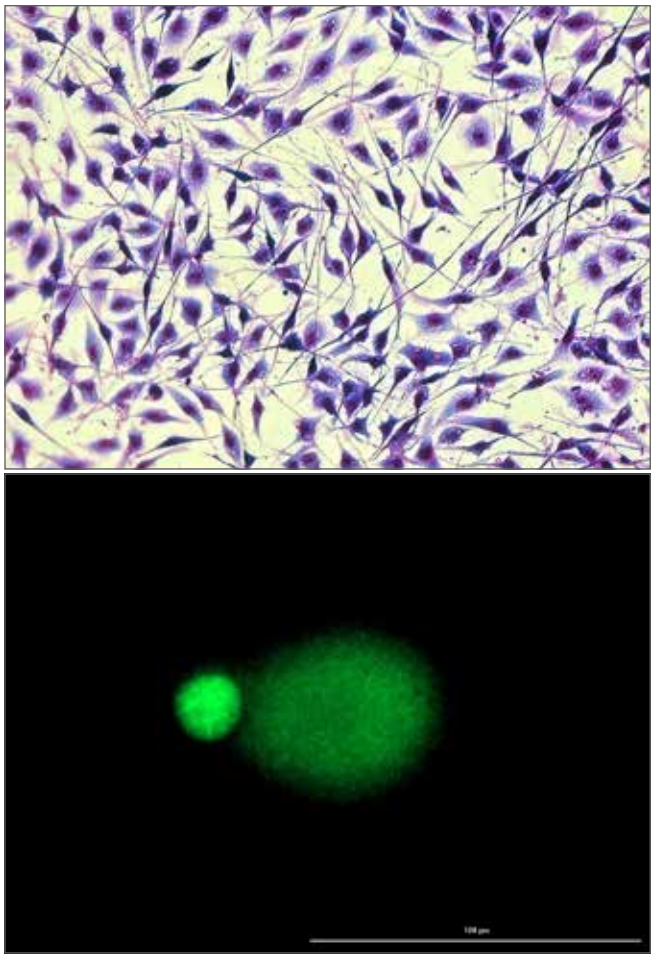


Figure 1: *On the top Human bronchial epithelial BEAS-2B cells forming a colony to assess the clonogenic potential of aerosols exposure. On the bottom DNA damage as assessed by Comet assay.*

Our research, in close collaboration with the other JMSC and HMGU research groups, has a strong international dimension, with the participation in international consortia funded by the European Commission (see report (08) in third party projects), the German-Israeli initiative aeroHEALTH with the associated partners Forchungszentrum Jülich (Germany) and Weizmann Institute of Science (Israel) (see reports in the aeroHEALTH section), as well as German and Bavarian funding programs (see report (08) in research area 1 and (03) in third party projects). In the newly funded EU-project ULTRHAS (see (01) in third party projects) as well as in projects funded by the Bavarian Ministry of Environment and consumer protection (see (10) in third party projects) deal with the cyto- and genotoxic effects of nanoparticles from traffic sources and a soot model generator.

Progress within the Aerosol Mutagenesis topic field will lead to an improved understanding of (epi)-genotoxic, mutagenic, and carcinogenic effects of environmental pollutants, including their potential adverse effects after atmospheric aging of aerosols. By using aerosol generators and ALI exposure systems it is also investigated whether gas-particle partitioning influences the mutagenic potential of semi-volatile organic compounds as well as how the persistency of oxidative stress and induced DNA damage may lead to a higher potential risk for human health.

Topic V: Comprehensive Separation

Topic Leader: Dipl.-Ing. Thomas Gröger

The Research Topic "Comprehensive separation" is performing research at the interface of the two main research areas "Enabling Analytical Technologies" and "Aerosols and Health" of the JMSC. The scientific goals are the development and translation of new and state-of-the-art analytical instrumentation and methods for their application in aerosol and health and other areas of environmental health. Key techniques are comprehensive two-dimensional gas chromatography (GC×GC) in combination with highly selective spectroscopic and mass spectrometric detection systems. Besides these, also thermal sample introduction techniques like direct insertion probe (DIP) and different thermal analysis (TA) approaches in combination with soft ionization techniques are established and utilized to expand the applied gas-phase ionization interfaces towards less- and non-volatile matrices. A major field of interest is also comprehensive and state-of-the-art data processing and data analysis of the generated multidimensional data.

Topic V offers a wide range of projects with a clear focus on the priorities of the Helmholtz Zentrum München and the JMSC. Internal and third party funded projects in the last years focused on diabetes research, allergy research, and aerosols and health. These interdisciplinary studies always aimed to bridge the gap between chemical analysis and biological response. In this context, tight interaction with other institutes on the HMGU Campus but also the JMSC topics "Aerosol Toxicology" and "Aerosol Mutagenesis" become more and more important.

A project on breath gas analysis in a diabetes study was successfully completed and a method for non-invasive respiratory gas testing using online proton transfer reaction mass spectrometry PTR-MS and NTD-GC×GC-TOFMS/VUV was presented and tested on individuals. Another study in the field of aerosols and health, which started in the reporting period, builds on a study on the chemical analysis of primary anthropogenic aerosols and their toxicology, which was successfully completed within the HICE project. The new study (see report 6 in the aeroHEALTH section) is part of the aeroHEALTH project (see (08) in third party projects) and focus on the formation of secondary aerosols, which are initially generated from individual precursors compounds in the laboratory and will be later investigated in large chamber experiments. As in the past, this study is interdisciplinary and will investigate the complex chemical composition of the aerosols based on GC×GC to enlighten and link possible health effects. In a third study (see report (09) in Research Area 1), different expertise within the JMSC as well as other institutes of the HMGU are merged to investigate the protective and adjuvant effects of bio- and anthropogenic aerosols in allergy research. Aerosols from a farming environment and aerosols from combustion processes will be chemically and toxicologically investigated in order to derive a link on immune-modulating effects.

Other more technologically/methodically orientated projects are based on close cooperation with industrial or medium-sized partners and are financed either by support programs for medium-sized companies or by direct industrial funding. These projects have a clear focus on the application of our state-of-the-art or novel analytical techniques towards highly complex matrices. In this context, petroleum-derived matrices, such as fuels, are acting as model substances (figure 1) to further develop the established techniques towards more sensitivity and selectivity (*e.g.* report (05) in research area 2). The aim is the later application of the developed techniques for aerosol and health research. An example of such successful cooperation and the subsequent translation of the field of application is the establishment of an analytical platform for the investigation of less volatile and extremely complex petroleum products. The platform allows the simultaneous application of different thermal methods like direct inlet probe (DIP), thermal analysis (TA) or high-temperature GC×GC coupled to a high-resolution time-of-flight mass spectrometer (see report (29) in third party projects).

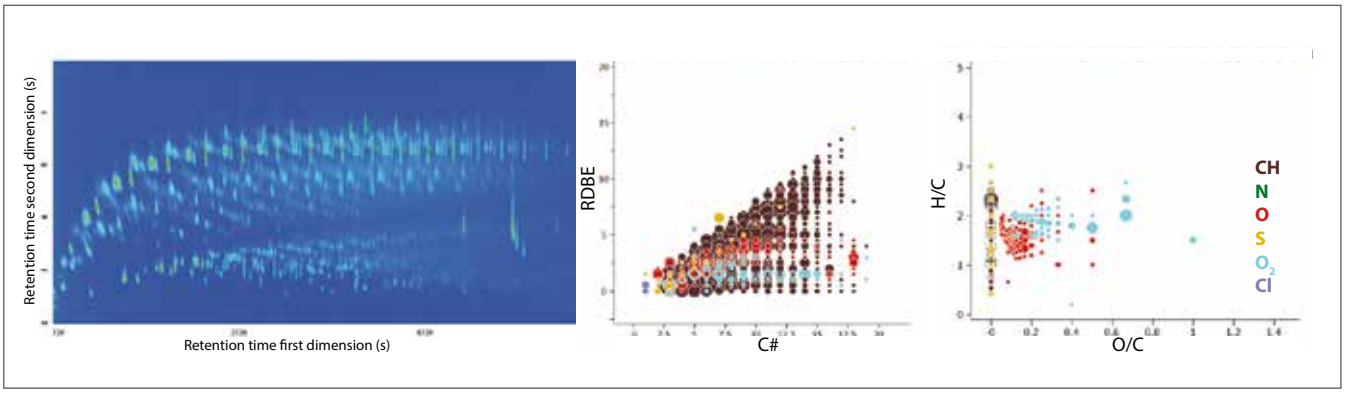


Figure 1: *The hyphenation of comprehensive two-dimensional gas chromatography with fast high-resolution time-of-flight mass spectrometry combines the strengths and selectivity of both techniques. GC×GC allows the separation of thousands of isomeric and isobaric compounds (left) and a subsequent quantification of a middle distillate. Mass spectrometric information on these compounds allows the calculation of the elemental composition and further classification (middle: ring and double bond equivalent; right: van Krevelen Plot).*

This successfully established platform will be applied in a new project to investigate pharmaceutical raw materials and identify pharmacologically active ingredients with similar complexity as petroleum-based matrices (see report (13) in third party projects). In this context, the cooperation with the manufacturers of analytical systems makes a decisive contribution to maintaining the analytical-technical level and the availability of state-of-the-art analytical platforms at the JMSC and HMGU. A unifying feature of all these projects, however, is the methodical approach of non-targeted and comprehensive chemical analysis.

Topic VI: Thermal Analysis and Photoionization Mass Spectrometry

Topic Leader: Dr. Thorsten Streibel

Thermal processes such as combustion and pyrolysis rank among the most important technical procedures with numerous applications for energy production, engine operation, and chemical product manufacturing. As a negative side effect to these processes, the formation of organic trace compounds, inorganic compounds, and particulate matter (PM) occurs. Therefore, a better understanding of the mechanisms that take place on a molecular level during the sequence of a thermal process bears a high relevance for prevention and remediation policies as well as optimization strategies. Moreover, combustion and pyrolysis processes exhibit a high dynamic behavior leading to rapid changes of concentration profiles within the reaction zone as well as in the exhaust or pyrolysis gases. Analytical methods providing fast means of detection are therefore required.

Photoionization mass spectrometry employing time-of-flight mass analyzers (PI-TOFMS) is such a feasible tool for the characterization of thermal operations. PI-TOFMS enables the fast generation of complete mass spectra with time resolutions below one second. Moreover, it counts among the soft ionization mechanisms in mass spectrometry minimizing fragmentation considerably. This proves advantageous when considering the large number of different organic trace compounds that can be present in exhaust gases, avoiding overlapping fragment ions.

The second route to unraveling the mechanisms of thermal processes consists in the analysis of the behavior of the fuels when subjected to thermal load. Methods of thermal analysis (TA) such as thermogravimetry and calorimetry are applied for these tasks. They are also hyphenated to PI-TOFMS providing evolved gas analysis of the evaporated chemical species. This concept can be extended to the investigation of the chemical composition of PM from combustion-related emissions. Particles sampled on filters are placed in an oven and subsequently heated to different temperatures under inert conditions. The evaporating organic compounds are guided to the PI-TOFMS system.

Special importance has to be attached to the sampling process for the investigation of the resulting rather complex gaseous mixtures. Cold spots in the sampling line have to be avoided to prevent condensation of semi-volatile substances and to ensure a representative mixture in the detection system. Heated interfaces and transport capillaries are therefore mandatory.

Both general types of measurement systems have been applied for the characterization of a variety of technical processes. This comprises online analyzes of the exhaust gases from a research ship diesel engine operated with diverse heavy fuel oils and distillate fuels. Crude oils and mineral fuels, the latter partially blended with biofuels were investigated with the TA-PI-TOFMS coupling (reports (11) in research area 1). Particles emitted from the combustion of ship diesel and passenger car gasoline engines as well as different wood types are characterized for their organic composition by thermal desorption coupled to photoionization mass spectrometry (report (07) in research are 2). Figure 1 depicts the different product patterns for three wood feedstocks. Solid materials, such as glass and carbon fiber composites, constitute a further object of scrutiny. The thermal behavior of such materials is of importance with respect to the possible formation of asbestos-like fibers after thermal treatment (reports (03) and (10) in research area 1). Finally, in the field of method development, an extension to liquid samples was undertaken. Aromatic compounds in the aqueous environment were detected utilizing a membrane inlet by extracting the organic substances by permeation through a PDMS membrane (see Third Party Project 30).

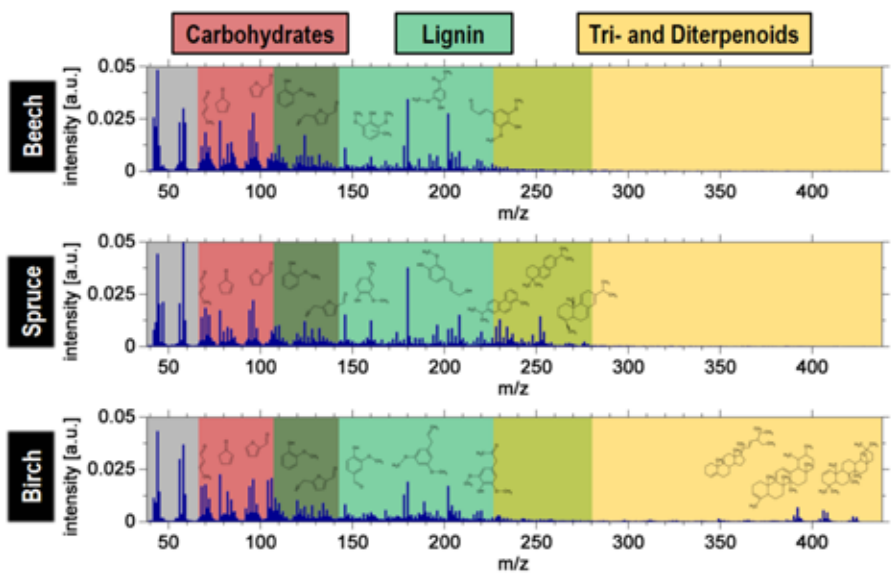


Figure 1: *Mass spectra generated by thermal desorption of PM from the combustion of beech, spruce and birch wood with subsequent detection by single-photon ionization-TOFMS. Carbohydrates from cellulose pyrolysis and phenolic species from lignin decomposition are visible. A distinctive feature is the appearance of terpenoids with birch PM.*

Topic VII: Aerosol- and Laser Mass Spectrometry

Topic Leader: Dr. Johannes Passig

Air pollution is one of the largest environmental risks to human health. The global deaths caused by exposure to ambient particulate matter with a diameter less than 2.5 µm (PM2.5) reached 4.2 million in 2015 and is further increasing with the rapid development of mobile industrial societies. However, current health risk assessment is still based on vastly simplifying parameters such as PM2.5 mass or particle number concentrations, discounting the enormous chemical and physical complexity of atmospheric aerosols. A substantial part of the health effects from air pollution is associated with toxic trace compounds, *i.e.* particle-bound carcinogenic polycyclic aromatic hydrocarbons (PAHs) as well as heavy metals and transition metals. An underestimated factor is the distribution of the harmful substances on the individual particles. Conventional methods cannot determine whether all particles contain low concentrations yielding a tolerable total dose or whether few particles could produce a high local cellular dose upon lung deposition (*i.e.* the internal or external mixture). Therefore, we believe that the single particle resolved analysis may be decisive to understand the surprisingly high toxicity of aerosol from many anthropogenic sources. Moreover, observations of the pollutant distribution, transport pathways, and degradation are crucial for risk assessment and mitigation, but so-far limited by technical challenges.

Topic VII establishes a basis for future health risk assessment and mitigation strategies by developing real-time technologies that acquire chemical information from individual, fine, and ultrafine particles. Key is the understanding and optimization of complex laser-matter interactions for efficient and non-destructive ablation, ionization, and detection of health-relevant trace components from particles. Several approaches have been developed, exploiting resonances between laser radiation and molecules or atoms to detect such species with masses in the order of 10-16–10-12 g per particle. In cooperation with the Institute of Physics in Rostock, the use of laser pulses with femtosecond duration is investigated. Such pulses interact with the particle on a timescale shorter than molecular vibration. It has been shown that they can ionize intact biomolecules from surfaces, which has so far not been realized for particles and bioaerosols (*e.g.* viruses, bacteria). All developments of Topic VII are demonstrated in real-life scenarios and measurement campaigns, providing *e.g.* the first single-particle distribution of PAHs in ambient air or revealing the sources and carriers of biologically relevant metals in the atmosphere, see figure 1.

The unique technologies of Topic VII are involved in many measurement campaigns of JMSC and cooperation projects, e.g. aeroHEALTH (see report (19) in third party projects), SAARUS (see report (17) in third party projects) LUKAS (see third party projects (06)) and MORE (see third party projects (11)). With their laser- and mass spectrometry expertise, all members of Topic VII provide technical assistance for other JMSC topics using comparable technology, in particular, Topics VII-X, AMMOTRACe (see third party projects (02)), ULTRHAS (see third party projects (01)). Topic VII focuses on streamlined dissemination of developments from the lab into real life, striving for rapid benefits for public health. Therefore, close cooperation with the JMSC spin-off Photonion (Topic X) already led to the development of commercial single-particle instruments and patent applications. Beyond that, Topic VII contributes the scientific part in several industrial projects with background in public health and civil safety, such as ZIM and KMU projects (Ship Profiler – report (05) third party projects, HazarDust – report (07) third party projects).

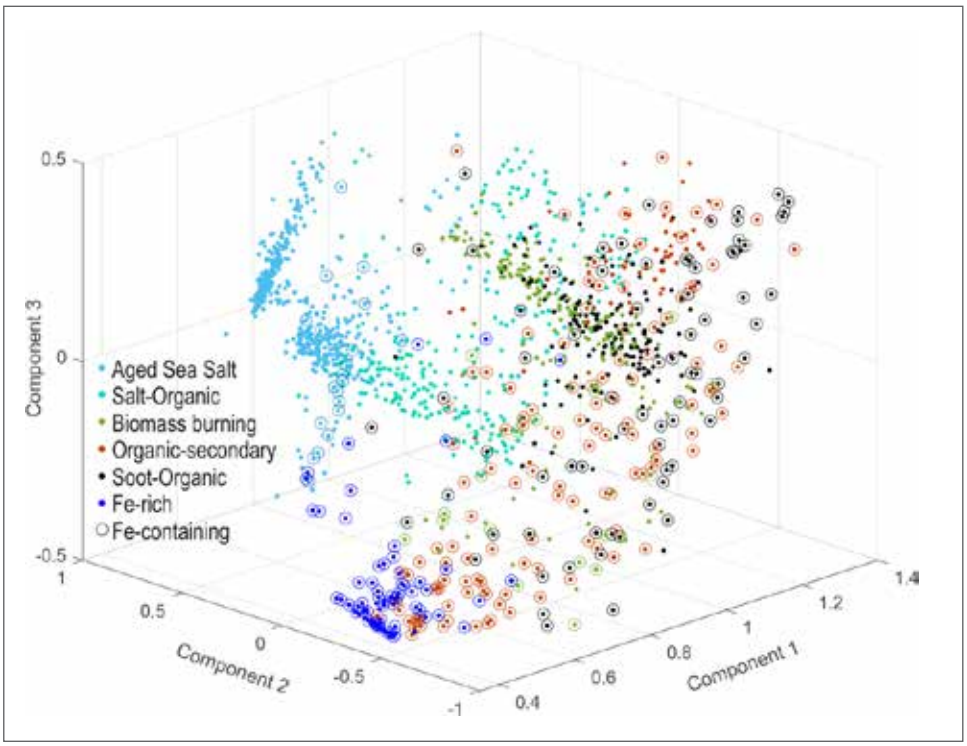


Figure 1: *Principal component analysis of single-particle chemical profiles from background aerosol measured at two days in autumn at the Swedish coast. The particles were classified from mass spectrometry data using a neuralnetwork algorithm (ART-2a). Exploiting laser-atom resonances, our new ionization approach reveals that organic aerosols from industrial/traffic combustion dominate the transport of biologically relevant iron (circulated) – affecting marine communities and human health. However, also mixtures with sea salt contribute to Fe transport. For details see Passig et al., Atmos. Chem. Phys., 20, 7139–7152, 2020.*

Topic VIII: Ultrahigh Resolution Mass Spectrometry

Topic Leader: Dr. Christopher P. Rüger

Chemical description of complex organic mixtures requires powerful analytical platforms. High-resolution mass spectrometry has proven to be a suitable approach for addressing the compositional space on the molecular level. High mass accuracy and mass resolving power allow attributing elemental compositions with sub-part-per-million (ppm) accuracy at the same time separating the vast complexity of signals at the mass-to-charge dimension (figure 1). Specific ionization schemes, such as laser-based photoionization, and coupling techniques, such as gas chromatography, allow targeting certain parts of the chemical space and adding further valuable structural information. Topic VIII ultrahigh resolution mass spectrometry summarizes the efforts of the JMSC in the field of Fourier transform mass spectrometry with a variety of instrumental developments (enabling technologies) as well as application fields.

The Topic VIII is working on multiple high-performance mass spectrometric platforms from multiple vendors. Central instrumentation is a Bruker solariX/apex Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) equipped with a 7 Tesla superconducting magnet. The platform consists of two mass spectrometer units: 1) a conventional solariX FT-ICR MS equipped with several direct infusion techniques (electrospray, atmospheric pressure chemical and photoionization (APCI/ APPI) and a matrix-assisted laser desorption ionization (MALDI) unit and 2) a modified apex FT-ICR MS coupled to either gas chromatography or thermogravimetry with APCI, APPI and atmospheric pressure laser ionization (APLI, Nd:YAG 266 nm, fluorine excimer 157 nm). The FT-ICR MS platform easily delivers resolving powers above 300,000 at *m/z* 400 with < 1 ppm mass accuracy. Moreover, Topic VIII runs two Orbitrap FT MS systems (a LTQ XL and an Exactive Orbitrap) and an Agilent gas chromatography mass spectrometer (GC-QMS) with electron ionization and PAL autosampler.

Apart from FT MS as linking mass spectrometric technology, the approaches and projects in Topic VIII are very diverse. In the field of *ambient air pollution and combustion emissions*, Topic VIII is involved in the aeroHEALTH project (see chapter 4), a DFG/ Russian Science Foundation project on wildfire emissions (see third party projects (14)), a DFG/ French Agence Nationale de la Recherche (ANR) project (see third party projects (04)), and the SAARUS project on shipping emissions (see third party projects (17)). Briefly, extraction procedures are developed and deployed for direct infusion electrospray measurements enabling to characterize high-polar constituents. The application field of petroleum and recycling is covered by the characterization of petroleum-based pharmaceutical products (Ichthyol cooperation (see third party projects (13)), the investigation of the pyrolysis behavior of composite materials and polymers and several projects focusing on deposits and fossil-, bio and pyrolysis oils (see third party projects (12)). The section *enabling technologies* is covered by two main attempts: 1) evolved gas analysis hyphenation, such as thermogravimetry (see Figure 2) or gas chromatography, and 2) laser-based photoionization. The latter is conducted within a European EUROSTARS project AerOrbi implementing laser photoionization at an Orbitrap high-resolution mass spectrometric platform (see third party projects (22)). The different sub-topics and application fields are connected through the joined development and optimization of the in-house software platform, which is also used in research projects at several other external partner institutions, such as the University of Eastern Finland, the High Magnetic Field Laboratory (Florida, USA) the University of Rouen (France) (see third party projects (23)) and the aeroHEALTH Helmholtz International Lab partner Weizmann Institute of Science (Rehovot, IL).

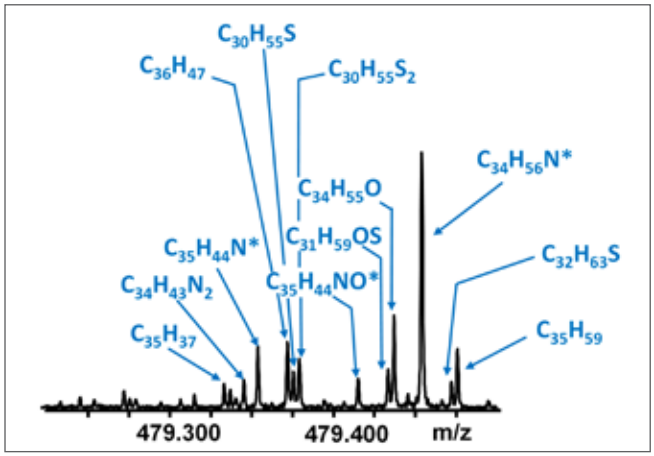
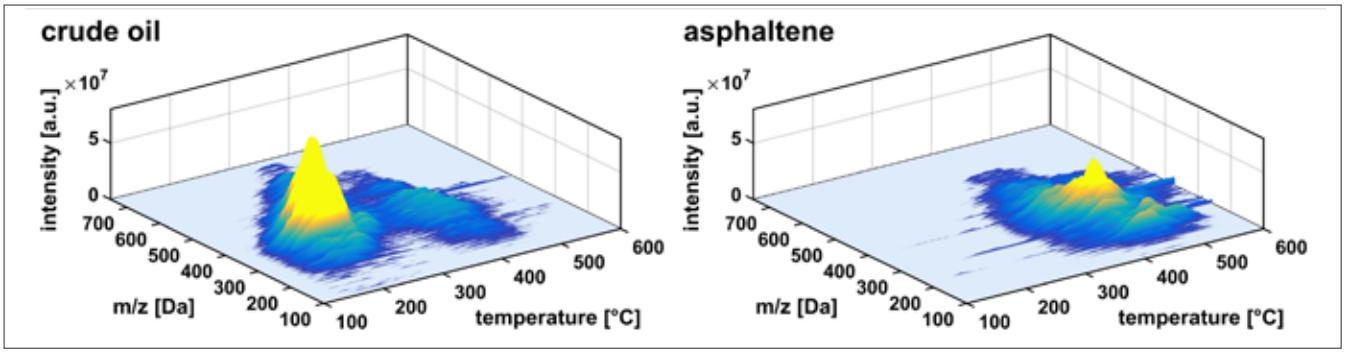


Figure 1: *Exemplary excerpt to ~250 mDa of a complex Petroleum sample investigated with FT-ICR MS. Attributed elemental compositions are given.*

Figure 2: *Survey diagram (temperature versus m/z) for a crude oil and asphaltene (high-aromatic fraction) investigated with thermogravimetry coupled to ultra-high resolution mass spectrometry equipped with soft atmospheric pressure chemical ionization preserving the molecular pattern (TG APCI FT-ICR MS). At elevated temperature pyrolysis products of larger constituents can be depicted.*



Topic IX: aeroHEALTH and Data Analysis

Topic Leader: Dr. Hendryk Czech

With the return of Dr. Hendryk Czech from his postdoc at the University of Eastern Finland and the inauguration of aeroHEALTH, the new topic aeroHEALTH and Data Analysis was launched at JMSC in 2019. The German-Israeli Helmholtz International Lab aeroHEALTH seeks to investigate and unravel complex relationships and mechanisms between physicochemical properties of primary and secondary aerosols and negative health effects arising from air pollution. aeroHEALTH is based on a holistic research approach combining the fields of measurement techniques and cell exposure systems, simulation of atmospheric chemistry in oxidation reactors, biological analysis from assay to multi-omics level and ultimately sophisticated data integration techniques. In addition to his responsibility for the aeroHEALTH workpackage "aerosol exposure" and characterization enabling a deep and comprehensive real-time view into dynamic aerosol systems, Dr. Hendryk Czech acts as project manager and mediator between the different fields in this highly multi-disciplinary project.

The second part of topic IX takes account for the research at the University of Rostock for "Habilitation", covering a broader range of research areas and topics at JMSC, such as (a) Aerosol Chemistry, (b) Laser Mass Spectrometry, and (c) Technology-Transfer and Application-oriented Research under the umbrella of Photonion, which are connected by advanced strategies of data analysis and chemometrics. It involves the application of basic uni- and bivariate statistics for hypothesis tests, regressions and error estimates, but also their generalization to multivariate analyses, machine learning and prediction modelling as well as deep learning techniques by means of artificial neural networks.

(a) A significant amount of aerosols relevant for human health is emitted by combustion, such as road traffic, maritime traffic, residential heating, and industrial production as anthropogenic sources and wildfires as example for a natural source. In order to identify relevant emission sources of an area, primary combustion aerosol sources are studied to compare their chemical fingerprints by pattern recognition and feature extraction techniques for the detection of chemical markers. Quantitative source contribution may be obtained by fitting of individual source profiles to the composition of ambient particulate matter or mathematical deconvolution.

(b) Single-particle mass spectrometry generates high amounts of data even at clean ambient air conditions. Mass spectra of particles are examined regarding their chemical similarities by clustering techniques which are based on different principles, for example hard clustering such as k-means with an unambiguous group membership of an object or soft clustering such as fuzzy c-means. Furthermore, relations between individual clusters are educible by hierarchical clustering. However, non-linear relations between single-particle mass spectra are better addressed by more sophisticated clustering techniques such as density-based spatial clustering of application with noise or adaptive resonance theory neural networks.

(c) Process control is essential for industrial production to maintain a constant and high level of product quality. Due to complex flavor formation mechanisms, coffee roasting is rather considered as art than trade. With multivariate statistics of data from photoionization mass spectrometric analysis of the coffee roast gas, it is possible to group and assign individual roast gas components according to their appearance during the roast. Ultimately, coffee properties relevant for the taste, human health, or simply reproducibility of the roasting, such as sensory attributes, polyphenolic content or roast degree, can be modeled and predicted from an on-line analysis at a time resolution of few seconds.

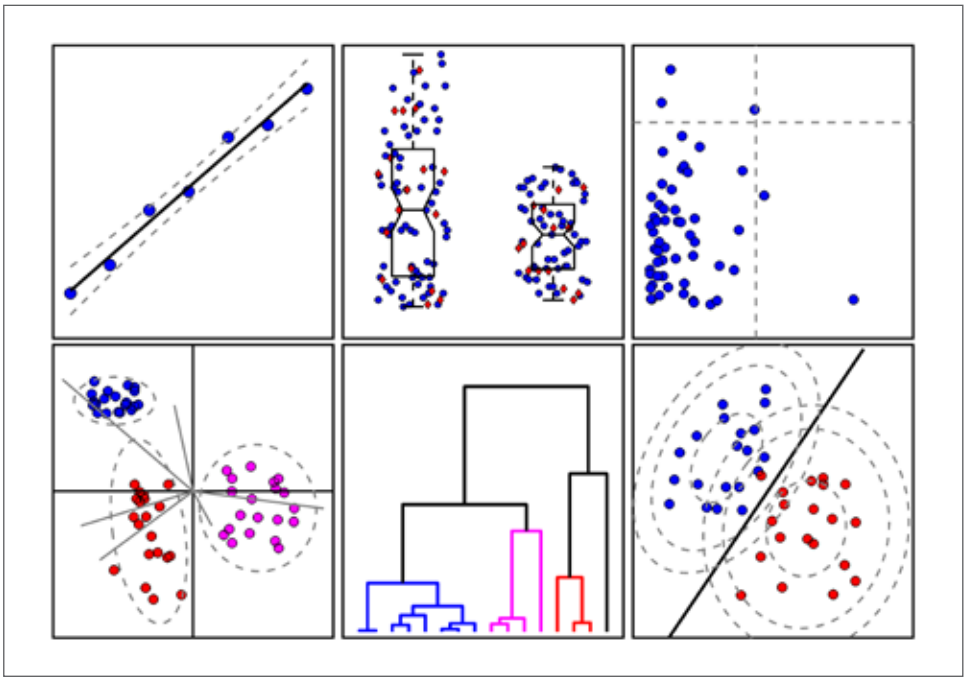


Figure 1: *Schematic representation of uni- to multivariate data analysis with supervised and unsupervised machine learning techniques and error estimation. Black lines represent functions from regression or discriminant analysis, dashed lines indicate error estimates, dots symbolize individual data points and colors refer to group memberships.*

Topic X: Transfer of technology and application-oriented research

Topic Leader: Dr. Sven Ehlert

Depending on the complexity and dynamics of the on-line monitored chemical process, robust and powerful analytical approaches are required for both, industrial or field measurement applications. With respect to the application and the research focus, different parameters as reaction kinetics, spatial or temporal resolution are of particular interest. We use photoionization mass spectrometry together with innovative sampling technologies to meet this challenge. The field of application ranges from the investigation of coffee roast gases to the smoke/vapor from smoking products, to the detection of security-relevant substances.

Figure 1: Coffee research scale-up from laboratory to industry using photoionization mass spectrometry for online investigation of coffee roast gases.

Photonion GmbH (Germany) is a company dedicated to providing customized solutions for the monitoring of chemical compounds and was founded in 2009. The portfolio of Photonion compromises powerful analysis devices for the chemical characterization of gases, liquids, and solids. The systems are based on mass spectrometric technology utilizing soft ionization methods as a key technique, in particular, photoionization approaches. Photonion is active in various sectors, which cover the identification of chemicals released during thermal analysis, standalone systems based on photoionization mass spectrometry are delivered as well as offering customized solutions, in terms of commissioned research.

One important application of the photoionization mass spectrometry (PIMS) gas-phase measurement in industry and research is the coffee roast gas analysis. Coffee is one of the most economically valuable beverages in the world. So the interest in understanding the roasting process is not surprising. During the roasting process, the green beans are transformed into roasted coffee, the basis of coffee enjoyment. In addition to the understanding of the process, the aim is of course to be able to adjust and influence this process in a targeted manner. At this point, on-line measurement (monitoring) is connected to the real-time process control.

The example of coffee roasting also illustrates the need to develop high-performance sampling strategies and methods. It is fundamental wisdom that analytical methods always create just a picture of reality. Now one can try to complete this picture with intelligent and powerful systems in order to validate the reliability of the results and the resulting conclusions. However, industrial processes also limit the number of procedures and methods. Parameters such as simplicity and robustness also play a role. The strategy in our research and development is now to use as much as necessary to obtain as much valid information as possible. The entire process from sampling and analysis to statistical evaluation of the results needs to be designed, developed, and evaluated. If the potential of a method can be confirmed, the next step is to prepare for commercialization. In the meantime, online coffee roasting gas analysis using photoionization mass spectrometry is also used in industrial R&D practice.

In cooperation with the other research topic areas, we support further industrial research projects, *e.g.* around the topic of fossil fuels. An actual project together with Topic VII is the development of an online single-particle mass spectrometer to detect dust particles carrying security-relevant substances. This security technology should be applied for monitoring freight shipments and luggage at airports. The aim of the "HazarDust" project is, to make the detection faster, more reliable, and less dangerous for the respective security personnel (see third party projects (07)). In addition, the collaboration with Topic VII generally covers developments regarding the online analysis of environmental aerosols using Single Particle Mass Spectrometry, a focus of the work at Photonion as well. A joint research project, LUKAS, for example, deals with the analysis of aerosol particles released from ship engines into the environment (see third party projects (06)). In addition to the topic related research work, it is always about the further development of measurement technology.

The further development of measurement technology also plays an important role in other applications in particle and gas-phase analysis. Regarding filter collected environmental particle samples the development and application of comprehensive mass spectrometric technologies such as TOCA-PIMS (Total Organic Carbon analyzer coupled to Photoionization Mass Spectrometer) is the key (see Third Party Projects (32)). Another technology under development to support time-resolved online mass spectrometric analysis is the FastGC approach. As a modular system, it can be applied to various systems and applications. It supports GC separation at a time scale below 30s full cycle time (see *e.g.* research area 1 report (19)).

In addition to academic research, technology transfer plays an important role. The further commercialization will then be handled in cooperation with our spin-off Photonion GmbH. Generally, the research in Topic X is third-party funded.

1.8 Historical outline on the Joint Mass Spectrometry Centre (JMSC) on the occasion of the 10th JMSC anniversary in 2018

Preamble:

In 1995, Dr. Zimmermann's working group at the Helmholtz Zentrum München started to conduct research and developments on the research field of instrumental analytical chemistry as well as on the research field of aerosols and fine dust, utilizing state-of-the-art mass spectrometry. This already resembles the current research foci "Aerosols and Health" and "Enabling Analytical Technologies" of the JMSC. Within the HMGU's focus on environmental health and diseases, the research area "Health impact of air pollution and aerosols" gained more importance over the years as a highly relevant, classic environmental health topic, which ideally combines both, the health and the environmental research expertise at HMGU. Furthermore, the research area is also well reflecting the "Helmholtz mission" of the Helmholtz Association of German Research Centers (HGF). In this sense, the orientation and vision of the joint research activities in JMSC have been continuously adapted and further developed. The cooperation of the Helmholtz Zentrum München (HMGU) and the University of Rostock (UR) was contractually set forth in December 2007 according to the model of a joint professorial appointment (*i.e.* "gemeinsame Berufung") and started to work in April of 2008 under the name "Joint Mass Spectrometry Centre (JMSC) of UR and HMGU.

The 10th anniversary of the HMGU-UR cooperation and the JMSC in April 2018 is covered by the reporting period of this progress report. Thus, in the following, a brief historical outline of the HMGU-UR cooperation and JMSC activities is given. In the framework of the cooperation, the concept to combine biological, medical, toxicological, environmental, and engineering research in a multidisciplinary approach to better understand the biological effects and mechanisms of the health effects of nanoparticles, combustion emissions, and photochemical SMOG was developed. The success of this strategy is demonstrated, among other things, by the acquisition of a HEF Helmholtz-Enterprise-Funds grant for the realization of the technology spin-off Photonion GmbH (2009, www.photonion.de) as well as the successful recruitment of the international, virtual Helmholtz Institute „HICE – Aerosol and Health" (2012–2017, https://www.helmholtz-muenchen.de/cma/HICE/) and the Helmholtz International Lab „aeroHEALTH" (2019–2027, www.aeroHEALTH.eu) at the HGF.

The development of the cooperation between the Helmholtz Zentrum München and the University of Rostock around the research activities of Prof. Dr. Zimmermann is presented chronologically. Please note, that the „Helmholtz Zentrum München, German Research Centre for Environmental Health" (HMGU) was known as „GSF – Research Centre for Environment and Health" before it was renamed in 2009. For simplicity, the name „Helmholtz Zentrum München" or HMGU is used throughout. Personal resources at the HMGU are stated as scientist equivalent positions.

The chronological sequence of events:

(a) Pre-history, induction of the work field at the Helmholtz Zentrum München

- **Since 1995 – Research Group at HMGU:** Dr. Zimmermann was habilitation candidate at the Technical University Munich (TUM) and led a junior research group at the Institute for Ecological Chemistry (IEC) of the Helmholtz Zentrum München. The main research areas were laser mass spectrometry, investigation of dioxin formation and analysis methods as well as aerosol and fine dust research. This period involved an intermittent PostDoc research stay on FT-ICR mass spectrometry at the University of Antwerp.

- **2000 – Call to the University of Augsburg and the bifa Environmental Institute:** A professorship in Analytical Chemistry was offered to Dr. Zimmermann by the University of Augsburg and the bifa – Environmental Institute in Augsburg as a joint professorial appointment.

- **2001 – 2008: University professor and cooperation with the University of Augsburg:** In January 2001, Dr. Zimmermann was appointed as Professor of Analytical Chemistry at the University of Augsburg and Head of the Department of Environmental Chemistry at the bifa – Environmental Institute. Cooperation between the University of Augsburg, the bifa, and the HMGU was established and contractually regulated. Thus, he continued to lead his constantly growing working group on aerosol and fine dust research at the Institute of Ecological Chemistry of the HMGU (at that time GSF Research Centre for Environment and Health).

- **2005 – New HMGU Managing Director (CEO):** Prof. Dr. G. Wess was appointed scientific director at HMGU and replaced Prof. Dr. E. – G. Afting.

(b) Development of the cooperation of the Helmholtz Zentrum München and the University of Rostock

- **2006 – Call to the University of Rostock for the Professorship in Analytical Chemistry (Full Professor, Chair-position):** Following the advertisement, Prof. Dr. Zimmermann applied for the chair position (C4) at the University of Rostock. After Prof. Dr. Zimmermann received the call to the full professorship at the University of Rostock, extensive negotiations between the management of the HMGU, the rectorate of UR, and Prof. Dr. Zimmermann were undertaken with the aim to establish a permanent cooperation in the style of joint professorial appointment.

- **2007 – Cooperation agreement and call acceptance:** After more than 9 months of negotiations, the HMGU-UR cooperation agreement was signed in December 2007. Prof. Dr. Zimmermann accepted the Full Professor Chair position at the UR and rejected the "offer of residence" (*i.e.* the so-called "Bleibeangebot") of the University of Augsburg and the bifa-Umweltinstitut. The cooperation agreement is formulated according to a joint professorial appointment, the joint activities established as: „Joint Mass Spectrometry Center of the Helmholtz Zentrum München and the University of Rostock" (JMSC). The contract is of unlimited duration and no ordinary termination of the contract before the final retirement of Prof. Dr. Zimmermann is foreseen (this is, including a senior professorship term, 2035). In the cooperation contract, it was agreed on a joint allocation of resources. Briefly, the joint JMSC activity is based on the following resources. A) Chair of Analytical Chemistry: Personal resources of 1 professor-, 2 senior-scientist-, 2 PhD-student-, 2 technician- and 0.5 engineer-positions, investments for two large instrument projects (FT-ICR and GCxGC-MS). B) HMGU cooperation group: Personal resources equalling 8.5 scientist positions and consumables/investment funds according to the rate specified in the cooperation contract. In addition, 2.5 further permanent scientist positions and ca. 24.000 € additional material resources per annum are explicitly assigned for the JMSC in the cooperation agreement. According to the cooperation contract, one HMGU scientist was permanently delegated to Rostock (Dr. Streibel) from the very beginning of the JMSC activities.

- **2008 – Establishment of the cooperation group at HMGU:** With the start of the cooperation on 1 April 2008, the cooperation group „Analysis of Complex Molecular Systems" at the Institute of Ecological Chemistry of the HMGU had 11 permanent scientist position (including the 2.5 additional permanent scientist positions due to the cooperation contract). The group had extensive premises in building 24 at HMGU and the HMGU management has made the commitment to renovate the premises in Building 24.

- **2008 – Establishment of the Chair/Cooperation Group at the UR:** At the beginning of the summer teaching term, the Chair of Analytical Chemistry started to operate on 1 April 2008 in newly renovated premises in the Dr. – Lorenz-Weg branch of the University. A new extension building for the Institute of Chemistry was in the preliminary planning phase to be realized in the mid-/end-2010s.

- **2009 – Change in the Rectorate:** Prof. Dr. W. Schareck took over the rector's office of the UR from Prof. Dr. T. Strothotte.

- **2009 – Acquisition of the LLM Research Building in Rostock:** New laboratory places in Rostock came in prospect by the successful recruitment of a new Research Building of the Department „Life, Light and Matter" (LLM) of the Interdisciplinary Faculty (INF) of the UR at the German Council of Science (*i.e.* the "LLM Forschungsbau"). The proposal for the LLM-Research Building was worked out by a professorial consortium under the direction of Prof. Dr. Meiwes-Broer and with the participation of Prof. Dr. Zimmermann.

- **2009 – Ceremony for the opening of the cooperation:** The ceremonial opening of the cooperation and the JMSC took place on July 3rd, 2009 at the historical university main building of Rostock. The opening was hosted by the Rector of the University of Rostock, Magnifizenz Prof. Dr. W. Schareck and the Scientific Director of the Helmholtz Zentrum München, Prof. Dr. G. Wess, in the presence of the representative Ministry of Education, Science and Culture of the state Mecklenburg-Western Pomerania, Mr. W. Venohr, the Deans of the Mathematical and Natural Sciences (MNF) and Interdisciplinary Faculty (INF), Spectabilities Prof. Dr. H. Schubert and Prof. Dr. U. Kragl, as well as colleagues from both institutions. During the meeting,

Prof. Dr. Wess and Rector Prof. Dr. Schareck agreed on a further deepening of the cooperation. The further expansion of the cooperation was planned according to the "Helmholtz Institute" model of the HGF (*i.e.* responsibility for infrastructure: the University of Rostock, representing funds of the state of Mecklenburg-Western Pomerania, responsibility for additional staff positions: Helmholtz Zentrum München representing funds of Federal Germany).

- **2009 – Approval of a Helmholtz Enterprise Fund (HEF) spin-off funding by HGF and foundation of Photonion GmbH:** After the successful acquisition of a Helmholtz Enterprise Fund project for spin-off funding from the HGF, the spin-off company „Photonion GmbH" originated and based on the research in Prof. Dr. Zimmermann's research group was entered in the commercial register on September 20th, 2009. Photonion GmbH, now based in Schwerin and Munich, develops, manufactures, and markets innovative photoionization mass spectrometers for the characterization of aerosols, for research, and for the industry with currently 5 employees.

- **2010 – Building infrastructure problems became immanent:** The detailed examination of the building structure of Building 24 at the HMGU showed that the refurbishment was not economically viable within the allocated budget. The search for alternative places for the CMA laboratories began.

- **2011 – Foundation of the CMA research unit at HMGU:** With the closure of the Institute of Ecological Chemistry at HMGU, the cooperation group „Analysis of Complex Molecular Systems" was transferred to the independent research unit „Comprehensive Molecular Analytics" (CMA). The permanent HMGU-funded personnel at CMA rose to 13.5 scientist equivalent positions.

- **2012 – Agreement for the relocation of personnel to Rostock:** Since sufficient modern laboratory space for aerosol characterization is not available in Munich, it was agreed between Prof. Dr. Wess, Prof. Dr. Schareck, and Prof. Dr. Zimmermann that the 1.5 scientist equivalent positions from the JMSC cooperation contract working in Munich were permanently relocated to Rostock after the completion of the new LLM Research Building. The building and equipment costs of the corresponding special laboratories (laser/aerosol laboratories and the required large-scale instruments) were covered by UR.

- **2012 - Approval of the virtual Helmholtz Institute „HICE-Aerosol and Health" by the HGF:** The international large-scale project „Virtual Helmholtz Institute HICE-Aerosol and Health" was funded by the HGF (2012-2017), coordinators of HICE were HMGU and UR, Prof. Dr. Zimmermann was the scientific director and spokesperson of HICE.

- **2015 – Commissioning of the new LLM Research Building in Rostock:** In November 2015 part of the working group moved into the new laboratories of the LLM Research Building of the INF, establishing the Competence Center Mass Spectrometry. As decided before by HMGU and UR, the 1.5 scientist equivalent positions were permanently sent from Munich to Rostock (currently: Dr. Passig, Fr. Kühl), joining Dr. Streibel who was sent already in 2008.

- **2015 – Occupation of the new laboratory in Munich-Sendling:** In October 2015, CMA moved into the new laboratory building in München Sendling. The laboratory space in Sendling was rented by HMGU, as no adequate facilities were available at the campus in Oberschleissheim. The refurbing and fitting-out of the laboratory cost more than one million euro. Nevertheless, some of the needed special infrastructures could not be realized in Sendling.

- **2015 – Agreement for the relocation of further HMGU employees to Rostock:** Due to the structural limitations at the Sendling site, some of the required special laboratories could not be realized on these premises. This applies, in particular, to aerosol laboratories (wood stoves/burners, etc.) in close proximity to exposure laboratories or biological laboratories for cell exposure experiments. As a result, an agreement was reached between UR and HMGU in accordance with the Helmholtz Institute Strategy: It was agreed that return for the inclusion of the expensive special laboratories required for the joint research in the development plan for the chemistry expansion building (funds of the University of Rostock, representing the state Mecklenburg-Western Pomerania), additional employees (up to 2.5 scientist equivalents) shall be sent permanently to the new laboratories upon commissioning of the extension building for chemistry (initially planned for 2019, now 2021).

- **2017 – Successful completion of the HICE funding period:** After a 5.5-years term, the HGF Presidential Fund Project Virtual Helmholtz Institute „HICE-Aerosol and Health" is successfully completed and excellently evaluated.

- **2017 – Transfer of HICE at HMGU:** Due to the success of HICE, the HMGU management decided to include the HICE activities into the basic financing of HMGU (*i.e.* "Verstetigung"). As part of the HMGU management's commitment to perpetuate HICE, CMA's personnel budget was permanently increased to 17.5 scientist equivalents. In addition, additional funds for material and consumable € 150,000 per annum were granted on a permanent basis.

- **2018 – Successful HGF review by CMA and the UR-HMGU cooperation:** Excellent performance of CMA and the HMGU-UR cooperation at the HGF reviews of health research was attested. The process of transferring the CMA department to a regular HMGU institute was started under CEO Prof. Wess.

- **April 1st, 2018 – 10th Anniversary of the UR-HMGU cooperation**

- **2018 – Contract with the Universität der Bundeswehr München and Deputy Head of CMA:** In the summer of 2018, the basis for the future close cooperation between the Universität der Bundeswehr Munich (UniBw M) and the HMGU was created by the signing of a cooperation agreement. Prof. Dr. T. Adam, Head of the Institute of Chemistry and Environmental Engineering at UniBW M, became the Deputy Head of CMA in secondary employment. By including UniBW M into the consortium, the strength and impact in JMSC research areas are further increased.

- **2018 – New HMGU Managing Director (CEO):** Prof. Dr. M. Tschöp took over the responsibilities as HMGU Managing Director from Prof. Dr. G. Wess on 1.8.2018. In October 2018, during a first meeting with the new CEO, Prof. Dr. Tschöp in Tel Aviv, he has pledged to support the already planned transfer of the research unit CMA into a regular institute of HMGU.

- **2018: Approval of the Helmholtz International Lab aeroHEALTH by HGF:** In a competitive, two-stage procedure, the Helmholtz International Lab aeroHEALTH was approved for 5 + 3 years under the direction of HMGU/CMA. Partners are the Weizmann Institute in Israel, the Jülich Research Centre as well as the Universities of Rostock and of Eastern Finland (the latter ones as associated partners). The speaker is Prof. Dr. Zimmermann. The HMGU pledges as its own participation a scientist position for habilitation for 8 years (Dr. Czech). After this period, the permanent employment of Dr. Czech as a topic leader is planned. This brings the current number of personnel at CMA to 18.5 permanently funded scientist equivalents.

- **2019 Ceremonial opening of the Helmholtz International Lab aeroHEALTH:** On April 1, 2019, the Helmholtz International Lab aeroHEALTH was inaugurated by the President of the Helmholtz Association, Prof. Dr. Otmar Wiestler and the President of the Weizmann Institute for Science (WIS), Prof. Dr. Daniel Zajfman in the presence of the project partners Prof. Dr. R. Zimmermann (HMGU/UR), Prof. Dr. Y. Rudich (WIS) and Prof. Dr. A. Kiendler-Scharr (FZJ) as well as other colleagues and employees in a small ceremony at the Weizmann Institute for Science in Israel.

- **2019 – Ceremonial opening of the first aeroHEALTH measurement campaign:** The CEO of HMGU, Prof. Dr. Tschöp welcomes the participants from Israel, Finland, and Germany (Jülich, Rostock, Munich) to the first aeroHEALTH measurement campaign at HMGU on December 5, 2019, in a small festive event. Experiments on the toxicity of secondary organic aerosols are carried out in the following weeks (see the aeroHEALTH section in this progress report for details).

- **2020 – Financial problems of the HMGU:** Due to financial problems at HMGU some of the commitments currently are suspended.

1.9 JMSC Highlights

In the following, selected highlights from the JMSC events and activities from 2018 – 2020 are reported. Due to the Corona pandemic, we naturally had less photogenic "highlight" occasions but accelerated publication activity and third-party funds – raising in 2020.

New HICE-Aerosols and Health Measurement Campaign, Finland, 2018
(January–February 2018, Kuopio, Finland)

With the successful conclusion of the Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health – HICE in 2017, permanent funding of the HICE topic was granted for JMSC by the Helmholtz Zentrum München. The actual scientific focus of this newHICE funding is a continuation of the HICE topic, namely understanding of the distinctions in adverse biological effects of different combustion emissions (fresh and atmospherically aged) on lung cells and tissue. The first newHICE campaign was performed together with our long-standing partners from the University of Eastern Finland in Kuopio in January and February 2018. One aspect of interest was, how pre-existing health conditions like COPD or chronic airway inflammation influence the biological effects of aerosol exposure. We investigated emissions from the combustion of wood and lignite in a stove and from a diesel engine and started a preliminary study to model atmospheric aging under realistic conditions both in a state-of-art smog chamber and using a novel and unique photochemical emission aging flow tube reactor (PEAR). The adverse health effects of these emissions were assessed using cell cultures and healthy mice as well as *in-vivo* and *in-vitro* disease models of chronic airway hyper-responsiveness. A TV team of the German federal television broadcasting (ARD, BR) accompanied the measurement campaign and produced and broadcasted a report on the campaign and the research (see "Section Media Coverage").

(Left) Prof. Jorma Jokiniemi and Prof. Ralf Zimmermann are looking into the ILMARI atmospheric aging chamber at the University of Eastern Finland, Kuopio.

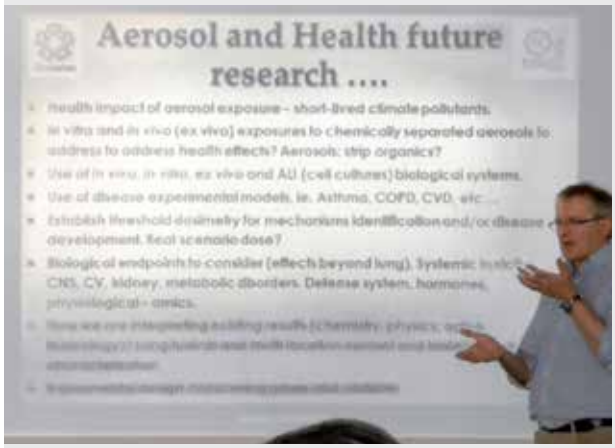
(Right) The German TV team of the ARD, BR accompanied the measurement campaign in Kuopio. Here shots inside the HICE MobiLab from the harvesting of aerosol exposed cells are taken.

Sino-German Workshop on Aerosol and Health, China, 2018
(March 19th–21st 2018, Guangzhou, China)

The workshop on "Bridging Aerosol Chemistry and Health Effects: Challenges and Opportunities for Analytical Chemistry" was organized by Prof. Xue Li (Jinan University) and Prof. Ralf Zimmermann (chairs) and funded by the German Science Foundation (DFG) after a successfully evaluated workshop proposal. Over 20 keynote speakers from Germany, China, USA, Australia, Hong Kong, Finland, and Macau were invited for overview lectures. The question arising from integrating the chemical characterization of aerosol with toxicological, epidemiological, and biological research on the health effects was intensively discussed during the 3-day event among the more than 70 participants. With the invited speakers, also a pre-event discussion meeting at the Polytechnic University of Hong Kong was held. The scientific sessions on the workshop were accompanied by a social program. This included a cruise on the river and a visit to the Chinese mass spectrometry company HEXIN Ltd., which develops single-particle mass spectrometers also in cooperation with JMSC and Photonion.

Participants of the Sino-German Workshop on Aerosol and Health held in Guangzhou, China in March 2018

Analytica Conference 2018: Aerosol and Health Conference Session and Workshop, Germany, 2018 (April 12th–13th 2018, Munich, Germany)



Prof. Ralf Zimmermann is discussing a workshop contribution with the auditorium.

In July 2018, CMA members visited the Analitika 2018 Conference in South Africa, which provides a forum for both users and developers of analytical chemistry methods and technologies to present their latest findings, ideas and applications. Beside results of different wood combustion experiments, Dr. Jürgen Orasche presented novel methods enabling the identification of health relevant compounds (out of 10000 compounds from wood smoke) potentially responsible for certain biological responses in human lung cells. Furthermore, Prof. Ralf Zimmermann gave a talk about innovative analytical approaches for the analysis of ambient air as well as emission- and workplace-aerosols and how we can address aerosol properties relevant for the health activity by instrumental analytical chemistry. Novel instrumentations from enabling technologies were introduced and first results from sampling in a platinum mine shown.

Project Meeting at the Environmental Research Station Schneefernerhaus, Germany, 2018 (June 2018, Zugspitze, Germany)

From the 12th–13th of June, our project partners from South Africa (Research group around Prof. Patricia Forbes, University of Pretoria) have been invited to a project meeting at the Environmental Research Station Schneefernerhaus (UFS). Besides the scientific exchange, aerosol samplers and portable optical aerosol detectors have been installed at the Zugspitzplatt and the UFS Schneefernerhaus in order to identify semi-volatile organic compounds (SVOC) as well as black carbon (BC). These data were used to obtain clean-air data at of the Mt. Zugspitze and to compare those with more polluted environments.

Delegation of CMA and project partners from the University of Pretoria, South Africa, at the Environmental Research Station Schneefernerhaus

Young Scientist Award for MSc. Anika Neumann, at 1st EU FT-ICR MS End User School, Finland, 2018 (August 20th–23rd 2018, Joensuu, Finland)

From the 20th to the 23rd of August, 2018, the first EU FT-ICR MS End User School took place at the Department of Chemistry at the University of Eastern Finland. Young scientists got the opportunity to present their research work in front of a larger audience. The research company Bruker Daltonics sponsored an award for the best young scientist presentation. The jury organized by the EU FT-CR MS consortium awarded Anika Neumann from the University of Rostock/JMSC for her scientific oral presentation.

End User School in Finland, 2018, Anika Neumann wins the Young Scientist Presentation Award at the 1st EU FT-ICR MS

Workshop of the US Environmental Protection Agency and the Helmholtz Zentrum München, USA, 2018 (October 9th–11th 2018, Washington DC, USA)

Prof. Zimmermann gives a lecture on the results of the Helmholtz Virtual Institute HICE on the HMGU-US EPA workshop.

The Helmholtz Zentrum München and the US Environmental Protection Agency (US EPA) have a longstanding cooperation in the field of air pollution and health, led from HMGU side currently by Prof. Annette Peters from the Institute of Epidemiology. Alternately workshops are conducted in Germany and the US and diverse joint projects were performed between the HMGU and US EPA partners. The workshop in Washington DC in 2018 was conducted on the occasion of the 20th anniversary of the HMGU – US EPA cooperation and a renewed cooperation contract was signed. Results on actual research activities in the field of air pollution and health, comprising work on epidemiological and aerosol toxicological investigations as well as aerosol characterization studies were discussed and new cooperations were agreed on during the 2-day workshop. The JMSC was able to win Dr. Ian Gilmore, a renowned USE EPA aerosol toxicologist, as member of the Scientific Advisory board of the Helmholtz International Lab aeroHEALTH.

Visit in Israel and Jordan with a Delegation of the HGF President, Israel and Jordan, 2018 (October 21st–26th 2018, Tel Aviv, Israel as well as Amman and Allan, Jordan)

On the occasion of the funding of the German-Israeli Helmholtz International Lab aeroHEALTH, Prof. Ralf Zimmermann and the other aeroHEALTH partners were invited to join a delegation visit of the president of the Helmholtz Association (HGF), Prof. Wiestler, to Israel and Jordan. In Tel Aviv, a new Helmholtz office was inaugurated followed by a reception at the German Embassy. The Partners of the Helmholtz International Lab aeroHEALTH, Prof. Astrid Kiendler-Scharr (Forschungszentrum Jülich), Prof. Yinon Rudich (Weizmann Institute of Science) and Prof. Ralf Zimmermann met in Tel Aviv, visited the Weizmann institute and discussed first steps into the aeroHEALTH program. From Tel Aviv the HGF-president's delegation moved on to Jordan, where different scientific organizations, such as the German-Jordanian University in Amman, were visited. A highlight was the visit at the "Synchrotron-Light for Experimental Science and Applications in the Middle East" (SESAME), a synchrotron-based research institute created under the auspices of UNESCO in Allen (close to Amman), Prof. Wiestler handed over symbolically the funding commitment of the Helmholtz Association for a new synchrotron beamline for SESAME. During a get-together, Jordan and German scientists discussed potential joint projects. A direct outcome is the Duststorm project initiative, led by Prof. Lips and Prof. Föhlisch (Helmholtz-Zentrum Berlin) as well as Prof. Zimmermann (HMGU) from the HGF side and Prof. Lausi from SESAME in Jordan. Duststorm is a coordinated approach to educate young Jordanian students during their PhD-studies in Germany under involvement of the German Academic Exchange Service (DAAD).

Left: Prof. Kiendler-Scharr (FZJ), Prof. Rudich (WIS) and Prof. Zimmermann clink glasses on the aeroHEALTH funding during the meeting in Tel Aviv.
Middle and Right: Meeting at the SESAME synchrotron in Allen, Jordan, where the Helmholtz president, Prof. Wiestler, handed over a funding commitment by the HGF for a new synchrotron beam line for SESAME.

Successful industry cooperation with LECO GmbH, Completion of 1st Contract, 2018, Germany (2018, Munich, Germany)

In 2018, JMSC has successfully completed a 5-year cooperation contract with the company LECO GmbH. The main subject was the evaluation of two gas chromatography platforms with high resolution Time-of-Flight (TOF) mass spectrometric detection (HRTMS), which were installed as beta-systems at the beginning of the cooperation and reached marketability shortly after. The cooperation with LECO continues and the two GCxGC-HRTMS now are overwritten to JMSC. The first system was put into operation in the new buildings of the working group in Munich immediately after the cooperation contract completion in 2018. A second system was first brought to the latest state of the art by the distributor and finally re-installed again in September 2020. JMSC now operates two of the most powerful two-dimensional gas chromatographs currently available on the market. A special feature is the very high mass resolution of R=50,000 at an unsurpassed repetition rate of 200 Hz, allowing for an uncompromised GCxGC resolution system. Due to the successful outcome of the first cooperation, further cooperation projects with LECO are currently in operation or being negotiated. This includes a recently funded third party project with Photonion, the company SIM GmbH and the Universität der Bundeswehr funded by the Ministry of Economics (BMWI) with the aim to develop an automated GC-MS system for monitoring of organic compounds in an ambient aerosol.

Prof. Zimmermann together with Dr. Uwe Käfer at the first LECO Pegasus HRT 4D System. The system is equipped with a direct insert probe, soft photoionization and a hyphenation for thermal analysis. Main applications will be the investigation of aerosols for the Helmholtz International Lab "aeroHEALTH".

Workshop on ultrafine particles, 2018, Germany (November 5th 2018, Munich, Germany)

On November 5, 2018, 30 experts in the fields of exposure, toxicology and epidemiology met at HMGU to discuss current issues related to ultrafine particles in the environment and their health effects. The meeting was themed „Thinking outside the box: how to use the existing science on ultrafine particles to protect against them?". One outcome of the workshop was the white paper "Ambient ultrafine particles: evidence for policy makers" was compiled, published under the umbrella of the European Federation of Clean Air and Environmental protection associations (EFCA) (https://efca.net/files/WHITE%20PAPER-UFP%20evidence%20for%20policy%20makers%20(25%20OCT).pdf) and made available to policy makers in Brussels and European capitals.

Group photo of experts in the fields of exposure, toxicology and epidemiology at the meeting at HMGU in November 2018.

Organization and Congress Chair of the Annual Meeting of the German Mass Spectrometric Society held at the University of Rostock, Germany, 2019

(March 10th–13th 2019, Rostock, Germany)

In 2019, the University of Rostock celebrated its 600-year anniversary, rendering it to one of the oldest, time-honored German universities. On the occasion of the University anniversary the Rektor of the University, Prof. Dr. Schareck, asked the faculties to invite national and international conferences to be held in Rostock preferably in the anniversary year. Prof. Michael Glocker and Prof. Ralf Zimmermann were able to bring the 52nd Annual Meeting of the German Mass Spectrometric Society (Deutsche Gesellschaft für Massenspektrometrie, DGMS) in 2019 to Rostock. The 52nd DGMS meeting attracted more than 350 scientists and was held in parallel with the spring meeting of the German Physical Society (DPG) with a partially interlinked scientific program (joint scientific sessions and plenary talks). In total, 60 scientific lectures and 130 poster contributions gave a cross-section on the field of mass spectrometry. The conference was accompanied by a social program. This included a guided city walk, a reception at the town hall, followed by a short sermon and organ concert in the St. Mary's Church (Marienkirche), the place where the University of Rostock was founded 600 years ago. The exhibition + poster session was set up in a heated tent in front of the main lecture halls at the campus in the Ulmenstrasse. During the conference dinner, which was held in the St. Nicholas' Church (Nikolaikirche) also the award ceremony was held.

The 52nd annual conference of the German Mass Spectrometry Society at the University of Rostock. From top/right in clockwise manner: The conference chairs, Prof. Glocker and Prof. Zimmermann are opening the 52nd annual DGMS conference, the rector of the university is giving a greeting address and a report on the 600-year history of Rostock University, Prof. U. Boesl from the TU Munich gives a plenary lecture on laser mass spectrometric separation of enantiomers, the logos of the DGMS and the 52nd annual DGMS conference, a view in the main lecture hall (plenary talk), Prof. Zimmermann is opening the conference dinner in the St. Nicholas' Church, Mattauch-Herzog- award ceremony at the conference dinner (from left) Dr. Möhring, Prof. Linscheid (head of the award committee) and the 2019 award winner Prof. Jürgen Hartler from the TU Graz. (Photos: © DGMS, photography by J. H. Gross)

Annual scientific meeting and get-together of all JMSC co-workers, Germany, 2019

(March, 13th–14th 2019, Rostock)

The annual meeting of the JMSC Group in 2019 took place from 13th–14th March in Rostock. The meeting was opened at the Ulmencampus in House 1 with a welcoming speech by Prof. Dr. Ralf Zimmermann and the introduction of our new colleagues in Rostock and Munich. In the following seminars, the JMSC researchers and PhD students and MSc students presented their work in lectures. The scientific topics and further plans of the JMSC were further discussed the next day. The annual meeting was concluded with a pizza dinner and a cozy get-together.

Group photo from the JMSC annual get-together in Rostock March 2019

Inauguration of the Helmholtz International Laboratory aeroHEALTH, Israel, 2019
(April 1st 2019, Rehovot/Israel)

On Monday, April 1st, 2019, the presidents of the Helmholtz Association and the Weizmann Institute in Israel, Prof. Otmar D. Wiestler and Prof. Daniel Zajfman, opened the joint Helmholtz International Lab in Rehovot near Tel Aviv. In the aeroHEALTH cooperation project, Helmholtz Zentrum München, Forschungszentrum Jülich and the Israeli Weizman Institute combine their expertise to elucidate the impact of air pollutants on human health with the focus on atmospheric processing by sunlight, ozone and nitric oxides. It is one of the first projects of an initiative launched by Helmholtz. „We are very pleased to be able to develop aeroHEALTH together with the renowned Weizmann Institute", said Helmholtz President Prof. Otmar D. Wiestler at the inauguration ceremony in Tel Aviv. „Air pollution is one of the greatest health risks of our time. However, there are still major gaps in our knowledge of the causes and underlying mechanisms. We now want to make another important contribution to fill this gap." The partners involved can draw on many years of cooperation and extensive joint preparatory work, are largely interdisciplinary and complement each other ideally. The research unit around Prof. Dr. Zimmermann provides expertise and infrastructure for the chemical characterization of airborne aerosols, the realistic exposure of biological systems to aerosols, and the investigation of toxicological and cellular effects of particles. The team is reinforced by the big data expertise of the group around Prof. Fabian Theis, also from the Helmholtz Zentrum Munich. The transformation and aging of aerosols through atmospheric processes is the field of the group centered around Prof. Astrid Kiendler-Scharr from the Forschungszentrum Jülich. Prof. Yinon Rudich from the Weizmann Institute is specialized in the *in-vivo* toxicology of air pollutants and their decomposition products, as well as physical aerosol characterization.

Joint laboratory for aerosol research opened: Helmholtz President Prof. Dr. Otmar Wiestler, Tropospheric Researcher Prof. Dr. Astrid Kiendler-Scharr, Weizmann President Prof. Dr. Daniel Zajfman, Project Coordinator and Aerosol & Health Researcher Prof. Dr. Ralf Zimmermann, and Aerosol Specialist Prof. Dr. Yinon Rudich (from right). (Photo: © Weizmann Institute, photography unit, Itai Belson)

Presentation of the Joachim-Jungius Award to Dr. Rüger, Germany, 2019
(July 5th 2019, Rostock, Germany)

Since 1992, the Society of Sponsors of the University of Rostock e.V. (GFUR) has annually awarded prizes for the best doctoral theses defended at the University of Rostock. In the course of the ceremonial presentation of the doctoral and postdoctoral degrees on July 5, 2019, (GFUR) awarded the Joachim Jungius sponsorship prizes for excellent doctorates. This time, four doctorates were awarded, each endowed with 2,000 €. One of them was our JMSC employee, Dr. Christopher Paul Rüger, Faculty of Mathematics and Natural Sciences with the subject of the dissertation: „Development and evaluation for innovative sampling, separation and ionization techniques for ultra-high resolution mass spectrometry – Analysis of petroleum and combustion aerosol samples".

Christopher Paul Rüger – one of four Jungius-Laureate 2019 (3rd from the left)
Jürgen H. Gross, ©Universität Rostock

16th International Congress on Combustion By-Products and Their Health Effects and presentation of Adel Sarofim Award, to Prof. Zimmermann, USA, 2019
(July 10th–12th 2019, Ann Arbor, USA)

On July 10th–12th, 2019, the University of Michigan hosted the 16th International Congress on Combustion By-Products and Their Health Effects. Local host is Prof. Angela Violi from the Department of Mechanical Engineering at the University of Michigan. For the past 28 years, this conference has served as an interdisciplinary platform for the discussion of the formation, environmental fate, health effects, policy, and remediation of combustion by-products. The conference was held on the University of Michigan campus in Ann Arbor. Scientific foci of the conference were among others health effects of mobile and stationary emission sources in urban environments, open fires, indoor air pollution, and halogenated pollutants. In the framework of the conference the renowned "Adel Sarofim Award for outstanding advancements in understanding combustion processes, formation of combustion by-products and mechanisms of their health effects" was handed to Prof. Dr. Ralf Zimmermann.

(Top) Ceremony of handing the Adel Sarofim award to Prof. Ralf Zimmermann. (Bottom) Group photo taken at the 16th International Congress on Combustion By-Products and Their Health Effects.

JMSC visits the European Aerosol Conference, Sweden, 2019
(August 25th–30th 2019/Gothenburg, Sweden)

One of the important annual scientific conventions is the EUROPEAN AEROSOL CONFERENCE (EAC). From 25 to 30 August 2019, the EAC 2019 was held in Gothenburg, Sweden, where the aeroHEALTH consortium was represented with 10 lectures. Stefanie Bauer, Christoph Bisig, Hendryk Czech, Sebastiano Di Bucchianico, Erwin Karg, Mohamed Khedr, Patrick Martens, Sebastian Öder, Jürgen Orasche, Johannes Passig, Julian Schade, Cao Xin, and Elias Zimmermann presented their results on the chemical composition and biological effects of allergy-relevant aerosols, DNA damage and changes in the cell by particulate matter, technologies for the remote detection of ship emissions and many more. The JMSC group also showed several posters. In 2020, the EAC was held online due to the Coronavirus-Pandemic. Nevertheless, our scientists presented novel inventions in the matter of gas spectrometry, mass spectrometry, the *in-vitro* air liquid interface model system and the mobile monitoring station with street view imagery (The online program of the EAC 2019 can be viewed at: https://eac2019.se/ the one of 2020 at: https://eac2020.de/). Furthermore, a preparation meeting for the first aeroHEALTH Helmholtz International Lab measurement campaign was held at the EAC in Gothenburg.

Some of JMSC members which visited the EAC 2019 in Gothenburg, Sweden in front of the booth of the JMSC spin-off company Photonion GmbH (from right): Mr. Martens, Dr. Czech, Prof. Zimmermann, Dr. Bisig, Dr. Bauer, Mr. Cao, Mr. Khedr, Dr. Di Bucchianico. Also, a Helmholtz International Lab aeroHEALTH meeting was conducted. Some of the partner representatives are shown in the photograph (from left), Prof. Rudich (WIS) and Dr. Hohaus (FZJ).

aeroHEALTH project discussions and inspection of the new reactor SAPHIR-STAR at the FZJ, Germany, 2019 (September 23rd 2019/Jülich, Germany)

Visit at the stirred-tank reactor SAPHIR-STAR which will be used in the Helmholtz International Lab aeroHEALTH

In 2019, Thorsten Hohaus and his colleagues from the IEK-8 at the Forschungszentrum Jülich have developed a new continuous stirred-tank reactor, called SAPHIR-STAR, in order to study the formation and chemical composition of atmospheric aerosols and their gas-phase precursors under different atmospheric conditions and oxidation regimes. Within the Helmholtz International Lab aeroHEALTH, SAPHIR-STAR will be employed to investigate the role of the chemical composition of atmospheric aerosols on their potential toxicity. On September 23rd 2019 Prof. Zimmermann visited the FZJ for a discussion with Prof. Kiendler-Scharr. In addition to a visit of the SAPHIR atmospheric simulation chamber also the SAPHIR-STAR reactor was inspected.

Measurement campaign on background aerosols at the Swedish coast, 2019, Sweden (October/November 2019/Onsala, Sweden)

With their newly developed laser ionization techniques in single-particle mass spectrometry, JMSC co-workers started the first field study to investigate the distribution of health-relevant polycyclic aromatic hydrocarbons and transition metals in background aerosols. In order to avoid the influence of local emissions and to detect ship plumes from distant marine traffic, the campaign was performed in the restricted area of a space observatory on a peninsula, belonging to the Chalmers University of Technology, Gothenburg, Sweden. The well-proven veteran truck from CMA served as measurement platform, transporting and housing all spectrometers and complex equipment. With the partners and hosts from the Chalmers University and the IVL Swedish Environmental Research Institute, a first comprehensive dataset of the single-particle distribution of health-relevant aerosol components was collected and has been submitted for publication.

Some Impressions from the measurement campaign in Sweden: (Left) JMSC truck with single-particle mass spectrometer, measuring background aerosols in remote environment at the Swedish coast. (Right) Arrival at the measurement site in the restricted area of the Onsala space observatory.

November 12th 2019 – The 600-year anniversary of the University of Rostock, Germany, 2019 (November 12th 2019/Rostock, Germany)

On the November 12th Anno Domino 1419 the ceremonial opening of the "Universitas Rostochiensis" (University of Rostock), founded by the dukes of Mecklenburg and the Johann IV. und Albrecht V. and the council of the Hanseatic city of Rostock as one of the oldest North European universities, was held in the St Mary's church (Marienkirche). 600 years later, on the same date and place, the University of Rostock, in keeping its motto "Traditio et Innovatio", held an academic celebration day as the highlight of the anniversary year.

(Left) Academic parade on the anniversary day from the min building to St. Mary's church. (Right) The rector of the university in his traditional academic gown and the anniversary logo (© University of Rostock).

aeroHEALTH Helmholtz International Lab inauguration ceremony at the HMGU, 2019, Germany (December 5th 2019/Munich, Germany)

After some preparation work at the JMSC premises in Sendling with finical help from the University of Rostock the aeroHEALTH Helmholtz International Lab went into practical operation in November 2019. On December 5th, 2019 the CEO of the HMGU Prof. Dr. Matthias Tschöp gave a welcome speech at an inauguration ceremony and unveiled the aeroHEALTH Helmholtz International Lab plaque. Prof. Tschöp welcomed the guests from Israel and Jülich, congratulated the consortium on the successful grant acquisition, ensured his support, and finally emphasized the importance of air pollution for human health and the need for interdisciplinary research, including "big data" science, in solving complex environmental health problems.

(top) CEO Prof. Dr. Matthias Tschöp talks to Prof. Dr. Kiendler-Scharr (FZJ) and Prof. Rudich (Weizmann Institute).

(right) The CEO of the HMGU Prof. Dr. Matthias Tschöp giving a welcoming speech at the opening of the aeroHEALTH International Lab at the premises of the JMSC in München Sendling.

First aeroHEALTH Helmholtz International Lab measurement campaign on health effects of secondary aerosols at the Helmholtz Zentrum München, 2019 – 2020, Germany (November 2019-February 2020/Munich, Germany)

The research of aeroHEALTH Helmholtz International Lab is organized in large measurement campaigns on specific research questions. The research topic of the first campaign was about the question whether secondary organic aerosols (SOA) from biogenic or anthropogenic precursors are more toxic and which chemical compounds are relevant for the differences in biological responses. After nearly half a year of preparation (including a renovation of the aerosol laboratory and set-up of flow tube reactor in the JMSC premises) the 10-week measurement campaign started in November 2019. Naphthalene and ß-Pinene were used as anthropogenic and biogenic SOA precursors, respectively, and fresh combustion soot was deployed as seed aerosol. After generation of the SOA in the oxidation flow tube, human lung cells (mono and co-culture lung models) were exposed at the air-liquid interface. Multiple aerosol toxicological investigations are accompanied by a comprehensive characterization of the aerosol properties and chemistry in order to address the scientific question (see also the last section in the report on the Helmholtz International Lab aeroHEALTH).

Some impressions of the first measurement campaign performed in at the HMHU in Munich: (Left) Mass spectrometers for online analysis of volatile organic components formed by aerosol aging (home build single-photon ionization mass spectrometer and a high resolution proton transfer reaction mass spectrometer). (Right) Aerosol sampling for off-line analysis and physical and chemical characterization of the secondary aerosol particles by scanning mobility particle sizer and counters and further aerosol mass spectrometry.

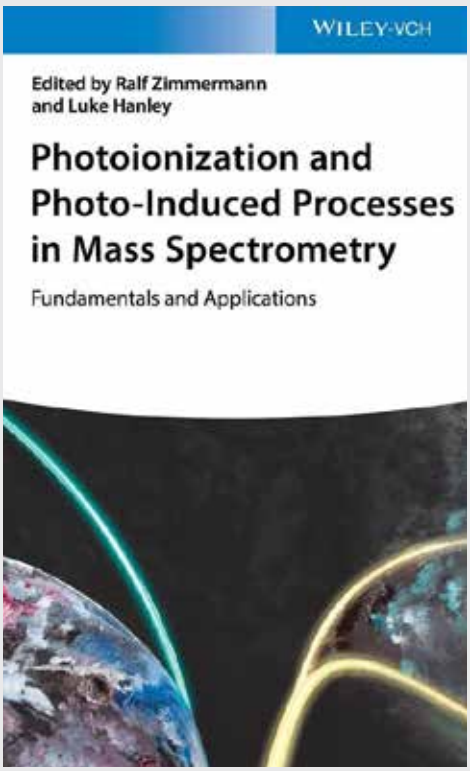
First ship diesel engine measurement campaign in the framework of the SAARUS project at University of Rostock, 2020, Germany (June–August 2020/Rostock, Germany)

In the framework of the SAARUS project (see also section third party projects (17)), the impact of sulfur scrubbers on the emission of ships is investigated. Furthermore, additional flue gas cleaning devices to reduce the emission of particles smaller than 1 µm are developed by the industrial partners and evaluated by JMSC researchers. The background of the studies is the fact, that with the 2020 sulfur cap legislation, the use of sulfur-rich heavy fuel oils is forbidden if no sulfur emission reduction device (sulfur scrubber) is used. Recent research concludes, however, that sulfur scrubbers are not efficiently reducing the emission of the health-effects causing, small, inhalable particulates. Therefore, additional flue gas treatment devices need to be installed and tested to minimize the public health risk of shipping. In the measurement campaign, which was run on ship diesel engine at the "Lehrstuhl für Kolbenmaschinen und Verbrennungsmotoren (LKV)" of the University of Rostock, the emission parameters for six different ship fuels and four different operating conditions were investigated in detail. In particular, the emission of cancer-causing Polycyclic Aromatic Hydrocarbons (PAH), lung toxic transition metals (*e.g.* V, Cu, Fe), and nano-particulate matter was determined. In a second campaign, the mitigation capacity of an optimized scrubber and additional flued gas cleaning devices, developed in the SAARUS projects consortium, will be studied.

Some impressions of the first measurement campaign performed at the LKV in Rostock. (Left) Central control unit for the experiments on the ship engine. (Right) Instrumentation for online measurements of gaseous and particulate emissions.

New Wiley-VCH Textbook on Photoionization and Photo-Induced Processes in Mass Spectrometry by Ralf Zimmermann and Luke Hanley (Editors/Authors) 2020 (2020/Weinheim, Germany)

In October 2020 the book "Photoionization and Photo-Induced Processes in Mass Spectrometry – Fundamentals and Applications" by Ralf Zimmermann and Luke Hanley (Editors) was published by the Wiley-VCH Verlag GmbH. On the occasion of an Analytica conference session organized by Ralf Zimmermann, he and Luke Hanley decided to fill a gap in scientific literature and write/edit a textbook on Photoionization and Photo-Induced Processes in Mass Spectrometry. The newly appeared book starts with a complete overview of the fundamentals of the techniques, covering the basics of the gas-phase ionization as well as those of laser desorption and ablation, pulse photoionization, and single-particle laser ionization. Numerous application examples from different analytical fields are described that showcase the power and the wide scope of photoionization in mass spectrometry. The book represents the first general reference book on photoionization techniques for mass spectrometry and examines technologies and applications of gas-phase resonance-enhanced multiphoton and single-photon ionization mass spectrometry (REMPI/SPI-MS) and gas-phase elemental resonance ionization mass spectrometry (RIMS) and provides complete coverage of techniques like MALDI or single-particle laser mass spectrometry. Current and potential applications of each technology, focusing on process and environmental analysis, are discussed. The book is intended for advanced students or PhD-students as well as practicing scientists (spectroscopists, analytical chemists, photochemists, physical chemists, and laser specialists). Four JMSC authors (H. Czech, J. Passig, T. Streibel, and R. Zimmermann) wrote/contributed to 5 of the 11 chapters of the book.



Cover of the textbook on Photoionization and Photo-Induced Processes in Mass Spectrometry by Ralf Zimmermann and Luke Hanley (Editors/Authors), ISBN (e-book/print) 978-3-527-68222-5/978-3-527-33510-7.

2 We are JMSC

2.1 JMSC Co-workers at the University of Rostock, Helmholtz Zentrum München, and UniBw (without MSc and PhD Students)

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- Director of the Institute of Chemistry & Environmental Engineering at the Department of Mechanical Engineering of the University of the Bundeswehr Munich (UniBwM).
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- Development, setup, and construction of a workplace aerosol simulation unit, consisting of an aerosol generator, a flow tube reactor, and an aerosol chamber.

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- Scientist
- Topic Leader "Technology Transfer: Photonion GmbH"
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- Organisation and implementation of lectures, seminars and analytical practical trainings for students of chemistry, agriculture, biotechnology, and for teacher trainees. The emphasis is on trace analysis with AAS, ICP, and polarography.
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- On-line measurements of Black Carbon (BC) by optical methods (Aethalometer AE 33, MA 200) and experimental aerosol (soot) production.
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Dr. Uwe Käfer, MSc.-Chem.

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Dr. Tamara Kanashova, Dipl.-Ing. Biosystemtechnik (left 07/2020)

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- Proteomic Investigation of the Impact of Combustion aerosols, particles, and gases on lung epithelial cells.

Erwin Karg, Dipl.-Met.

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- Characterization of particulate and gaseous atmospheric components; characterization of particle surface area; experimental aerosol production, instrumentation, and analysis; the impact of particulates on human health.
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Sophie Klingbeil,
Dipl. Math. Oec (left 9/2020)

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- Processing and preparation of statistical tools and data evaluation.

Dr. Vesta Kohlmeier,
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- Postdoc
- Characterization of dynamic aerosols generated from semi-volatile organic compounds (SVOC) at workplaces and their impact on human health; investigation of denuders as gas-particle partitioning tools for SVOC aerosols and their inclusion into personal aerosol samplers.

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- UR
- Development of electronics from CAD –Design up to complete devices; Programming in Matlab, Labview, C/C++, etc.; technical and scientific steering of research projects, bachelor-, master- and graduation thesis; Hardware support for electrical chemical measurement devices of all groups; Repair and service for electrical lab equipment (sensors, pumps, mixer, heat guns, etc.).
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- Senior Scientist/Works council
- Characterization of ambient aerosol by physical and chemical analysis employing on-line particle counters, impactor probing, and aerosol mass spectrometry (AMS).

Dr. Toni Miersch,
MSc.-Chem. (left 01/2020)

- UR
- Postdoc
- Investigation of the chemical nature of the carbonaceous fraction of ambient particulate matter (PM) and those originating from combustion processes using soft photoionization time-of-flight mass spectrometry hyphenated to a modified thermo/optical carbon analyzer.

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Dr. René Reiss,
MSc.-Chem. (left 10/2018)

- University of Rostock
- Postdoc
- Development of a measurement system to be used for the fast and reliable detection of low volatile security-relevant substances on surfaces.

Dr. Christopher Paul Rüger,
MSc.-Chem.

- UR
- Habilitation candidate
- Team leader "Ultra HR-MS"
- Development of analytical techniques based on high-resolution mass spectrometry, particularly, evolved gas analysis coupling (thermogravimetry, gas chromatography) and (laser-based) photoionization techniques. Comprehensive analysis of complex organic mixtures, such as environmental samples (organic aerosols), in Petroleomics and material science (*e.g.*, polymers). Special focus on data processing, statistics, and software development for high-resolution mass spectrometry and ion mobility spectrometry.
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Dr. Martin Sklorz,
Dipl.-Geoök.

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- Senior Scientist
- Topic leader "Aerosol Physics"
- Application, optimization, and development of analytical tools for physical and chemical characterization of complex systems. Special interest in finding clever solutions for the elucidation of environmental and health-related processes.
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Dr. Thorsten Streibel,
Dipl.-Chem.

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- Assistant Director UR/Senior Scientist
- Topic Leader "Thermal Processes & Photoionisation Mass Spectrometry"
- Improving the comprehension of the formation of organic trace compounds originated from thermal processes such as pyrolysis and combustion. Investigation of the thermal behavior and characterization of fossil and regenerative fuels.
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Michael Wendler

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- Support with various laboratory measurements (EC/OC, Pyro/GC-MS, and photometry), preparation, and implementation of internships.
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Prof. Dr. Ralf Zimmermann,
Dipl.-Chem.

- HMGU/UR
- Professor of Analytical Chemistry
- Head of the Cooperation Group Comprehensive Molecular Analytics (CMA)
- Head of Joint Mass Spectrometry Centre (JMSC)
- Spokesperson of the Helmholtz Virtual Institute of Complex Molecular Systems in
- Environmental Health (HICE)
- Main research interests on the one side are the development and application of novel analytical approaches, in particular in the field of general mass spectrometry, photoionization mass spectrometry, aerosol mass spectrometry and high resolution mass spectrometry as well as in hyphenated instrumental analysis systems and comprehensive multidimensional separation approaches. On the other side, the second dominating research interest lies in Aerosol and Health research, which includes the chemical and physical characterization of ambient-, emission- and model-aerosols as well as the analysis of biological and health effects of aerosol exposure on cellular model systems and humans. The aim is to better understand the reasons and mechanisms of aerosol induced health effects.
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2.2 New Habilitants

Dr. Hendryk Czech

Hendryk Czech studied Chemistry at the University of Rostock and received his M. Sc. and Dr. rer. nat. degrees at the chair of analytical chemistry (Prof. Ralf Zimmermann) of the University of Rostock. He investigated the chemical composition of primary and aged combustion aerosols with advanced mass spectrometric analyses and chemometric techniques for data mining. During his doctoral studies, he was granted a scholarship for two years from "Landesgraduiertenakadamie" of the University of Rostock for in-depth aerosol particle analysis with photoionization mass spectrometry.

In 2018, he joined the group Fine Particle and Aerosol Technology Laboratory (FINE) of Professor Jorma Jokiniemi, University of Eastern Finland (UEF) at Kuopio, as a postdoctoral researcher focusing on fundamental aspects of aerosol aging in oxidation flow reactors, laboratory aging of biomass burning aerosol and its effect on toxicological aerosol properties.

In 2019, he moved to Prof. Zimmermann's group "Comprehensive Molecular Analytics" (CMA) at Helmholtz Centre Munich (HMGU) and became a project manager and research associate of the Helmholtz International Lab aeroHEALTH and topic leader aeroHEALTH and data analysis at JMSC within the research area "Aerosols and Health". In parallel, he started as a habilitation candidate at the chair of analytical chemistry (Prof. Zimmermann) and lecturer for the study programs "Chemistry" (BSc/MSc) and "Medical Biotechnology" (BSc) at the University of Rostock (UR). Besides his embedment in major research projects between HMGU, UR, and UEF, he is heading a DFG-funded project on ultra-fast gas chromatography photoionization mass spectrometry and co-leads together with Dr. Chrisopher Rüger a DFG-RFBR-funded project on the characterization of Siberian wildfire aerosol emissions, their atmospheric transformation, and effects on the environment and human health. Furthermore, he supports the development of strategies for data analysis in single-particle mass spectrometry and for industrial process control and optimization.

In his habilitation, he seeks to foster the cross-talk between the fields of natural science and mathematics, specifically combustion and atmospheric chemistry, physical and chemical measurement techniques, botany, molecular biology, chemometrics, and bioinformatics.

Dr. Hendryk Czech has published 25 documents in peer-reviewed journals and one book chapter. His h-index amounts to 12 as calculated by Scopus resulting in 374 citations in 288 documents.

Dr. Christopher Paul Rüger

Christopher P. Rüger studied Chemistry at the University of Rostock and received his Master of Science and Dr. rer. nat. degree in the working group of Prof. Ralf Zimmermann in 2013 and 2018, respectively. During the Master thesis, he focused on unraveling the chemical complexity of ship diesel aerosol emissions utilizing laser desorption ionization and ultra-high resolution mass spectrometry. He started his doctoral studies with a two-year postgraduate scholarship from the European social fund administrated by the "Landesgraduiertenakademie" of the University of Rostock. During his doctoral studies, Dr. Rüger did a 3-month research visit in the working group of Prof. Peter O'Connor and Dr. Mark Barrow at the University of Warwick, UK. He received his doctoral degree with distinction and in 2019 he was awarded the annual Joachim-Jungius-Preis of the University of Rostock for the best doctoral thesis in natural sciences.

From May 2018 till July 2019, he was a postdoctoral scientist in the Analytical Chemistry group at the University of Rouen-Normandy, headed by Prof. Carlos Afonso, working at Fourier transform ion cyclotron resonance (FT-ICR) and ion mobility mass spectrometry. This Postdoc was performed in close cooperation with Total SE and the International Joint Laboratory – iC2MC: Complex Matrices Molecular Characterization. Furthermore, he was involved in the prototype development and testing of novel ion mobility high-resolution mass spectrometric platforms from Bruker Daltonics and Waters Corporation with the motivation to describe the size/shape of isomers in ultra-complex mixtures.

In July 2019, he rejoined the working group of Prof. Ralf Zimmermann at the University of Rostock becoming the topic leader for ultra-high resolution mass spectrometry in the research area "Enabling Analytical Technologies" at the same time starting to work for his habilitation thesis. He lectures in Bachelor and Master of Science courses for Chemists and future teachers. Dr. Rüger is involved in various research proposals, such as the European FT-ICR MS network, the SAARUS project, and the recycling of wind energy composite materials. Moreover, he scientifically leads several projects, such as the Eurostars AerOrbi project and a joined project between the German and French science foundation (TIMSAC, DFG-ANR). Furthermore, he is strongly involved in the organization of the competence centre mass spectrometry at the Interdisciplinary faculty, department life, light and matter (LLM).

His habilitation is focused on the further development of high-resolution mass spectrometry. Particularly the hyphenation of evolved gas analysis techniques, such as thermal analysis and gas chromatography, as well as implementing novel laser-based ionization schemes is foreseen. Together with ion mobility technology, he seeks to unravel ultra-complex organic mixtures from the field of Petroleomics (bio- and recycling fuels, asphaltenes, and shipping fuels), describing

Dr. Hendryk Czech

Dr. Christopher Paul Rüger

Dr. Sebastiano Di Bucchianico

recycling processes (composite materials), and understanding carbonaceous aerosol emissions on the molecular level. A particularly interesting aspect is the connection between complex fuel composition and the molecular pattern of the corresponding emissions (including primary and secondary aerosol particles) as well the health implications. The development of software-applications for automated data analysis/processing is a key aspect and will be performed as a partner of iC2MC sharing a post-doctoral scientist.

To date, Dr. Christopher Rüger has published 25 documents in peer-reviewed journals and has an h-index of 10 (365 citations in 309 document).

Dr. Sebastiano Di Bucchianico

Sebastiano Di Bucchianico received his M. Sc. degree in Biological Sciences and a Ph.D. degree in Cell and Molecular Biology in Professor Anna Poma's and Dario Botti´s groups, respectively, at the University of L´Aquila (Italy). He was working in genetic toxicology as well as conducting cytogenetic studies on the 3D chromosomal structure by means of Atomic Force Microscopy and molecular tools to generate single-cell genetic probes. During both master and doctoral studies, he was granted EU Erasmus scholarships to study at the Sorbonne University Pierre et Marie Curie and at the Institute Jacques Monod – University Paris Diderot (France), respectively.

In 2010, he was recruited to the group of Professor Lucia Migliore, University of Pisa (Italy), as a post-doctoral fellow focusing on genetic toxicology of nanomaterials and conducting experiments in the frame of two European projects, NanoReTox and SanoWork. These projects were aimed at identifying the potential risks to human health posed by free-engineered nanomaterials and at identifying a safe occupational exposure scenario at all stages of nanomaterial production, use, and disposal.

In 2014, he joined Professor Hanna Karlsson's group at the Karolinska Institute (Stockholm, Sweden) investigating whether nanoparticles-containing metals can cause DNA and chromosomal damage in cells as well as elucidating underlying molecular mechanisms and whether is the nanoparticles or the released ions responsible for the induced genotoxicity and mutagenicity. Another focus was to investigate various methods to study nanoparticle-cell interactions upon exposure to airborne nanoparticles at Air-Liquid Interface or by assessing short-term vs long-term nanoparticle exposure effects on lung cells.

In 2017, Dr. Di Bucchianico moved to the Comprehensive Molecular Analytics unit led by Professor Ralf Zimmermann at Helmholtz Zentrum München, as a scientist working in the aerosol toxicology topic led by Sebastian Öder. At that time, he was implementing the CMA bio-lab with new instruments and equipment (*e.g.* real-time PCR, optical microscope, OMICS dedicated software) as well as introducing genetic toxicology assays to the group, planning and conducting measurement campaigns in the frame of HICE-Aerosol and Health project. He was involved in planning and performing experiments in the frame of the C^3 project (toxicity of Carbon Concrete Composite) aimed at investigating genotoxicity and fibrotic changes induced by inhalable particles and fibers at working places. Together with his passion to plan and conduct experiments he was supervising PhD students working on understanding how gas-particle partitioning influences the genotoxicity of semi-volatile organic compounds as well as working on assessing DNA bases oxidation and epigenetic changes by liquid chromatographic tandem mass spectrometric methods following particulate matter in vitro exposures. In course of his involvement in the Helmholtz International Lab aeroHEALTH, Dr. Di Bucchianico was honored in 2019 to lead the new CMA research topic "Aerosol Mutagenesis", and he was selected as Work Package leader for the EU granted project Biogenic Organotropic Wetsuits (BOW, lead beneficiary HMGU).

Dr. Di Bucchianico published more than 25 documents including peer-reviewed journal articles and book chapters, and he has an h-index of 12 as calculated using Scopus resulting in 616 citations in 534 documents.

2.3 Current PhD Students

Lukas Anders

Lukas Anders studied physics at the University of Rostock and received his Master of Science degree in November 2019. His current research focuses on the optimization of time-of-flight mass spectrometry techniques for individual aerosol particles to investigate air pollution from ships. Ship emissions are known to have severe impacts on human health and the marine environment. Relevant aerosol components are sulfur, polycyclic aromatic hydrocarbons, and metal oxides bound to airborne particles. Therefore, a new two-step photoionization technique permits the investigation of the particles' chemical composition using tailored laser ionization schemes. This technique shall reveal characteristic chemical fingerprints of emissions from several ship fuels and the impact of scrubber technology on emissions.

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Stephanie Binder

Stephanie Binder studied Biology at Salzburg University and received her Master's degree in March 2018. Her research is concerned with the adverse health effects of semi-volatile organic compounds (SVOCs) at the workplace. SVOCs constitute a gas-particle-composition in which both components occur in coexistence. This fact challenges the risk assessment of the exposure of those hazardous substances at the workplace. Using the in vitro air-liquid interface (ALI) model, this challenge will be overcome by detecting both components separately. It allows furthermore the investigation of how far the aggregate phase of a hazardous substance affects the respiratory system and on which cellular level these effects will be reflected. Her PhD project is financed by the „German Social Accident Insurance" DGUV.

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Xin Cao

Xin Cao completed his Master's degree in chemistry at Beijing Forestry University in 2016. He is enrolled at the University of Rostock and works at Helmholtz Zentrum Muenchen since 2016. At present, he is a member of TOPIC IV „Aerosol Mutagenesis" group at Comprehensive Molecular Analytics (CMA). His research focuses on analyzing markers of cell metabolisms such as oxidatively damaged nucleobases and lipid oxidation products by using liquid chromatography tandem mass spectrometry combined with different sample preparation methods such as solid-phase extraction and liquid-liquid extraction. His research goal is to study the impact of aerosol on human health. He is currently investigating the in vitro effects of different reference particulate matter (urban dust, diesel combustion particles, and traffic-related particles) on epigenetics of modified DNA bases by LC-MS/MS.

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Lukas Friederici

Lukas Friederici studied Chemistry at the University of Rostock and received his Master of Science degree in September 2019. His research can be classified as the topic of material science. His focus is on the analysis of different sorts of composite materials, ranging from glass/carbon fibers, polymers, biomass up to pyrolysis chars from the recycling of heterogenic polymer waste. The main interest is the analysis of products from a new build pyrolysis reactor, within the cooperation with TAB Maschinen- und Stahlbau GmbH, for the recycling of rotor blades from the demolition of wind turbines. Therefore, a high-resolution mass spectrometer (FTICR MS) is applied for the detailed description of evolved gas from a thermal balance. With this technique, the pyrolysis process can be mimicked. As a second separation method, gas chromatography is used for the analysis of evaporable isomeric components.

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Nadine Gawlitta

Nadine Gawlitta completed her Master's Degree in Analytical Chemistry at the Leibniz University Hanover in 2017. In April 2018 she started her PhD at the Helmholtz Zentrum München. She is working in close cooperation between CMA and the Institute for Asthma and Allergy Prevention (IAP, Head: Prof. von Mutius) to link allergy prevalence and environmental exposures. In this context, she is sampling in

possibly allergy-protective farming environments, following chemical characterization of the aerosol composition using GCxGC-TOFMS. With the means of chemometric analysis, she is trying to identify components in the air that might be responsible for the protective farm effect. Moreover, she is sampling anthropogenic aerosols from diesel exhaust as an example for allergy-adjuvant surroundings and is analyzing these samples likewise. The biological analysis follows and aims to connect chemical components with biological responses being highly relevant in allergy development.

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Barbara Giocastro

Barbara Giocastro studied at the University of Messina and graduated in Pharmacy in 2016. She joined CMA in November 2019 for five months applying GC×GC-MS to analyze semi-volatile organic compound emissions collected from food cooking. Barbara started her PhD already in October 2017 at the University of Messina with the research project „Use and evaluation of novel thermal and flow modulators for comprehensive 2D GC“. Her research focuses on the analysis of volatiles and semi-volatile substances in complex samples using comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GC×GC-MS). Concerning mass spectrometry, rapid-scanning single quadrupole, triple quadrupole, and high-resolution time-of-flight devices have been used in this research. In the field of GC×GC, she has carried out applications, method optimization studies, and in-depth studies on novel thermal and flow modulation systems.

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Christoph Grimmer

Christoph Grimmer studied chemistry at the University of Rostock and received his Master‘s degree in 2015. The goal of his research is to identify the composition of petrochemical substances, which are analyzed with difficulty by usual methods. Using thermogravimetry, substances can either be evaporated or broken down into volatile fragments. Resulting heat flow or typical reaction temperatures may allow conclusions on the initial structures. Subsequently, the gaseous substances are ionized by laser radiation with defined wavelength and energy density and measured by a mass spectrometer. Switching between selective ionization (resonance-enhanced multiphoton ionization) or universal ionization (single photon ionization) yields further information about the analytes.

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Elena Hartner

Elena Hartner completed her Master‘s Degree in Environmental Science at the University of Bayreuth in 2019. In August 2019 she started her PhD at CMA at the Helmholtz Zentrum München. In her work, she is characterizing the chemical composition of primary and secondary organic aerosols with a focus on the particle and gas phase using comprehensive two-dimensional gas chromatography and spectroscopic techniques. The research is done within the framework of the aeroHEALTH project, which aims to understand the biological and health effects of atmospheric aerosols. To investigate how atmospheric aging affects the health impact of aerosols and which aging products are the most health-relevant, aerosol aging is simulated under atmospheric relevant conditions in oxidation flow reactor experiments.

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Jan Heide

Jan Heide studied Chemistry at the University of Rostock and reached his Master‘s degree in September 2016. His research topic is the online analysis of coffee roast gases. Coffee roasting is a very complex process being influenced by the used beans, roaster technique, and various conditions. The aim is to obtain an understanding of the roasting process and to achieve a model for different coffee roasters and roasting conditions. Due to the high complexity of the investigated matrix, especially soft photoionization techniques such as SPI as well as REMPI will be used combined with the fast and reliable TOF-MS technology to achieve time-resolved information of the roasting process.

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Seongho Jeong

Seongho Jeong studied environmental engineering at the RWTH Aachen and received his Bachelor‘s and Master‘s degree in September 2016 and April 2019. His research

addresses the relationship between physical and chemical parameters of combustion aerosols and their biological responses to human lung cells. Mainly diesel exhaust particles are investigated, which exist in wide size ranges from a few nanometers up to some micrometers. Their physical and chemical characteristics are analyzed, depending on fuel type, combustion parameters, and emission control measures. To elucidate the corresponding toxicological effects, in vitro cell exposures in an „Air Liquid Interface (ALI)“ are planed. This work is conducted in the framework of the SAARUS project at the University of Rostock, investigating the effects of secondary treatment for reducing Sulfur- and particle emissions from ship diesel engines.

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Mohamed Khedr

Mohamed Khedr studied Chemistry at the Tanta University (Egypt) and worked in the field of generic pharmaceuticals‘ formulation and quality control. In 2017, he received his Master‘s degree at the University of Siegen (Germany); majoring in Analytical Chemistry with a focus on applications of Gas Chromatography-Mass Spectrometry (GC-MS) in investigating trace analytes (down to the ranges of ng/kg) within a diversity of matrices, such as biological samples, beverages, foodstuff, and food contact materials. His current research focuses on developing sensitive methods for determining semi-volatile organic compounds involved in personal exposure to particulate matter in ambient air. In order to validate the scientific scouts to be developed within the SmartAQnet project; and to perform source apportionment for detectable species. SmartAQnet project aims at characterizing air quality in the urban part of Augsburg and is funded by the German Federal Ministry of Traffic and Digital Infrastructure – Bundesministerium für Verkehr und digitale Infrastruktur (BMVI).

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Arne Koch

Arne Koch studied Chemistry at Leipzig University and received his Master’s degree in 2018. His research focuses on the chemical and morphological analysis of aerosols formed during the processing of Carbon Concrete Composites (C^3), a newly designed fiber-reinforced concrete material. To avoid health impairments as they were observed for asbestos concrete, C^3 processing should be investigated prior to mass production. Since C^3 reinforcement materials contain organic polymers as well as inorganic carbon fibers, knowledge about organic compounds and the morphology of fibers contained in the inhalable particulate matter of aerosols from dry cutting processes and thermally treated C^3 materials shall help to understand and minimize possible health impairments.

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Paul Kösling

Paul Kösling studied physics at the University of Rostock and received his Master’s degree at the end of 2018. His PhD project is part of the EUROSTARS cooperation project „AerOrbi“, which includes the technical hardware modification of a commercial Exactive Orbitrap mass spectrometer implementing resonance-enhanced multiphoton ionization (REMPI). This high-resolution mass spectrometric platform is foreseen to be used for the chemical characterization of carbonaceous aerosols. High mass accuracy and resolving power, and the selectivity towards aromatic constituents due to the REMPI process are key factors. These characteristics allow unraveling the chemical composition of complex organic mixtures. Carcinogenic substance classes, such as polycyclic aromatic hydrocarbons, can be measured at trace levels. The PhD project is in close cooperation with an international team of companies: Photonion GmbH (Germany), Aerosol d.o.o. (Slovenia) and Spectroswiss SARL (Swiss).

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Xiansheng Liu

Xiansheng Liu studied Environmental Sciences at the Nanjing Normal University (Nanjing, China) and received his Master‘s degree in June 2017. His research topic comprised the ecological restoration of water, in particular odorous compounds, using gas chromatography-mass spectrometry. His PhD project focuses on the spatial variability, source contribution, and influencing factors of ambient particulate matter composition, answering key questions concerning ambient air pollution. The main goal of the project is the investigation and characterization

of organic constituents of ambient particulate matter for health-related epidemiological and toxicological studies.

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Patrick Martens

Patrick Martens has studied chemistry at the University of Rostock and received his Master's degree in September 2019. In October 2019, he started his PhD at the University of Rostock. His research focuses on the characterization of aerosols from the combustion of lignite and wood in small-scale heating appliances for domestic use. Although solid fuels for residential heating have been replaced in many developed countries, they are still a common source of energy in many parts of the world and, in particular, wood may gain more importance in developed countries in the nearer future again. Lignite on the other hand, although uncommon in developed countries, is still of high importance in many parts of the world, *e.g.* eastern Europe and China. Nevertheless, information on emissions from lignite combustion in small-scale heating appliances typical for European end-users is scarce. Therefore, on-line monitoring of gaseous emissions during the combustion with a low-fragmenting single-photon-ionization time-of-flight mass spectrometer (SPI-ToF-MS) is used to provides insights into occurring processes, *e.g.* volatilization and thermal degradation, and their characteristic emission patterns. Furthermore, a thermal-optical carbon analyzer coupled to a photoionization (PI) mass spectrometer is used to investigate the chemical nature of the carbonaceous particle fraction as well as to provide insights into the organic fraction of particulate matter on a molecular level.

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Anika Neumann

Anika Neumann studied chemistry at the University of Rostock and received her Master of Science degree in September 2016. Since October 2016, she is PhD student in the subgroup for high-resolution mass spectrometry. Her research is focused on the chemical characterization of high complex petroleum-derived material, such as bitumen and asphaltenes, by thermal analysis coupled to Fourier transform ion cyclotron resonance mass spectrometry. Additionally, she worked on the further development of ionization techniques for a better understanding of the ionization schemes as well as to expand their application.

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Dac-Loc Nguyen

Dac-Loc Nguyen has started his PhD project in Germany in October 2016. He has established his expertise in gas chromatography (GC) mass spectrometry (MS) techniques, both in one dimensional GC and two dimensional GC with in situ derivatization methods. The focus of his thesis is applications of comprehensive GC-MS techniques to characterize chemical properties/profiles of near-source, chamber, and atmospheric-related biomass burning agricultural residuals aerosols. The characterization of aerosols released from biomass combustion sources and ambient/chamber aerosol can help drawing implications of air quality and environmental impacts in Vietnam. It seeks to determine the chemical properties of near-source fresh aerosols originating from biomass burning sources such as agricultural burning practice, domestic cooking, and the secondary aerosol under different controlled conditions, in chamber studies and ambient air.

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Svenja Offer

Svenja Offer completed her Master's degree in molecular biology with the major subject of medical biology at the University of Lund (Sweden) in 2018. Her work contributes to the international and interdisciplinary research project „AeroHEALTH", which aims to understand the detailed physio-chemical characteristics of aged aerosols and their human health effect. She is elucidating the toxicological effects on the cellular and systemic level with molecular biological approaches, *e.g.* how transcriptome and proteome can give insights into health-relevant mechanisms of induced toxicological effects. By working with ALI-systems and developing new cell culture and tissue models, the effects of air pollutants in and beyond the lung can be assessed in a more realistic scenario.

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Jana Pantzke

Jana Pantzke completed her Bachelor's degree in Chemistry at the Free University Berlin in 2016 and her Master‘s degree in Toxicology at the University of Potsdam in 2018. In July 2019, Jana started her PhD in the research project „Toxicity of Wind Mill Fibers“ at the University of Rostock (working place at HMGU). She is investigating the toxicity of products arising during the recycling process of wind turbine blades in order to analyze whether structural and morphological changes occurring during the decomposition of fibrous material can increase health risks. To ensure especially occupational safety, it is inevitable to reduce toxicological concerns of the intermediate and end products of the recycling process. To do so, Jana is developing and applying suitable cell culture models and experimental techniques.

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Ellen Iva Rosewig

Ellen Iva Rosewig has studied chemistry at the University of Rostock and received her Master of Science degree in October 2020. Her research focuses on on-line aerosol mass spectrometry. She analyzes particulate matter with single-particle time-of-flight mass spectrometry and aerosol mass spectrometry to obtain information about harmful components, like polycyclic aromatic hydrocarbons or transition metals on individual particles. These studies comprise also safety-relevant powders and dusts. Her research aim is the development of novel technologies for air pollution monitoring, health risk estimates, and civil security. Examples are the remote evaluation of ship emissions and the automated detection of powders from drugs and explosives.

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Julian Schade

Julian Schade studied Chemistry at the University of Rostock and received his Master of Science degree in September 2016. His research is focused on photoionization mass spectrometry, specifically the development of a bipolar single-particle time-of-flight mass spectrometer coupled with novel photoionization techniques. To obtain information on the most harmful components of individual particles, resonance effects between laser light and these substances are used. Thus, the information about the particles‘ inorganic composition and organic adsorbates is combined for source apportionment and health risk evaluation. This setup allows for obtaining detailed information about single particles concerning their chemical composition as an on-line measurement technique.

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Marco Schmidt

Marco Schmidt studied Chemistry at the Heinrich Heine University Düsseldorf and received his Master‘s of Science degree in October 2019. His research topic focuses on femtosecond laser photoionization time of flight mass spectrometry. This comprises the adaption of new femtosecond laser ionization techniques such as pump-probe on single particles and aerosols and the optimization of aerosol time-of-flight mass spectrometers. The novel laser ionization approaches target a soft, precise, and fragment free ionization scheme for aerosols and individual airborne particles. Therefore, femtosecond laser ablation permits the investigation of the ionization mechanism, the mass spectral signatures, and the chemical composition of the particles.

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Eric Schneider

Eric Schneider studied Chemistry at the University of Rostock and received his Master’s degree in September 2020. The DFG project he is involved in, together with the Moscow State University and the Technical University Munich, includes investigations of the impact of aerosolized particulate matter from aged wildfire plumes on the environment and human health. By using the world’s largest aerosol chamber in Tomsk (Russia) and the understanding of the simulation of wildfires and aging of the emitted aerosols, sufficient fine particles can be collected to study both the biological effects and the physical-chemical parameters responsible for these outcomes. Within this project, the aim is to develop a method for extraction of aerosol filter samples to characterize the chemical composition of wildfire particle emissions as well as the influence of aerosol aging. For this purpose, electrospray ionization (ESI) and Fourier-transform ion cyclotron resonance mass spectrometry hyphenated to liquid chromatography (LC FT-ICR MS) is applied to enable the chemical characterization of these ultra-complex mixtures.

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Lukas Schwalb

Lukas Schwalb studied analytical chemistry and quality assurance at the university of applied science Bonn-Rhein-Sieg and received his Master's degree in October 2020. His PhD Thesis started in 2020 and is part of a cooperation project with the University Rostock as an academic partner and Ichthyol-Gesellschaft Cordes Pharma GmbH as an industrial partner. During his PhD project, he will analyze pharmaceutical raw material and precursor substances derived from shale oil to characterization the complex organic matrices. For this purpose, he will apply dedicated analytical techniques like comprehensive two-dimensional gas chromatography and develop analytical workflows to enable the measurement of highly sulfonated and polar active compounds. For further investigation of the sulfonation process, he will also carry out the synthesis of the intermediate products based on fractionized raw material.

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Ole Tiemann

Ole Tiemann studied chemistry at the University of Rostock and received his Master's degree in September 2020. In his PhD project, he will analyze shale oil and sulfonated shale oil materials as part of the cooperation with the Ichthyol Gesellschaft Cordes Pharma GmbH. Those high complex organic mixtures will be addressed with ultra-high resolution Fourier-transform ion cyclotron resonance mass spectrometry (FTICR MS). In order to look at different parts of the chemical space, various ionization techniques, such as electrospray ionization (ESI), will be deployed. ESI is an ambient ionization technique, which is capable of ionizing large and polar compounds that cannot be vaporized due to their size or thermal instability. In contrast, other ambient ionization approaches, such as atmospheric pressure photoionization (APPI) and atmospheric pressure chemical ionization (APCI), will be used to focus on a broader chemical space. Besides the chemical characterization, also the distinction between counterfeit products and the sulfonated shale oils is part of his PhD.

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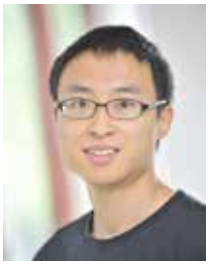
Xiao Wu



Xiao Wu studied Environmental Health and Occupational Health at the Shandong University (China) and received his Master's degree in 2012. As a PhD student, he is in the cooperation group CMA at Helmholtz Zentrum München. The main goal of his research is to investigate the oxidative stress induced by particulate matter (PM) exposure on the human body with LC/ LC-MS methods. The work runs in the frame of the „Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health – HICE" and „Cooperative Health Research in the Augsburg Region – KORA". Robust LC/ LC-MS methods to determine oxidative stress-related biomarkers have been developed, optimized, and established. They could be successfully applied on three different projects: 1) a cohort of travelers has been recruited. Urine samples were collected before and after travel and analyzed to investigate the effects of air pollution; 2) Urine samples from volunteers of the Augsburg region have been collected and analyzed to investigate the association between the long term ultrafine particulate matters (UFPM) exposure and human health (KORA cohort); 3) samples from cigarette smoke-exposed mice were collected and oxidative stress burden was investigated.

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Elias Zimmermann

Elias J. Zimmermann completed his Master's degree in molecular microbiology, microbial ecology, and immunobiology at the University of Vienna in 2017. He is interested in cellular mechanisms and their responses to different (unfavorable) conditions induced by *e.g.* pathogens or aerosols. Since January 2018, he is doing his PhD in aerosol sciences at the Helmholtz Zentrum Munich, where he is investigating cellular responses at the air liquid interface (ALI). The main focus lies on responses of in vitro mammalian cells, especially lung cells, to ambient (bio-) aerosols with a particular focus on allergens. By working with ALI-systems, the effects of allergens and other ambient particles can be assessed in a more realistic scenario, illustrating their role in the sensitization of lung cells and thus the development of allergic diseases. To incorporate also large (bio-) particles/ fibers into the study, his work includes the development of a new exposure- and laser-based detection method.

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2.4 Awarded Doctorates in Natural Sciences (Dr. rer. nat.)

Dr. Hendryk Czech

- Graduated on 5th December 2017 at the University of Rostock with Prof. Dr. Zimmermann with the grade "summa cum laude"
- Thesis title: "Primary and secondary combustion aerosols from wood stoves and marine engines"
- External referees: Prof. Dr. Joakim Pagels (Lund University, Sweden) and Prof. Dr. Wolgnag Rogge (University of California at Merced, USA)
- Co-supervisor JMSC: Dr. Thorsten Streibel

Dr. Tamara Kanashova

- Graduated on 23rd January 2018 at the University of Rostock with Prof. Dr. Zimmermann
- Thesis title: "Applying quantitative mass-spectrometry based proteomics on relevant questions of combustion as-induced health effects"
- External referee: Prof. Dr. Barbara Rothen-Rutishauser (University of Fribourg, Switzerland)
- Co-supervisor: Prof. Dr. Gunnar Ditmar (Luxemburg Institute of Health)
- Co-supervisor JMSC: Dr. Sebastian Öder

Dr. Christopher Rüger

- Graduated on 9th January 2018 at the University of Rostock with Prof. Dr. Zimmermann with the grade "summa cum laude"
- Thesis title: "Development of characterization techniques for petroleum and combustion aerosol utilizing high resolution Fourier transform mass spectrometry"
- External referee: Dr. Janne Jänis (University of Eastern Finland, Finland)
- Co-supervisor JMSC: Dr. Martin Sklorz

Dr. Fengxia Li

- Graduated on 3rd July 2018 at University of Rostock with Prof. Dr. Zimmermann
- Thesis title: "Chemical Characterisation and Source Apportionment of Atmospheric Quasi-Ultra-Fine Particulate Matter ($PM_{0.36}$): Seasonality and Spatio-Temporal Variability"
- External referee: Magda Claeys (Emeritus Professor at the Department of Pharmaceutical Sciences at the University of Antwerp, Belgium)
- Co-supervisor JMSC: Dr. Jürgen Schnelle-Kreis

Dr. Sarah Padoan

- Graduated on 29th January 2019 at Ca'Foscari University of Venice, Italy
- Thesis title: "Environmental Markers in Remote Areas and Urban Sites. Investigation of the Contribution of Natural and Anthropogenic Sources"
- Co-supervisor JMSC: Dr. Jürgen Schnelle-Kreis

Dr. Vesta Kohlmeier

- Graduated on 5th February 2019 at University of Rostock with Prof. Dr. Zimmermann
- Thesis title: "Development and Application of Novel Portable Personal Aerosol Samplers for Partitioned Sampling of Aerosolized Semi-Volatile Organic Compounds in Workplace Environments"
- External referee: Prof. Dr. Thomas P. Knepper (Institute for Analytical Research (IFAR), Hochschule Fresenius, Idstein, Germany)
- Co-supervisors JMSC: Dr. George Dragan, Dr. Martin Sklorz

Dr. Frank Hauser

- Graduated on 5th February 2019 at the University of Rostock with Prof. Dr. Zimmermann
- Thesis title: "Novel analytical approaches to detect and assess clandestine amphetamine laboratories"
- External referee: Prof. Dr. Thomas Knepper (Institute for Analytical Research (IFAR), Hochschule Fresenius, Idstein, Germany)
- Co-supervisor: Dipl.-Chem. Michael Pütz/BKA

Dr. Toni Miersch

- Graduated on 2nd July 2019 at the University of Rostock with Prof. Dr. Zimmermann
- Thesis title: "Composition of carbonaceous fine particulate emissions of a flexible fuel DISI engine under high velocity and municipal conditions"
- External referee: Prof. Dr. Christian Focsa (University of Lille, France)
- Co-supervisor JMSC: Dr. Hendryk Czech

Dr. Theo Schwemer

- Graduated on 28th January 2020 at the University of Rostock with Prof. Dr. Zimmermann
- Thesis title: „Entwicklung und Optimierung einer Methode zur Characterisierung unbekannter Substanzen mittels Gaschromatographie – ultra-hochauflösender Massenspektrometrie und chemischer Ionisierung unter Atmosphärendruck"
- External referee: Prof. Dr. Andreas Römpp (University of Bayreuth, Germany)
- Co-supervisor JMSC: Dr. Christopher Rüger

Dr. Uwe Käfer

- Graduated on 21st January 2020 at the University of Rostock with Prof. Dr. Zimmermann
- Thesis title: "Integration of different sample inlets for high resolution time-of-flight mass spectrometry to investigate heavy petroleum compounds"
- External referee: Prof. Dr. Carlos Afonso (Université de Rouen-Normandie, France)
- Co-supervisor JMSC: MSc.-Math. Sciences Thomas Gröger

Dr. Sascha Müller-Münster

- Graduated on 7th January 2020 at the University of Rostock with Prof. Dr. Zimmermann
- Thesis title: “Novel approaches for impurity and isotopic profiling of synthetic cannabinoids”
- External referee: Prof. Dr. Heiko Hayen (University of Münster, Germany)
- Co-supervisor: Dipl.-Chem. Michael Pütz/BKA

Dr. René Reiss

- Graduated on 7th January 2020 at the University of Rostock with Prof. Dr. Zimmermann
- Thesis title: “Investigation and development of analytical techniques for trace level detection of drugs, explosives and their precursors”
- External referee: Prof. Dr. Heiko Hayen (University of Münster, Germany)
- Co-supervisor JMSC: Dr. Sven Ehlert

Dr. Christian Gehm

- Graduated on 30th June 2020 at the University of Rostock with Prof. Dr. Zimmermann
- Thesis title: “Resonance enhanced multiphoton ionization of polycyclic aromatic hydrocarbons”
- External referee: Prof. Dr. Christine Achten (Univeristy of Münster, Germany)
- Co-supervisor JMSC: Dr. Thorsten Streibel

Dr. Julian Schade

- Graduated on 19th January 2021 at the University of Rostock with Prof. Dr. Zimmermann
- Thesis title: “Investigation of Airborne Particles by On-Line Single-Particle Mass Spectrometry with Novel Ionization Approaches”
- External referee: Dr. Thomas Leisner, (Karlsruhe Institute of Technologie, Germany)
- Co-supervisor JMSC: Dr. Johannes Passig

Dr. Anika Neumann

- Graduated on 26th January 2021 at the University of Rostock with Prof. Dr. Zimmermann
- Thesis title: “Investigation of heavy petroleum-derived material with thermogravimetry coupled to high resolution Fourier transform ion cyclotron resonance mass spectrometry”
- External referee: Dr. Pierre Giusti (Total Research and Technology Gonfreville, France)
- Co-supervisor JMSC: Dr. Christopher Rüger

2.5 Scientific Training: B. Sc. and M. Sc. Students

B. Sc. Students at the Chair of Analytical Chemistry, University of Rostock:

- Ellen Iva Rosewig, 2018, Thesis: Determination of aromatic pollutants in water samples with membrane-inlet mass spectrometry
- Eric Schneider, 2018, Thesis: Characterization of petrochemicals by thermogravitmetry hyphenated to mass spectrometry
- Rike Thomsen, 2018, Thesis: Analysis of conventional and new smoking devices using an oa-SPITOF-MS
- OleTiemann, 2018, Thesis: Gaschromatography and high resolution mass spectrometry of polycyclic aromatic hydrocarbons – Basic study on the chemical and laser-based two-photon ionization
- Martin Bauer, 2019, Thesis: Mass spectrometric investigations of particulate emissions from a ship engine
- Christian Bremert, 2019, Thesis: Combination of an orthogonal acceleration time-of-flight mass spectrometer with a vacuum pulsed ion source for monitoring of the coffee roasting process
- Sören Iwe, 2019, Thesis: Real-time investigation of commercial tobacco products with photo-ionization mass spectrometry
- Lars Kurths, 2019, Thesis: Water samples analysed in real time with membrane-inlet mass spectrometry
- Fabian Carl, 2020, Thesis: Determination of amino acid spectra in fishery products to detect foreign proteins

M. Sc. Students at the Chair of Analytical Chemistry, University of Rostock:

- Abdulghani Ebtini, 2019, Thesis Real-Time Measurements of Polycyclic Aromatic Hydrocarbons on Individual Air Pollution Particles using Mass Spectrometry
- Lukas Friederici, 2019, Thesis: Studies of selected petrochemical solids
- Patrick Martens, 2019, Thesis: Mass spectrometric analysis of primary and secondary organic aerosols from solid fuel combustion
- Ellen Iva Rosewig, 2020, Thesis: Single-Particle Mass Spectrometry of Ship Emissions
- Eric Schneider, 2020, Thesis: Thermal analysis of complex petrochemical materials with soft ionisation and detection by mass spectrometry
- Ole Tiemann, 2020, Thesis: Characterization of petroleum products by gas chromatography and high-resolution mass spectrometric techniques – Shale oil and Shale oil derivatives
- Kevin Schnepel, 2021, Thesis: Hyphenation of ultra-fast gas chromatography and photo-ionization mass spectrometry fort he investigation of ambient aerosols
- Rike Thomsen, 2021, Thesis: Investigation of the capability of photoionization mass spectrometry for process control in industrial coffee roasting

Internships and Exchange students

- Michael Mang (HMGU, 06.18 – 10.18 Aerosol Physics)
- Ahmed-Burhan Tasci (UniBW, 10.18 – 12.18 Aerosol Physics)
- Elena Bialas (HMGU, 11.18 – 12.18 Aerosol Toxicology)
- Martin Otto Leichenauer (HMGU, 06.19, Aerosol Chemistry)
- Anni Hartikainen (U Rostock, 03.18 – 11.18, High Resolution Mass Spectrometry)

3 What did JMSC

3.1 Selected Research Projects Research Area 1: Aerosol and Health

(01) A Single-Particle Mass Spectrometry Study using Adaptive Resonance Theory (ART-2a) Clustering: Chemical Composition of Particles released from a Major Fire Incident at a Discarded Metal Storage in Rostock, Germany

H. Czech (HMGU/UR), J. Passig (UR/HMGU), J. Schade (UR), R. Irsig (UR/Photonion), R. Zimmermann (UR/HMGU)

In epidemiological studies, short-term exposure to high levels of particulate matter (PM), especially from combustion processes, has been found to increase the number of cardiovascular diseases and respiratory ailments (Samoli *et al.*, 2016). Apart from scheduled events, such as New Year's Celebration or Bonfire Night, natural events such as sand storms, wildfires, and volcano eruptions or incident fires are inevitable and may promptly affect public health. Furthermore, the PM composition is not considered in current EU regulation, assuming equal toxicity of PM from different origins. Here we describe high ambient concentrations of particulate heavy metals over seven hours without exceeding the daily PM10 limit, analyzed by single-particle mass spectrometer (SPMS).

Single-particle mass spectrometry, sampling site, and data analysis

We investigated PM emissions from a major fire incident on 4th July 2018 in Rostock (Germany) by a bipolar SPMS and laser desorption ionization (LDI) in both positive and negative mode at 248 nm (Li *et al.*, 2011). The scattering unit of the SPMS allows the detection of particles in the size range from 200 nm to 2000 nm, which belongs to the range of respirable PM.

In this incident, about 4000 t of discarded metal in a temporary storage facility were burning at an industrial area in the harbor area in central Rostock. Because of the prevailing winds from the north-northwest, combustion aerosols were transported to the city center and university site in the south (figure 1). Data from the frequent ambient measurements on 26th June 2018 of the same daytime and similar weather conditions were selected as a reference for the urban PM to separate background exposure from additional PM released from discarded metal burning. On 26th June, the dominant wind direction was also northwest, which is used to be associated with relatively clean air masses of marine origin. In addition to SPMS mass spectra, PM10 data is available from an air quality monitoring station at an urban traffic site ("Holbeinplatz"). To identify particle sources, single-particle mass spectra of both days were clustered by adaptive resonance theory (ART-2a) neural networks (Carpenter *et al.*, 1991).

Figure 1: *Left: City map of Rostock with locations of a fire incident, air quality monitoring station "Holbeinplatz", and university sampling site. The orange cone represents the transport and dispersion of fire-related PM from the incident site due to variation in wind direction. Right: Dense smoke in the city harbor during the evening, approximately 4 km southeast from the fire incident.*

Detection rate and composition of discarded metal burning particles

In total, more than 75,000 particles were detected from 2.43 pm to 9.43 pm, which equals to > 10,000 particles per hour. In the late evening, the wind direction changed that noticeably less particles reached the city center and the detection frequency declined to 300 particles per hour. In more than 50 % of the particles in positive LDI (+LDI), the toxic heavy metal lead was detected (broad peak spanning the m/z range from 206 to 208 in figure 2). Note, that lead was not found in the urban background of 26th June. Additionally, substantial intensities of zinc, iron, and possibly copper and aluminum ions were spotted. At the applied wavelength of 248 nm, the detection of iron is augmented by resonance effects in LDI, which also improves the sensitivity for other trace metals (Passig *et al.*, 2020). In the negative LDI (-LDI) mass spectra, ions of halogens and pseudohalogens were detected, such as chlorine, nitrate, and sulfate. Probably due to the high particle load of metals and chlorine, complex ions such as $[ZnCl_3]^-$ are formed, which was derived from comparison with the theoretical isotopic pattern (figure 2).

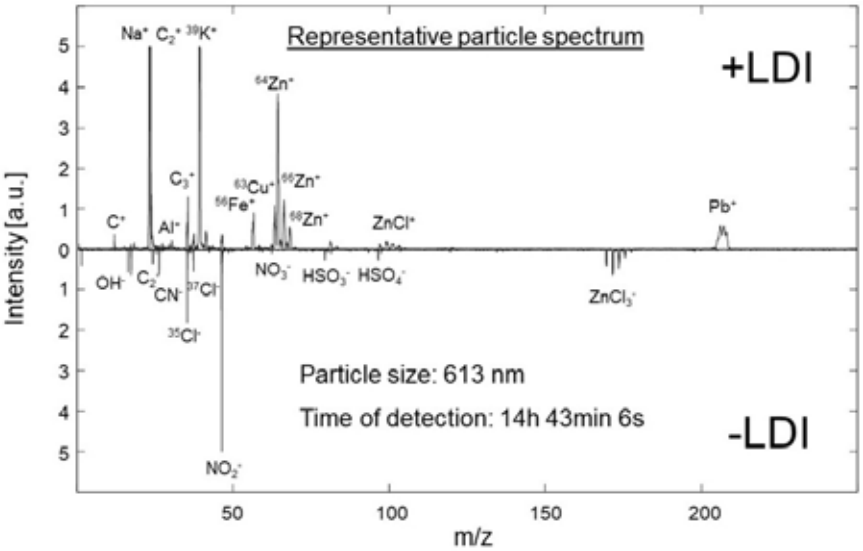

Figure 2: *Representative +LDI and −LDI mass spectrum of particles of 4th July 2018 with significant loads of zinc, lead, iron, and copper.*

Particle classification by ART-2a Clustering

Based on the findings from manual inspection of the mass spectra, all m/z in the range from 203 to 211 were binned to one variable, accounting for the lead signal. We evaluated the quality of the clustering result over several conditions in ART-2a by calculating a self-developed score. The best score with 91 % of the particles sorted into seven clusters was achieved using 50 maximum iterations, a vigilance factor of 0.7, and a learning rate of 0.1. The cluster centers (figure 3) were assigned to the following particle types: Three groups of discarded metal fire (with relative contributions of 33 %, 32 %, and 17 %), two groups of road traffic (10 % and 2 %), one group of maritime traffic (2 %), and one group of aged soot (2 %). Therefore, the incident fire accounted for > 80 % of ambient PM levels. The relative contribution of the three metal clusters (metal 1-3) to the total particle count remained constant, indicating different types of discarded metal burning. Particles of sorted to soot clusters (soot 1-2) contain carbon clusters as dominant peaks and originate from local emissions sources, such as road traffic. A small fraction of soot particles, accounting for 4 % of the total detected particles, were transformed by atmospheric aging and contain enhanced intensities of nitrate-related peaks (NO_3^-, NO_2^- and NO^+) from the oxidation of nitrous gases and oxygen-containing fragments of oxygenated organics, such as CHO+ and C_2H_3O+. Remarkably, ART-2a analysis even resolved the contribution of the marine fuel combustion. The most abundant metals in heavy marine fuels are vanadium, nickel, and iron, which are the dominating peaks in the ship cluster (Streibel *et al.*, 2017). Further evidence for ship emissions is gained from a small peak of HSO_4^- because marine fuels contain orders of magnitude more sulfur than fuels for road traffic.

In the illustration of the size distribution (figure 3), particle modes of all cluster centers are located between 450 and 500 nm with rapid declines in particle number towards 200 and 2000 nm, which is caused by the sensitivity of the SPMS for the size mode between ultrafine and coarse particles. Nevertheless, it is apparent that fresh combustion particles from the fire incident appear at lower particle sizes in relation to other cluster centers containing particles of longer atmospheric residence time.

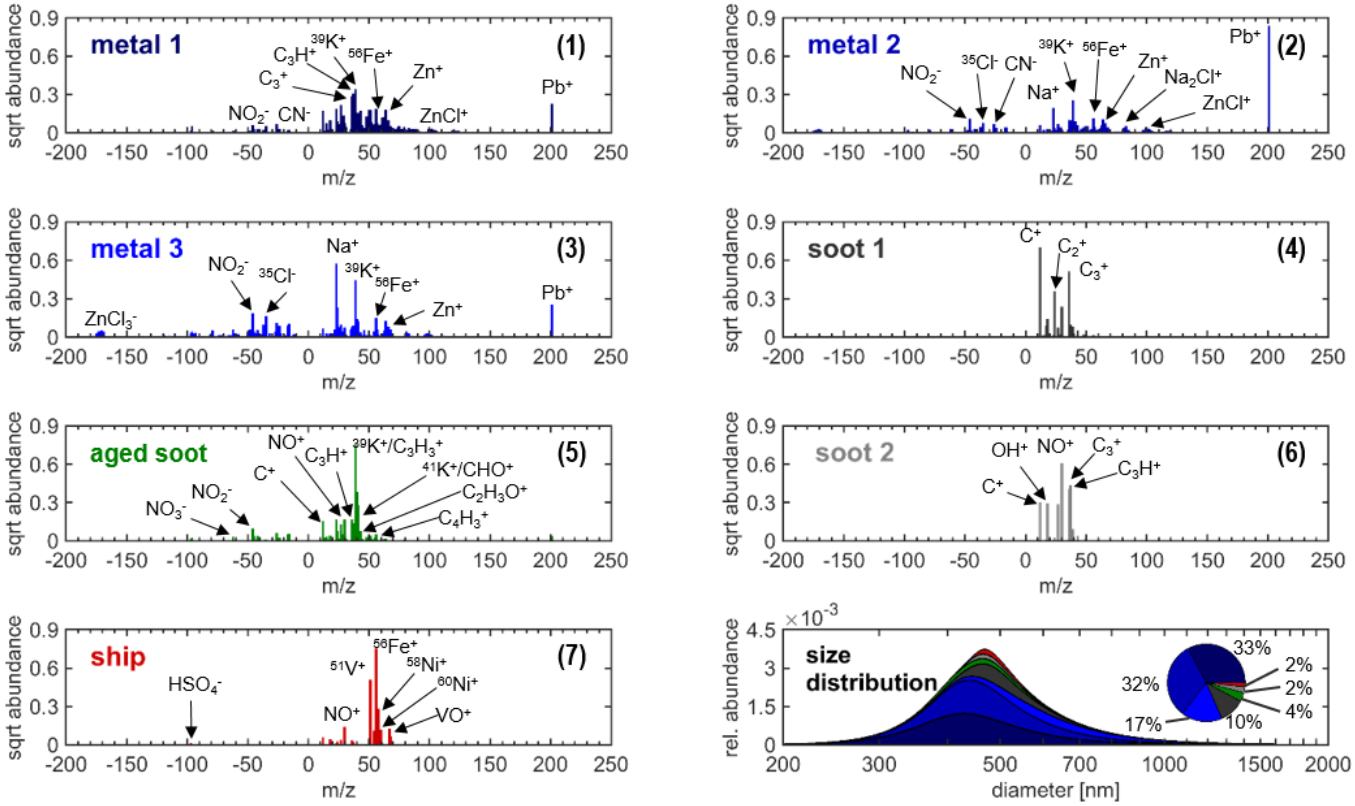

Figure 3: Seven cluster centers obtained from ART-2a analysis of in total 93,000 particle mass spectra.

Local PM10 monitoring for EU limit values

The local monitoring station close to the city center determined a daily mean PM10 concentration of 35 µg m^{-3} which is clearly below the limit of 50 µg m^{-3} from EU legislation. Since all particles of significant lead and zinc content appear in a size range, that can penetrate deep into the lung, our results represent a typical example, showing that the PM mass concentration is not a suitable legal metric to assess air pollution and air pollution-related health effects. Furthermore, the redox cycling activity of inhaled transition metals such as iron induces oxidative stress and is involved in severe health effects from air pollution (Fang *et al.*, 2017). Therefore, our measurements emphasize the need for a more sophisticated metric than particle mass concentration, which include particle number and particle composition, for an improved relation between PM levels and expected health risks.

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(02) Impact of Photochemical Aging on Toxic Polycyclic Aromatic Hydrocarbons (PAH) and Oxygenated PAH (Oxy-PAH/OH-PAH) in Logwood Stove Emissions: A Photochemical Emission Aging flowtube Reactor (PEAR) Study

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Small-scale residential heating with firewood is the main source for ambient organic aerosol (OA) in many parts of the world. In European cities, its relative contribution to ambient OA has been estimated to further increase in the future due to the encouraged use of renewable sources of energy. On the other hand, OA emissions from other sources, such as industry and traffic, are decreasing. Furthermore, total OA emission and its composition strongly depend on combustion conditions and procedure, combustion technology, and type of firewood (Hartikainen *et al.*, 2020).

Once emitted into the atmosphere, OA from wood combustion undergoes substantial physical and chemical conversion processes, known as atmospheric aging. At daytime, hydroxyl radicals (OH) dominate this aging process, oxidizing organic vapors and increasing their functionalization. Some of these oxidized secondary organic species condense as new particles or on existing particles, resulting in an enhancement of ambient OA concentrations. The class of volatile aromatic compounds has been found to be important SOA precursors. However, the atmospheric fate of low-volatile aromatic compounds, incl. toxic and carcinogenic polycyclic aromatic hydrocarbons (PAH) such as benz[a]pyrene, depends on a variety of conditions and is not well understood. Moreover, the aging of PAH may lead to the formation of hydroxy- and oxygenated PAH (OH-PAH/Oxy-PAH), which may be even more potent carcinogenic agents than their PAH precursors (Miersch *et al.*, 2019).

Wood combustion experiments, aging in PEAR, and sampling of PM1

We burned five consecutive batches of 2.0 kg of spruce logwood in a non-heat-retaining iron stove over 4 h. During the last 30 min, the channels for secondary air supply were closed, according to the instructions of the manufacturer. The combustion aerosol was further fed into the "Photochemical Emission Aging flowtube Reactor" (PEAR), referring to an oxidation flow reactor operated with an external feed of ozone and minimum UV light wavelength of 254 nm (Ihalainen *et al.*, 2019), in order to simulate atmospheric aging of the aerosol in the laboratory (figure 1). On average, the photochemical aging in the PEAR was equivalent to approximately 2 days of photochemical aging in the atmosphere. Since batch wise wood combustion may produce highly variable emissions, parallel sampling of respirable particulate matter (PM1) on quartz fiber filters was conducted for experiment-wise evaluation of the effect of photochemical aging on combustion aerosol emissions.

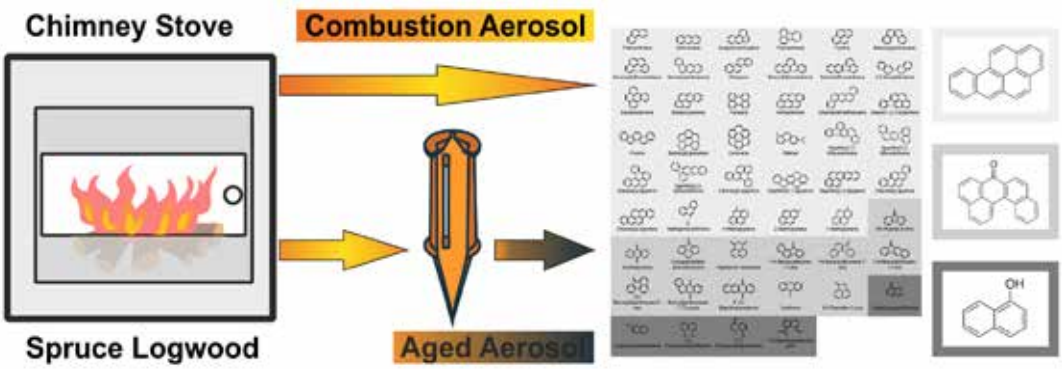

Figure 1: *Spruce logwood combustion, photochemical aging in PEAR, and analysis of PAH, Oxy-PAH and OH-PAH.*

Targeted and untargeted chemical analysis of aromatic compounds in PM1

Filter samples of PM1 were analyzed by in situ derivatization thermal desorption (IDTD) gas chromatography (GC) time-of-flight mass spectrometry (TOFMS) with electron ionization (EI) (Orasche *et al.*, 2011) for 35 PAH, 11 Oxy-PAH, and 5 OH-PAH targets. Additional untargeted analysis of aromatic particle constituents

and quantitative analysis of organic (OC) and elemental carbon (EC) was conducted by thermal-optical carbon analysis (TOCA) hyphenated to TOFMS with resonance-enhanced multi-photon ionization (REMPI) (Diab *et al.*, 2015).

Effect of photochemical aging on primary organic (OC) and elemental carbon (EC) emissions

Primary wood combustion emissions revealed (9±6) mg MJ^{-1} of OC, which is comparable with previous studies of spruce logwood combustion in modern wood stoves. However, emission factors of (43±20) mg MJ^{-1} of EC were three to four times higher, which might be caused by different combustion technologies, such as the feed of secondary air or the heat-retaining or non-heat-retaining stove material. The resulting ratio of OC to EC clearly appears below one, which indicates efficient high temperature combustion. After passing the PEAR, OC increased by (35±8) % indicating secondary organic carbon formation. In contrast, EC decreased by 2 %, which is lower than the measurement uncertainty of the TOCA. However, in one experiment with the 50 % less primary OC, EC decreased by 10 %. Hence, even EC may be aged in PEAR if the layer organic coating of wood combustion particles becomes less.

Effect of photochemical aging on particle-bound polycyclic aromatic compounds

PAH emission factors from all primary spruce logwood combustion experiments span a range of one order of magnitude with a mean emission factor of 400 µg MJ^{-3}, emphasizing the need for pairwise evaluation of primary combustion with the subsequent individual aging experiment (figure 2). The photochemical aging significantly ($p = 0.029$; paired t-test) reduced total PAHs by more than 70 % to 70 µg MJ^{-1} on average. Also for Oxy-PAH a reduction in emission factors was observed after aging, however, the difference between primary and aged particles was 50 % on average and statistically insignificant ($p = 0.078$). Oxy-PAHs may be a first-generation oxidation product of PAH, but also undergo further oxidation as well. The same holds for OH-PAHs, but for OH-PAHs we observe a distinct net formation, in particular for naphthaldehydic acid, which we propose as a marker for first-generation SOA. Untargeted particle analysis by TOCA-REMPI-TOFMS revealed also a significant reduction of aromatic compounds on the particles, but a relative enrichment of larger PAHs. Smaller PAHs with three or four aromatic rings may evaporate to the gas phase in which homogeneous reactions with atmospheric oxidants are faster than heterogeneous reactions between oxidants in the gas phase and PAHs in the particle phase.

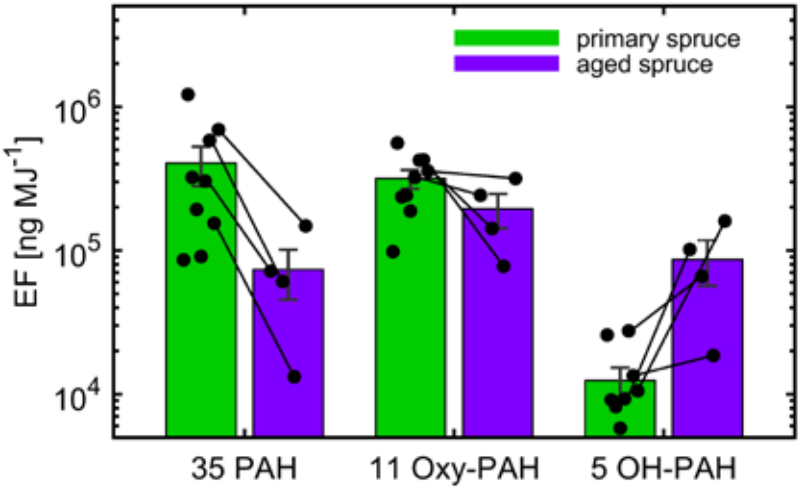

Figure 2: *Emission factors of PAH, Oxy-PAH, and OH-PAH for primary and aged spruce combustion aerosol. For four experiments, both primary (green) and aged particles (purple) were analyzed and connected by black lines. Further dots in the figure (•) denote additional combustion experiments with only primary particle sampling. Error bars represent the standard error of the mean.*

PAH toxicity equivalents (PAH-TEQ)

PAHs are known as carcinogenic compound class. However, the carcinogenic potential of individual PAHs may differ by several orders of magnitude. In the concept of PAH toxicity equivalents (PAH-TEQ), the carcinogenic of individual PAHs is determined and set into relation to benz[a] pyrene (Nisbet and LaGoy, 1992). Larger PAHs tend to be more potent carcinogens than smaller ones, in particular, if they exhibit specific structure characteristics such as the so-called "bay region" or "fjord-region" (Broyde *et al.*, 2010).

PAH-TEQs of primary spruce combustion PM1 were comparable to literature data and significantly reduced by one order of magnitude after photochemical aging (figure 3). Although wood smoke was classified as "likely carcinogenic" (class 2A) by the International Agency for Research on Cancer (IARC), PAH-TEQ emission factors of aged wood combustion particles were still one order of magnitude higher than for emissions from a diesel engine ("carcinogenic", class 1). Based on our data, we estimate that wood smoke has to be processed in the atmosphere over more than 4 days to reach PAH-TEQ levels comparable to fresh diesel exhaust particles. However, we want to emphasize that a) atmospheric aging may also lead to the generation of mutagenic compounds,

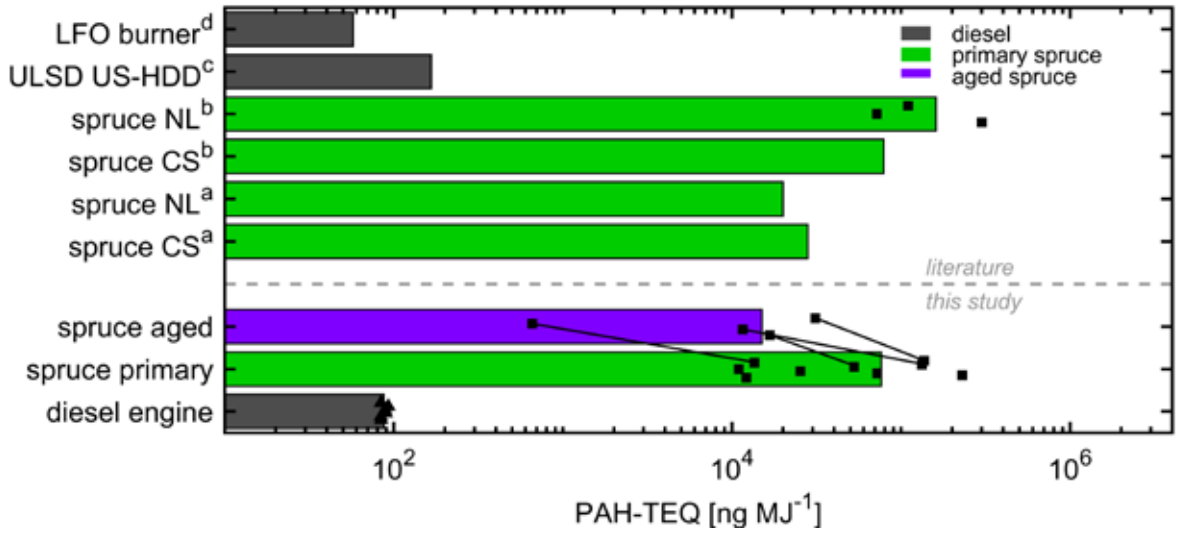

Figure 3: *PAH-TEQ emission factors for primary (green) and aged (purple) spruce logwood combustion particulate matter (PM) as well as PM from a non-road 24.5 kW diesel engine (grey) in comparison with literature data from a) Orasche et al. (2012, DOI: 10.1021/ef301295k), b) Orasche et al. (2013, DOI: 10.1021/ef301506h), c) Lin et al. (2011, DOI: 10.1016/j.energy.2010.10.045), and d) Kaivosoja et al. (2013, DOI: 10.1016/j.atmosenv.2013.05.014). Triangles (diesel) and squares (wood) denote individual experiments. It is apparent that the PAH-TEQ burden of residential wood emissions (green bars) is considerably higher than the emissions of diesel engines, light fuel oil (LFO) burners, or trucks operated with ultra-low sulfur diesel (ULSD) in the US-heavy duty driving cycle (US-HDD).*

b) protective particle constituents such as polyphenolic species may play a role and c) interactions with the gas phase are not taken into account by the PAH-TEQ concept, which is purely based on concentrations of individual particle bound PAHs. The Helmholtz International Lab aeroHEALTH and Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health, therefore, strive to unravel relations between combustion conditions, atmospheric transformation processes, and biological mechanisms of toxicity for a comprehensive assessment of health effects from air pollution.

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(03) Toxicological and physicochemical characterization of inhalable dust from dry cutting processes of carbon concrete composites and their reinforcement materials

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Abstract

Carbon Concrete Composites (C^3) are newly designed construction materials with a good resistance against corrosive environments. However, during the life-cycle of these construction materials, they encounter different stress situations, which could lead to the formation of harmful fibers or organic substances. We investigated the chemical composition and morphology of inhalable particulate matter (PM) formed during dry cutting simulations of rod-shaped (carbon rod) reinforcement materials as well as unstressed and thermally stressed C^3 materials. We exposed human lung cells and a co-culture of human lung cells and fibroblasts at the air-liquid interface (ALI) to these PM and examined the adverse effects on the cells regarding cytotoxicity, genotoxicity, and pro-inflammatory effects. Acute adverse effects were determined immediately after a 2 h continuous exposure to the PM. Long-term effects were examined after a 24 h post-incubation period. While harmful fibers according to the WHO definition were only observed in abrasive dust of thermally stressed C^3, organic substances were only found in abrasive dust of carbon rods due to pyrolysis of the organic matrix at the edge of the saw blade. The relative cytotoxicity of all three abrasive dust PM was significantly higher immediately after the 2 h exposure than after the post-incubation period. However, secondary genotoxic effects on the fibroblasts were apparent only after the 24h post-incubation period.

Introduction

Carbon Concrete Composites (C^3) are a promising alternative to the widely used construction material steel-reinforced concrete. Due to the resistance of carbon fibers against alkaline and corrosive environments such as the concrete matrix itself or marine air, this newly designed material exhibits higher longevity. Furthermore, five-fold higher tensile strength can be achieved, which facilitates the use of less resources and thus resource-saving constructions (Bienkowski *et al.*, 2017, Böhm *et al.*, 2018).

During their lifetime, construction materials underlie different stress situations, such as cutting, thermal recycling, or domestic fires, which could lead to the formation of harmful fibers as in asbestos concrete aerosols, or harmful organic compounds. While no studies on thermal recycling and domestic fires of C^3 materials have been carried out yet, only a few studies on aerosols from cutting processes of C^3 materials can be found. Bienkowski *et al.* (2017) and Hillemann *et al.* (2018) found no asbestos-like fibers according to the WHO definition (> 5 µm L, < 3 µm Ø, L / Ø > 3) (BAuA 2020) in aerosols from dry cutting processes. However, they found fibers with 7 µm in diameter, which are contained in the C^3 material due to the production process. Furthermore, Hillemann *et al.* (2018) found traces of organic compounds in the gas phase of the aerosols. The adverse effects of inhalable carbon nanotubes on the pulmonary system, such as sustained inflammation, fibrosis, and tumor formation are well known (Kobayashi *et al.*, 2017). Many *in-vivo* studies have demonstrated that the interaction of such poorly soluble particles and fibers with one lung cell type can even cause secondary DNA-damage in another cell type (Evans *et al.*, 2017). For the investigation of these possible adverse effects, we developed an *in-vitro* system where human lung cells and a co-culture of human lung cells and fibroblasts (figure 1) are exposed to the abrasive dust from the dry cutting process of carbon rods, C^3 and thermally stressed C^3 materials at ALI conditions. At the same time, we collected the emerging inhalable PM on filter substrates to investigate its organic composition as well as the occurrence and morphology of fibers.

Materials and Methods

One commercially available rod-shaped C^3 reinforcement material coated with an epoxy resin (carbon rod), a C^3 material with a grid-shaped C^3 reinforcement material coated with an SBR polymer as well as the same C^3 material, which was thermally stressed at 700 °C for 3 hours with a ramp rate of 1.5 K min^{-1} were investigated. Particulate matter was produced in a dry cutting simulation (figure 2A) using an "Abrasive Dust Generator" (Vitrocell, Waldkirch, Germany) equipped with a diamond saw blade. Cutting of the

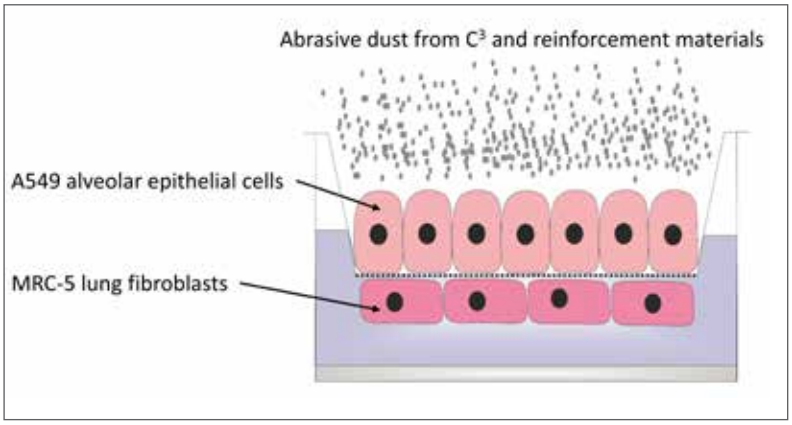
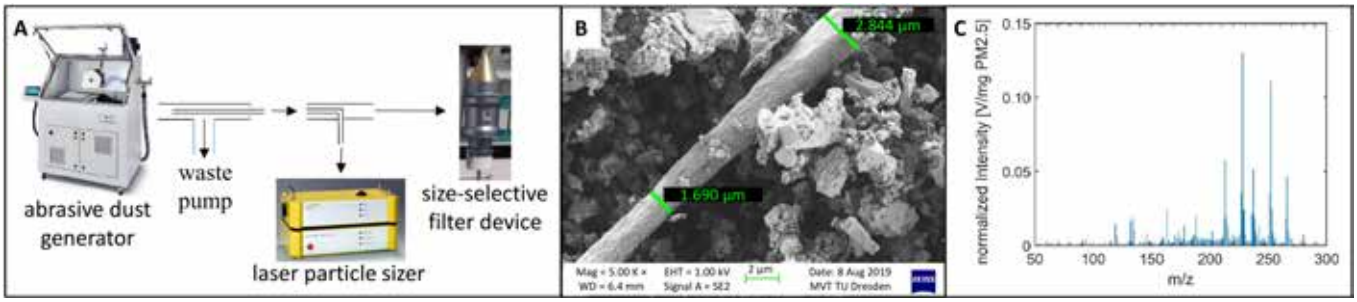


Figure 1: *Schematic drawing of the cell culture model utilized to test the toxicity of the abrasive dust from C³ material, thermally stressed C³ material, and carbon rods reinforcement material. For the A549 monoculture, no MRC-5 lung fibroblasts were cultured on the basolateral side of the cell culture insert.*

materials was performed along the carbon fibers with a saw blade rotation of 50 Hz and a cutting speed of 0.2 mm sec^{-1} and 0.1 mm sec^{-1} for carbon rods and concrete plates, respectively. It was always ensured to cut through the reinforcement material in its full depth to produce the highest possible amount of harmful organic compounds and fibers. The produced abrasive dust was extracted at the saw blade edge. The inhalable particulate matter fractions PM2.5 and PM10 were either collected via impactors on methylcellulose and quartz fiber filters for morphological and chemical analysis, or they were guided to the Vitrocell® Automated Exposure Station (Vitrocell, Waldkirch, Germany) for the *in-vitro* exposure experiments. Cells were continuously exposed to the abrasive dust from the three materials for a duration of two hours at 37 degrees with 85 % rH. After the 2 h exposure, one half of the cells was given a post-incubation period of 24 h in a humidified incubator at 37 degrees with 5 % CO_2. In parallel to the sample collection or exposure, the particle size distribution was measured via an isokinetic sampling line using a laser particle sizer "LAP 322" (Topas, Dresden, GER). Fiber occurrence and morphology were investigated by SEM with a "GeminiSEM 300" (Zeiss, Oberkochen, Germany). The chemical composition of the samples was investigated using a carbon analyzer (EC/OC) "Model 2001 A" (Desert Research Institute, Reno, USA) hyphenated to a time-of-flight (TOF) mass spectrometer equipped with the soft photoionization techniques REMPI (resonance enhanced multi photon ionization, 248 nm) and SPI (single-photon ionization, 118 nm) (BAuA 2020, Grabowsky 2011). In addition, compounds were evaluated using a GC×GC-HRTOFMS device "Pegasus GC-HRT 4D" (Leco, Mönchengladbach, Germany).

We used an *in-vitro* model consisting of either alveolar epithelial cells (A549, ACC 107, DSMZ, Braunschweig, Germany) or a co-culture of A549 cells and MRC-5 lung fibroblast cells (ATCC® CCL-171, LGC Standards, Wesel, Germany) (figure 1) to assess the adverse effects of the particles and fibers produced during the dry cutting process. MRC-5 cells were seeded on the basolateral side of semipermeable Transwell inserts (PET, 0.4 µm pore size, 4.76 cm2 membrane growth area, Corning, NY, USA) until they had attached. Then, A549 cells were seeded in the apical side of the inserts (figure 1), or only A549 cells were used for the A549 monoculture and cultured for three days prior to the exposure. Cells were adjusted to air-liquid interface conditions gradually to encourage the development of the appropriate cell polarity and epithelial tissue properties. We assessed the cytotoxic effects of the abrasive dust samples using the lactate dehydrogenase (LDH)-assay using the Roche Cytotoxicity Detection KitPLUS (Merck, Darmstadt, Germany) according to the manufacturer's recommendations. As positive control we used cells, which were lysed completely using a 2 % Triton-X-100 solution in serum-free culture medium. The number of viable cells was calculated as the percentage relative to completely lysed cells. Absorbance was measured at 492 nm in a multiplate reader (Varioskan LUX, ThermoFisher Scientific, Dreieich, Germany). For the COMET assay, the cells from the A549/MRC-5 co-culture were harvested separately and DNA-damage was assessed in each cell type according to methods described previously [Di Bucchianico *et al.*, 2017]. The concentration of the pro-inflammatory chemokine CXCL8 was assessed

Figure 2: *(A) The C³ dust was produced by an abrasive dust generator and collected on filters using a size-selective filtering device. In parallel, the particle size distribution was measured via isokinetic sampling using a laser particle sizer. (B) Fibers with critical dimensions according to the WHO definition could only be observed in dust from thermally stressed C³. (C) Some aromatic, organic compounds could be found in PM2.5 of carbon rods using REMPI-TOFMS (248 nm) hyphenated to an Thermo-Optical Carbon Analyzer (TOCA) for EC/OC analysis (140°C–280°C).*



from the exposure and post-incubation medium by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (DuoSet ELISA, DY208, R&D Systems, Abington, UK).

Results

While dry cutting of carbon rods led to the formation of about 90 % ultrafine particles with scattered light equivalent diameters of 0.2 to 0.5 µm, equal amounts of particles with diameters of 0.2-0.5 µm, 0.5-1.0 µm and 1.0-2.5 µm were formed during dry cutting of the C³ material without thermal treatment. Thermally stressed C³ led to a higher amount of particles with a diameter of 0.2-0.5 µm (approx. 50 %). For all materials, > 99 % of the particles had a diameter < 10 µm and were thus in the inhalable size range. Approximately 97 % for the carbon rods and 94 % for the C³ materials even had a diameter of < 2.5 µm. Asbestos-like fibers according to the WHO definition were not found in PM10 from carbon rods or C³ without prior thermal treatment. However, splint-like fibers with critical diameters < 3 µm were observed in PM10 from the thermally stressed C³ material (figure 2B).

In C³ abrasive dust with and without prior thermal treatment, no aromatic compounds were observed. In contrast, we found several aromatic organic compounds in the PM2.5 and PM10 from dry cutting processes of carbon rods (figure 2C). By comparison with the NIST database, we could classify them to be bisphenol A and its derivatives and fragments. Epoxy resins used to produce the carbon rods often use bisphenol A as a monomer, hence these compounds are most likely pyrolysis products of the organic coating material of the carbon rods. Bisphenol A is well known to impair the mammal reproduction system and has previously exhibited genotoxic effects *in-vitro*, too.

The toxicological effects of the three investigated abrasive dust samples differed significantly not only between the samples but also depending on the time point (directly after exposure vs. 24h post-incubation) and cell type. The relative cytotoxic effects of all samples were significantly higher directly after 2 h exposure compared to 24 h post-incubation for both cell models (figure 3A). In the A549 monoculture after 2 h exposure, the cytotoxic effects of both C³ materials were considerably higher than those of the carbon rods. In the A549/MRC-5 co-culture, carbon rods and thermally stressed C³ material exhibited higher effects than the non-stressed C³ material (figure 3A). In the COMET assay, we saw a significant increase in the amount of DNA in the tail in A549 cells for the abrasive dust from the carbon rods and the thermally stressed C³ material both after the 2 h exposure and the 24 h post-incubation period (figure 3B). For the MRC-5 cells, the same increase was observed after the 24 h post-incubation period only. MRC-5 cells grown on the basolateral side of the cell culture inserts are never in direct contact with the particles and fibers from the abrasive dust samples,

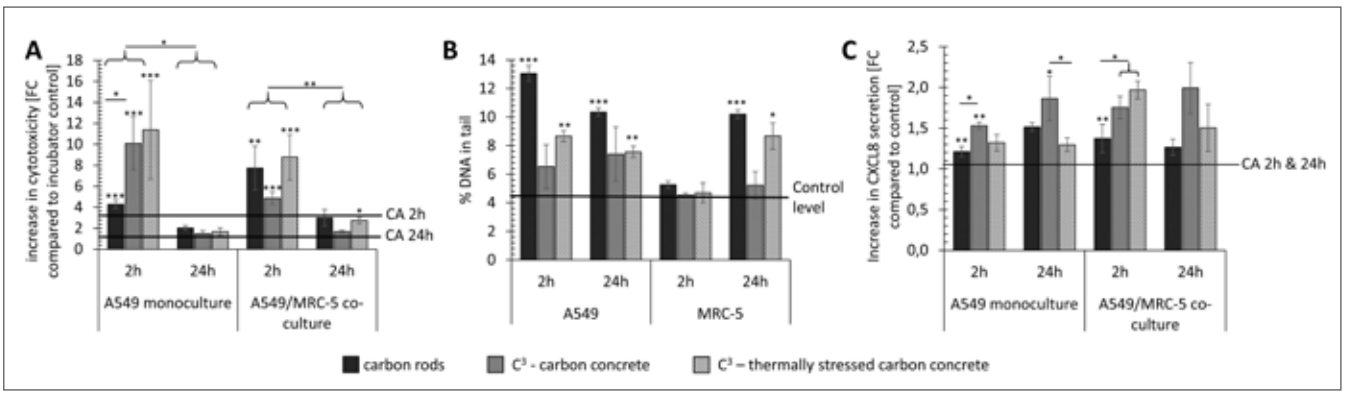


Figure 3: *Results from the toxicological assessment of the three abrasive dust samples. (A) Relative cytotoxicity expressed as fold-change compared to the incubator control assessed by LDH-assay. (B) Genotoxic effects assessed by the COMET assay and expressed as percentage of DNA in the tail of the comets. (C) Secretion of the pro-inflammatory mediator CXCL8 expressed as fold-change compared to incubator control. CA: clean air control, *: significance at 0.05-level, **: significance at 0.01-level, ***: significance at 0.001-level as assessed by Tukey's range test.*

thus, an increase in the amount of DNA in the tail of the comets indicates the occurrence of secondary genotoxic effects in these non-target cells. Secondary genotoxic effects are generally driven mainly by the secretion of reactive oxygen or nitrogen species or inflammatory reactions of the target/exposed cells (Shins and Knaapen, 2007, Åkerlund *et al.*, 2019). Therefore, we investigated the extent of pro-inflammatory reactions in the two cell culture models by measuring the concentration of the chemokine CXCL8 in the exposure medium both directly after the 2 h exposure and after the 24 h post-incubation period. In A549 cells, the non-stressed C^3 material caused the highest secretion of CXCL8 both after 2 h and after the 24 h post-incubation period (figure 3C). The difference to the effects from the carbon rods was significant directly after the 2 h exposure, the difference to the effects of the thermally stressed C^3 material was significant after the 24 h post-incubation period (figure 3C). In the A549/MRC-5 co-culture, we detected significance from cells exposed to clean air only for the carbon rods reinforcement material. Nonetheless, at the 2 h time point, the amount of CXCL8 secreted by the cells after exposure to both C^3 materials was significantly higher than that of the carbon rods reinforcement material. These results indicate that mainly other processes than inflammation drive the secondary genotoxic effects in MRC-5 fibroblasts.

Conclusion

In this study, the inhalable particulate matter fractions PM2.5 and PM10 formed during dry cutting processes of unstressed and thermally stressed C^3 materials and rod-shaped reinforcement materials were investigated towards the occurrence and morphology of fibers and organic compounds. Additionally, we evaluated their acute and long-term toxicological impact on human alveolar epithelial cells and a co-culture of human alveolar epithelial cells and lung fibroblasts. We observed strong effects of the abrasive dust from the carbon rods and thermally stressed C^3 material regarding primary and secondary genotoxicity as well as acute cytotoxicity, whereas the secretion of the inflammatory mediator CXCL8 was comparably low for all tested abrasive dust samples. The dry cutting processes of thermally stressed C^3 and carbon rods lead to the formation of a high fraction of ultrafine particles, which likely are the driving force for the adverse effects we have seen here. Furthermore, we found harmful fibers according to the WHO in PM10 from the dry cutting of thermally stressed C^3. Thus, we recommend to wear particle masks and to reduce the amount of inhalable dust by using wet cutting processes and suction devices during the treatment of C^3 materials. Due to the formation of derivatives and fragments of bisphenol A during dry cutting of epoxy resin-coated carbon rods, we further recommend to use non-bisphenol A-based coating materials in the production of C^3 reinforcement materials. To understand in more detail the driving mechanisms behind the secondary genotoxic effects we have seen here, we will conduct further studies, including the comprehensive transcriptome and proteome analysis, as well as the determination of oxidative stress markers of exposed cells. Future experiments will also include the behavior of thermally stressed C^3 materials at lower temperatures during the dry-cutting process.

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(04) Seasonal and spatial variation of organic composition and source contributions of ultrafine and accumulation mode ambient particulate matter

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The health effects of smaller particles have been a concern in the study of airborne particulate matter, particularly ultrafine particulate matter (UFP) which often referring to PM < 100 nm and quasi-UFP referring to particulate matter (PM) up to several hundred nanometers. Short term ultrafine particle exposure was found to be associated with cardiovascular recurrent events in an epidemiological study in Augsburg, Germany. Although UFP contribute substantially to particle number counts, due to minor contribution to mass (figure 1 A) they are not regulated by mass-based legislation. Moreover, their characteristics are also not well studied compared to fine or coarse particles (PM2.5, PM10). Chemical characteristics, sources, and adverse health effects of UFP/quasi-UFP are intrinsically associated with each other. PM0.25 ROS activity was found to be associated with its organic carbon (OC), and water-soluble carbon (WSOC) and transition metal concentrations and also its Dithiothreitol (DTT) activity associated with chemical species like PAHs, hopanes, etc. Moreover, it was found that differences in source contributions to PM0.25 OC are the driving force of oxidative potential differences at various locations. However, little is known about the contributions of secondary organic aerosols (SOA) to ambient UFP. Using measured chemical markers as input variables, source apportionment studies have been widely performed to identify and apportion the sources of fine and coarse ambient PM. However, due to the high requirement of sampling and measurement strategies, the goal of doing source apportionment analysis based on the chemical speciation of UFP/quasi-UFP is difficult to achieve, especially when a long-term study is desired. To address this problem, long-term field samples of size-separated PM including quasi-UFP and chemical speciation were conducted in a UFP characterization project in which the CMA collaborated with the Institute of Epidemiology (Prof. A. Peters, Dr. J. Cyrys).

Sampling, chemical speciation, and positive matrix factorization (PMF) analysis

For automated sampling, 3-stage rotating drum impactors (RDI) are sequentially connected to particle filter samplers (Li *et al*., 2016). PM0.36 was sampled on 47 mm quartz fiber filters after the RDI. Continuous unattended week-long sampling of daily samples was achieved. To study the spatiotemporal variability, samples were collected at a reference site located on the campus of the University of Applied Sciences Augsburg (Germany) and meanwhile parallel at one of 5 mobile sites (T1-T4 and B1). Successive sampling at each of these mobile sites was performed 2 weeks each in summer, winter, and spring or fall. 294 PM0.36 samples were analyzed for carbon fractions (IMPROV A protocol) using a thermal-optical carbon analyzer and for organic composition using in-situ derivatization thermal desorption gas chromatography coupled with time of flight mass spectrometry (IDTD–GC–TOFMS). EPA PMF 5.0 was applied to determine the contributions of the main sources to the OC of collected PM0.36.

Results

March, June, September, and December are set as the starting month of spring, summer, autumn, and winter. Regarding OC fractions, seasonality of OC^2 concentration was found to be in the order of spring > summer > autumn, winter (Li *et al*., 2018a). For OC^3, it was summer > spring > autumn, winter. OC^4 concentration was very high in winter, but comparably low during the other 3 seasons, indicating different chemical components and sources contributing to the OC4.

Regarding representative compounds, the highest concentration of high molecular weight (HMW) PAHs (MW > 228), levoglucosan, and vanillic acid were found in winter, and then autumn > spring > summer. Hopanes demonstrate much less seasonal variability with slightly higher concentrations in spring and winter than in summer and autumn. The cis-pinonic acid level rises from spring to peak values in summer and drops from summer to winter. Maximum concentrations of 2-methytetrols (2-MT) were also found in summer (July and August) and they were not detected from late October to late April during which temperature was low. 4-nitrophenol and 4-nitrocatechol showed high values in winter.

Source apportionment by PMF analysis revealed five factors explaining the variability in PM0.36 composition. These source factors are: (1) biogenic secondary organic aerosol (bioSOA), which is characterized by cis-pinonic acid, 3-hydroxyglutaric acid, 3-methy-butyltricarboic acid, malic acid, dicarboxylic acids, OC^2, and OC^3, showing typical seasonality of summer > spring > autumn > winter (figure 1 B); (2) isoprene derived SOA (isoSOA), which is characterized by 2MT and was found in summer; (3) traffic, characterized by hopanes and showed less seasonal variation; (4) biomass burning (BB), associated with biomass combustion marker compounds such as levoglucosan and vanillic acid and displaying seasonal contribution of winter > autumn > spring > summer; (5) biomass burning SOA (bbSOA), characterized by 4-nitrophenol and 4-nitrocatechol and was found at high levels during the coldest period in winter.

On average, contribution of the source factors to OC is in the order of traffic > bioSOA > BB > isoSOA > bbSOA. Biogenic emission originated SOA (including bioSOA and isoSOA) was found to be the main contributor to semi-volatile organic carbon in PM0.36, especially in summer. Semi-volatile fractions OC^3 and OC^2 are modeled much better than OC^4, which is reasonable because low volatile species that belong to OC^4 fraction cannot be analyzed with the GC method used in this study.

Particle size depended particle number concentrations were found to have source-specific patterns (figure 1 C). Specifically, the traffic factor is mainly characterized by the smallest particles. Traffic explained number concentrations decrease

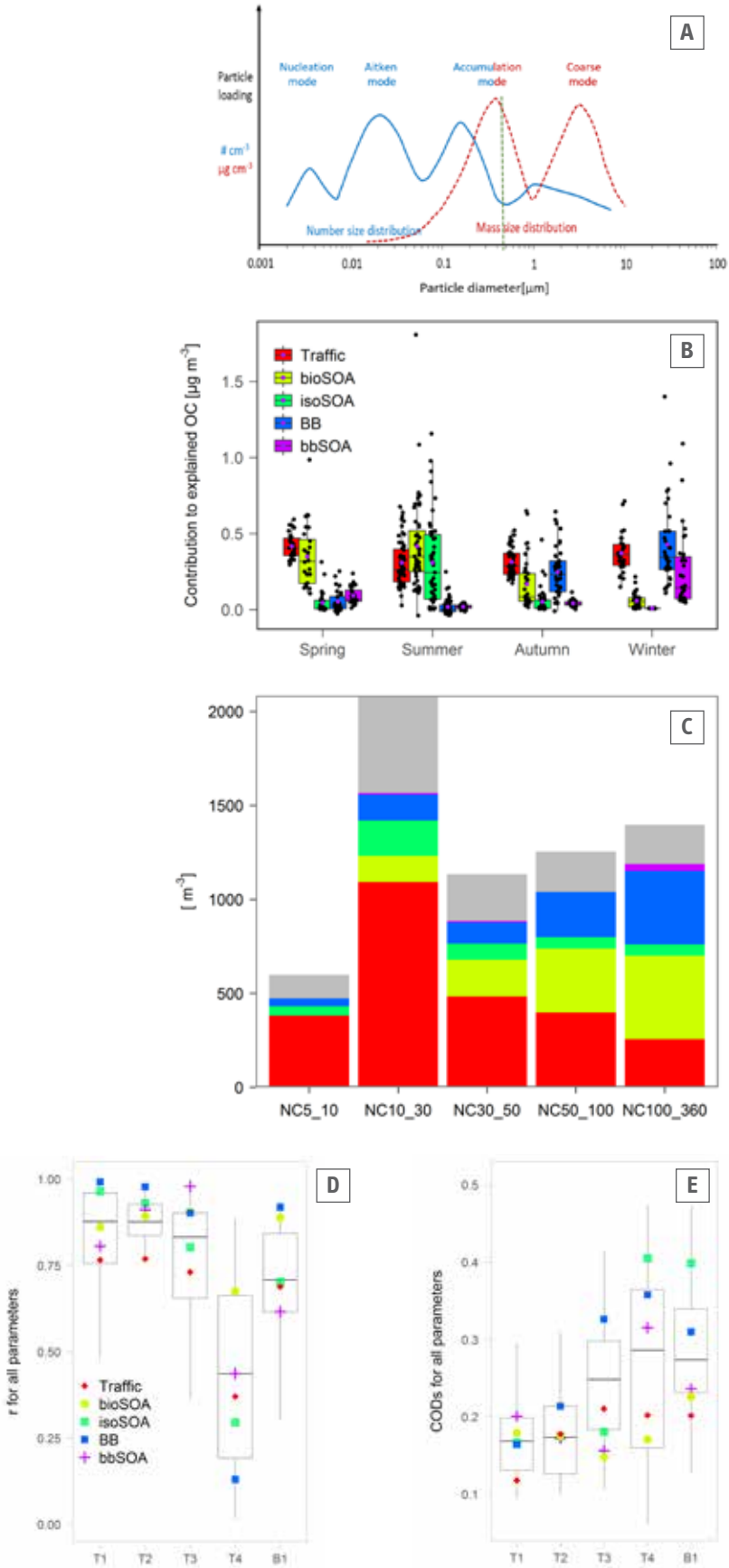

Figure 1: A: Typical size distribution of urban aerosols. Dashed green line cut-off of the last stage of the RDI; B: Organic carbon explained by identified source factors in each season. C: Contributions of identified source factors to particle number concentrations in different size classes; D: Calculated Pearson correlation (r) and E: Coefficient of divergence (COD) between the reference site and the monitoring sites for identified source factors.

from 81 % to 22 % as the size increases from 5 – 10 to 100 – 360 nm. On the other hand, bioSOA and BB are mainly associated with accumulation mode particles probably due to BB emission size distribution and gas to particulate partitioning. Only 5 – 12 % of particles in different size ranges are explained by isoSOA and very few are explained by bbSOA.

Temporal correlation (represented by Pearson correlation coefficient r, figure 1 D) between reference and each mobile site of BB, bioSOA, bbSOA, and isoSOA are in general high (Li *et al.*, 2018b). Traffic generally shows a lower temporal correlation than other factors indicating stronger local impacts on the variability. Spatial variability (represented by COD: coefficient of divergence, figure 1 E) of BB demonstrate local influences with higher COD at T3 and B1, and also higher COD of isoSOA at B1. No high COD were observed for bioSOA and traffic source indicating a dominating impact of background concentrations. However, high traffic contributions at specific periods at T1 were observed by time series pattern and mean contribution comparison.

Summary

Long-term sampling, chemical speciation, and PMF source apportionment were done for atmospheric quasi-UFP. Seasonal and spatiotemporal variability of chemical species as well as source contributions could be resolved revealing higher spatial variability as was found for larger size fractions of ambient PM. However, future studies must achieve a better separation of ultrafine and accumulation mode particles, especially to better characterize the spatial variability and thus the exposure of affected individuals.

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(05) Detection and Evaluation of Air Pollution from Distant Ships by Single-Particle Mass Spectrometry

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According to the WHO, ambient air pollution is the largest environmental risk to public health (WHO 2016). While great efforts were made to reduce land-based emissions, the majority of ships is not equipped with any exhaust cleaning system and low-grade fuels are commonly used. Consequently, ships emit large amounts of sulfur, organic aerosols, and metals with substantial impacts on climate and public health (Corbett *et al.* 2007) (see figure 1). Between 60,000 and 400,000 annual deaths from cardiopulmonary diseases and lung cancer as well as 14 million cases of childhood asthma were attributed to ship emissions (Sofiev 2018). Mitigation strategies focus on the sulfur aspect, *e.g.* by a global 0.5 % S standard for ship fuels in 2020 and by the implementation of Sulfur Emission Control Areas (SECA, < 0.1 % S in fuel). However, neither the emitted particle mass or number concentration nor the most health-relevant particle components such as metals, soot, and the organic contents are yet regulated. These particles are transported from the open sea and harbors to densely populated coastal cities and residential areas. The real-time monitoring and risk assessment of the health-relevant PM2.5 components from shipping remains a key challenge for analytical technology and public health research.

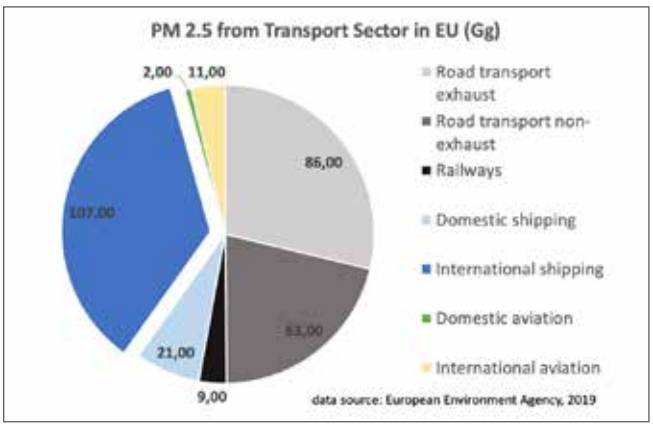

Figure 1: *The PM2.5 emission from the transport sector in the EU. Shipping is responsible for a huge fraction of the emissions.*

Our workgroup meets this challenge by developing and applying novel technologies addressing this open research gap. In detail, we developed a new technology for single-particle mass spectrometry (SPMS) that reveal the carcinogenic Polycyclic Aromatic Hydrocarbons (PAH) and toxic metals on individual particles with unprecedented sensitivity (see Research Area 2, Enabling Analytical Technologies, (03) and (04) for details).

Figure 2: *Remote detection of ship plumes in coastal air using SPMS. (a) Air mass origin (top row: > 12 h, bottom row < 12 h) according to back trajectory analyses. (b) Measured wind data. (c) The time series of particle counts from general particle classes shows regional/long-range transported air pollution and nighttime secondary organic aerosol formation. (d) The same data as (c), but normalized to total particle counts illustrate the contribution of each particle type. (e) The temporal behavior of metal-containing particles from residual fuel combustion reveals emissions from individual ships during onshore winds. Figure from Passig et al., submitted to Atmos. Meas. Tech. 2020.*

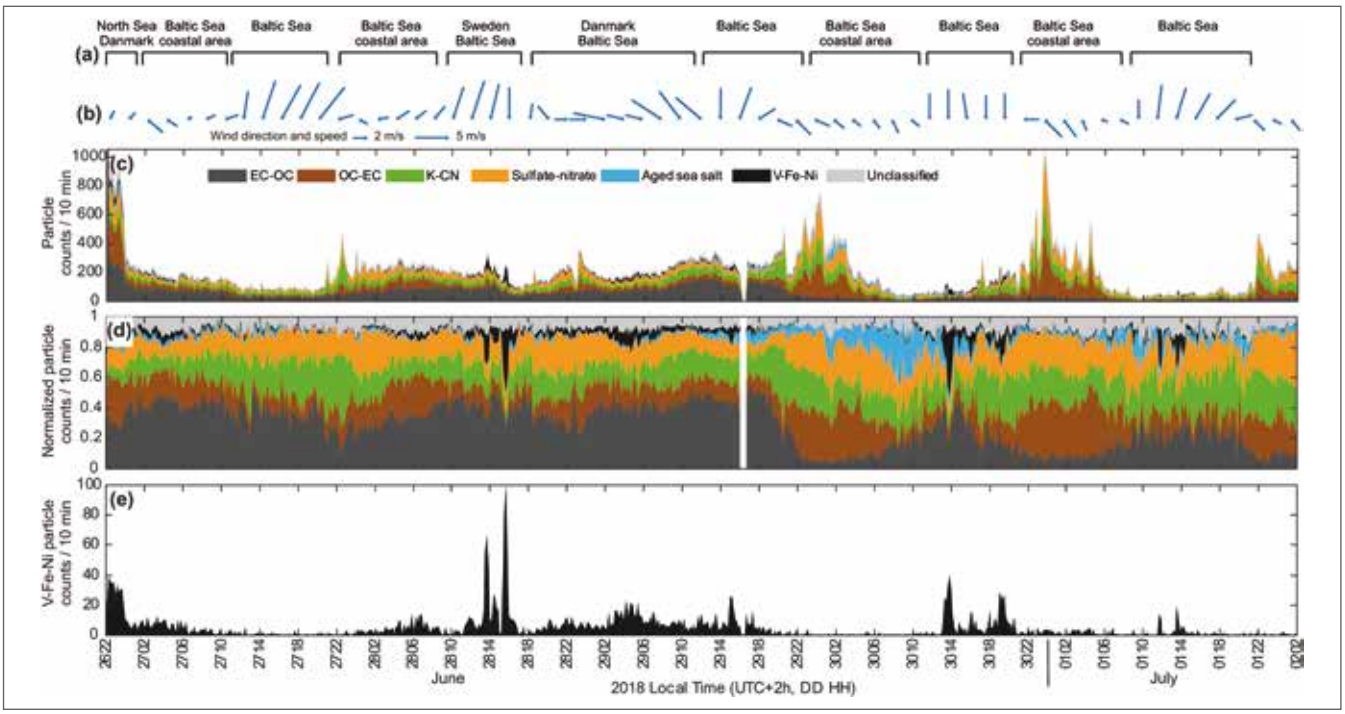

Figure 2 illustrates the potential of our method for ship emissions surveillance and health risk estimates. From the laboratory in Rostock, we detected the signatures of individual ships running on heavy fuel oils with high metal- and sulfur content on the Baltic Sea.

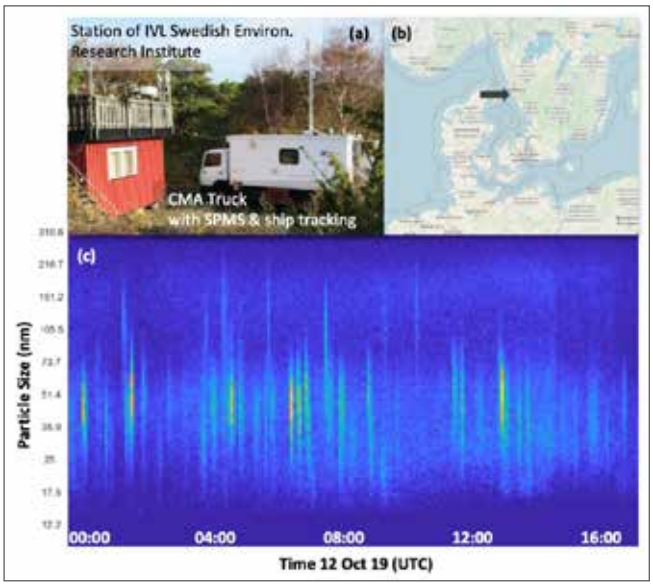

Figure 3: *(a, b) Joint field campaign with IVL Swedish Environmental Research Institute and Chalmers University of Technology, Gothenburg. (c) Signatures of ship plumes from the Kattegat at onshore wind, measured by a scanning mobility particle sizer and correlated with ships by tracking their transponder signals.*

In autumn 2019, we used the CMA measurement truck to set up our SPMS at a remote peninsula at the Swedish coast, in a restricted area without local emissions, see figure 3 a, b. With support from our Swedish partners (Chalmers University of Technology, Gothenburg, and IVL Swedish Miljöinstitut), we recorded the physical signatures of more than a hundred individual plumes of ships, passing at a distance of 30–50 km – much more than in any study before, see figure 3c. The dataset is currently merged with results from the Lund University to better estimate the contribution of ships to air pollution in coastal areas (Ausmeel *et al.* 2020). With our new SPMS approach, we recorded individual mass spectra from millions of individual particles (Passig *et al.* 2020). However, most ships in emission controlled areas (like the North and Baltic Sea) use distillate fuels, such as marine gas oil (MGO). Their signatures equal land-based emissions, even in SPMS. However, our unique approach yields detailed PAH-spectra from single particles, and the PAH-mass spectra of ship emissions running on such fuels were shown to exhibit a unique PAH profile (Czech *et al.* 2017). The field study yielded many thousand PAH-spectra – the first comprehensive dataset of PAHs on individual particles in ambient air. The spectra were analyzed using neural network algorithms and provide deep insight into atmospheric processing and transport of PAHs (see Research Area 2 (03)). The algorithm also yielded a distinct type that matches PAHs signatures from ship exhaust particles in laboratory experiments, see figure 4. Tracking ship transponder signals and calculations of air mass trajectories, we could attribute this particle class to a ferry passing at a distance of 15 km, representing the first case of ship plume detection and evaluation on basis of the health-relevant PAHs.

Within the first measurement campaign of the SAARUS project in summer 2020 (see Research Area 1 Report (11) and Third Party Projects (17)), we recorded single-particle PAH spectra from a ship engine running on different fuels. As apparent from figure 4, the PAH-pattern from MGO combustion resembles the ambient air data attributed to the ferry, proving the source apportionment potential of the single-particle PAH approach. Our developments on SPMS-based detection of ship emissions provide the scientific and technological basis for the DTEC-Proposal “LUKAS” (see third party projects (06)).

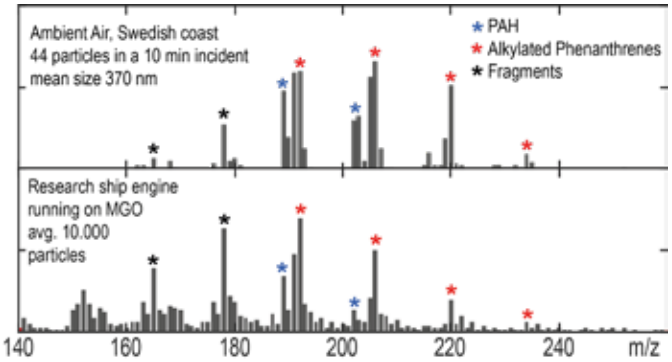

Figure 4: *top: Mass spectra of aromatic molecules from a transient event in Sweden (17th Oct 19, 00:30). bottom: Mean particle spectra from the research ship engine in Rostock, running on Marine Gas Oil (MGO). Dominant signals of alkylated phenanthrenes in the respective m/z-region are indicative for MGO combustion on ships.*

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(06) Toxicity of combustion-derived aerosols *in-vitro* and *in-vivo:* the newHICE project

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Background

The Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health – HICE, successfully ended in 2017 and revealed novel insights regarding the toxicity of various combustion-derived aerosols. The use of diesel fuel in marine engines, for example leads to similar effects as the use of heavy fuel oil (Oeder *et al.*, 2015, Sapcariu *et al.*, 2016). Additionally, the aerosols from various small-scale wood combustion appliances, fed with different types of logwood or pellets have hugely different toxicological effects *in-vitro* (Kasurinen *et al.*, 2017 & 2018, Dilger *et al.*, 2016, Kanashova *et al.*, 2018). We also discovered that the gas phase of combustion-derived aerosols could be responsible to a large extent for the observed effects in some endpoints *in-vitro* (Ihantola *et al.*, 2020). Due to the complexity of both the aerosol composition and deposition and the biological response, many research questions are still open even at the end of the intense HICE research period. The follow-up project newHICE aims to answer two of those questions: (1) how well do *in-vitro* and *in-vivo* experiments detect the same effects of aerosol exposure? and (2) how does a prior respiratory condition affect the strength of these effects? Therefore, we conducted a first joint measurement campaign with our long-standing cooperation partners from the ILMARI chamber at the University of Eastern Finland. Further campaigns are in preparation after being postponed in 2020 due to the COVID-19 pandemic.

Methods

Logwood and lignite bricks were combusted in a masonry heater and compared with emissions from a stationary diesel generator (figure 1). Human respiratory cells (A549) and mouse alveolar macrophages (RAW264.7) were exposed at air-liquid interface (ALI) conditions for four hours (Mühlhopt *et al.*, 2016; system from Vitrocell® GmbH, Waldkirch, Germany). Additionally, female C57BL/6 mice were exposed to the same aerosols for four hours each on three consecutive days. Murine macrophages RAW264.7 and animals were either directly exposed or pre-treated with bacterial lipopolysaccharide (LPS) to induce airway hyperresponsiveness, in order to discriminate how this condition affects the aerosol exposure. For the *in-vitro* experiments,

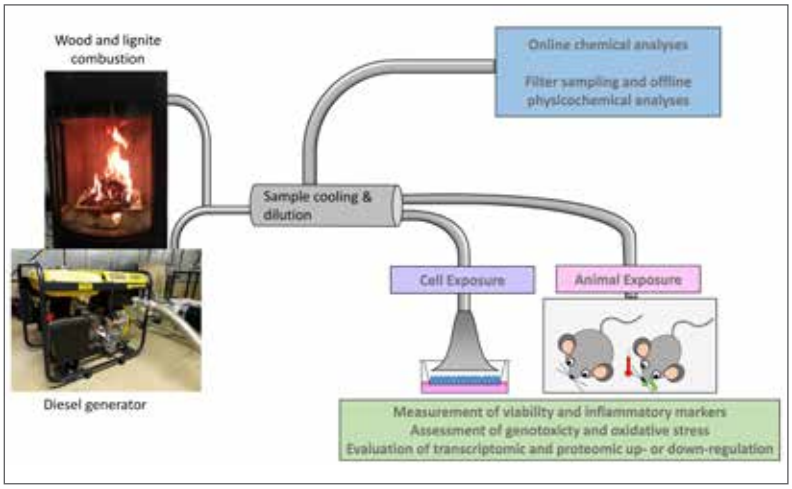

Figure 1: *Schematic overview over the 2018 newHICE campaign. After production of the combustion aerosols, filter sampling, online chemical analyses, and cell and animal exposures were conducted at the same time.*

the cytotoxicity of the aerosols was measured with the lactate dehydrogenase (LDH) assay and the cell viability with the AlamarBlue (AB) assay. The number of circulating immune cells in the bronchoalveolar lavage fluid (BALF) of the mice was counted and their viability assessed using trypan blue (TB).

Results

The LPS pre-treatment had little effect on the overall cytotoxicity of the tested emissions in RAW264.7 cells (figure 2A). We saw an increase in the cytotoxicity compared to the clean air control only for the 1:60 diluted diesel emissions. The 1:30 diluted logwood emissions and the 1:60 diluted lignite brick emissions were even less cytotoxic than the clean air exposure. This effect is probably caused by the higher humidity of biomass combustion emission compared to clean air or diesel emission exposure and the overall fact, that macrophages are less suitable for ALI exposure experiments than epithelial cells (figure 2A). In A549 cells, none of the emissions caused a significant increase or decrease of the cytotoxicity compared to the clean air exposure (figure 2A). However, with a high voltage deposition enhancement (HV), we observed a significant increase in cytotoxicity for the logwood combustion. Exposure to the 1:60 diluted diesel emission decreased the cell viability in A549 significantly (figure 2B).

With HV, the viability of A549 cells after the

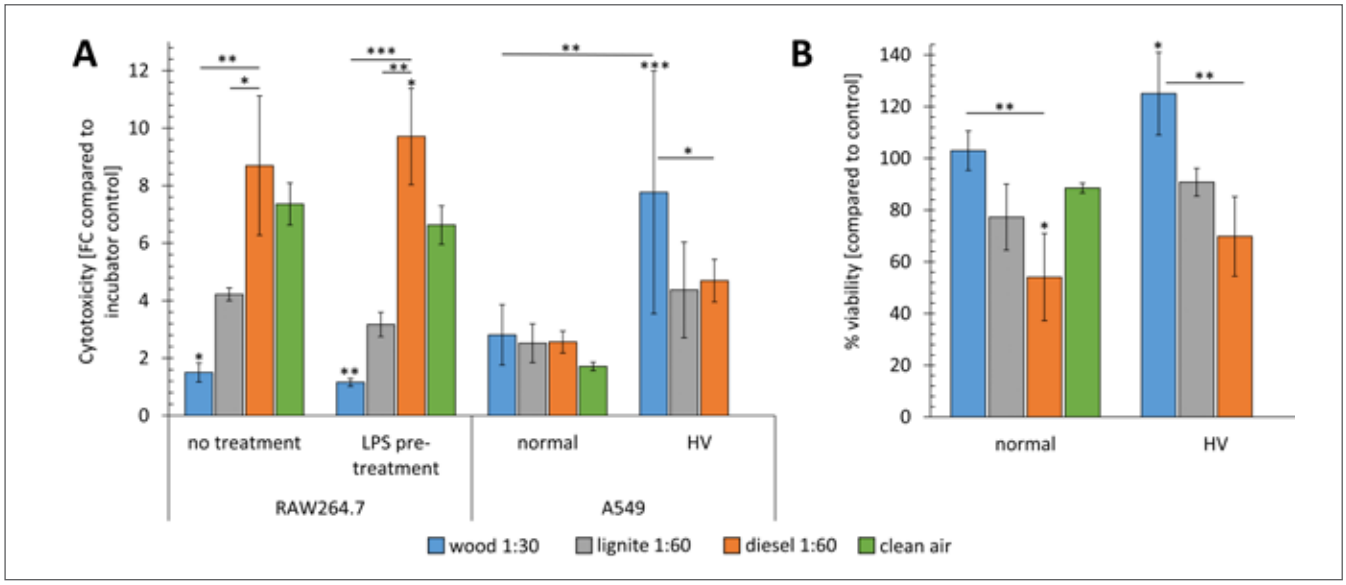

*Figure 2: (A) Cytotoxicity of combustion aerosols in A549 and RAW264.7 cells after a 4 h exposure at ALI conditions. RAW264.7 cells were pre-treated with 5 ng/mL lipopolysaccharide (LPS) 24 h prior to the exposure or a sham control, no pre-treatment was applied for A549 cells. (B) Viability of A549 cells after a 4 h exposure to various combustion aerosols with or without high-voltage deposition enhancement. HV: high voltage deposition enhancement * denotes significance at 0.05 level, ** at 0.01 level, and *** at 0.001 level determined by Tukey's range test.*

logwood exposure seemed to increase compared to clean air exposure, but this effect is likely caused by optical interference of the deposited particles with the AB assay itself. We found generally higher numbers of circulating immune cells in the BALF of LPS-treated animals, but the difference to healthy animals was not significant (figure 3A). However, this indicates that the induction of airway hyperresponsiveness was successful. Only the 1:10 diluted diesel emissions in the LPS-treated animals reduced the viability of those cells significantly to animals, which were exposed to clean air (figure 3B). This decreased viability was also significantly lower than that of BALF cells in mice exposed to the other combustion-derived aerosols.

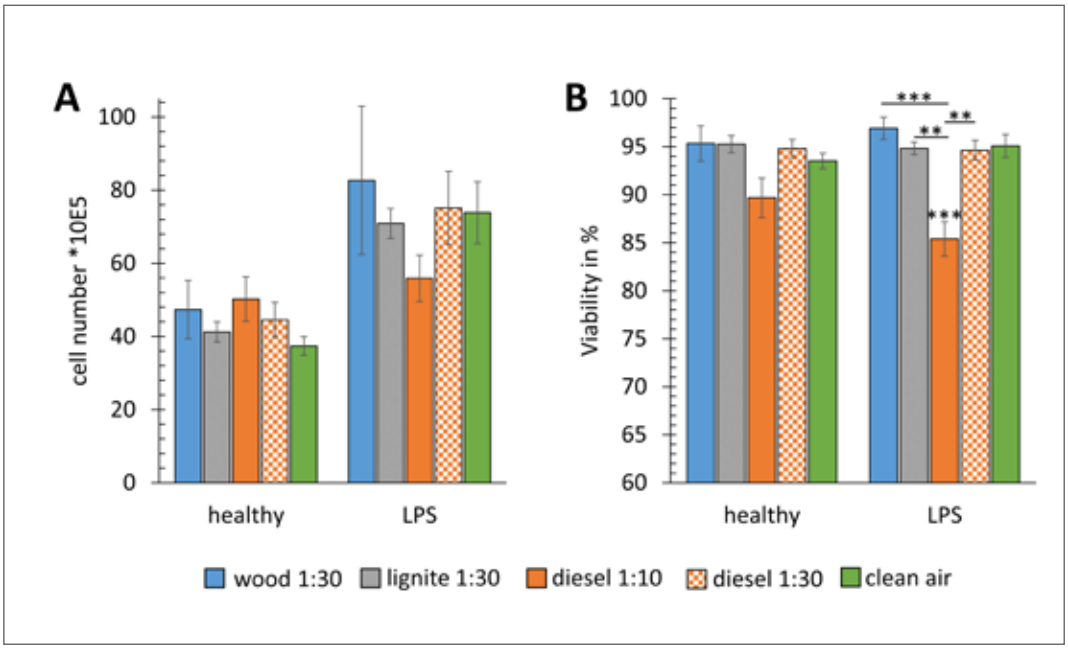

Figure 3: (A) Total cell number in the bronchoalveolar lavage fluid (BALF) of healthy and LPS-instilled mice. (B) Viability of cells in the BALF of healthy and LPS-instilled mice after a 4h exposure to various combustion aerosols on three consecutive days. denotes significance at 0.05 level, ** at 0.01 level and *** at 0.001 level determined by Tukey's range test.*

Conclusion

The results of the 2018 newHICE campaign show that combustion-derived emissions cause different effects in the cell models and animal models. While logwood emissions seemed to have cytotoxicity-protecting effects in RAW264.7 cells, they caused the higher cytotoxic effects in A549 cells after electrostatically enhanced particle deposition. In the animal model, however, the logwood emissions did not cause any significant effects regarding the cell number in BALF or their viability, which is in line with the theory of a potential protection by wood smoke antioxidants (Kanashova *et al.*, 2018). Contrarily, the diesel emissions caused the highest cytotoxicity in RAW264.7 cells with or without LPS-treatment, but they had no significant effect in A549 cells with or without particle deposition enhancement. In the animal model, the diesel emissions were the only aerosol to reduce the viability of BALF cells significantly in LPS-treated animals, however only at a 1:10-dilution. Further analyses are underway and will examine the transcriptome and proteome of A549 and RAW264.7 cells as well as different tissue samples from the animals. The detailed physicochemical analysis of the combustion-derived emissions and the data integration with all toxicological endpoints should then reveal why the aerosols exhibit such different behaviors in the different test systems.

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(07) Development of an automated *in-vitro* exposure system for longterm lung cell exposures to analyze the effects of air pollution

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Current *in-vitro* cell exposure systems are versatile tools to describe the (adverse) effects of all kinds of aerosols (*e.g.* engine and biomass combustion emissions, cigarette smoke, and more) on lung cells. Such exposures usually last between 2–6 h and typically involve high concentrations of the emissions. However, the recent environment- and health-awareness of the public and politics calls for more research under normal ambient conditions.

In-vitro exposures to ambient air should be performed as realistically as possible, *i.e.* culturing and exposing the lung cells at the air-liquid-interface (ALI) to allow direct contact between the aerosol and the cells. They should also last as long as possible, *i.e.* more than 4 h, because humans constantly breathe air. Additionally, ambient pollution levels are lower than emission levels, which also calls for longer exposures.

The automated exposure station (Vitrocell®, Waldkirch, Germany) is such an instrument allowing our group to perform these controlled *in-vitro* ALI cell exposures (figure 1). In previous experiments with this system and A549 lung cells, we were able to perform 48 h exposures. Applying diffusion-flame generated diesel particles twice for 6 h during the 48 h significantly increased DNA-strand breaks without induction of cytotoxicity. Even longer exposure times were not possible due to limitations in the exposure station and the cell culture system, therefore this project was initiated.

Required adjustments in the automated exposure station are the humidity generation, the airflow over the cells, exchange of the culture medium, remote software control, and temperature-controlled air-intake. Such changes are best implemented with strong customer feedback. Vitrocell®, therefore, collaborates with HMGU to develop the best possible new techniques. A new humidity generation system and control units are available now and will be tested in early 2021. Additionally, a new aerosol inlet, which should reduce the intrinsic stress of the airflow is currently evaluated by Vitrocell®. The new technique could be ready mid-2021 for first experiments.

ALI-cultures lack nutrients from the top, therefore many cell models can dry out and remain viable only for a few days. Öhlinger *et al.* (2019) cultured A549 cells at ALI for up to 6 d and achieved a monolayer by initially seeding only a few cells. With similar parameters, we also observed a viable monolayer with very few dead cells after 6 d. However, this could only be achieved with the incubator controls, cells put in the automated exposure station for 4 h on three

Figure 1: *Automated exposure station from Vitrocell®. A versatile tool to assess in-vitro effects of aerosols at the air-liquid-interface. Exposures of up to 48 h were possible without increased cytotoxicity.*

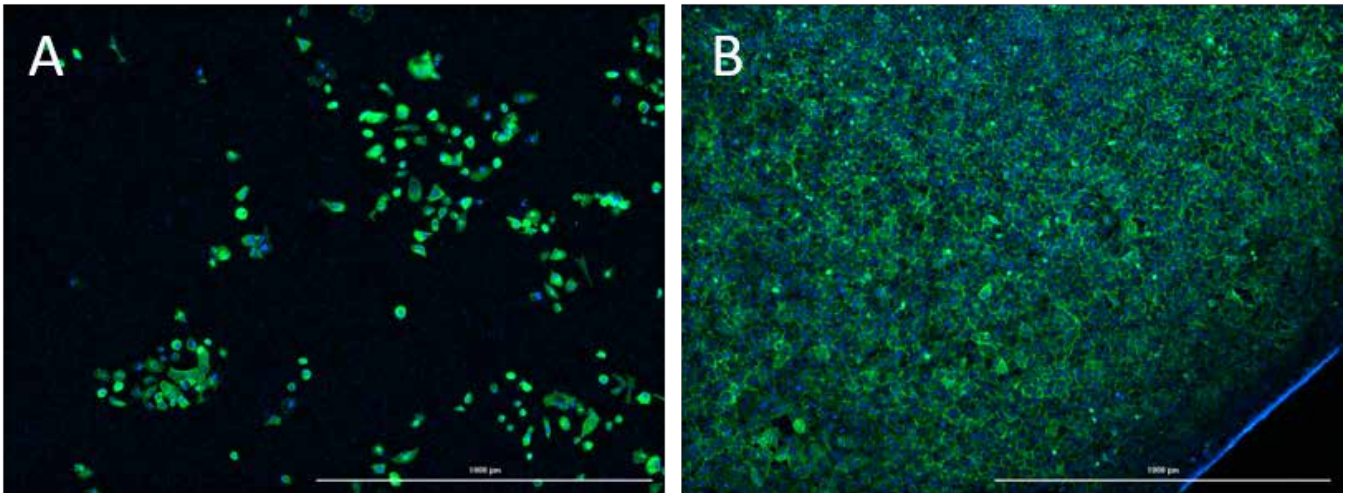

Figure 2: *Alveolar epithelial cells cultured for 4 days at ALI. A: repeated exposure to filtered ambient air and B: incubator controls. This first result suggests that under the current settings a longterm exposure with A549 cells is not possible. The aerosol inlet will be improved, which should decrease the cellular stress making longterm exposures with A549 probable. Blue: nuclei stained with 4',6-diamidino-2-phenylindole; Green: f-actin cytoskeleton stained with Phalloidin A488; scale bar 1mm.*

consecutive days died. Since these exposures were done before the cells reached confluence (day 3-5), they were especially susceptible to any type of stress (figure 2). Further experiments will include other seeding densities, time points, and media to enhance the durability of A549 cells.

More recently, Braakhuis *et al.* (2020) showed that another cell line, the bronchial epithelial cell line CALU-3, is suitable for long term exposures. This cell line can be cultured up to 3 weeks as a confluent monolayer with important tight junctions.

The project presented here will further optimize the use of A549 cells as a model for long term exposures at the ALI. Additionally, the most promising respiratory cell line according to literature, CALU-3, will be tested. The improved cell cultures will then be used in combination with the new technical adjustments of the automated exposure station, with the aim to suggest protocols for long term ambient air exposures.

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(08) Determination of genotoxicity of the semi-volatile environmental and workplace contaminant dibutyl-phthalate on A549 alveolar epithelial cells exposed at the air-liquid interface

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Introduction and objective

Phthalates are ubiquitous environmental and workplace pollutants that are used in a broad range of consumer products and industrial branches due to their beneficial properties. On the other hand, their potential adverse health effects are of great concern for our health and environment (Wormuth *et al.*, 2006). Several epidemiological, *in-vivo*, and *in-vitro* studies have associated phthalate exposure with an increased risk of developing asthma and allergy, and even cancer, and have uncovered potential genotoxic effects and oxidative-stress related toxicity. Even though, the effects of inhalable phthalates on the development and promotion of lung diseases remain elusive.

This study focused on the potential genotoxic effects of the semi-volatile organic compound (SVOC) di-n-butyl phthalate (DBP), which is one of the majorly detected phthalates in gas and particle fractions in workplace and indoor environments (Szewczyńska *et al.*, 2020; Chi *et al.*, 2017). Knowledge of real exposure concentrations and cellular doses is an important criterion to mimic reality exposure scenarios and assess dose-driven cellular responses. This study aimed to consider the direct interaction of airway epithelium with inhaled air in an *in-vitro* exposure at the air-liquid interface (ALI) to unravel potential effects of the semi-volatile compound DBP in the development and promotion of lung diseases.

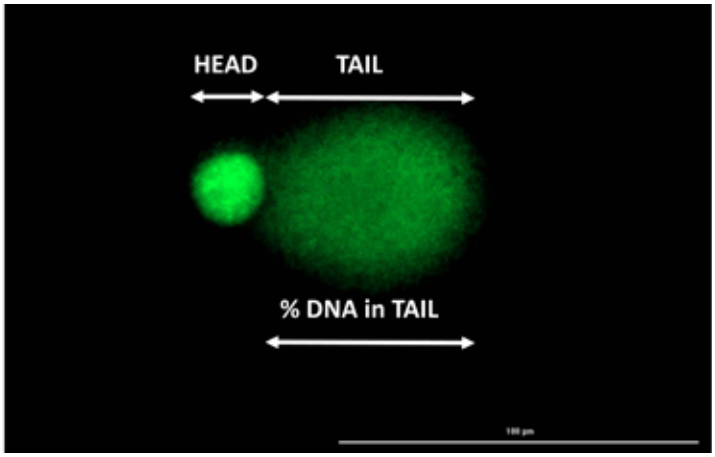

Figure 1: *Representative microscopic appearance of Comet assay. A549 nucleoid with highly damaged DNA upon 30 µM H_2O_2 (positive control) treatment.*

Methods

A549 lung epithelial cells were cultured onto an insert membrane to a confluent monolayer. Cells were exposed for 9 minutes to different concentrations of DBP at the air-liquid interface in the Vitrocell® Cloud 6 cell exposure system. Cytotoxicity and cell viability were measured by lactate dehydrogenase release into the cell culture medium (LDH assay) and AlamarBlue reagent, a resazurin-based indicator of cell health, following 4h and 24h post-exposure. The oxidative stress biomarker malondialdehyde and cellular deposition of isotope labeled DBP-D4 in the exposure system were analyzed via HPLC-MS/MS. The Comet assay, single-cell gel electrophoresis, was utilized to evaluate induced single and double DNA strand breaks after 4h and 24h post-exposure (figure 1).

The cytokinesis-block micronucleus cytome assay (CBMN Cyt, OECD 487) was performed after 48 hours of treatment for a comprehensive assessment of cytotoxicity, cytostasis, and chromosomal instability. The distribution of mononucleated, binucleated, and multinucleated cells (figure 2a) allows the calculation of a proliferation index indicating the average number of cell cycles while the number of mitotic figures (figure 2b), apoptotic (figure 2c), and necrotic cells (figure 2d) indicate cytostasis and cytotoxicity. Chromosome breakage and loss, which are expressed as micronuclei in mononucleated cells (figure 2e) or in once-divided binucleated cells (figure 2f), are evaluated to distinguishing between aneuploidogenic and clastogenic effects, respectively. Finally, chromosome rearrangements are evaluated by scoring nucleoplasmic bridges (figure 2g) and nuclear buds (figure 2h) are evaluated as a biomarker of amplified DNA and/or DNA repair complexes elimination.

Results

The measured total cell-specific deposition efficiency of DBP in the Vitrocell® Cloud system corresponded to about 3 %. The selected exposure concentrations were oriented at real exposure scenarios and covered several magnitudes of orders, ranging from 0.02-18 ng cm^{-3}. Comet assay and CBMN Cyt assay revealed that DBP

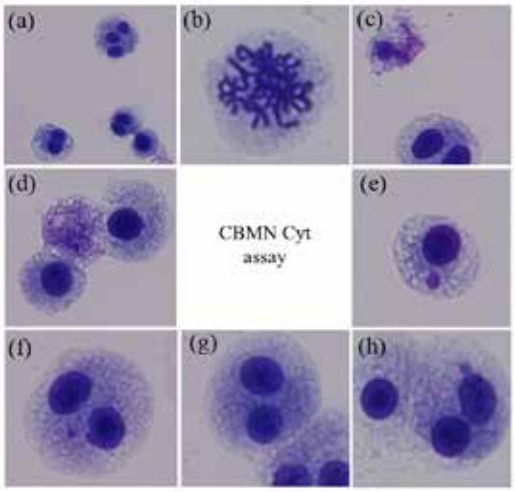

Figure 2: CBMN Cyt assay pictures showing different biomarkers of cytotoxicity, cytostasis, and chromosomal instability.

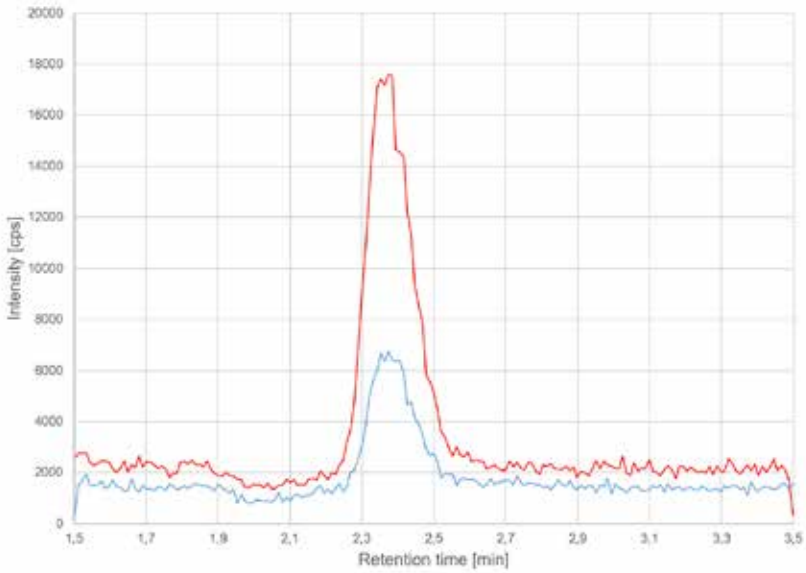

Figure 3: Typical LC-MS/MS chromatogram of an internal standard sample (red) and the measured MDA intensity (blue).

induces genotoxicity on DNA and chromosome level in A549 cells at ALI conditions. None of the exposures showed cytotoxic levels higher than 10 % in comparison to solvent control. After 4 and 24 hours, DBP leads to highly significant DNA damage from the lowest tested concentration, 0.07 µM, up to the highest, 50 µM. Notably, DNA damage in terms of DNA strand breaks was elevated at the later time point compared to the earlier time point. Additionally, chromosomal instability measured as the number of micronuclei in mono- and binucleated cells increased with increasing treatment concentration. Furthermore, oxidative stress marker malondialdehyde (MDA) significantly increased upon 48h DBP exposure to 0.6 and 5.6 µM indicating a persistent oxidative stress condition possibly leading to the observed chromosomal damage. In figure 3, an exemplary chromatogram of the MDA assay positive control, 700 µM tert-butyl-hydroperoxide, and the respective internal standard used to quantify MDA content are shown.

Conclusions

Considering the different magnitudes of orders of applied DBP concentrations, the used concentrations are in the range of occupational exposure levels estimated by the European Chemicals Agency (ECB 2004). At the air-liquid interface, DBP induced genotoxicity at DNA and chromosomal level under sub-cytotoxic conditions, which indicates a direct connection to potential pathogenic events in the lung upon inhalation. Furthermore, DBP treatment led to a persistent increase of MDA concentrations, indicating a potential role of oxidative stress in the observed genotoxic effects. The Vitrocell® Cloud system emerged as a useful tool to study dose-specific cell responses and allowed for the assignment of cell-specific deposition of DBP. Thereby, a dose-response relationship between respiratory semi-volatile organic compound exposures and associated adverse health effects might be evaluated in a reproducible exposure system. Prospective studies will reveal the influence of the cellular metabolic activity of DBP as well as the influence of the different aggregation states of DBP and its underlying mechanisms of toxicity.

Acknowledgements

We like to thank the German Social Accident Insurance, DGUV, for funding as part of the project FP425. Additionally, we would like to thank Dr. Jutta Lintelmann for her support.

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(09) Adjuvant or protective effects in Allergy – Impact of the chemical composition of pollutants and shed aerosols on biological effects in lung epithelial cells

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In the last decades, the prevalence of allergy and asthma has risen immensely worldwide. Because of the short time period and the drastic increase of allergic diseases, possible associated environmental factors are in focus. In 2017, the Helmholtz Zentrum München initiated several collaboration projects with the aim to bring together the complementary expertise of different institutes on allergy research. The CMA and Institute of Allergy Research (IAF) together with partners from the Institute of Asthma and Allergy Prevention (IAP) and the Institute of Lung Biology and Disease (ILBD) initiated and participate in "Project 1: HMGU Allergy Cluster Air" on health risks caused by interactions of biologic components of ambient air with pollutants and protective environments (figure 1). In total, 4 PhD Projects are conducted in close cooperation within this Allergy Research Cluster.

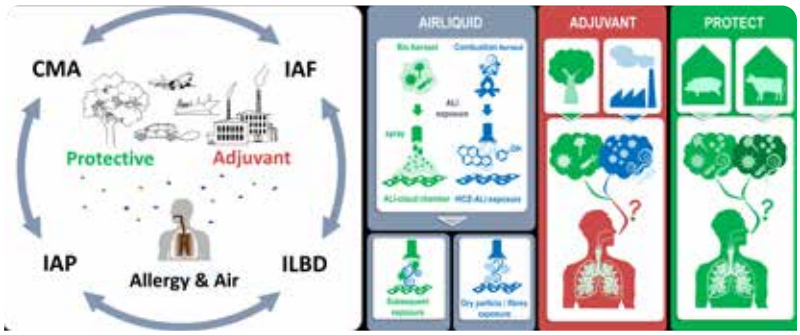
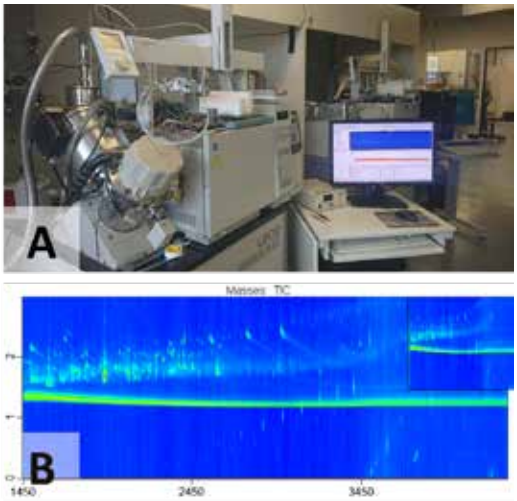


Figure 1: left: The interaction of the HMGU Allergy Cluster Air with the four key partners (Institutes). Right: Topical focus of the HMGU Allergy Cluster "Air": Development and application of adapted ALI and cloud technology for bio- and combustion aerosols for the investigation of adjuvant effects using biological/disease models and investigation of aggravating of protective effects of specific microbiomes or atmospheres.

Figure 2: A: Modified Vitrocell© cloud chamber with a laser detection system for quantification and distribution measurements; B: Pollen Sedimentation Chamber for intact pollen exposure; C, D: Sampling sites in cow and sheep sheds; E: Sampling station with high volume sampler and denuder.

CMA participates with two research projects, which are realized within two PhD theses. The first thesis has a focus on the biological responses of cell systems when they are exposed to anthropogenic aerosols and/or biogenic aerosol extracts to investigate possible adjuvant and protective allergic effects. *In-vitro* cell exposures were carried out based on the Air-Liquid-Interface (ALI) and cloud chamber technology, both established at CMA (figure 2A) (Mülhopt *et al.*, 2016, He *et al.*,2020). A complementary approach with a focus on the interaction of intact pollen is carried out at IAF with another exposure unit for pollen (figure 2 B). The combination of the expertise of CMA in the aerosol generation and aerosol cell exposure and the expertise of the IAF on whole pollen exposure enabled a study of the adjuvant effects. In a joint measurement campaign, human lung cells were subsequently exposed with biogenic and anthropogenic aerosols. A first exposure regime comprises the exposure of cells in the cloud chamber and subsequent exposure with allergen extracts and anthropogenic combustion aerosol (*e.g.* generated by propane and diesel CAST generators). For the second regime, the cells were first exposed to CAST aerosols and afterwards to whole birch pollen. To get a comprehensive picture of the underlying mechanisms, viability tests, genotoxicity tests, and transcriptomic analysis were conducted on the exposed cell models. In general, cytotoxicity could not be observed, but subsequent exposures showed stronger effects on cells as these increased both stress and redox pathway expression as well as showing significant genotoxic effects. The expression of allergy relevant genes was mainly upregulated in earlier time points and declined with time.

Figure 3: A: GCxGC-TOFMS; B: GCxGC-Chromatogram, over 1000 different peaks and, thus, over 1000 different components can be detected per run.



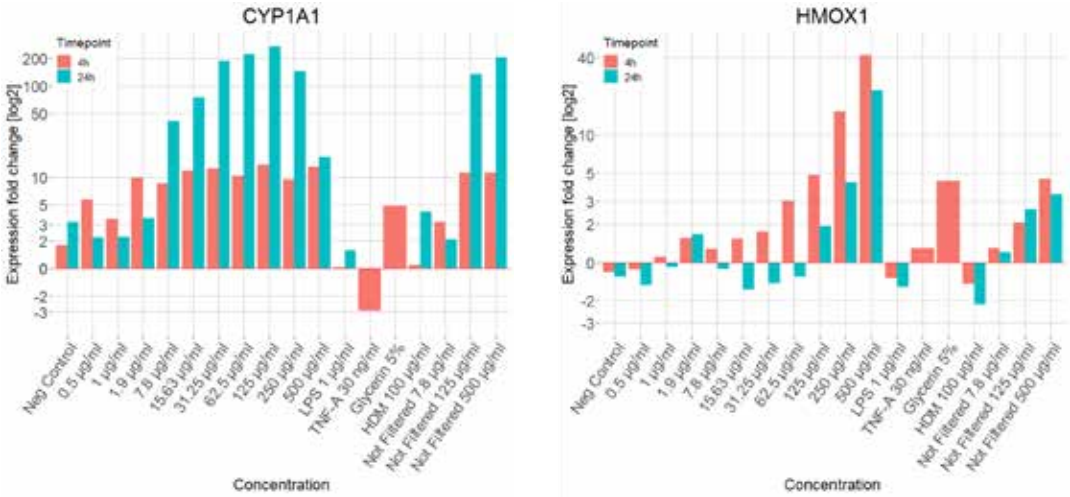


Figure 4: *Gene expression fold change in human bronchial epithelial lung cells (BEAS-2B) treated with different cow shed extract concentrations for 4h or 24h. Left: Xenobiotic metabolism marker CYP1A1. Right: Oxidative stress marker HMOX. Controls: untreated negative control (Neg. Control), lipopolysaccharide (LPS), tumor necrosis factor – alpha (TNF-A), Glycerin 5 %, house dust mite extract (HDM).*

The partner institute IAP focuses on the protective effects of farming environments and was already able to prove a protective effect of the cow shed environments/aerosol in animal experiments with mice (Peters *et al.*, 2006). The second PhD thesis at CMA tackles this issue and focuses on a potential protective effect of the semi-volatile fraction of such "farming" aerosols (figure 2 C-E). Aerosol sampling was carried out in two cowsheds, which have previously shown a protective impact in epidemiologic studies, and two sheep sheds as a reference to evaluate the differences of the organic semi-volatile aerosol composition from the distinct livestock. For comparison, gas-phase samples and respirable particulate matter up to a diameter of 2.5 µm were collected and analyzed by (comprehensive two-dimensional) gas chromatography time-of-flight mass spectrometry ((GCx) GC-TOFMS) (figure 3 A,B). The detailed chemical analysis revealed significant changes in the chemical profile and the most significant semi-volatile compounds already showed some possible link to allergic mechanism based on literature studies. Subsequent *in-vitro* analyses with size segregated extracts (molecular weight cut off < 3000 Dalton) and different allergy-relevant endpoints are currently in progress. This unique combination of reproducible sampling with chemical characterization and biological response testing is valuable and suggests fostering the implementation of the Air liquid interface (ALI) technology for allergy relevant (biological) aerosols.

After establishing the different methods and focusing on the two main questions regarding adjuvant and protective effects, including their underlying mechanisms, the next step will be to combine biological and chemical analysis seeking a unified risk assessment system for allergy. An interesting further research route tackles the impact of biological and/or anthropogenic combustion-related aerosols of (viral) infections. The ILBD investigated the virus reactivation reactions due to cell exposure by environmental particles and the following exacerbation of chronic lung diseases. First studies focused on the reactivation of murine gamma herpes virus-68 (MHV-68) by mice exposure to carbon nanoparticles and the establishment of an *in-vitro* inhalation system for virus reactivation. For *in-vitro* virus reactivation, bone marrow derived macrophages (ANA-1 cells) were infected with MHV-68 and exposed to environmental relevant particles via cloud chamber (Sattler *et al.*, 2017). A study attenuating viral reactivation after *in-vitro* exposure to possibly protect livestock environments following particle exposure is currently scheduled between CMA and ILBD.

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(10) *In-vitro* toxicity of recycled fiber-containing composite materials from wind power stations

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Since wind energy is a clean, renewable, and efficient source of energy, the wind power industry is a rapidly growing sector worldwide. In 2019, 130,000 wind turbines supplied 15 % of the electricity in the EU (WindEurope 2020). The first generation of turbines was installed in the 1990s and with an estimated life span of 20 to 25 years, many turbines are nearing the end of their life cycle and up to 12,000 turbines could be dismantled within the next five years. Wind turbine blades are made of composite materials, like glass or carbon fibers embedded in a polymer matrix. When composites get cured, the polymers undergo an irreversible crosslinking process, which makes fibers hard to recycle (Chen *et al.*, 2018; Jensen and Skelton 2018).

In this project, we are investigating the recycling of the wind turbine blades by pyrolysis, a process in which the material undergoes thermal decomposition by applying high temperatures in an inert atmosphere. During the shredding of the turbine blades and their treatment with high temperatures the dimensions of the glass and carbon fibers could change, and the formation and release of harmful fibers must be excluded. Whether a fiber is able to exert toxic effects depends on several factors, such as the dosage, their durability, and their dimensions. Macrophages easily engulf short fibers and clear them from the lung. However, larger fibers, which exceed the size of the macrophages, can trigger a process called frustrated phagocytosis, which increases the biopersistence of the fibers. During the clearance of fibers from the lung, macrophages release reactive oxygen species, inflammatory mediators, growth-factors, and chemo-attractants, which recruit other cells, such as fibroblasts. Those cells play a major role in wound-healing processes, secreting extracellular matrix constituents like collagen. Especially chronic exposure to hazardous fibers can lead to an overload of lung clearance mechanisms as well as deregulation of wound-healing processes. This imbalance can subsequently cause pulmonary fibrosis, resulting in decreased tissue elasticity and lung function, as well as lung cancer or mesothelioma (Lippmann, 1990; Donaldson *et al.*, 2011).

Previous studies on carbon fiber-reinforced composite materials showed that elevated temperatures could lead to a decrease in fiber diameter due to pitting and local degradation of the carbon fibers (Feih and Mouritz, 2012; Eibl, 2017), and thus, to the formation of harmful fibers. It is likely, that these degradation processes are dependent on the atmosphere during treatment at elevated temperatures (Yatim *et al.*, 2020). Scanning electron microscopy (SEM) will help to understand the morphology of the fibers after wind turbine recycling processes and their impact on the toxicity.

To investigate the toxicity of recycled fiber materials from wind power stations, we established multi-cellular culture systems, which resemble the physiological conditions in the human lung and allow the examination of the interplay between different cell types.

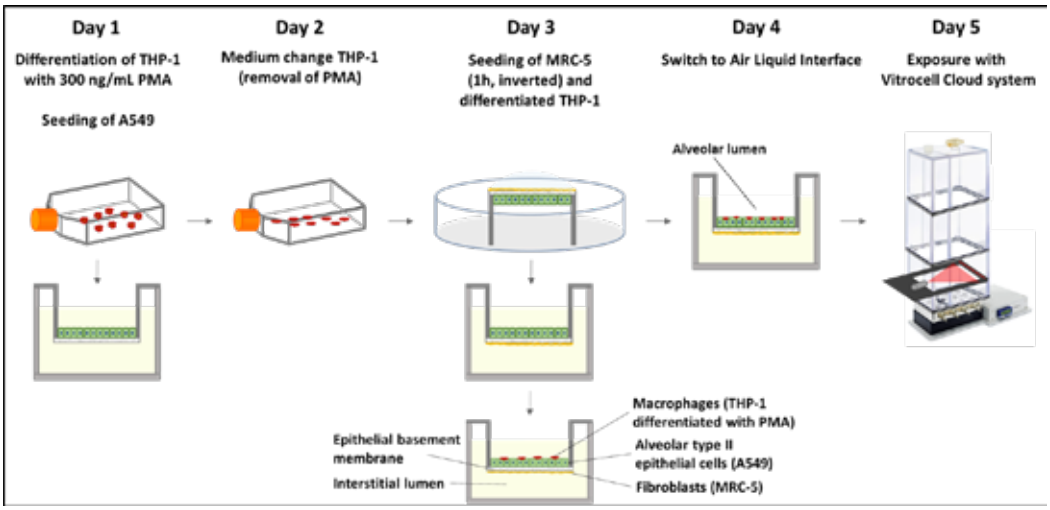

Figure 1: *Experimental set-up of the triple culture system comprising alveolar epithelial cells, fibroblasts, and macrophages.*

The development of alternative *in-vitro* model systems in contrast to animal models is not only necessary to reduce ethical concerns, but also to overcome inter-species differences like the dimensions of the respiratory system or the deposition of fibers in the nasal region. The development of fibrosis-specific *in-vitro* systems includes a co-culture of alveolar epithelial cells (A549) and fibroblasts (MRC-5) as well as a triple culture of A549, MRC-5, and macrophages (THP-1) (figure 1). These advanced *in-vitro* systems can be established and applied in every laboratory due to their cost-effective and easy experimental design.

We used multi-walled carbon nanotubes (MWCNTs) as reference material to validate our *in-vitro* systems, addressing endpoints known to play a role in fiber toxicity. MWCNTs are needle-like shaped fibers with a high aspect ratio, high respirability, and high biopersistence. Due to these factors, MWCNTs cause adverse health effects similar to those of other harmful fibers like asbestos. These adverse effects range from cytotoxicity or inflammation to fibrosis and immunotoxicity or even genotoxicity and tumor development (Dong and Ma, 2015).

The toxicological analyses of MWCNTs were performed in three independent experiments. After exposure, the release of lactate dehydrogenase (LDH) into the cell culture medium was analyzed as well as the colony-forming efficiency (CFE), indicating cytotoxic effects. Furthermore, the genotoxic potential, describing the ability of MWCNTs to induce DNA strand breaks, was assessed by the comet assay. The comet assay was additionally modified for the detection of oxidized bases using the endonuclease formamidopyrimidine-DNA glycosylase (Fpg). Moreover, cells, as well as cell culture medium, were collected to detect oxidative stress markers like glutathione (GSH) and malondialdehyde (MDA) via liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and to perform enzyme-linked immunosorbent assays (ELISA) and quantitative Polymerase Chain Reaction (qPCR) analyses targeting oxidative stress- and fibrosis-related markers. The sampled medium is also analyzed using the Sircol™ collagen assay to monitor the collagen production of MRC-5 cells after exposure to the MWCNTs (figure 2, II). Preliminary results show a non-significant increase of LDH in the cell culture medium following 24 h as well as 48 h exposure to MWCNTs implying a cytotoxic effect with the two highest concentrations (25 µg/mL and 50 µg/mL). This is also supported by a decreased colony-forming efficiency of A549 cells detected with the two highest concentrations applied and could be related to oxidative stress. Genotoxicity in A549 cells could not be observed after 24 h exposure (figure 2, III).

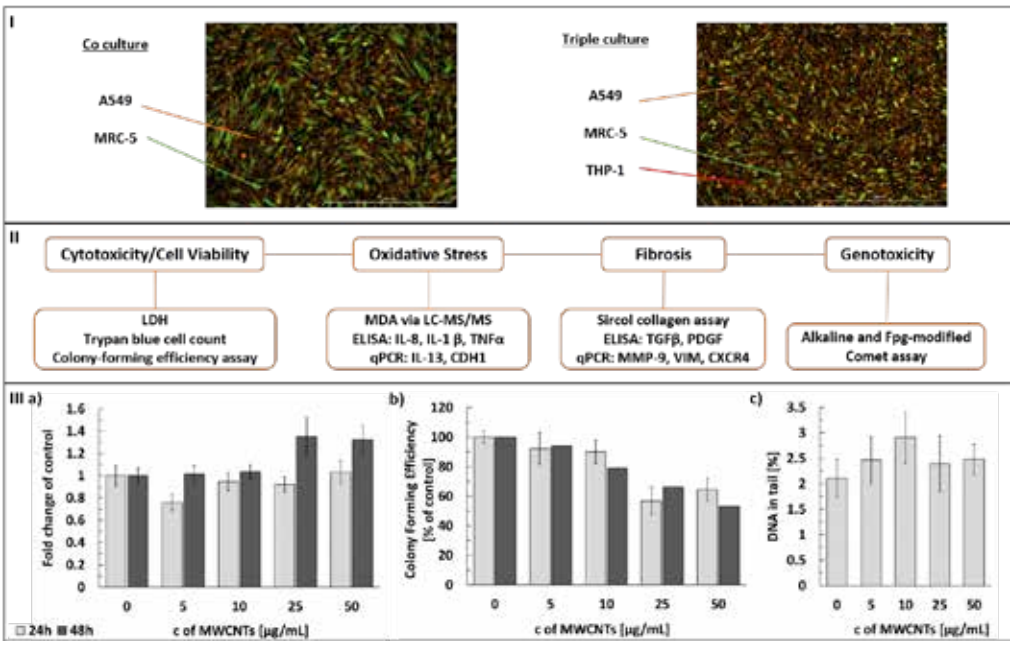

Figure 2: *Summary of established cell culture models (I), addressed endpoints (II) and preliminary results of the LDH assay (III, a), CFE assay (III, b) and alkaline comet assay (III, c).*

Upcoming studies will include the analysis of the recycled fiber-containing composite materials within the established cell models as well as the use of models containing primary or diseased cells to receive a comprehensive understanding of the mechanisms involved in fiber toxicity.

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(11) Optimization of marine after-treatment technology for reduction of harmful ship emissions (SAARUS)

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Introduction

Worldwide, ship emissions pose a significant risk to health and the environment and can account for up to 50 % of the total PM in coastal areas. According to epidemiological studies, 60,000 annual deaths from lung and cardiovascular diseases are linked to ship engine PM.
Ship engines are using various fuels: low-grade heavy fuel oil (HFO), marine gas oil (MGO), diesel fuel (DF), renewable hydrotreated vegetable oil (HVO), and other types including blends.
These fuels vary in the content of organic and inorganic compounds and have different physical-chemical properties which lead to different emission characteristics and toxicity. For example, HFO emissions typically contain a high concentration of harmful sulfur oxides (SOx), polycyclic aromatic hydrocarbons (PAHs), and heavy metals (Oeder *et al.*, 2015).
In comparison to emissions from the industry and road traffic, ship emissions had not been in the focus of legislative regulations, although shipping is one of the biggest contributors to anthropogenic SOx and NOx emissions. Since 2005 the International Maritime Organization (IMO) has started regulating SOx emissions by limitation of the sulfur content in heavy fuel oil.
Modern fuels and blends with lowered sulfur contents can result in other emission patterns compared to conventional fuels. For instance, fuels with high contents of aromatic compounds can lead to higher emissions of PAHs and PM due to higher ignition delay which leads to incomplete combustion, emission of unburned fuel, and higher possibility of pyrogenic formation of PAHs (Käfer *et al.*, 2019). Therefore, unconventional new fuels could result in even more harmful emission profiles, although they meet the latest IMO criteria. Hence, a comprehensive characterization of the fuel and its related emission profile is of great interest.
Since merchant vessels have lifetimes of more than two decades and technical upgrades due to new legislative regulations might lead to severe problems for ship owners IMO agreed to use fuels with higher amount of sulfur if the Exhaust Gas Cleaning Systems (EGCS), *e.g.* "scrubbers" would be installed and efficiently in use. Thereby, wet scrubbers use water for removal of SOx, NOx, and particles, whereby open-loop scrubber systems discard the contaminated water into the sea, which can cause maritime pollution in turn.

Figure 1: *Team of the first SAARUS measurement campaign. The campaign was successfully carried out in compliance with COVID-19 pandemic regulations and measures.*

SAARUS project objectives

The SAARUS project mainly focuses on the evaluation and optimization of the scrubbing technology in terms of decreasing particulate emissions and non-regulated gas-phase compounds. Experiments will unravel how the physical properties and chemical composition of the emissions change upon the use of a scrubber in combination with different filter technologies. Moreover, different modern and conventional fuels will be comprehensively tested and compared. In addition, contamination of the wash water, as well as the effectiveness of water cleaning technologies will be evaluated as well.

The consortium of the SAARUS project consists of 8 partners from public research institutions (Universität der Bundeswehr München (UniBW M), University of Rostock – Chair of Analytical Chemistry (CAC) and Chair of Piston Engines and Internal Combustion Engines (LKV), Leibnitz Institute for Baltic Sea Research (IOW)) and from the industry (SAACKE GmbH, RVT Process Equipment GmbH, SULT GmbH, GEA Westfalia Separator Group GmbH, AVL Deutschland GmbH). UniBW M is responsible for the chemical characterization of gaseous emissions, including investigation of ozone precursors and real-time analysis of polycyclic aromatic hydrocarbons (PAH), aerosol sampling (UniBw M), and inorganic particle analysis. The University of Rostock is responsible for the comprehensive chemical and physical characterization of particulate emissions (CAC).

First measurement campaign (summer 2020): Comprehensive characterization of marine engine emissions from different conventional nd modern fuels

The first measurement campaign took place

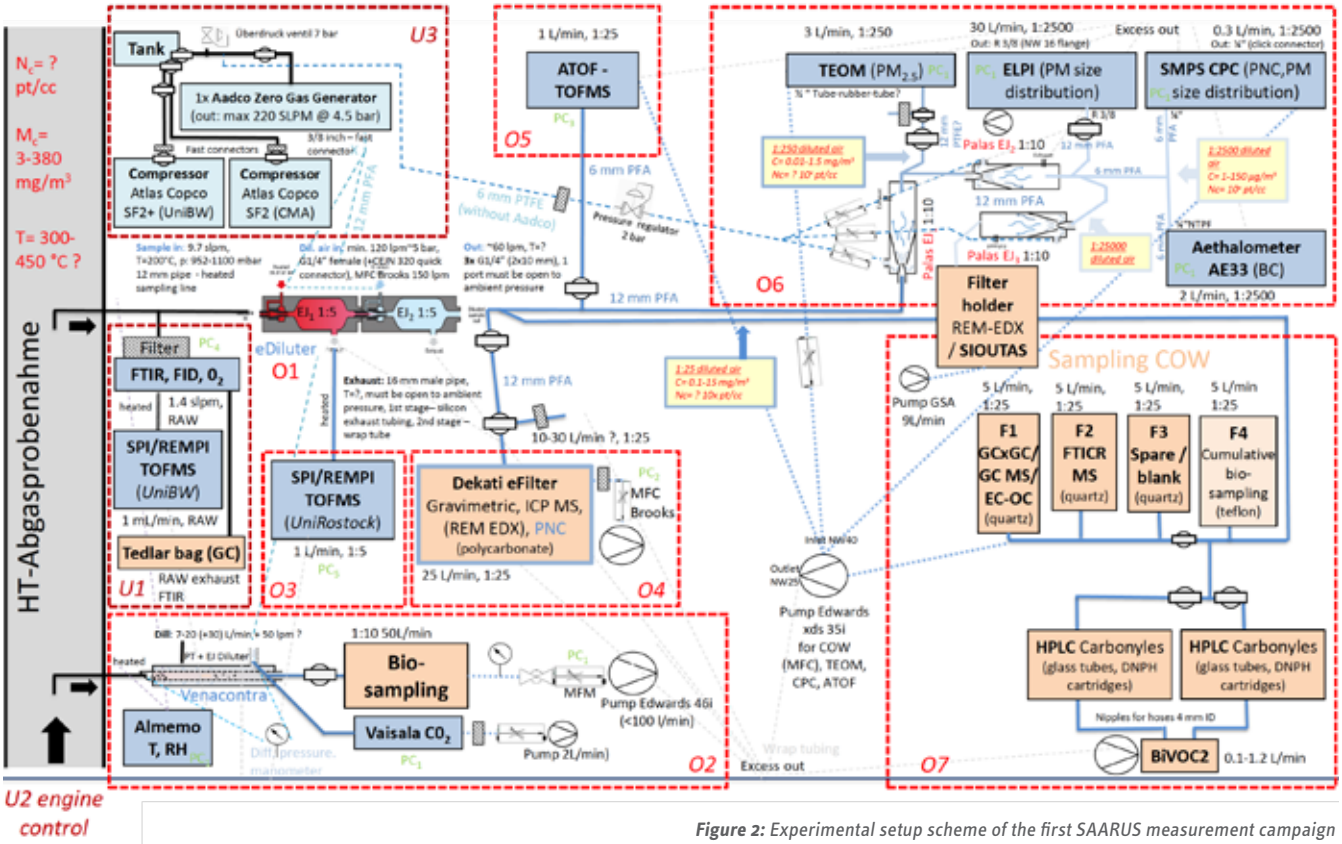

Figure 2: Experimental setup scheme of the first SAARUS measurement campaign

at the LKV department of the University in Rostock from 29.6.–21.8.2020. Particulate and gaseous emissions from 7 different fuels were comprehensively characterized in controlled experiments with the ship engine operating under different engine loads and conditions. The set of fuels compiled traditional as well as new compliant marine fuel.

Methods

The four-stroke, one-cylinder common ship diesel engine (80 kW) was applied for comparison of emissions from 7 fuels: Diesel Fuel (DF), Marine Gas Oil (MGO, S < 0.1 %), Hydrotreated Vegetable Oil (HVO), high-aromatic Heavy Fuel Oil (HFO, S = 0,06 %), compliant HFO (S = 0.53 %), HFO S = 1.34 % and HFO S = 2.4 % at 4 different loads (20 kW, 40 kW, 60 kW, 80 kW) and usually 5 replicates of IMO testing cycle. Both, gas and particulate phase were analyzed using on-line techniques for particle size distribution (SMPS, ELPI), particle mass (TEOM), black carbon (Aethalometer), gas analysis (FTIR, FID), organic compounds including polycyclic aromatic hydrocarbons and monoaromatics (VUV Fast GC, SPI/REMPI-TOFMS). Single particle mass spectrometer (SPMS) was used for particle characterization. Off-line filter analysis comprises of metals (ICP MS), organic and elemental carbon (OC/EC), SVOC (GCxGC MS and GC MS), microscopy (REM EDXA), and gravimetric analysis. Gas phase samples were also analyzed off-line for carbonyls and VOC by HPLC and GC. Additional samples will be used for FT ICR MS and bio-analysis (figure 2).

Preliminary results

On-line measurements confirmed significant differences between emission profiles of the 7 different fuels. DF, HVO, and MGO produced the highest particle mass and number concentration at 40 and 60 kW while all HFO emitted the highest concentrations at 20 kW. The particle size distribution of HFO 0,06 % S is shifted into the smaller sizes (20 kW – 27 nm mode) compared to MGO (20 kW – 64 nm), HFO 2.4 % S (20 kW – 56 nm), and other fuels.

At the same time, the particle number concentration of HFO 0,06 % S is approximately half that of HFO 2.4 % S, and the mass concentration is about 15 %, respectively. REMPI-MS graphs show higher signals for HFO 0,06 % S. Therefore, we can expect a higher number of PAHs bonded on 30 nm nanoparticles.

While tracheobronchial and alveolar deposition efficiency is the highest at 10-20 nm (around 0,7), this is very unfavorable regarding the health effects. Therefore, mass concentration (even PM1) does not pose a sufficient marker of health risks and fuels can be harmful even if they meet the new IMO sulfur requirements. This is important especially for harbors, coastal areas, and ship passengers.

Samples and other data from the first campaign are currently being analyzed.

Acknowledgement: This study is a part of the project SAARUS 03SX483Z supported by Federal Ministry for Economic Affairs and Energy BMWi (Germany).

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(12) The Nature of Light-absorbing Carbon in Particle Emissions from a Marine Engine operated with Heavy Fuel Oil, Marine Gas Oil or Diesel Fuel

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In addition to Black Carbon (BC) particles, a brown-colored form of carbonaceous particulate matter (PM) absorbs sunlight in the range of UV and visible UV, contributing to Earth's radiative forcing. This so-called brown carbon (brC) (Andrea and Gelencsér, 2006) has been considered as a complex, liquid-like mixture of light-absorbing organic molecules that are volatilizable, miscible with non-light absorbing PM components such as organic aerosol, and soluble in common organic solvents. In previous works, brC has been mainly associated with different types of biomass burning.

Insoluble light-absorbing carbon (LAC) has a substantially different environmental fate to soluble brC. For example, insoluble LAC may accumulate on snow and ice surfaces during the melt, absorbing sunlight and accelerating melt rates. Fundamental differences in physical properties also affect the interpretation of fundamental LAC measurements and treatment in model simulations. Insoluble LAC species include BC as well as the "tarball" brC previously identified by electron microscopy in biomass burning samples, which has fundamentally different properties to both BC and soluble brC.

We investigated the optical properties of LAC in the emissions from a marine engine operated on various loads and three different fuels. As marine engines are usually operated without flue gas cleaning devices, the particulate emission from shipping is considered a severe threat to public health (Corbett *et al.*, 2007). In the past decade, the fuel sulfur content of marine fuels has been stepwise lowered to today's limit of 0.1 % in sulfur emission control areas, incl. Baltic Sea, the North Sea, and North American coastal waters, and a global limit of 0.5 % for other territories. This regulation dismisses the frequent use of heavy fuel oil (HFO), a low-cost fuel originating from blending of the vacuum residue from crude oil distillation with middle distillate and high sulfur content, and either promotes the use of controversially discussed sulfur scrubbers, rare low-sulfur HFO or diesel-like low-sulfur fuels, such as marine gas oil (MGO). The primary motivation for this sulfur limit was to reduce SOx emission and prevent the use of HFO. However, the impact on the PM emission is supposedly small and further impacts on the environment and human health are unknown. Recent studies suggest that emissions of both, residual as well as distillate fuels exhibit a comparable high acute toxicity (Oeder *et al.*, 2015).

Brown Carbon in emissions from a marine engine

In our first study on marine engine emissions, we defined brC operationally by attributing all infrared light absorption to BC, assume an Absorption Ångström Exponent (AAE) value of 1.0, and assigning excess absorption from BC to brC. The AAE value of for a pair of wavelengths λi is calculated by

$$AAE(\lambda_1, \lambda_2) = -\ln \frac{b_{ATN,\lambda_1} / b_{ATN,\lambda_2}}{\lambda_1 / \lambda_2}$$

with bATN being the light attenuation coefficient measured by an aethalometer (Magee model AE33). The wavelength-dependent mass absorption coefficient of organic matter (MACOM) was obtained from the determination of the absorption at 780 nm (babn,λ) by Cavity Attenuation Phase Shift PM single scattering albedo monitor (CAPS PMssa), combining it with AAE from aethalometer and finally dividing by the concentration of organic matter (OM):

$$MAC_{OM,\lambda} = \frac{b_{abn,OM,\lambda}}{c_{OM}}$$

The split between BC and brC was based on the assumption that brC absorption is negligible at a longer wavelength (λ ≥ 780nm).

We calculated AAEs, emission factors (EF) of OM and MACOM at 370 nm for engine emissions from the two marine fuels HFO and MGO as well as EN590 diesel fuel (DF) as reference for fossil diesel with lowest available fuel sulfur content of maximum 10 ppm. It is evident by AAE up to 2.3 that HFO-PM from ship contains significant amounts of brC, in particular at low loads. Ships operate their engine in "slow steaming" mode so that the overall fuel consumption is reduced. Similar to AAE, also the emission factor for OM increases towards lower engine loads, so more brC is emitted. Finally, HFO-OM absorbs orders of magnitude more strongly at 370 nm, illustrated by MACOM, 370 nm, than low sulfur fuels, having increasing EF of OM for low loads as well, but show constantly low UV light absorption overall engine operating conditions.

Another side effect of switching to low-sulfur distillate fuel lies in the content of incombustible metals. Vanadium, which is used to be the most abundant transition metal in HFO, is enriched in

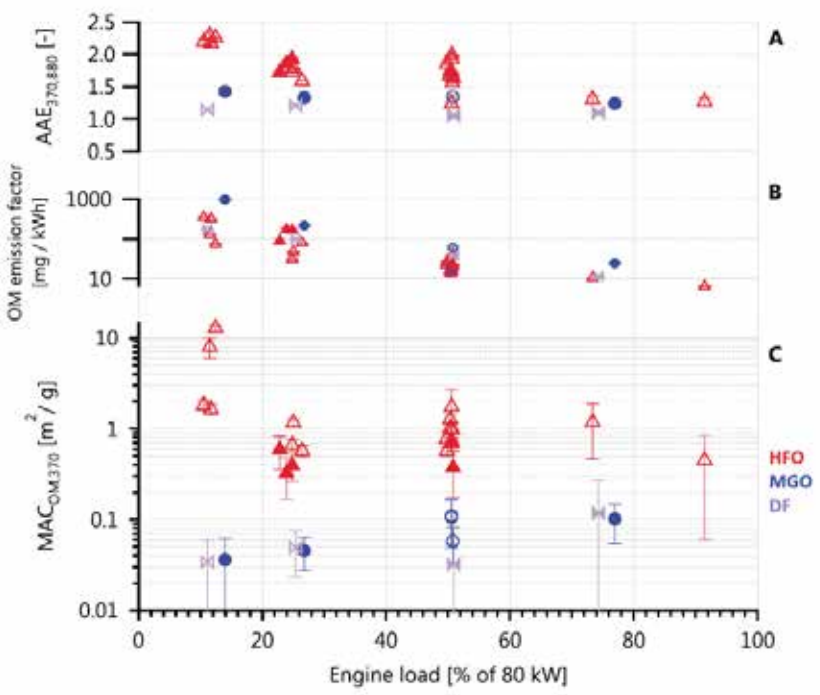


Figure 1: *(A) Angström Absorption Exponent (AAE), (B) emission factors (EF) of organic matter (OM) and (C) mass absorption coefficient (MAC) at 11 %, 25 %, 50 %, 75 % and 91 % engine load (reprinted with permission from Creative Commons Attribution 4.0 International License from Corbin et al. (2018))*

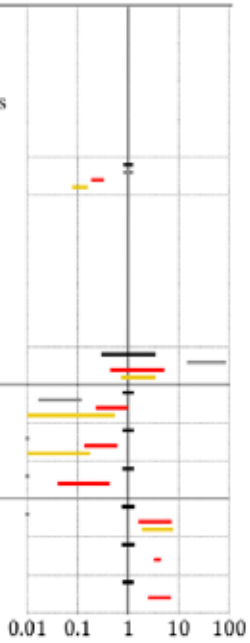
the soot-mode of PM2.5 from HFO combustion. Since diesel-like fuels have an intrinsically lower content of heavy metals, fuel switching could lower oxidative stress in lung tissue associated with ship-derived PM.

Tar brC, an insoluble light-absorbing carbon species in ship particulate matter

We investigated the physical nature of brC in ship PM by combining a variety of analytical techniques. First, we pursue the hypothesis that aromatic hydrocarbons and their derivatives are significantly responsible for the absorption properties. In contrast to diesel-like MGO, HFO-OM consists of substantial amounts of unburned fuel, which is characterized by multiple substituted aromatics with the backbone of polycyclic aromatic hydrocarbons or thiophenes (Czech *et al.*, 2018). TOCA-REMPI-TOFMS refers to a sensitive technique to determine aromatic compounds in PM samples. However, after extraction of the HFO-PM with water, hexane, and toluene, the remaining signals in the REMPI mass spectrum are poor, but AAEs significantly greater than 1.0 maintained. Despite its different origin, we found that this insoluble brC is comparable in nature to previously described tarballs from biomass burning (Li *et al.*, 2019): it is insoluble in water and organic solvents, refractory, and absorbs in the near-infrared, similarly to BC, but has a higher sp3/sp2 carbon bonding ratio and AAE than BC. Hence, we suggest to label this LAC species in ship PM as tar brC. An optical model demonstrated that tar brC is the major light-absorbing species in HFO-PM, in particular at low engine loads, and even to the near-infrared spectral region at approximately 1000 nm. Overall, the optical properties of tar brC in HFOm-PM agree well with brown carbon spheres of unknown origin observed in the East Asian outflow (Alexander *et al.*, 2008). Moreover, the combination of properties we identify for HFO-PM tar brC means that the majority of common analytical techniques for quantifying airborne or in-snow LAC are substantially biased in its presence. Our results call for a refined classification of atmospheric LAC (figure 2) and the re-interpretation of multiple atmospheric-aerosol and snow-darkening-by-LAC studies (Corbin *et al.*, 2019).

Figure 2: Properties are sorted by their degree of overlap between LAC types. The ranges of continuous properties are plotted relative to soot BC (right). The morphology diagrams in columns 2–4 depict aggregated spherules, porous cenospheres, and solid spheres. This table combines literature data with experimental and modeling results from the present work (reprinted with permission from Creative Commons Attribution 4.0 International License. Please see Corbin et al. (2019) for details).

Property	LAC type: Soot BC ●	Char BC ●	Tar brC ●	Soluble brC ●
Solubility[a]	Negligible solubility in common solvents			Soluble
Light absorption	300–1000 nm [detected as eBC at NIR λ]			300–600 nm
Chemical state	Contorted graphene layers		Amorphous	Distinct molecules
Carbon bonding	sp^2 dominated		sp^2 and sp^3	sp^2 and sp^3
Vapourization at[b]	~ 4000 K [EC, rBC]		~ 1000 K [EC]	< 600 K
Produced by	Flame synthesis	Fuel-droplet pyrolysis	Partial pyrolysis	Oxidation, pyrolysis, ...
Morphology				Spheres or coatings
Diameter[c] [μm]	0.02–0.2	1–5	0.03–0.3	0.05–0.2
MAE (370 nm)[d] [m^2/g]	11.1 ± 1.8	0.2–1.2	2.7–9.9	$\ll$0.1–6.0
MAE (550 nm)[d] [m^2/g]	7.5 ± 1.2	0.2–1.3	1.1–4.1	$\ll$0.1–1.2
MAE (880 nm)[d] [m^2/g]	4.7 ± 0.8	0.2–1.5	0.2–1.8	n.a.[e]
AAE (370, 530 nm)[d]	0.8–1.2	-0.3 to -0.1	1.7–6.5	2–7
AAE (370, 950 nm)[d]	0.8–1.2	-0.2 to 0.0	3.5–4.0	n.a.[e]
AAE (880, 950 nm)[d]	0.8–1.1	-0.3 to 0.0	2.5–6	n.a.[e]



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(13) Carbonaceous aerosol composition in air masses influenced by large-scale biomass burning: A case-study in Northwestern Vietnam

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Biomass Burning (BB) is among the important sources emitting trace gases and particles into the atmosphere on both global and regional scales (Elsasser *et al.*, 2012). After primary emission (POA), smoke plumes undergo complex physical and photochemical processes during transport, which results in the formation of secondary organic aerosol (SOA). Biomass burning aerosols contain large quantities of genotoxic compounds such as polycyclic aromatic hydrocarbons (PAH) or oxidized PAHs (oxy-PAH) (Kim *et al.*, 2018; Ihantola *et al.*, 2020). Northern Southeast Asia is well known for intense and recurring BB emissions from forest wildfires and post-harvest crop residue burning during the pre-monsoon season from February to April, influencing the large-scale atmospheric circulation and composition in Northern Southeast Asia (Lin *et al.*, 2013). We investigated the aerosol composition at a remote site Pha Din in Northern Vietnam, a place where data availability is generally scarce, and show that BB is the dominating aerosol contributor.

Aerosol chemical speciation, trace gas analysis, and backward trajectory modelling

A field campaign was performed at Pha Din remote mountainous station during the biomass burning season from late March to early April in 2015. PM2.5 was collected on 47 mm-quartz fiber filters by MiniVol TAS samplers (Airmetrics, USA) with a 24 h sampling protocol from 23rd March to 12th April 2015. Air mass backward trajectory (BWT) analysis combined with BB locations as retrieved from the Moderate Resolution Imaging Spectroradiometers (MODIS) from the Terra and Aqua platform (MODIS Collection 6 Hotspot/ Active Fire Detections MCD14DL). computed with the FLEXTRA trajectory model driven by 3-hourly meteorological analysis fields (1° x 1° resolution) of the operational Integrated Forecast System (IFS) of the European Center for Medium-range Weather Forecast (ECMWF). Additionally, the atmospheric trace gases CO, CO_2, and O3 as well as meteorological conditions were monitored throughout the sampling period.

Filter samples were analyzed by using in-situ derivatization thermal desorption gas chromatography time-of-flight mass spectrometry (IDTD-GC TOFMS) (Orasche *et al.*, 2011). Organic and elemental carbon fractions (OC and EC) were obtained by thermal-optical carbon analysis (Improve A protocol) with a DRI model 2001 carbon analyzer.

Connecting organic and elemental carbon to biomass burning: a levoglucosan and soot-based approach

During the sampling campaign, the concentrations of OC and EC were constantly elevated and peaked on 5th and 6th April before ambient aerosol was scavenged on 7th April by rainfall. Levoglucosan, a well-established marker for BB originating from the pyrolysis of cellulose, correlated with both OC and EC (Pearson' r > 0.97) (figure 1a). Furthermore, the char- vs. soot-EC diagram shows that higher EC concentrations contain higher amounts of char-EC, which is typical for BB-related EC (Han *et al.*, 2007).

Classification of sampling days by biomass burning influence: a statistical approach

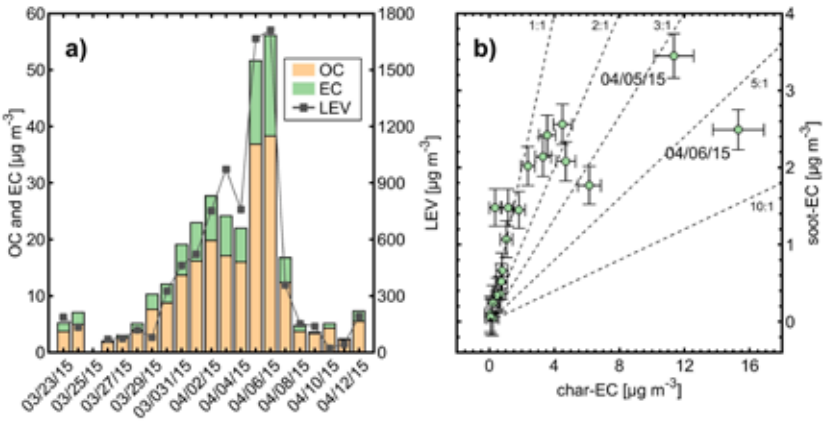

Figure 1: *a) Concentrations of organic (OC) and elemental carbon (EC) and correlation with levoglucosan (LEV). b) Char-EC and soot-EC, linked- to BB and engine-related EC, with measurement uncertainty (reprinted with CC BY 4.0 License from Nguyen et al. (2020)).*

We grouped the targets of the IDTD-GC TOFMS analysis in the compound classes anhydrous sugars, resin/lignin compounds, n-alkanes, fatty acids, PAH, oxy-PAH, and nitrophenols, and performed a combined hierarchical cluster analysis on sampling days and compound classes. The resulting clustergram revealed three distinct clusters of low (beginning and end of sampling period), medium (middle of sampling period), and high influence of BB (5th and 6th April). Anhydrosugars and methoxyphenols, pyrolysis products of lignin, appear closely together with OC and to a lesser extent with fatty acids and alkanes, indicating a substantial contribution of BB to the organic aerosol level. PAH, o-PAH and

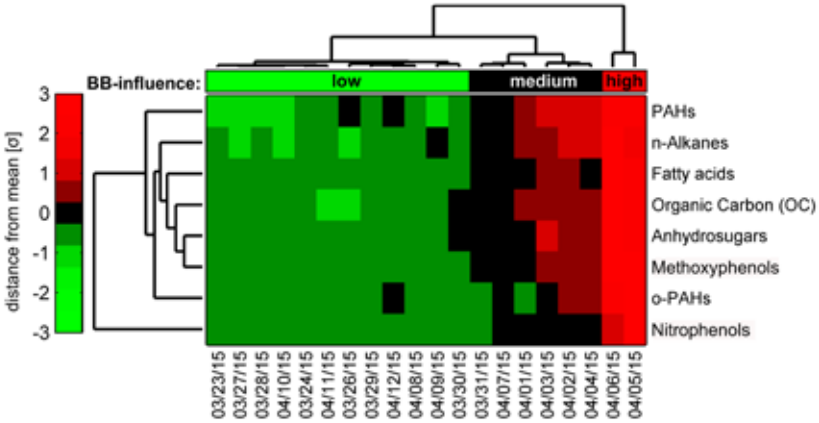

Figure 2: *Clustergram consisting of two dendrograms for compound classes (left) and sampling days (top) together with a heatmap displaying the distance from campaign mean expressed in multiple of the standard deviation σ (reprinted with CC BY 4.0 License from Nguyen et al. (2020))*

nitrophenols seem to be released by additional sources, such as road traffic (for PAH and o-PAH) and atmospheric aging (o-PAH and nitrophenols).

Insights into the aerosol composition by molecular marker and indices

The high level of chemical speciation from analysis by IDTD-GCTOFMS enables increasing confidence in the apportionment of aerosol sources. In addition to levoglucosan, n-alkanes and methoxyphenols may give insights into the type of BB. N-alkanes from C20 to C33 with a strong dominance of odd-numbered carbon chain lengths are emitted by epicuticular plant wax. The carbon preference index (CPI) account for the ratio of alkanes with odd to even carbon numbers, increases during medium- and high-BB days, and correlates with the observed concentrations of levoglucosan. Methoxyphenols refer to monomers of the lignin backbone of woody biomass, having different composition in gramineae, angiosperms, and gymnosperns. The highest relative abudnances of hydroxybenzoic acids and syringol derivatives point toward burning of gramineae, *e.g.* from burning of agricultural residues, and angiosperms, *e.g.* hardwood. Despite release during BB, nitrophenols are substantially formed from aromatic VOCs by atmospheric aging and rather considered as an early secondary compound from BB. Significant levels of nitrophenols were observed during the high-BB days, indicating short-term atmospheric aging.

Type of biomass burning and plume age estimation by backward trajectories and trace gas analyses

A common metric to assess the type of is provided by the modified combustion efficiency MCE = $\Delta CO_2 / (\Delta CO_2 + \Delta CO)$. On days with high-BB influence, hourly MCE dropped down to 0.84, appearing in the upper range reported for wildfires. From the excess $\Delta O3/\Delta CO$ ratio, the plume age on the high BB-influenced days was estimated to less than two days (Jaffe and Widger, 2012). Although this ratio is very sensitive to the method for background level determination, it agrees well with the BWT analysis showing that air masses crossed fire areas less than 36 hours before arriving the sampling site.

Conclusion

We characterized a smoke plume arrival at a remote mountainous site in Northern Vietnam. Individual approaches for aerosol source apportionment unambiguously linked increased levels of PM2.5 to BB from agricultural and wildfires and agrees well with the finding from Bukowicki *et al.* (2019), who based source apportionment of optical aerosol properties. Our chemical speciation of organic PM2.5 constituents add valuable data for a region with scarce data availability and may help to understand the impact of BB aerosol exposure on the Vietnamese population.

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(14) Is the aerosol particle deposition dose in an Air Liquid Interface cell exposure facility comparable to the human lungs?

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Background

Air Liquid Interface (ALI) devices are used to test the toxicity of aerosols. Here a layer of human lung cells is exposed to an aerosol and changes in the cell properties are determined (figure 1). ALI exposure experiments are relatively simple to perform compared to animal studies.

The relation between the toxicity measured with a single cell line and the toxicity in the human lung is an important question. One parameter is the mass or number of particles deposited on the surface of the cells either in the ALI or in the lung. The aim of this study is to compare the particle deposition in the ALI (Vitrocell® 6 well set-up, 100 mL/min flow, (Oeder *et al.*, 2015)) with the particle deposition in the human lungs to determine conversion factors (Karg and Ferron, 2012).

Modeling the Deposition

The ALI deposition by Comouth *et al.* (2013) uses an equation approximating measured experimental data for the size range from 29 nm to 2 µm. Deposition in the human airways is calculated with the Hygroscopic Particle Lung Deposition (HPLD) model developed at the HMGU (Ferron *et al.* 2013, Karg and Ferron, 2012). This model uses a lung structure model of the nose or mouth, trachea and bronchi up to the alveolar ducts, particle deposition equations for diffusion, sedimentation and impaction, and an algorithm to calculate the deposition in lung regions and the total lungs. Respiration is modeled for a sitting male subject and mouth breathing (Karg *et al.*, 2020).

Deposition Modeling Results

Figure 2 shows the deposition being strongly dependent on particle size for both the ALI and lung regions. A minimum is found at a diameter of about 0.4 µm. Values for the deposition at the minimum are 0.0010 for the ALI, 0.0016 for the mouth, 0.03 for the tracheobronchial (TB) region, 0.09 for the alveolar (AL) region, and 0.12 for the total lungs (TL). Note the steeper slope of the deposition curve of the ALI with respect to the lung deposition curves. Albeit the deposition efficiency in the range of 100 nm to 1µm in the ALI system is relatively low (figure 2) we need to take the cell surface area of the ALI unit with respect to the lung surface into account (Table 1) in order to estimate the relative doses measures as "deposition per cell surface area".

Deposition per Cell Surface Area as a Comparison Parameter

The ratio of the particle number deposition per cell surface area in a lung region and in the ALI is shown in figure 3 as a function of particle diameter. The surface areas of lung regions and ALI are listed in Table 1. The maximum of the ratio for the deposition per cell surface area of ET and ALI is about 0.09, of TB and ALI is 0.08, of AL and ALI is 0.0009 and of AL and ALI is 0.0012. Particle size ranges between 40 and 450 nm are identified (and marked by horizontal bars in figure 3), where this ratio varies by a factor of less than two. In this size region, which is assumed to be important for particle-associated health effects, the deposited particle numbers are ca. by a factor 1000 larger in the ALI than in the human lung. Outside this range, the deposition in the ALI is much larger than in a lung region and strongly depends on particle size. This information is important for conditioning the exposure aerosol (*e.g.* precipitation of the larger particles by a pre-impactor) and calculating the exposure dose. As in most cases, short-term exposures (1-4 h) and diluted aerosols are tested, the found deposition efficiency is very reasonable for aerosol toxicological studies.

Region i	***Surface area A_i***
Air Liquid Interface Device ALI (6-well format)	4.7 cm²
Human Extrathoracic region ET	89.5 cm²
Human Tracheo-Bronchial region TB	0.33 m²
Human Alveolar region AL	74.7 m²
Human Total Lungs TL	75 m²

Table 1: *Surface area of the cell layer at the ALI, and of lung regions and the total lungs.*

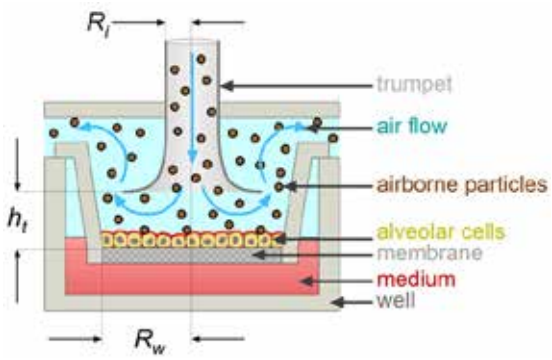

Figure 1: *Simplified scheme of an Air Liquid interface ALI exposure setup (with aerosol inlet and trumpet; insert with membrane and cell layer, medium, aerosol outlet).*

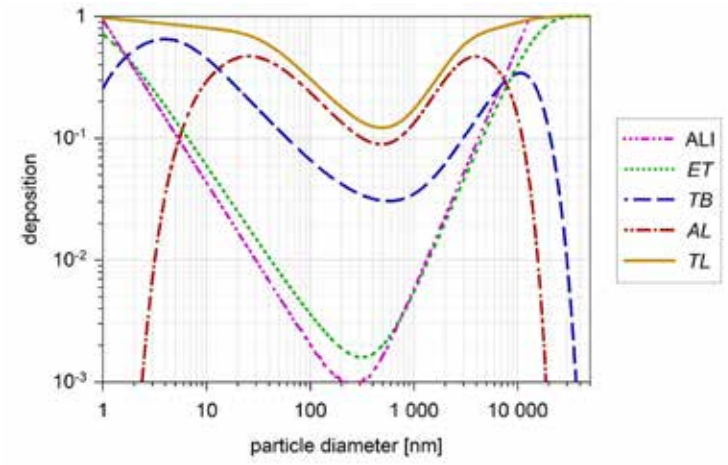

Figure 2: *Deposition in the ALI and in the extrathoracic (ET), tracheobronchial (TB), alveolar (AL) regions, and the total lungs (TL). Note the similar slope for the curves of ALI and ET region and the different slopes for ALI and TB, AL and TL. Deposition in the ALI is calculated with an equation based on experimental data in the size range of 29 to 2000 nm (Comouth et al., 2013). Deposition in lung regions and the total lungs are calculated with the HPLD model (Ferron et al., 2013). Values of the deposition below 5 nm are questionable, since these particles may have gas-like properties.*

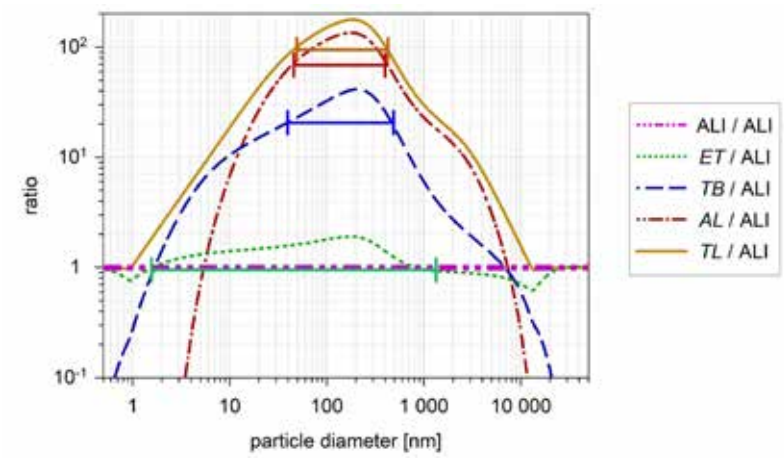

Figure 3: *Ratio of the deposition per cell surface area in a lung region and in the ALI. Horizontal bars show the particle size range between 40 and 450 nm, where the ratio differs less than a factor of two from the maximum value. Then the mean ratio for TB and ALI has a value of about 0.06, AL and ALI a value of 0.0006, and TL and ALI a value of 0.0009. The difference is caused by the larger area of the alveolar region compared to the ALI.*

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3.2 Selected Research Projects (Topic 2: Enabling Analytical Technologies)

(01) A new mass spectrometric approach for direct analysis of toxic environmental pollutants in water

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Introduction

Adverse effects of (polycyclic) aromatic hydrocarbons ((P)AHs) on aquatic flora and fauna as well as on human health are well known, and thus make their determination in aquatic environments indispensable (Bai *et al.*, 2014; Manzetti, 2013). Very low concentrations, however, render their analytical detection and monitoring difficult. In general, extensive enrichment steps are required to obtain the desired information from the corresponding water samples (Witt, 2002). Therefore, the development of new analytical techniques for the fast and reliable determination of (P)AHs in the aqueous matrices is of crucial importance. A promising approach is the direct analysis of water by means of membrane-inlet mass spectrometry (MIMS) in combination with resonance-enhanced multiphoton ionization (REMPI) (Oser *et al.*, 2007; Soni *et al.* 1998).

In MIMS, compounds are transported selectively through a semipermeable membrane into the vacuum region of the mass spectrometer. The most common membranes used today are made of silicone and are particularly selective for non-polar compounds due to their lipophilic character.

In REMPI, molecules are ionized by the sequential absorption of at least two photons via real intermediate states. The ionization efficiency of REMPI depends on the spectroscopic properties of the individual compounds (Gehm *et al.* 2018). When using intense UV radiation, REMPI allows the efficient and selective ionization of (P)AHs in complex gas mixtures. In combination with time-of-flight mass spectrometers (TOFMS). REMPI is known as one of the most sensitive methods for the real-time analysis of (P)AHs in the gas phase.

Experimental Design

For the direct and fast analysis of AHs in environmental waters two different external membrane inlet devices were developed, utilizing sheet as well as hollow fiber membranes (figure 1) (Gehm *et al.*, 2019, 2020).

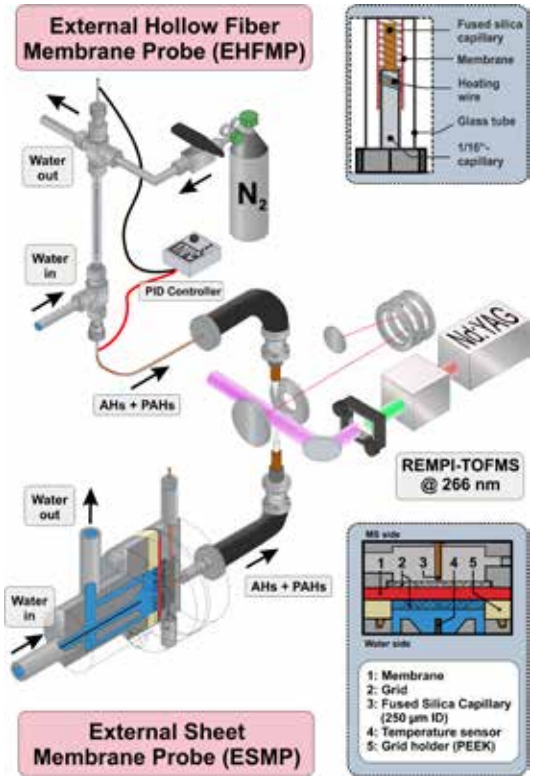

Figure 1: *Overview of the developed membrane inlets for direct and fast analysis of aromatic hydrocarbons (AH) in water by resonance-enhanced multiphoton ionization (REMPI) coupled to time-of-flight mass spectrometry (TOFMS).*

The setup of the external hollow fiber membrane probe consists of a hollow fiber membrane with a thickness of 250 µm, which is fitted with both ends on stainless steel capillaries. The membrane is placed inside of a 6 mm glass tube, which is connected to the water stream via Swagelok connectors. The inner side of the membrane is supported by a thin nickel wire, used to additionally heat the membrane's inner surface.

The external sheet membrane probe consists of a thin sheet membrane (150 µm) placed between two stainless steel flanges. The MS-side as well as the waterside of the membrane is supported by stainless steel grids. Thermalized water (80 °C) is guided orthogonally to the center of the membrane, which leads to increasing pervaporation rates through the membrane. The

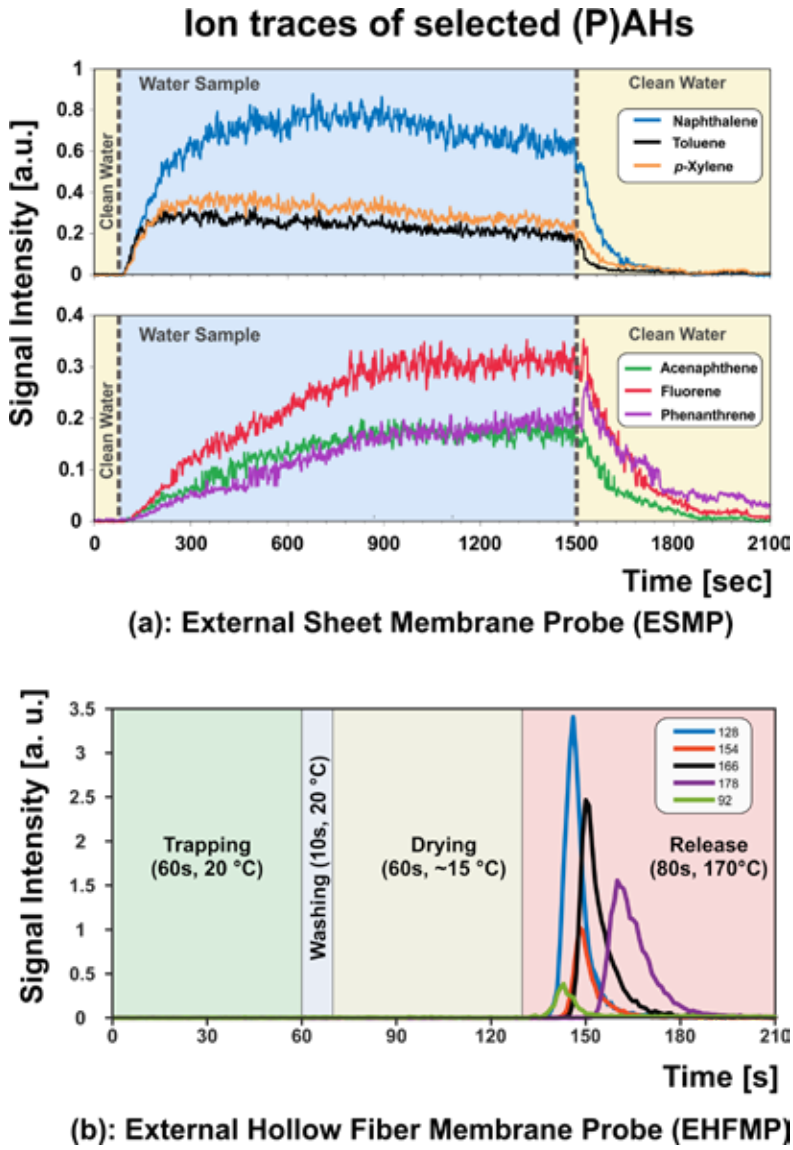

Figure 2: Ion traces of selected (polycyclic) aromatic hydrocarbons ((P)AH) obtained by: (a) external sheet membrane probe. Fast response can be observed for volatile (P)AHs, whereas response times are considerably longer for higher boiling point compounds.; (b) external hollow fiber membrane probe using T&R at a trapping time of one minute. In order to improve the performance and repeatability of T&R, a washing and drying step was subsequently applied after trapping.

MS-side of the ESMP can be differentially pumped by a diaphragm pump down to 300 mbar.

In both setups, the pervaporating molecules are transferred into the ion source of the TOFMS via heated fused silica capillaries (inner diameter = 250 µm) placed in close vicinity to the membrane. The subsequent ionization is performed by UV-laser pulses with a wavelength of 266 nm at a repetition rate of 10 Hz.

Results

With both systems, measurements of low concentrations of selected (P)AHs in different simulated (tap water, artificial seawater) and real-world water samples were performed without any sample preparation. An excerpt of initial laboratory experiments using spiked tap water samples is presented in figure 2.

For the sheet membrane assembly (figure 2a), the membrane is heated indirectly by tempering the inflowing water. With increasing water temperature improvement of response times and signal heights can be achieved. For volatile compounds, response times from 30 seconds up to 2.5 min were measured, while semi-volatile substances show increasing response times up to 15 min. Limits of detection (LOD) in the range of 10-60 ng/L^{-1} for selected (P)AHs were determined. For the hollow fiber membrane setup, the trap-and-release technique (T&R) can be utilized for direct determination of the selected (P)AHs in water samples down to ng/L-range in less than 5 minutes (figure 2b). Here, the pervaporating compounds are trapped inside the membrane tube for one minute and are released as a sharp peak into the MS by fast heating of the membrane up to temperatures of 150-250 °C. The application of T&R enables fast and sensitive detection of higher boiling point compounds (figure 2b). In addition, the analysis time is reduced for these compounds and improved signal-to-noise ratios (S/N) were achieved. For EHFMP, LODs were estimated based on S/N=3 and are in the range of 6-50 ng/L for 1 min trapping time. The performance of T&R-EHFMP can be additionally improved by using longer trapping times.

With the developed systems, detection of AHs in environmental waters is accessible without any sample pre-treatment. Figure 3 shows ion traces of selected AHs for both inlet designs as well as the corresponding mass spectrum either averaged over 100 s (ESMP) or assembled based on maximum signal intensity over the desorption period (EHFMP). For this purpose, water from the Warnow river estuary was collected and measured using the developed setups. Besides

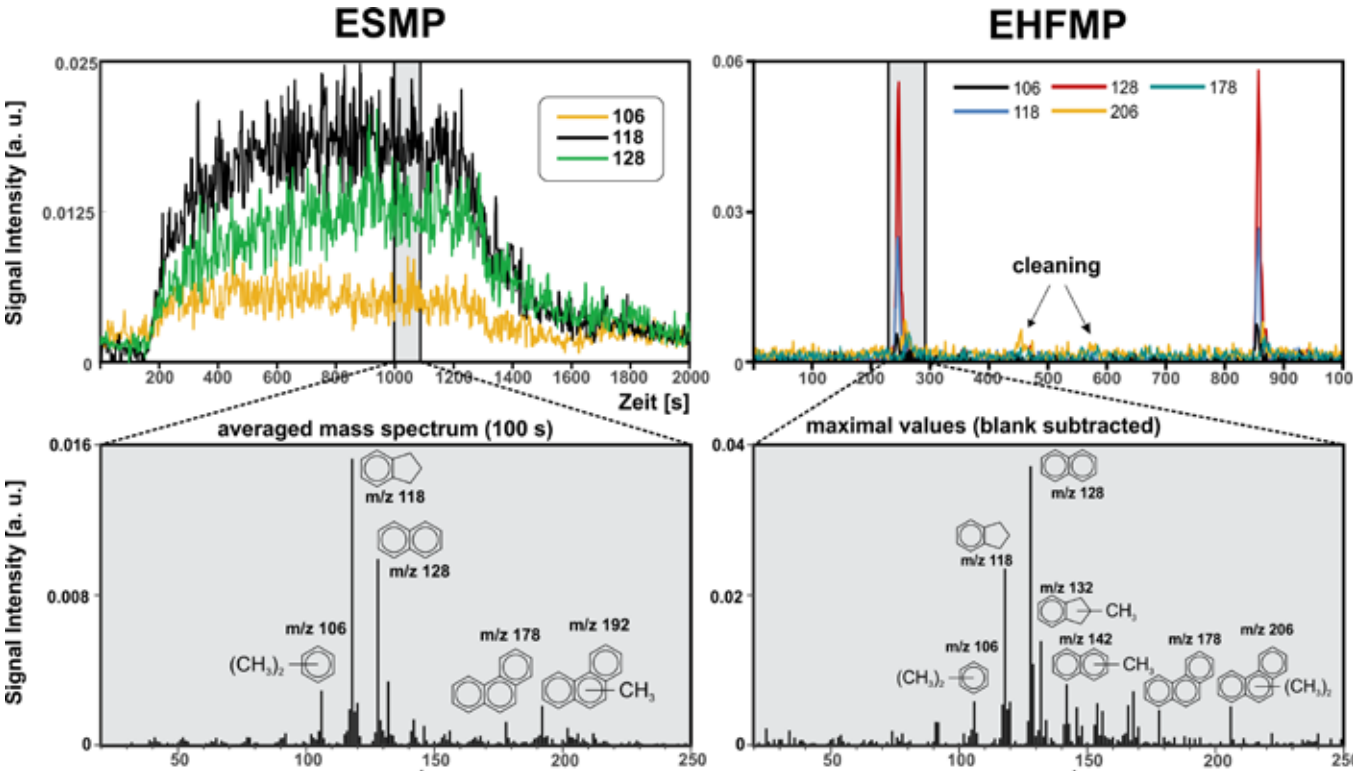

Figure 3: Selected ion traces of aromatic hydrocarbons (AH) found in environmental water (Warnow estuary) and the corresponding mass spectra obtained by external sheet membrane probe (ESMP, left) and one-minute trap-andrelease (T&R) provided by external hollow fiber membrane probe (EHFMP, right). With both inlet design similar mass spectra can be recorded, whereas higher molecular weight compounds show more intense signals if T&R-EHFMP is applied. Between each T&R-measurement, the membrane was cleaned by heating.

indane and xylene, PAHs such as naphthalene, acenaphthene, fluorene, phenanthrene, and their alkylated derivatives were detected in the water as well. Parallel to the ESMP-REMPI-TOFMS measurements, these samples were additionally characterized by GC-MS. In a direct comparison of the determined concentrations, an excellent agreement between REMPI-MIMS (121 ng/L^{-1}) and GC-MS (115 ng/L^{-1}) was found for naphthalene.

Summary and Outlook

In the framework of a DFG-funded project, membrane-inlets for laser mass spectrometry were developed, which utilize sheet as well as hollow fiber membranes. Both inlet designs offer the possibility of fast, direct, and powerful detection of (P)AHs in water on a minute time-base, which enables first steps towards a sensitive real-time analysis of (P)AHs in marine environments. With the sheet membrane setup, monitoring of volatile AHs dissolved in water can be performed continuously, whereas with hollow fiber design sensitive detection of semi-volatile (P)AHs can be achieved by utilizing the T&R-approach. However, the developed prototypes still offer the potential for further methodological optimization. This includes, for example, the use of laser systems with higher repetition rates as well as different laser wavelengths. The focus of future work lies in the development of a robust and versatile analysis system that can be placed on marine research vessels for continuous on-board measurements of seawater. In particular interesting is the investigation of water pollution by marine technology or ships. This includes, among others, oil spills or discharge of (P)AH containing wastewater from ship engine flue gas cleaning devices (scrubber). A further application may be the detection of anthropogenic pollutants and marker compounds of thawing permafrost regions in river water.

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(02) Atmospheric pressure photo and laser ionization ultrahigh resolution mass spectrometry for complex mixture analysis

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Introduction and Motivation

Ionization of the analytes is one of the most critical aspects of mass spectrometry. Each ionization technique covers a specific compositional space and by applying a certain technique, particular chemical classes can be highlighted, reduced, or even completely neglected. Selective ionization techniques can reduce matrix effects, commonly observed in atmospheric pressure ionization schemes, even without additional sample pre-treatment. Thus, the development of novel ionization schemes is of high analytical interest. In this respect, atmospheric pressure photoionization (APPI) was shown to cover a broad compositional space with rather low matrix effects. Technically, APPI is realized by using the VUV-radiation of an electrodeless microwave discharge lamp filled with a noble gas, most often Krypton (Kr-APPI, 10.6 and 10 eV / 117 and 124 nm) (Syage *et al.* 2000; Kauppila *et al.*, 2014). Note, that the photoexcitation under ambient pressure cause photoionization products (i.e., radical cations) as well as photochemically induced chemical ionization products (i.e., quasi molecular- and adduct-ions). In this report, the further development of APPI by applying other wavelengths, then commonly available, as well as the introduction of a new laser-based ionization technique, atmospheric pressure single-photon laser ionization (APSPLI), is shown. The expansion of the assessable chemical space and the control of matrix effects in atmospheric pressure ionization is needed for the characterization of ultra-complex petrochemical samples and fuels as well as the comprehensive characterization of the ambient particular matter and secondary aerosols (SOA) in the framework of aerosol and health research.

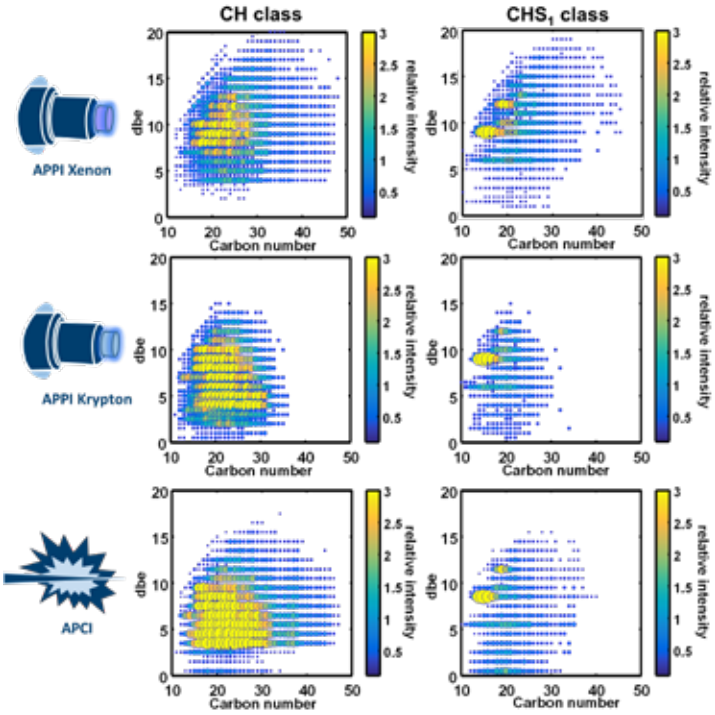

Figure 1: *Double bond equivalent plots for the CH- and CHS1 compound class of a light crude oil analyzed with APCI-, Kr-APPI and Xe-APPI FT-ICR MS. The sample material is introduced by thermal analysis coupling and temperature-averaged data are depicted. Data is color-coded with the relative abundance.*

Results and Discussion

For direct single photoionization generating radical cations $[M]^{\bullet+}$, the ionization energy of a molecule has to be below the photon energy of the light source. Here, the common approach of APPI was adapted by the installation of a microwave discharge lamp filled with Xenon (Xe-APPI), emitting radiation with less energy/ higher wavelength (8.4 and 9.6 eV / 148 and 129 nm). The ionization energy of polycyclic aromatic hydrocarbons (PAHs) was found to be below the direct photoionization limit of the Xenon lamp. The ionization behavior of this adapted setup is evaluated by the exemplary investigation of a light crude oil. The results of the APPI experiments are directly compared to atmospheric pressure chemical ionization (APCI) conducted under the same conditions.

All three ionization techniques (Kr-APPI, Xe-APPI, and APCI) were able to ionize compounds from the CH-, O1-, S1- and N1-class. Comparison of the techniques revealed, that Kr-APPI ionizes CH-class species with higher relative abundance compared to Xe-APPI. Most interestingly, APCI primarily generates protonated ions $[M+H]^+$, whereas Kr-APPI produces both radical $[M]^{\bullet+}$ and protonated molecular ions $[M+H]^+$, and Xe-APPI predominantly results in radical ions $[M]^{\bullet+}$ (Rüger *et al.* 2018). Consequently, the double bond equivalent (DBE), a measure for aromaticity, was found to be half-integer (even electron configuration) for APCI, mixed (half-integer/integer, mixed electron configuration) for Kr-APPI, and integer (odd electron configuration) for Xe-APPI. Another promising effect is depicted in figure 1. Xe-APPI is predominantly able to ionize aromatic compounds with DBE values greater or equal to 4. Both techniques, Xe-APPI and APCI, covered different DBE ranges. Xe-APPI revealed constituents with a DBE up to 19 (*e.g.*, Coronene, $C_{24}H_{12}$) not detected with classical Kr-APPI or APCI. Thus, Xe-APPI could be identified as a valuable ionization approach for the selective ionization and detection of aromatic constituents from complex mixtures, technically realized by a cheap and robust electrodeless discharge lamp setup.

Plasma discharge lamps emit radiation with high divergence and, thus, photon densities are comparatively low. Consequently, the reported limit of detection for APPI approaches was found to be rather high. Due to the high photon density, laser-based approaches are able to overcome this limitation. Atmospheric pressure laser ionization (APLI) is technically realized by UV-radiation deploying solid-state, *e.g.*, 4th harmonic of an Nd:YAG (266 nm) or Excimer laser, *e.g.*, KrF (248 nm), radiation. APLI is therefore based on a multiphoton ionization mechanism and selectively ionizes mostly aromatic compounds (Schmidt *et al.*, 1999; Kersten, *et al.*, 2011). We aimed to combine the advantages of both techniques—APPI covering a broad chemical space and APLI resulting in low detection limits—by introducing atmospheric pressure single-photon ionization (APSPLI). Technically, we realized APSPLI by the installation of a fluorine excimer laser

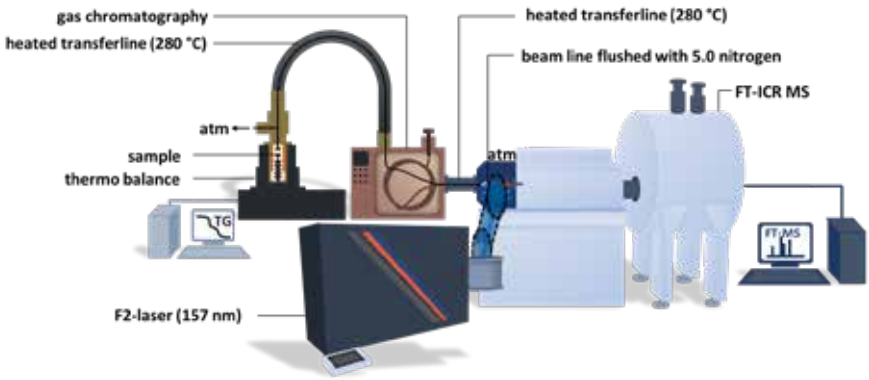

Figure 2: Scheme of the thermal analysis and gas chromatographic FTICR MS setup equipped with APSPLI as ionization technique, carried out by a fluorine excimer laser emitting photons of 157 nm (7.9 eV) (Rüger et al., 2020).

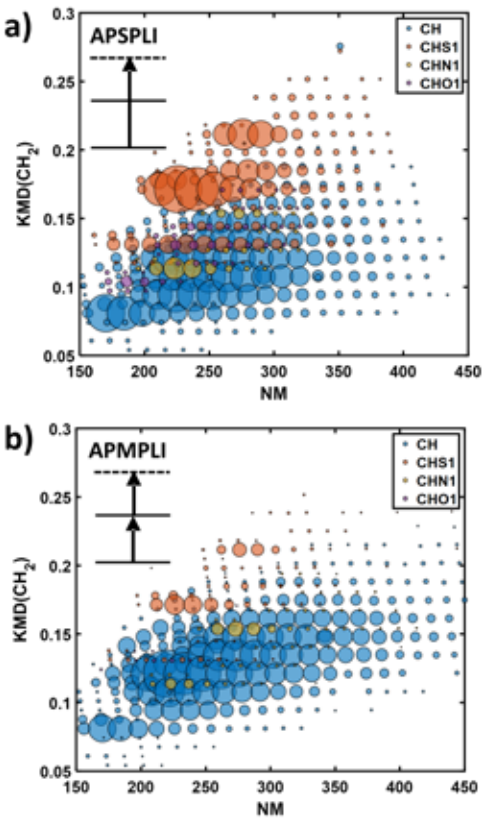

Figure 3: CH_2-based Kendrick mass defect (KMD) diagram of a light crude oil (CPC Blend, Kazakhstan) subjected to the ionization source by thermal analysis coupled to-FT-ICR MS: a) Deploying APSPLI (157 nm) and b) atmospheric pressure multiphoton laser ionization (APMPLI, 266 nm). The nominal mass (NM) is depicted at the abscissa. Elemental composition attribution is color-coded according to the attributed compound class and size coded according to the summed abundance (Rüger et al., 2020).

emitting radiation with an energy of 7.9 eV (157 nm). The novel ionization technique is evaluated by PAH standard mixtures as well as a light crude oil, serving as a complex matrix.

We found that the amount of oxygen in the atmospheric pressure ionization volume is critical. UV radiation with wavelengths below 240 nm causes photodissociation of oxygen. The installation of oxygen and water filter cartridges was necessary to reduce the abundance of oxidized ionization artifacts. This development step leads to spectra with the radical cation the base peak and ionization artifacts below 10 %. The radical cation is hypothesized to be formed by a direct single-photon ionization mechanism and, thus, species with higher ionization energy, such as naphthalene (8.1 eV), could not be detected. Beneficially, PAHs with lower ionization energy could be detected with a high signal to noise and low detection limits. For the investigation of the complex matrix with this novel ionization approach, we found that APSPLI is able to efficiently ionize CHS-class constituents. In comparison to APLI (266 nm), we could conclude, that APSPLI is advantageous for unraveling the molecular pattern of N-, O-, and S-containing species (Rüger *et al.*, 2020).

Conclusion and Outlook

Atmospheric pressure photo and laser ionization schemes revealed great potential for novel developments further expanding the capabilities of high-performance mass spectrometry platforms. Adaptation and variation in the photon wavelength utilized for APPI revealed great potential in the field of enabling technologies. In this respect, we could show that Xe-APPI selectively targets aromatic constituents, enabling to detect high-aromatic species even within highly complex organic mixtures. Moreover, generating purely radical ions will lower the spectral complexity and, thus, simplify elemental composition attribution. Installation of VUV excimer laser technology to an atmospheric pressure ionization volume allowed us to introduce a novel ionization approach, APSPLI. This technique generates radical cations with high sensitivity by a single-photon mechanism. Most importantly. APSPLI is able to highlight heteroatom-containing aromatic constituents and covers therefore a broader chemical and compositional space.

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(03) Real-Time Speciation of Polycyclic Aromatic Hydrocarbons and Inorganic Compounds in Aerosols on a Single-Particle Basis

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous and toxic organic trace compounds originating from natural and anthropogenic combustion processes. Bound on airborne particles, they are transported over long distances. Because of their well-documented carcinogenicity and mutagenicity, they play a key role in health effects from air pollution (Agudelo-Castañeda *et al.*, 2017). Observations on the impact of particular sources, the distribution pathways, and the degradation processes are crucial for risk assessment. However, a large part of this information is lost in conventional PAH-measurements by mixing, averaging, and degradation on filters prior to time-consuming laboratory analyses (Nozière *et al.*, 2015). Studies on PAHs in aerosols, their monitoring, and risk management would greatly benefit from online detection of PAHs along with analysis of the carrying particles to identify the source.

Among the aerosol mass spectrometers that can obtain chemical information in real-time (Laskin *et al.*, 2018), single-particle mass spectrometry (SPMS) stands out for characterizing individual particles (Passig and Zimmermann, 2020), see figure 1 for the working principle.

Individual particles are exposed to a laser pulse that desorbs and ionizes material from the particle (laser desorption/ionization, LDI), see figure 2(a). The ions are extracted in a dual-polarity mass analyzer, revealing mostly inorganic particle compounds, such as metals, salts, elemental carbon, etc.

However, molecules are typically fragmented by the intense LDI laser pulse. This problem can be circumvented by a two-step scheme, where an IR laser pulse heats the particle producing a gaseous plume of the organic molecules prior to the UV ionization pulse (figure 2b). The second pulse can resonantly ionize the PAHs, yielding single-particle PAH-spectra, as shown in our joint experiments with the Weizmann Institute of Science in the framework of the Helmholtz International Lab aeroHEALTH focusing on "tarball" aerosols(Li *et al.*, 2019). The important disadvantage of this LD-REMPI approach is that the inorganic particle composition, and thus information on the particle source and toxic elements, such as iron or lead is lost. Our group invented techniques to overcome this limitation. In a first approach, successive LD-REMPI and LDI were capable to detect both PAHs and inorganic ions from the same particles for the first time

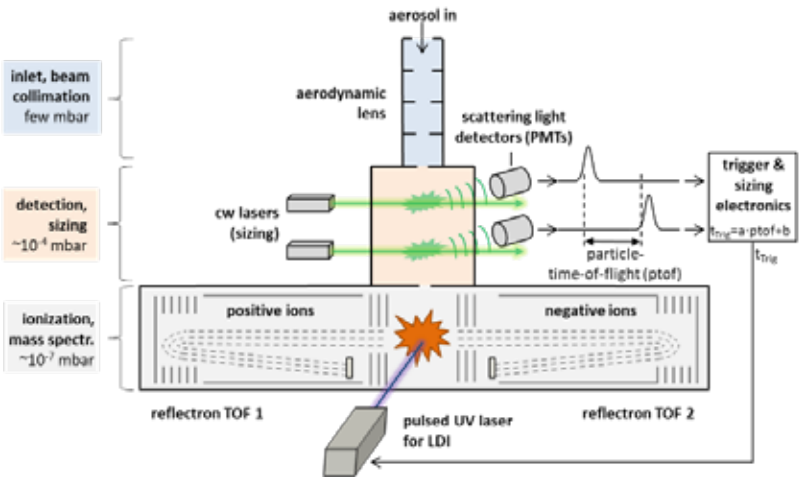

Figure 1: Working principle of conventional SPMS: Particles are introduced into vacuum and optically detected and sized. A UV laser pulse is triggered when the particle enters the ion source of a mass spectrometer, producing positive and negative ions via laser desorption/ ionization (LDI).

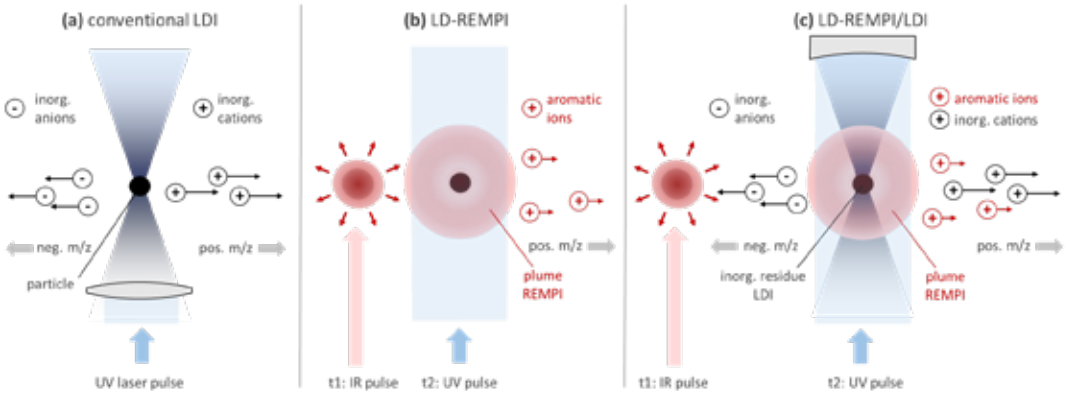

Figure 2: Laser ionization schemes. (a) Conventional laser desorption/ionization (LDI) producing positive and negative ions from inorganics and fragments. (b) Two-step LD-REMPI process, yielding only molecular ions from PAHs. (c) Novel two-step approach that produces PAH ions as well as positive and negative ions from LDI in a spatially shaped ionization laser pulse.

(Passig *et al.*, 2017). However, this method is very complex and addresses only a part of the inorganic composition because only positive ions can be detected. Recently, we could solve these problems by developing a new, robust, and field-deployable method (Schade *et al.*, 2019). A spatially formed laser pulse profile is utilized to ionize PAHs after LD. The refractory particle residue is hit by a more intense part of the beam, thus inducing LDI and REMPI within a single laser shot, see figure 2(c). To measure the low ion signal of PAHs along with the much more intense inorganic ions, the mass analyzer's transmission is modulated to achieve sufficient sensitivity and dynamic range. Figure 3 shows examples of two individual particles.

sources in Europe. Several analysis strategies for this new kind of data structure are currently under evaluation, based on adaptive resonance theory (ART-2a) neural networks. The study will provide new insights into the sources, distribution and photochemical degradation of PAHs in Europe and the manuscript for this unique experiment is under preparation. The method was also used to measure the single-particle composition of ship engine emissions in the framework of the SAARUS project, see Third Party Projects (17). Different ship fuels were used to analyze the chemical composition of the resulting emissions, see Research Area 1 (05) for the background. As our method acquires PAH profiles, it can help to better estimate health risks from ship

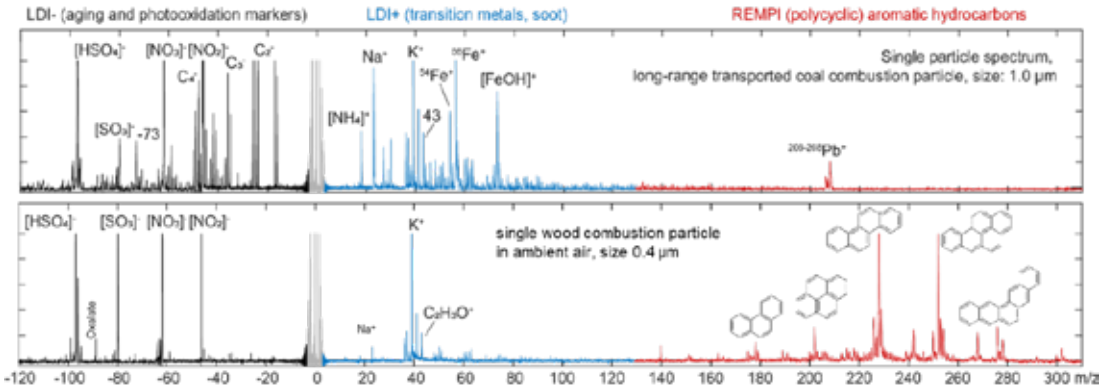

Figure 3: *Exemplary mass spectra from two individual particles (top) If PAHs are absent, the technique yields bipolar mass spectra similar to conventional, LDI-based SPMS. (bottom) For PAH-containing particles, both inorganic ions, as well as a full-fledged PAH-spectrum is recorded, containing information on the particle's origin and aging processes during atmospheric transport (Schade et al., 2019).*

The new method was patented and is now marketed by the HMGU spin-off Photonion GmbH. Several application experiments with the new approach have already been performed. In an extended measurement campaign at the Swedish west coast in autumn 2019 (see Research Area 1 (05)), the first set of single-particle data on PAHs combined with information on the particle class and origin as derived from the inorganic composition was recorded. The dataset contains individual chemical information from more than 20.000 PAH-containing particles from various

emissions. Moreover, the PAH patterns can act as molecular markers indicating the particle source The unprecedented single-particle sensitivity of our approach can therefore open new avenues for source apportionment and environmental monitoring. Figure 4 shows exemplary single-particle mass spectra from a diesel car and a ship engine running on marine gas oil. The inorganic composition revealed from LDI (black and blue) is not source-specific with respect to different diesel engines. However, the PAH-pattern shows a distinct difference, with dominating signals

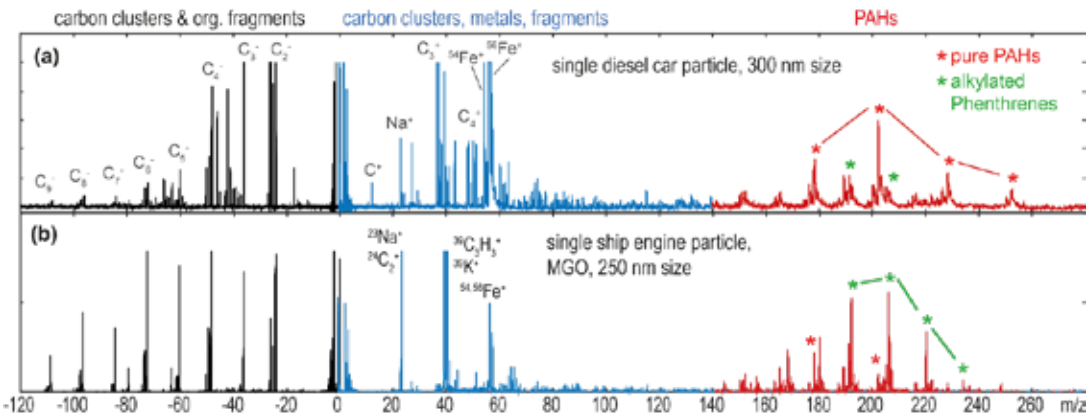

Figure 4: *Exemplary mass spectra from (a) a diesel car particle and (b) a particle from the research ship engine at LKV in Rostock, running on marine gas oil. The inorganic composition alone is no unequivocal indicator for any specific engine or fuel type. However, the alkylated phenanthrenes dominating the PAH spectrum have been found to point on ship emissions (Czech et al., 2017). Lines are guide to eyes. See also Research Area 1 (05) for identifying ship exhaust particles in ambient air.*

from alkylated phenanthrenes exclusively for the ship engine (Czech *et al.*, 2017). Although these substances can also stem from lube oil residues of any engine, further signals from LDI (*e.g.* Ca^+/K^+-ratio) can be used to identify and exclude lube oil particles (Toner *et al.*, 2006), demonstrating the unprecedented source apportionment potential of combining organic and inorganic particle composition as realized with our new method.

Within the SAARUS-measurement campaign in 2020, thousands of individual mass spectra were recorded for each of six different fuel types and different engine loads. Two manuscripts are under preparation from this data. A particular important aspect for health-related aerosol studies is that the compound classes, which are believed to dominate the toxicity of ambient particulate matter, namely PAHs and transition metals, can be analyzed simultaneously by the new single-particle mass spectrometry method.

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(04) Trace Detection of Environmental Health Relevant Metals in Individual Aerosol Particles

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Aerosols of both natural and anthropogenic origin play a key role in global climate and biogeochemical cycles. While sulfate and carbonaceous aerosols are in the research focus of their environmental and climate effects (Wang *et al.*, 2016; Bond *et al.*, (2013), the particle-bound metals affect ecosystems and human health. Inhaled transition metals such as iron (Fe) induce oxidative stress and are involved in health effects from air pollution (Ye *et al.*, 2018; Fang *et al.* 2017). Furthermore, metal-containing aerosols are important sources of marine micronutrients (Mahowald *et al.* 2018; Jickells *et al.* 2005). In addition to natural mineral dust, the highly soluble and thus bioavailable Fe from anthropogenic aerosols affects primary production and carbon fixation in the world's oceans (Ito and Shi, 2016). Beyond Fe, further trace metals show health effects (Gaur and Agnihotri, 2019) or can modulate ecosystems, *e.g.* as enzyme cofactors (Mahowald *et al.*, 2018). Zinc is also associated with toxicological responses to wood combustion aerosols (Kanashova *et al.*, 2018). However, the magnitude and variability of anthropogenic sources of bioavailable metals are poorly characterized, in particular, because of limited observations and a lack of real-time technology.

Single-particle mass spectrometry (SPMS) based on Laser Desorption/Ionisation (LDI) is capable to obtain a chemical profile from individual particles in real-time, see figure 1 for the working principle (Pratt and Prather, 2012). In contrast to other aerosol mass spectrometry methods, it also detects metals, although with highly variable efficiency. The detection difficulties account for different metals, are associated with humidity and the particle main composition, and result from several poorly determined interactions at the particle surface during LDI, the standard ionization technique in SPMS.

With our experiments, we were the first group using a tunable laser system (optical parametric oscillator, OPO) for LDI in SPMS. By changing the wavelength, we found that the ionization efficiency for metals depends strongly on the overlap between the photon energy and absorption

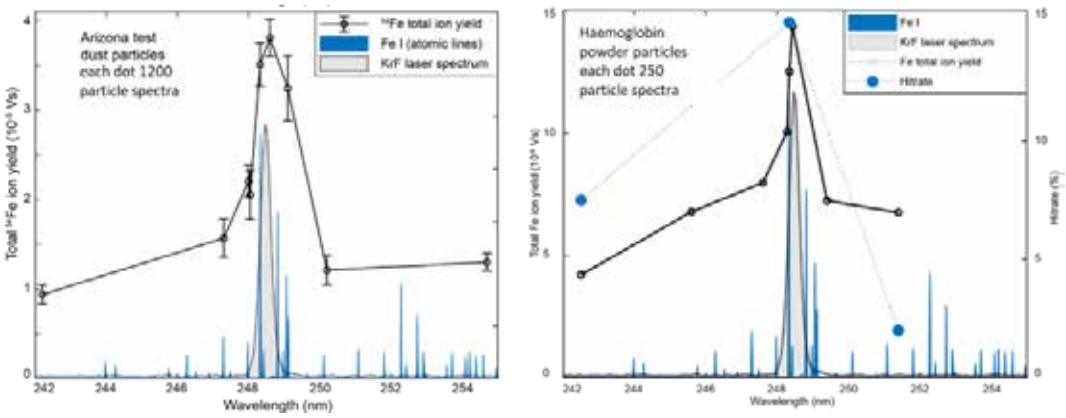

Figure 2: *Wavelength-dependent ion signal of 54Fe+ from (left) desert dust particles and (right) hemoglobin powder. The signal peaks for wavelengths that match a major atomic transition of Fe (blue lines) and coincide with the KrF-excimer laser spectrum (grey)[12].*

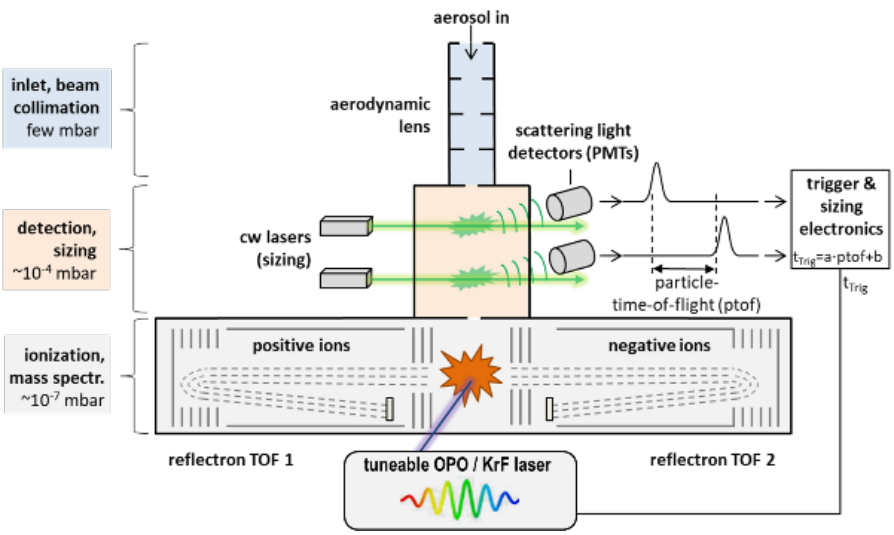

Figure 1: *Working principle of SPMS: Particles are introduced into vacuum and optically detected and sized. In the current experiments on resonant ionization of metals, a tunable UV laser pulse is triggered when the particle enters the ion source of a mass spectrometer, producing positive and negative ions via resonant Laser Desorption/Ionization (LDI).*

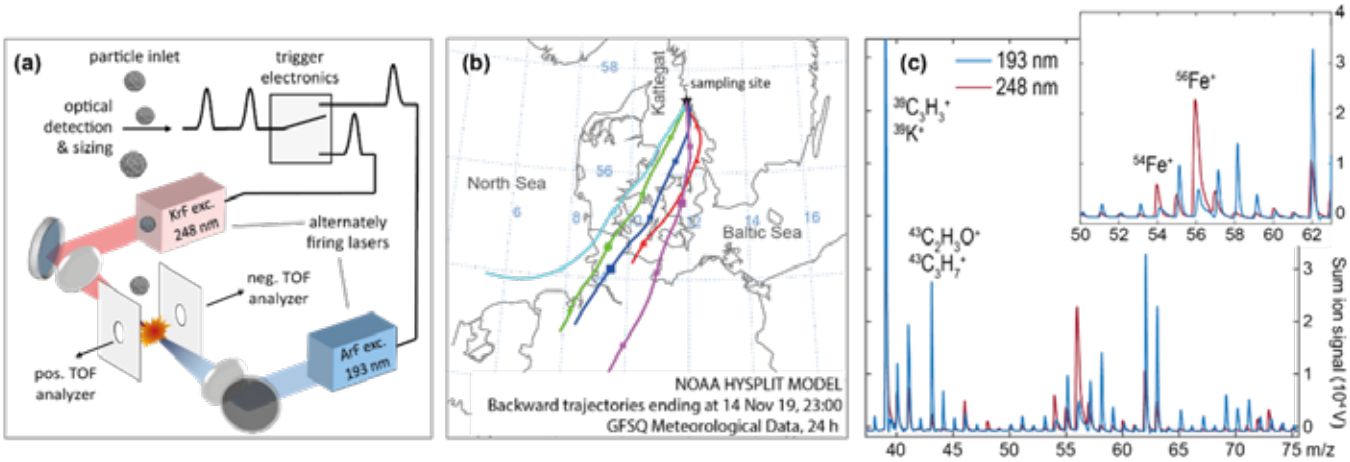

Figure 3: Setup for direct comparison of non-resonant and resonant ionization of Fe in ambient air particles using the same mass spectrometer. (b) Back trajectories ending at the sampling site during the experiment on long-range transported particles. (c) Accumulated cation mass spectra (n=15 000) show a Fe+-signal enhancement for resonant ionization (248 nm, red) versus the non-resonant case (193 nm, blue) (Passig et al., 2020).

lines of free atoms instead of the particle's bulk absorption properties (see figure 2).

We investigated these resonance effects in LDI and introduced the technique in SPMS (Passig *et al.*, 2020). The primary application is the detection of biologically relevant metals in atmospheric aerosols while gathering information about the particle's origin as well as the metal's bioavailability, *e.g.* through adsorbed sulfuric acid. While the resonance-enhanced ion yield for laboratory particles is moderate (figure 2), we found that the resonance can dramatically improve the metal detection efficiency in real-world aerosols. Therefore, we performed a field experiment on ambient air in Sweden (see Research Area 1 (05)) with two alternately firing excimer lasers of different wavelengths (figure 3a). Herein, resonant LDI with the KrF-excimer laser (248.3 nm) revealed iron signatures for many more particles of the same aerosol ensemble compared to the common ArF-excimer laser line of 193.3 nm (non-resonant LDI of iron), see figure 4.

A key finding is that the resonant ionization provides a more secure and universal detection of metals, with less dependency on the main particle composition (figure 4). Our experiment indicates that previous SPMS studies might

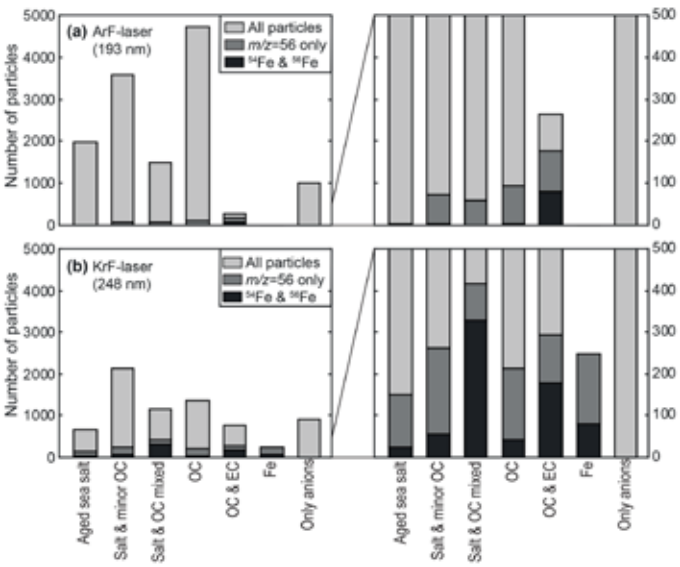

Figure 4: Number of particles within main classes. Dark grey: particles with Fe signatures. Black: particles with additional 54Fe+ isotope. (a) If non-resonantly ionized (λ=193 nm), strong fragmentation leads to dominating organic carbon (OC). Fe-signals are almost exclusively observed for particles with soot signatures. (b) Although fewer particles produce ion signals if exposed to 248 nm pulses, the particle fraction showing Fe-signatures is much larger. Fe-signals are not limited to soot-containing particles but occur for all classes. This suggests that the resonant ionization allows a more universal and secure detection of Fe [12].

have underestimated the role of organic aerosols and heavily mixtures particles as atmospheric Fe-carriers (Dall'Ostro *et al.*, 2016). The resonant ionization in SPMS allows better estimates of the sources and transport pathways of bioavailable metals to the oceans, as well as improved risk assessment and monitoring of health-relevant air pollutants. With our new method, SPMS is further developed to a potential key technology for observations of biogeochemical cycles and to constrain aerosol contributions in climate research. In conjunction with the developed approach to include the information of the Polycyclic Aromatic Hydrocarbons (PAH, see section (Enabling Analytical Technologies (03)) in SPMS, the resonance-enhanced LDI of health-relevant transition metals moreover may be used to establish a method to analyze the key toxicants (PAH, Fe, etc.) in aerosols on a single-particle basis.

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(05) The application of complementary high-resolution mass spectrometric techniques for complex matrices – a case study on petrochemical matrices in the context of new IMO regulations for ship fuels

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New IMO regulations & emerging unconventional marine fuels

Marine transportation is one of the main contributors to anthropogenic PM, SOx, and NOx emissions globally, accounting for a high number of premature deaths in coastal regions (figure 1, Corbett *et al.*, 2007). To reduce environmental sensitive SOx emissions and the use of "dirty" residual fuels (Heavy Fuel Oils, HFO), new regulations for the fuel composition were introduced in January 2020 (IMO 2020), to limit the total sulfur content of the fuel and/or related emission to < 0.5 %. To meet the high demand for such compliant fuels, but also targeting economic aspects, unconventional production pathways and feedstocks are explored nowadays. The resulting so-called "compliant fuels" and blends, which are emerging on the market, lead to a higher fluctuation in fuel compositions. In order to enable a smooth transition to the new generation of fuels and to cope with a widely diversified spectrum of fuels, a comprehensive chemical description is mandatory to correlate possible incompatibilities with ship engines, negative impact on the storage stability, or arising potential new threats to the environment and health.

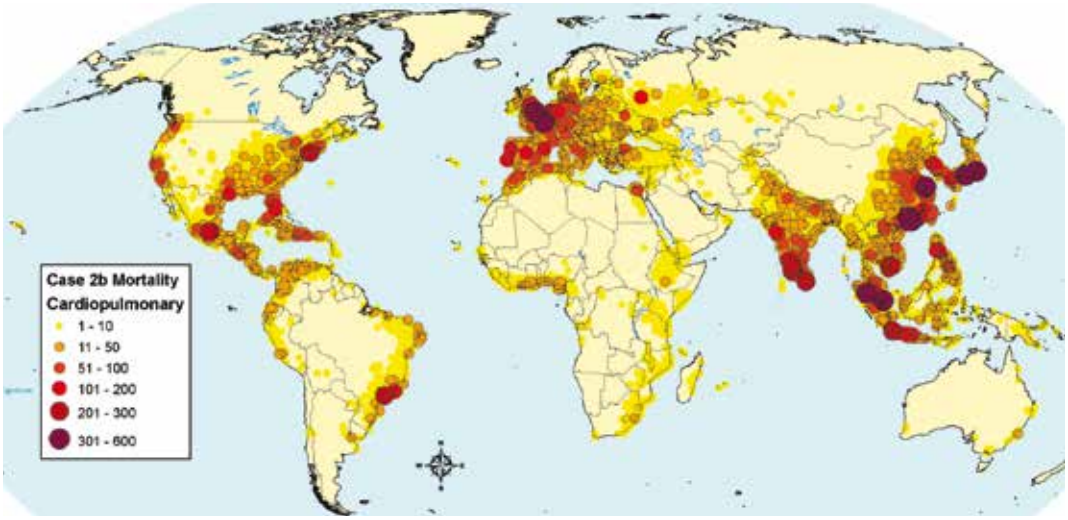

Figure 1: *Cardiopulmonary mortality (No/100,000) attributable to ship emissions PM2.5 worldwide for all PM constituents (Reprinted with permission from Corbett et al., 2007. Copyright 2007 American Chemical Society)*

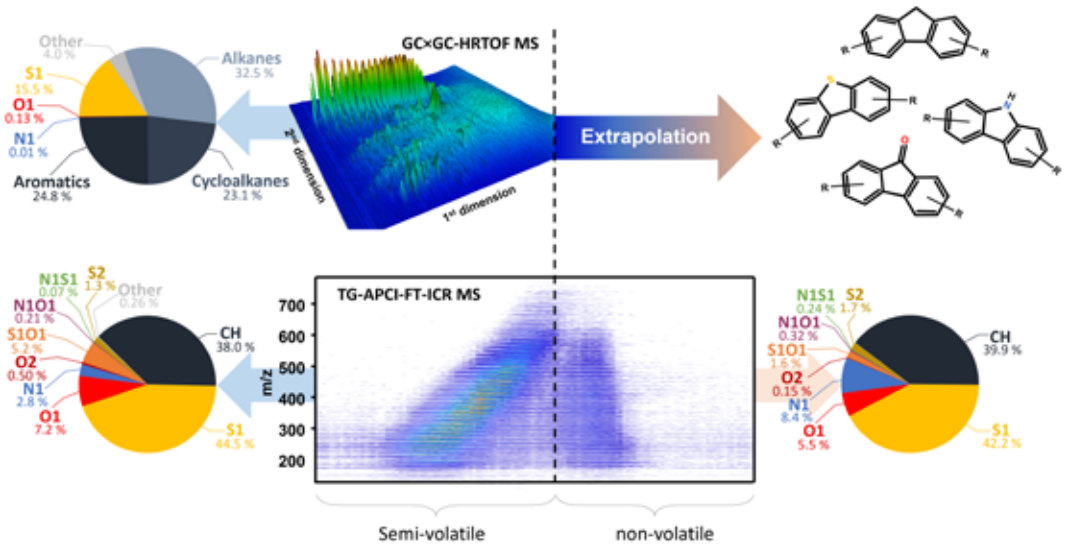

Figure 2: *Method integration of GC×GC–HRTOFMS (top) and TG-APCI-FT-ICR MS (bottom) for the analysis of a broad chemical space. The combined approach conducted by JMSC groups in Rostock and Munich integrate the strengths of both techniques (isomeric separation and ultra-high mass resolution). GC×GC–HRTOFMS allows the assignment of mass spectra and structural information. Structures found within the semi-volatile region (e.g. small R) could be extrapolated to larger or non-volatile structures (e.g. large R), where the mass resolution of applied HRTOFMS is not sufficient to assign unique elemental compositions. These structural information could be also used to interpret sum formulas and thermal break down fragment found with FTICR approach.*

Analytically challenging samples require complementary methodological approaches

Due to the tremendous chemical complexity of petroleum-derived matrices, potent analytical methods have to be applied for a detailed chemical description. The application of high-resolution mass spectrometry with different ionization techniques is very common to classify compound groups in such matrices. Therefore, the unmatched mass resolving power of Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is an essential tool for a comprehensive analysis of complex matrices (Rüger *et al.* 2015) and was applied in this study. As a complementary technique, comprehensive two-dimensional gas chromatography (GCxGC) coupled to high-resolution time-of-flight mass spectrometry (GC×GC-HRTOFMS) enables separation and identification of the isomeric composition. However, residual high boiling compounds are discriminated due to volatility limitations of the gas chromatograph. In contrast, thermal separation techniques under atmospheric (thermogravimetric analysis, TGA) and reduced pressure conditions (Direct inlet probe, DIP) also enables the analysis of the nonvolatile parts of the samples (Käfer *et al.*, 2019) and can be hyphenated to the same HRTOFMS platform. In a combined approach we applied the different mass spectrometric approaches from both JMSC facilities in Rostock and Munich for a comprehensive investigation of conventional and modern ship fuels (figure 2).

While a high content of aliphatic structures typically leads to high cetane indices and more complete and "cleaner" combustion, there are known issues regarding the compatibility with conventional HFOs, which may cause problems for blending, fuel switching, or storage in contaminated tanks. In contrast, also extremely aromatic fuels (figure 3) may cause problems in terms of engine incompatibility. Although such compliant fuels may meet the IMO 2020 specification, they could negatively impact human health due to the emission of toxic PAHs and particulate matter. Therefore, further studies connect the chemical profiles of arising unconventional compliant fuels, efficient exhaust after-treatment (*e.g.*, SCRUBBER technologies), and the biological effects of corresponding emissions.

Within the JMSC framework, the tightly integrated cooperation between the University of Rostock and the Helmholtz Center Munich allows the combination of such highly sophisticated approaches for complementary analytical studies and their biological interpretation. This is especially important to identify future challenges from newly appearing complex chemical matrices and their impact on health and the environment.

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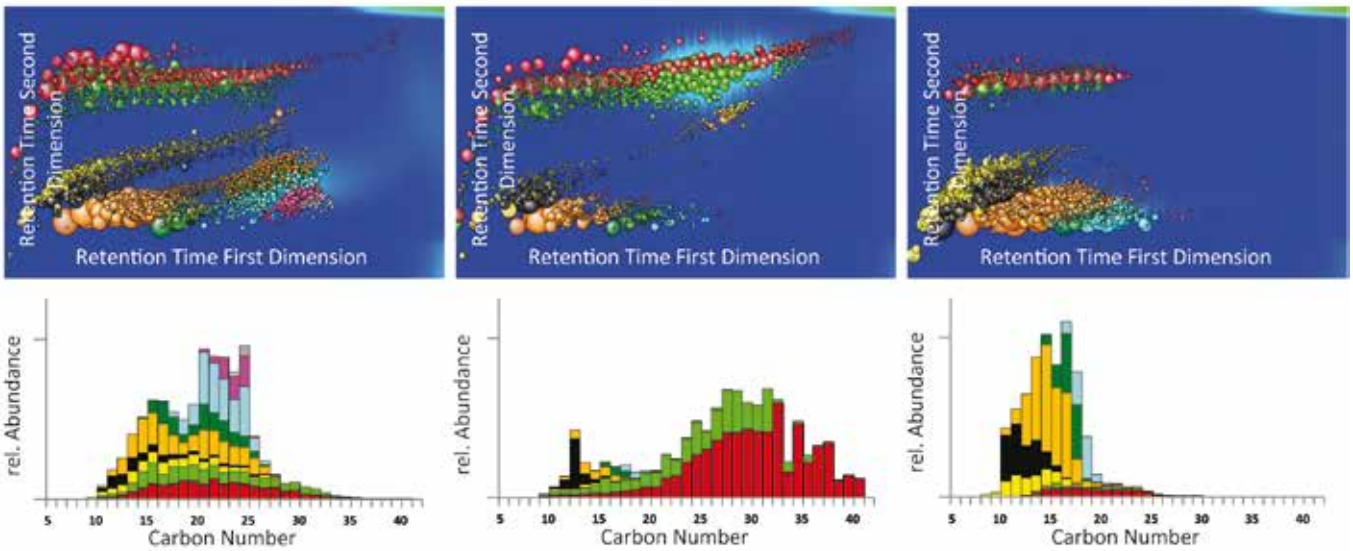

Figure 3: Chemical profiles of a HFO (left) and two "unconventional" compliant fuels.
(• Paraffines, • Naphthenes, • Benzenes, • Benzonaphthenes, • 2-ring aromatics, • 3-ring aromatics, • 4-ring aromatics, • 5-ring aromatics, • 6-ring aromatics)
The chemical profile differs significantly. While the middle compliant fuel is enriched in paraffin and naphthenes the right compliant fuel is dominated by aromatics.

(06) Linking roast gas composition and antioxidant capacity: A chemometric approach with photoionization mass spectrometry for optimization of health benefits from coffee

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Coffee beverage is known to contain cancer-causing compounds in significant amounts. However, epidemiological results show that there is no increased incidence of cancer diseases in coffee consumers with respect to non-consumers. This effect is referred to as the "coffer cancer paradox" (Bøhn *et al.*, 2014). It is believed that protective compounds present in coffee may play a role in this effect. Coffee beverages are known to contain substantial amounts of antioxidants, such as polyphenolic acids, stilbenes, lignans, tannins, and flavonoids, depending on the coffee cultivar, geographic origin, and roast conditions In particular, these polyphenolic species gained attention in food research because foods rich in polyphenolics have been associated with the prevention of inflammation-related diseases, cancer, cardiovascular diseases, neurodegenerative diseases, diabetes, obesity, and osteoporosis. Although polyphenols and their metabolites may directly scavenge free radicals, negligible importance was attributed to this mode of action in the human body (Ludwig *et al.*, 2014). More likely, their ability to act as chelating agents for redox-active metals and modulatory actions are the key mechanisms. For example, polyphenols may affect the enzymatic activity of the gut microbiota or act on protein kinase and lipid kinase signaling pathway, preventing glucose intolerance and diabetes. Therefore, product optimization toward healthy foodstuffs or conversion into so-called functional foods may be beneficial for human health.

Photoionization time-of-flight mass spectrometry at different wavelengths has been demonstrated as a powerful tool for process control, identification of roast stadia and temporal evolution of roast gas components (Czech *et al.*, 2016). Here, we linked photoionization mass spectra to polyphenolic content in order to generate a tool for online prediction of health-relevant coffee properties in real-time.

Roast experiments and roast gas analysis

Green Arabica coffee beans (Coffea Arabica) from Colombia were roasted with an electrically heated single drum roaster having approximately 100 g batch size (figure 1a). First, 20 roasts of different roast degrees were conducted with roast gas analysis by single-photon ionization (SPI) time-of-flight mass spectrometry (TOFMS). Based on the experiences in this pilot study (Heide *et al.*, 2020), in total 84 roasts with resonance-enhanced multi-photo ionization (REMPI) TOFMS at 248 nm and 69 roasts with REMPI-TOFMS at 266 nm were performed (figure 1b). While REMPI selectively ionizes aromatic compounds (and also aliphatic amines at 248 nm), SPI is a more universal ionization technique.

All roasts started with an outer drum temperature of (180 ± 2) °C. In order to avoid too steep ascent in bean pile temperature and too fast aroma development, the damper was opened after approximately 6 min causing a higher airflow

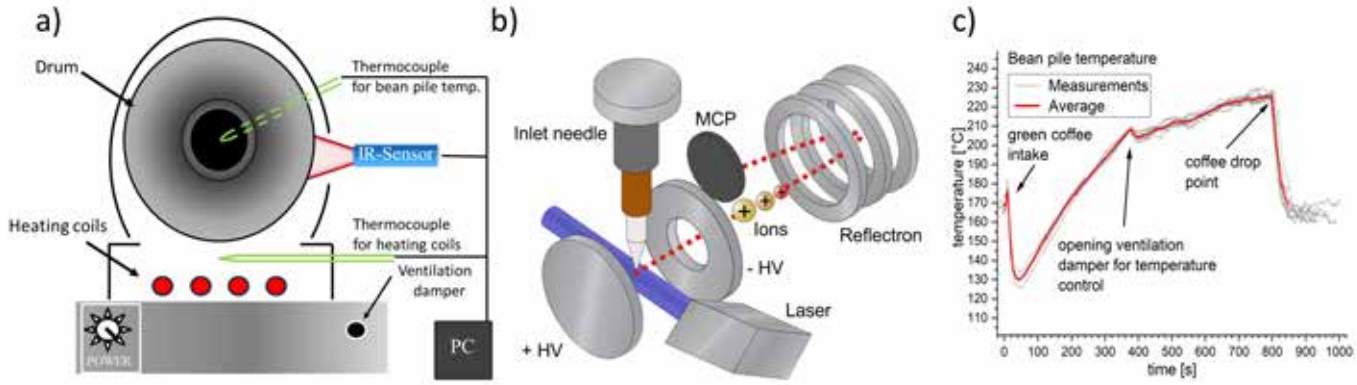

Figure 1: *a) Coffee drum-roaster with a batch size of 100 g and thermocouples, b) setup of the laser based photoionization time-of-flight mass spectrometer and c) roast profile (reprinted with CC BY 4.0 License from Czech et al., 2020)*

with the resulting roasting profile depicted in figure 1c.

Determination of antioxidant capacity and roast degree by Folin-Ciocalteu (FC) Assay

The FC assay was used as an antioxidant measurement, which is in particular sensitive to (poly)phenolic compounds, and results are expressed as gallic acid equivalent (GA eq.). Ground coffee was brewed in a French press, filtered and diluted, then set to pH of 10 and mixed with the FC reagent. The resulting blue complex was photometrically measured at 765 nm and related to an external calibration with anhydrous gallic acid in deionized water.

Statistical modeling with PLS regression and model refinement by Monte Carlo techniques

About 80 % of the mass spectra at the coffee bean drop of each REMPI method were paired up with antioxidant capacity using PLS regression. The PLS regression models were validated by Monte Carlo cross-validation and external validation using the remaining 20 % of the mass spectra. In order to remove redundant variables and simplify the model, we applied competitive adaptive reweighted sampling (CARS) (Li *et al.*, 2009).

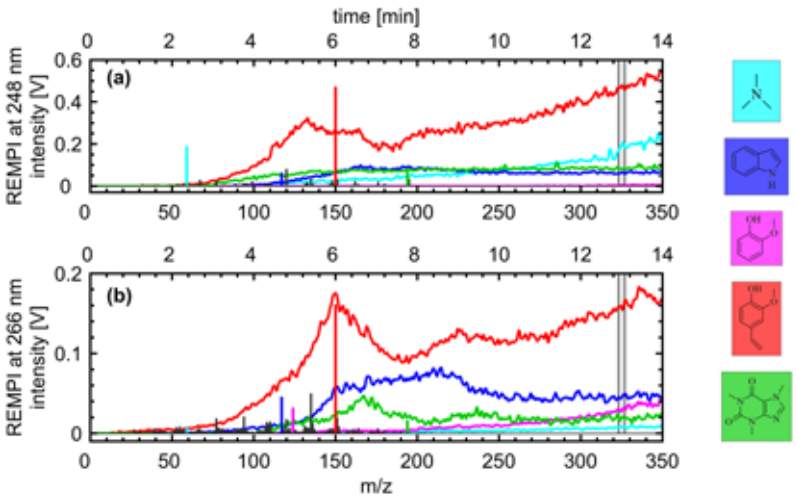

Figure 2: Combined mass spectra and time traces of individual roast gas components using REMPI at a) 248 nm and b) 266 nm. Grey-shaded area denotes the time-period referring to the displayed mass spectrum (reprinted with CC BY 4.0 License from Czech et al., 2020).

Roast gas composition and monitoring of evolving VOCs

In both mass spectra of REMPI at 248 nm and 266 nm (figure 2) from a roast of 14 min (medium/dark roast), the base peak appears at m/z 150, which refers to 4-vinylguaiacol from the decomposition of chlorogenic acids. Further phenolic species appear at the m/z 124, 122, 110, 108, and 94, which can be assigned to guaiacol, dimethylphenol, benzenediol, methylphenol, and phenol, respectively. Peaks occurring at m/z 194, 117, and 59 can be assigned to the N-containing compounds caffeine, indole, and C^3-amines, respectively. Overall, REMPI at 248 nm leads to higher intensities for most of the peaks, in particular for smaller N-containing compounds and five-ring heterocycles, such as furans and pyrroles. In contrast, REMPI at 266 nm is more selective for heterocycles and phenolic compounds with a higher degree of substituents at the aromatic ring. Despite the fact that most of the polyphenolic antioxidants are not volatile enough to be detected in the roast gas, the assigned compounds are products of chemical reactions which are involved in antioxidant formation.

Model performance and online prediction of antioxidant capacity in real-time

For REMPI at 248 nm and 266 nm, PLS regression models with one and two PLS components were obtained with root-mean-square error in prediction (RMSEP) of 80.3 and 151 GA-eq mg L-1, and explained covariance (R2P) of 82 and 45 %, respectively (Figure 3 top). Therefore, it is competitive with the precision of the photometric measurement with a residual standard deviation of 106 GA-eq mg L 1. Limits of detection (LODs) calculated with different approaches varied between 100 and 600 GA eq mg L-1, hence FC values in coffees with roast degrees appropriate for the market can be determined. However, REMPI at 248 nm clearly led to both lower LODs and RMSEP. The FC values in the calibration dataset from early to late coffee bean drops allow the application of the model directly within the data acquisition software of the TOFMS and prediction of health-relevant coffee properties during the roast in real-time (figure 3 bottom). These results suggest that photoionization mass spectrometry process monitoring can be utilized in industrial coffee roasting to optimize the content of health-relevant antioxidants.

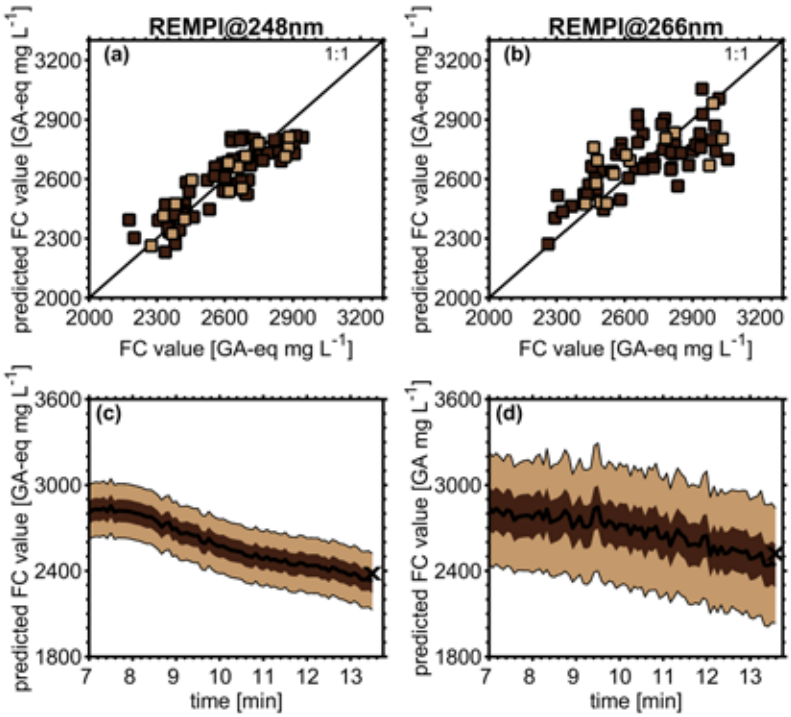

Figure 3: *Top: Relation between measured and predicted FC value for REMPI at 248 nm (a) and 266 nm (b for calibration (dark brown) and external validation data set (light brown). Bottom: Model application on online data for FC value prediction in real-time with REMPI at 248 nm (c) and 266 nm (d) with predicted RMSEP (dark brown), prediction band (light brown), and measured FC value at drop of coffee beans (black cross).*

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(07) Insights into the molecular composition of the organic carbon (OC) fraction in spruce logwood and lignite briquette combustion aerosol particles by thermal-optical carbon analysis hyphenated to photoionization mass spectrometry (TOCA-PIMS)

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Although being partly replaced by other fuels in most European countries, solid fuels such as wood or coal remain important fuels for residential heating with boilers or stoves in many households. Emissions from the combustion of lignite can be of utmost local importance since its consumption varies considerably among countries. Especially in Eastern Europe, people still heavily rely on burning solid fossil fuels for the production of heat in households. Junninen *et al.* (2009) showed that domestic coal combustion may contribute heavily to the local air burden in areas of eastern Europe where a lot of coal is consumed, even in urban areas with additional substantial emission contributions from traffic and industry. However, emissions of Eastern Europe may be carried westward into central and western Europe and affect the air quality there. Furthermore, wood combustion was and partially still is promoted *e.g.* in Germany as supposedly environmentally friendly (*i.e.* carbon neutral) fuel. Due to the large PM emission factors solid state combustion has generally a large contribution to the ambient PM (Brandt *et al.*, 2011; Lin *et al.*, 2019).

Thermal-optical carbon analysis (TOCA) for the determination of OC and EC is known as a standard technique in atmospheric and combustion science. The unique hyphenation of TOCA to photoionization mass spectrometry (PIMS) provides rapid molecular insights into the composition of evolving gases during TOCA (Diab *et al.*, 2015) without any time-consuming sample pretreatment and risk of contamination. Here we present molecular features of respirable particulate matter (PM1) from the combustion of lignite briquettes in comparison to spruce logwood.

Combustion experiments

PM1 from several batches of lignite briquettes and spruce logwood combustion in a modern non-heat-retaining iron stove were sampled over 4 h on quartz fiber filters at the ILMARI facility at the University of Eastern Finland. Spruce logwood combustion started with a cold stove, whereas two batches of spruce logwood were burned prior to lignite briquette combustion due to its worse ignition properties.

Thermal-optical carbon analysis and photo-ionization mass spectrometry (TOCA-PIMS)

TOCA gives quantitative information on elemental carbon (EC) and organic carbon (OC) content of particles, which are bulk parameters typically monitored in source apportionment studies and emission inventories. The organic carbon fraction was analyzed with a resonance-enhanced multi-photon ionization (REMPI) time-of-flight mass spectrometer (TOFMS) coupled to the carbon analyzer (figure 1) for direct analysis of the evolving gases, affording the chemical composition of the individual OC fractions.

For REMPI we used ultraviolet radiation (248 nm, equal to a photon energy of 5.0 eV) provided by KrF excimer laser. With moderate light intensities, ionization of thermally desorbed particle constituents takes place in a two-photon process: The photon excites the molecule to a transition state, while a second photon conducts the ionization. In this way, aromatic compounds are selectively ionized out of a complex mixture because only aromatic compounds provide a sufficiently long lifetime of the transition state.

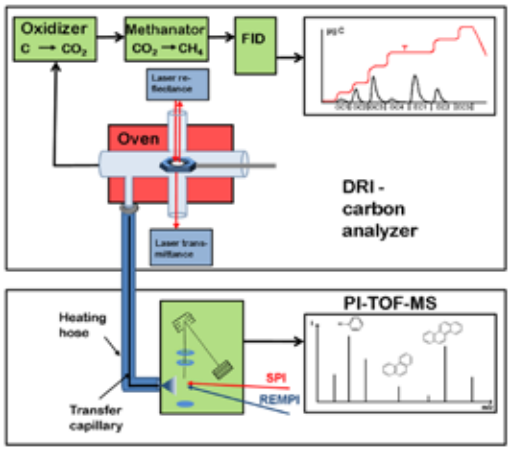

Figure 1: *Thermal-optical carbon analyzer coupled to a photoionization time-of-flight mass spectrometer (TOCA-PIMS; reprinted with CC Attribution 3.0 License from Diab et al., 2015).*

Carbon emission factors from spruce logwood and lignite briquette combustion

With 16 mg OC MJ-1 and 8 mg OC MJ-1, lignite briquette and spruce logwood combustion produced similar amounts of organic carbon (OC) in the emitted particulate matter. However, we observed significantly higher emission factors

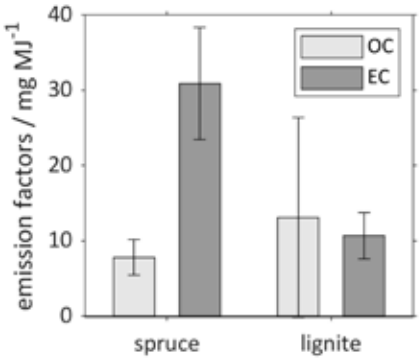

Figure 2: OC and EC emission factors from the combustion of spruce logwood and lignite briquettes. Bars and whiskers represent mean emission factors with one standard deviation.

for EC in spruce logwood (30 mg OC MJ^{-1}) than in lignite briquette combustion (8 mg OC MJ^{-1}) (figure 2), which might be a consequence of the different starting temperatures of the stove or different fuel composition. Furthermore, we obtained a one order of magnitude higher content of ash for lignite briquettes than for spruce logwood, indicating that OC and EC contribute to a lower fraction of total PM for lignite than spruce.

Insights into the molecular aromatic compound composition of lignite and spruce PM

In the accumulated REMPI-mass spectra of OC1 and OC2 (figure 3), it can be seen that spruce and lignite exhibit different molecular patterns. While the evolved gas in the analysis of spruce (top) showed fewer but more intense signals, there are substantially more signals in the mass spectrum from lignite (bottom). Especially signals in the higher m/z area are of interest because marker compounds originating from incomplete combustion and providing the opportunity to be traced back to chemical structures present in the unburned fuel can be found here. In emissions from lignite, picenes, derived from the multi-angled 5-ring PAH picene, appear in the higher m/z range of 300 to 400, which are known as coal combustion markers. During the formation of lignite from biomass, pentacyclic triterpenoids may be transformed into picenes upon decarboxylation, demethylation, and aromatization.

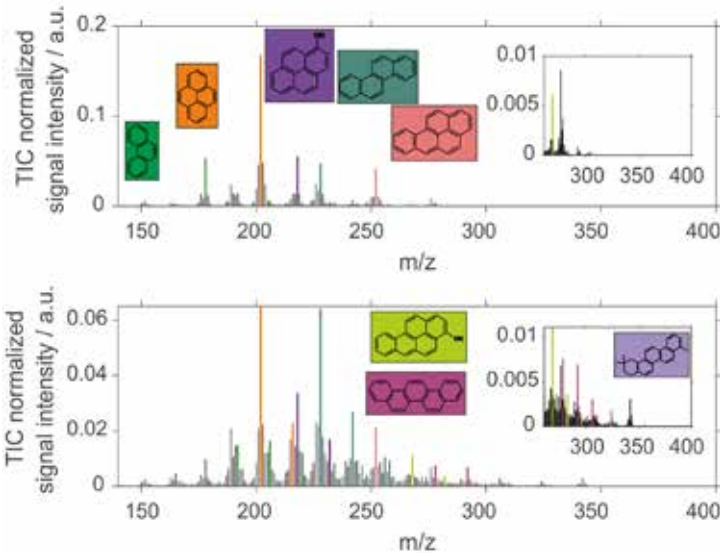

Figure 3: Normalized REMPI mass spectra accumulated over the first two organic carbon fractions OC1 and OC2 of spruce logwood (top) and lignite briquettes combustion PM (bottom). Typical structures and their alkylation series are indicated for the most abundant polycyclic aromatic compounds. Note, the applied REMPI ionization for mass spectrometric fingerprinting selectively addresses the evolved aromatic structures of interest.

However, picenes are only one example of high-molecular weight aromatic with higher abundance in lignite than in wood combustion emissions. With increasing ring number, PAHs tend to increase in their carcinogenic potential (Nisbet and LaGoy, 1996) being rapidly metabolized in the so-called "bay-region" or "fjord region" to the active diols and diol epoxides. Hence, REMPI mass spectra reveal a first indication of a likely toxicologically more potent emission from lignite combustion than spruce logwood. However, PAHs represent only one class of compounds known to be carcinogenic and individual PAH with the same ring number may vary by one order of magnitude, which motivates further analysis for higher chemical speciation, such as the implementation of ultra-fast gas chromatography (Fischer *et al.*, 2015) coupled in between TOCA and TOFMS.

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(08) Thermal analysis hyphenated to ultrahigh resolution mass spectrometry (FT-ICR MS)

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Introduction and Motivation

For molecular-level analysis, solid and high-viscous sample materials with poor solubility cannot be approached by classical gas chromatographic or direct infusion approaches. Consequently, those matrices remain a significant analytical challenge. In particular, unraveling the chemical composition of high-boiling point petroleum mixtures, such as asphaltenes or bitumen, is in some respect limited by the currently available analytical techniques. Chemical specification of those materials is crucial for a variety of application fields, such as the development of refining strategies, ensuring the best utilization of resources, the design of polymers with improved physicochemical properties, and the recycling of composite materials. The diversity and width of application fields with a strong economic and ecological background create a high demand for novel analytical approaches and strategies. Thermal analysis, employing thermal gravimetry, coupled to mass spectrometry was previously shown by us to be a powerful hyphenation targeting solid and high-viscous materials. High-boiling point and high-molecular-weight constitutes are intentionally pyrolyzed and the evolved gas mixture can be chemically analyzed. Those pyrolysis products allow drawing conclusions on the initial molecular composition and structure. Nonetheless, the enormous compositional and structural complexity requires powerful mass spectrometric platforms. Therefore, ultrahigh resolution mass spectrometry, known for its unbeaten mass resolving power and mass accuracy as well as diversity in ionization techniques, featuring certain parts of the chemical space, was identified as a highly suitable platform. This report summarizes our results and developments in the field of thermal analysis coupled to ultrahigh resolution mass spectrometry with special emphasis towards heavy petrochemical fractions. The technologies, however, are interesting for the analysis of heavy fuels and lubricants relevant for particulate emission from ship engines and other compliances.

Results and Discussion

In 2015, we presented the first hyphenation of thermal gravimetry (TG) to ultrahigh resolution mass spectrometry (FT-ICR MS) (Rüger *et al.*, 2015). A heated interface between the emission chamber of the thermobalance and ionization source of the mass spectrometer was developed, which allows direct sampling of the evolved gas mixture by a deactivated fused silica capillary. The sample material evolved from the thermobalance was transported by a slight over-pressure of several mbar into the atmospheric pressure ionization source. Here, atmospheric pressure chemical ionization (APCI) was utilized. The system was evaluated and tested subjecting various lignocellulosic biomass samples and petrochemicals. In brief, we were able to resolve the isobaric complexity of the evolved gas pattern at the molecular level attributing elemental compositions with sub-part-per-million (ppm) accuracy. The basic concept of the hyphenation is presented in figure 1 A.

For the newly introduced hyphenation technique, asphaltenes, the most aromatic and complex petroleum fraction, which is often related to severe problems in petroleum exploration, transport, refining, and storage, served as the first analytical challenge. Figure 1 B exemplarily depicts a typical temperature-resolved mass spectrum of TG-FT-ICR MS data. As also shown in figure 2 A/B, spectra can be divided for petrochemicals in the desorption phase (below 300 °C) and the pyrolysis phase (starting between 300-350 °C). Non-evaporable material remains as a thermogravimetric residue in the TG crucible. The first study on asphaltenes precipitated from their parent heavy oils with different paraffinic solvents showed that TG-FT-ICR MS is able to characterize ultracomplex petrochemical materials. More specifically, the different fractionation procedures revealed differences in the material occluded on the asphaltenes released during the desorption phase. This occluded material is critical for the formation of aggregates and the deposition formation of asphaltenes. Furthermore, physicochemical properties and the molecular response were correlated for the heavy oils and asphaltene samples, finding a strong correlation between sulfur content and abundance of CHSx-class compounds as well as between double bond equivalent (DBE) and API gravity. Collision-induced dissociation (CID) gave access to structural information. CID mainly leads to dealkylation and, thus, the aromatic core structures of the evolved gas pattern could be traced temperature-resolved (Rüger *et al.*, 2017).

Knowledge of the molecular architecture of asphaltenes is essential for predicting their physicochemical behavior. Generally, two architecture models are discussed in the literature: Single-core/ island-type and multi-core/ archipelago-type structures. For further investigation on this critical aspect, a specially precipitated and washed asphaltene was distributed within the Asphaltene Characterization Interlaboratory Study for the PetroPhase 2017 conference. Three different thermal analysis mass spectrometry hyphenations with five diverse ionization techniques varying in selectivity were deployed: (1) thermal desorption/pyrolysis gas chromatography electron ionization (TD/Pyr-GC-EI-QMS), (2/3) thermo-gravimetry single-photon/ resonance-enhanced multiphoton ionization time-of-flight (TG-SPI/REMPI-TOFMS), and (4/5) thermogravimetry atmospheric pressure photo-/ chemical ionization ultrahigh-resolution mass spectrometry (TG-APPI/APCI-FT-ICR MS). In contrast to the non-washed asphaltenes from the previous study, the investigated asphaltene revealed no mass loss below 300 °C, and virtually all occluded material was removed. Combining the information of all techniques (figure 3), including the average asphaltene mass obtained by field desorption experiments and aromatic core size received by collision-induced dissociation, the archipelago-type molecular structure seems to be dominant for the investigated asphaltene (Rüger *et al.*, 2018).

The latest study focused further on the ability of TG FT-ICR MS to investigate the molecular architecture of asphaltenes. For

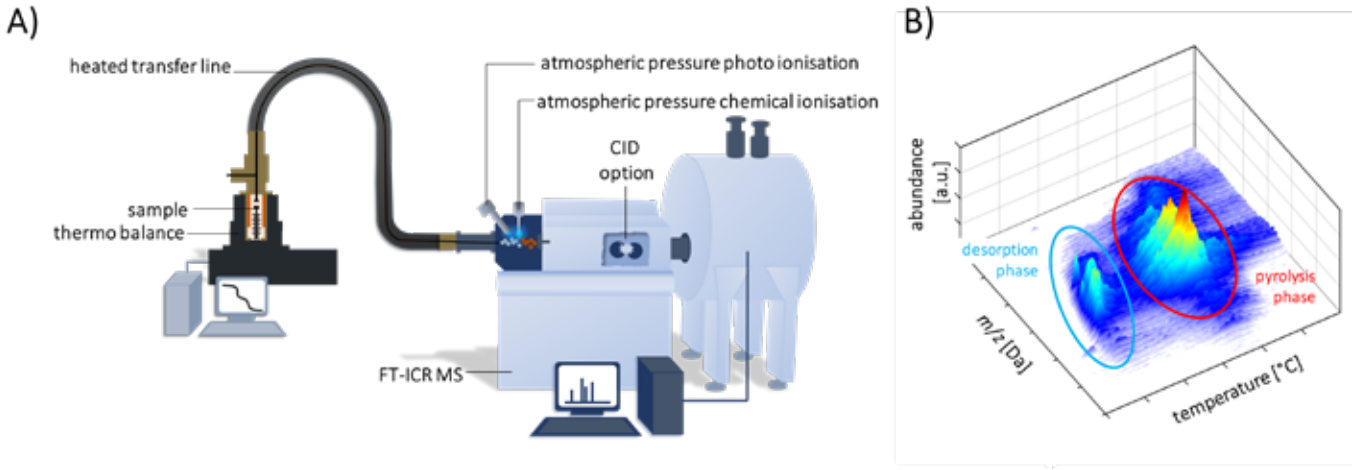

Figure 1: A) Schematic set-up of TG-FT-ICR MS. The thermobalance is coupled to the ionization source of the FT-ICR MS by a heated transfer line. For ionization, any atmospheric pressure gas-phase ionization technique can be applied. Alternating collision-induced dissociation reveals information on the molecular structure. (not to scale) B) Temperature resolved mass spectrum given as survey diagram. The spectrum can be divided into the desorption phase (below 300 °C) and the pyrolysis phase (starting between 300-350 °C).

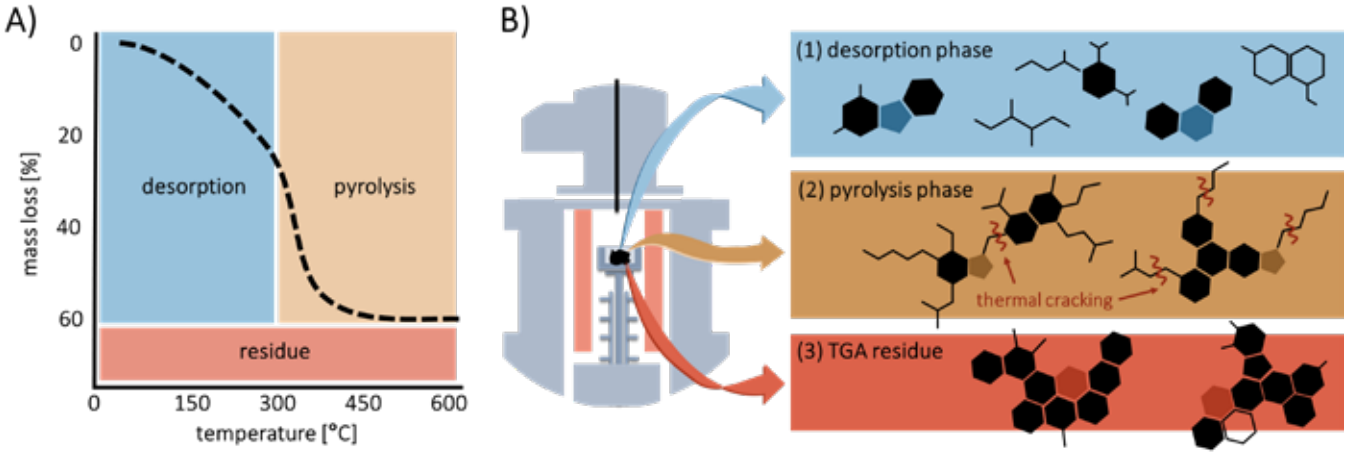

Figure 2: A) Typical mass loss diagram obtained by thermogravimetric measurements. The TG process can be divided into two phases, the desorption and the pyrolysis phase, whereas non-evaporables remain as residue. B) The three main pathways petroleum-derived materials undergo during the thermal gravimetric analysis. Smaller components are intactly desorbed, whereas high-molecular compounds are decomposed into smaller pyrolysis products. The non-evaporable material remains as coke in the TGA residue.

this purpose, island- and archipelago-enriched asphaltenes and their extrographic fractions were investigated (Neumann *et al.*, 2020). The island-type enriched sample revealed a significantly higher coke residue after the pyrolysis process compared to the archipelago-type enriched sample. Visualization of the double bond equivalent (DBE) and carbon number (#C) of the gas mixture evolved during the pyrolysis phase revealed specific compositional trends: compounds with high DBE values and short alkylation likely to be originated from island-type asphaltenes, whereas species with low DBE values and high carbon numbers likely derive from archipelago-type asphaltenes. With this study, we could show that TG FT-ICR MS serves as an additional technique for the characterization of asphaltenes and is in agreement with results obtainable by other approaches, such as direct infusion mass spectrometry. The DBE versus #C diagrams in combination with the mass loss information can serve as a fingerprint measure for identifying the dominant structural motif of these ultracomplex organic mixtures (figure 4).

Conclusion and Outlook

Thermal analysis coupled to ultrahigh resolution mass spectrometry equipped with soft atmospheric pressure ionization schemes allowed for the molecular description of complex high-viscous or solid sample materials. Soft atmospheric pressure chemical and photoionization preserved the molecular information of the thermal emission profile. In this short note, exemplarily, the results in the field of petrochemical materials, more specifically on asphaltenes were summarized. Furthermore, we were able to deploy this hyphenation for the description of other matrices, such as aged bitumen (Neumann *et al.*, 2020b) or polymers (Dhahak *et al.*, 2020). Currently, the platform is heavily involved in the project for the recycling of composite materials from wind power plants and the characterization of petroleum-based pharmaceutics. Moreover, further studies on bitumen additives and aging products are planned to enable a molecular-level evaluation of the potential environmental

impact of abrasive road dust. Recently a direct insertion probe (DIP) for the atmospheric pressure ion source was acquired, allowing the analysis of smaller sample sizes. This hopefully will enable the expansion of the TA-FTICR MS approach to reduced mass loading, *e.g.*, to analyze the ambient particulate matter.

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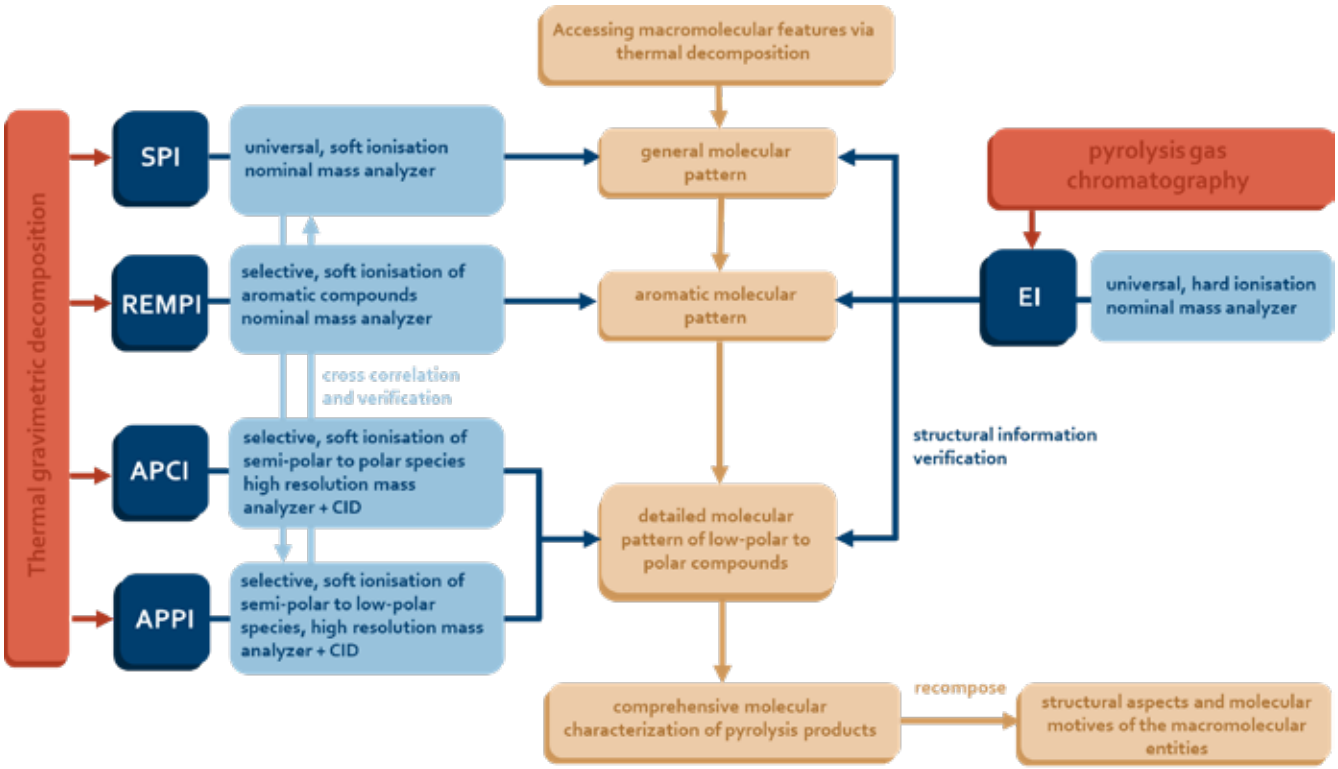

Figure 3: Flowchart for the integration of the TG-MS data and verification by GC-MS. For a comprehensive description of asphaltene pyrolysis products, three different mass spectrometric techniques, as well as 5 different ionization techniques, were applied. The comprehensive molecular characterization of the pyrolysis products enabled the recomposition of structural aspects as well as suggesting an average molecular motif for the PetroPhase 2017 asphaltene.

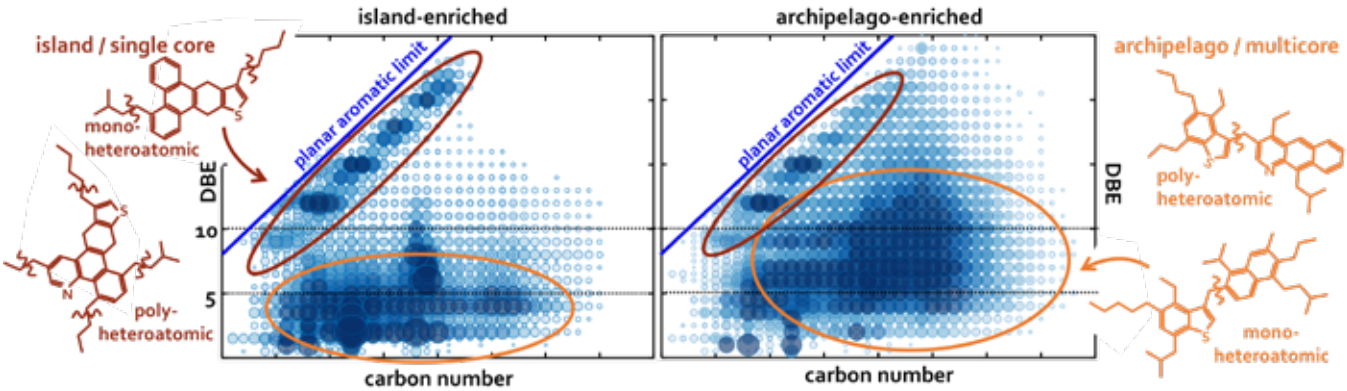

Figure 4: DBE vs. #C diagrams of the acetone fraction pyrolysis phase of island-enriched and archipelago-enriched asphaltenes measured by TG-APCI-FT-ICR MS. All compound classes are overlaid. Island-type asphaltenes decompose into highly aromatic core structures with short alkylation and pyrolysis products with DBE values below five and comparatively high alkylation. Archipelago-type asphaltenes revealed high abundant thermal fragments with DBEs of less than 15 and high carbon numbers /alkylation.

(09) Resonance-enhanced multiphoton ionization ultrahigh resolution Orbitrap mass spectrometry for evolved gas analysis

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Introduction and Motivation

Laser-based photoionization at reduced pressure allows for a soft and sensitive ionization of organic constituents. Depending on the deployed laser-wavelength, single- and resonance-enhanced multi-photon ionization can be differentiated (SPI and REMPI). The latter (REMPI) is particularly selective for aromatic and polycyclic aromatic hydrocarbons, whereas single-photon ionization (SPI) operated at VUV-wavelength is practically a universal ionization scheme for organic compounds (Hanley and Zimmermann, 2009). State-of-the-art REMPI mass spectrometric platforms are based on time-of-flight technology with detection limits in the ppt-region and high acquisition rate (up to 100 Hz), such as the devices from Photonion GmbH. Nonetheless, these systems, which are designed for rapid analyses of organic mixtures, lack in resolving power and mass accuracy which are needed for resolving isobaric interferences (same nominal mass but different exact mass). Thus, chemical attribution of the detected molecular pattern is most often based on the optical selectivity (REMPI, threshold selectivity in case of SPI) or literature knowledge, specific properties/origin of the sample. Consequently, there is a demand for improved analytical solutions for cases where unambiguous molecular identification is required. Feasible approaches are the coupling of fast chromatographic separation techniques (see Research Area 2, report (10)), which allows isomeric separation, or certain mass spectrometric fragmentation techniques. In this study, the mass spectrometric analyzer/detector system is targeted and Fourier-transform mass spectrometry (FTMS), employing Orbitrap technology, is foreseen for the construction of a new generation of photoionization mass spectrometers (PIMS). FTMS delivers unbeaten resolving power and part-per-million (ppm) mass accuracy can easily be achieved (Kanawati and Schmitt-Kopplin, 2019). Therefore, we hypothesize, that this system will have unique capabilities for process- and evolved-gas analysis. It could be a valuable tool for various application fields, such as the chemical description of carbonaceous aerosols. One aim is to apply the technology for a single-particle mass spectrometry approach (see Research Area 2, reports (03) and (04)). The here shown development was done in the framework of the EUROSTARS AerOrbi project, a cooperative effort between Aerosol d.o.o. (Slovenia), Spectroswiss SARL (Swiss), Photonion GmbH, and the University of Rostock. Thermo Fisher Scientific is acting as an external partner contributing specific details on the hardware and software architecture.

Results and Discussion

A Thermo Fisher Scientific Exactive Orbitrap high-resolution mass spectrometer was used as a basis for the construction of the vacuum photoionization Orbitrap prototype. A majority of the state-of-the-art high-resolution mass spectrometric platforms are dedicated towards life science and biochemical applications utilizing atmospheric pressure ionization techniques. Hence, the Exactive Orbitrap features electrospray ionization is followed by several stages of ion lenses and multipoles for ion transmission and step-wise reduction of the pressure. Finally, the ions from the continuous ionization source are directed into a curved ion trap, the so-called C-trap, from which ion packages are subjected to the Orbitrap mass analyzer for measurement. Vacuum photoionization does not require the complex setup of ion optics and the photoionization can take place directly in front of the Orbitrap, in the C-trap.

Figure 1 visualizes the realized instrumental setup for the prototype. REMPI is performed with the 4th harmonic of an Nd:YAG solid-state laser (266 nm) (figure 1 A). The maximum pulse repetition rate is 20 Hz, but commonly limited in the given setup by the comparable slow mass analyzer and, thus, slowed down to 1-2 Hz. The radiation is guided by a mirror geometry into the mass spectrometer through a Magnesium-Fluoride window installed on a modified flange on the HCD-cell side of the instrument (opposite side of the atmospheric pressure ionization part). The complete front part of the ion optics (atmospheric pressure inlet till C-trap entrance) is removed considerably lowering the hardware complexity of the system. The components were replaced by a stainless steel vacuum cube for installation of an additional turbopump, pressure gauge, and the heated sample inlet (figure 1 B). The heated inlet is realized by a long brass tip containing a deactivated fused silica capillary directly facing the C-trap entrance. This heated gas-phase inlet can virtually be

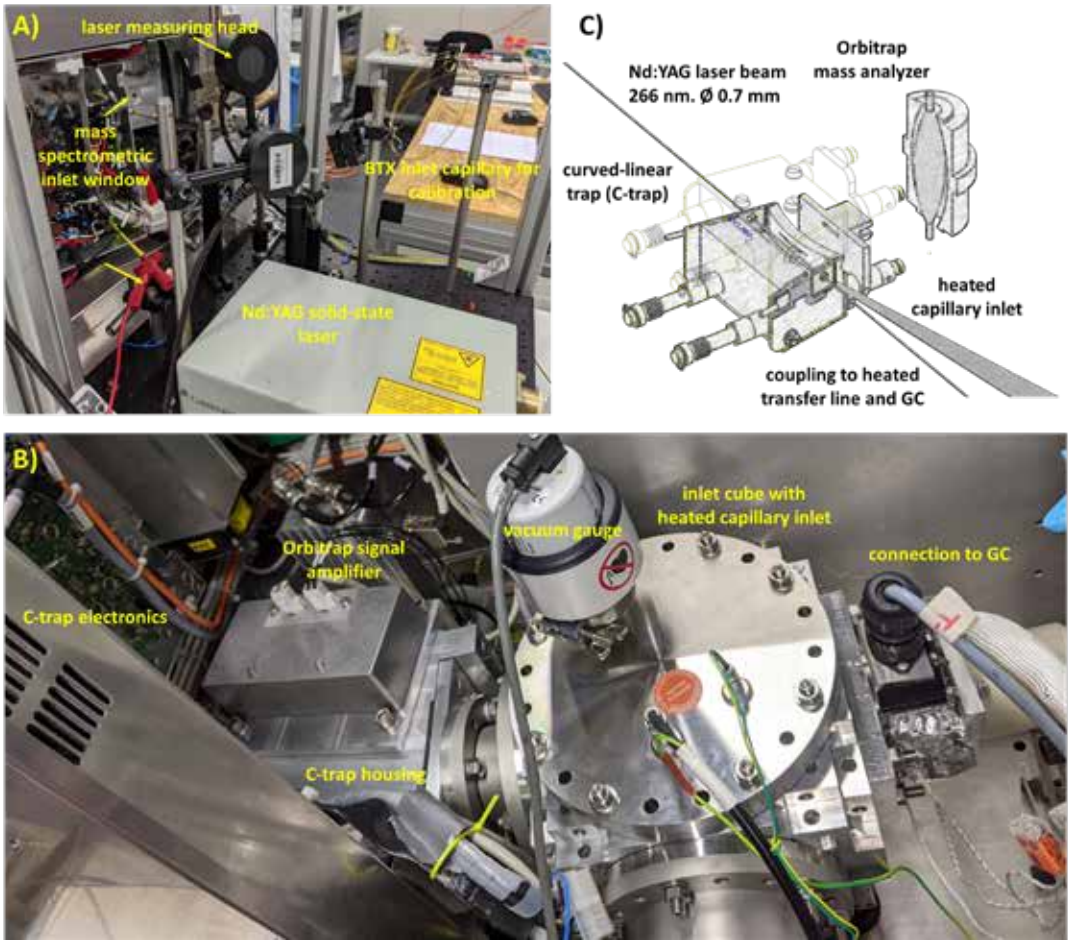

Figure 1: *Scheme and photographs of the laser-based photoionization high-resolution mass spectrometer (REMPI Orbitrap MS). A) View on the laser-system depicting the Nd:YAG solid-state laser (266 nm) and the inlet window into the C-trap housing. B) View on the sample inlet side showing the inlet cube with heated capillary, connection to the gas chromatograph (GC), and C-trap housing with attached Orbitrap analyzer (behind).*

coupled to any evolved gas emission device, here a gas chromatograph. Figure 1 C gives a detailed impression of the ionization taking place in the C-trap by the UV-radiation and sample introduction from opposite sides.

For calibration and evaluation purposes a non-heated PEEK-capillary is mounted directly to the C-trap volume. Figure 2 A) depicts the REMPI spectrum for a calibration gas mixture (10 ppmv) composed of benzene, toluene, p-xylene, and 1,2,4-trimethylbenzene (BTX) introduced via the non-heated inlet capillary. Parts-per-million mass accuracy with resolving power of at least 180,000 and above 200,000 < m/z 100 is achieved with high signal-to-noise values. The single scan limit of detection can be estimated to the low ppbv level. The signal stability is mainly limited by the fluctuation of the laser power and is within ~10 % standard deviation for the total ion count (figure 2 B). Further long-term tests, acquiring data over several hours showed a stable ion yield, a requirement for the prototype to be operated during field campaigns. The exact mass drift of the system was found to be minimal and the same m/z-calibration, achieving ppm-accuracy, could be used for several weeks.

The evaluation of the gas chromatographic coupling via the heated capillary inlet was done by a variety of PAH standard mixtures and several complex petrochemical sample materials. In this respect, marine gas oil (MGO), heavy fuel oil (HFO), recycling pyrolysis oils based on tire and plastic waste, were analyzed. In particular shipping fuels, such as MGO and HFO, are of interest as they are a feed matrix for the respective ship engine aerosol emissions (Streibel *et al.*, 2016). Except for the high-boiling six-ring PAHs, all constituents of the PAH standard mixtures could be detected and the gas chromatographic coupling enabled a limit of detection in the low nanogram range (injected on column). Preliminary data analysis of the petrochemical materials revealed a reduced isobaric complexity compared to comparable sample materials analyzed by atmospheric pressure photoionization (APPI). This effect is mainly caused by the selective ionization of aromatic constituents drastically narrowing down the observed compositional space. Nonetheless, the majority of nominal masses showed several isobaric signals, exemplarily the O/CH4 mass split (36.4 mDa) should be mentioned here. At m/z 300-400 resolving power of at least 10,000 is required, easily achieved by the Orbitrap mass analyzer. Mass resolving power of above 150,000 at m/z 200 with attribution errors below 1 ppm were found. These findings point out the capability of the system of preventing chemical miss interpretation.

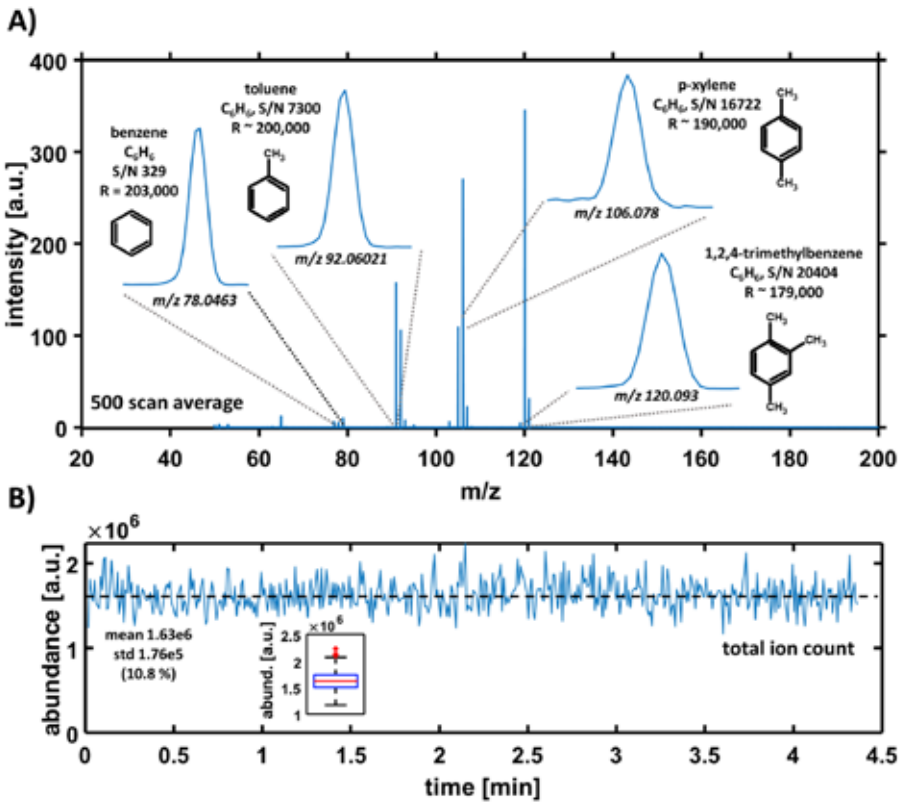

Figure 2: *A) REMPI spectrum at 266 nm for the calibration gas mixture (10 ppmv) introduced directly into the C-trap via a non-heated inlet capillary composed of benzene, toluene, p-xylene, and 1,2,4-trimethylbenzene (BTX). Parts-per-million mass accuracy with resolving power of at least 180,000 is achieved with high signal-to-noise values. The differences in relative intensity are caused by the different ionization cross sections. B) Signal stability of the BTX measurement for 500 scans. The standard deviation in the total ion count of below 11 % is due to the laser power stability.*

Conclusion and Outlook

A commercial Thermo Fisher Scientific Exactive Orbitrap high-resolution mass spectrometer was successfully reconstructed and vacuum resonance-enhanced multiphoton ionization was installed. The prototype system allowed for a rapid, sensitive, and selective description of the aromatic constituents. The limit of detection for BTX was estimated to be in the lower v-ppb level. The heated inlet enabled coupling of evolved gas analysis (gas chromatography) and detection of heavier components. The REMPI-Orbitrap-MS system will be further evaluated with different complex matrices introduced by the gas chromatographic coupling. Furthermore, the installation of other laser-systems, such as a Krypton-Fluoride-Excimer laser (248 nm) is foreseen. The PI-Orbitrap-MS instrument, developed in the framework enabling analytical technologies, will finally be utilized for the chemical description of carbonaceous aerosols. This will be performed either offline by evolved gas analysis, realized by coupling a newly developed filter thermal desorber unit, or the Thermal-Optical Carbon Analyzer (see Research Area 2, report (07)). An on-line filter sampling/desorption system for online in field-experiments is currently developed by the partner SMU Aerosol d.o.o. The parts-per-million mass accuracy and high-resolving power will add a further cornerstone to the PIMS, allowing to unravel the isobaric complexity. This is of particular interest if we extend the ionization to SPI. The final aim is to use the technology also in a combined laser desorption ionization and laser desorption/REMPI scheme in single-particle mass spectrometry (see Research Area 2, reports (03) and (04)), allowing to resolve, *e.g.*, isobaric aromatics and carbon clusters or inorganic species.

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(10) Hyper-fast gas chromatography photoionization mass spectrometry for the detection of secondary organic aerosol precursors from coniferous trees

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Oxidative gas to particle conversion of volatile organic compounds (VOC) is a fundamental atmospheric process and responsible for a substantial fraction of ambient particulate matter (PM). On a global scale, plants emit the vast majority of VOC, whereas anthropogenic VOC releases use to increase regionally with the degree of urbanization or industrialization. Among the biogenic VOC, the class of monoterpenes, structurally derived from two isoprene units, are the most abundant VOC species and predominantly emitted by coniferous plants (Pinophyta). In forests, ambient monoterpene concentrations may even reach 100 µg m^{-3}. Generally, monoterpenes react rapidly with atmospheric oxidants, including hydroxyl radicals, ozone or nitrate radicals, forming secondary organic aerosol (SOA). However, between the monoterpene isomers structural differences appear, such as the position of double bonds or the identity as a cyclic or aliphatic molecules, determining the individual chemical fate of a monoterpene and its atmospheric lifetime in the range of few minutes to three hours. Additionally, coniferous plants have their own monoterpene signature, which even depends on the season, age of the plant, part of the plant (needles or twigs) or insect infestation. Hence, the determination of biogenic VOC emission profiles is essential to understand SOA formation. This is particularly important as SOA is suspected to contribute significantly to the health effects of ambient respirable PM. The question, whether SOA from plant derived VOC is less toxic compared to SOA from anthropogenic sources is a key question of the Helmholtz International Lab aeroHEALTH.

Monoterpenes are appropriate targets in gas chromatography (GC), but common run times for GC are in the range of 30-60 min. Here we demonstrate a hyper-fast GC (fastGC; runtime < 1 min) method with single-photon ionization (SPI) time-of-flight mass spectrometry (TOFMS) for rapid resolution and detection of eight individual monoterpenes in needles of individual coniferous trees.

Setup of hyper-fast gas chromatography single-photon ionization time-of-flight mass spectrometry

The instrumental setup of the fastGC-SPI-TOFMS is based on the developments by Fischer *et al.* (2015) and Wohlfahrt *et al.* (2016), which has been extended by a 2-way-valve in order to decouple sample gas phase and GC carrier gas. VOCs are sampled through a heated transfer capillary inside the headspace unit and trapped on the loop of a Peltier modulator, which is cooled to -55 C, and thereby trapped on a GC column. After the sampling, the Peltier modulator heats to 300°C within 2 s, injecting the trapped VOCs with small peak width on the GC column of 3 m length. Due

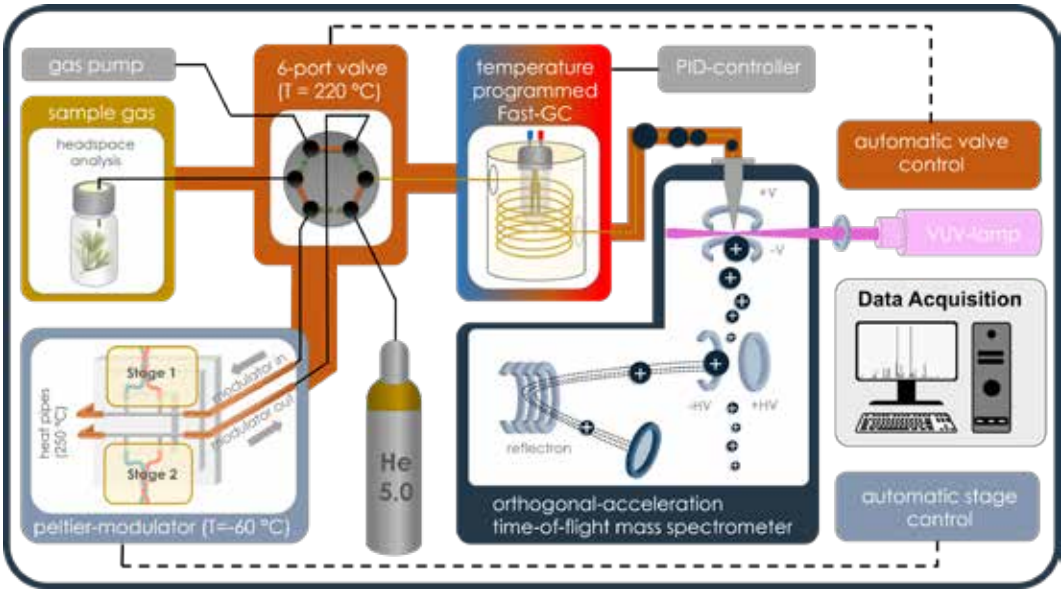

Figure 1: *Schematic setup of the hyper-fast GC-SPI-TOF-MS system for headspace analysis of needles with sampling unit, 6-port-2-way valve for changing to helium as GC carrier gas, Peltier modulator, optically heated fastGC, VUV-lamp for single-photon ionization (SPI) and oa-TOFMS.*

to two stages inside the Peltier modulator, VOCs can be continuously sampled and analyzed. The GC is flexibly heated by a halogen lamp, starting with 50 °C and increasing to 270 °C within the total fastGC run time of 25 s. At the end of the fastGC run, the fastGC column is cooled by blowing room air for 5 s. Monoterpenes are detected by TOFMS using a deuterium lamp with a peak wavelength at 124 nm for SPI.

Standard of monoterpene mixture

A monoterpene standard was prepared from α-pinene, β-pinene, Δ3-carene, α-terpinene, camphene, limonene, myrcene and γ-terpinene in methanol and injected in the fastGC. Peaks of all eight monoterpenes were separated and detected in less than 15 s (figure 2). However, the chromatographic resolution of α-terpinene and limonene could only be improved with the cost of other peak interferences, hence the temperature program was kept and these two individual monoterpenes were resolved by deconvolution of the mass spectra, exploiting differences in their fragmentation patterns. Therefore, we run non-negative matrix factorization (NMF) with a two-factor solution on the unresolved peak and sorted the two factors according to their maxima appearing before or after the maximum of the unresolved peak. The mean result from 100 NMF runs clearly reveals two individual peaks of α-terpinene and limonene as shown in the zoom-in for the retention time around 9 s. Additional confidence is obtained by the high correlations of > 0.99 of the NMF factor loadings and the pure spectra of α-terpinene and limonene.

Despite SPI being a soft ionization technique, generating for most analytes predominantly molecular ions, we may observe low to substantial fragmentation for the investigated rather labile terpenoids. Substituted six-rings containing two double bonds, such as α-terpinene and γ-terpinene, have the lowest fragmentation, whereas the only aliphatic monoterpene myrcene shows the lowest yield on molecular ions at m/z 136. However, the fragmentation patterns at m/z 121, 107, 94, 93, 92 and 80 could be used to identify the order of monoterpene elution.

Analysis of monoterpenes released from coniferous tree needles

To demonstrate applicability of the setup for environmental samples, we measured VOC emissions from four needles of coniferous trees by headspace analysis at room temperature. Pine (Black pine, Pinus nigra) and spruce (Serbian spruce, Picea omorika) are important trees in forestry and the dominating coniferous tree species in Europe. Black pine is also preferably grown in shelter belts. In contrast, wood from larch (European larch, Larix decidua) and yew (European yew, Taxus baccata) trees are the most valuable conifers due to high dense wood, but less abundant conifer species. In particular, recognized that yew trees account for the majority of coniferous trees in city parks ("Lindenpark", "Barnstorfer Wald" and "Wallanlagen"), back and front yards of Rostock. All monoterpenes were identified in the needle chromatograms based on their retention time and characteristic fragments at m/z 136, 121, 107, 94 93, 92 and 80 with a correlation coefficient of > 0.85.

The chromatograms of the four needles revealed apparent both qualitative and quantitative

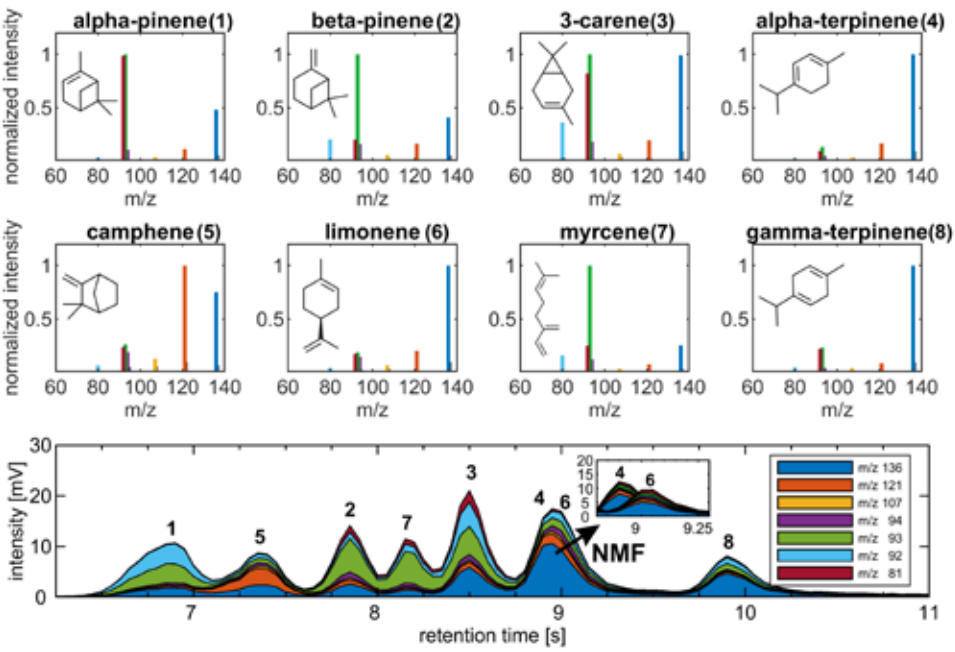

Figure 2: SPI mass spectra of eight individual monoterpenes (top) and chromatogram of the monoterpene mixture (bottom). The overlapping peaks of α-terpinene and limonene are deconvolved by non-negative matrix factorization (NMF) due to slight differences in fragmentation patterns.

differences (figure 3). Black pine emits apparently high amounts of α-pinene, accounting for > 85 % of the total monoterpenes. Furthermore, β-pinene, myrcene, limonene, 3-carene and despite high interfering peak of α-pinene also camphene were detected together with two unknown peaks with retention times at 9.45 s and 10.45 s. The same monoterpenes could be identified for Serbian spruce, but with highest intensity for limonene and an additional small peak for γ-terpinene. In contrast to Black pine, monoterpene did often account for the total intensity. Therefore, Serbian spruce must emit significant amounts of other VOCs. The most peaks before the first eluting monoterpene α-pinene could be identified for European larch. Regarding monoterpenes, again α-pinene was the dominating species, followed by myrcene, 3-carene and β-pinene. Minor intensities were obtained for camphene, limonene and γ-terpinene. Surprisingly, intensities were close to the limit of detection except α-pinene.

Peaks of retention times beyond the eight targeted monoterpenes showed highest correlations with the fragment-poor mass spectra of α-terpinene, γ-terpene and limonene. Thus, the mass spectral signature point towards monocyclic monoterpenes from the methane class, such as α-, β-phellandrene or terpinolene. The peak at retention time 9.45 s gives a clear contribution from the molecular monoterpene ion at m/z 136, but more substantial fragmentation, giving indication for an aliphatic or bicyclic monoterpene species.

We determined a limit of detection for monoterpenes of approximately 1 ppb based on the noise of 20 chromatograms and a ratio of signal to noise (S/N) of 3, which is better or competitive to other fastGC approaches with soft ionization mass spectrometers (Materic *et al.*, 2015; Lacko *et al.*, 2019). With 25 s the time for analysis is substantially shorter than reported before (80 s and 140 s). Finally, more peaks with higher S/N were obtained for the optically heated fastGC with Peltier modulator and SPI-TOFMS.

Application in online mass spectrometry for real-time analysis of VOCs

Beyond its application for rapid headspace analysis, we seek to equip online photoionization TOFMS with fastGC for enhanced chemical speciation of VOCs, but keep a beneficial level of time resolution. Such research fields at JMSC for which high time resolution is required are all types of combustion emissions, their atmospheric aging and process control for the production of foods and semi-luxury foods with health benefits, *e.g.* coffee with maximized content of polyphenolic antioxidants. Furthermore, the new fastGC system may improve our unique approach to analyze the composition of individual fractions in thermal-optical carbon analysis with photoionization TOFMS (Diab *et al.*, 2015). Together with our recent development for optical aerosol particle characterization, the extended setup may add knowledge to individual compounds of light-absorbing ("brown") carbon and will support the work in aeroHEALTH on health effects of SOA from single biogenic SOA precursors and natural toxic effects from plant-derived SOA from atmospheric oxidation of different monoterpene mixtures.

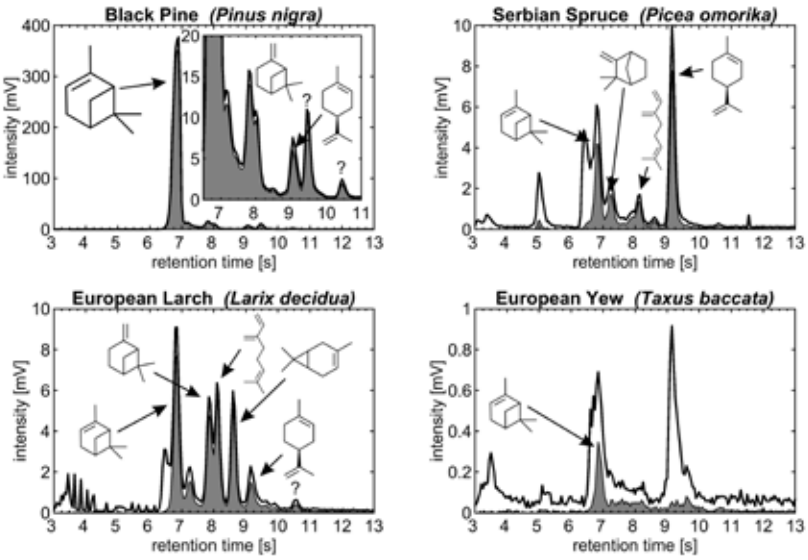

Figure 3: *fastGC chromatograms of accumulated monoterpene-related m/z 136, 121, 107, 94, 93, 92 and 80 (gray), of needles for four coniferous trees black pine, Serbian spruce, European larch and European yew, depicting the diversity of the SOA-precursor "cocktail" from different plant species. The additional white peak area represents the total measured intensity from SPI-TOFMS, giving evidence for the release of none-monoterpene VOCs from coniferous trees.*

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(11) Cyclic ion mobility spectrometry coupled to high-resolution time-of-flight mass spectrometry equipped with atmospheric solid analysis probe for the molecular characterization of combustion particulate matter

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The immense molecular complexity of particulate matter (PM) is a result of primary organic aerosol (POA) emitted from various sources as well as secondary organic aerosols (SOAs). The detailed analysis of the health effects of primary and secondary aerosols for the research focus in aerosol and health research in JMSC and the aeroHEALTH program requires an in-depth understanding of the molecular inventory of aerosols. For the molecular level characterization of carbonaceous aerosols, most often hyphenated chromatographic and mass spectrometric approaches are applied. On the one side, liquid chromatography requires a liquid aerosol extract but quartz fiber filter (QFF) extraction can be a tedious process with a high risk of contamination. On the other side, gas chromatography is inherently limited to volatilizable and temperature stable compounds and thus also in the accessible chemical space. In this context, direct inlet probe (DIP) techniques have proven to be a complementary approach for rapid complex mixture analysis of high-viscous or solid materials. Nonetheless, the isomeric information gained by chromatographic separation is lost deploying direct insertion high mass resolution MS techniques such as High-Resolution TOF mass spectrometry or Fourier Transform Ion Cyclotron Resonance mass spectrometry. Ion mobility is an approach that can help to characterize isobaric molecules based on structural properties in connection with mass spectrometry and thus reduce the drawbacks of the missing chromatographic separation. In the following study, the feasibility of a direct inlet approach (DIP) is in conjunction with ion mobility spectrometry- high-resolution mass spectrometric (IMS-HRMS) detection for characterization was evaluated. From the ion mobility drift time profiles, the collision cross-section (CCS) can be calculated, an intrinsic property of ions directly related to their structure. For the first time, an atmospheric solid analysis probe (ASAP, an atmospheric pressure DIP source) with ion mobility spectrometric detection for chemical characterization of combustion aerosols was applied (Rüger *et al.*, 2021). The particulate matter samples were generated during a measurement campaign at the University of Eastern Finland in 2018. In this study, fresh and aged PM emissions from a diesel generator and a logwood stove feed with spruce are investigated. Moreover, emissions from lignite combustion in the stove were analyzed. Aging was realized by a novel high-volume photochemical emission aging flow tube reactor (PEAR, Ihalainen *et al.* 2019). The aerosol particles were directly analyzed without any sample pretreatment. For this purpose, the PM is loaded to a glass capillary of a commercial Waters® atmospheric solid analysis probe (ASAP) by pressing the open side onto the brittle filter material (figure 1). This concept was introduced previously for the investigation of biomass pyrolysis products (Castilla *et al.* 2020). The evolved material was ionized by atmospheric pressure chemical ionization and analyzed by cyclic ion mobility high-resolution mass spectrometry. Details on the instrumental setup are given elsewhere (Giles *et al.*, 2019; Riches *et al.* 2019).

The approach was evaluated with non-aged fresh diesel emissions. Immediately after inserting the probe into the ionization volume, a high response was acquired, lasting for several minutes. Depending on the QFF aerosol loading the signal declined after 3-10 min. Constituents with higher volatility, such as pure hydrocarbons, are evaporated rapidly, whereas oxygenated species evolve with a delayed and more flat profile. The high-resolution mass spectrometric detection allows for elemental composition attribution with part per million mass accuracy. For the diesel, a repetitive pattern of CH_2 building blocks was found, characteristic for petroleum-related emissions. Several alkylated series of CH-class species dominate the spectrum belonging to double bond equivalents (DBE) of 4-7. Those series can be tentatively attributed to benzene and naphthenic derivatives.

In the following, the approach was used for discrimination of combustion sources and types (aged versus non-aged). Figure 2 depicts the DBE versus carbon number (#C) diagrams size-coded according to abundance and color-coded according the the ion mobility drift profile apex time. Diesel emissions revealed the broadest mass spectrometric profile, whereas the lignite and spruce emissions showed a less structured, i.e., less dominated by homolog series, mass spectrum with a narrower distribution. Regarding the isomeric diversity, measured by the ion mobility drift time profile, the lignite emission shows a lower isomeric diversity than the diesel. The spruce aerosol is structurally strongly linked to the lingo-cellulosic biomass. Hence, the isomeric diversity accessed based on the ion mobility peak width is comparatively low. The high-complex data can be visualized based on specific diagrams frequently used in high-resolution mass spectrometry, *e.g.*, figure 2. This representation nicely points out the fingerprinting capabilities bringing together the IMS

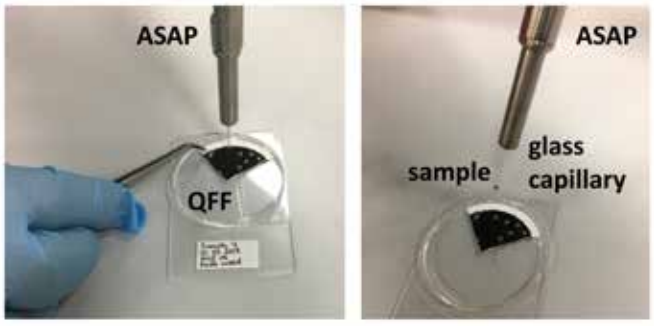

Figure 1: *Photographic description of the particulate matter sampling from quartz-fiber filters for deploying to the atmospheric solid analysis probe. A tiny aliquot of the homogenously sampled aerosol is loaded to a pre-cleaned glass capillary and immediately transferred to the ASAP probe and into the ionization chamber, preventing contamination, at the same time starting data re-cording.*

and MS data. For studying the effect of photochemical aging, a more targeted approach was chosen. The mass range was limited to m/z 250 ± 50 and the ion mobility resolution was increased by allowing the ions to separate in the cyclic device for three passes. Figure 3 displays three exemplary attributed elemental compositions with a clear change in the isomeric profile. $C_{18}H_{11}O_2$, most likely an oxidized fourring polycyclic aromatic ion species, revealed a strong increase for the complete isomeric profile. Other targets, such as pure PAHs are mostly affected in abundance rather than in the isomeric profile. Certain oxygenated species also drop in abundance ($C_{21}H_{11}O_1$). Highly oxidized aromatics resulted from the aerosol aging are most likely not detectable with the heated probe due to thermal decomposition or low vapor pressure, a drawback of the evolved gas analysis concept. Nonetheless, the more targeted approach with increased ion mobility resolution is able to separate groups of isomers and, thus, address changes in the molecular structure directly within minutes.

Conclusion and Outlook

ASAP with ion mobility separation and high-resolution mass spectrometric (ASAP IMS-MS) detection allowed for rapid chemical characterization of combustion aerosols with almost no sample preparation. The approach serves as fingerprinting techniques and is able to distinguish aerosol emissions sources and types (aged versus non-aged). Isobaric and isomeric differences can be directly addressed on the molecular level. Important molecular species such as PAHs and oxygenated PAHs can be monitored. Hence, ASAP IMS-MS is a promising technology for a rapid as-sessment of a high number of PM samples with respect to their general molecular composition and the presence of toxicological relevant compounds and thus may help in solving challenges in environmental health and climate change research.

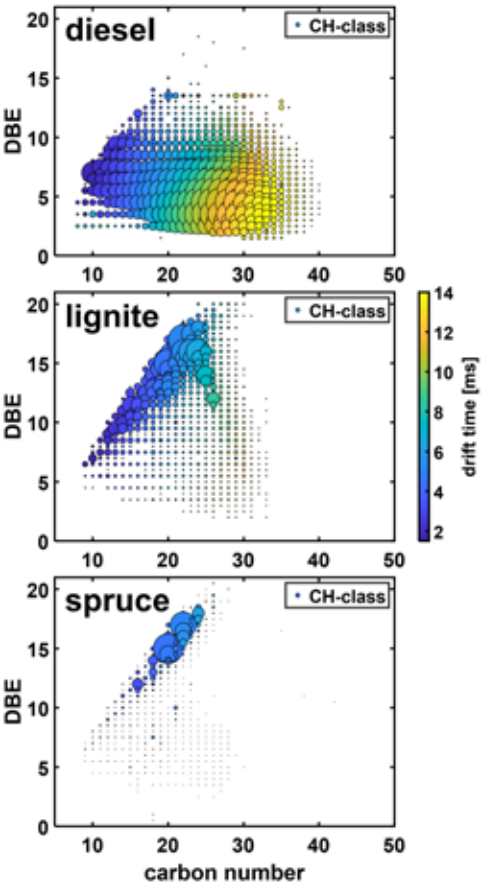

Figure 2: Molecular responses of the direct inlet probe approach given as carbon number versus DBE (CH-class) for the diesel, lignite, and spruce emissions. The apex peak position of the drift time (ion mobility response) is deployed for color-coding, whereas the size is coded according to the summed abundance of the respective elemental composition.

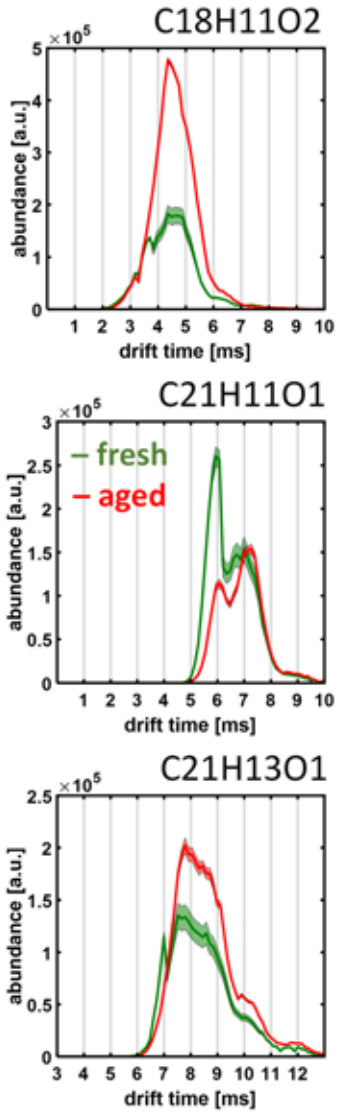

Figure 3: Selected drift time profiles based on species found in fresh (green) and aged (red) spruce emissions. Differences in the isomeric profile can be depicted. The shaded area is giving the standard deviation of three technical replicates.

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[&] *TOT: Total Refining and Chemicals, France, Belgium*
[§] *WAT: Waters Corporation, United Kingdom*
[#] *UEF: University of eatsren Finland, Finland*
[$] *URN: University of Rouen-Normandy, France*

3.3 Cooperation Partners

Industrial Partners

Aerosol d.o.o.
SI-1000 Ljubljana, Slovenia
Dr. Martin Rigler, Dr. Asta Gregorič
www.aerosol.si

Airsense Analytics
Schwerin, Germany
W. Münchmeyer, Dr. A. Walte
www.airsense.com

ASG Analytik-Service Gesellschaft mbH
Neusäß, Germany
Dr. T. Wilharm
www.asg-analytik.de

AVL Deutschland GmbH
Mainz-Kastel, Germany
S. Seidl
www.avl.com

Azul Kaffee GmbH & Co. KG
Bremen, Germany
Jörg Biess
www.azul.de

Biodevice Systems sro.
Praha, Czech Republic
Dr. V. Goranov
www.biodevicesystems.com

Bruker Corporation
Billerica (MA), USA
Dr. Mark Ridgeway
www.bruker.com

CASC4DE
Strasbourg, France
Marc-André Delsuc
www.casc4de.eu

Centrum Techniki Morskiej (CTM)
Gdynia, Poland
Dr. Izabela Kazmierczak
www.ctm.gdynia.pl

C^3 – Carbon Concrete Composite e. V.
Dresden, Germany
Dr. – Ing. M. Lieboldt
www.bauen-neu-denken.de

Deutsche Post AG
Bonn, Germany
Johannes Abresch
www.dpdhl.com

Decodon GmbH
Greifswald, Germany
M. Kolbe
www.decodon.com

Eberspächer Climate Control Systems GmbH & Co. KG
Esslingen, Germany
Dipl.-Ing. W. Pfister
www.eberspaecher.com

Environmental & Marine Engineering
Applied Development & Innovation
Zwijndrecht, Belgium – D. Durt
www.deme-group.com

ERC Additiv GmbH
Buchholz, Germany
Dr. M. Müller
www.erc-online.de/erc-additiv

Fasmatech Science and Technology
Athens, Greece
Dr. Dimitris Papanastasiou
www.fasmatech.com

Flughafen Rostock-Laage GmbH
Laage, Germany
Dörthe Hausmann
www.rostock-airport.de

GEA Westfalia Separator Group GmbH
Oelde, Germany
Heiko Sims
www.gea.com

GRIMM Aerosol Technik Ainring GmbH & Co. KG
Ainring, Germany
M. Pesch
www.grimm-aerosol.com

Guangzhou Hexin Instrument Co., Ltd
Guangzhou, China
Dr. Zhen Zhou
www.tofms.net

HansaBioMed Life Science Ltd
Tallin, Estonia
Dr. N. Zarovni
www.hansabiomed.eu

ICHTHYOL-GESELLSCHAFT, Cordes, Hermanni & Co. (GmbH & Co.) KG
Hamburg, Germany
Dr. G. Gayko
www.ichthyol.de/de/home/

Innolas Laser GmbH
Krailling, Germany
Dr. Christian Menhard
www.innolas-laser.com

Lablicate GmbH
Hamburg, Germany
Dr. P. Wenig
www.lablicate.com

LECO Instruments
Mönchengladbach, Germany
Dr. R. Löscher
www.leco-europe.com

Lohmeyer GmbH
Karlsruhe, Germany
Dr. Ingo Düring
www.lohmeyer.de

MAN Energy Solutions SE
Advanced Engineering & Exhaust Aftertreatment
Augsburg, Germany
Dr. D. Struckmeier
www.man-es.com

MLase AG
Germering, Gemany
J. Junger, T. Dickenhorst, J. Piel
www.mlase.de

ParteQ GmbH
Malsch, Germany
M. Seipenbusch
www.parteq.net

PROBAT-Werke von Gimborn Maschinenfabrik GmbH
Emmerich am Rhein, Germany
T. Koziorowski
www.probat.com

Rigenerand srl
Medolla (MO), Italy
Dr. M. Dominici
www.rigenerand.it

RVT Process Equipment GmbH
Steinwiesen, Germany
Dr. Christian Geipel
www.rvtpe.com

SAACKE GmbH
Bremen, Germany
Niels Homburg
www.saacke.com

SABIC
Riad, Saudi Arabia
F. Cuoq
www.sabic.com/corporate/en/

SICK Engineering GmbH Dresden
Ottendorf-Okrilla
Kai Kindler
www.sick.com

Scientific Instruments Manufacturer GmbH
Oberhausen, Germany
Rolf Eichelberg
www.sim-gmbh.de

Spectroswiss SARL
Lausanne, Switzerland
Dr. Yury Tsybin
www.spectroswiss.ch

SULT GmbH
Nufringen, Germany
Harald Schlosser
www.sult.de

TAB GmbH
Barth, Germany
Peter Thum, Holger Keuer
www.tab-barth.de

The J.M. Smucker Company
Schwerin, Germany; Orwell, OH, USA
Dr. J. Howell
www.smuckers.com

Thermo Fisher Scientific
Bremen, Germany
Dr. Alexander Makarov, Dr. Kyle Fort
www.thermofisher.com

Topas GmbH
Dresden, Germany
A. Rudolph, S. Große
www.topas-gmbh.de

TOTAL GmbH
Berlin, Germany
Dr. U. Mayer
www.total.de

Harfleur, France
Dr. Pierre Giusti

Seneffe-Feluy, Belgium
Dr. Gaëtan Burnens
www.total.com/en

Vitrocell Systems GmbH
Waldkirch, Germany
T. Krebs, M. Berger
www.vitrocell.com

VUV Analytics
Cedar Park, USA
Dr. D. Harrison
www.vuvanalytics.com

Waters Corporation
Wilmslow, United Kingdom
Dr. Eleanor Riches
Dr. Martin Palmer
Dr. Martin Wells
www.waters.com

International and National Research Institutions

Bifa Environmental Institute
Augsburg, Germany
Prof. Dr. W. Rommel
www.bifa.de

Center for Colloid and Surface Science
Firenze, Italy
Prof. Dr. P. Bergese
www.csgi.unifi.it

Desert Research Institute
Division of Atmospheric Sciences
Reno, Nevada, USA
Prof. Dr. J. C. Chow, Dr. D. Lowenthal
www.dri.edu/das

iC2MC – International Complex Matrices Molecular Characterization
Harfleur, France
Dr. P. Giusti, Prof. Dr. C. Afonso, Prof. Dr. B. Bouyssiere, Dr. R. Rodgers
c2mclab.wordpress.com

Finnish Institute for Health and Welfare
Helsinki, Finland
Prof. Dr. O. Hanninen
www.thl.fi

Forschungszentrum Lausich GmbH
Institute for Energy and Climate Research IEK-8: Troposphere
Prof. A. Kiendler-Scharr
www.fz-juelich.de/iek/iek-8/

Fraunhofer Research Institution for Large Structures in Production Engineering IGP
Rostock, Germany
Dr. Nikolai Glück
www.igp.fraunhofer.de/en.html

German Society for Petroleum and Coal Science and Technology (DGMK)
Hamburg, Germany
Dipl.-Ing. J. Ludzay
www.dgmk.de

GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel
Prof. Dr. E. Achterberg
www.geomar.de/forschen/fb2/fb2-ch/arbeitsgruppen/team-water-column-biogeochemistry

Helmholtz-Zentrum Geesthacht Zentrum für Material- und Küstenforschung
Chemistry Transport Modelling
Dr. Volker Matthias
www.hzg.de/institutes_platforms/coastal_research/biogeochemistry_in_coastal_seas/chemistry_transport_modelling/index.php.en

Helmholtz Zentrum München
Munich, Germany

Institute of Allergy Research (IAF)
Prof. Dr. C. Schmidt-Weber
www.helmholtz-muenchen.de/en/institute-of-allergy-research

Institute of Asthma and Allergy Prevention (IAP)
Prof. Dr. E. von Mutius,
www.helmholtz-muenchen.de/en/iap

Institute of Computational Biology (ICB)
Prof. Dr. F. Theis
www.helmholtz-muenchen.de/icb

Institute of Environmental Medicine (IEM)
Prof. Dr. C. Traidl-Hoffmann
www.helmholtz-muenchen.de/en/institute-of-environmental-medicine/

Institute of Epidemiology (EPI)
Prof. Dr. A. Peters, Dr. J. Cyrys
www.helmholtz-muenchen.de/epi

Institute of Lung Biology and Disease (iLBD)
Dr. A. Önder Yildirim
www.helmholtz-muenchen.de/ilbd

Genome Analysis Center (GAC)
Prof. Dr. J. Adamsky/ Dr. C. Prehn
www.helmholtz-muenchen.de/gac

IVL Svenska Miljöinstitut
Gothenburg, Sweden
Dr. J. Moldanova
www.ivl.se

Institute for Heat and Oil Technique (IWO)
Hamburg, Germany
Dipl.-Ing. oec. L. Lucks
www.zukunftsheizen.de

Institute of Oceanology
Polish Academy of Sciences
Department of Marine Chemistry and Biochemistry (IOPAN)
Sopot, Poland
Dr. hab. J. Beldkowski
www.iopan.pl

Karlsruhe Institute of Technology (KIT)
Karlsruhe, Germany

Institute of Catalysis Research and Technology (IKFT)
Prof. Dr. E. Dinjus, PD. Dr. N. Dahmen
www.itc-cpv.kit.edu/english/index.php

Institute of Toxicology and Genetics (ITG)
Dr. S. Diabaté, Prof. Dr. U. Strähle, Dr. C. Weiss
www.itg.kit.edu/index.php

Technology for Pervasive Computing (TECO)
Dr. T. Riedel
www.teco.kit.edu/

Institute of Technical Chemistry (ITC)
Eggenstein-Leopoldshafen, Germany
S. Mülhopt
www.itc-tab.kit.edu

Institute of Meteorology and Climate Research
Garmisch-Partenkirchen, Germany
Prof. Dr. S. Emeis
www.imk-ifu.kit.edu/english/index.php

Leibnitz Institute for Baltic Sea Research Warnemünde
Warnemünde, Germany
Prof. Dr. D. Schulz-Bull
www.io-warnemuende.de

Leibnitz Institute for Tropospheric Research
Leipzig, Germany
Prof. Dr. Hr. Herrmann, Prof. Dr. A. Wiedensohler
www.tropos.de

Luxemburg Institute of Health
Quantitative Biology Unit
Strassen, Luxemburg
Dr. G. Dittmar
www.lih.lu

Max Delbrück Center for Molecular Medicine (MDC)
Berlin-Buch, Germany
Dr. Philipp Mertins
www.mdc-berlin.de/en/index.html

Max-Planck Institute for Polymer Research
Mainz, Germany
Prof. Dr. K. Landfester
www.mpip-mainz.mpg.de/en/home

National High Magnetic Field Laboratory
Tallahassee (FL), USA
Dr. Ryan Rodgers, Dr. Martha Liliana Chacon Patino
https://nationalmaglab.org/

National Research Council of Italy
Roma, Italy
Dr. A. Bongiovanni
www.cnr.it

Norwegian Institute of Public Health
Oslo, Norway
Prof. Dr. J. Øvrevik
www.fhi.no

Oel-Waerme-Institut GmbH
Herzogenrath, Germany
Dr.-Ing. K. Lucka, Dr.-Ing. R. Edenhofer
www.owi-aachen.de

Paul Scherrer Institute
Laboratory of Atmospheric Chemistry
Villingen, Switzerland
Prof. Dr. U. Baltensperger, Dr. A. Prévôt, Dr. E. A. Bruns
www.psi.ch/lac

Skolkovo Institute of Science and Technology (Skoltech)
Mass Spectrometry Laboratory
Moscow, Russia
Prof. Dr. Evgeny Nikolaev, Dr. Alexander Zherebker
www.skoltech.ru/massspeclab/

Swiss Federal Laboratories for Materials Science and Technology (EMPA)
Laboratory of Air Pollution and Environmental Technology
Dübendorf, Switzerland
Dr. Simone M. Pieber
www.empa.ch/de/web/s503/team

Synchrotron-light for Experimental Science and Applications in the Middle East
SESAME
Allan, Jordan
Prof. Dr. A. Lausi
www.sesame-org.jo

Techniologie und Förderzentrum
Straubing, Germany
Dr. H. Hartmann, Dr. E. Remmele
www.tfz.bayern.de

V. E. Zuev Institute of Atmospheric Optics (IAO)
Russian Academy of Science Siberian Branch
Tomsk, Russia
Dr. Valerii S. Kozlov, Dr. Michail Panchenko
www.iao.ru/en

Weizmann Institute of Science
Earth and Planetary Science
Rehovot, Israel
Prof. Dr. Yinon Rudich
www.weizmann.ac.il/EPS/Rudich/home

National and International Universities

Cardiff School of Biosciences
Cardiff, United Kingdom
Prof. Dr. K. BéruBé
www.cardiff.ac.uk/biosi

Chalmers University of Technology
Gothenburg, Sweden
Prof. Johan Mellqvist
www.chalmers.se/en/staff/Pages/johan-mellqvist.aspx

Freie Universität Berlin
Berlin, Germany
Prof. Dr. K. Lips
www.fu-berlin.de

Friedrich-Alexander University Erlangen-Nuremberg
Institute and Polyclinic for Occupational, Social and Environmental Medicine
Erlangen, Germany
Prof. Dr. H. Drechsler, Prof Dr. S. Schmitz-Spanke
www.ipasum.med.fau.de

Hong Kong Polytechnic University
Research Centre for Environmental Technology & Management Department
Kowloon, Hongkong, China
Prof. (Frank) Shung Cheng Lee
www.cse.polyu.edu.hk/04environmental.php

Department of Civil & Environmental Engineering
Kowloon, Hongkong, China

Prof. Xiangdong Li
www.polyu.edu.hk/cee/web/lixiangdong

Institute of Technology Sligo
Sligo, Ireland
Dr. N. Touzet
www.itsligo.ie

Ludwig Maximilian University, Munich
Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, Munich, Germany
Prof. Dr. D. Nowak, Dr. S. Karrasch
www.klinikum.uni-muenchen.de/Institut-und-Poliklinik-fuer-Arbeits-Sozial-und-Umweltmedizin/en/index.html

Moscow State University (MSU)
Skobeltsyn Institute of Nuclear Physics
Moscow, Russian Federation
Prof. Dr. Olga Popovicheva
www.sinp.msu.ru/en/users/260

Örebro University
School of Science and Technology
Prof. Tuulia Hyötyläinen
www.oru.se

Swiss Federal Institute of Technology (ETH)
Zurich, Switzerland
Prof. Dr. P. Arosio
www.ethz.ch

Technical University of Berlin
Institute of Environmental Technology
Prof. A. Held
www.uc.tu-berlin.de/menue/ueber_uns/team/

Technical University of Dresden
Arbeitsgruppe Mechanische Verfahrenstechnik (MVT)
Dresden, Germany
Dr.-Ing. L. Hillemann
www.tu-dresden.de

Institut für Baubetriebswesen (IBB)
Dresden, Germany
Dipl.-Ing. C. Weller
www.tu-dresden.de

Technical University of Munich
Center of Allergy & Environment – Zentrum Allergie & Umwelt (ZAUM)
Munich, Germany
Prof. Dr. J. Buters, R. Effner
www.zaum-online.de

Chair of Analytical Chemistry and Water Chemistry
Prof. Dr. M. Elstner
www.ws.chemie.tu-muenchen.de

Physics Department
Garching bei München
PD Dr. A. Ulrich
www.ph.tum.de

Research group remote sensing of trace gases
Munich, Germany
Prof. Dr. M. Wenig
www.meteo.physik.uni-muenchen.de/~mark.wenig/

University of Augsburg
Augsburg, Germany
Chair of Resource Strategy
Prof. Dr. A. Reller
www.mrm.uni-augsburg.de/de/gruppen/reller/

Institute of Geography
Physical Geography and Quantitative Methods
PD Dr. A. Philipp
www.geo.uni-augsburg.de/en/chairs_professorships/phygeo/

Univerität Basel
Departement Umweltwissenschaften
Prof. Markus Kalberer
https://duw.unibas.ch/en/atmospheric-sciences/

Universitätsklinikum Würzburg
Würzburg, Germany
apl. Prof. Dr. med. Stephan Hackenberg
www.ukw.de/en

University Bayreuth
Bayreuth center of Ecology and Environmental Research
Bayreuth, Germany
Prof. Dr. A. Nölscher, Dr. S. Kernchen
www.bayceer.uni-bayreuth.de

University of California, Merced
School of Engineering
Prof. Rogge
engineering.ucmerced.edu

University of California, Davis
Department of Chemistry
Davis, California, USA
Prof. P. B. Kelly
www.chem.ucdavis.edu

University of Copenhagen
Department of Plant and Environmental Sciences
Copenhagen, Denmark
Prof. Dr. Jan Christensen
www.ku.dk/english/

University of Eastern Finland (UEF)
Inhalation Toxicology Laboratory
Kuopio, Finland
Prof. Dr. M.-R. Hirvonen
www.uef.fi/intola

Fine Particle and Aerosol Technology Laboratory
Kuopio, Finland
Dr. O. Sippula, Prof. Dr. J. Jokiniemi
Dr. P. Tiitta
www.uef.fi/en/web/fine

Aerosol Physics Research Group
Kuopio, Finland
Dr. A. Buchholz
https://uefconnect.uef.fi/en/group/aerosol-physics-research-group/

Faculty of Science and Forestry, Department of Chemistry
Joensuu, Finland
Prof. Dr. Janne Jänis, Dr. Timo Kekäläinen
https://uefconnect.uef.fi/en/group/mass-spectrometry-and-bioanalytical-chemistry-research-group/

University of Fribourg
Fribourg, Switzerland
Prof. Dr. B. Rothen-Rutishauser
www.unifr.ch

Universitätsklinikum Freiburg
Forensic Toxicology
Freiburg, Germany
Prof. V. Auwärter
www.uniklinik-freiburg.de/

University of Liege
Faculté des Sciences
Département de chimie (sciences)
Chimie analytique, organique et biologique
Molecular Systems (MolSys)
Dr. B. Weggler
www.uliege.be

University of Messina
Messina, Italy

Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali Department
Prof. P. Dugo
www.sepsci.unime.it/en/

Facoltà di Farmacia, Dipartimento Farmaco-Chimico
Prof. L. Mondello
www.farmacia.unime.it

University of Luxembourg
Luxembourg Centre for Systems Biomedicine
Esch-Belval, Luxembourg
Dr. K. Hiller
wwwen.uni.lu/

University of Mainz
Institute for Inorganic and Analytical Chemistry
Prof. Dr. T. Hoffmann
www.ak-hoffmann.chemie.uni-mainz.de

University of Nevada, Las Vegas
Environmental and Occupational Health
Las Vegas, NV, USA
Prof. Dr. L. W.-A. Chen
www.unlv.edu/people/lung-wen-chen

University of Potsdam
Potsdam, Germany
Prof. Dr. A. Föhlisch
www.uni-potsdam.de

University of Pretoria
Department of Chemistry
Pretoria, South Africa
Prof. Dr. E. Rohwer, Dr. P. Forbes
www.up.ac.za/chemistry

University of Rouen
Mont Saint Aignan, France
Prof. Dr. Carlos Afonso, Dr. Julien Maillard
www.univ-rouen.fr/university-of-rouen-normandy/

University of Rostock
Rostock, Germany

Department of Chemistry
Prof. Dr. U. Kragl, Prof. Dr. R. Ludwig
www.chemie.uni-rostock.de/en/

Department of Physics
Prof. Dr. O. Kühn, Dr. S. Bokarev
web.physik.uni-rostock.de/quantendynamik/index.html

Department of Piston Engines and Internal Combustion Engines
Prof. Dr.-Ing. B. Buchholz,
Dr. U. Schümann, Dipl.-Ing. C. Fink,
Dipl.-Ing B. Stengel
www.lkv.uni-rostock.de/en

University of Santiago de Compostela
Santiago de Compostela, Spain
Prof. Dr. J. Rivas Rey
www.usc.es

University of Warwick
Coventry, Great Britain
Prof. Dr. P. O'Connor
warwick.ac.uk/fac/sci/chemistry/research/oconnor/oconnorgroup

Dr. M. Barrow
warwick.ac.uk/fac/sci/chemistry/research/barrow/barrowgroup

Public and State Institutions

Aerosol academy e.V.
Neuherberg, Germany
H. Grimm
www.aerosol-akademie.de

Augsburg City
Augsburg, Germany
T. Gratza
www.augsburg.de

BAUA, Bundesanstalt für Arbeitsschutz und Arbeitsmedizin
Gruppe 4.4 "Gefahrstoffmessungen"
Dr. G. Dragan
www.baua.de/DE/Die-BAuA/Organisation/Fachbereich-4/Gruppe-4-4.html

Bavarian Environment Agency (Bayerisches Landesamt für Umwelt)
Augsburg, Germany
Dr.-Ing. C. Marb, Dr. W. Reifenhäuser
www.lfu.bayern.de/index.htm

Bavarian Research Foundation
Munich, Germany
Prof. Dr. S. Mayer
www.forschungsstiftung.de

Bundesamt für Seeschifffahrt und Hydrographie
Hamburg
Dr. Andreas Weigelt
www.bsh.de/EN/TOPICS/Shipping/Environment_and_shipping/environment_and_shipping_node.html

Bundeswehr Research Institute for Protective Technologies and CBRN Protection (WIS)
Munster, Germany
Dr. R. Hetzer
www.baainbw.de/portal/poc/baain?uri=ci%3Abw.baain.diensts.wis

Bundeszollverwaltung
Bonn, Germany
Ramona Daesler
www.zoll.de

Federal Criminal Police Office (BKA)
Wiesbaden, Germany
Dr. M. Pütz
www.bka.de

Federal Environment Agency Germany (Umweltbundesamt)
Global Atmosphere Watch (GAW) – Globale Überwachung der Atmosphäre
Garmisch-Partenkirchen, Germany
Dr. L. Ries
www.umweltbundesamt.de

Federal Institute for Materials Research and Testing (BAM)
Berlin, Germany
Dr. K. Urban
www.bam.de

Federal Office of Civil Protection and Disaster Assistance (BBK)
Bonn, Germany
M. Drobig
www.bbk.bund.de

Fire brigade Frankfurt (FF)
Frankfurt, Germany
Dr. U. Annel
www.feuerwehr-frankfurt.de

IFA, Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung
Berlin, Germany
Prof. Dr. D. Breuer
www.dguv.de/ifa/wir-ueber-uns/organisation-des-ifa/fachbereich-2/index.jsp

Institute for Fire and Disaster Prevention Heyrothsberge (IBK)
Berlin, Germany
Dr. Jan Voigt, Frank Schuppe
www.inneres.sachsen-anhalt.de/bks-heyrothsberge/bks_neu/

Marinekommando
Rostock, Deutschland
TRDir M. Potschantek
www.Deutsche-Marine.de

State Office of Criminal Investigations (LKA)
Berlin, Germany
S. Rosenkranz
www.berlin.de/polizei/dienststellen/landeskriminalamt/

Mainz, Germany
Dr. S. Zörntlein
www.polizei.rlp.de/de/startseite/

3.4 Third Party Funding

3.4.1 List of Funded Third Party Projects of the JMSC (January 2018 – December 2020)

In the following list, the running and newly acquired third party projects of JMSC in the reporting period are tabulated. Externally (co-)funded projects with a funding sum above 50.000 € are represented as short, one-page project profiles in the section Third Party Projects Short Profiles.

Funding Institution	Grant period	Project Title (Number of short presentation)	Funding amount
European Commission	10/2021 – 09/2025	ULTRHAS – ULtrafine Particles from Transportation – Health Assessment of Sources (01)	782 170 € (HMGU) 698 172 € (UR) 458 000 € (UniBW M)
European Commission (ERA-NET-Cofund MarTERA)	09/2021 – 08/2024	AMMOTRACe – Ammunition exploration by surface- and underwater-based laser mass spectrometric tracing technology (02)	225 000 € (UR)
Deutscher Akademischer Austauschdienst (DAAD)	07/2021 – 06/2024 *	DUST-STORM initiative – Inhalable airborne desert DUST: A comprehensive Study On physical micro-structure, chemical composition and Respiratory toxicity of fine Mineral and anthropogenic dust (03)	~ 55 000 € (UR)
Deutsche Forschungsgemeinschaft (DFG), Agence Nationale De La Recherche (ANR)	03/2021 – 02/2024	TIMSAC – Thermal analysis and ion mobility coupled to high-resolution mass spectrometry for organic aerosol characterization (04)	236 850 € (UR)
Bundesministerium für Wirtschaft und Energie (BMWi)	06/2021 – 12/2023	SEP-3AP – Ship Emission Profiler – Advanced Analyzer for Aerosol Particles (05)	219 528 € (UR)
Deutsche Bundesregierung	01/2021 – 12/2024	LUKAS – Mobile air pollutant warning system for health, environmental & disaster protection by real-time monitoring of atmospheric aerosols including pollutant source localization (06)	3 900 000 € (UniBW M)
Bundesministerium für Bildung und Forschung (BMBF)	01/2021 – 12/2023	HazarDust – Echtzeitdetektion toxischer Substanzen in Staubpartikeln zur Identifikation sicherheitsrelevanter Inhalte aus Sendungen und Gepäckstücken (07)	542 502 € (UR)
Europäische Kommission (EU HORIZON 2020)	11/2020 – 10/2024	BOW – Biogenic Organotropic Wetsuits (08)	342 591 € (HMGU)
Bayerisches Staatsministerium für Umwelt und Verbraucherschutz (StMUV)	11/2020 – 10/2023	Methodenoptimierung zur chemischen Analyse von UFP (09)	203 895 € (HMGU)
Bayerisches Staatsministerium für Umwelt und Verbraucherschutz (StMUV)	11/2020 – 10/2023	Biologische Antwort auf Partikel in einem Lungenmodell (10)	416 018 € (HMGU)
Deutsche Bundesregierung	10/2020 – 12/2024	Munich Mobility Research Campus (MORE): Implementation of a model campus for future mobility at the Universität der Bundeswehr München (11)	4 000 000 € (UniBW M)
Arbeitsgemeinschaft industrieller Forschungsvereinigungen (AiF) über Deutsche Gesellschaft für Erdöl, Erdgas und Kohle e.V. (DGMK)	10/2020 – 03/2023	Einfluss der Brennstoffzusammensetzung auf die Rückstandsbildung bei der Verdampfung flüssiger Brennstoffe in porösen Medien (12)	200 611 € (UR)

Funding Institution	Grant period	Project Title (Number of short presentation)	Funding amount
Ichthyol-Gesellschaft	10/2020 – 09/2023	Untersuchung der molekularen Zusammensetzung von Ölschieferdestillaten und deren sulfonierter Produkte mittels multidimensionaler Chromatographie und hochauflösender Massenspektrometrie (13)	420 000 € (UR)
Deutsche Forschungsgemeinschaft (DFG)	10/2020 – 09/2023	IMPAERO – An interdisciplinary study on the impact of aerosolized particulate matter from aged wildfire plumes on environment and human health (14)	334 200 € (UR)
Bundesministerium für Wirtschaft und Energie (ZIM Kooperationsprojekte)	10/2020 – 03/2023	Development of a mobile and full automated measurement system for the simultaneous chemical analysis of organic compounds in the gas phase and particulate matter of environmental aerosols. (15)	219 000 € (UniBW M)
Bundesministerium für Wirtschaft und Energie (ZIM Kooperationsprojekte)	12/2019 – 11/2021	ALIAS-LUNG – Stabilisierung der Zellsysteme für Langzeitexposition und Durchführung biologischer Messreihen (16)	190 000 € (HMGU)
Bundesministerium für Wirtschaft und Energie (BMWi)	06/2019 – 05/2022	SAARUS – Optimierung der Scrubber-Abgaswäsche Technologie zur Reduktion von Schiffsemissionen (17)	567 336 € (UR) 562 000 € (UniBW M)
Europäischer Fonds für regionale Entwicklung (EU-EFRE) via Technologie Beratungsinstitut GmbH (TBI)	06/2019 – 05/2022	Chemische Charakterisierung und toxikologische Untersuchung für Thermolysereaktor (18)	382 110 € (UR)
Helmholtz-Gemeinschaft e.V. Impuls- und Vernetzungsfonds	04/2019 – 03/2024	aeroHEALTH – Impact of Atmospheric Aersols on Human Health (19)	1 000 000 € (HMGU)
Deutsche Gesetzliche Unfallversicherung e.V.	02/2019 – 01/2022	Gas-Partikelmischungen am Arbeitsplatz (20)	594 180 € (HMGU)
Bundesministerium für Bildung und Forschung (BMBF)	08/2018 – 07/2020	Carbon Concrete Composite C^3 – V3.2: Gesundheit II: TP1: Exponierung von Lungenzellen mit Stäuben aus der thermischen und mechanischen Belastung von Carbonbetonmaterialien (21)	370 320 € (UR)
Europäische Kommission (EU HORIZON 2020 Eurostars) via Bundesministerium für Bildung und Forschung (BMBF)	07/2018 – 06/2021	Verbundprojekt: Aerosol Orbitrap-Massenspektrometrie mit weicher Photoionisierung (AerOrbi); Teilprojekt: Systementwicklung und -aufbau sowie Evaluation und Testung in Labor- und Feldexperimenten (22)	234 213 € (UR)
Danube Strategic Project Fund (DSPF)	01/2018 – 01/2019	CONSPIRO – Breathing together: Decreasing Air Pollution from Local Heating Systems	9 777 € (HMGU)
Europäische Kommission (EU HORIZON 2020), INFRAIA	01/2018 – 12/2021	EU FT-ICR MS – European Network of Fourier-Transform Ion-Cyclotron-Resonance Mass Spectrometry Centers (23)	410 285 € (UR)
Deutsche Forschungsgemeinschaft (DFG)	10/2017 – 12/2020	Kopplung von schneller Gaschromatographie an Photoionisierungsmassenspektrometrie (24)	313 500 € (UR)
Bundesministerium für Bildung und Forschung (BMBF)	07/2017 – 02/2020	PPK – Prozessanalyse und –steuerung der industriellen Röstung von Lebens- und Genussmitteln mittels Photoionisationsmassenspektrometrie am Beispiel von Kaffee (25)	210 000 € (UR)
Zentrales Informationsprogramm Mittelstand (AiF/ZIM)	06/2017 – 02/2020	Entwicklung eines alltagstauglichen und mobilen Messgerätes zur Echtzeit-Untersuchung von einzelnen Nanopartikeln (Feinstäuben) hinsichtlich ihrer detaillierten chemischen Zusammensetzung und ihrer Partikelgröße (26)	106 470 € (UR)

Funding Institution	Grant period	Project Title (Number of short presentation)	Funding amount
Sabic Global Technologies BV	06/2017 – 05/2019	Quantification of Diels-Alder fouling vs. Radical fouling using TGA/DSC/PI-MS	60 000 € (UR)
Bundesministerium für Bildung und Forschung (BMBF)	05/2017 – 04/2021	MTSD – Neuartige Monitoring Technologien für eine nachhaltige Entwicklung (27)	66 634 € (HMGU)
Bundesministerium für Verkehr und digitale Infrastruktur (BMVI)	04/2017 – 09/2020	Verbundprojekt Smart Air Quality Network (28)	226 143 € (HMGU)
Bundesministerium für Wirtschaft und Energie (ZIM Kooperationsprojekte)	06/2016 – 05/2019	Entwicklung eines Screening-Verfahrens für fossile und biogene Schwerflüchter (29)	186 577 € (HMGU)
Deutsche Forschungsgemeinschaft (DFG)	05/2016 – 04/2019	Entwicklung und Optimierung eines Membraneinlasses – Photoionisierungsmassenspektrometers für die Echtzeitanalytik (poly)aromatischer und halogenierter Kohlenwasserstoff (30)	203 072 € (UR)
Bundesministerium für Bildung und Forschung (BMBF)	12/2015 – 03/2018	Entwicklung und Anwendung von analytischen Methoden zur chemischen Charakterisierung von Partikeln und zur biochemischen Untersuchung von Zellkulturen (31)	599 973 € (UR)
Deutsche Forschungsgemeinschaft (DFG) und U.S. National Science Foundation (NSF)	10/2014 – 03/2018	CHARCOAL – Chemical composition and origin of atmospheric brown carbon aerosol (32)	180 000 € (UR)
Deutsche Gesetzliche Unfallversicherung e.V.	09/2014 – 05/2018	Dynamisches Verhalten von semi-volatilen Mehrkomponenten-Gefahrstoff-Aerosolen bei der Probenahme am Arbeitsplatz (33)	696 269 € (HMGU)

List of Funded Scholarships of the JMSC (January 2018 – December 2020)

Name of beneficiary	Funding institution	Title
Xiansheng Liu	Nanjing Normal University, China; Chinese Scholarship Council (CSC)	Spatial variability of ambient particulate matter – composition, source contributions and influencing factors
Xin Cao	Beijing Forestry University, China; Chinese Scholarship Council (CSC)	Development and application of LC-MS methods for the investigation of the metabolom of different cell lines
Dac-Loc Nguyen	Deutscher Akademischer Austauschdienst (DAAD)	Characterization of wildfire aerosols
Dr. Christoph Bisig	Swiss National Science Foundation	Combustion-derived aerosols, what are the drivers of toxicity? Development of an optimized *in-vitro* exposure protocol and performance of in-depth toxicological analysis.

3.4.2 Third Party Projects

(01) ULTRHAS – ULtrafine Particles from TRansportation – Health Assessment of Sources

Project consortium: J. Øvrevik (NIPH), R. Zimmermann (UR/HMGU), T. Streibel (UR/HMGU), B. M. Rothen (UFR), B. Buchholz (UR), M. – R. Hirvonen (UEF), J. Jokiniemi (UEF), T. Adam (UniBw M), O. Hanninen (THL)

Funded by the European Union

Funding organization: European Commission, Horizon 2020

Funding period:
10/2021 – 09/2025

Funding amount:
782 170 € (HMGU),
698 172 € (UR),
458 000 € (UniBW M)
Total funding: 4 037 501 €

This project is an international cooperation project funded by the European Commission. The Coordinator of the Project is the Norwegian Institute of Public Health (NIPH), the HMGU has the co-lead. Partners are in addition to HMGU and the University of Rostock the University of Eastern Finland (UEF), the University of Fribourg, Switzerland (UFR), the Finnish Institute for Health and Welfare (THL), and the Universität der Bundeswehr München (UniBw M).

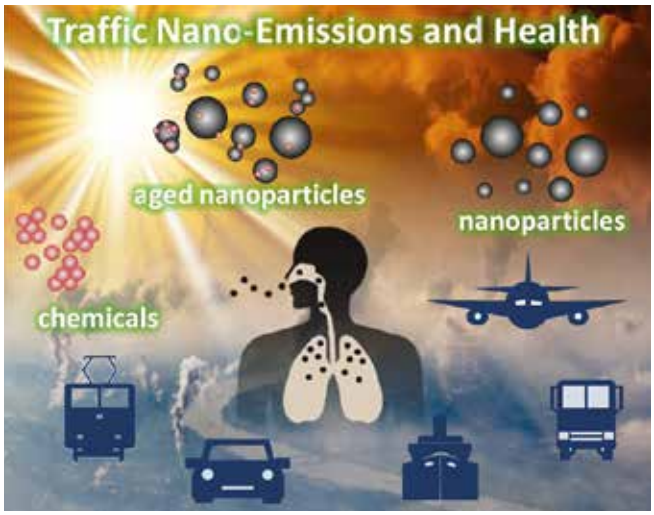
Background: According to the State of Global Air 2019 report, air pollution ranked fifth among all mortality risk factors in 2017, accounting for 5 million deaths and 147 million years of life lost, worldwide. An increasing number of studies indicate a role of chemical constituents from combustion emissions in cardiopulmonary effects. A 'Trojan horse effect' has therefore been postulated with combustion nanoparticles acting as carriers of volatile and semi-volatile chemicals and enabling access to organs and cellular compartments they otherwise could not reach.

Transport-related nanoparticles (particles ≤ 100 nm) from the exhaust and non-exhaust emissions, in atmospheric sciences referred to as ultrafine particles (UFPs), are variable entities with highly source-dependent properties. The health risks posed by different types and sources of UFPs and interactions between particles and gaseous components are not sufficiently captured by current mass-based regulations on the ambient air particulate matter (PM). Many primary exhaust components have a short half-life and are altered by atmospheric aging processes. The health impact of near-source and background pollutants, therefore, likely differ even when originating from the same source.

Aim of the project: ULTRHAS aims to determine the impact of UFPs from different transport mode emissions on human exposures and health, and clarify the importance of physicochemical characteristics and atmospheric processes applying cutting-edge exhaust generation and exposure approaches. The overall objective is to improve risk assessment of air pollutants and to advise policy-makers and regulators on more targeted mitigation measures against the emission components and sources that contribute the most to adverse effects. This will allow for more efficient initiatives to improve urban air quality and promote health and wellbeing. Briefly summarized, the project pursues the following main objectives:

- Generation of a comprehensive and publicly available registry on the physicochemical characteristics of nanoparticle emissions from a unique test battery of different transport modes (exhaust and non-exhaust from the road, rail, aviation, and shipping, as well as different fuel types), including both primary and secondary volatile and non-volatile UFPs and the impact of atmospheric aging processes.
- Optimization of a toxicity testing strategy based on advanced lung and secondary tissue models in combination with air liquid interface exposure and the Adverse Outcome Pathway framework for the assessment of aerosol toxicity in the lung and beyond, validated by targeted animal studies.
- Improvement of the mechanistic understanding of the biological responses leading to adverse effects in multiple relevant tissues of transport mode emissions, focusing on carcinogenic and inflammatory responses.
- Hazard ranking of different transport mode emissions based on *in-vitro* and *in-vivo* toxicity outcomes
- Identification of the main physicochemical characteristics driving the biological effects induced by transport emissions.
- Health impact assessment of the various transport modes emissions based on *in-vitro* and *in-vivo* toxicological data in combination with integrated exposure pathway modeling and the intake-DALY approach.
- Socio-economic evaluation of the possible future impact of new policies in this area on public health and well-being of citizens.
- Contribution to the current discussion which metric is best suited to address the health effects of UFP-transport emissions and ambient aerosols.

Figure 1: *Knowledge need for contribution of different transport modes to human health effects.*



(02) AMMOTRACe – AMMunitiOn exploration by surface- and underwater-based laser mass spectrometric TRACing technology

Project realization: E. Achterberg (GEO), R. Zimmermann (UR), D. Schulz-Bull (IOW), J. Beldkowski (IOPAN), D. Durt (DEME), A. Walte (Photonion), C. Menhard (IN), I. Kazmierczak (CTM)

Funded by the European Union

MarTERA ERA-NET COFUND

Funding organization:
European Commission (MarTERA, ERA-NET-Cofund)

Funding period:
09/2021 – 08/2024

Funding amount:
225 000 € (UR),
Total funding: 2 112 000 €

AMMOTRACe is an international cooperation project funded by MarTERA, an ERA-NET Cofund scheme of the European Commission. This project draws together European companies and research organizations that develop analytical techniques and instruments for environmental contaminant measurements, design and build hardware for underwater operations in marine systems, assess the presence of historic munitions in marine waters and sediments, and conduct underwater munition clearance operations. The project is coordinated by GEOMAR Helmholtz Centre for Ocean Research (GEO, Germany). Academic partners within the project are: the Institute for Baltic Sea Research Warnemünde (IOW, Germany), the University of Rostock (UR, Germany), as well as the Institute of Oceanology Department of Marine Chemistry and Biochemistry (IOPAN, Poland). Company partners include Photonion, Innolas (IN, Germany), Dredging, Environmental & Marine Engineering (DEME, Belgium), and Centrum Techniki Morskiej (CTM, Poland).

Background: After WWI and WWII, large amounts of conventional and chemical weapons were dumped in European seas to eliminate the overwhelming quantities of war remnants. Today, European waters are strongly contaminated with underwater munition (UM), including hundreds of thousands of de-fused shells, mines, and aerial bombs. Furthermore, unexploded ordnance (UXO), sunken underwater mine barriers, and wrecks of military vessels carrying munitions contribute to the problem as well. It is estimated that the German portions of the North Sea and Baltic Sea alone contain approx. 1.6 Mio. tons of UM, which can be often found in relatively shallow waters, in areas of active fishing, or near major shipping routes. Today, all dumping sites represent a serious threat to marine environments, human activities, and seafood provision. Explosion risks, in particular, apply to increasing ship traffic and water sports in general as well as to intensified dredging and offshore work. Moreover, munition shells corrode over time, allowing the release of munition compounds (MC) as well as the slow alteration of explosive materials upon seawater exposure, making them more sensitive to detonation. Released chemicals associated with conventional explosives and chemical warfare agents have cytotoxic, genotoxic, and carcinogenic properties and can enter into the marine food chain up to human consumption. Therefore, safe, reliable, and cost-effective localization and clearance of UM in coastal waters are ecologically, economically, and socio-politically important. Commonly applied techniques for the localization of dumping sites include geophysical techniques, discrete sample collection with subsequent lab analysis, and chemical sensing technologies. However, despite the clear need for fast and reliable as well as in situ chemical detection technologies, successful methods for broad application remain unrealized.

Aim of the project: AMMOTRACe is a transdisciplinary project, involving science, engineering, and companies across a range of disciplines to develop new solutions beyond disciplinary perspectives. The project aims to design, develop, prototype, and demonstrate complete technology solutions for new real-time ship-board and in situ analyzers for conventional and chemical MCs in coastal systems. For this, new laser photoionization mass spectrometers (PIMS), including latest laser developments as well as state of the art ion mobility spectrometers (IMS) will be combined with innovative membrane inlet (MI) sampling to provide highly selective and sensitive detection of MCs in marine systems. The key objectives of the project are as follows:

- Design and development of ship-board as well as a submersible PIMS/IMS prototypes for rapid and direct analysis of MCs in seawater
- Design and develop Nd:YAG-laser – optical parametric oscillator (OPO) systems with tuneable wavelengths as sources for photoionization, for ship-board and in situ submersible use
- Design and develop components and interfaces for the underwater deployment of the submersible PIMS/IMS device
- Demonstration of the prototypes alongside traditional chemical and geophysical measurements at munition dumping sites and regions with the presence of munitions.

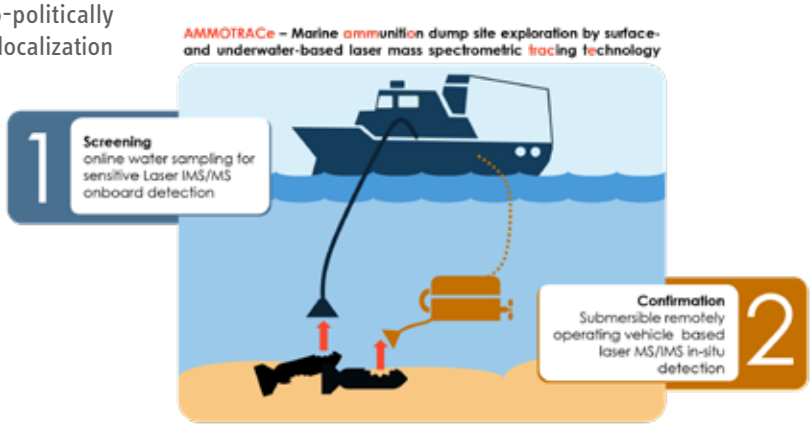


Figure 1: Within AMMOTRACe two submersible and ship-based laser mass spectrometer devices with membrane inlet systems are developed for the detection of hazardous compounds such as explosives or chemical warfare agents from ammunition dumpsites in seawater.

(03) DUST-STORM – Inhalable airborne desert DUST: A comprehensive STudy On physical micro-structure, chemical composition and Respiratory toxicity of fine Mineral and anthropogenic dust

Project realization: S. Di Bucchianico (HMGU), A. Föhlisch (University of Potsdam), A. Lausi (SESAME, Jordan), K. Lips (Free University of Berlin), R. Zimmermann (UR/HMGU)

Funding organization:
German Academic Exchange Service (DAAD)

Funding period:
07/2021 – 06/2024

Funding amount:
3.5 year PhD stipend
~55 000 € (UR)

The DUST-STORM project initiative is born as a joint interdisciplinary initiative of German and Jordan Universities with the support of the German Academic Exchange Service (DAAD) to reinforce international academic co-operation and the training of excellent young MSc level Jordanians with PhD-projects in Germany. The idea to investigate inhalable airborne desert dust in its physico-chemical characteristics and related respiratory toxicity was created during a scientific delegation journey of German scientists organized by the Helmholtz Association of Germany (HGF) to the Synchroton-Light for Experimental Science and Applications in the Middle East (SESAME) laboratory located in Allan (Jordan) and Jordan Universities. The DUST-STORM initiative is thematically linked to the Helmholtz International Lab. aeroHEALTH and aims to establish and deepen cooperation between German partners, SESAME, and Jordan Universities in the field of aerosol and health. Dust storms frequency has been rising in the last decade as a response to the effects of climate change and a number of infectious and non-infectious diseases have been associated with dust exposure including chronic obstructive pulmonary diseases, asthma, and pulmonary fibrosis (Schweitzer *et al.*, 2018). Dust particles initiate inflammatory immune responses, which play an important role in the pathogenesis of different cardiopulmonary diseases. However, recognizing desert dust particles induced cellular and molecular effects by taking into consideration the role of anthropogenic activities derived particles, *e.g.* soot from wood combustion or traffic emission, that potentially agglomerate with desert dust derived particles is an unexplored topic. Dust storm project aims to characterize the inhalable dust fraction in Jordan from a different area, determine the relative toxicity in lung cell model systems, identify reactive oxygen and radical species, analyze and quantify organic components as well as determine the structural and electronic properties of mineral dust grains down to the atomic level.

Expected Results: In the framework of this project, we will elucidate the key drivers within structural and electronic properties of mineral dust particles as well as within the chemical variety of organic adsorbates, which contribute to the toxicity of both collected and standard dust particles. The scientific approach comprises the comparison of locally collected airborne mineral dusts (particulate matter PM10), desert dust samples, standard dusts (Arizona Test Dust), NIST urban dust, and soot. Electronic structure, chemical properties, and biological effects will be investigated. Submersed exposure of human pulmonary epithelial cells and other relevant cell models, including disease-oriented co-culture models, to the collected aerosol particles, will reveal their typical effect strengths via analysis of cytotoxic, inflammatory and primary- as well as secondary genotoxic and mutagenic responses. It will be deciphered which aerosol components and properties lead to direct or inflammation-driven secondary mechanisms of genotoxicity before and after thermal removal of organics from the dust particles leading to a better understanding of the relative contribution of different components in the observed biological outcomes. The first call for students was handled during 2020. A Jordan biologist fulfilled the criteria and will likely join the team at JMSC. A next call is planned for 2021.

Reference

1. Schweitzer, M. D. *et al.*, (2018). Lung health in era of climate change and dust storms. Environ Res., 163, 36-42.

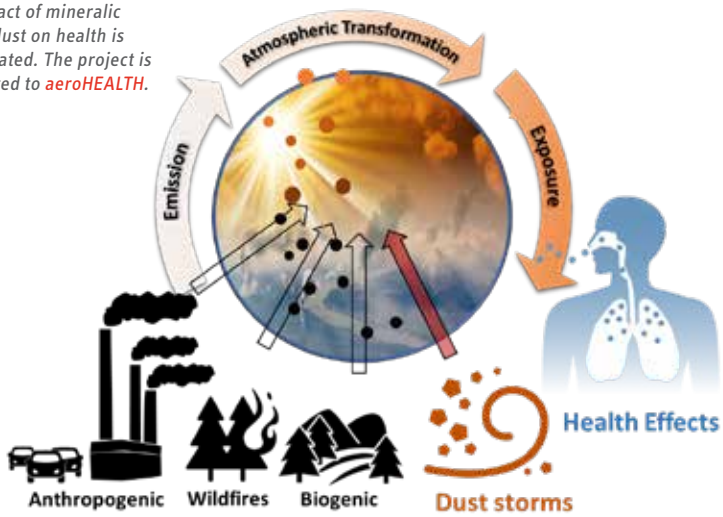


Figure 1: *In DUST-STORM the impact of mineralic desert dust on health is investigated. The project is associated to aeroHEALTH.*

(04) TIMSAC – Thermal analysis and ion mobility coupled to high-resolution mass spectrometry for organic aerosol characterization

Project realization: C. Rüger (UR), C. Afonso (URO), R. Zimmermann (UR/HMGU)

Background: The major impact on humankind and the molecular complexity of organic aerosols motivates the development of novel analytical instrumentation approaches. Recently, high-resolution mass spectrometry (HRMS) and ion mobility spectrometric (IMS) platforms have shown great potential. HRMS allows unraveling high-complex organic mixtures and attributing elemental compositions, whereas IMS directly gives access to structural information.

Aim of the project: In this three-year corporate project between the University of Rostock and the University of Rouen-Normandy (France), novel evolved gas analysis (EGA) techniques coupled to state-of-the-art mass spectrometry (MS) platforms for the detailed chemical description of primary and secondary organic aerosols will be developed. Fourier-transform ion cyclotron resonance (FT-ICR) and high-resolution time-of-flight MS will allow for molecular-level insights. Three main EGA approaches will be utilized for hyphenation: Atmospheric solids analysis probe (ASAP/DIP), thermal-optical carbon analyzer (TOCA), and gas chromatography (GC). Ion mobility spectrometry (IMS) will serve as an additional separation technique for determining the size and shape of the evolved constituents. The project addresses the carbonaceous aerosol fraction of various primary aerosol sources, but also aged emissions. Specific parts of the chemical space can be selectively addressed by sample preparation strategies (derivatization, tagging) and ionization schemes.

Data fusion from the different approaches aims to reach three main achievements: 1) insights into the isomeric complexity of organic aerosols, 2) description of the chemical moieties at the molecular level, and 3) chemical comparison of primary emissions and those formed by chemical reactions (secondary, aged aerosols). Structural information is gained by combining GC retention time, selective ionization techniques (*e.g.*, photoionization targeting aromatic species), fragmentation pattern (both thermal fragments by DIP and ASAP as well as by tandem mass spectrometry), and ion mobility response. Adapted data processing strategies will be developed, including the theoretical computation of collision cross-sections (CCS). A molecular repository for different types of aerosol sources, with a particular focus on shipping emissions, will be set up. This molecular library is foreseen to be suitable to link human health aspects.

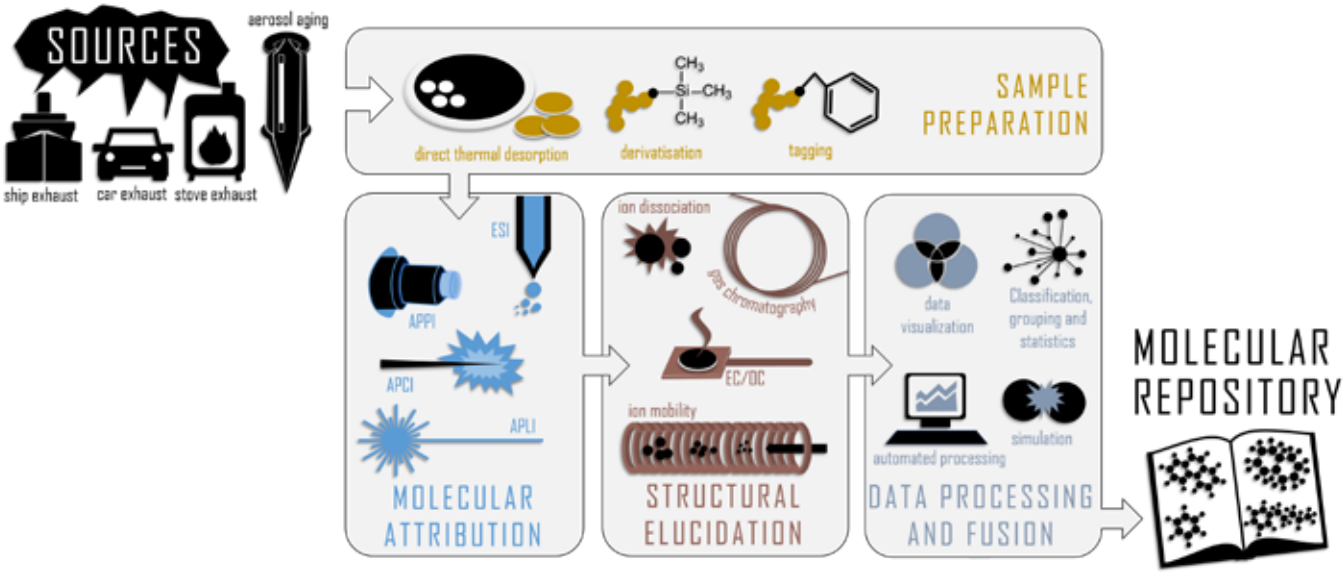
Cooperation partners: University of Rouen-Normandy (URO), University of Rostock (UR)

Funding Organization:
Deutsche Forschungsgemeinschaft (DFG), Agence Nationale De La Recherche, France (ANR)

Funding period:
03/2021 – 02/2024

Funding amount:
236 850 € (UR)

Figure 1: *Project outline visualizing the 1) Sample material and customized preparation, 2) Molecular attribution, 3) Structural elucidation, and 4) Data evaluation, processing, and fusion, finally giving novel instrumental approaches and the anticipated molecular library for organic aerosols.*



(05) SEP-3AP – Ship Emission Profiler – Advanced Analyzer for Aerosol Particles

Project realization: R. Irsig (UR/Photonion), J. Passig (UR/HMGU), I. Düring (Lohmeyer), E.-I.-Rosewig (UR), S. Ehlert (Photonion/UR), T. Adam (UniBW M/HMGU), R. Zimmermann (UR/HMGU)

Funding Organization:
Bundesministerium für Wirtschaft und Energie

Funding period:
3/2021 – 9/2023

Funding amount:
219 528 € (UR)

Background: Ships are the main contributors to global air pollution. Up to 400,000 annual deaths by cardiopulmonary diseases and lung cancer and 14 million cases of childhood asthma were attributed to ship emissions (Sofiev *et al.*, 2018). Regulations focus on fuel's sulfur content in Emission Control Areas (*e.g.* Baltic Sea, North Sea) and by a global 0.5 % standard since 2020. Compliance monitoring is so-far limited to gas-phase measurements in harbors and surveillance flights.

Aim and first results of the project: The project aims to realize the remote detection of ship plumes from the open sea, based on single-particle mass spectrometry (SPMS). Therefore, wind-transported particles are detected by land-based SPMS systems. Air trajectory analysis and ship transponder data are used to assign the aerosol data to individual ships (Passig *et al.*, 2021). The project brings our SPMS developments into application, see Research Area 2 (03) and (04). In preparatory work, we could demonstrate the fuel-specific detection of ships from many km distance, see figure 1 and (Passig *et al.*, 2021). New threads emerge from new fuels following the 2020 global regulations. Some of the fuels have very high contents of toxic aromatic compounds. With the unique PAH-detection of our SPMS, the contribution of ships to particular health-relevant air pollution will be investigated.

The Bundeswehr University Munich will optimize the SPMS system for ultra-fine particles and robustness for field studies, integrating our novel laser ionization. Further equipment will be installed to detect potential ship plumes by an increase of particles in the ultra-fine mode.

Photonion GmbH will develop a new software to control the work mode of the SPMS between in-attendance and full-acquisition mode during the presence of a plume. A patented system allows multiplexing between synchronized triggering to larger particles and simultaneous unsynchronized operation for ultra-fine particles. Photonion will integrate the system and create software for data pre-processing, reduction, and pattern identification.

The partner Lohmeyer GmbH is responsible for the calculation of air trajectories and source assignment. Real-time and forecast weather data will be integrated into wind field and trajectory calculations. Ship positions from an own receiver of transponder data will be matched with air trajectories to identify potential sources.

References

1. Passig, J. *et al.* (2021) Detection of Ship Plumes from Residual Fuel Operation in Emission Control Areas using Single-Particle Mass Spectrometry, Atmos. Meas. Tech. Disc. (submitted) DOI: 10.5194/amt-2020-482.
2. Sofiev, M. *et al.* (2018) Cleaner fuels for ships provide public health benefits with climate tradeoffs. Nat. Commun. 9, 406.

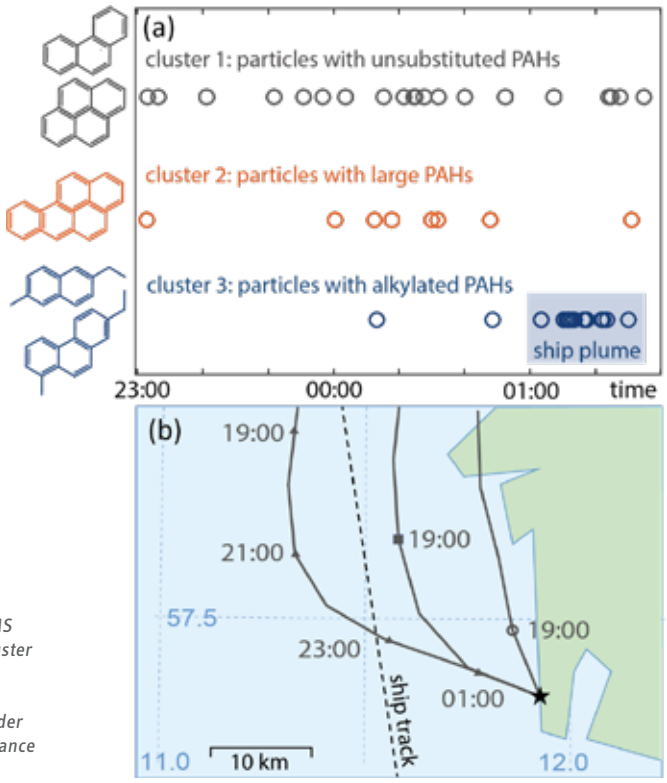


Figure 1:
(a) Detection of PAH-containing particles with specific signatures as function of time during SPMS measurements at the Swedish coast. The third cluster exhibits a ship-specific PAH-profile; its transient increase indicates a ship plume.
(b) Matching of air trajectories and ship transponder data indicates a ferry passage in about 15 km distance as particle source.

(06) LUKAS – Mobile air pollutant warning system for health, environmental & disaster protection by real-time monitoring of atmospheric aerosols including pollutant source localization

Project realization: Institute of Chemistry and Environmental Engineering: T. Adam (UniBW M/HMGU); Cooperation at JMSC: J. Passig (HMGU/UR), R. Irsig (UR/Photonion), R. Zimmermann (UR/HMGU)

The aim of the LUKAS project is to develop a novel mobile measurement and warning system for monitoring and detecting particulate pollutants in the atmosphere. The system is capable of analyzing single airborne particles in the nanometer and micrometer size range in real-time for physical properties and their chemical composition, whereby the database of known chemical patterns is continuously evolved (artificial intelligence). Real-time data analysis and meteorological modelling are used to simultaneously locate the pollutant source, forecast pollutant dispersion, and provide a prospective warning message to a base or to the public, *e.g.* via a web app. The used key measurement technology is a novel laser-based single particle mass spectrometry (SPMS) technology for the detection of inorganic and organic airborne aerosol particle components in combination with conventional gas and particle analyzers. LUKAS is suitable for application in disaster prevention, health and environmental protection, and civil defense. The novel LUKAS system is developed and initially tested by monitoring ship and coastal emissions in collaboration with the German Marinekommando and the Bundesamt für Seeschifffahrt und Hydrographie (BSH). Subsequently, LUKAS is being tested together with various national institutions such as the Bundeskriminalamt (BKA), the Bundesamt für Bevölkerungsschutz und Katastrophenhilfe (BBK), the Umweltbundesamt (UBA), and the Bayerische Staatsministerium für Umwelt und Verbraucherschutz (StMuV) for further applications in the fields of disaster prevention, counter-terrorism, and crime prevention, and environmental monitoring.

LUKAS is funded for 4 years (2021 – 2024) by the Federal Government of Germany with a total sum of 3.9 Mio €. The steering committee consists of the LUKAS project leader, Thomas Adam (UniBW M/HMGU), and the UniBW M researchers Christian Kähler, Heinrich Ruser, and Annika Sehl. In addition to this, 5 early-career scientists complete the LUKAS core team. Key partners of LUKAS are the JMSC (Helmholtz Zentrum München and University of Rostock), Helmholtz Zentrum Geesthacht, Photonion GmbH, and further German KMUs.

German project title:
Mobiles Luftschadstoffwarnsystem für den Gesundheits- Umwelt- & Katastrophenschutz durch Echtzeitüberwachung & -evaluation atmosphärischer Aerosole sowie Ortung der Schadstoffquelle (LUKAS)

Funding organization:
Deutsche Bundesregierung

Funding period:
01/2021 – 12/2024

Funding amount:
3 900 000 € (UniBW M)

Figure 1: *Partners of the LUKAS project.*

(07) HazarDust – Real-time detection of Hazardous Dust particles to identify security-relevant content from shipments and baggage

Project realization: S. Ehlert (Photonion/UR), R. Irsig (UR/Photonion), J. Passig (UR/HMGU), J.Schade (UR), M. Seipenbusch (ParteQ), H. Schanzmann (BKA), M. Pütz (BKA), A. Walte (Photonion), R. Zimmermann (UR/HMGU)

Bundesministerium für Bildung und Forschung

German project title:
Echtzeitdetektion toxischer Substanzen in Staubpartikeln zur Identifikation sicherheitsrelevanter Inhalte aus Sendungen und Gepäckstücken (HazarDust)

Funding organization:
Bundesministerium für Bildung und Forschung (BMBF), KMU-Innovativ Programm.

Funding number:
FKZ: 13N15569

Funding period:
01/2021-12/2023

Funding amount:
542 502 € (UR)
Total funding: 1 289 019 €

Growing mobility, globalized world trade, and the rapidly increasing online shipping poses enormous technological and logistical challenges for the detection of hazardous substances in goods transport, baggage handling, and transit. In addition to the threat of explosives, in recent years, *e.g.*, the illegal transportation of synthetic opioids increased in the wake of the so-called "opioid crisis." These substances, especially fentanyl and its derivatives, are synthesized and transported as powders. There is a particular risk for employees of the fire brigade or the police, as well as for personnel who is checking goods or shipping through damaged packaging, which can lead to serious incidents, *e.g.*, reported in the United States and Canada. The combination of the high toxicity of fentanyls and their derivatives with the exponential increase of the drugs on the black market requires new protective measures.

These safety-relevant substances (or illegal precursor products) also occur when shipping luggage, *e.g.*, during baggage handling at the airport. While most of the target substances are not very volatile for detection in the gas phase or are present in particulate form, the detection of adhering or released residues and dust is in principle possible using aerosol particle analysis techniques. Particularly promising for this is single particle mass spectrometry (SPMS), a method that has so far only been used in research on aerosol particles from combustion emissions and in the atmosphere, which provides a chemical signature of individual microscopic particles in real-time. Recently developed, new ionization methods for SPMS allow a greatly improved detection of organic compounds. The approach of single-particle analysis enables extremely high sensitivity, which makes even the smallest amounts of material that are whirled up detectable.

In the context of the present project, this research method for the fast and reliable detection of synthetic opioids, narcotics, as well as their pre- and preprecursors, but also other low-volatility hazardous substances in baggage/shipping security checks will be applied and further developed. For sample preparation, aerodynamic devices are set up to whirl up and concentrate the particles. Various laser ionization processes for SPMS, some of which have only recently been developed, are tested and optimized on simulants, pure substances, and real samples and combined with known processes in order to obtain a large spectrum of detectable, safety-relevant substances. Pattern recognition algorithms or artificial neural networks, which are implemented in the application software, are used to recognize typical patterns in the mass spectra.

Finally, the developed SPMS technology and the verification systems will be tested under realistic conditions in the baggage security check of a regional airport and in a package distribution center.

Project partners: Photonion GmbH, ParteQ GmbH, Universität Rostock, Bundeskriminalamt (BKA)

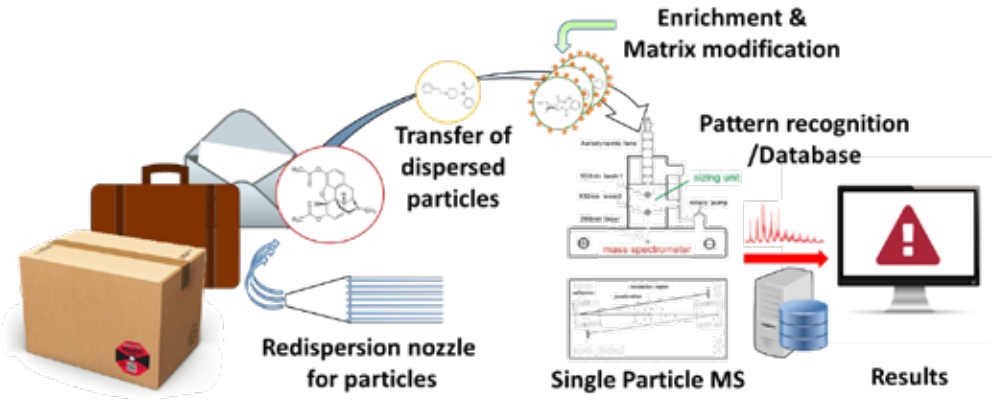


Figure 1: *Schematic drawing of the detection concept from the redispersion of the surface-bound particles to the output of the results.*

(08) BOW – Biogenic Organotropic Wetsuits

Project realization: S. Di Bucchianico (HMGU), M. Rehberg (HMGU), P. Bergese (Center for Colloid and Surface Science), T. Stöger (HMGU-iLBD), R. Zimmermann (UR/HMGU)

Funded by the European Union

Funding organization:
European Commission, Horizon 2020 research and innovation programme under grant agreement no. 952183.

Funding period:
11/2020 – 10/2024

Funding amount:
342 591 € (HMGU-CMA)
307 766 € (HMGU-iLBD)
Total funding: 4 442 551€

Targeted delivery of therapeutic agents by nanoparticles has been featuring strongly in the last few years. BOW envisions bringing these innovative approaches to the next level, by aiming at developing a technology that streamlines the assembly of biogenic-synthetic hybrid nanodevices in which a theranostic synthetic core is camouflaged/functionalized by a "wetsuit" made by an extracellular vesicle (EVs) membrane. EVs are intercellular and inter-organismal agents, "made by cells for cells", to shuttle lipids, proteins, and nucleic acids, which mediate physiological processes and help to spread various diseases, including cancer and infections. The exquisite circulation, targeting, and trafficking ability of EVs are determined by their membrane properties, *e.g.* integrin associated proteins, which may be augmented by nano-engineering.

BOW proposes nanotechnologies that reinvent these EVs to naturally tailor, rather than synthetically tinker, the biological identity of implantable autonomous nanodevice by sweeping paradigm-shift in clinical translation of nanoparticles. Human mesenchymal stromal/stem cells and microalgal species will be the wetsuits EV sources. Nanodevices based on superparamagnetic iron-oxide beads (MBDs) spanning the typical size range of small EVs, 50 – 250 nm, will be used as the synthetic cores to be cloaked by the EV derived wetsuits (evBOW). MBDs will consist of two components, magnetic nanobeads, and a silica or polymeric shell that encapsulates them.

HMGU-CMA is work-package leader for the evaluation of biological performances of evBOW products in terms of *in-vitro* and *in-vivo* nanotoxicity, immunological activity, and *in-silico* modeling by a mathematical evaluation of potential risk. CMA and iLBD will provide *in-vitro* pre-screening information of evBOW products as well as their cellular uptake, cytotoxicity, phagocytic clearance, biodegradation, genotoxicity, and mutagenic potential of evBOWs, fulfilling REACH information requirements. Biokinetics and bio-distribution of the different evBOW products will be determined *in-vivo* by investigating the ability of different EV coatings, with or without functionalization, to avoid immune clearance and facilitate an organotropic (brain, cancer, lung), or cell type specific targeting. Special attention will be paid to the role of evBOW in lung, since lung accumulation and protective effects of exosomes in a fibrosis model have been recently reported.

The membrane engineering for augmented immune escape and targeting towards brain, lung and mammary tumors coupled to the *in-vitro* and *in-vivo* testing will ensure the validation of the potential clinical applications of BOW products as enhanced photothermal ablators of cancer cells and as multimodal imaging agents.

BOW is highly interdisciplinary and involves team members coming from different geographical areas and covering different disciplines including aquatic biological sciences, molecular biology, nanomedicine, green chemistry, physical chemistry, genetics, biochemical engineering, and biotechnology. BOW shall pave the road for the development of aerosolized vesicles as inhalable transport vehicles of therapeutic agents in future projects.

Consortium: Industrial Partners: HansaBioMed Life Science Ltd, Estonia; Biodevice Systems sro., Czech Republic; Rigenerand srl., Italy; National and International Research Institutions: HMGU CMA and iLBD, Germany; Center for Colloid and Surface Science, Italy; National Research Council of Italy, Italy; Max-Planck Institute for Polymer Research, Germany; National and International Universities: University of Santiago de Compostela, Spain; Institute of Technology Sligo, Ireland; Swiss Federal Institute of Technology, Switzerland

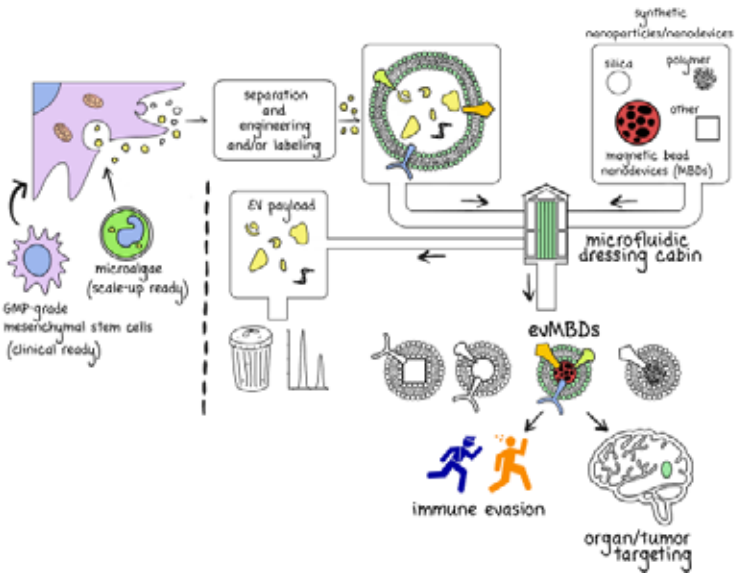


Figure 1: *EV membrane wetsuit recapitulates key biological functions to synthetic nanomaterials and nanodevices. MBDs enter the microfluidic dressing cabin together with the raw or engineered EVs. The device outputs the magnetic core functionalized by the biomimetic and organotropic capabilities of the EV membranes.*

(09) Method optimization for the chemical analysis of ultrafine particles

Project realization: J. Schnelle-Kreis (HMGU), M. Sklorz (HMGU), J. Orasche (HMGU), A. Nölscher (UBT), S. Kernchen (UBT), R. Zimmermann (UR/HMGU)

Background: Knowledge of sources of ultra-fine particles (UFP), their distribution in the atmosphere near the ground, and their chemical composition are key factors, needed to estimate whether they pose a risk to human health. However, a risk assessment is currently limited by the quality and comparability of the methods used to characterize UFP in the environment. Therefore, the aim of this project is to optimize methods for the separation, collection, and analysis of UFPs in such a way that a reference for standardized measurements of UFPs is created and an evaluated, flexibly applicable mobile module enables comparable measurements at different sites. Both approaches were conducted with high-quality analysis of the chemical composition of the ultrafine particles. The measurements on temporal and spatial variability allow the identification of specific source and exposure situations and enable conclusions on potential risk factors for human health.

Aim of the project: This project is planned over a period of 3 years and is divided into four work packages: (1) Method optimization, (2) Set up and evaluate mobile UFP measurement reference module, (3) Set up and evaluate the mobile module, and (4) Develop and perform fingerprint analysis. In particular, the newly developed mobile module will, after the evaluation and characterization phase, also be used in projects of the UFP research network partners, a framework of in total six Bavarian Universities, and research institutes funded by the StMUV.

(1) Method optimization: In the first year of the project, different methods for sampling UFP in the laboratory and outdoor air will be tested, compared, and optimized. In doing so, we will compile established separation and collection methods (*e.g.* Moudi or Sioutas impactors) and new technologies (*e.g.* Aerodynamic Aerosol Classifier, AAC), see figure 1.

(2) Set up and evaluate the mobile UFP measurement reference module: The methods identified as the technical optimum are set up as a reference module and evaluated at a main site (years 2+3). The long-term observations of UFP at the Augsburg site traces seasonal trends and dependencies.

(3) Set up and evaluate the mobile UFP measurement module: Parallel to the reference module, a second module is set up as a flexible traveling module. This module is designed to be easy to use and to run automatically and shall move between different Bavarian measuring-stations. In this way, comparability between the different locations can be achieved and the local UFPs characteristics will be examined for fingerprints, typical local sources, and formation processes in the environment.

(4) Develop fingerprint analysis: Simultaneously with the development of methods for separating and collecting UFP, the chemical composition of the collected UFP is characterized offline using a variety of analytical methods. Methods established by the project partners will be used to provide additional insights, *e.g.* into chemistry (*e.g.* by TD-GC-MS, ICP-MS, HPLC-MS), morphology (scanning electron microscope), or functionality (Raman Imaging, STXM).

The overall objective of the fingerprint analysis is to obtain a source attribution for atmospherically relevant UFPs, but also to trace marker components identified as health-critical in samples from different characterized environments.

Project partner: University of Bayreuth (UBT), Prof. Dr. A. Nölscher, Atmospheric Chemistry

Bayerisches Staatsministerium für Umwelt und Verbraucherschutz

German project title:
Methodenoptimierung zur chemischen Analyse von ultrafeinen Partikeln

Funding organisation:
Bayerisches Staatsministerium für Umwelt und Verbraucherschutz (StMUV) via an onlending agreement with the University of Bayreuth

Funding period:
11/2020 – 10/2023

Funding amount:
203 895 € (HMGU)

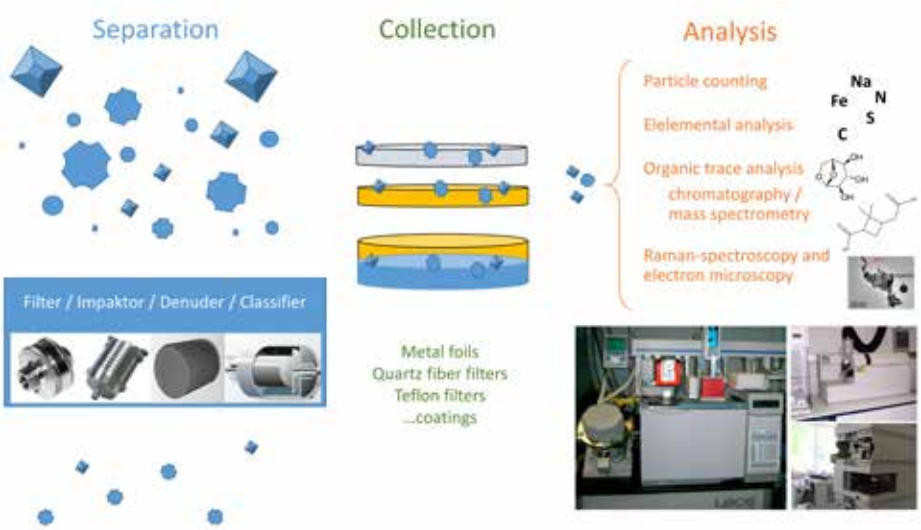


Figure 1: *Examples of the separation, collection, and analysis procedures that will be combined and optimized, depending on the individual target parameter. The following instrumental techniques will be applied for characterization of UFP: Scanning mobility particle sizers and aerosol classifiers in combination with condensation particle counters for measuring particle number and -size distribution, inductive coupled plasma mass spectrometry and carbon analysis for the determination of the elemental composition, thermodesorption gas chromatography/mass spectrometry for analysis of semi-volatile organic trace components (e.g. PAH) and their fingerprints, high performance liquid chromatography for quantitative determination of known polar aerosol components (e.g. anhydrous-sugars) as well as Raman spectroscopy and electron microscopy for morphological analysis of UFP.*

(10) Biological impact of particles in a lung model

Project realization: S. Öder (HMGU), S. Schmitz-Spanke (FAU), S. Di Bucchianico (HMGU), M. Sklorz (HMGU), J. Schnelle-Kreis (HMGU), R. Zimmermann (UR/HMGU)

Bayerisches Staatsministerium für Umwelt und Verbraucherschutz

German project title:
Biologische Antwort auf Partikel in einem Lungenmodell

Funding organization:
Bayerisches Staatsministerium für Umwelt und Verbraucherschutz (StMUV) via an onlending agreement with the University of Erlangen

Funding period:
11/2020 – 10/2023

Funding amount:
416 018 € (HMGU)

Background: Due to their extremely small aerodynamic diameter (< 100 nm), ultrafine particles (UFP) can penetrate very deep into the human respiratory system, even up to the alveolar region. Their retention time in the human alveoli can be significantly longer than that of bigger particles. Upon their deposition there, the UFP can induce sustained inflammatory and immunological responses as well as cause direct DNA damage. Especially polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs associated with UFPs from combustion sources have been shown to affect the cellular metabolism negatively and induce oxidative stress responses already at very low concentrations. However, it remains somewhat unclear how UFPs exert those effects and which of the UFP's inherent properties exactly are responsible for these effects.

Current scientific status regarding UFP: In their review from 2019, Ohlwein *et al.* concluded that most of the current studies investigating the adverse effects of UFPs did not even investigate UFPs per se, but mostly particles with an aerodynamic diameter > 100 nm. However, even here, they found clear evidence that fine and ultrafine particles induce short-term inflammatory processes in the lung and can even lead to changes in heart rate variability and increased blood pressure. Supposedly, disturbances in the intracellular concentration of reactive oxygen and nitrogen species and an imbalance of intracellular antioxidants set off a signaling cascade, which releases pro-inflammatory mediators and critically damages alveolar immune cells. These effects can eventually lead to sustained systemic inflammation and even cause fibrotic damage in the respiratory tissue of the alveoli (pulmonary fibrosis).

Aim of the project: In this project, we will reproducibly generate UFPs using Combustion Aerosol Standard (CAST) generators using either propane or propane/diesel fuels. With these CAST generators, we are not only able to produce UFP aerosols with a narrow size range below 100 nm but also control the exact carbon composition through various techniques (Ohlwein *et al.*, 2019). We will also include thermal denuders to remove volatile compounds or particle filters to exclude particles of a certain size range from the aerosols. The model aerosols will be characterized in cooperation with the other project led by Prof. A. Nölscher (see Third Party Project (9)). We will expose advanced *in-vitro* models, consisting of respiratory cells and immune cells, directly to those aerosols at the air-liquid interface. This setup corresponds very well to the actual physiological conditions in the human respiratory system. Both the exposure duration and UFP concentration will be varied to find an exposure-response relationship. We will assess the adverse effects of the UFP exposure on the cells with various "classic" toxicological assays addressing endpoints such as oxidative stress response, intracellular calcium flux, cell viability, measurement of the mitochondrial membrane potential, xenobiotic metabolism, DNA-damage and mutagenesis, and inflammation. To investigate the potential of the UFPs to induce pulmonary fibrosis, we will expose alveolar fibroblasts with the culture medium after the exposure. Here, we will assess the extent of the transformation of fibroblasts into myofibroblasts, a key event in the onset of pulmonary fibrosis. For all cell types, we will additionally conduct an in-depth analysis of metabolomics and transcriptomic changes to find early key events and adverse outcome pathways associated with the UFP exposure. Together with the cooperation partner Prof. S. Hackenberg, from the University Hospital Würzburg, also primary nasal cells as a model for the upper airways will be included in the investigation.

Partners: Prof. Dr. med. Simone Schmitz-Spanke, Friedrich-Alexander University Erlangen Nürnberg, Institute and Policlinic for Occupational, Social and Environmental Medicine, Erlangen, Germany

References:

1. Mueller L., *et al.* (2015). Online determination of polycyclic aromatic hydrocarbon formation from a flame soot generator. Anal Bioanal Chem, 407(20); 5911-5922.
2. Ohlwein S., *et al.* (2019). Health effects of ultrafine particles: a systematic literature review update of epidemiological evidence. Int J Public Health, 64(4); 547-559.

(11) MORE – Munich Mobility Research Campus: Implementation of a model campus for future mobility at the Universität der Bundeswehr München

Project realization: Institute of Chemistry and Environmental Engineering: T. Adam (UniBW M/HMGU), J. Bendl (UniBW M), S. Padoan (UniBW M, HMGU), A. Mudan (UniBW M); Cooperation at JMSC: M. Sklorz (HMGU), J. Passig (HMGU/UR), R. Zimmermann (UR/HMGU)

Funding organization:
Deutsche Bundesregierung

Funding period:
10/2020 – 12/2024

Funding amount:
4 000 000 € (UniBW M)
Total funding: 43 000 000 €

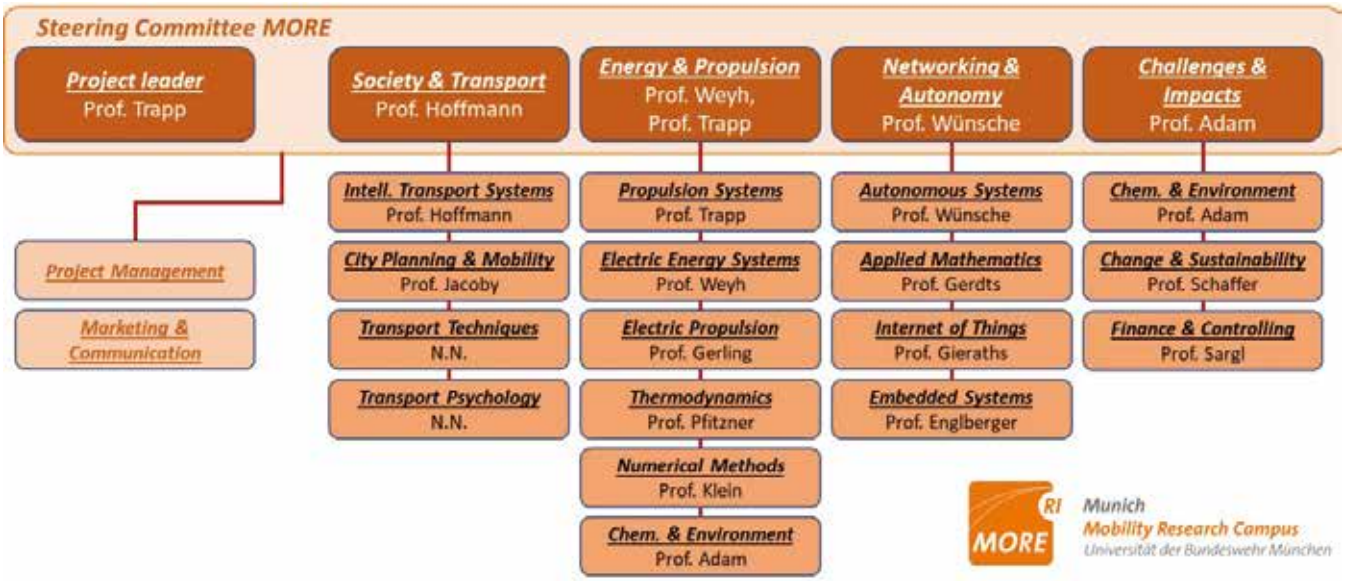
MORE is a unique and interdisciplinary approach to evaluate sustainable, efficient, and safe mobility technologies of the future. Within MORE the campus of the Universität der Bundeswehr München (UniBW M) is transformed into a model city and different innovative mobility concepts are developed and implemented on site.

Based on future mobility requirements of society, MORE tackles problems and challenges in the 4 research fields: 'Energy & Propulsion', 'Society & Transport', 'Networking & Autonomy', and 'Challenges & Impacts'. Activities range from the planning of modern cities, energy returned on investment (EROI) evaluations of "clean" energy sources, research projects on innovative propulsion systems (*e.g.* electromobility, hydrogen, and biofuels), implementation of autonomous driving concepts on the campus including the connection of transport infrastructure and users as well as evaluation of the overall impacts for human and environment.

MORE is funded for 4.5 years (2020 – 2024) by the Federal Government of Germany with a total sum of 43 Mio €. In total, 17 UniBw professors and 42 researchers from various disciplines, *i.e.* engineering, natural sciences, economics, and social sciences work closely together with numerous industrial partners and other research institutions to establish this worldwide unique approach.

The steering committee consists of the project leader of MORE Prof. Dr. Trapp and the leaders of the 4 research fields. Prof. Dr. Thomas Adam, director of the UniBw's institute of chemistry and environmental engineering and deputy director of HMGU's CMA, is heading the research field 'Challenges & Impacts' and is a member of the steering committee.

Figure 1: *Organigram of MORE.*



(12) Influence of the fuel composition on the formation of deposits during the evaporation of liquid fuels in porous media

Project realization : T. Streibel (UR/HMGU), A. Neumann (UR), C. Rüger (UR), H. Czech (HMGU/UR), R. Zimmermann (UR/HMGU)

German project title:
Einfluss der Brennstoffzusammensetzung auf die Rückstandsbildung bei der Verdampfung flüssiger Brennstoffe in porösen Medien

Funding organization:
Arbeitsgemeinschaft industrieller Forschungsvereinigungen (AiF) über Deutsche Gesellschaft für Erdöl, Erdgas und Kohle e.V. (DGMK)

Funding period:
10/2020 – 03/2023

Funding amount:
200 611 € (UR)

Background: Devices for the combustion of heating oil often exhibit the phenomenon that solid deposits are forming on the inner surfaces (see for example figure 1). This leads to an impairment of the operation up to a complete shutdown of the device. The underlying reasons for this disturbance are not completely understood and are sometimes unpredictable. Two heating oils with similar physical properties can show completely different behavior in this respect, one forming a lot of depositing material, the other almost none. The situation gets even more complicated when heating oils with different content of regenerative fuels such as FAME are regarded. Since their share in the utilized heating oil is expected to be increasing steadily in the future, a solid prediction of their tendency to form deposits should be known beforehand to ensure the safe operation of the combustion devices. Moreover, the storage of the fuels may also have an influence depending on its duration.

Aim and first results of the project: Since the tendency to form solid deposits obviously is not correlated to general physical properties of the fuels such as density, viscosity, or heat capacity, it is hypothesized that differences in the chemical composition may give valuable hints in this respect. Therefore, an analytical approach was chosen, consisting of conducting thermal analysis of the different fuels combined with a detailed chemical characterization of the evaporating chemical compounds. The respective product patterns will be compared to test facility experiments with fibrous-web combusters taken for evaporation of heating oils, where the deposit formation is evaluated. The latter experiments are carried out by the project partner Öl-Wärme-Institut. Chemical analysis of the deposits is attached afterwards.

It is expected that the differences in the product pattern of the evaporated substances are marginal, therefore a detailed chemical characterization is called for involving extensive statistical data analysis. The planned analytical setup will consist of a thermal balance (combined with a calorimeter) that is hyphenated to a time-of-flight mass spectrometer (TOF) operating with soft photoionization. A further enhancement of the method is supposed to be achieved by implementing a fast-gas chromatography unit between the thermal balance and the mass spectrometer. The additional step of separation will help to distinguish isobaric compounds and strengthen the assignment of peaks in the mass spectra to actual distinct chemical species. One alternative for photoionization is single-photon ionization (SPI), which is able to ionize a wide variety of organic compounds, as long their ionization energies are below the photon energy of the light source. Previous studies however have already suggested that differences in the pattern of polyaromatic compounds in the fuel are possible indicators for deposit formation. Therefore an additional ionization method, resonance-enhanced multi-photon ionization (REMPI) will be implemented as well. REMPI is well known for being very selective as well as sensitive for aromatic species and should thus provide an improved means of detection for such substances. In addition to TOF also ultra-high resolution Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometry will be applied. The Rostock FT-ICRMS unit is equipped with several thermal inlet techniques such as a thermal balance coupling and a direct insertion probe (DIP). Furthermore, different ionization techniques are available for FT-ICRMS as well.

Project partner: Öl-Waerme-Institut GmbH, Herzogenrath, Germany

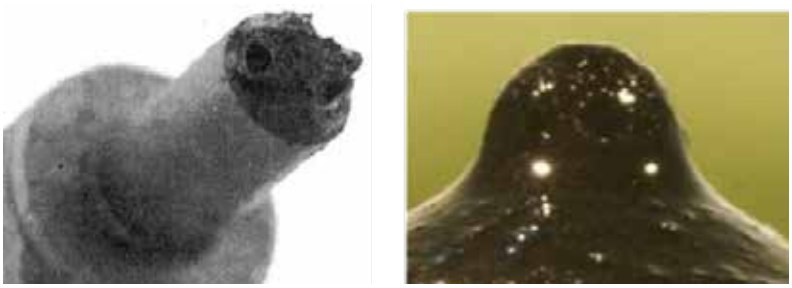


Figure 1: *Examples for deposit formation in devices for combustion of heating oil. Source: Öl-Wärme-Institut*

(13) Application of complementary high-resolution mass spectrometric approaches for an in-depth investigation of highly complex pharmaceutical precursor matrices

Project realization at JMSC: L. Schwalb (UR/HMGU), O. Tiemann (UR), U. Käfer (UR/HMGU), C. Rüger (UR), T. Gröger (HMGU), R. Zimmermann (UR/HMGU)

ICHTHYOL® substances are raw materials for pharmaceutical products and formulations. The precursor material and basis for the product is shale rock, which is mined in France. From the rock material, crude shale oil is derived by a dry distillation process. The shale oil is further distilled and purified and finally sulfonated and neutralized to form a water-soluble pharmaceutical active matrix which belongs to the best-documented active ingredients from nature today. Their versatile actions and their good tolerance are substantiated by clinical and toxicological studies, respectively. However, due to the complexity of the raw material and the multi-stage processing, a comprehensive chemical characterization was not possible so far, and only the main compound groups were identified. The industrially financed project started in October 2020 and aims to advance the chemical characterization of ICHTHYOL® substances further using state-of-the-art mass spectrometric methods. For this purpose, two PhD Students apply high-resolution mass spectrometry and different chromatographic techniques. The topic "High-resolution mass spectrometry" applies a top-down approach and utilizes Fourier-transform ion cyclotron resonance and Orbitrap mass spectrometer with a resolution above 100.000 at m/z 400 and a mass accuracy below 1 ppm for the direct investigation of the sulfonated water-soluble product as well as of the precursor raw materials. The focus is the direct inlet of the sample and an adaption of the ionization technique and sample preparation for the highly sulfonated compounds (Tiemann, 2020). Matrix-assisted laser desorption-ionization (MALDI), as well as electrospray-ionization (ESI), is applied, in particular, targeting thermally not vaporizable and polar compounds. Atmospheric ionization techniques based on chemical (APCI) and photoionization (APPI) are applied to target less-polar and aromatic structures. Hyphenated techniques like thermogravimetry and chromatography allow pre-separation while structural aspects of the found compounds are investigated by fragmentation techniques. The topic "comprehensive separation" in Munich applies a button-up strategy to investigate the chemical complexity of the sulfonated product. Comprehensive two-dimensional gas chromatography (GCxGC) (Käfer *et al.*, 2019) is applied to investigate the non-sulfonated precursor matrix. High-performance and flash chromatography allow a break-down of the complex matrix into different fractions containing specific functional groups and thus narrowing the chemical space. Furthermore, derivatization techniques are deployed to make polar fractions accessible to gas chromatography. First preliminary measurements on the distillates and water-soluble sulfonated products (figure 1) already show the applicability of the proposed mass spectrometric techniques and the complexity of the matrix. GC×GC is able to investigate the matrix on an isomeric level while FTICR is able to target also non-vaporizable and very polar constituents. The results of this project may help to better understand the anti-inflammatory and anti-microbial properties of ICHTHYOL® and to confirm the minor significance of any hazardous compounds present in the multi-component mixture.

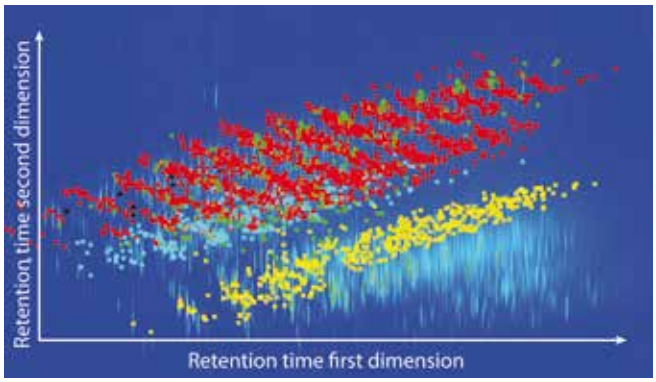


Figure 1: *Preliminary measurements with comprehensive two-dimensional gas chromatography of the Ichthyol matrices reveals a very complex isomeric pattern of sulfur compound classes. The graphs show a part of a typical two-dimensional separation with focus on sulfur classes. Each dot represents an individual compound, those dots are grouped according to their chemical functionality. (■ Thiophenes, ■ Tetrahydrothiophenes, ■ Dihydrothiophenes, ■ Naptha-/olefino- thiophenes, ■ Benzothiophenes)*

German project title:
Untersuchung der molekularen Zusammensetzung von Ölschieferdestillaten und deren sulfonierter Produkte (Arzneistoffe) mittels Multidimensionaler Chromatographie und hochauflösender Massenspektrometrie.

Funding: ICHTHYOL-GESELLSCHAFT Cordes, Hermanni & Co. (GmbH & Co. KG), Hamburg

Funding period:
10/2020 – 09/2023

Funding amount:
420 000 € (UR)

Partner: ICHTHYOL-GESELLSCHAFT Cordes, Hermanni & Co. (GmbH & Co. KG), Hamburg; G. Gayko (Ichthyol)

References:

1. Käfer U. *et al.* (2019) Detailed Chemical Characterization of Bunker Fuels by High-Resolution Time-of-Flight Mass. Spectrometry Hyphenated to GC × GC and Thermal Analysis. Energy and Fuels
2. Tiemann, O. (2020) Characterization of petroleum products by gas chromatography and high-resolution mass spectrometric techniques – Shale oil and Shale oil derivatives, Master Thesis, University of Rostock

(14) IMPAERO – An interdisciplinary study on the impact of aerosolized particulate matter from aged wildfire plumes on environment and human health

Project realization: H. Czech (HMGU/UR), C. Rüger (UR), J. Buters (TUM), O. Popovicheva (MSU), J. Schnelle-Kreis (HMGU), R. Zimmermann (UR/HMGU)

Funding organization:
Deutsche Forschungsgemeinschaft

Funding period:
10/2020 – 09/2023

Funding amount:
334 200 € (UR)

This project is a binational cooperation project funded by the German (DFG) and Russian Science (RFBR) foundations. The cooperation partners are the Technical University Munich (TUM, Prof. Dr. J. Buters) and the Moscow State University (MSU, Dr. O. Popovicheva).

Background: Emissions from wildfires (WF) are major contributors to the global budget of ambient particulate matter (PM) affecting air quality, absorbing and scattering incoming radiation, forming haze and clouds. Due to climate change (CC) wildfires in many regions become a serious problem. Together with thawing permafrost, the increased incidence of wildfires, in particular also in Siberia, is becoming a self-reinforcement component of CC. Furthermore, several adverse health effects of wildfire emissions are discussed, including induction of diseases via inflammatory and gene-toxic pathways. By using the world largest aerosol aging chamber (LAC) in Tomsk, Russia, the expertise to simulate wildfires, and the aging of the emitted aerosols, it will be possible to collect sufficient mass of fresh and aged PM to study biological effects of fine particles which also can reveal the physicochemical parameters responsible for the adverse effects.

Aim of the project: Great variety of gas-aerosol processes and multicomponent smoke characteristics evolving in wildfire plumes and their numerous dangerous impacts on environment and health concerns to one of the most complicated problems of fundamental research. Scientific questions are related to simulation of combustion at the conditions of real-world environment, realistic aging of emitted aerosols, as well as aerosol characterization, and its biological effects. In this project, the effects of aging of wildfire emission plumes on the chemical composition, microphysical properties, and toxicological effects on human lung cells are investigated. For this purpose, wildfires are simulated according to existing procedures in the world's largest aerosol aging chamber. The emissions are aged under dark and light conditions, to simulate day- and nighttime, and tracked by on-line analytical methods. The large volume of the LAC chamber of 1800 m3 allows collecting sufficient amounts of aged wildfire PM2.5 for combined thorough chemical, microphysical and toxicological investigations (Popovicheva *et al.*, 2016). The project consortium will address the following hypotheses: (I) the phase of biomass combustion, open flaming or smoldering, significantly affects the PM composition and the health relevance. (II) Although peat fires will increase largely due to climate change, nearly nothing is known about composition, aging, and health effects of peat fire emissions. Likely, the higher maturity of peat compared to biomass alters the balance of oxidizing and anti-oxidative compounds in peat fire PM2.5 with respect to forest fires. This will have an impact on the observed biological/health outcomes: The type of fuel (peat/biomass) controls the PM composition and the health relevance. (III) With aging and type of aging, depending on aging time and type of biomass burning, the related PM2.5 might be toxified or de-toxified. (IV) The balance on oxidative-acting and anti-oxidative compounds is important for relevant toxicological and allergological endpoints, but affected during atmospheric aging. In spring 2020, the LAC was setup with UV-lamps for the photochemical aerosol aging experiments. The LAC will be first evaluated with non-volatile model aerosol, secondary organic aerosol (SOA) precursors under various photochemical conditions, and NO_x photolysis rates to assess wall losses, SOA yields, and finally its comparability to other established environmental chambers.

Consortium: University of Rostock (UR), Technical University Munich (TUM) and Moscow State University (MSU), Moscow, Russia, and V. E. Zuev Institute of Atmospheric Optics (IAO), Tomsk, Russia

Reference

1. Popovicheva, O. B. *et al.* (2016) Optical-Microphysical and Physical-Chemical Characteristics of Siberian Biomass Burning: Experiments in Aerosol Chamber. Atmos. Oceanic Opt. 29, 492–500.

Figure 1: *Realization of the illumination for the photochemical aerosol aging experiments in the LAC with 198 UV-lamps.*

(15) Development of a mobile and full automated measurement system for the simultaneous chemical analysis of organic compounds in the gas phase and particulate matter of environmental aerosols.

Project realization: B. Giocastro (UniBW M/HMGU), M. Saraji (Photonion/UniBW M), S. Ehlert (Photonion/UR), T. Gröger (HMGU/UniBW M), J. Orasche (HMGU), J. Schnelle-Kreis (HMGU), M. Sklorz (HMGU), R. Zimmermann (UR/HMGU), T. Adam (UniBW M/HMGU)

Background: Epidemiological studies have revealed clear relationships of gaseous pollutants and particulate matter (PM) with adverse health effects. It could be associated with a variety of different implications like cardiovascular and respiratory diseases and a modeled mortality of about four million deaths per year on a global level (Shiraiwa 2017). Although the exact role of the different constituents of the aerosol have not yet been fully elucidated, there are strong indicators that the organic-chemical loading of aerosols is a crucial factor. (Kraus 2011). However, the comprehensive chemical investigation of the chemical composition of aerosols is costly in terms of personnel and time. Therefore, it would be desirable to have a standalone online analytics, for a detailed chemical analysis of the volatile and semi volatile organic fraction of aerosols to close the gap between exposure and biological response in interdisciplinary health related studies.

Aim of the project: The project aims to develop and build up a demonstration system for a fully automated sampling of PM and gaseous phase of aerosols and subsequent fully integrated analysis by gas chromatography – mass spectrometry. The system is supposed to operate autonomous and remotely with a minimal personal service direct at the point of exposure. The development builds on an established but semi-automated and laboratory based method for the analysis of PM collected on quartz fiber filter (Orasche 2011). A first field of application will be the highly time resolved long-term sampling and analysis of environmental aerosols. A modular design will also allow the application towards other types of emission. A subsequent further development of an prototype and commercialization is to be carried out via the participating mid-caps for industrial, academic and governmental end user.

The Universität der Bundeswehr München participates as the academic partner with a Postdoc and will be supported by the JMSC. Main tasks will be the further methodological development of an existing method (Orasche 2011) regarding robustness of the applied chemical reactions and an improvement of the overall duty cycle. Key aspects will be a comprehensive derivatization of polar organic compounds and the reduction of analysis time due to the application of fast and sensitive time-of-flight mass spectrometric detection system, which will be provided by LECO GmbH. The chromatographic concept should also enable the simultaneous determination of gas and particle phase. Scientific Instruments Manufacturer GmbH (SIM) is responsible for the development of a desorption and reaction unit for the PM and collected gaseous phase. Derivatization will be integrated as individual unit or combined with desorption. In addition, SIM robotics will be used to handle the collected samples. Photonion GmbH is responsible for the overall system integration and especially the collection of the PM and gaseous phase. The implementation of an progressive concept will allow an optimal utilization of the duty-cycle with overlapping sampling and analyzing steps.

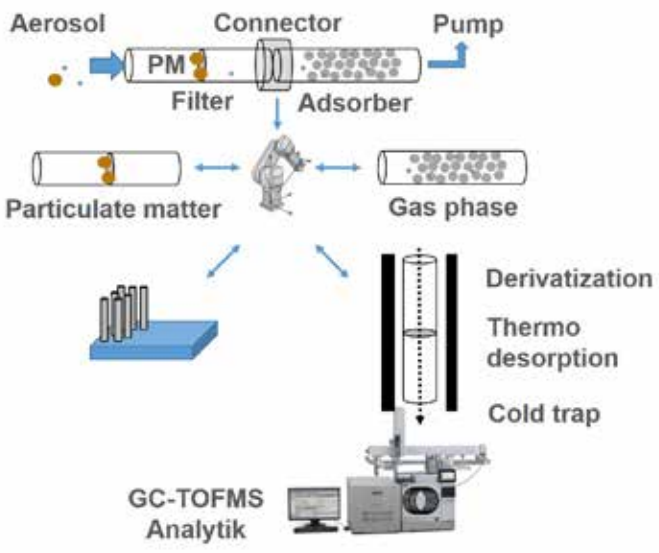


Figure 1: *Concept for a simultaneous sampling and analysis of particulate and gas phase of (environmental) aerosols. Main components of the system will be a unit for sampling, desorption and chromatographic-mass spectrometric analysis.*

German project title:
Entwicklung eines mobilen, automatisierten Messsystems für die zeitgleiche Analytik von Partikeln und Gasphase aus Aerosolen für die fortgeschrittene Umweltanalytik – Online-Aerosol-Analytik

Funding organization:
ZIM Bundesministerium für Wirtschaft und Energie (ZIM Innovation program for the development of new methods and methodologies)

Funding period:
10/2020 – 3/2023

Funding amount:
219 000 € (UniBw M)

Project Partner: Scientific Instruments Manufacturer GmbH, Germany

References

1. Kraus, U., *et al.* (2011). "Particle-associated organic compounds and symptoms in myocardial infarction survivors." Inhalation Toxicology 23(7): 431-447.
2. Orasche, J., *et al.* (2011). "Technical Note: In-situ derivatization thermal desorption GC-TOFMS for direct analysis of particle-bound non-polar and polar organic species." Atmos. Chem. Phys. 11(17): 8977-8993.
3. Shiraiwa, M., *et al.* (2017). "Aerosol Health Effects from Molecular to Global Scales." Environmental Science & Technology 51(23): 13545-13567.

(16) ALIAS-Lung – Development of an automated *in-vitro* exposure system for long-term lung cell exposures to analyze the effects of air pollution

Project realization: C. Bisig (HMGU), A. Huber (HMGU), S. Oeder (HMGU), S. Di Bucchianico (HMGU), C. Schlager (Vitrocell), B. Gutmann (Vitrocell), T. Krebs (Vitrocell), R. Zimmermann (UR/HMGU)

Background: Air pollution is associated with respiratory and cardiovascular diseases leading to premature deaths worldwide. Nowadays, the main tool to investigate the (adverse) effects of ambient pollution are epidemiological studies, which are time-consuming and confounding factors (*i.e.* smoking) that can lead to biased outcomes (Alexis *et al.*, 2004). Lung cell cultures grown at the air-liquid interface could be a reliable addition to these epidemiological studies. Bisig *et al.*, (2018) have previously exposed lung cell cultures to ambient air, showing no effect of summer air with low PM concentrations. Therefore, long-term exposures using both reproducible cell cultures and a reliable exposure system are needed.

Aim of the project: In this two-year project we are collaborating closely with Vitrocell Systems GmbH to implement a complete package for the long-term toxicological evaluation of ambient air. Firstly, the cell cultures conditions need optimization, because cells at the air-liquid interface lack nutrition from the top, making them vulnerable for weeks of cultivation. The current approach is to test the available cell lines, such as A549 and CALU-3, with different media, seeding densities, and media exchange rate to achieve good cell viability after 1 week of exposure. Additionally, the automated exposure system will be improved to ensure uninterrupted exposures of up to 1 week, ideally without local supervision. See figure 1 for an overview.

Partner: Vitrocell Systems GmbH, Waldkirch, Germany.

References:

1. Alexis, N., *et al.* (2004) Health effects of air pollution. J Allergy Clin Immunol 114: p. 1116-23.
2. Bisig, C., *et al.* (2018). A realistic in vitro exposure revealed seasonal differences in (pro-) inflammatory effects from ambient air in Fribourg, Switzerland. Inhalation Toxicology 30(1), 40-48.

German project title:
Entwicklung einer Expositionseinheit zur automatisierten Langzeit-*In-Vitro*-Zellexposition zur Analyse schädlicher Effekte der Luftverschmutzung auf Lungenzellen

Funding organization: ZIM Bundesministerium für Wirtschaft und Energie (ZIM Innovation program for the development of new methods and methodologies)

Funding period:
12/2019 – 11/2021

Funding amount:
190 000 € (HMGU)

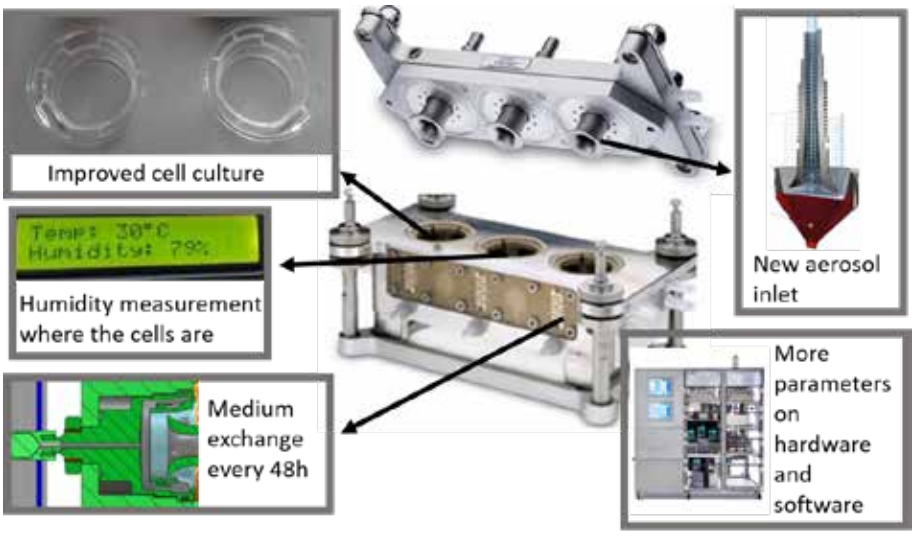


Figure 1: *Selection of adjustments, which are in progress or planned. A long-term exposure needs a reliable and viable cell culture. A new aerosol inlet will reduce the intrinsic stress by the airflow over the cells. An improved medium exchange will enable surveillance of cell viability during an exposure. Measurement of the relative humidity at the cell culture allows for a more accurate reading. Further adjustments of the hardware, such as temperature resistant casing, and software, such as the possibility for remote control, are planned for 2021.*

(17) SAARUS – Optimization of Scrubber-Exhaust Gas Cleaning Technology for the reduction of polluting ship emissions

Project realization at JMSC: T. Streibel (UR/HMGU), C. Rüger (UR), U. Käfer (UR/HMGU), T. Gröger (HMGU), M. Sklorz (HMGU), G. Jakobi, U. Etzien (LKV), B. Buchholz (LKV), J. Passig (HMGU/UR), S. Jeong (UR/HMGU), L. Anders (UR), T. Adam (UniBW M/HMGU), J. Bendl (UniBW M), R. Zimmermann (UR/HMGU)

Background: Pollutants emitted by ocean-going ships are a considerable problem with respect to climate, environment, and human health. Most of the regulations implemented by the International Maritime Organization (IMO) only concern the sulfur content of the fuels. Since January 2020, ships are only allowed to burn fuels with a maximum sulfur content of 0.5 %. In special designated areas called Sulfur Emission Control Areas (SECA), fuel sulfur content is even limited to 0.1 %. The regulations offer an additional choice, *i.e.* to still use fuel with a higher sulfur content than 0.5 % with the installation of a sulfur scrubbing unit to reduce the sulfur dioxide content of the exhaust gas.

Aim of the project: Construction of a downscaled scrubber unit (figure 1): The SAACKE Company will develop and build a scrubber unit that can be adapted to a one-cylinder ship diesel research engine for conducting extensive evaluation and performance studies with different fuels:

- Hydrogenated Vegetable Oil (HVO), a regenerate fuel
- Marine Gas Oil (MGO) as standard distillate fuel
- Compliant Heavy Fuel Oil (HFO), a new brand of HFO that fulfills the fuel sulfur content requirements
- Heavy Fuel Oil with 1.35 % sulfur
- Heavy Fuel Oil with 2.4 % sulfur
- Heavy Fuel Oil with 0.06 % sulfur, but a very high aromatic fraction

Analytical monitoring of the ship engine experiments: A large variety of analytical equipment is employed to assess as many parameters as possible. Special emphasis is set on the effects of the scrubber operation with respect to the emitted particulate matter (PM). The scrubber unit is able to remove approximately 80 % of particle mass from the exhaust as well, but nanoparticles, the fractions most hazardous for human health, remain in the exhaust. Therefore, additional exhaust gas after-treatment measures in the form of particle filters will be integrated.

A first extensive measurement campaign has been conducted without the scrubber unit to establish the current situation with respect to exhaust gas constituents. While the focus is set on the particulate emissions, gas-phase analyses were also performed (see Research Area 1 Report (10)).

Project partners: SAACKE GmbH, Germany, GEA Westfalia Separator Group GmbH, Germany, RVT Process Equipment GmbH, Germany, AVL Deutschland GmbH, Germany, Department of Piston Engines and Internal Combustion Engines of the UR, Leibnitz Institute for Baltic Sea Research, Germany, Sult GmbH, Germany

Bundesministerium für Bildung und Forschung

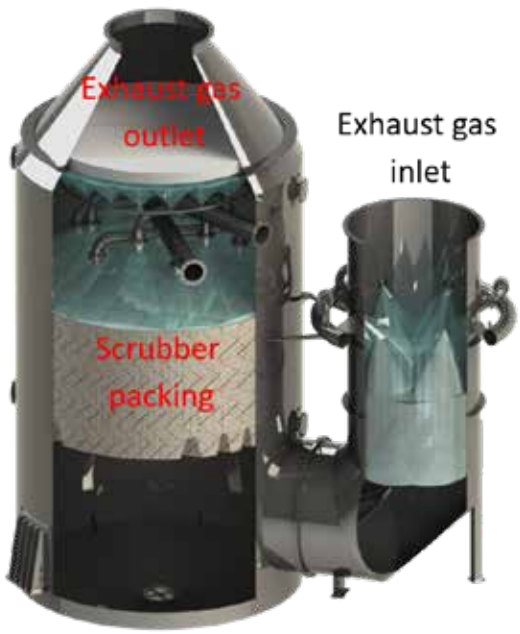
German project title:
Optimierung der Scrubber-Abgaswäsche Technologie zur Reduktion umweltschädlicher Schiffsemissionen

Funding organization:
German Ministry for Economy and Energy (BMWi)

Funding period:
06/2019 – 05/2022

Funding amount:
567 336 € (UR)
562 000 € (UniBW M)

Figure 1: *Model of the scrubber unit to be constructed by SAACKE Company (Source: SAACKE)*



(18) Chemical characterization and toxicological investigations for a thermolysis reactor

Project realization at the JMSC: T. Streibel (UR/HMGU), L. Friederici (UR), A. Koch (UR), P. Martens (UR), C. Rüger (UR), J. Pantzke (HMGU), S. Bauer (HMGU), S. Di Bucchianico (HMGU), R. Zimmermann (UR/HMGU)

EUROPÄISCHE UNION
Europäischer Fonds für regionale Entwicklung

German project title:
Chemische Charakterisierung und toxikologische Untersuchungen für einen Thermolysereaktor

Funding organization:
Europäischer Fonds für regionale Entwicklung (EU-EFRE) über Technologie Beratungsinstitut GmbH

Funding period:
06/2019 – 05/2022

Funding amount:
382 110 € (UR)

Background: In the vein of increasing the share of regenerative sources in energy production, wind power plants play an ever-expanding role. However, there is no general solution about how to deal with the installations, especially the rotor blades, once the units are dismantled after exceeding their lifetime. The current procedure is based on separating the different components (for an average composition see figure 1) and disposing or recycling them. One important constituent of rotor blades is glass fiber or carbon fiber reinforced polymer material (GFRP or CFRP), which is after separation combusted in kilns for cement production. The question arises if there could be a better and more sustainable strategy based on material recycling of those fiber reinforced polymer materials.

Aim and first results of the project: Within the scope of the project comprises the design and construction of a new reactor for the thermolysis of glass fiber reinforced plastic and carbon fiber reinforced plastic. This part is executed by the project partner TAB GmbH. Parallel to the reactor design process, laboratory experiments are carried out to chemically characterize the feedstock material and evaluate its thermal behavior. The acquired data will help optimizing the design parameters of the reactor, especially temperature profiling. Additional data will be generated by experiments at an existing thermolysis plant, which allows access to product samples and side products such as coke and condensates. This will also include the application of modern laser mass spectrometric on-line process analytical technologies. A further concern in the recycling of fiber-containing materials is the potential inhalation toxicity of inhalable fiber dust (after WHO definition: persistent inorganic fibers with a length > 5 µm a diameter < 3 µm and a length-to-diameter ratio of > 3:1) or toxic chemicals that may occur in the in different stages of the recycling process or in the products. It is *e.g.* well known that carbon fibers could shrink under thermal stress to dimensions deemed critical according to the WHO definitions. For testing the products or process dusts for potential chemical or fiber toxicity in addition to chemical analyses for toxicants also *in-vitro* toxicological studies on human lung cell cultures will be performed and cytotoxic and genotoxic endpoints are determined.

An important parameter for the successful performance of the recycling process is the thermolysis temperature. It must be high enough to decompose and remove the organic coating material, but should be as low as possible to preserve the tensile strength of the recycled glass or carbon fibers. Thermogravimetry (TG) investigations of different feedstock materials containing GFRP revealed the majority of thermal decomposition at temperatures around 400 °C. The first results on the evolved gas analysis of the released chemical compounds during TG experiments showed bisphenol A and its pyrolysis products, typical degradation substances of epoxy resin. Investigating thermolysis products of the feedstock material from the existing plant will help to determine if all organic substances have been successfully removed during the process. With this, an optimal temperature window for the thermolysis process will be assessed for each feedstock. For the investigation of toxicological properties of the formed recyclates and solid byproducts, a novel cell culture system will be designed, assembled, and tested. It is about a triple cell culture system consisting of lung epithelial cells (A549), fibroblasts, and macrophages. Currently, the triple cell culture was successfully assembled and tested with *e.g.* carbon nanotubes.

Project partner: TAB GmbH, Germany

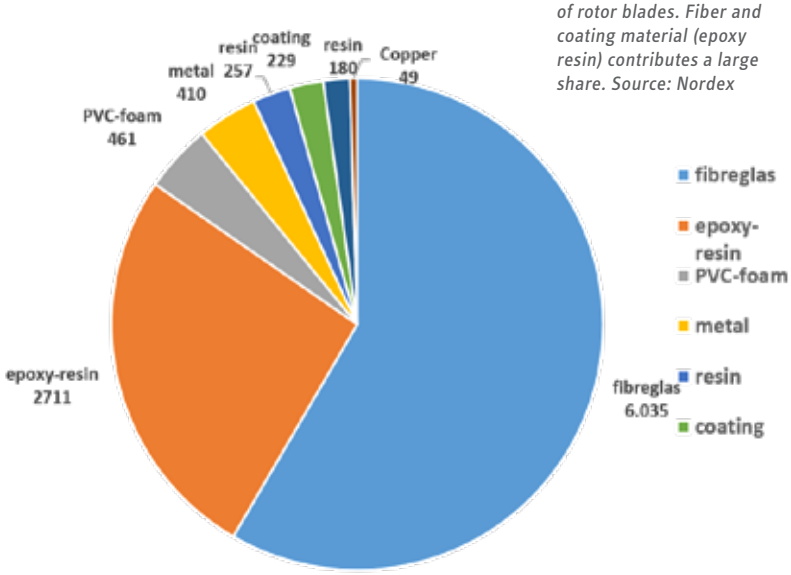


Figure 1: *Incoming amounts of the different components of rotor blades. Fiber and coating material (epoxy resin) contributes a large share. Source: Nordex*

(19) aeroHEALTH – German-Israeli Helmholtz International Laboratory: Impact of Atmospheric aerosols on Human HEALTH

Project realization: R. Zimmermann (UR/HMGU), H. Czech (HMGU/UR), E. Hartner (HMGU), S. Offer (HMGU), Y. Rudich (WIS), M. Levin (WIS), D. Gat (WIS/HMGU), C. Li (WIS), A. Kiendler-Scharr (FZJ), T. Hohaus (FZJ), A. Paul (FZJ), F. Theis (HMGU), A. Marsico (HMGU) C. Ogris (HMGU), Y. Hu (HMGU) and further co-workers of HMGU, WIS and FZJ

HELMHOLTZ
RESEARCH FOR GRAND CHALLENGES

Funding organization:
Helmholtz Association of Research Centers (HGF)

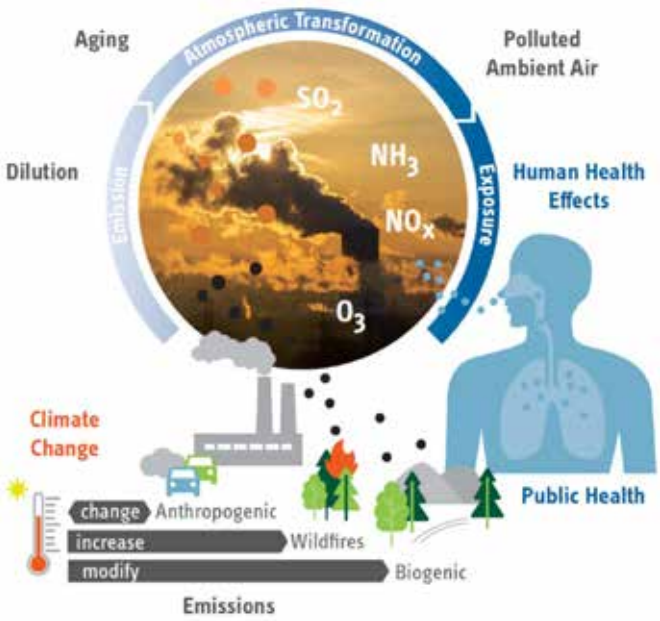
Funding period:
04/2019 – 3/2024

Funding amount:
1 000 0000 € (to HMGU by HGF) + guaranteed own contribution by HMGU of 1 000 000 €.
The Weizmann Institute covers its own funding

Background: After the successful evaluation and permanent adoption of the HICE program at the HMGU, the question arose about how to include international partners in the aerosol and health research. From a consultation between the HMGU and HGF management boards, we were directed to the competitive Helmholtz International Lab call. The question of the effects of atmospheric processing and oxidative aging of aerosol emissions on health effects suggested a consortium consisting of the Weizmann Institute of Science in Israel (Yinon Rudich's research group), the Helmholtz Zentrum München (Ralf Zimmermann's and Fabian Theis's research groups) as well as the Forschungszentrum Jülich (Astrid Kiendler-Scharr's research group). We prevailed a competitive internal selection of our proposal at the leading Helmholtz center (HMGU) and became eligible to officially apply. The subsequent two-stage selection process was based on an international peer-review of the proposal text and a defense session in front of a selection panel headed by the president of the HGF, Prof. Wiestler in the summer of 2018. The Helmholtz International Lab aeroHEALTH was finally funded for an initial 5-year period and was officially started by an inauguration ceremony at the Weizmann Institute on April 1st, 2019.

Aim of the project: In the last years, many studies pointed out that the chemical composition of aerosol emissions matters for the observed health effects. However, the question if atmospheric photochemical aging of the emitted aerosols and the associated increase or change in the composition of the organic aerosol fractions is influencing the aerosol-induced health effects is a societally very important and yet not well-addressed research task. In particular, it is important to know, whether secondary organic aerosols (SOA), which are originated from anthropogenic emissions, exhibit different toxicity than secondary aerosols originated from natural, biogenic sources. Selected further research questions are whether the photochemical age of an aerosol is altering adverse health outcomes, which compounds are important for the health effects, and if indicator compounds for prediction of those can be identified. A final goal for the originally anticipated 8-year funding period is to further develop the aerosol toxicological approaches used in aeroHEALTH, namely the realistic exposure of human lung cell models at the Air-Liquid-Interface followed by cyto- and genotoxicological as well as molecular biological response analysis, to enable a monitoring and air pollution risk analysis for ambient air. Among other anthropogenic sources, biomass burning (*e.g.* for residential heating) and traffic-related combustion aerosols from road or ship traffic are of particular interest. Regarding natural emission sources, the release of a volatile organic compound from plants as SOA precursors, wildfire emission, and dust are put in the focus. The complex aeroHEALTH research questions can only be addressed if realistic aerosol aging experiments, using existing and new oxidation flow reactors or atmospheric simulation chambers, are accompanied by comprehensive analyses of chemical, physical and biological effects. This requires that within aeroHEALTH the expertise of toxicologists, biologists, engineers, environmental scientists, chemists, aerosol physicists, data scientists, and bio-informaticians is merged into truly joint multidisciplinary research. An important aspect of the multidisciplinary strategy of aeroHEALTH is to generate data within joint measurement campaigns, making all involved scientists a part of the experiment. In addition to excellent research, aeroHEALTH also strives to undertake special measures for the education of young scientists (*e.g.* PhD students) and early career scientists in view of tenure track positions or postdoctoral qualifications ("Habilitation").

Project Partners: Weizmann Institute of Science (Israel), Helmholtz Zentrum München (Germany), Forschungszentrum Jülich (Germany), University of Rostock (associated, Germany), University of Eastern Finland (associated, Finland).



(20) Aerosols at workplaces: Investigation of gas- and particle distribution of semi-volatile organic compounds (SVOCs)

Project realization: N. Rastak (HMGU), S. Binder (HMGU), E. Karg (HMGU), G. A. Ferron (HMGU), S. Di Bucchianico (HMGU), C. Bisig (HMGU), J. Orasche (HMGU), M. Sklorz (HMGU), R. Zimmermann (UR/HMGU)

German project title:
Gas-Partikel-Mischungen am Arbeitsplatz: Entwicklung von neuartigen personengetragenen Probenahmesammlern und Untersuchung der unterschiedlichen Toxizität von Gasen und Partikeln

Funding organization:
Deutsche Gesetzliche Unfallversicherung (DGUV)

Funding period:
02/2019 – 01/2022

Funding amount:
594 180 € (HMGU)

The German Social Accident Insurance (Deutsche Gesetzliche Unfallversicherung, DGUV) is interested in health-related hazards caused by aerosols at workplaces. At work, chemical substances can be emitted as aerosols through industrial manufacturing processes. The inhalation of these aerosolized substances may cause respiratory tract irritation, occupational diseases or even cancer. SVOCs are present in both, particulate and gaseous phase. From a toxicological point of view, it makes a huge difference if a substance in the air is inhaled as a particle or as vapor, even if the concentration is the same. This project targets the development of analytical and aerosol toxicological methods to differentiate the composition and biological effects of SVOCs in the particle or vapor phase and has three main objectives: 1) Development of personal aerosol samplers, 2) Construction of a cell exposure system, 3) Toxicological evaluation of the gas and particle phase.

1) Samplers: (Gesamtstaub-Gas-Probenahme, GGP) are mobile samplers to analyze the aerosols at workplaces (figure 1). Here, we add charcoal denuders to the samplers to remove the gas phase. First, the denuder absorbs the gas phase, the filter collects the particles and the adsorber recovers what evaporates from the particles. The other line measures the total concentration and the difference obtains the gas concentration.

2) Cell exposure system: In order to do cell exposures to the gas and particle phase of SVOCs, separately we use the exposure part of the Air-Liquid Interface (ALI, Mülhopt 2016) in a tight climate chamber suitable for exposures to highly toxic compounds. To distinguish between gas- and particle-phase, charcoal denuders and filters will be located in the system (figure 2).

3) Toxicological evaluation: In the next step, the biological effect of gas- and particle-phase of dibutylphthalate (DBP, ECB 2003) on cells will be analyzed using the new exposure system.

References:

1. ECB (2003): European Union Risk Assessment Report–DibutylPhthalate. Volume 29
2. Mülhopt *et al.* (2016). Toxicity testing of combustion aerosols at the air–liquid interface with a self-contained and easy-to-use exposure system. Journal of Aerosol Science, 96, 38-55.

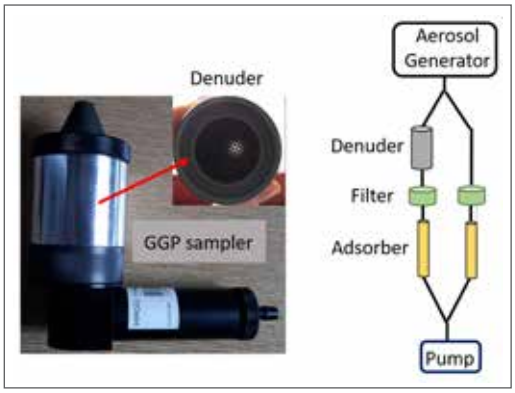


Figure 1: *The GGP sampler and the set up to investigate the gas and particle phase of samples.*

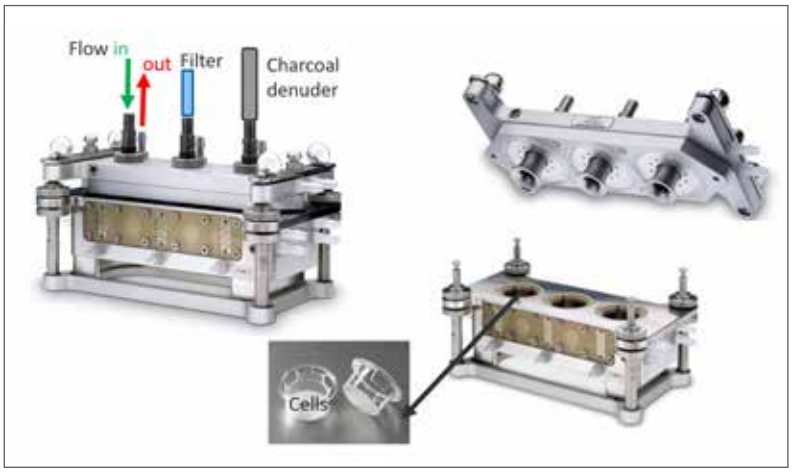


Figure 2: *Exposure part of ALI. The trumpet guides aerosol flow to the cells.*

(21) Carbon Concrete Composite C³ and health: Exposure of lung cells with dust particles from the thermal and mechanical processing of carbon concrete materials

Project realization at JMSC: T. Streibel (UR/HMGU), T. Kanashova (UR/MDC), A. Koch (UR), S. Bauer (HMGU). S. Di Bucchianico (HMGU), R. Zimmermann (UR/HMGU)

Bundesministerium für Bildung und Forschung

German project title:
Carbon Concrete Composite C³ und Gesundheit: Exponierung von Lungenzellen mit Stäuben aus der thermischen und mechanischen Belastung von Carbonbetonmaterialien

Funding organization:
Federal Ministry for Education and Research (BMBF)

Funding period:
08/2018 – 07/2020

Funding amount:
370 320 € (UR)

Background: In a preceding project (Third Party Project (31)) a measurement system has been developed for the comprehensive characterization of dust particles produced during the processing of carbon concrete materials. This approach is applied in this project to investigate newly developed materials such as rod-shaped reinforcement construction components. Moreover, the question remains what could happen to carbon fibers when carbon concrete composites are subjected to high temperatures, *e.g.* during thermal recycling processes or fires. Carbon fibers tend to shrink under thermal stress, bearing the danger of reaching dimensions deemed critical under WHO guidelines for inhalation fiber toxicity.

Aim and results of the project: The central aim of the project consists of establishing the effects of thermally stressed carbon concrete composite with respect to the properties of dust particles from abrasive cutting. In the first approach, a relatively high temperature of 700 °C was chosen for a thermal pretreatment of carbon concrete materials to cover extreme cases such as fire accidents in carbon concrete structures. Afterwards, samples kept at room temperature and thermal pretreated ones are processed with the cutting module. Generated dust particles were either sampled on filters for chemical and morphological analyses or guided directly to an Air-Liquid Interface (ALI) cell exposure system to assess potential toxicological effects (see Research Area 1 (3)). The chemical characterization of untreated material revealed a high concentration of bisphenol A (figure 1) and its pyrolysis products in carbon concrete reinforcement material, stemming from the epoxy resin coating. Concentrations in carbon concrete itself are much lower due to the high content of mineral particles. The morphological investigation by scanning electron microscopy confirmed the absence of WHO critical fibers in the particulate matter from the cutting of carbon concrete at room temperature. However, the 700 °C pretreatment led to the sporadic appearance of fibers exhibiting critical dimensions.

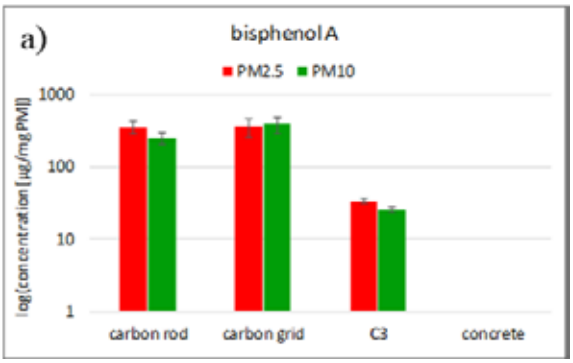


Figure 1: Bisphenol A concentrations in dust particles from the abrasive cutting of carbon concrete material. The reinforcement units (carbon rod and carbon grid) exhibit relative high concentrations, carbon concrete itself shows a lower content, concrete none at all.

The project aimed to model the cellular interplay between lung epithelial and fibroblast cells using an *in-vitro* co-culture system for the assessment of indirect mechanisms resulting from aerosol exposures of one cell type, *e.g.* epithelial cells, causing secondary genotoxic effects in another, *e.g.* fibroblasts. For this scope, the ALI exposure system used a confluent monolayer of A549 lung epithelial cells cultured onto an insert membrane and lung fibroblasts MRC-5 cultured in the downside of the insert membrane as a feeding layer (figure 2). Comet assay was performed on both cell types immediately following 2 hours exposures and after 24 hours post-incubation. Carbon rods and thermally treated carbon concrete aerosols, but not carbon concrete per se, induced DNA damage in A549 cells both after exposure and post-incubation. Fibroblast cells did not show genotoxicity following 2 hours exposure, but a statistically significant increase of DNA damage was noticed following post-incubation, thus highlighting secondary genotoxicity mechanisms of action due to cell-cell signaling. Special attention should be paid to hazard mitigation when working with pure reinforcement materials due to their organic content. Whenever materials are put under thermal stress, substances with potentially adverse health effects could be released. If possible, different coating materials than epoxy resin should be employed.

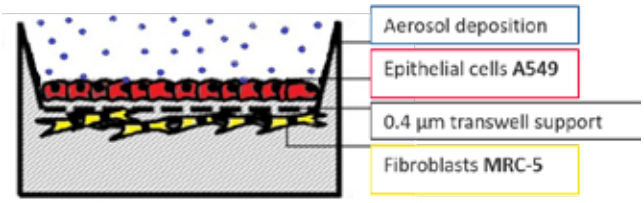


Figure 2: Co-culture model of lung epithelial A549 cells (red) and MRC-5 fibroblasts (yellow) cultured in the downside of an insert membrane.

Project partners: Technical University Dresden, Germany, Vitrocell Systems GmbH, Germany, Topas GmbH, Germany

(22) AerOrbi – Aerosol soft photoionization Orbitrap mass spectrometry

Project realization: C. Rüger (UR), P. Kösling (UR), J. Schade (UR), J. Passig (UR), S. Ehlert (Photonion/UR), R. Irsig (UR/Photonion), Y. Tsybin (Spec), A. Kozhinov (Spec), M. Rigler (Aero), A. Gregorič (Aero), R. Zimmermann (UR/HMGU)

Funded by the European Union

eurostars™

Funding organization:
Horizon 2020 Eurostars

Funding period:
07/2018 – 06/2021

Funding amount:
234 213 € (UR)
Total funding: 1 605 713 €

Background: Aerosol characterization is essential for environmental health and climate research and, thus, represents an attractive market for analytical instrumentation and sector for innovations in enabling technologies. In this respect, there is a need for high-performance online instrumentation unraveling the aerosol chemical composition on the molecular level. The idea of the Eurostars AerOrbi project is to address a niche in the existing field-deployable instrumentation sector by the development of a new hyphenated instrument. The new device will combine soft photoionization, preserving the molecular finger-print, and Orbitrap-based high-resolution mass spectrometry, allowing to attribute distinct elemental composition to the constituents.

Aim of the project: The aim of the AerOrbi project is the development of a functional prototype of an innovative transportable instrument for aerosol chemical specification, which is capable of gas-phase measurements and single-particle analysis. A direct gas-phase inlet, developed by the University of Rostock and Photonion GmbH, will allow determining the chemical composition of the gaseous phase of an aerosol. A second alternative will be composed of an additional aerosol thermal desorption system, invented by Aerosol d.o.o., mimicking the well-established EC/OC technology. Currently, there is no instrument commercially available on the market addressing the molecular complexity of carbonaceous aerosols, which can be used in field experiments. The third approach will be a single particle module. This version will introduce the particle phase (PM2.5) directly into the system, and a cascade of lenses and sizing units enable the mass spectrometric measurement of single nanometer-size particles. The final product is able to detect toxic substances, emission sources, and relevant environmental parameters. Chemical characterization on the single-particle level allows for source apportionment, *e.g.*, primary emission sources and transport paths of air pollution.

In the AerOrbi prototype, resonance-enhanced multiphoton ionization (REMPI), a soft photoionization scheme, will be deployed. This technique is realized via a 266 nm Nd:YAG solid-state and a 248 nm Excimer pulsed ns-laser. The key benefit of REMPI is the high sensitivity towards aromatic constituents, including the toxic and carcinogenic polycyclic aromatic hydrocarbons. The laser technique enables a fast repetition and, thus, a rapid acquisition rate. The last structural component of the instrument is the mass analyzer. An Orbitrap high-resolution mass analyzer will be installed with certain beneficial features, such as a high resolving power and ppm mass accuracy. Carbonaceous aerosol consists of a high number of different chemical species; therefore, a mass analyzer with high resolving power improves signal detection and assignment. Overlaps and peak distortion, which could lead to a wrong interpretation, are minimized. The highly complex data of the Orbitrap analyzer have to be detected and processed efficiently online. For this purpose, a high-sophisticated DAQ-system will be developed and installed by Spectroswiss SARL and Photonion GmbH. This system transcripts the raw data in a series of processing steps, including fast Fourier transformation, signal detection, and correction. The benefit of this high-level DAQ-system is a quasi-instant and in-line response of the measured data, which enables a fast interpretation of the sample with the highest achievable sensitivity and dynamic range. High-performance mass spectrometry of the prototype results in unambiguous chemical information.

The EUROSTARS AerOrbi project brings together the unique expertise of four partners: one academic institution and three SMEs. Thermo Fisher Scientific, the sole vendor of Orbitrap-based mass spectrometric solutions, acts as an external partner, contributing in-depth know-how on the hardware and software architecture of the mass analyzer platform.

Cooperation partners: Aerosol d.o.o. (Aero), Spectroswiss SARL (Spec).

Figure 1: *Consortium of the Eurostars AerOrbi project combining expertise in the aerosol specification (Aerosol d.o.o.), photoionization mass spectrometry (Photonion GmbH), data processing and software (Spectroswiss SARL), and high-resolution mass spectrometric instrumentation and application (University of Rostock)*

(23) EU FT-ICR MS - European Network of Fourier-Transform Ion-Cyclotron Resonance Mass Spectrometry Centers

Project realization at JMSC: C. Rüger (UR), A. Neumann (UR), T. Kröger-Badge (UR), M. Sklorz (HMGU), R. Zimmermann (UR/HMGU)

Funded by the European Union

Funding organization:
Europäische Kommission (EU Horizon 2020), INFRAIA

Funding period:
01/2018 – 12/2021

Funding amount:
410 285 € (UR)

Background: Mass spectrometry (MS) is one of the most ubiquitous analytical techniques in use today, providing information on the composition and structure of substances from a complex mixture. MS is characterized by the existence of a large range of instruments combining different ionization sources, mass analyzers, and ion fragmentation methods. The performance of MS mainly relies on the intrinsic properties of the mass analyzed in terms of mass resolving power, mass accuracy, dynamic range, acquisition speed, and mass range. In this respect, Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) delivers unparalleled mass accuracy and mass resolving power, enabling the analysis of ultra-complex organic mixtures. Nonetheless, there are two major drawbacks of FT-ICR MS. First, the high price for the instrumentation of over 1 Million € limiting its implantation, and, second, the necessity of having very qualified engineers and scientists for obtaining satisfactory results.

Aim of the project: The objective of the EU FT-ICR MS project is to develop a European state-of-the-art network of Fourier Transform Ion Cyclotron Resonance mass spectrometry centers in collaboration with an instrument manufacturer (Bruker Daltonics, Bremen, Germany) and association with an SME software company (CASC4DE, Illkirch, France). The EU FT-ICR MS network encompasses ten FT-ICR MS centers from eight different European countries and one third-country (Russia). The project focuses on four objectives. First, providing the European academic, SME, and industrial communities' with access to world-class FT-ICR MS centers. This objective is realized by trans-national access (TNA). TNAs are free of charge and travel funding is granted based on a transparent and fair application process. Second, an EU community of end-users and FT-ICR MS scientists will be created by short-courses, advanced user schools, and end-user schools. Moreover, a scientist exchange between the centers will be conducted to stimulate building up the EU FT-ICR MS community. The third objective is to develop an open-access platform for the data as well as an open-source software for handling the complex spectral information. The fourth objective is to strengthen the FT-ICR MS application fields by promoting innovative and cooperative research between the centers as well as to included private companies.

In the research activities at the University of Rostock, novel ionization schemes and hyphenation techniques are explored. In this respect, particularly, thermal analysis coupling for the investigation of ultra-complex viscous and solid sample materials as well as photoionization, conducted by different gas-discharge lamps or VUV/UV-laser systems, are promoted. Among others, trans-national access projects allowed to monitor the slow pyrolysis of polyethylene terephthalate (Dhahak *et al.*, 2020) and to identify aging processes in bitumen at the molecular level (Neumann *et al.*, 2020).

Project Partners: EU Horizon 2020 INFRAIA Project for Starting Communities. Cooperation partners: Centre National de la Recherche Scientifique (CNRS), Universite de Lille (UL), Universite Paris Sud (UPS), Universite de Rouen-Normandie (URO), Universite de Liege (LIEG), Czech Academy of Science – Institute of Microbiology (PRAG), University of Rostock (UR), University of Eastern Finland (UEF), Università di Roma La Sapienza (ROMA), Universidade de Lisboa (FC-LISB), Skolkovo Institute of Science and Technology (MOSC), University of Warwick (WARW), CASC4DE (CASC4DE), Absiskey (AK)

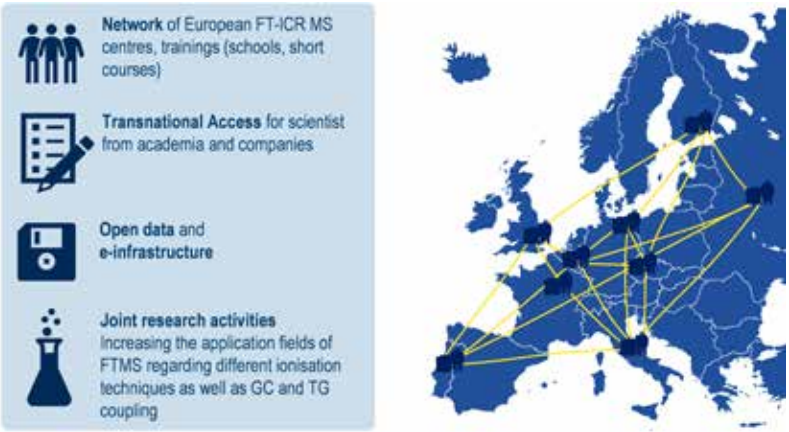


Figure 1: *Schematic representation of the European Network of Fourier-Transform Ion-Cyclotron Resonance Mass Spectrometry Centers (EU FT-ICR MS) in terms of the central work packages and aims (left) and the geographical distribution over the entire European continent (right).*

References:

1. Dhahak, A. *et al.* (2020) Real time monitoring of slow pyrolysis of polyethylene terephthalate (PET) by different mass spectrometric techniques Waste Management 106, 226-239
2. Neumann, A. *et al.* (2020) Investigation of Aging Processes in Bitumen at the Molecular Level with High-Resolution Fourier-Transform Ion Cyclotron Mass Spectrometry and Two-Dimensional Gas Chromatography Mass Spectrometry Energy Fuels 34(9), 10641–10654

(24) Development of hyper-fast gas chromatography (fastGC) for online photoionization mass spectrometry

Project realization: H. Czech (HMGU/UR), C. Gehm (UR), K. Schnepel (UR), T. Miersch (UR), S. Ehlert (Photonion/UR), R. Zimmermann (UR/HMGU)

German project title:
Entwicklung einer ultraschnellen Gaschromatographie für on-line Photoionisationsmassen-spektrometrie

Funding organization:
Deutsche Forschungs-gemeinschaft (DFG)

Funding period:
10/2017 – 12/2020

Funding amount:
313 500 € (UR)

Background: Online time-of-flight mass spectrometry (TOFMS) with photoionization (PI) under vacuum conditions offers many advantages for analyses of complex samples with high time resolution. In contrast to hard electron ionization, PI does not produce a significant number of fragment ions, improving the interpretability of the mass spectra. Compared to other so-called soft ionization techniques at atmospheric pressure, the analyte gives the same signal independent from the composition of the sample matrix and appears solely as a molecular ion without adduct formation to quasi-molecular ions. Complex samples from different scientific disciplines contain isomeric compounds, appearing at the same exact mass, which cannot be resolved by increasing the resolution of the mass spectrometer. The implementation of ultra-fast gas chromatography to PI-TOFMS enhances the chemical specificity of the analysis and keeps the level of time resolution appropriate for many various applications.

Aim of the project and setup: This project targets the implementation and improvement of recently developed advanced devices for fastGC for online PI-TOFMS. A consumable-free high-performance electrical modulator enables parallel sampling and injection of volatile organic compounds (VOC) onto a GC column with a peak width of less than 10 % of the chromatographic peak width (Wohlfahrt *et al.*, 2016). GC run times of 30 s and even down to 15 s with custom-temperature programs can be achieved with a novel optically-heated GC column based on multiple reflections of heat radiation from a halogen lamp. Additionally, the low thermal mass of the GC unit allows rapid cooling in a few seconds by blowing room air (Fischer *et al.*, 2015). In order to the peak capacity and guarantee repeatable GC conditions, we installed a 6-port-2-way valve to provide helium 5.0 as a carrier gas for the chromatographic separation. The performance of the entire setup is demonstrated by headspace and online analyses in real-time of VOCs released from needles of coniferous trees and biomass burning (see Research Area 2: Enabling Analytical Technologies (10)).

Preliminary analytical figures of merit: We followed guidelines for proper analytical method evaluation and determined required figures of merit. We can show that our fastGC system has a linear response over a dynamic range of several orders of magnitude and a limit of detection in the sub-ppb range for p-xylene based on a signal to noise ratio of three. The analyte range with modulator settings of -65°C in sampling mode is determined by its trapping efficiency, which was found to increase asymptotically for aromatic compounds with increasing m/z. While benzene (m/z 78) is trapped with an efficiency of 22 %, almost 80 % of p-xylene (m/z 106) condenses on the modulator stage.

Applicability of real sample VOC analysis: The application domain for the fastGC instrument is dedicated to two broader fields: offline headspace analysis and direct online analysis. Headspace analysis is an established technique in many areas and measures evolving compounds from a sample at room or higher temperature. Our developed fastGC system enables many measurement repetitions in the same analysis time of a conventional GC-based instrument, thus increasing the confidence of the result. Direct online analysis by fastGC may be added to SPI-TOFMS for studies in which 30 s of time resolution is still sufficient, such as biomass burning or in laboratory-aging of combustion emissions or individual VOC, with the benefit of substantially enhanced chemical space.

Outlook: Both headspace and online analysis by fastGC may be of interest for all research topics of our group in which VOCs are analyzed. Furthermore, evolved gas analysis from thermal gravimetric analysis or, in the case of aerosol filter samples, from thermal-optical carbon analysis by PI-TOFMS (Diab *et al.*, 2015) would benefit from the enhanced chemical speciation. For that, we aim to shift the range of the analytes towards lower volatility in order to analyze aerosol particle constituents. Because of expected lower chromatographic resolution, a vital part of the project is the implementation of sophisticated peak detection and deconvolution strategies. Finally, we seek to optimize the system towards lower detection limits for VOC analysis in ambient air.

References:

1. Diab, J. *et al.* (2015) Hyphenation of a EC / OC thermal-optical carbon analyzer to photo-ionization time-of-flight mass spectrometry: An off-line aerosol mass spectrometric approach for characterization of primary and secondary particulate matter. Atmospheric Measurement Techniques, 8(8), 3337–3353.
2. Fischer, M. *et al.* (2015). Optically heated ultra-fast-cycling gas chromatography module for separation of direct sampling and online monitoring applications. Analytical Chemistry, 87(17), 8634–8639.
3. Wohlfahrt, S. *et al.* (2016). Dual-Stage Consumable-Free Thermal Modulator for the Hyphenation of Thermal Analysis, Gas Chromatography, and Mass Spectrometry. Analytical Chemistry, 88(1), 640–644.

(25) PPK – Process analysis and control of industrial roasting of coffee using photoionization mass spectrometry

Project realization: J. Heide (UR), H. Czech (HMGU/UR), R. Reiss (UR); S. Ehlert (Photonion/UR), R. Zimmermann (UR/HMGU), T. Koziorowski (Probat), J. Piel (MLase)

Background: The roasting process of coffee is a food technology process with high added value and economic importance. The trading volume of roasted coffee in Germany is 3.5 billion euros. The typical aromatic substances are produced by mild pyrolysis of the green coffee beans at 250 to 380 ° C. In order to research the roasting process and to optimize the yield of the desired flavoring substances, reliable online process analysis is necessary, which records the relevant substances and their flavors in real-time.

Aim and results of the project: The aim of the PPK research project was to develop a novel method with which a coffee with special properties can be produced during roasting. A process analyzer must be set up for this purpose, which was tested in several measurements and test campaigns at Probat on small and medium-size roasters as well as in an industrial roasting plant. The measured molecular signals, the predicted process, and product properties are correlated in real-time in order to regulate the roasting process.

In accordance with the objective of building a sensitive and selective process analyzer based on photoionization mass spectrometry (PIMS) that is suitable for industrial online use, developments on sampling, ionization, detection, evaluation, and control were necessary. In addition, it was also necessary to expand the general knowledge for the identification of relevant chemical markers in the roasting process and essential measurement parameters.

Even before roasting, raw green coffee beans contain around 300 volatile compounds but lack both the right color and the characteristic aroma for which roast coffee is prized. The most important chemical reaction paths that occur during roasting are the Maillard reaction, the Strecker degradation and caramelization reactions. They give coffee its characteristic color and aroma. PI-TOFMS enables monitoring of the temporal evolution of volatile and semivolatile compounds in the roasting off-gas. The excellent time resolution achieved by photoionization allows the release profiles of individual compounds to be selectively investigated over the entire roasting process. One of the most interesting aspects of coffee roasting is the degradation or conversion of chlorogenic acids. In addition to a number of other compounds, thermal degradation products such as 4-vinylguaiacol, vanillin, phenols, vinylcatechol, and ethylcatechol can be detected. The release profiles allow conclusions to be drawn about the reactions taking place and how they are affected by the roasting conditions.

Within the project, a greater understanding of coffee roasting and the processes involved could be achieved. It can form the basis for targeted interventions to create certain tastes or avoid or enhance specific properties such as the resulting polyphenol content, which can be seen as a marker for the antioxidant content. Real-time analysis in combination with modeling the coffee roasting process would not replace trained coffee roasters but to provide them with an additional tool to get the best out of the green bean and promote potential positive health effects.

Consortium: Photonion GmbH, Germany; MLase AG, Germany; University of Rostock; PROBAT-Werke von Gimborn Maschinenfabrik GmbH, Germany (associated); Azul Kaffee GmbH & Co. KG, Germany (associated)

Bundesministerium für Bildung und Forschung

German project title:
Carbon Concrete Composite C³ und Gesundheit: Exponierung von Lungenzellen mit Stäuben aus der thermischen und mechanischen Belastung von Carbonbetonmaterialien

Funding organization:
Federal Ministry for Education and Research (BMBF)

Funding period:
08/2018 – 07/2020

Funding amount:
370 320 € (UR)

Figure 1: *Schematic representation of the project goal as a real-time measurement and control system for coffee roasting.*

(26) Development of a Robust and Mobile Single-Particle Mass Spectrometer for Real-Time Characterization of the Size and Chemical Composition of Nanoparticles and Aerosols

Project realization: R. Irsig (UR/Photonion), J. Passig (HMGU/UR), S. Ehlert (Photonion/UR), J. Piel (MLase), R. Zimmermann (UR/HMGU)

Background: Single-Particle Mass Spectrometry (SPMS) is a unique particle measurement technology yielding a chemical fingerprint and the size of individual aerosol particles, see also Research Area 1, Aerosol and Health (01) and (05) and Research Area 2, Enabling Analytical Technologies (03) and (04). However, this technique is complex with large and fragile instrumentation that requires delicate adjustments. Moreover, the minimum particle size that can be analyzed by active detection and triggering to individual particles is limited to about 150 nm by the wavelength of the particle detection lasers.

Aim and results of the project: The aim of this project was the development and construction of a field-deployable and powerful on-line analyzer for single aerosol particles that can also obtain chemical information on particles smaller than 100 nm. These nanoparticles are assumed to be particularly important for air pollution-related health effects. Furthermore, the system should provide the technology for ultra-sensitive detection of polycyclic aromatic hydrocarbons and metals as recently developed by JMSC, see reports in Research Area 2 (03 & 04).

Within the project, the partner MLase developed an excimer laser operating in the UV spectral range. The wavelength of this system can be changed faster and more easily than for any commercial system, thus allowing maximum flexibility for different ionization schemes in measurement campaigns. Moreover, the pulse repetition rate of the system reached 2 kHz, thus it can fire with a much higher frequency than comparable lasers. This is the key for measurements on ultra-fine particles by firing into the aerosol beam without active triggering, accidentally hitting particles that are too small for optical detection ("free-running" mode). The partner Photonion developed a new software that is performing a fast pre-check of signal presence before obtaining a full bipolar mass spectrum, thus it records only spectra from particles that were fully hit by the high-repetition laser. This new software architecture features fully automated single-particle data acquisition with high resolution at more than 500 Hz repetition rate in the current configuration. The partners jointly designed the overall instrumental setup. The whole, improved SPMS system, including the new excimer laser source and the bipolar mass spectrometer is made in a compact and mobile design with low power consumption. The analyzer is currently used for several JMSC research activities and measurement campaigns, where also its unique capabilities for ultra-fine particles are used, *e.g.* in the SAARUS project (see Third Party Projects (17)).

German project title:
PPK – Brozessanalyse und -steuerung der industriellen Röstung von Lebens- & Genussmitteln mittels **P**hotoionisations-Massenspektrometrie am Beispiel Baffee

Funding organization:
Bundesministerium für Wirtschaft und Energie

Funding period:
06/2017 – 02/2020

Funding amount:
106 470 € (UR)

Figure 1: *Photograph of the mobile single-particle mass spectrometer with high-repetition excimer laser for characterization of ultra-fine particles.*

(27) MTSD – Novel monitoring technologies for sustainable development: Linking the Bioeconomy and Resource Management to Air Quality

Project realization at JMSC: J. Orasche (HMGU), G. Jakobi (HMGU), G. C. Dragan (HMGU), V. Kohlmeier (HMGU), R. Zimmermann (UR/HMGU)

Background: Environmental health and the search for cleaner, environmentally friendly fuels are two of the most important concerns modern society is dealing with. The combustion of fuels can release high amounts of particulate matter (PM), especially soot, as well as semi-volatile organic compounds (SVOCs), which in turn will increase the risk of health complications for the population exposed to such pollutants. One open question related to this issue is whether a transition from mineral resources to biofuels will considerably help reduce the amount of PM, SVOCs, and greenhouse gasses emitted to the atmosphere. Such an emission reduction will reduce human health risks associated with exposure via inhalation and also contribute to a decrease of pollutants released into the atmosphere. The project is also strengthening collaborations between young scientists of both working groups in Germany (HMGU) and South Africa (University of Pretoria).

Aim of the project: This project aims to determine whether a transition from mineral fuels to the bioeconomical exploitation of crop residues and the use of biofuels will significantly lead to reductions in emissions of harmful substances, and thus to healthier, sustainable development. The aim will be achieved via the monitoring of air pollutants at various sites, including in the mining, agricultural and biorefinery industries. Emissions were measured from motorized equipment utilized in underground platinum mines in South Africa. Here pollutants emitted from diesel engines were compared to the use of biofuels. Air pollutant monitoring was also carried out during the burning of sugarcane crop residues in South Africa. Air emissions monitoring will include a sampling campaign at the pilot plant of a biorefinery in Europe, which converts biomass to fuels. In this manner, we aim to explore how the conversion of biomass waste into fuels could serve as an alternative to combustion, minimizing the emission of harmful air pollutants. To support field measurements lab experiments were done. Diesel fuel and biofuels were combusted with a diesel-CAST (combustion aerosol standard) at the CMA laboratories in Munich. Furthermore, emission factors of sugar cane leaf burning were determined within the frame of Eurochamp 2020 at the ILMARI facilities of the University of Eastern Finland in Kuopio under controlled conditions. Here we extended the original aims of the project to investigate the question of the formation of secondary organic aerosols from sugar cane burning aerosols under realistic conditions in the ILMARI smog chamber.

Project Partners: University of Pretoria (South Africa), South African Sugarcane Research Institute (SASRI), Impala Platinum Pty (Ltd) (South Africa), University of Eastern Finland (Kuopio, Finland)

Consortium: Photonion GmbH, Germany; MLase AG, Germany; University of Rostock; PROBAT-Werke von Gimborn Maschinenfabrik GmbH, Germany (associated); Azul Kaffee GmbH & Co. KG, Germany (associated)

Bundesministerium für Bildung und Forschung

EUROCHAMP 2020

German project title:
Neuartige Monitoring-Technologien für eine nachhaltige Entwicklung: Verknüpfung von Bioökonomie und Ressourcenmanagement mit der Luftqualität

Funding organization:
Bundesministerium für Bildung und Forschung (BMBF), Eurochamp 2020

Funding period:
05/2017 – 04/2021

Funding amount:
66 634 € (UR)

Figure 1: *Sampling campaign underground in a South African platinum mine.*

Figure 2: *Field measurements at an open agricultural fire in South Africa and under lab conditions at the ILMARI facilities in Kuopio, Finland.*

(28) SmartAQnet – Smart Air Quality Network

Project realization: M. Khedr (HMGU), X. Liu (HMGU), J. Schnelle-Kreis (HMGU), J. Cyrys (HMGU-EPI), A. Peters (HMGU-EPI), T. Riedel (KIT-TECO), S. Emeis (KIT-IMK-IFU), M. Pesch (Grimm), R. Zimmermann (UR/HMGU)

Gefördert durch:

Bundesministerium für Verkehr und digitale Infrastruktur

aufgrund eines Beschlusses des Deutschen Bundestages

mFUND

Funding organization:
Bundesministerium für Verkehr und digitale Infrastruktur

Funding period:
04/2017 – 09/2020

Funding amount:
226 143 € (HMGU-CMA)
261 951 € (HMGU-EPI)

In this project, CMA worked with HMGU-EPI (Dr. Cyrys, Prof. Peters) on a joint project together with Karlsruher Institut für Technologie (KIT), Institut für Telematik, Lehrstuhl für Pervasive Computing Systems/TECO (KIT-TECO, Dr. Till Riedel) and Institut für Meteorologie und Klimaforschung, Institut für Atmosphärische Umweltforschung (KIT-IMK-IFU, Prof. Dr. Emeis), Universität Augsburg, Institut für Geographie, Lehrstuhl für Physische Geographie und Quantitative Methoden (IGUA, Dr. A. Philipp), Stadt Augsburg, Umweltamt, T. Gratza and GRIMM Aerosol Technik GmbH & Co. KG; Markus Pesch.

Background: Air quality and the associated subjective and health-related quality of life are major issues of our time. Nevertheless, it is very difficult for many cities to take measures geared to today's mobility, living, and working needs because a consistent database with fine-grained data on effect chains is lacking. The "SmartAQnet" project is based on a pragmatic, data-driven approach, which for the first time merges existing data sets and combines them with a networked mobile measurement strategy. By combining open data and new mobile measurement approaches a novel measurement and analysis concept is being created and tested within the Augsburg model region. In addition to novel analyses, a technology stack is to be created as a prototype, which can enable a scalable area-wide application through modern analytical methods combined with Big Data and Internet of Things (IoT) technologies.

Aim of the project and current results: The SmartAQnet combines existing in situ and remote sensing data sets with a networked mobile measurement strategy for air pollutants (PM10, PM2.5) in the urban space. By connecting open data, such as weather data, remote sensing of influencing factors, and new mobile measurement approaches, such as participatory sensing with low- and mid-cost sensor technology a novel measuring and analysis concept is created within the model region of Augsburg, Germany. The subproject being worked on by the HMGU shall demonstrate that the measuring devices developed within the project (scientific scouts) deliver air quality data with sufficient accuracy and significance to be used in the newly established measurement network and thus, above all, the desired high spatial and temporal resolution of air quality data can be achieved. By comparing stationary and mobile measurements of the developed scientific scouts with reference measurements and source analyses, data is obtained to ensure the quality of the measurements. For the stationary reference measurements, the measuring instruments of the HMGU aerosol measuring station and reference instruments at two more sites within Augsburg are used. For the mobile measurements, three trolleys were equipped with reference measurement instruments for particles. The trolleys were used in Augsburg to study the temporal and spatial variability of PM2.5 and other PM metrics (figure 1). As one result of the investigations, it was found that photographs of the immediate surroundings together with meteorological data are suitable for estimating exposure to black carbon. At present, the data and samples obtained in the mobile and stationary measurements are evaluated in-depth in order to investigate the influence of different particle properties or sources on the quality of the measurement results of the low-cost measurement technology developed by the Grimm company.

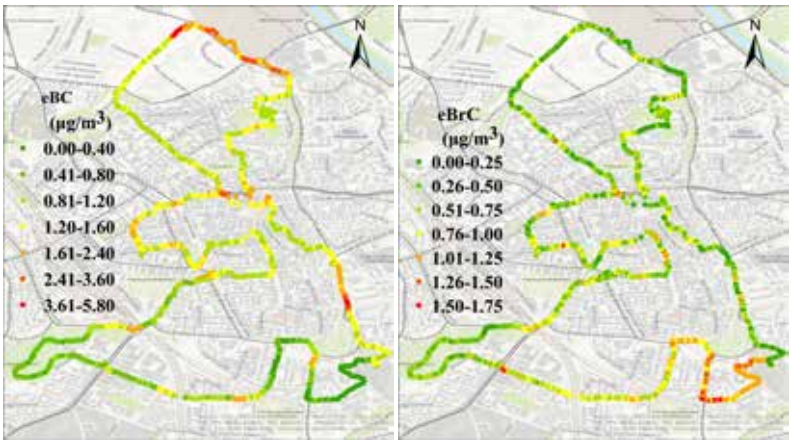


Figure 1: *Equivalent Black (eBC, left) and equivalent Brown Carbon (eBrC, right) concentrations along the walking path in Augsburg (example from 21.11.2019).*

(29) Investigation of the chemical complexity of bitumen and its chemical alteration during aging

Project realization: U. Käfer (UR/HMGU), A. Neumann (UR), T. Gröger (HMGU), C. P. Rüger (UR), R. Zimmermann (UR/HMGU)

Millions of tons of bitumen are produced and exposed to the environment every year, making it the most important binder for asphalt concrete. However, specifications and quality criteria for bitumen are limited to a few physical and chemical parameters and potential environmental threats of this industrial product are still not extensively studied. The chemical composition of bituminous material is extremely complex, containing highly aromatic species and compounds enriched in heteroatoms, such as sulfur, nitrogen and oxygen as well as metals. During the lifetime of the pavement, the binder undergoes aging processes, resulting in hardening, cracking and embrittlement of the material. Short-term aging occurs during the mixing and paving process, while long-term aging occurs during the service-time of the pavement.

In a three-year ZIM cooperation project, we worked together with ASG as industrial partner to develop potent analytical techniques, investigate the chemical characteristics of bitumen, and study aging mechanisms. In a first step, we developed a direct inlet probe method hyphenated to high-resolution time-of-flight mass spectrometry (DIP-HRTOFMS) to differentiate several bitumen samples and relate their chemical profile to physical properties, provenance, and production pathway (Käfer et al, 2019). In further studies, we traced chemical changes during short-term aging in bitumen at the molecular level. Aging-related alterations were comprehensively investigated by thermal gravimetry coupled to Fourier transform ion cyclotron resonance mass spectrometry (TG-FT-ICR MS) as well as two-dimensional gas chromatography high-resolution time-of-flight mass spectrometry (GC×GC-HRTOFMS) (Neumann *et al.* 2020, figure 1)

Oxidation effects were found as prevalent mechanism during the short-term aging of bitumen, which is indicated by the increase of O-, SO- and NO-containing compounds. In general, a strong decrease of non-aromatic sulfur components and N-containing species were observed.

The combination of TG-FT-ICR MS and GC×GC-HRTOFMS was shown to provide valuable information on aging processes in bitumen at the molecular level. The successfully completed industrial-academic co-operation project again shows the advantage of the complementarity within the JMSC network to be able to apply state-of-the-art technologies independent of location and institutional affiliation. Road, tire and break wear are other important contributors to urban inhalable fine dust. Future studies will take the formation, composition and health effects of these sources and road dust formed by abrasion of the bitumen containing asphalt due to traffic into account.

Partner: ASG Analytik Service Gesellschaft mbH, Neusäß, Germany.

German project title:
Entwicklung eines Screening-Verfahrens für fossile und biogene Schwerflüchter zur erstmalig validierten Bestimmung von spezifischen Parametern in Bitumen-Matrizen zur qualifizierten Alterungsprognostik und Schadensanalytik z.B. im Straßenbau

Funding organization:
ZIM Innovation program for the development of new methods and methodologies

Funding period:
06/2016 – 05/2019

Funding amount:
186 577 € (HMGU)

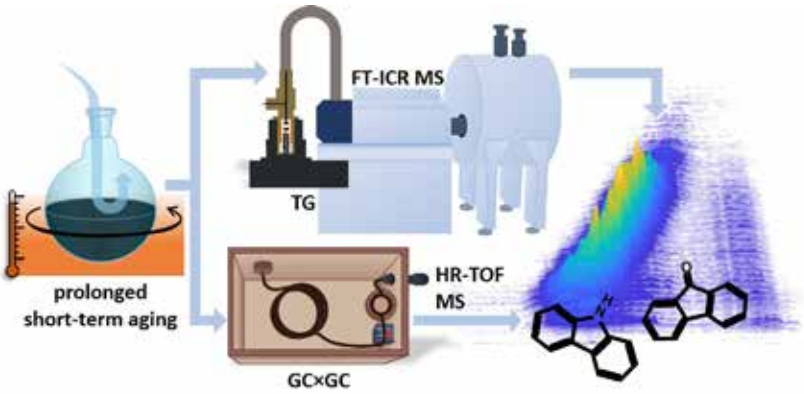


Figure 1: *Bitumen samples were artificially aged and investigated by TGA-FT-ICR-MS and GC×GC-HRTOFMS to reveal chemical alterations caused by aging.*

References:

1. Käfer, U. *et al.* (2019). Direct inlet probe–High-resolution time-of-flight mass spectrometry as fast technique for the chemical description of complex high-boiling samples. Talanta, 202, 308-316.
2. Neumann, A. *et al.* (2020). Investigation of Aging Processes in Bitumen at the Molecular Level with High-Resolution Fourier-Transform Ion Cyclotron Mass Spectrometry and Two-Dimensional Gas Chromatography Mass Spectrometry. Energy & Fuels, 34(9), 10641-10654.

(30) Development and optimization of a membrane-inlet-photoionization mass spectrometer for the real-time analysis of (poly)aromatic and halogenated hydrocarbons in aquatic systems

Project realization at JMSC: C. Gehm (UR), T. Streibel (UR/HMGU), D. Schulz-Bull (IOW), R. Zimmermann (UR/HMGU)

German project title:
Entwicklung und Optimierung eines Membraneinlass – Photoionisierungs-Massenspektrometers für die Echtzeitanalytik (poly)aromatischer und halogenierter Kohlenwasserstoffe in aquatischen Systemen

Funding organization:
German Research Foundation (DFG)

Funding period:
05/2016 – 04/2019

Funding amount:
203 072 € (UR)

Background: Very low concentrations of aromatic compounds and especially polycyclic aromatic hydrocarbons (PAH) in marine systems impede their analytical detection and monitoring. Therefore, the development and implementation of a measurement system for real-time analysis of (poly)aromatic substances would take the assessment of their concentration levels and distribution patterns one step further. The utilized approach consists of the application of a polydimethylsiloxane membrane inlet time-of-flight mass spectrometer in combination with laser based resonance enhanced multiphoton ionization (REMPI).

Aim and results of the project: The first aim of the project was the development of external membrane modules that are suitable for direct coupling with a mass spectrometer. For this purpose, two different approaches were selected and implemented; one being an external sheet membrane probe (ESMP) and the other an external hollow fiber membrane probe (EHFMP). After construction, the optimization of the modules took place with tap water samples (occasionally spiked with diesel fuel) for analytical parameters such as temperature, flow rate, and response time.

The schematic setup of the EHFMP is shown in figure 1a. The hollow fiber membrane has a wall thickness of 250 µm. Compounds transferring the membrane are transported to the mass spectrometer via an inert gas flow. The gas flow can be applied continuously (open-end capillary, oec) with constant heating of the membrane or it can be interrupted leading to trapping of the analytes on the inside followed by their subsequent release by fast heating (trap and release, T&R). The respective response behavior for some analytes is shown in figure 1b. Real-life water samples were investigated as well, comprising water from the Warnow river estuary and the Baltic sea. One- to three-ring aromatic species were detected and quantified. Limits of detection are in the low ng/l range.

One big challenge in the future is the translation and modification of the apparatus for usage on seagoing ships to carry out continuous measurements in ocean water. The technology could be used for industrial process monitoring purposes and marine environment applications. It might also be applied for the detection of pollutants from disposed ammunition and other hazardous waste dumpsites.

References:

1. Gehm, C. *et al.* (2019) Development and Optimization of an External-Membrane Introduction Photoionization Mass Spectrometer for the Fast Analysis of (Polycyclic)Aromatic Compounds in Environmental and Process Waters. Analytical chemistry, 91, 15547–15554.
2. Gehm, C. *et al.* (2020) External trap-and-release membrane inlet for photoionization mass spectrometry: Towards fast direct analysis of aromatic pollutants in aquatic systems. Rapid Commun Mass Spectrom. DOI: 10.1002/rcm.8863

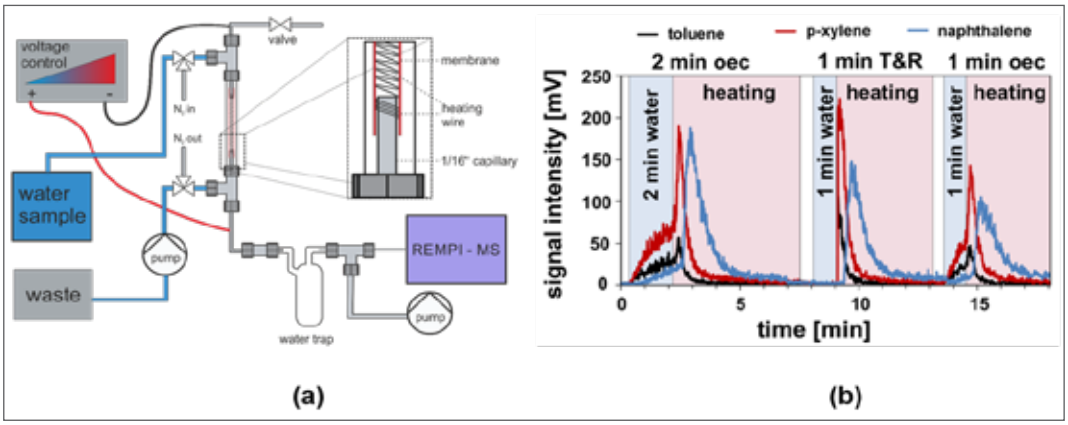


Figure 1: a) Experimental setup external hollow fiber membrane probe. b) Response behavior for toluene, p-xylene and naphthalene when applying the two variations of operation of the probe, open-end capillary or trap and release.

(31) Carbon Concrete Composite and health: Development and application of analytical methods for the chemical characterization of particles and the biochemical investigation of cell cultures

Project realization at JMSC: T. Streibel (UR/HMGU), T. Kanashova (UR/MDC), C. Knizia (UR), S. Öder (HMGU), S. Bauer (HMGU), S. Di Bucchianico (HMGU), R. Zimmermann (UR/HMGU)

Bundesministerium für Bildung und Forschung

German project title:
Carbon Concrete Composite und Gesundheit: Entwicklung und Anwendung von analytischen Methoden zur chemischen Charakterisierung von Partikeln und zur biochemischen Untersuchung von Zellkulturen

Funding organization:
Federal Ministry for Education and Research (BMBF)

Funding period:
12/2015 – 03/2018

Funding amount:
599 973 Euro (UR)

Background: The utilization of carbon-based reinforcement materials instead of steel for concrete composites (Carbon Concrete Composite, C^3) offers an interesting and promising alternative for the future in construction. However, one must not disregard possible effects on human health, safety, and the environment. Hence, questions arise for the identification of potential health hazards during the application of carbon fibers and carbon concrete composite containing such fibers. In particular, during the life cycle of carbon concrete one has to be aware of releasing fiber-like particles in the environment and the breathing air of humans. This incorporates production, processing, decomposition, and recycling or disposal.

Aim and results of the project: The first central aim of the project was the development and construction of a reliable measurement system to assess the toxicological effects of the processing of carbon concrete materials, especially the fiber-containing particle ensemble. For this, abrasive cutting of carbon concrete was chosen as a demonstration process. The project partner Vitrocell Systems developed a new cutting module with a diamond saw in combination with an Air-to-Liquid Interface (ALI) cell culture exposure unit (figure 1). Particles generated during the cutting process could directly be guided to the cell culture inserts. The chemical composition of the particles was determined by thermal desorption/photoionization mass spectrometry, morphology by electronic microscopy. Pure concrete was investigated as reference material.

Concerning the chemical composition, clear differences were observed between carbon concrete and concrete. Carbon concrete particles contain hydrocarbons not present in concrete particles, such as styrene and butadiene. These compounds derive from the organic polymer used as a coating material for the carbon fiber grid inserted in the concrete. Electronic microscopy revealed no critical fibers according to WHO guidelines to identify material with asbestos-like fiber toxicity. For the cell culture exposure, lung epithelial cells A549 and human monocyte cells THP1 were employed for four-hour exposure with the particles. Subsequent toxicological analyses showed no significant difference between carbon concrete and concrete, both exhibiting relatively minor toxic effects (see figure 2). A deeper biological analysis on the transcriptome and proteome levels also showed comparable behavior of carbon concrete and concrete with again weak effects in total. The results indicate that the replacement of steel-reinforced concrete with carbon concrete likely does not introduce additional hazards for human health.

Project partners: Technical University Dresden, Germany; Vitrocell Systems GmbH, Germany; Topas GmbH, Germany

Figure 1: The newly developed cutting module (left) and the cell exposure unit (right) for the abrasive cutting experiments to assess the toxicological potential of particles released during the cutting process.

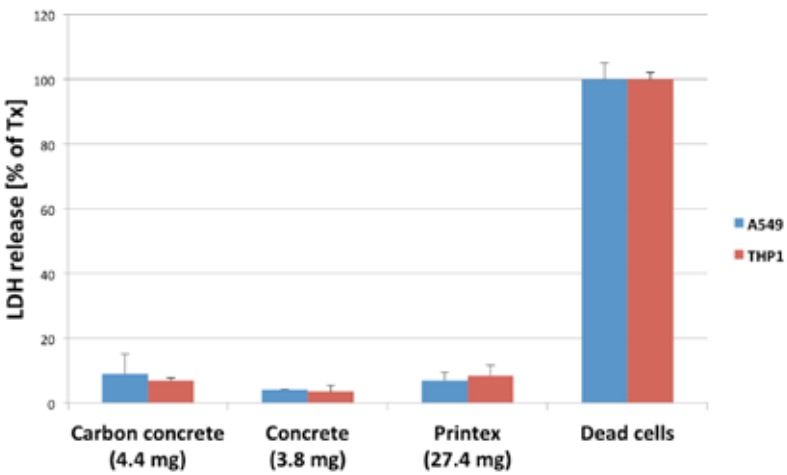


Figure 1: Toxicological response (LDH release) of carbon concrete and concrete particles produced during abrasive cutting. Printex soot particles were taken as reference. Cell death is the respective endpoint. No significant difference is seen between carbon concrete and concrete particles and both depict minor toxic effects.

(32) CHARCOAL – CHemical composition and origin of Atmospheric bRown CarbOn AerosoL (CHARCOAL)

Project realization : T. Miersch (UR), H. Czech (HMGU/UR), A. Chen (UN-LV), J. Chow (DRI), J. Watson (DRI), T. Streibel (UR/HMGU), R. Zimmermann (UR/HMGU)

Funding organization:
German Research Foundation (DFG) and U.S. National Science Foundation (NSF)

Funding period:
10/2014 – 03/2018

Funding amount:
180 000 € (UR)

This project is a DFG-NSF binational cooperation project with partners from Desert Research Institute DRI (Prof. Dr. Judith C. Chow, Dr. John G. Watson) and the University of Nevada at Las Vegas (Prof. Dr. L. – W. Antony Chen)

Background: Carbonaceous aerosols account for 10-40 % of the global aerosol mass and affect the Earth's climate, tropospheric chemistry, visibility, and public health. The sources, sinks, and spatiotemporal distributions of carbonaceous aerosol have therefore drawn attention to the scientific community because of its role in urban air pollution and the global atmospheric environment. It has been proposed that carbonaceous aerosol covers a continuum of substances, which may be classified by thermochemical, optical, and molecular properties. Black Carbon (BC), defined by optical methods, and Elemental Carbon (EC), defined by thermal-optical methods, have a graphite-like structure and are the dominant light-absorbing component in the troposphere. However, the fraction of organic matter, i.e., organic carbon (OC), also contains species absorbing light in the visible UV range, which are called brown carbon (brC) and might be an unrecognized species of relevance for Earth's radiative forcing.

Aim of the project: This project aims to bridge optical and thermal-optical carbon analysis (TOCA) by the addition of multi-wavelength transmittance measurements to TOCA. Furthermore, we seek to investigate the association of spectral particle properties with their molecular composition using the hyphenation of TOCA to photoionization mass spectrometry (PIMS) (Diab *et al.*, 2015)

Results: A multi-wavelength module with laser diodes, emitting light from visible UV to near-infrared (NIR) region, was successfully implemented in the DRI thermal-optical carbon analyzer and demonstrated with filter samples containing particulate matter from various sources (Chen *et al.*, 2015). With the combined information on spectral particle properties and carbon speciation, mass absorption efficiencies for EC of various combustion sources could be calculated for seven different wavelengths in visible UV to NIR as well as the Angström Absorption Exponent α. In particular, biomass burning organic aerosol is known to contain significant amounts of brC, which is also water-soluble. However, an insoluble brC species call "tarballs" has gained attention in samples from biomass burning. In our study, we were able to unambiguously link brC and "tar" for the first time to emissions from marine engines and revealed that the high content of light-absorbing semi- to low-volatile aromatic compounds are negligible for the intense absorption of tar (Corbin *et al.*, 2019). In order to identify known brC species in aerosol particles, we investigated the fragmentation behavior of common particle constituents in the TOCA-PIMS instrument operated with single-photon ionization (SPI). In particular, levoglucosan and other carbohydrate derivatives as major constituents of particles affected by biomass burning cannot be re-volatilized from the filter without thermal decomposition. It thermally decomposes into well-known carbohydrate-related compounds, such as furfuryl alcohol (m/z 98) and 1,4:3,6-Dianhydro-α-D-glucopyranose (m/z 144), and fragment at m/z 60 ($C_2H_4O_2^+$) (figure 1a). In contrast, the aromatic brC species 3-methyl-4-nitrophenol, mainly formed by atmospheric oxidation of phenol or benzene in the presence of NOx, shows a distinct molecular ion at m/z 153 and minor fragmentation (figure 1b). TOCA-PIMS is a novel, worldwide exclusive technique developed at JMSC and uniquely combines established and quantitative thermal-optical and thermal methods with advanced mass spectrometric techniques in order to maximize the information extractable from one individual filter sample. First systems currently are made available to other researchers via the JMSC spin-off company Photonion GmbH.

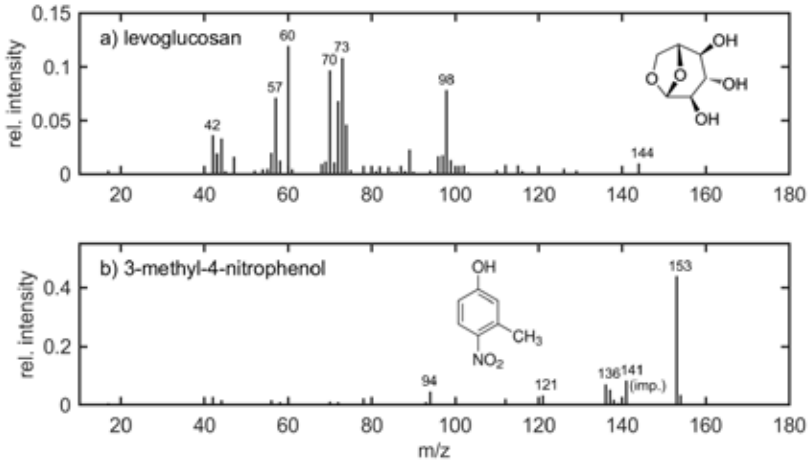


Figure 1: *SPI mass spectra of a) levoglucosan and b) 3-methyl-4-nitrophenol.*

References

1. Chen, L. – W. A. *et al.* (2015) Multi-wavelength optical measurement to enhance thermal/optical analysis for carbonaceous aerosol. Atmospheric Measurement Techniques, 8(1), 451–461.
2. Corbin, J. C. *et al.* (2019) Infrared-absorbing carbonaceous tar can dominate light absorption by marine-engine exhaust. npj Climate and Atmospheric Science, 2(1), 3985–3994.
3. Diab, J. *et al.* (2015) Hyphenation of a EC / OC thermal–optical carbon analyzer to photo-ionization time-of-flight mass spectrometry. Atmospheric Measurement Techniques, 8(8), 3337–3353.

(33) Dynamic behavior of hazardous semi-volatile multi-component aerosols during sampling at the workplace

Project realization: E. W. Karg (HMGU), J. Schnelle-Kreis (HMGU), V. Kohlmeier (HMGU), G. A. Ferron (HMGU), G. C. Dragan (HMGU), R. Zimmermann (UR/HMGU)

German project title:
Verhalten von semi-volatilen Mehrkomponenten-Gefahrstoff-Aerosolen bei der Probenahme am Arbeitsplatz

Funding organization:
Deutsche Gesetzliche Unfallversicherung (DGUV)

Funding period:
09/2014 – 05/2018

Funding amount:
696 269 € (HMGU)

The DGUV (German Social Accident Insurance) is the umbrella association of the accident insurance institutions for the industrial and public sectors. The Institute for Occupational Safety and Health (IFA) of the DGUV supports the institutions and organizations in solving scientific and technical problems related to occupational safety and health. The DGUV-IFA funded a three-year project FP371 on the sampling of semi-volatile organic compounds (SVOC) at the workplace. FP371 ended in May 2018.

Hazardous SVOC exist in both particulate and vapor phase. The sampling of SVOCs is challenging as both phases dynamically interfere with surrounding substances. Particle and vapor phase have a different exposure risk, as particles and gas molecules behave differently during inhalation. The project addressed the challenges of accurately measuring SVOC aerosols at the workplace (Dragan 2020). SVOC aerosols of several polar and non-polar substances and particles of various diameters were generated, diluted with particle-free nitrogen, and monitored for particle evaporation loss. The aerosols were quantified using the off-line methods (workplace filter and denuder samplers) and the on-line methods (optical particle sizer and flame-ionization detector) in parallel for development and quality test. The most-accurate off-line samplers used three substrates in series, a denuder, a filter, and an adsorber. The denuder stripped the gas-phase from the aerosol, the filter collected the particles from the air and the adsorber yielded information about erratic SVOC loss from denuder and filter.

A computer model was developed to compare the measurement with theory. Prototypes of new personal samplers were found to be appropriate for workplace measurements, with over 96 % collection efficiency of the vapor phase and particle losses below 5 % (Kohlmeier 2018). The denuder-filter-adsorber configuration was characterized in a series of controlled laboratory tests with aerosols of different particle-vapor ratios (Dragan 2020) and subsequently used for a field sampling campaign in an underground platinum mine. Measurements revealed a predominance of alkanes in the air, mainly from engine emissions, and PAHs in a substantial amount in both phases (figure 1).

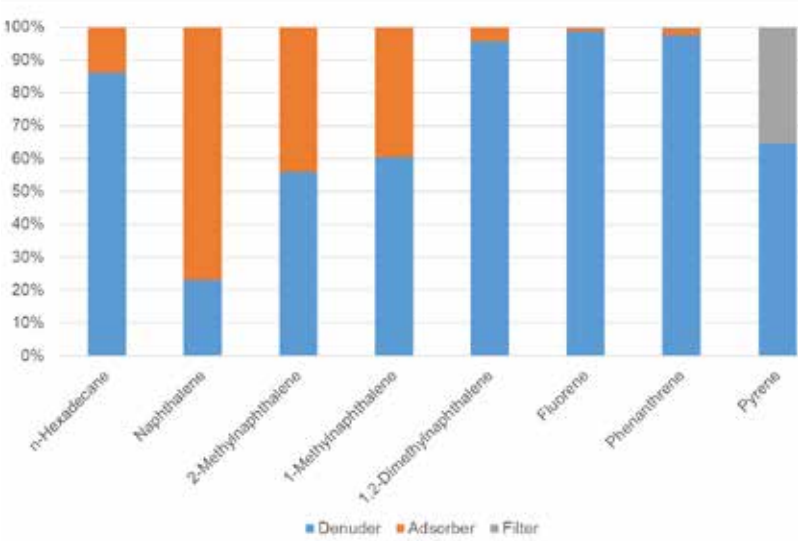


Figure 1: *Particle-vapor-partitioning for different polyaromatic hydrocarbons from a personal sampler, carried by the driver of a diesel-powered transport vehicle in the platinum mine.*

References

1. Dragan, G. C., *et al.* (2020) Development of a Personal Aerosol Sampler for Monitoring the Particle–Vapour Fractionation of SVOCs in Workplaces Annals of Work Exposures and Health, 64(8), 903-908.
2. Kohlmeier, V., *et al.* (2018) Carbonaceous Monolithic Multi-Channel Denuders as Vapour–Particle Partitioning Tools for the Occupational Sampling of Semi-Volatile Organic Compounds. Annals of Work Exposures and Health, 62(7), 899-903.

3.5 International and National Conferences and Workshops

2020

Analytica Conference, 21.10.2020
- Gröger, T. *et al.*: The application of direct inlet techniques in combination with electron ionization – high resolution time-of-flight mass spectrometry. (Lecture)
- Rüger, *et al.*: Direct thermal analysis methods as sample introduction for high-resolution mass spectrometry – Molecular level description of heavy petroleum fractions. (Lecture)

University of Lille, France, 15.09.2020
- Zimmermann, R. *et al.*: Aerosols and Health: Accessing the chemical composition and the toxicity of fine ambient particulate matter. (Invited lecture, fully funded).

European Aerosol Conference (online), Aachen, Germany, 31.8. – 4.9.2020
- Hartner, E.: A novel hybrid direct insertion probe/two-dimensional gas chromatography – ultra high-resolution time-of-flight mass spectrometer for aerosol characterisation: Detailed chemical investigation of semi-volatile SOA compounds from biogenic or anthropogenic precursors. (Poster)
- Orasche J. *et al.*: Emission factors and formation of secondary aerosols related to sugar cane harvesting using field fires. (Poster)
- Binder, S. *et al.*: An in vitro air liquid interface model system to predict inhalation toxicity of semi-volatile environmental contaminants. (Lecture)
- Liu, X. *et al.*: Spatial characteristics and determinants of black carbon in Augsburg, Germany: Combination of mobile monitoring in predefined micro-environment and street view imagery. (Poster)
- Passig, J. *et al.*: Resonance Effects in Laser Ionization of Metals in Single-Particle Mass Spectrometry. (Poster)
- Passig, J. *et al.*: Tailored Laser Ionization Schemes Reveal the Key Compounds for Health Effects in Aerosols via Single Particle Mass Spectrometry. (Lecture)

GDCh-lecture, Rostock, Germany, 2.7.2020
- Zimmermann, R. *et al.*: Aerosols and Health: How to access knowledge on the chemical composition and the toxicity of ambient particulate matter. (Invited lecture, via internet).

Separation Science 2020 (https://www.sepscience.com/), 11.6.2020 – open
- Gröger, T. *et al.*: Characterization of Organic Components on the Particulate Matter of Aerosols by GC- and GCxGC-TOFMS for the Evaluation of its Impact to Health and the Environment. (Lecture)

69th American Society for Mass Spectrometry conference (ASMS), Philadelphia, 6. – 10.6.2020
- Friederici *et al.*: Rapid Chemical Description of Organic Aerosols with a Direct Inlet Probe Coupled to Trapped Ion Mobility Time-of-Flight Mass Spectrometry. (Poster)
- Passig, J. *et al.*: Resonance Effects in Laser Ionization of Metals in Single-Particle Mass Spectrometry. (Poster)
- Rüger *et al.*: Atmospheric Pressure Photo and Laser ionization (APPI/APLI) – Aspects on complex Mixture Characterization. (Lecture)
- Rüger *et al.*: Cyclic ion mobility spectrometry coupled to high-resolution mass spectrometry –Prospects for complex mixture analysis. (Poster)

Workshop "Ultrafeine Partikel", Klinik Großhardern, München, Germany, 13.3.2020
- Zimmermann, R. *et al.*: Chemische und Biologische Charakterisierung von Ultrafeinen Partikeln. (Lecture)

DRI (Desert Research Institute), Reno, NV, USA, 9.3.2020
- Zimmermann, R. *et al.*: Adding the chemical dimension: Coupling of the DRI thermo-optical carbon analyzer to photoionisation mass spectrometry. (Lecture)

Pittcon 2020, Chicago, IL, USA, 1. – 6.3.2020
- Käfer, U., ..., Zimmermann, R.: The Complementary of different gas-phase introduction methods on a high resolution Time-of-Flight mass spectrometer platform. (Lecture)
- Zimmermann, R. *et al.*: Monitoring of the Industrial Coffee Roasting Process by Photo Ionization-Mass Spectrometry (PIMS): Towards an Optimized Coffee Quality. (Lecture)

- Passig, J., ..., Zimmermann R.: On-line Single Particle MS for Resonance Enhanced Laser Ionization of Polycyclic Aromatic Hydrocarbons and Transition Metals. (Invited Symposium lecture, fully funded).

53rd annual conference of the DGMS, Germany, 01.03. – 04.03.2020
- Friederici *et al.*: Description of polymers and composite materials with thermal analysis hyphenated to photoionization mass spectrometry. (Poster)
- Gehm, C. *et al.*: Development and optimization of a membrane – inlet – photoionization mass spectrometer for fast analysis of (polycyclic)aromatic compounds in aquatic systems. (Lecture + Poster)
- Heide, J. *et al.*: Setup of an orthogonal acceleration time-of-flight system (oa-tof) with simultaneously on-line vacuum single- and multi-photon ionization. (Poster)
- Rüger *et al.*: Cyclic ion mobility spectrometry coupled to high-resolution mass spectrometry –Prospects for complex mixture analysis. (Lecture)
- Schade, J. *et al.*: Resonance-Enhanced Detection of Metals in Aerosols using Single-Particle Mass Spectrometry (Poster)

XXII-SASP (Symposium on Atomic, Cluster and Surface Physics), St. Moritz, Switzerland, 2. – 7.2.2020
- Passig J., ..., Zimmermann, R.: Simultaneous detection of polycyclic aromatic hydrocarbons as well as inorganic ions in single particle mass spectrometry using molecular and atomic resonances. (Lecture)

16th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology, Ghent, Belgium, 29. – 30.1.2020
- Weggler, B. *et al.*: Establishing a Unique Open-Source Benchmark Dataset for the Comprehensive Evaluation of GCxGC Software. (Lecture)
- Käfer, U., ..., Zimmermann, R.: Extending the Application Range of a GC×GC High-Resolution TOF-MS platform for Fuel Analysis by Hyphenation to Direct Inlet Thermal Analysis Techniques: Shipping fuels. (Lecture)

2019

4EU+ Project "CONSPIRO – Breathing Together for Cleaner Air" meeting at Charles University, Czech Republic, 11. – 13.12.2019
- Bendl, J., *et al.*: Alternative Methods of Air Quality measurements. (Lecture)

UC Merced, Merced, CA, USA, 11.12.2019
- Zimmermann, R. *et al.*: New methods and instruments for accessing the composition, sources and health-relevance of aerosols: From lung cell exposure based biological effects analysis to single particle laser mass spectrometry. (Invited lecture, fully funded).

AGU (American Geophysical Union) Fall Meeting, San Francisco CA, USA, 9. – 13.12.2019
- Passig J., ..., Zimmermann, R.: A new single particle chemical analysis approach for characterization of atmosphere-marine biosphere interactions: Detection and source apportionment of iron containing aerosols. (Lecture)
- Zimmermann, R., Czech, H. *et al.*: Impact of photochemical ageing on the composition and toxicity of wood combustion aerosols: A combined cell exposure and analytical-chemistry approach. (Lecture)

Fourth aerosol particle organic analytical training course of the Calibration Centre for the analysis of organic tracers and particle-phase constituents (OGRTAC CC), Leipzig, 2. – 6.12.2019
- Hartner, E. *et al.*: Chemical Characterization of Secondary Organic Aerosols by GCxGC-TOFMS. (Lecture)

DGMK – Jahrestreffen der Analytiker, Hamburg, Germany, 28.11. – 29.11.2019
- Rüger, C. P. *et al.*: Structural analysis of heavy oil fractions compounds refractory to hydrodenitrogenation reactions by high-resolution tandem mass spectrometry and ion mobility spectrometry. (Lecture)

Key Laboratory of Aerosol Chemistry and Physics, Chinese Academy of Sciences, Xi'an, China; 28.11.2019
- Zimmermann, R. *et al.*: New methods and instruments for accessing composition, sources and health-relevance of aerosols: From lung cell exposure-based toxicological analyses to photo ionisation mass spectrometry. (Invited lecture, fully funded).

National Sun Yat-sen University, Kaohsiung, Taiwan, 26.11.2019
- Zimmermann, R. *et al.*: On-line Single Particle Mass Spectrometry: Methodology, Applications and new Perspectives for Assessing Health Relevant Aerosols as well as Aerosol Particle Sources. (Invited lecture)

4th Mediterranean Shipping Conference, Piraeus, Greece, 20.11.2019
- Passig *et al.*: Ship Emissions and Health. (Lecture)

1st International Symposium on Aerosols-Characterization and Therapies, Kaohsiung, Taiwan, 29. – 31.10.2019
- Zimmermann, R. *et al.*: Innovative methods for accessing the composition, sources and health-relevance of aerosols: From cell exposure at the air-liquid-interface to innovative single particle laser mass spectrometry. (Invited plenary lecture, fully funded)

Seminar at Zhejiang University, Hangzhou, China, 25.10.2019
- Zimmermann, R. *et al.*: Application of Photo Ionisation Mass Spectrometry (PIMS) to analyse complex mixtures. (Invited lecture, fully funded).

4EU+ Project "CONSPIRO – Breathing Together for Cleaner Air" meeting at Heidelberg University, Germany, 23. – 24.10.2019
- Bendl, J., *et al.*: Health Effects of Atmospheric Aerosols. (Lecture)

37th Annual Conference of American Association for Aerosol Research (AAAR), Portland, Oregon (USA), 14. – 18.10.2019
- Bendl, J., *et al.*: Comprehensive Mobile Measurements of Aerosols from Residential Heating in Small Settlements for Temporal/ Spatial Variability and Hot-Spots Identification: Cross Border Study. (Lecture)
- Czech, H., ..., Zimmermann R.: Implications of Photochemical Ageing for Toxicity and Source Apportionment of Wood Combustion Aerosols: A Combined Biological and Chemical Study. (Lecture)
- Oeder, S., ..., Zimmermann, R.: The toxicity of gasoline automobile engine emissions depends on fuel type (gasoline and ethanol): A combined biological and aerosol composition study. (Lecture)
- Passig, J., ..., Zimmermann, R.: Resonances in Laser Desorption/Ionization of Particle-Bound Metals Feature Remote Sensing of Ship Emissions. (Lecture)
- Zimmermann, R., Passig, J. *et al.*: Aerosol Mass Spectrometer for On-Line Detection of Polycyclic Hydrocarbons as well as Inorganic Cations and Anions from Single Particles. (Lecture)

Workshop zu Ultrafeinen Partikeln am Bayerischen Staatsministerium für Umwelt und Verbraucherschutz, München, Germany, 2.10.2019
- Zimmermann, R. *et al.*: Charakterisierung von Ultrafeinen Partiklen (UFP) am JMSC und dem aeroHEALTH International Lab im Helmholtz Zentrum München. (Lecture)

Treffen der "Fachgruppe FT-MS und hochauflösende Massenspektrometrie" der Deutschen Gesellschaft für Massenspektrometrie (DGMS), Berlin, Germany, 19.09. – 20.09.2019
- Rüger, C. P. *et al.*: Direct thermal analysis methods as sample introduction for high-resolution mass spectrometry – Molecular level description of heavy petroleum fractions. (Lecture)

University of Poznan, Poznan, Poland, 19.9.2019
- Zimmermann, R. *et al.*: Hyphenation of Thermal Analysis to Photoionisation Mass Spectrometry (PIMS): Method, Applications and new concepts Seminar on Thermal Analysis. (Invited lecture, fully funded).

4EU+ Project "CONSPIRO – Breathing Together for Cleaner Air" meeting at Copenhagen University, Denmark, 16. – 22.9.2019
- Bendl, J., *et al.*: Mobile measurements of residential heating emissions. (Lecture)

Leco GC×GC Symposium, Berlin, Germany, 16. – 18.9.2019
- Käfer, U. *et al.*: High resolution time-of-flight mass spectrometry for the chemical description of complex matrices. (Lecture)

Cannabis Science Conference, Portland, OR, USA, 4. – 6.9.2019
- Zimmermann, R. *et al.*: On-line puff-by-puff time-resolved analysis of THC and other smoke constituents in the smoke of cannabis products using photoionization mass spectrometry. (Lecture)

Shipping and the Environment Conference, Gothenburg, 4. – 6.9.2019
- Käfer, U. *et al.*: Detailed chemical investigation of bunker fuels. (Poster)
- Passig, J. *et al.*: Remote Detection and Evaluation of Ship Emissions in Real-Time using Novel Particle Mass Spectrometry Techniques. (Lecture)

European Aerosol Conference – EAC 2019, Gothenburg (Sweden), 25. – 30.8.2019
- Bauer, S. *et al.*: In vivo and in vitro toxicity assessment of a stationary diesel generator. (Lecture)
- Bisig, C. *et al.*: A Step towards Standardisation of Air Liquid Interface Exposures using a Model Diesel Aerosol. (Poster)
- Czech, H. *et al.*: Implications of photochemical ageing for source apportionment and health effects of wood combustion aerosol. (Lecture)
- Czech , H. *et al.*: Single-particle Analysis from a Major Incident Fire of Discarded Metal in Rostock, Germany. (Lecture)
- Di Bucchianico, S. *et al.*: In Vitro Assessment of Secondary Genotoxicity by Air-Liquid Interface Exposures. (Lecture)
- Karg, E. W. *et al.*: Emission Particulate Deposition at the Air-Liquid-Interface and in the Human Lungs – A Computer Model Approach. (Lecture)

- Khedr, M. *et al.*: Pedestrians' exposure to ambient organic particulate matter in Augsburg, Germany. (Poster)
- Martens, P. *et al.*: Emission profiles from domestic burning of various wood fuels and household waste in a logwood stove. (Poster)
- Oeder, S. *et al.*: Toxicity of automobile engine emissions depends on fuel type and speed cycle. (Lecture)
- Orasche *et al.*: Novel techniques for chemical characterization of organic particulate matter. (Lecture)
- Passig, J. *et al.*: Aerosol Mass Spectrometer for On-Line Detection of Polycyclic Hydrocarbons as well as inorganic Cations and Anions from Single Particles. (Lecture)
- Schade, J. *et al.*: Resonant Ionization Enhancement in Laser Desorption/Ionization features Remote Sensing of Ship Emissions with Single Particle Mass Spectrometry. (Poster)
- Xin, C. *et al.*: Determination of DNA Damage and Modifications in Cells Exposed to Particulate Matter. (Poster)
- Zimmermann, E. J. *et al.*: Allergy-adjuvant or -protective aerosols – combining chemical composition and biological effects. (Poster)
- Zimmermann, R. *et al.*: Adding a chemical dimension: Coupling of the DRI thermo-optical carbon analyzer (TOCA) to photoionisation mass spectrometry (PIMS). Invited lecture. (Magee Scientific Workshop, 28.8.2019) (Lecture)

PIC 2019 -16th International Congress on Combustion By-Products and Their Health Effects, Ann Arbor, MI, USA, 10. – 12.7.2019
- Zimmermann, R. *et al.*: Chemical composition and toxicological effects on ALI-exposed human lung cells of freshly emitted and atmospherically aged combustion aerosols. (Lecture)
- Passig J., ..., Zimmermann, R.: New Developments and Applications in Single Particle Aerosol Mass Spectrometry: Simplified Simultaneous Detection of Polycyclic Aromatic Hydrocarbons and Elements. (Lecture)

5th International CoCoTea Congress 2019, Bremen, Germany, 25. – 26.6.2019
- Gehm, C., ..., Zimmermann, R.: MIMS for coffee – Membrane Inlet photoionization Mass Spectrometry (MI-PIMS) as an on-line investigation tool for liquid coffee. (Poster)
- Zimmermann, R. *et al.*: Investigation of the coffee roast-gas composition and on-line process control in real-time using photoionization-mass spectrometry. (Lecture)
- Heide, J. *et al.*: On-line Coffee Roast Control using Photoionization Mass Spectrometry: Prediction of Bean Color and Antioxidant Capacity (Lecture)

23rd ETH conference on combustion generated nanoparticles, Switzerland, 17. – 20.6.2019
- Bisig, C. *et al.*: A Step towards Standardisation of Air-Liquid Interface Exposures using a Model Diesel Aerosol (lecture)
- Karg, E. W. *et al.*: Why Detoxing All Combustion Engines? A Computer Model Approach to Regional Lung Deposition. (Poster)
- Streibel, T. *et al.*: Implications of photochemical ageing for health effects of wood combustion aerosol. (Poster)

GDCh-Fresenius Lecture at the Heinrich Heine Universität Düsseldorf, 18.6.2019
- Zimmermann, R. *et al.*: Aerosols, coffee, cigarettes & ship emissions: Analytical applications of photoionisation – mass spectrometry ... and beyond. (Invited GDCh-Fresenius lecture, fully funded).

67th ASMS Conference on Mass Spectrometry and Allied Topics, Atlanta, GA, USA, 2. – 6.6.2019
- Gröger, T. *et al.*: Extending the Application Range of a GC×GC High-Resolution TOF-MS platform for Fuel Analysis by Hyphenation to Thermal Analysis Techniques. (Lecture)
- Heide, J. *et al.*: Monitoring of Coffee Roasting by Vacuum Photoionization TOF-MS: Towards a Prediction Model for Bean Color and Antioxidant Capacity. (Lecture)
- Käfer, U., ..., Zimmermann, R.: Extending the Application Range of a GC×GC-High-Resolution TOF-MS platform for Heavy Fuel Analysis by Hyphenation to Thermal Analysis Techniques. (Lecture)
- Rüger, C. P. *et al.*: Direct thermal analysis methods as sample introduction for high-resolution mass spectrometry – Molecular level description of heavy petroleum fractions. (Lecture)
- Rüger, C. P. *et al.*: Structural study of analogues of Titan's haze by trapped ion mobility coupled with a Fourier transform ion cyclotron mass spectrometer. (Poster)
- Rüger, C. P. *et al.*: Photoionization (APPI/PI) – Bridging the Gap between Academic and Industrial Research. (Lecture in the Photoionization Workshop Session)
- Rüger, C. P., ..., Zimmermann, R.: Thermal Analysis Mass Spectrometry For Complex Mixture Analysis (Lecture in the Workshop "Energy, Petroleum, and Biofuels MS: Targeted analysis, fingerprinting and speciation in complex mixtures").

11th Asian Aerosol Conference (AAC) 2019, City University of Hong Kong, China, 27. – 30.5.2019
- Karg, E. W. *et al.*: Ageing of Particles by Coagulation: Changes in Regional Lung Deposition. A Computer Model Approach. (Lecture)
- Czech, H. *et al.*: Implications of photochemical ageing for source apportionment and health effects of wood combustion aerosol. (Lecture)
- Passig, J. *et al.*: Remote Sensing of Ship Emissions by Single Particle Mass Spectrometry with Ionization Enhancement in Laser Desorption/Ionization. (Lecture)

- Passig, P., ..., Zimmermann, R.: A New Multiple-Ionization Single Particle Aerosol Mass Spectrometer: Rapid On-line Analysis of Toxic Polycyclic Aromatic Hydrocarbons (PAH), Metals (Pb, Zn, etc.) as well as Anionic Source Marker Compounds on Individual Airborne Aerosol Particles (Lecture)
- Streibel, T. *et al.*: Chemical Characterization of Particulate Matter from Combustion Devices Related To Wood Combustion And Internal Combustion Engines. (Lecture)
- Zimmermann, R. *et al.*: Fresh and aged combustion aerosols: Chemical composition and molecular biological/toxicological effects on air-liquid-interface exposed human lung cells. (Lecture)

PEFTEC, Rotterdam, Netherlands, 22. – 23.5.2019
- Käfer, U. *et al.*: High resolution time-of-flight mass spectrometry coupled to GC×GC, direct inlet probe (DIP) and thermogravimetry (TGA) for the study of marine fuels. (Lecture)
- Rüger, C. P. *et al.*: Direct insertion probe high-resolution mass spectrometry for the rapid chemical description of petroleum heavy ends – Analysis of vacuum gas oils, bitumen, and asphaltenes. (Lecture)

43rd International Symposium on Capillary Chromatography, 16th GCxGC Symposium, Fort Worth, TX, USA, 13. – 17.5.2019
- Zimmermann, R. *et al.*: Application of one- and comprehensive two-dimensional gas chromatography for the targeted and non-targeted analysis of combustion aerosols. (Poster)
- Gröger, T. *et al.*: High resolution time-of-flight mass spectrometry coupled to GC×GC, direct inlet probe (DIP) and thermogravimetry (TGA) for the study of marine fuels (Heavy Fuel Oils). (Lecture)

16th GCxGC–Symposium and 43rd ISCC, Fort Worth, TX, USA, 13. – 16.5.2019
- Käfer, U., ..., Zimmermann, R.: High resolution time-of-flight mass spectrometry coupled to GC×GC, direct inlet probe (DIP) and thermogravimetry (TGA) for the study of marine fuels (Heavy Fuel Oils). (Invited lecture, fully funded).

Faraday Discussion: Challenges in analysis of complex natural mixtures, Edinburgh, United Kingdom, 13.05 – 15.05.2019
- Rüger, C. P. *et al.*: Data processing in Petroleomics: Petroinformatics approaches and CERES – a Matlabbased platform. (Poster)

1st EU-FT-ICR MS Advanced-User school, Lisbon, Portugal, 14. – 18.4.2019
- Sklorz, M.: Atmospheric pressure ionization. (Lecture)

Seminar at University of Pretoria, Gauteng, South Africa, 10.4.2019
- Gawlitta, N. *et al.*: Characterization of the chemical Composition of (Bio-) Aerosols concerning their Allergic Traits. (Lecture)

12th International Conference on Carbonaceous Particles in the Atmosphere (ICCPA), Vienna, 3. – 6.4.2019
- Czech, H. *et al.*: Implications of photochemical ageing for source apportionment and health effects of wood combustion aerosol. (Lecture)
- Padoan, S. *et al.*: Spring aerosol in urban atmosphere: analytical and statistical assessment for source impacts. (Poster)
- Nguyen, D. – L. *et al.*: Primary and secondary organic aerosol at Global Atmosphere Watch (GAW) regional station in North Western Vietnam during biomass burning period. (Poster)

Analytica Vietnam, Ho Chi Minh City, Vietnam, 3. – 4.4.2019
- Zimmermann, R. *et al.*: Air pollution and Health: Novel biological and chemical analysis techniques. (Invited lecture. fully funded).

Lecture in the framework of the official inauguration of the aeroHEALTH Helmholtz International Lab. by the President of the Helmholtz Association and the President of the Weizmann Institute, Weizmann Institute of Science, Rechovot, Israel, 1.4.2019
- Zimmermann, R. *et al.*: aeroHEALTH: The newly funded Helmholtz International Lab. (Lecture)

Anakon, Münster, Germany, 25. – 28.3.2019
- Gehm, C. *et al.*: Entwicklung und Optimierung eines Membraneinlass-Photoionisierungsmassenspektrometers für die Echtzeitanalytik (polycyclischer) aromatischer Kohlenwasserstoffe in aquatischen Systemen. (Lecture)
- Grimmer, C. *et al.*: Description of complex petrochemical samples by thermal analysis mass spectrometry. (Lecture)
- Gawlitta, N. *et al.*: The Chemical Characterisation of Aerosols with either Allergy-Adjuvant or-Protective Effects and Subsequent Cell Exposure. (Lecture)
- Gröger, T. *et al.*: Vacuum Photoionization ToF-MS A powerful tool for the investigation of coffee roast gas composition and on-line process control in real-time. (Poster)
- Gröger, T. *et al.*: From e-cigarettes to joints – Puff resolved online investigation of smoke products using Photoionization Mass Spectrometry. (Poster)
- Gröger, T. *et al.*: Die integrative Anwendung verschiedener gaschromatographischer- und thermischer Einlassmethoden für die massenspektrometrische Charakterisierung der flüchtigen und nicht-flüchtigen Bestandteile von Schweröl. (Lecture)

- Öder, S., ..., Zimmermann, R.: and further HICE co-workers, Analysis of the toxicity of fresh and aged combustion aerosols: Chemical composition and molecular biological effects on directly exposed lung cells.(lecture)
- Passig, J., ..., Zimmermann, R.: New Developments in Single Particle Aerosol Mass Spectrometry. (Lecture)

21st annual conference of the JCF, Frühjahrssymposium, Bremen, Germany, 20.03 – 23.03.2019
- Friederici, L. *et al.*: Studies on the deposit formation potential of biofuel/heating oil blends by selective evolved gas analysis. (Poster)
- Grimmer, C. *et al.*: Characterisation of heavy petrochemicals by thermal analysis hyphenated to (selective) mass spectrometry. (Poster)

Pittcon 2019, Philadelphia, PA, USA, 17. – 21.3.2019
- Ehlert, S., ..., Zimmermann, R.: Investigation of the coffee roasting process, aroma profile and antioxidant capacity by time-resolved vacuum photoionization TOF-MS. (Lecture)
- Passig, J., ..., Zimmermann, R.: A New Multiple-Ionization Single Particle Aerosol Mass Spectrometer: Rapid On-Line Analysis of Toxic Polycyclic Aromatic Hydrocarbons, Metal-Cations as well as Anionic Source Marker Compounds on individual Airborne Aerosol Particles. (Lecture)

52th DGMS, Rostock, Germany, 10.–13.3.2019
- Czech *et al.*: Single-particle Analysis from a Major Fire Incident of Discarded Metal in Rostock, Germany. (Poster)
- Gehm, C. *et al.*: Development of a membrane-introduction photoionization mass spectrometer for real-time analysis of aromatic and polycyclic aromatic hyrdocarbons in aquatic systems. (Poster)
- Grimmer, C. *et al.*: Description of complex petrochemical samples by thermal analysis mass spectrometry. (Lecture)
- Heide, J. *et al.*: Monitoring of Coffee Roasting by Vacuum Photoionization TOF-MS:Towards a Prediction Model for Bean Color and Antioxidant Capacity. (Lecture)
- Käfer, U. *et al.*: Extended application range for a GCxGChigh-resolution time-of-flight mass spectrometer platform by hyphenation to thermal analysis. (Poster)
- Neumann, A *et al.*: Comparison of different atmospheric pressure photo ionisation techniques with atmospheric pressure chemical ionisation for gas phase ionisation high resolution FT-ICR MS. (Poster)
- Passig, J. *et al.*: Remote Detection of Ship Emissions using Single-Particle Mass Spectrometry. (Lecture)
- Rüger, C. P. *et al.*: Structural analysis of heavy oil fractions by combination of high-resolution tandem mass spectrometry and ion mobility spectrometry. (Lecture)

10th Multidimensional Chromatography Workshop, Liege, Belgium, 21. – 23.1.2019
- Käfer, U. *et al.*: Comprehensive study of heavy fuel oils by high resolution mass spectrometry hyphenated to GCxGC, thermogravimetric analysis and direct inlets. (Lecture)
- Gawlitta, N. *et al.*: Evaluation of two newly available software packages for the processing and statistical analysis of comprehensive two-dimensional gas chromatography data. (Lecture)

2018

AGU (American Geophysical Union) Fall Meeting 2018, Washington DC, USA, 9. – 12.12.2018
Passig, J., ..., Zimmermann, R.: Do we need a new metric for ambient aerosol toxicity prediction? – On-line determination of the internal-/external-mixture of toxic chemicals in ambient air particles by a novel single particle MS technology. (Lecture)

Ichthyol-Cordes Pharma GmbH, Hamburg, Germany, 5.12.2018
- Zimmermann, R.: Untersuchung der molekularen Zusammensetzung von Ölschieferdestillaten und deren sulfonierter Produkte (Arzneistoffe) mittels multidimensionaler Chromatographie und hochauflösender Massenspektroskopie. (Invited lecture).

DGMK-Jahrestreffen der Analytiker, Hamburg, 29. – 30.11.2018
- Gröger, T. *et al.*: The combined and comprehensive analysis techniques based on high resolution mass spectrometry for the investigation of medium-heavy and heavy petroleum matrices. (Lecture)

CMSC 2018 (Chinese Mass Spectrometry Conference), Guangzhou, China, 24. – 25.11.2018
- Zimmermann, R. *et al.*: Aerosol particles, tobacco smoke, crude oil, coffee roasting and industrial processes: Analysis of complex materials, thermal processes and aerosols by Photoionisation Mass Spectrometry (PIMS). (Invited lecture, fully funded).
- Passig, J., ..., Zimmermann, R.: New perspectives in on-line single particle mass spectrometry. (Lecture at the Aerosol Mass spectrometry Workshop, Hexin Inc., 23.11.2018)

Mini Symposium on Aerosols and Mass Spectrometry, Hong Kong City University, Hong Kong, 21.11.2018
- Zimmermann, R. *et al.*: How to address the influence of atmospheric aging in research aerosol-induced health effects and what is the right aerosol parameter to measure? (Lecture)

Workshop on ultrafine particles, Munich, Germany, 05.11.2018
- Schnelle-Kreis J. *et al.*: Personal exposure to ultrafine particles in the environment – sources, composition, spatial and temporal variability. (Lecture)

Seminar at University of Rouen, France, 3.11.2018
- Zimmermann, R. *et al.*: Anthropogenic aerosols: Chemical analysis and health effects assessment. (Invited lecture)

Workshop of the US EPA and the Helmholtz Zentrum München, Washington DC, USA, 9. – 11.10.2018
- Zimmermann, R. *et al.*: Influence of atmospheric aging on aerosols health effects AND What is the right parameter to measure? (Lecture)

Seminar at SABIC Inc., Geleen, Netherlands, 3.10.2018
- Rüger, C. P., ..., Zimmermann, R.: Multi-methodological mass spectrometric investigation of heavy petrochemical samples. (Invited lecture, fully funded).

9th International Conference on Nanotoxicology, Neuss (Germany), 18. – 21.09.2018
- Di Bucchianico, S. *et al.*: Genotoxic and epigenetic effects following long-term low-dose exposure of BEAS-2B cells to Ni and NiO nanoparticles. (Poster)
- Gliga, A. *et al.*: Silver nanoparticles modulate LPS-induced secretion of pro-inflammatory cytokines in human lung and macrophage-like cells. (Lecture)

27th ASIC (Association for science and information on coffee) Congress, Portland, OR, USA, 16. – 20.9.2018
- Zimmermann, R.: Resolving coffee roasting phases by vacuum photoionization TOF-MS process monitoring: Towards coffee with optimized aroma profile and antioxidant-capacity. (Lecture)

"Luftqualität: Emissionsminderung im Fokus", Frankfurt, Germany, 11.9.2018
- Zimmermann, R. *et al.*: Gesundheitseffekte von Feinstäuben und Gasen: Biologische Wirkung von Abgasen aus Verbrennungsprozessen HKI-Forum (Industrieverband Haus-, Heiz und Küchentechnik e.V.). (Invited lecture, fully funded).

10th International Aerosol Conference (IAC 2018) Saint Louis, MO, USA, 2. – 7.9.2018
- Karg, E. W. *et al.*: Is the particle deposition in a cell exposure facility comparable to the lungs? (Lecture)
- Passig, J., ..., Zimmermann, R.: Single Particle Aerosols Mass Spectrometry: Detection of polycyclic aromatic hydrocarbons as well as positive and negative inorganic ions from individual airborne particles. (Lecture)
- Streibel, T. *et al.*: Chemical Characterization Of Combustion Aerosols Related To Wood Combustion And Internal Combustion Engines. (Poster)
- Zimmermann, R. *et al.*: Application of air-liquid-interface (ALI) based *in-vitro* exposure of human or murine lung cells and validation by selected animal exposure tests in the framework of the HICE consortium to investigate fresh and aged combustion aerosols. (Lecture)

12th ESTAC (European Symposium on Thermal Analysis and Calorimetry), University Transilvania, Brasov, Romania, 27. – 30.8.2018
- Gröger, T. *et al.*: Thermal Analysis coupled to High Resolution Time-of-Flight Mass Spectrometry for in-depth Analysis of high boiling Petroleum Constituents. (Poster)
- Zimmermann, R., Gröger, T. *et al.*: *et al.*: Chemical profiling of crude oil and asphaltene samples by Thermal Analysis coupled to photoionization mass spectrometry and other TA-MS Methods. (Lecture)

XXII International Mass Spectrometry Conference, Florence, 26. – 31.08.2018
- Czech *et al.*: Direct Infusion Resonance-Enhanced Multiphoton Ionization Mass Spectrometry (DI-REMPI-MS) of Liquid Samples under Vacuum Conditions. (Poster)
- Gehm, C. *et al.*: Development and optimization of a membrane – inlet – photoionization mass spectrometer for real-time analysis of (poly)aromatic compounds in aquatic systems. (Lecture)
- Käfer, U. *et al.*: Direct inlet probe – high resolution time-of-flight mass spectrometry for the description of high boiling petroleum fractions. (Lecture)
- Rüger, C. P. *et al.*: Comprehensive high-resolution mass spectrometric evolved gas analysis in the context of Petroleomics. (Lecture)

1st EU-FT-ICR MS End-User school, Joensuu, Finnland, 20. – 24.8.2018
- Neumann, A. *et al.*: Comparison of atmospheric pressure chemical and photo ionization for evolved gas analysis high resolution FT-ICR MS for petroleum-derived material. (Lecture)

- Sklorz, M.: Atmospheric pressure ionization for FT-ICR MS (Lecture)

Analitika 2018, Mookgophong, Limpopo, South Africa; 22. – 25.7.2018
- Orasche *et al.*: Analysis of Semi Volatile Organic Compounds (SVOC) with In-situ Derivatization Thermal Desorption Two-dimensional Gas Chromatography and Time of Flight Mass Spectrometry (IDTD-GCxGC-ToF-MS). (Lecture)
- Zimmermann, R. *et al.*: Innovative analytical approaches for analysis of ambient air as well as emission- and workplace-aerosols. (Invited lecture, fully funded).

19th Annual Conference on Petroleum Phase Behavior and Fouling, The Chateaux, Deer Valley, UT, USA, 8. – 12.7.2018
- Gröger, T. *et al.*: Comprehensive Gas Chromatography and Thermoanalytical techniques coupled to High Resolution Mass Spectrometry for in-depth Analysis of Crude Oils and Bitumen. (Lecture)
- Rüger, C. P., ..., Zimmermann, R.: Comprehensive mass spectrometric evolved gas analysis (EGA) in the context of Petroleomics. (Lecture)
- Zimmermann, R. *et al.*: Comprehensive Gas Chromatography and Thermoanalytical techniques coupled to High Resolution Mass Spectrometry for in-depth Analysis of Crude Oils and Bitumen. (Lecture)

RAIS 2018, WS on Urban Air Pollution and Health, Hong Kong Polytechnic University, Hong Kong, 30.6.2018
- Zimmermann, R. *et al.*: Accessing the toxicity of aerosols: Single-particle aerosol mass spectrometry for detection of aromatics and metals as well as biological testing of air-liquid-interface exposed lung cells. (Invited lecture, fully funded).

DIN-FAM Arbeitsgremien NA 062-06-14 AA "Chromatographische Analyse", Berlin, 20.06.2018
- Gröger, T. *et al.*: The application of ASTM D8071 for Oxyblends and non-specified matrices. (Lecture)

22nd ETH Conference on Combustion Generated Nanoparticles, Zürich, Switzerland, 18. – 21.6.2018
- Czech, H. *et al.*: Primary and Secondary Aerosol Emissions from Modern Small-scale Wood Combustion Appliances with Advanced Secondary Air Supply. (Lecture)

CONCAWE Workshop, Munich, Germany, 14. – 15.6.2018
- Käfer, U. *et al.*: Thermal Analysis – High Resolution Mass Spectrometry for the analysis of high boiling petroleum fractions. (Lecture)
- Gawlitta, N. *et al.*: Alternative software packages for untargeted analysis of GCxGC-TOF Data. (Lecture)
- Gröger, T. *et al.*: Introduction to GC×GC. (Lecture)
- Gröger, T. et al: Vacuum Ultraviolet Absorption Spectroscopy. (Lecture)
- Gröger, T. *et al.*: High Temperature GC×GC. (Lecture)
- Streibel, T. *et al.*: Application of Thermal Analysis. (Lecture)

CEECHE 2018 (Central and Eastern European Conference on Health and the Environment), Krakow, Poland; 10. – 14.6.2018
- Zimmermann, R. *et al.*, Analysis of the Toxicity of Combustion Aerosols: Chemical Composition of Different Combustion-Emissions and their Molecular Biological Effects on Air/Liquid-Interface Exposed Lung Cells. (Invited lecture, fully funded).

66th ASMS Conference on mass spectrometry and allied topics, San Diego, USA, 3. – 7.6.2018
- Käfer, U. *et al.*: High-resolution Time-of-Flight Mass Spectrometry as Versatile and Investigative Tool for the Hyphenation with Different Sample Introduction and Ionization Techniques. (Poster)
- Rüger, C. P. *et al.*: Comprehensive mass spectrometric evolved gas analysis (EGA) in the context of Petroleomics. (Poster)
- Zimmermann, R.: Vacuum photoionisation: SPI and REMPI. (Lecture at the Workshop “Photoionisation Mass Spectrometry”, 6.6. 2018)

Guangzhou Institute of Geochemistry, CAS, Guangzhou, China, 2.6.2018
- Zimmermann, R. *et al.*: Single-particle aerosol mass spectrometry for detection of aromatics and transition metals and biological testing of air-liquid-interface exposed lung cells. (Invited lecture).

3rd International Conference on Atmospheric Dust, Bari, Italy, 29. – 31.5.2018
- Orasche *et al.*: The impact of electrostatic precipitators and secondary heat exchangers on particulate matter and accompanied inorganic and organic species emitted by wood combustion. (Lecture)
- Orasche *et al.*: Dust research under extreme conditions – Personal Sampling and chemical characterization of aerosols in a platinum mine. (Poster)
- Orasche *et al.*: Novel techniques for characterization of organic composition of fine dust. (Lecture)

42nd International Symposium on Capillary Chromatography, 15th GCxGC Symposium, Riva del Garda, Italy, 13. – 18.5.2018
- Gawlitta, N. *et al.*: New Platform-independent Data Analysis Software with build-in chemometric Tools for the Data Analysis of comprehensive Two-Dimensional Gas Chromatography. (Poster)

- Zimmermann, R., Gröger, T. *et al.*: The combination of different thermal and chromatographic analysis techniques based on high resolution mass spectrometry for the investigation of medium-heavy and heavy petroleum matrices. (Invited lecture, fully funded).

13th EFTMS Workshop, Germany, 24. – 27.4.2018
- Neumann, A. *et al.*: Comparison of Xe/Kr atmospheric pressure photo ionization (APPI) to atmospheric pressure chemical ionization for the analysis of complex petrochemical mixtures. (Poster)
- Rüger, C. P. *et al.*: Comprehensive high resolution mass spectrometric evolved gas analysis in the context of Petroleomics: Analysis of bitumen, heavy crude oils and asphaltenes. (Lecture)

Sulphur Cap 2020 Conference & Exhibition, Amsterdam, Netherlands, 17. – 18.4.2018
- Zimmermann, R. *et al.*: Why the sulphur cap is important – Health, Economical and Environmental considerations. (Invited lecture, fully funded).

Petromass 2018, Bled, Slowenien 15.04. – 18.04.2018
- Gröger, T. *et al.*: Mass spectrometry as selective and sensitive detector for thermal analysis – A versatile hyphenation for the analysis of complex and high boiling petroleum products. (Lecture)

Analytica Conference, Munich, Germany, 10. – 13.4.2018
- Czech, H. *et al.*: Profiles of Carbonaceous Aerosol Emissions and Secondary Organic Aerosol from a Marine Engine and Modern Wood Combustion Appliances. (Invited lecture at the Session "Aerosol & Health", fully funded)
- Zimmermann, R. *et al.*: Analysis of composition and biological activity of emissions from engines and wood combustion. (Invited lecture, fully funded).
- Zimmermann, R.: Introduction to the Session "Aerosols and Health: Characterization of the Composition and the Toxicological Effects of Air Pollution". (Invited session organizer, fully funded).
- Zimmermann, R. *et al.*: Analysis of composition and biological activity of emissions from engines and wood combustion. (Invited lecture, fully funded).

Sino-German Symposium on „Bridging Aerosol Chemistry and Health Effects: Challenges and Opportunities for Analytical Chemistry", Guangzhou, China, 19. – 23.3.2018
- Passig, J. *et al.*: On-line Laser Mass Spectrometry of PAHs and Inorganics from Individual Particles. (Lecture)
- Streibel, T. *et al.*: On- and offline chemical characterization of combustion aerosols. (Lecture)
- Sklorz, M. *et al.*: High resolution mass spectrometry screening – Data processing and data mining. (Lecture)
- Zimmermann, R. *et al.*: Towards understanding of health effects of air pollution (aerosols): Studying biological effects and physicochemical properties of combustion aerosol emissions within the HICE project. (Funded by DFG; Sino-German Workshop; chair with Prof Xue Li)

Seminar at Hong Kong Polytechnic University, Hong Kong, 16.3.2018
- Zimmermann, R. *et al.*: The Virtual Helmholtz Institute HICE: Interdisciplinary research on aerosols. (Lecture)
- Sklorz, M. *et al.*: High resolution mass spectrometry for aerosol analysis. (Lecture at the Workshop on "Air Pollution and Health")
- Streibel, T. *et al.*: EC/OC coupled to Photo-Ionization Mass Spectrometry for the chemical characterization of particulate matter. (Lecture at the Workshop on "Air Pollution and Health")

51st European Mass Spectrometry Conference, Saarbrücken, Germany, 11. – 15.3.2018
- Czech, H. *et al.*: A chemometric investigation of aromatic emission profiles from a marine engine in comparison with residential wood combustion and road traffic: Implications for source apportionment inside and outside sulphur emission control areas. (Poster)
- Gehm, C. *et al.*: Development and optimization of a membrane-inlet-photoionization mass spectrometer for real-time analysis of (poly)aromatic compounds in aquatic systems. (Lecture)
- Käfer, U. *et al.*: High Resolution Mass Spectrometry hyphenated to different Inlet Techniques for the Characterization of high boiling Petroleum fractions. (Poster)
- Neumann, A. *et al.*: Comparison of Xe/Kr atmospheric pressure photo ionization (APPI) to atmospheric pressure chemical ionization for the analysis of complex mixtures. (Poster)
- Passig, J., ..., Zimmermann, R.: A New Single Particle Aerosol Laser Mass Spectrometer for Dual TOFMS Analysis of the Individual Airborne Aerosol Particles: Detection of Polycyclic Aromatic Hydrocarbons and Inorganic Compounds. (Keynote lecture)
- Rüger, C. P. *et al.*: Comprehensive mass spectrometric evolved gas analysis in the context of Petroleomics. (Lecture)

Seminar at the Oregon State University, Corvallis, Oregon, USA, 5.3.2018
- Zimmermann, R. *et al.*: Addressing air pollution-related biological &health effects: Exposure of lung cells and animals to combustion aerosol emissions followed by biological response analysis and comprehensive aerosol characterization. (Lecture)

Leco User Meeting, Berlin, Germany, 28.1 2018

- Käfer, U. *et al.*: Investigation of petrochemical samples in the transition Region toward nonvolatility -Applying LECO HRT technology with different inlet methods for the discrimination of heavy fuel oil. (Lecture)

GDCh-Fresenius Lecture at the Univerität Bayreuth, 17.1.2018

- Zimmermann, R. *et al.*: Kaffeeröstung, Erdöl und Aerosole: Einsatz der Photoionisations-Massenspektrometrie und anderer Analysemethoden zum Verständnis komplexer Prozesse und Stoffsysteme. (Invited GDCh-Fresenius lecture, fully funded).

GDCh-Fresenius Lecture at the Univerität Regensburg, 12.1.2018

- Zimmermann, R. *et al.*: Anthropogenic aerosols: Analysis as well as biological and health effects. (Invited GDCh-Fresenius lecture, fully funded).

Doktorandenseminar Hohenroda, Hohenroda, Germany, 7. – 9.1.2018

- Käfer, U. *et al.*: Hochauflösende Flugzeit-Massenspektrometrie mit verschiedenen Probenein-lässen und Ionisationsmethoden für die Analytik hochsiedender Rohölfraktionen. (Lecture)

3.6 Peer-Reviewed Publications and Book Chapters

2021 (Period covered until editorial deadline on 31.01.2021)

127. Bauer, S. *et al.*: In vivo and in vitro toxicity of emissions from a non-road diesel engine. (in process of submission)

126. Binder, S. *et al.*: In vitro genotoxicity of dibutyl phthalate on A549 lung cells at air-liquid interface in exposure concentrations relevant at workplaces (in process of submission)

125. Cao, X. *et al.*: Adenine derivatization for LC-MS/MS epigenetic DNA modifications studies on monocytic THP-1 cells exposed to reference particulate matter. Analytical Biochemistry DOI: 10.1016/j.ab.2021.114127 (2021)

124. Cao, X. *et al.*: A comparative study of persistent DNA oxidation and chromosomal instability induced in vitro by oxidizers and reference airborne particles. (in process of submission)

123. Castilla, C. *et al.*: Comparison of the pyrolysis products of five biomass samples by direct introduction high resolution mass spectrometry and ion mobility. Journal of Applied Pyrolysis (submitted)

122. Di Bucchianico, S. *et al.*: System analysis of respiratory response to wood pellet combustion reveals inflammatory effect. (in process of submission)

121. Gehm, C. *et al.*: Advanced VOC speciation in headspace and on-line analysis by combining hyper-fast gas chromatography and single-photon ionization time-of-flight mass spectrometry with integrated electric modulator-based sampling. Analyst. (submitted)

120. Gat, D. *et al.*: The biogeography of dust in the Eastern Mediterranean, Communications Earth & Environment (submitted)

119 Grimmer, C. *et al.*: Characterization of Polyethylene Branching by Thermal Analysis-Photoionization Mass Spectrometry. J. Am. Soc. Mass. Spectr. DOI: 10.1021/jasms.0c00291 (2021)

118. Heide, J. *et al.*: Puff-resolved analysis of chemicals in vapors and smoke of E-Cigarettes, Heat-not-Burn devices and conventional cigarettes using single-photon ionization time-of-flight mass spectrometry (SPI-TOFMS): A comparative study. Nicotine Tob. Res. (submitted)

117. Helin, A. *et al.*: Variation of Absorption Ångström Exponent in Aerosols from Different Emission. Journal of Geophysical Research (submitted)

116. Koch, A. *et al.*: Investigation of chemical composition and fiber occurrence in PM2.5 and PM10 from dry cutting processes of carbon concrete composites and their reinforcement materials. Aerosol Science and Engineering (submitted)

115. Kohlmeier *et al.*: Portable aerosol sampling techniques for airborne particulate health relevant compounds in an underground platinum mine. SAIMM (submitted)

114. Kösling, P. *et al.*: PhotOrbi: a platform for resonance-enhanced multiphoton ionization of gas-phase molecules inside the C-trap and a high-resolution ion detection with an Orbitrap mass analyzer, Anal. Chem. (submitted)

113. Li, F. *et al.*: Chemical profiles, sources, and PAH toxicity of PM2.5 in Beijing in the fall-winter transit season-individual and interactive effects of home heating, pollution control measures, and meteorology. Chemosphere 276, 130143 (2021)

112. Liu, X. *et al.*: Air pollution in Germany: spatio-temporal variations and their driving factors based on continuous data from 2008 to 2018. Environmental Pollution DOI: 10.1016/j.envpol.2021.116732 (2021)

111. Liu, X. *et al.*: Spatiotemporal characteristics and determinants of black carbon in Augsburg, Germany: Combination of mobile monitoring and street view imagery. Environ. Sci. Technol. DOI: 10.1021/acs.est.0c04776 (2021)

110. Liu, X. *et al.*: Analysis on mobile monitoring data from the microAeth® MA200 and an assessment of data treatment methods for measuring changes in black carbon on the roadside in a German city. Atmos. Meas. Tech. Disc (preprint, DOI: 10.5194/amt-2020-517)

109. Liu, X. *et al.*: Exposure assessment models for black carbon in urban environments: A combination of land use and street view image approach. (in process of submission)

108. Momenimovahed, A. *et al.*: Comparison of black carbon measurement techniques for marine engine emissions using three marine fuel types, Atmospheric Environment (submitted)

107. Neumann, A. *et al.*: Investigation of island/single-core and archipelago/multicore enriched asphaltenes and their solubility fractions by thermal analysis coupled to high resolution Fourier transform ion cyclotron resonance mass spectrometry. Energy & Fuels DOI: 10.1021/acs.energyfuels.0c03751 (2021)

106. Nguyen, D. L. *et al.* Organic aerosol composition analyzed in air masses influenced by open biomass burning plumes: a case-study in Northwestern Vietnam. Atmos. Chem. Phys. Disc. (preprint, DOI: 10.5194/acp-2020-1027)

105. Offer, S. *et al.*: Atmospheric Aged Aerosol Toxicity of Anthropogenic and Biogenic Emissions in Human Airway Barrier Model Systems after Air-Liquid Interface (ALI) Exposures. Environ. Health Perspect. (submitted)

104. Pardo, M. *et al.*: Toxicity of water- and organic-soluble wood tar fractions from biomass burning in lung epithelial cells Chemical Research in Toxicology (submitted)

103. Passig, J., *et al.*: Detection of Ship Plumes from Residual Fuel Operation in Emission Control Areas using Single-Particle Mass Spectrometry, Atmos. Meas. Tech. Disc., (preprint, DOI: 10.5194/amt-2020-482)

102. Reiss, R. *et al.*: Comparison of three analytical methods for the on-site analysis of traces at clandestine drug laboratories. Applied Sciences (submitted)

101. Rüger, C. P., *et al.*: Atmospheric pressure laser ionization mass spectrometry with a 157-nm Krypton-Fluoride excimer laser for sensitive detection of polycyclic aromatic hydrocarbons. Anal. Chem. DOI: 10.1021/acs.analchem.0c04740 (2021)

100. Rüger, C. P. *et al.*: Exploring complex mixtures by cyclic ion mobility high-resolution mass spectrometry – Application towards Petroleum. Analytical Chemistry (submitted)

99. Rus, C. M. *et al.*: Key triggers determination for metabolomics analysis from dried blood spots. Metabolites (submitted)

98. Smita Gupta, S. *et al.*: An integrative workflow to prioritize toxicity initiation events from air-liquid-interface exposure of human lung cells to combustion aerosols: First application to emissions from a heavy fuel oil or diesel fuel operated ship engine. Research Square (preprint, DOI: 10.21203/rs.3.rs-152521/v1)

97. Sun, F. et al: Mass Transfer-Limited Biodegradation – Evidence from Reactive Transport Modeling of Isotope Profiles in a Mesoscale Aquifer. Environmental Science and Technology (submitted)

96. Weggler, B. A., *et al.*: A unique data analysis framework and open source benchmark data set for the analysis of comprehensive two-dimensional gas chromatography software. Journal of Chromatography A 1635:461721. DOI: 10.1016/j.chroma.2020.461721 (2021)

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95. Boesl, U. and Zimmermann, R; Fundamentals and Mechanisms of Resonance-Enhanced Multiphoton Ionization (REMPI) in Vacuum and its Application in Molecular Spectroscopy, in Zimmermann, R. and Hanley, L. “Photoionization and Photo-Induced Processes in Mass Spectrometry”, Wiley-VCH Verlag, Weinheim, Germany, 2020 DOI: 10.1002/9783527682201.ch2 (2020)

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92. Czech, H. *et al.*: Smart online coffee roasting process control: Modelling coffee roast degree and brew antioxidant capacity for real-time prediction by resonance-enhanced multi-photon ionization mass spectrometric (REMPI-TOFMS) monitoring of roast gases. Foods 9:627 (2020)

91. Dhahak, A. *et al.*: Real time monitoring of slow pyrolysis of polyethylene terephthalate (PET) by different mass spectrometric techniques. Waste Manag. 106, 226-239 (2020)

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4. Reiss, R. *et al.*: Ambient pressure laser desorption-chemical ionization mass spectrometry for fast and reliable detection of explosives, drugs, and their precursors. Appl. Sci. 8:933 (2018)

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2. Rüger, C.P. *et al.*: Combination of Different Thermal Analysis Methods Coupled to Mass Spectrometry for the Analysis of Asphaltenes and Their Parent Crude Oils: Comprehensive Characterization of the Molecular Pyrolysis Pattern, Energy & Fuels, 32, pp. 2699-2711 (2018)

1. Shen, R. *et al.*: Seasonal variability and source distribution of haze particles from a continuous one-year study in Beijing. Atmos. Pollut. Res. 9, 627-633 (2018)

3.7 Seminars

Seminars of the Joint Mass Spectrometry Centre, the Helmholtz Virtual Institute HICE, and the Helmholtz International Lab aeroHEALTH.

In addition to weekly internal research progress seminars with PhD-students and PostDoc-researchers, JMSC organizes a weekly seminar with invited experts during teaching terms. The seminar takes place at the Institute of Chemistry, Albert Einstein Str. 3a, 18059 Rostock. These lectures are broadcasted to the Helmholtz Zentrum München as well as to the partners of the Helmholtz Virtual Institute HICE via video conference.

Some of the lectures are supported by the Gesellschaft deutscher Chemiker [*] or were invited by Leibnitz Institute for Baltic Sea Research Warnemünde (IOW) [**].

Winter teaching term 2017 / 2018			
#	Date / Time	Speaker	Title
1	24.10.17	**Dr. Heinz Renner** Linseis GmbH Selb, Germany	Thermogravimetry under extreme conditions-measurements at high pressures and high temperatures
2	14.11.17	**Prof. Dr. Mikhail Yavor** Institute for analytical instrumentation of the Russian Academy of Sciences St. Petersburg, Russia	Multi-pass time-of-flight mass analyzers: advances in ion-optical design
3	21.11.17	**Dr. Jonathan Symonds** Cambustion Ltd. Cambridge, GB	Novel methods of aerosol particle classification by size and mass
4	05.12.17	**Prof. Dr. Joakim Pagels** Lund University Lund, Sweden	Black Carbon Research at Lund University – From Formation and Oxidation in the Cylinder to Atmospheric Aging and Health Effects
5	09.01.18	**Prof. Dr. Janne Jänis** University of Eastern Finland Joensuu, Finland	High-resolution mass spectrometry for chemical fingerprinting of complex mixtures
6	16.01.18	**Dr. Volker Matthias** Helmholtz-Centre Geesthacht GmbH Institute of Coastal Research Geesthacht, Germany	How shipping and agriculture impact air quality in coastal regions
7**	22.01.18	**Prof. Dr. Hartmut Herrmann** Leibniz Institute for Tropospheric Research (TROPOS) Leipzig, Germany	Atmospheric multiphase SOA formation: isoprene oxidation and cloud processing
8*	23.01.18	**Prof. Dr. Frank-Michael Matysik** University of Regensburg Regensburg, Germany	Electrochemistry – the flexible tool box for the construction of hyphenated instrumental systems
9**	29.01.18	**Prof. Dr. Anton Eisenhauer** Helmholtz-Centre for Ocean Research Kiel, Germany	Theory and Application of Calcium and Strontium Isotope Fractionation During Calciumcarbonate Precipitation

Summer teaching term 2018			
#	**Date/ Time**	**Speaker**	**Title**
1	24.04.18	**Dr. Daniel Reiser** **Dr. Alois Fendt** AIR LIQUIDE Research & Techn logy Center Frankfurt, Germany	Analytical approaches in the process industry
2	08.05.18	**PhD Asta Gregorič** AEROSOL d.o.o. Ljubljana, Slovenia	Urban Black Carbon emissions: ambient measurements, sources and effect on climate
3	19.06.18	**Prof. Dr. Robert David** Reference and Translation Center for Cardiac Stem Cell Therapy University of Rostock Rostock, Germany	Cardiac Pacemaker Cells from pluripotent Stem Cells
4	03.07.18	**Prof. Dr. em. Magda Claeys** University of Antwerp Antwerp, Belgium	Molecular characterization of α-pinene ozonolysis secondary organic aerosol and formation mechanism

Scheduled lectures for 29.05.18 and 25.06.18 had to be postponed to the next teaching term due to illness of the speakers.

Winter teaching term 2018 / 2019			
#	**Date/ Time**	**Speaker**	**Title**
1	30.10.18	**Thomas Koziorowski** Probat-Werke von Gimborn Maschinenfabrik GmbH Emmerich, Germany	Aroma development and on-/offline analysis on industrial coffee roasters – possibilities and development potentials
2	13.11.18	**Johannes Piel** MLase AG, Gemmerich, Germany **Dr. Johannes Passig** University of Rostock, Rostock, Germany	Recent developments in excimer laser technology and its application in particle mass spectrometry
3	26.11.18	**Prof. Dr. Joachim Wegener** Fraunhofer EMFT and University Regensburg Regensburg, Germany	Cells in Contact to Nanomaterials: A Label-Free and Integrative Perspective
4	27.11.18	**Dr. Anders Holmen Pedersen** GEA Process Engineering A/S Soeborg, Denmark	Industrial Instant Coffee Processing and the Impact on Coffee Flavour
5	04.12.18	**Dr. Dimitris Papanastasiou** Fasmatech Athens, Greece	Recent advances in omnitrap technology coupled to ion mobility spectrometry, orthogonal TOF and orbitrap mass analyzers
6	18.12.18	**Dr. Jürgen H. Gross** Institute of Organic Chemistry of the University of Heidelberg Heidelberg, Germany	Fundamentals, Instrumentation, and Applications of Field Ionization and Field Desorption Mass Spectrometry

Winter teaching term 2018 / 2019			
#	Date/ Time	Speaker	Title
7*	08.01.19	**Dr. habil. Michael Maiwald** Federal Institute for Materials Research and Testing (BAM) Berlin, Germany	Compact NMR Spectroscopy: A Versatile Tool for Automated Continuous-Flow Production of Chemicals and Pharmaceuticals
8	15.01.19	**Dr. Harald Saathoff** Karlsruhe Institute of Technology (KIT) Karlsruhe, Germany	Mass spectrometric characterization of chemistry and mixing state of aerosols in simulation chamber and field studies
9	22.01.19	**Prof. Dr. Philipp Weller** Mannheim University of Applied Sciences Mannheim, Germany	Profiling, Data Mining, Data Fusion – modern analytical strategies against food fraud of honeys and olive oils
10	05.02.19	**Prof. Dr. Thomas P. Knepper** Hochschule Fresenius, Institute for Analytical Research (IFAR) Idstein, Germany	Highly polar organic water contaminants in raw and tap water – challenging not only the analytical chemist

Summer teaching term 2019			
#	Date/ Time	Speaker	Title
1*	29.04.19	**Dr. Hellmut Mahler** Criminal Science and Technology Instituteof the State Criminal Police Office of North Rhine-Westphalia Düsseldorf, Germany	Gifte und Vergiftungen, Drogen und Rauschzustände. Die Forensische Toxikologie in der Kriminaltechnik
2**	29.04.19	**Prof. Dr. Martin Elsner** Technical University of Munich, Institute of Hydrochemistry Munich, Germany	Reactive Tracers in Chemical Substances: Analysis of Stable Isotopes to Elucidate Processes in Complex Systems
3	29.04.19	**Prof. Dr. Klaus Lips** Helmholtz Centre Berlin, Institute for Nanospectroscopy Berlin, Germany	Electron Paramagnetic Resonance-on-aChip (EPRoC) – a Paradigm Shift in Spin-Radical Analysis
4	29.04.19	**Dr. Guido Gayko** ICHTHYOL-GESELLSCHAFT Cordes, Hermanni & Co. (GmbH & Co.) KG Hamburg, Germany	Highly Sulfuric Oil Shale as a Source of Substances of Complex Chemical Composition – History, Current Uses and Outlook
5	29.04.19	**Dr. Anne-Kathrin Bergmann** **Dr. Matthias Fritzsche** Merck KGaA Darmstadt, Germany	Central analytics@Merck – a concept of how mass spectrometry can be applied in an industrial environment
6	01.07.19	**Dr. Jessalin Howell** The J.M. Smucker Company Orrville,(Oh)/USA	The Use of Single-Photon Ionization Time-of-Flight Mass Spectrometry as Tool for Flavor Prediction During Coffee Roasting: An Industry Perspective
7	02.07.19	**Prof. Dr. Cristian Focsa** PhLAM-Laboratory of Physics of Lasers, Atoms and Molecules University of Lille Villeneuve d'Ascq cedex, France	Mass spectrometry studies of combustion-generated particles

Winter teaching term 2019 / 2020			
#	Date/Time	Speaker	Title
1	21.10.19	**Prof. Dr. Ronald Redmer** Institute of Physic University of Rostock Rostock, Germany	Matter under Extreme Conditions: Planets, Exoplanets, Brown Dwarfs
2*	05.11.19	**Prof. Dr. Gertrud Morlock** Justus Liebig University Giessen, Germany	How to analyze unknown unknowns, the ones we do not know we do not know?
3	12.11.19	**Dr. Andreas Mayer** **Mr. Thomas W. Lutz** TTM- Technik Thermische Maschinen Niederrohrdorf, Switzerland	A new particle filter system for marine engines
4**	18.11.19	**Prof. Dr. Thorsten Reemtsma** Helmholtz Centre for Environmental Research-UFZ Leipzig, Germany	Mass spectrometric approaches to study contaminants in the environment and in biota: particles, highly polar compounds and transformation products
5*	02.12.19	**Prof. Dr. Rainer Weinkauf** Heinrich-Heine University Düsseldorf, Germany	Circumventing spin selection rules in molecular chromophores by radical anion photodetachment photoelectron spectroscopy
6	17.12.19	**Prof. Dr. Alexander Föhlisch** Helmholtz Centre Berlin Berlin, Germany	Photochemical Pathways from X-ray Spectroscopy
7	07.01.20	**Prof. Dr. Heiko Hayen** University of Münster Münster, Germany	Analysis of (glyco-) lipids by hyphenated mass spectrometric techniques
8**	14.01.20	**Prof. Dr. Eric Achterberg** GEOMAR, Helmholtz Centre for Ocean Research Kiel, Kiel, Germany	Application of mass spectrometric techniques to trace ocean biogeochemical cycles
9	21.01.20	**Prof. Dr. Carlos Afonso** University of Rouen Mont Saint Aignan, France	Characterization of highly complex organic mixtures by high-resolution mass spectrometry coupled to ion mobility spectrometry and planar chromatography

The already planned lectures for the summer semester 2020 had to be canceled due to Corona. The lectures to follow took place predominantly as non-presence events.

Winter teaching term 2020 / 2021			
#	**Date/Time**	**Speaker**	**Title**
1	10.11.20	**Prof. Dr. Stefan Oswald** Institute of Pharmacology and Toxicology, Rostock University Medical Center, Rostock, Germany	Mass spectrometry-based absolute quantification of clinically relevant proteins
2	17.11.20	**Dr. – Ing. Nikolai Glück** Fraunhofer Research Institution for Large Structures in Production Engineering IGP, Rostock, Germany	Characterization of wind turbine rotor blade materials
3	24.11.20	**Prof. Dr. Dominik Kraus** Institute of Physics, High Energy Density, Physics, University of Rostock, Rostock, Germany	Warm Dense Matter: From Giant Planets and Stars to Nanoparticles
4	01.12.20	**Prof. Dr. Udo Kragl** Institute of Chemistry, University of Rostock, Rostock, Germany	Hydrogels and polymerized ionic liquids: New materials for applications in medicine and engineering
5	08.12.20	**Dr. Christopher Rüger** Institute of Chemistry, University of Rostock, Rostock, Germany	Insights into isomeric and isobaric diversity of complex organic mixtures by ion mobility and high resolution mass spectrometry
6	15.12.20	**Prof. Dr. Marcus Rieker** HORIBA Europe Ltd., Oberursel, Germany	Measurement and Characterization of Ultrafine Particles in Engine Exhaust
7**	05.01.21	**Prof. Dr. Andreas Macke** Leibniz Institute for Tropospheric Research Leipzig, Germany	Exploring the marine troposphere from ship-based and coastal observations and modeling
8	12.01.21	**Dr. Annette Sethmann** ICHTHYOL-GESELLSCHAFT Cordes, Hermanni & Co. (GmbH & Co.) KG Hamburg, Germany	Pharmacological activity of sulfur-rich oil shale derived medicinal products
9	19.01.21	**Prof. Dr. Thomas Leisner** Karlsruhe Institute of Technology Karlsruhe, Germany	Ion traps and mass spectrometry as a tool for studying meteoric smoke aerosol and noctilucent clouds
10	26.01.21	**Dr. habil. Pierre Giusti** TRTG – TOTAL RAFFINAGE CHIMIE – Normandie Harfleur, France	Contribution of separation sciences and mass spectrometry for molecular characterization and elemental speciation of petroleum products & new feedstock for energy

3.8 Media Coverage

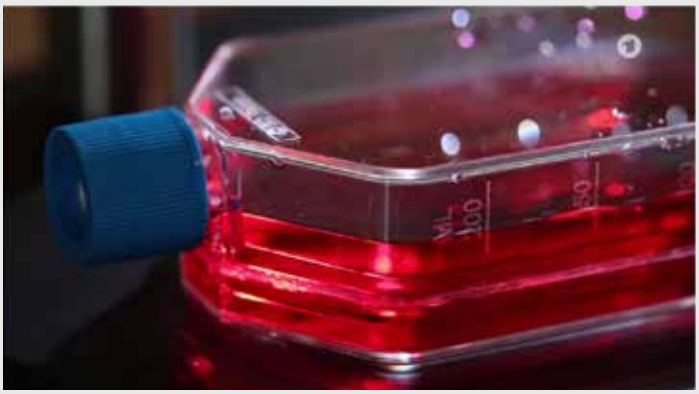
In addition to several recognitions of JMSC's research activities and progress on the topic Aerosol and Health in print media we had several visits of TV teams during measurement activities or interview appointments. See below an overview of selected TV reports.

Health effects of stove emissions (20.01.2020)

The cozy warmth, the gently flickering flames and the image of wood as a CO_2-neutral and therefore environmentally friendly source of energy make stoves a popular living accessory in Germany. At the same time, stoves have become one of the largest sources of fine dust in the country. In the TV show "45 min" (Kaminöfen: Gemütlich, aber schädlich?) on NDR on 20th of January, 2020, Prof. Dr. Ralf Zimmermann and Dr. Jürgen Schnelle-Kreis were available as experts to answer questions about the harmfulness of stoves. As specialist for assessing pollutants in the air, Dr. Jürgen Schnelle-Kreis was invited to measure fine dust in a stove-heated living room and in the ambient air outside the heated house. While no fine dust could be measured inside, the fine dust values escalated in the smoke plume outside the house. Furthermore, Prof. Dr. Ralf Zimmermann is interested in the question of how fine dust affects human health in detail. Therefore, he led the television team to the University of Eastern Finland in Kuopio, where chemical and biological experiments have been carried out. Lung cells were exposed to a daily dose of the exhaust gases. The biological studies revealed DNA-damages and inflammation of the lung cells and showed that the harmfulness of fine dust from wood-burning stoves is in no way inferior to diesel exhaust gases.

Link to the TV broadcast:
https://www.ndr.de/fernsehen/sendungen/45_min/Kaminoefen-Gemuetlich-aber-schaedlich,sendung990872.html

Ship emissions under scrutiny (15.7.2019)



Air quality in port cities is poor due to the high density of cruise shipping. The values of ultrafine dust exceed the values of many large cities in the world. In the TV Broadcast "Die Story im Ersten" (Traumschiffe als Luftverschmutzer?) on ARD from July 15th, 2019, Prof. Dr. Ralf Zimmermann and his team gave journalists insights into their research on the impact of marine emissions on human health. They address questions, such as: "how do ship emissions differ from other emissions" and "how does heavy oil work in comparison with other fuels". Prof. Dr. Ralf Zimmermann and his team have already been able to prove that fine dust is harmful to human health. Zimmermann explains, however, that these studies strongly depend on the type of exposure. Until now, lung cells in liquid medium were exposed to pollutants in liquid form, which is unnatural. The peculiarity of his research is that the lung cells are exposed to the gaseous pollutants, as in reality.

Link to the TV broadcast:
https://www.youtube.com/watch?v=5SeLIR5Rz3Q

Health effects of cruise ship emissions (25.2.2019)

Residents of port cities on the North, Baltic or Mediterranean Sea are daily exposed to health pollution from ship exhausts. While the residents complain more and more about the negative effects of ship emissions, the cruise industry is experiencing a boom – even in 2019. In the TV Broadcast "45 min" on NDR from February 25th, 2019, Prof. Dr. Ralf Zimmermann explains what harmful effects ship exhaust emissions can have. While particles larger than 10 µm are filtered by the nose, ultra-fine particles (< 2.5 µm) penetrate the fine branches of the lungs causing dangerous inflammation. Those particles can even reach the bloodstream, causing a heart disease and heart attacks, or the brain, causing strokes. The realistic effect of ship exhaust gases on living human cells can be investigated experimentally with the help of cell cultures. Because the components of the fine dust in exhaust gases can be identified individually, the effects on humans can be described so precisely. In the department of Prof. Dr. Ralf Zimmermann, other processes are examined as well, such as exhaust gases from wood combustion or car exhausts. However, ship exhausts have been found to have the most harmful effects among the mentioned ones.

Link to the TV broadcast:
https://www.ndr.de/fernsehen/sendungen/45_min/rueckschau/Wie-gesundheitsgefaehrdend-sind-Schiffsabgase, kreuzfahrtschiffe131.html

Czech TV team accompanies ambient aerosol measurements (24.11.2018)

Measuring- and recording device for capturing particle matter of different sizes, particle number concentrations, aerosol particle size distribution, black and brown carbon.

Emissions from residential combustion can cause pulmonary diseases in humans. Especially in small settlements in Middle and Eastern Europe the population is severely exposed to harmful substances. Low-quality fuel and boilers and improper operation of such are leading to an exceedance of particulate matter limits, resulting in respiratory problems or even cancer and premature deaths. In the Czech TV broadcast from November 2018, the group around Prof. Dr. Ralf Zimmermann introduced a device that consists of various measuring- and recording devices with which parameters such as particle matter of different sizes, particle number concentrations, aerosol particle size distribution, black carbon and brown carbon are measured. The emission studies were carried out in two small neighboring locations – Bayerisch Eisenstein in Germany and Železná Ruda in Czech Republic. With this equipment, our scientists could on the one hand, counteract the lack of data and on the other hand perform a comprehensive physical-chemical characterization of aerosols.

Link to the TV broadcast (only in Czech language):
www.ceskatelevize.cz/ivysilani/

Fine Dust in the idyllic Alps (15.03.2018)

"When using wood-burning stoves without filters, wood burning leads to harmful emissions. These emissions endanger human health, especially in autumn and winter, as the inversion weather conditions are smog-like." Prof. Dr. Ralf Zimmermann and his group contributed to news about health risks of the environmental pollution by residential wood combustion in the TV broadcast quer on BR Television) from March 15th, 2018.

Link to the TV broadcast:
https://www.helmholtz-muenchen.de/fileadmin/CMA/Quer/Quer_Holzofen.mp4

Emission of fine particles from wood combustion (03.03.2018)

Compared to fossil fuels, wood is considered to be more environment-friendly. Consequently, the conversion to wood heaters was also promoted by the state. However, the emissions associated with the burning process are often not taken into account. Today, privately operated wood heaters such as wood stoves are a significant source of particulate matter, which has been proven to be directly related to negative health effects. The knowledge magazine "gut zu wissen" of the Bayerischen Fernsehen took up this topic in an episode of 03.03.2018 and reported, among other things, on a measurement campaign of the JMSC in Kuopio, Finland. Prof. Dr. Ralf Zimmermann reports on the objectives of this interdisciplinary study to investigate emissions caused by wood combustion. Using a complex experimental set-up at the University of Eastern Finland, the aerosol produced during combustion was first examined physically and chemically before human lung cells were exposed at the same time.

Link to the TV broadcast:
https://www.br.de/mediathek/video/gut-zu-wissen-03032018-ansteigende-meeresspiegel-feinstaub-schleuder-holzofen-av:5d306c7cfe462a001a667abe

aeroHEALTH

A Helmholtz International Laboratory

4 aeroHEALTH

4.1 Overview

aeroHEALTH – Facts in brief

- The Helmholtz International Laboratory aeroHEALTH is a cooperation of the **Helmholtz Zentrum München** (HMGU, Germany), the **Forschungszentrum Jülich** (FZJ, Germany) and the **Weizmann Institute of Science** (WIS, Israel) with the associated partners **University of Rostock** (UR, Germany) and **University of Eastern Finland** (UEF, Finland).
- aeroHEALTH addresses the understanding of the impact of ambient aerosols on human health. On the one hand, the formation of aerosols by the atmospheric transformation of biogenic and anthropogenic precursor emissions is in focus. On the other hand, particulate emissions from biomass burning and other anthropogenic sources, dust and wildfires are considered.
- aeroHEALTH uses state-of-the-art aerosol analytical approaches, oxidation flow reactors, and aerosol chambers to generate aerosols of atmospheric relevance and investigate their chemical composition down to the molecular level.
- aeroHEALTH applies advanced cell exposure techniques, molecular biological approaches, and bioinformatic measures to elucidate the adverse effects of the aerosols.
- aeroHEALTH is funded by the Helmholtz-Gemeinschaft e.V. (HGF) via its Impuls- und Vernetzungsfonds with 1.500.000 €. This funding is matched by the Helmholtz Zentrum München and the Forschungszentrum Jülich as well as by the Weizmann Institute of Science. The total available funding is 4.500.000 €.
- aeroHEALTH started in April 2019 as a five-year project (2019–2024).
- aeroHEALTH information is available via the website: www.aeroHEALTH.eu

aeroHEALTH – Motivation and Introduction

The World Health Organization WHO states, that air pollution by particulate matter (PM) is the largest environmental health risk in Europe, causing a substantial disease burden. It is estimated that in 2014 more than 90% of the world population lived in places where WHO air quality guidelines were not met, causing more than 3 million premature deaths due to exposure to ambient air pollution and making it the fourth-highest risk factor for death globally[1] (figures 1 and 2). The WHO estimates that in 2016, some 58% of outdoor air pollution-related premature deaths were due to ischemic heart disease and strokes, 18% due to chronic obstructive pulmonary disease (COPD) and acute lower respiratory infections and 6% due to lung cancer[2]. A recent study on the U.S. Medicare population concluded that exposure even below WHO guideline levels to air pollution lead to serious health effects[3]. It also draws public awareness to the increasing number of smog alarms in European cities (*e.g.* Paris, Milan, Athens, Stuttgart) and the recent diesel car emission issues. However, there is a substantial knowledge gap regarding

[1] 'Ambient Air Pollution. A Global Assessment of Exposure and Burden of Disease', WHO report, 2016
[2] WHO (https://www.who.int/news-room/fact-sheets/detail/ambient-(outdoor)-air-quality-and-health)
[3] Di, Q. *et al.*, N. Engl. J. Med. 376(26):2513-2522, 2017
[4] Hallquist, M. *et al.*, Atm. Chem. Phys. 9, 5155-5236, 2009
[5] Heald, C. L. & Spracklen, D. V. Chem. Rev., 115(10): 4476:4496, 2009
[6] Bergstrom, R. M., Atm. Chem. Phys., 14(24): 13643-13660, 2014
[7] Kanashova, T. *et al.*, J. Mol. Clin. Med. 1(1):23-35, 2018

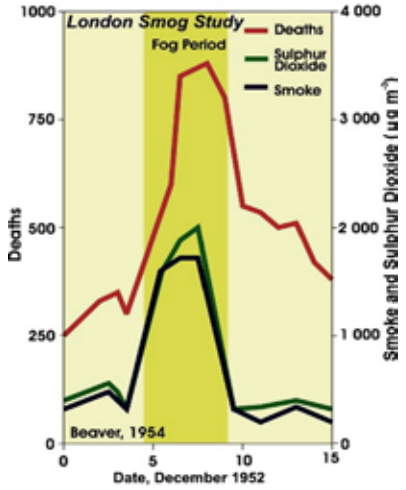


Figure 1:
(left) During the "terrible smog period" in London in 1952 the daily deaths in Greater London was peaking due to acute air pollution effects (after: Environ. Health Perspect. 2004, 112 6–8)
(right) According to the WHO the worldwide annual death toll due to air pollution is in the range of seven million premature deaths (after: Ambient Air Pollution: A global assessment of exposure and burden of disease. WHO report, 2016). For comparison: In the first year of the Corona pandemic worldwide two million COVID 19-related deaths were observed.

the drivers and underlying mechanisms of the health effects and, as a result, a lack of data that can be used for burden assessment and mitigation policies. Despite extensive research, it is still unclear which aerosol elements or compound classes cause adverse health effects, what are the prevailing biological mechanisms behind them, and how these effects depend on atmospheric chemistry. The scientific research questions behind this complex problem are highly interdisciplinary and addressing them requires health-, geo- and environmental sciences, engineering, physics, chemistry, biology, statistics, and computing/modeling to work together. Atmospheric aerosols are complex mixtures of directly emitted (primary) aerosols and those formed in the atmosphere by chemical reactions of gaseous precursors (secondary aerosols). Up to 80% of the fine particulate matter forms through atmospheric chemistry processes, where oxidation of gas-phase precursors form multi-functional condensable products that partition to the particle phase[4]. Changes in atmospheric composition are expected through emission changes from natural and anthropogenic sources that will lead to complex and variable secondary particle composition and exposure[5,6]. Moreover, a warmer climate enhances wildfire and high pollution events, while changes in atmospheric flows will transport dust into new regions. While most air quality standards rely on PM mass, recent evidence suggests that different chemical aerosol components produce vastly different health effects[7]. From 2012 on, the Helmholtz Virtual Institute HICE – Aerosol and Health, investigated biological effects of combustion aerosols. One result of the HICE program was the proof that different combustion emissions, depending on their different chemical composition due to fuel type or combustion compliance, cause also very different adverse molecular biological effects in exposed lung cell models and animals. First experiments with an oxidation flow tube reactor moreover showed the strong impact of atmospheric aging processes on the toxicity of

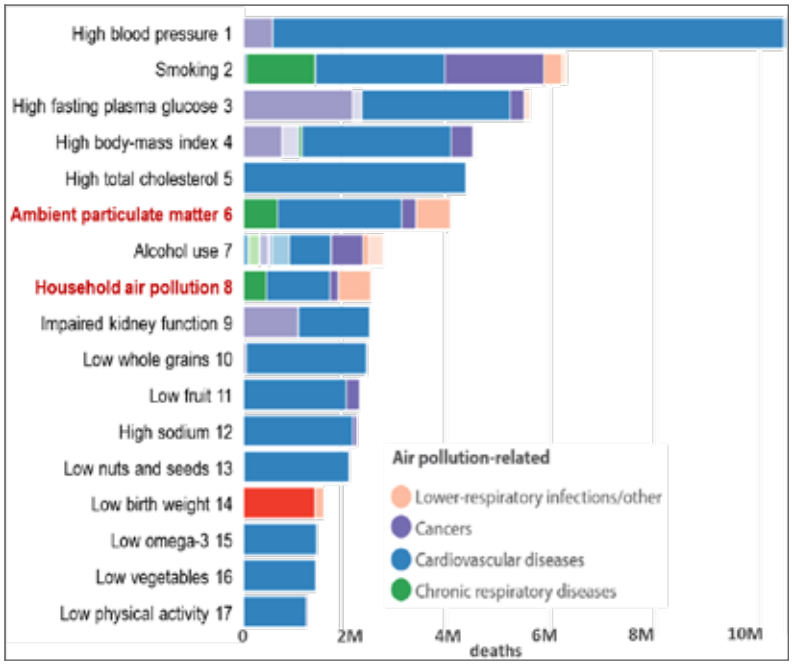


Figure 2: Why we need the aeroHEALTH initiative? The graph shows causes for premature deaths (annual): Ambient particulate matter and air pollution are the most important environmental factors for premature deaths (after Health Effects Institute: State of Global Air 2018. Report. Boston, MA).

combustion emissions. Thus, an important goal for further studies, namely the understanding of how varying atmospheric processes affect the potency of aerosol emission and ambient air to induce adverse biological effects, was identified. The research question of the impact of atmospherically aged emission on health led to the formation of the aeroHEALTH consortium (figure 3), an international cooperation of the Helmholtz Zentrum München (HMGU, Germany) the Forschungszentrum Jülich (FZJ, Germany), and the Weizmann Institute of Science (WIS, Israel) as well as further associated partners. The consortium applied to the Helmholtz Association in 2018 for funding of a Helmholtz International Lab. After a competitive selection process, the Helmholtz International Lab aeroHEALTH got co-funded by the Helmholtz Association (HGF, Germany) and started its research activities in 2019 with an inauguration ceremony at the Weizmann Institute in Israel (figure 4). Unique simulation facilities to study aging at different time scales and simulation complexity (*e.g.* SAPHIR aerosol chamber, oxidation flow tube reactors) coupled to advanced exposure systems for biological models (air liquid interface exposure units for exposing cell lines, co-culture tissue models, or knockout cell lines as well as *in-vivo* models) will be used for studying the health effects of complex aerosols and to elucidate how they change during atmospheric aging. Various exposure models in combination with state-of-the-art biological investigations (of functional and "omics"-level) will be implemented for deciphering the biological effects. aeroHEALTH establishes a direct link between emission scenarios, chemical composition, atmospheric aging and health effects. By combining facilities and expertise, the aeroHEALTH consortium will be enabled to address these complexities on a level that was not possible before. The obtained knowledge will finally be essential for science-based strategies and guidelines for policy makers as well as the development of abatement strategies and technologies. In addition to the scientific program, aeroHEALTH provides also an interdisciplinary educational platform for young and early career scientists. With the start of the aeroHEALTH project, the aeroHEALTH web page (https://www.aeroHEALTH.eu) went on-line. To inform about the activities, an aeroHEALTH-flyer was published (figure 5). In the context of a delegation visit of the HGF-President to Israel and Jordan in 2018, an aeroHEALTH project meeting was held and contact to Jordan scientists from the "Synchrotron-Light for Experimental Science and Applications in the Middle East" (SESAME), a synchrotron-based research institute, created under the auspices of the UNESCO in Allen, was established. A direct outcome is the "DUSTSTORM" project initiative, led by Prof. Lips and Prof. Föhlisch (HZB) as well as Prof. Zimmermann (HMGU) from the HGF side and Prof. Lausi from SESAME in Jordan. DUSTSTORM is a coordinated approach to educate young Jordanian students during their PhD-studies in Germany under the involvement of the German Academic Exchange Service (DAAD) and involves them also in aeroHEALTH project research, in particular on the topic of the toxicity of airborne desert dust. A first DUSTSTORM PhD student will join the team in mid-2021.

Figure 4: *Inauguration of the Helmholtz International Lab. aeroHEALTH at the Weizmann Institute in Israel: Helmholtz President Prof. Dr. Otmar Wiestler, Tropospheric Researcher Prof. Dr. Astrid Kiendler-Scharr, Weizmann President Prof. Dr. Daniel Zajfman, Project Coordinator and Aerosol & Health Researcher Prof. Dr. Ralf Zimmermann and Aerosol physicist and toxicologist Prof. Dr. Yinon Rudich (from right, photo: © Weizmann Institute, photography unit, Itai Belson).*

Figure 3: *Formation of the consortium: An aeroHEALTH planning meeting, spring 2018 at the Weizmann Institute in Israel. From right: Prof. Rudich (WIS), Prof Kiendler-Schrar (FZJ) and Prof. Zimmermann (HMGU).*

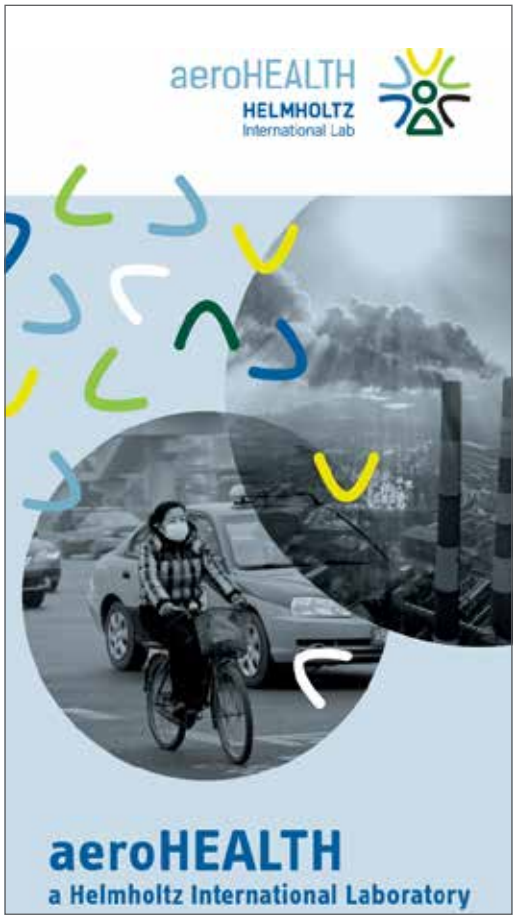


Figure 5: *The aeroHEALTH-project flyer. It can be downloaded at the aeroHEALTH-web page (https://www.aeroHEALTH.eu) and is available as print version as well.*

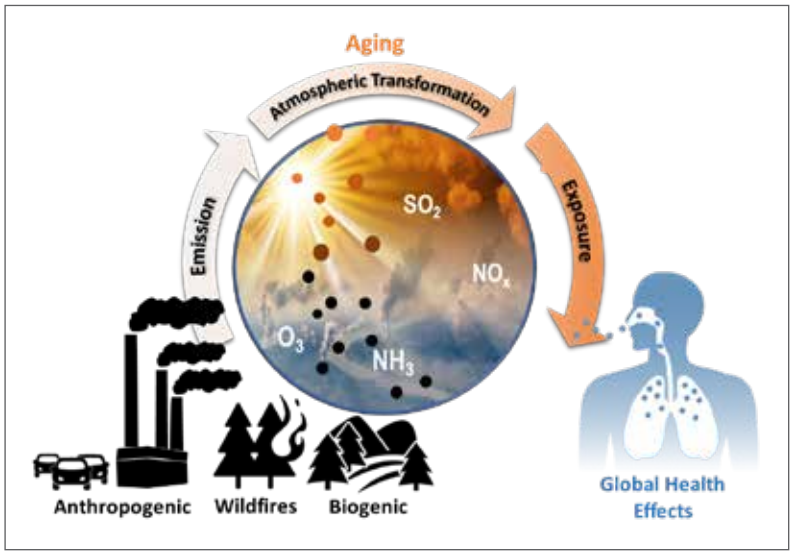


Figure 6: *aeroHEALTH aims to study the biological and health effects of secondary organic aerosols from anthropogenic and biogenic precursors and wants to unravel the underlying biological mechanisms. By this improved metrics and biomarkers for aerosol toxicological assessment of emissions and ambient air shall be established. The aeroHEALTH studies include Investigation of the health impact of relevant fresh and aged aerosol sources such as engine emissions, biomass burning, and wildfires as well as dust and the dust microbiome.*

The goal of the Helmholtz International Laboratory aeroHEALTH is to understand the biological and health effects of atmospheric aerosols in a mechanistic way by combining information on primary emissions as well as on secondary and ambient aerosols. The aeroHEALTH concept and infrastructure are partly based on preliminary work of the Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health – Aerosol and Health (HICE), *e.g.* on the mobile exposure laboratory (HICEMobiLab), the air-liquid interface exposure approach (ALI), advanced high-volume oxidation flow reactors for photochemical aging (PEAR) and novel aerosol characterization approaches (single particle mass spectrometry, SPMS). In addition, profound expertise on atmospheric transformation and analysis as well as aerosol aging chamber capabilities are coming from FZJ. The Weizmann Institute adds further capabilities in the field of aerosol transformation and exposure of animal models as well as aerosol toxicology and in-depth omics facilities. The Institute of Computational Biology (ICB) of the HMGU finally adds bioinformatics and artificial intelligence approaches for solving big data issues arising from comprehensive chemical analysis and multi-omics data. While HICE targeted primary anthropogenic combustion emissions, aeroHEALTH extends this approach to secondary and ambient aerosols. Aerosol emissions undergo a rapid conversion ("aging") by complex multiphase- and photochemistry, which alter the physico-chemical properties. Although the association between different aerosols and health effects is well established, the effect of atmospheric aging on aerosol toxicity has been sparsely investigated. Thus, understanding their toxicological potential is the challenging key topic of aeroHEALTH. In order to realize the goals of aeroHEALTH, the atmospheric processing of biogenic and anthropogenic emissions is simulated under as realistic as possible conditions on shorter and longer time scales to connect laboratory observations with the observed health impacts from field experiments. Thereby, the research benefits from the expertise in various disciplines, including analytical chemistry, physics, biochemistry, biology, medicine, engineering, statistics, and informatics. The aeroHEALTH research mission and objectives are stated in the following and are partly reflected in figure 6.

aeroHEALTH research mission and objectives

1. Studying the effects of secondary and aged organic aerosols from anthropogenic, biogenic, or mixed precursors and the atmospheric aging conditions on human health by advanced and comprehensive aerosol analysis and realistic exposure of *in-vitro* and *in-vivo* models.

2. Unravelling the biological mechanisms and different roles of aerosol constituents for health effects by a unique integration of aerosol analytics and biological effects assessment by multi-omics approches and functional assays with multi-layer bioinformatics models.

3. Development of improved metrics and biomarkers for aerosol toxicological assessment of emissions and ambient air in environmental health.

4. Investigation of properties and assessments of health impact of relevant fresh and aged aerosol sources. In particular, in focus are: i) biomass burning and wildfires, ii) dust and the dust microbiome as well as iii) engine emissions.

Consortium and Infrastructure

The multi-disciplinary aeroHEALTH consortium consists of leading research groups of two Helmholtz centers in Germany and the Weizmann Institute for Science in Israel (figure 7). HMGU brings forward the expertise and infrastructure from the HICE initiative for aerosol characterization and toxicology based on air-liquid-interface cell exposure, extended by "Big Data" analytical competence. The FZJ enters the aeroHEALTH consortium with its unique infrastructure for simulation of atmospheric aging and physical characterization approaches. WIS contributes by *in-vivo* toxicological expertise, aerobiology, and further complimentary aerosol characterization approaches to aeroHEALTH. In brief, the aeroHEALTH consortium possesses outstanding and complementary abilities by simulation of realistic and long-term atmospheric aging, including extreme endpoints, comprehensive physico-chemical and biological aerosol analysis, exposure approaches, and state-of-the-art *in-vivo* and *in-vitro* toxicology. World-class biological services are provided by the infrastructure to investigate the biological outcomes. Finally, incorporation of new "Big Data" and Artificial Intelligence methods will be developed to elucidate the connection between environmental exposure and health effects.

Figure 7: The aeroHEALTH consortium consists of principal investigators and co-PI's form research groups of the Helmholtz Zentrum München, the Forschungszentrum Jülich and the Weizmann Institute for Science in Israel. PI are: aeroHEALTH-spokesperson Prof. R. Zimmermann and Prof. F. Theis/Prof. A. Marsico for HMGU, Prof. A. Kiendler-Scharr for FZJ and Prof. Y. Rudich for WIS. Co-PI are Dr. H. Czech and Dr. C. Ogris (HMGU), Dr. T.Hohaus (FZJ) and Dr. M. Levin (WIS). Furthermore Dr. D. Gat from the Weizmann Institute joined as a second co-PI by funding from the HMGU.

Partner details:

- Helmholtz Zentrum München, Germany. CMA – Comprehensive Molecular Analytics, PI Prof. R. Zimmermann, co-PI Dr. H. Czech as well as ICB – Institute of Computational Biology, PI's Prof. F. Theis and Prof. A. Marsico and co-PI Dr. C. Ogris

- Forschungszentrum Jülich, Germany. Institute of Energy and Climate Research IEK-8: Troposphere, PI Prof. A. Kiendler-Scharr and co-PI Dr. T. Hohaus

- Weizmann Institute of Science, Israel. Department of Earth and Planetary Sciences, PI Prof. Y. Rudich, co-PI Dr. M. Levin and Dr. D. Gat

- University of Rostock (UR), Chair of Analytical Chemistry (associated partner)

- University of Eastern Finland (UEF), Department of Environmental and Biological Science (associated partner)

Structure of the joint work in aeroHEALTH – The work package structure

The Helmholtz International Lab aeroHEALTH divides its interdisciplinary research into one project management work package (Work Package 1) and four interlinked scientific work packages. In the following, a brief description of the work package structure and the respectively applied methods and concepts is given.

Work Package 1: Management (AeroMAN)

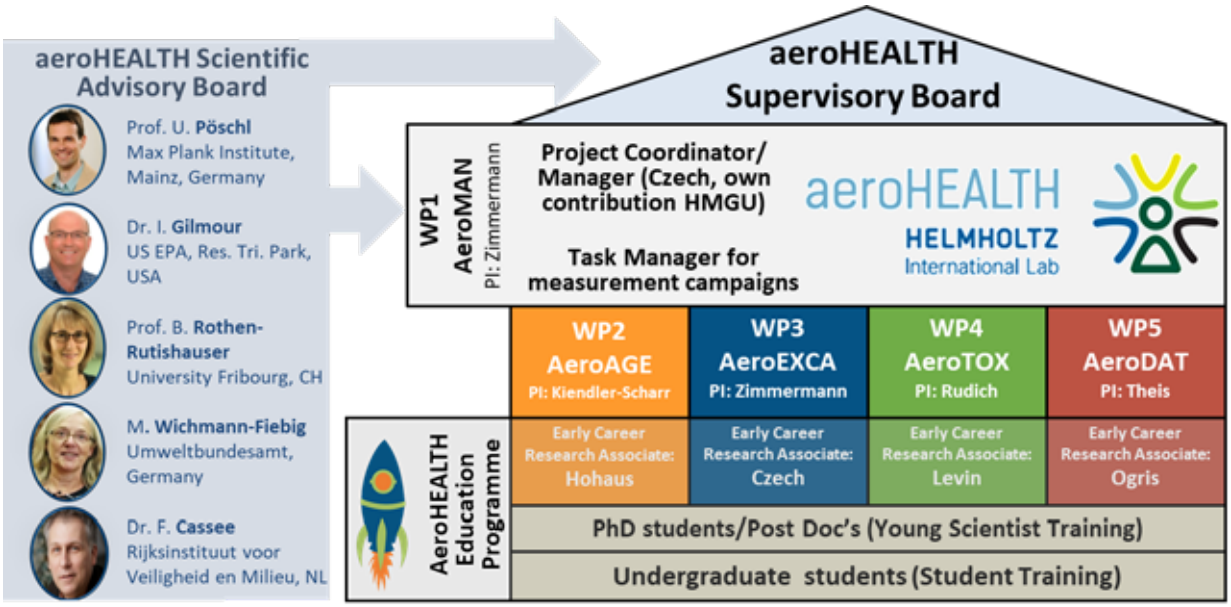


Figure 8: aeroHEALTH management structure, including the WP structure, teaching program and the scientific advisory board (SAB).

The WP 1 (AeroMAN), headed by HMGU (Zimmermann/Czech), coordinates and manages the overall activities of aeroHEALTH. The joint management is conducted by the Work Package 1 (AeroMAN) and supervised by the Supervisory Board (SB). The management structure of aeroHEALTH is depicted in figure 8. The PI and co-PI are directly involved in organizing the work within the scientific WP 2 – 5. Within WP1 the main decision-making body of aeroHEALTH, namely the Supervisory Board (SB), is organized. The SB members are the PI and co-PI: R. Zimmermann, H. Czech, A. Kiendler-Scharr, T. Hohaus, Y. Rudich, M. Pardo, F. Theis/A. Marsico, C. Ogris. The SB is chaired by R. Zimmermann. A project manager (H. Czech, funded by the financial own contribution of the HMGU) coordinates the joint research activities. In addition to conducting a challenging cutting-edge scientific work program, the aeroHEALTH Helmholtz International Laboratory program is devoted to support young scientists on all education and career levels. Firstly, the careers of four excellent early career scientists (the co-PI's) at the partner institutes are supported by the direct involvement in the organization of the activities in the aeroHEALTH work packages as Early Career Research Associates. Secondly, young scientists on the PhD-student or postdoc levels are supported by direct contribution to the research and by intense mentoring. A highly profiled, international aeroHEALTH Scientific Advisory Board (SAB) supports and advises the aeroHEALTH management and scientific bodies. The aeroHEALTH-SAB comprises experts from academic research institutes (Prof. U. Pöschl, Max-Plank Institute Mainz, D, and Prof. B. Rothen-Rutishauser, University Fribourg, CH) and from authority near research or administrate entities (Dr. I. Gilmour, US-Environmental Protection Agency; Dr. M. Wichmann-Fiebig, Umweltbundesamt, D, and Prof. F. Cassee from the Dutch National Institute for Public Health at the Environment Ministry of Health, Welfare and Sport, NL). The SAB meets annually.

Figure 9: The two and a half day initial extended aeroHEALTH steering board and scientific meeting with the scientific advisory board was held in 2019 on the research station UFS Schneefernerhaus at the highest German mountain, the Zugspitze. Top: Group photo during the tour on the scientific infrastructure of the UFS. From right: Prof. Zimmermann (HMGU) Prof. Pöschl (Max-Plank Institut Mainz), Dr. Gat (WIS), Dipl.-Ing. Gröger (HMGU), Prof. Kiendler-Scharr (FZJ), Dr. Hellack (Umweltbundesamt), Dr. Levin (WIS), Dr. Di Bucchianico (HMGU, Dr. Streibel (HMGU), Dr. Hohaus (FZJ), Dr. Öder (HMGU), Dr. Sklorz (HMGU), Dr. Gilmour (US Environmental Protection Agency), Prof. Adam (HMGU), Dr. Sippula (UEF), Dr. Chowdhury (WIS), Dr. Ogris (HMGU). Dr. Czech (HMGU), Prof. Rudich (WIS), Dr. Müller (HMGU), Dipl.-Met. Karg (HMGU), Dr. Stöger (HMGU), Dr. Jakobi (HMGU). Bottom: Intense scientific discussions helped to design an optimal program for the first joint measurement campaign.

Work Package 2: Aerosol aging and transformation (AeroAGE)

In WP2 (AeroAGE), headed by FZJ (Kiendler-Scharr/Hohaus), the consortium's expertise and resources for simulating and understanding the aging and atmospheric processing of anthropogenic and biogenic emissions are exploited to simulate current and future aerosol composition. The formation and chemical aging of different types of ambient aerosols (*e.g.* SOA, mineral dust, and biomass burning aerosols) is studied. Realistic conditions of the oxidation of gaseous precursors and subsequent SOA formation, biomass burning emissions, and coated mineral dust particles in the atmosphere are crucial to understand the underlying processes and their chemical evolution and impacts. Therefore, a comprehensive set of simulation experiments are conducted representing typical atmospheric oxidation processes using oxidation flow tubes that allow simulation of long exposures, a continuous stirred tank reactor, and an outdoor atmosphere simulation chamber studying the aging processes for a wide range of atmospheric relevant parameters and conditions (including daytime and nighttime). The chemical transformation of ambient aerosols, containing an enormous number of organic compounds, is comprehensively investigated and characterized. Additional to the direct biogenic (vegetation) and anthropogenic emissions (*e.g.* combustion), oxidative processing (aging) of volatile organic compounds (VOC) vastly increase its chemical complexity[8]. Herein, the formation of oxidation products, organic coatings on dust particles, transformation of biomass burning emissions, and secondary organic aerosols (SOA) are heavily dependent on parameters such as oxidant, NOx concentrations, and their precursors[9]. Therefore, we study representing systems of ambient aerosols under controlled conditions. This comprises comprehensive physical and chemical analyses (in conjunction with WP3) and estimated future atmospheric compositions. Available simulation environments (see below) enable us to examine a large parameter range from

Figure 10: Aging experiments under realistic conditions can be performed in the SAPHIR aerosol chamber at the FZJ.

hours to days. For these tasks, oxidation flow reactors (OFR) and simulation chambers will be used. SAPHIR[10], an atmosphere simulation chamber (figure 10) will be used to study chemical aging close to real atmospheric conditions concerning precursor concentration and SOA yields by using natural sunlight. SAPHIR will be utilized to investigate simple systems using single biogenic or anthropogenic precursors up to highly complex real plant emissions using the plant chamber SAPHIR-PLUS[11,12]. In addition, SAPHIR++, a continuous stirred tank reactor (CSTR), is designed to maintain steady-state conditions and allows for controlled variations of multiple atmospheric parameters and introduction of primary aerosol particles like mineral dust to determine the effect of aging on the organic coatings of primary aerosol. Chemical and physical characterization of both gas and particle phase will be carried out using a comprehensive set of instrumentation. *E.g.* chemical bulk parameters like oxygen-to-carbon-ratio (O:C) will be measured using high-resolution time-of-flight aerosol mass spectrometry (HR-TOF-AMS)[13] coupled to thermal desorption aerosol gas chromatography (TAG)[14] providing additional molecular marker identification. The OFR at WIS, HMGU, and UEF allow extending the aging time to about 1 week, complementing the capabilities of the SAPHIR and SAPHIR++ chambers. Various organic and precursors such as naphthalene and ß-pinene are used in OFR experiments.

Work Package 3: Aerosol Exposure and Characterization (AeroEXCA)

In WP3 (AeroEXCA), headed by HMGU (Zimmermann/Czech), the expertise in aerosol exposure and characterization of the consortium is bundled. The objective of WP is to characterize the fresh and aged aerosols and to develop and apply innovative and novel techniques in aerosol enrichment, air-liquid interface (ALI) cell exposure, as well as ALI exposure-dose measurement. Comprehensive chemical and physical characterization of fresh and aged aerosols are performed in order to unravel their chemical composition and changes upon aging. In addition to the application of high-end chemical and physical characterization of the exposure aerosol, innovative new aerosol mass spectrometric methods will be developed and applied to reveal the internal and external mixing within particle ensembles. HMGU, WIS, and FZJ contribute to WP3. Large Facilities of the Helmholtz International Laboratory in WP3 are the deep organic characterization Laboratory at HMGU featuring ultra-high resolution mass spectrometry (FT-ICR MS) and special PM analysis adapted high-end chromatographic-mass spectrometric instruments. Furthermore, mobile on-line gas- and particle-phase mass spectrometric systems are available for joint research activities (HMGU and FZJ). These instruments represent the state-of-the-art in terms of characterization of atmospheric particulate matter and gas-phase species. In WIS, high resolution time-of-flight aerosol mass spectrometer and aerodynamic aerosol classifier will be used to correlate between aging, chemical composition, and density. Innovative air-liquid interface (ALI) aerosol exposure systems (2 different types and approaches) and approaches for human and murine lung cells, co-cultures and tissue models will be applied and further optimized. This includes the application of on-line particle enrichment and dose determination approaches. Crucial is the improvement in the practical realization of long-time and multiple ALI-cell exposure scenarios in order to increase the biological effect detection efficiency required for addressing the diluted chamber aged aerosol and ambient air directly. Large Facilities of the International Lab: Europe's first

Figure 11: *(left) The HICE-MobiLab during a measurement campaign for testing the biological activity and toxicity of shipping engine emissions (right) Mobile automated air-liquid interface (ALI) exposure station for direct exposure of cell culture and co-culture tissue models to aerosols.*

[8] Goldstein, A. H. *et al.*, Environ. Sci. Techn. 41(5): 1514-1521, 2007.
[9] Shrivastava, M. *et al.*, Rev. Geophys. 55(2): 509-559, 2017
[10] Rohrer, F. B. *et al.*, Atm. Chem. Phys. 5: 2189-2201, 2005
[11] Hohaus, T. et al, Atm. Meas. Techn. 9(3): 1247-1259, 2016
[12] Bergstrom, R. M., Atm. Chem. Phys., 14(24): 13643-13660, 2014
[13] Canagaratna, M. R *et al.*, Mass Spectrom. Rev. 26(2): 185-222, 2007
[14] Williams, B. J. *et al.*, Aerosol Sci. Technol. 48(4): 358-370, 2014

mobile, field-deployable S2-biosafety laboratory (HICE-Mobilab) equipped with the newest generation of mobile automated ALI-exposure-station systems with in total 60 simultaneously exposable transwell-positions (HMGU, figure 11) is used for on-site lung cell ALI-exposure studies during the consortium wide measurement campaigns for chamber and flow reactor based aging experiments. State-of-the-art and novel approaches for aerosol characterization are applied, including: in situ derivatization thermal desorption multidimensional gas chromatography-mass spectrometry (DTD-GC-TOFMS, GCxGC-TOFMS)[15], ultra-high resolution mass spectrometry (FT-ICR MS)[16] and aerosol single particle TOF-mass spectrometry (SPMS)[17]. Furthermore, indicator parameters for the identified, health-relevant aerosol fractions are determined by comprehensive statistical approaches in cooperation with WP5. The characterization of the aged aerosols is directly linked to WP2. Another challenge of WP3 is the optimization of ALI technology to allow for longer exposure times than the typically used 4 h period in order to cope with the low PM concentrations (~30 – 100 µg m^{-3}) of ambient aerosols or aging chamber aerosol (WP 2). In addition to the mentioned measures more robust cell cultures and test approaches (including disease models and acute as well as subacute endpoints) are developed together with WP4.

Work Package 4: Aerosol Toxicology (AeroTOX)

In WP4 (AeroTOX), headed by the Weizmann Institute of Science (Rudich/ Levin), efficient read-out of the aerosol induced mechanisms of biological and toxicological effects induced by different types of aerosol (SOA, biomass burning, and dust) with advanced exposure models will be developed, optimized and applied. Crucial for predicting the effects of ambient aerosol health effects is the use of a comprehensive set of cell cultures, silenced cell lines, co-cultures, tissue cultures, and *in-vivo* experiments accompanied by state-of-the-art molecular biological and toxicological effect analyses. This includes omics and functional assays (figure 12). By developing lung cell culture models to co-cultures with cells from other organs, the covered effects will be extended beyond the lung and enable a better prediction of human health effects. The partners WIS and HMGU contribute to this WP. The cell and tissue culture-based results are tested and validated with careful use of *in-vivo* models, thus adhering to the guiding principle for the ethical use of animal testing (3R principle: reduce, replace, refine). WP4 makes use of the animal facilities, the core omics characterization and biological labs at WIS as well as the toxicological and biological laboratories at HMGU. New infrastructures such as advanced electron microscopies and imaging of metabolites and cell features will be used by all three partners as they become available in WIS.

The objectives of this WP are to get a deeper insight in the biological effects of the atmospherically transformation of anthropogenic and biogenic aerosols. In particular, we address question of whether anthropogenic emissions are toxic upon aging in the atmosphere with respect to acute cytotoxicity, genotoxic or epigenetic effects. Therefore, WP4 will develop and apply disease-relevant, sensitive, and validated *in-vitro* lung cell and tissue models as well as suited animal models for validation of the observed effects. By improving and adapting toxicological and omics methods as well as developing simpler biological assays a routine toxicological monitoring of ambient air pollution effects is a long-term goal.

The molecular biological effects following exposure to aerosols on human cells and tissue models will be comprehensively investigated. The respiratory and oral tracts along with the skin are the common routes by which humans are exposed to a wide variety of ambient pollutants. Therefore, we will study the effects of exposure on human lung, liver (represents a secondary organ of exposure), and skin (epidermis) cells. Subsequent to

[15] Orasche, J., et.al. Atm. Chem. Phys. 11(17): 8977-8993 (2011)
[16] Kourtchev, I., *et al.* Atm. Chem. Phys. 15(10): 5683-5695 (2015)
[17] Passig, J. *et al.*, Anal. Chem. 89, 6341-6345 (2017)

the aerosol exposure (ALI exposure or off-line exposure using extracts) the human cell culture models and *in-vivo* models will be subjected to an innovative and refined molecular biological analysis scheme for highly sensitive, high throughput, and comprehensive detection of adverse cellular and whole tissue responses. State-of-the-art cell culture and 3D tissue models are used in: i) adaptation of the cell culturing and exposure protocols representing different tissues, ii) development of cell co-culture models and usage of lung 3D tissues for a better description of disease-specific health endpoints, iii) cells silenced for specific mechanisms, such as the Nrf2-related protection pathways, and iv) *in-vivo* exposure using mice for systemic evaluation of aerosol exposure. By including macrophage cultures as immune-competent cells, a better description of inflammatory responses for enduring long-term exposures is envisaged. For specific endpoints, in particular beyond the lung, existing co-culture expertise is used for setting up disease-directed co-culture models using lung cells (alveolar or bronchial epithelial cells and/or macrophages (for inflammatory endpoints) in conjunction with *e.g.* fibroblasts (fibrosis induction) or endothelial cells (secondary toxic effects). Different endpoints (*e.g.* secretion of specific interleukins, cell viability and integrity, gene screen analysis, mitochondrial function, tissue histology, and advanced imaging) are performed. For specific endpoints (such as mitochondrial function, metabolism, and cell imaging), the seahorse analyzer and state of the art confocal microscopy with specific "reporters" will be used. Gene microarray and advanced bioinformatics are used, in addition to gene-specific function using silenced gene techniques (shRNA) for cell cultures. This will provide the basis for the subsequent deep molecular biological analyses required to cope with the low concentrations of ambient aerosols. The studies provide a comprehensive characterization of biological toxicity mechanisms in regard to the respective emission sources and their aging mechanisms, which will be connected to the relative to health risk within WP5.

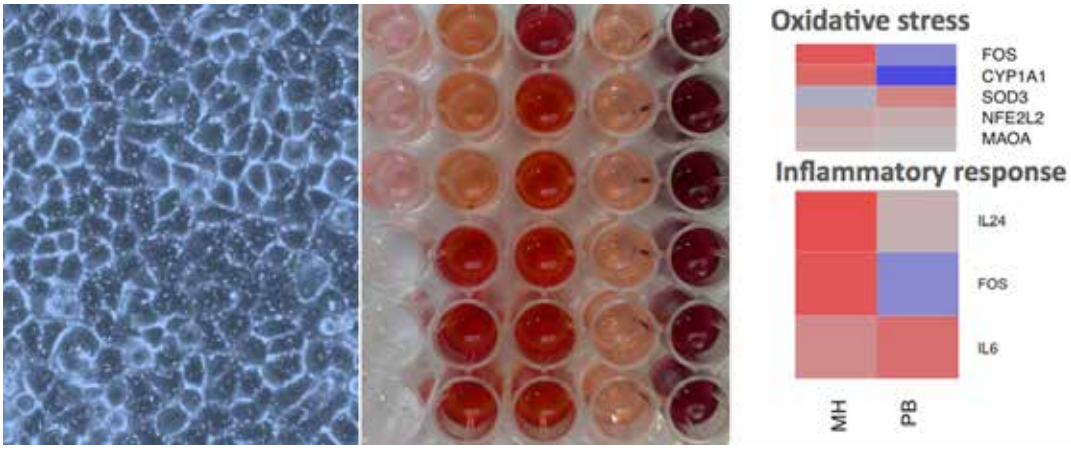


Figure 12: *left) Confluent A549 cells seen in a light microscope, middle) LDH release measurement after exposures for determining cell integrity, right) Transcriptomic result from the comparison of aerosol emissions of a masonry heater (MH) and a pellet burner (PB) (both wood combustion). Different gene activation patterns are observed.*

Work Package 5: Aersol Data (AeroDAT)

In WP5 (AeroDAT), headed by HMGU (Theis/Marsico/Ogris), aerosol and biological response data, obtained in WP2–WP4 are integrated and analyzed in order to identify the aerosol parameters, that are critical for the health effects. All partners contribute to WP5. For this decisive task, bioinformatic and chemometric expertise is bundled and accompanied by software developing capacity. The overall aim is to improve risk management by identifying the components that likely contribute the most to the development of adverse health outcomes. This WP will heavily make use of the bioinformatics services at the Weizmann Institute. HMGU has funded an extra position for Dr. D. Gat at WIS to support WP5. This will provide identifying guidelines for which sources to regulate in order to optimize public health benefits. The toxicological results will be integrated with the 'omics and epigenetic data, while the biological responses will be put in context to the exposure data (*i.e.* the comprehensive analysis of the chemical and physical properties of

the exposure aerosol) to link aerosol source characteristics via observed biological effects in the cell cultures with potential heath-effects in humans (figure 13). With the ever-increasing complexity and volume of data in life sciences in general, and in genomics in particular, it is clear that analysis methods constantly need to be developed and adapted. The ICB pioneers in this challenging field and contributes constantly to the scientific community and are part of various consortia and for instance, recently launched and coordinates a single cell omics network in Germany.

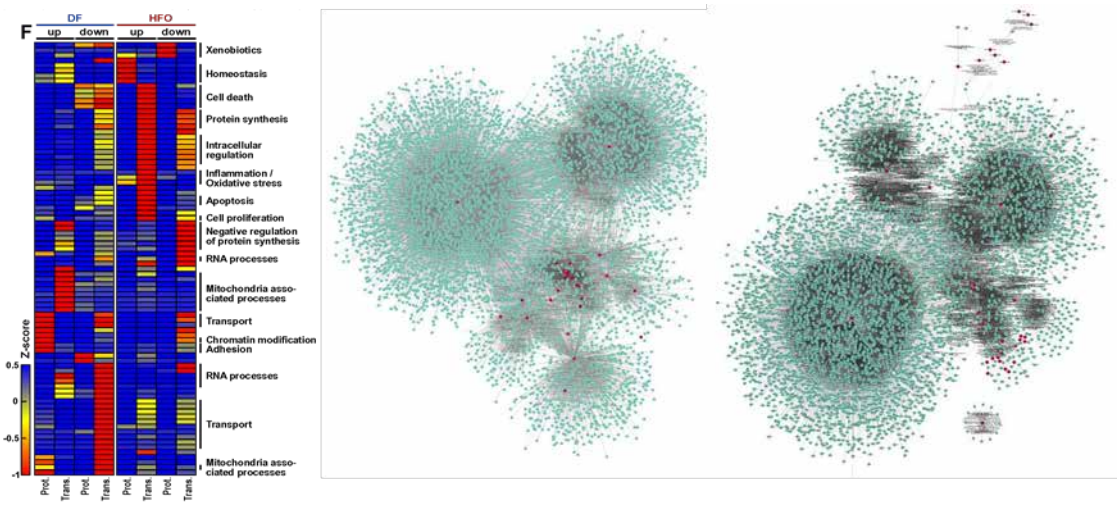


Figure 13: *left) Illustrative integration of transcript-omics and proteomics data for human epithelial cells exposed with aerosols from a heavy fuel oil (HFO) & diesel fuel (DF) operated ship engine. Biological response network for (Middle) HFO and (right) DF exhaust exposure of Beas2B cells. The networks are generated after merging individual chemical-gene/protein network for the chemicals present in high concentration in one exhaust compared to another one (Chemicals-red nodes, gene/proteins – green nodes).*

The objectives of WP5 are the establishment of an interpretable integrative network linking toxicity parameters with molecular omics data and delineating toxicity effects of cell culture and 3D models on pathway level. WP5 supports individual WPs for the analysis of all aeroHEALTH generated omics data with state-of-the-art preprocessing, quality assessment, and normalization.

To develop a method to integrate toxicity with molecular measurements we use regression analysis and mixed effect modeling allowing accounting for technical covariates. We use existing data and synthetic data to develop and fine-tune the methods, in order to apply the methods to aeroHEALTH data once it becomes available. For the integration of epigenetic, transcriptomic and, proteomic data, we use prior knowledge to allocate interaction pairs subjected to correlation analysis. This multi-omics network will enrich the aeroHEALTH knowledge graph of toxicity effects. Integrated data analysis will identify the biological impact of epigenetic modifications in the exposed cell systems. For the second objective, we investigate biological effects in multi-omics data through a combination of similarity networks based on omics-data profiles, independent component analysis (ICA), and machine learning (ML), allowing the deconvolution of mixed signals from the multi-cell cultures. The extracted features will then be used as inputs to ML techniques, *e.g.*, random forests, for predicting the biological effects of air pollution. Methods bridging the “omics” findings to a specific mechanism of toxicity will be applied to strengthen the link between particle composition/source apportionment and human health effects. By this, further steps towards a cell-culture result-based risk analysis will be done. During the course of the project, in-depth investigations of biological associations between the newly obtained cell culture-based results and animal model data are performed. Apart from prediction models, a key outcome will be the identification of biological pathways affected by different ambient aerosol parameters (*e.g.*, chemical compounds, particle size) and emission sources (*e.g.*, traffic, wood combustion, mineral dust), which are implicated in the induced biological and adverse health effects.

Status of the work in AeroHEALTH

The projects started in April 2019. Luckily, we succeeded in performing the initial, first large measurement campaign on the toxicity of secondary aerosols from anthropogenic and biogenic precursors before practical work was heavily impacted by the Covid-19 pandemic. The preparation of the first campaign, which was performed at the HMGU started in Summer 2019. Pre-measurements could be performed from November 2019. On 5th December the HMGU CEO Prof. M. Tschöp unveiled the aeroHEALTH plaque in the CMA building in Sendling in a small ceremony and opened the first aeroHEALTH measurement campaign, which lasted till the beginning of March 2020.

Figure 14: *Top row) left: Inauguration of the aeroHEALTH Helmholtz International Lab and the first aeroHEALTH measurement campaign at the CMA building in Sendling on 5th December 2019 by the HMGU CEO Prof. M. Tschöp, unveiling the aeroHEALTH plaque during a small ceremony; middle: Prof. M. Tschöp in Discussion with Prof. A. Kiendler-Scharr (FZJ) and Prof. Y. Rudich (WIS); right: aeroHEALTH co-worker click glasses upon the occasion;*
Bottom row) left: Dr. S. Di Bucchianico explains an automated lung cell model air-liquid-interface exposure unit; middle: Prof. R. Zimmermann in scientific discussion with Dr. Ö. Yildirim and Dr. T. Stöger; right: the aerosol characterization setup for the produced soot and SOA aerosols.

The second large measurement activity, which was planned for autumn 2020, is now rescheduled for November 2021 due to Covid-19 restrictions. Thus we can estimate that we will face a 9–12 months delay in the aeroHEALTH program schedule due to the Corona pandemic.

4.2. Selected aeroHEALTH research reports

In the following, nine selected research reports from the aeroHEALTH initiative are given (aeroHEALTH 1–9). Albeit the start of the practical work was heavily impacted by the Covid-19 pandemic, we succeeded in performing the initial large measurement campaign.

The first three reports are covering pre-work which led to the setup of the aeroHEALTH consortium as well as some early aeroHEALTH work. This includes the development of an improved oxidation flow tube reactor (1) by the aeroHEALTH partner UEF, HMGU, and FZJ, a HICE study with *in-vivo* and *in-vitro* toxicology (2) by the aeroHEALTH partner UEF and HMGU as well as aerosol toxicological pre-work by the aeroHEALTH partners WIS and HMGU (3). The following aeroHEALTH reports (4 – 9) are covering the setup and first results of the initial aeroHEALTH measurement campaign at the HMGU in Munich. This campaign, which focusses on a comparison of the biological effects of SOA from anthropogenic and biogenic precursors condensed of soot particles, lasted for more than 3 months. All aeroHEALTH partners were involved in planning, preparation, performing the practical experiments in December 2019 till March 2020, data analysis as well as in the result analysis and discussion. We were lucky that we could conclude the practical experimental work (ALI-exposures) just 10 days before the first Corona lockdown brought many activities to a standstill for about 6 months.

(aeroHEALTH 1) Establishing a novel high-volume Photochemical Emission Aging flow tube Reactor (PEAR) for aerosol chemical and toxicological studies

H. Czech (UEF/HMGU/UR), M. Ihalainen (UEF), P. Tiitta (UEF), P. Yli-Pirilä (UEF), A. Hartikainen (UEF), M. Kortelainen (UEF), J. Tissari (UEF), B. Stengel (UR), M. Sklorz (HMGU), H. Suhonen (UEF), H. Lamberg (UEF), A. Leskinen (FMI§), A. Kiendler-Scharr (FZJ), H. Harndorf (UR), R. Zimmermann (UR/HMGU), J. Jokiniemi (UEF), O. Sippula (UEF)*

Once released into the atmosphere, aerosols undergo a complex chemical and physical transformation, which we call atmospheric "aging". The aging process changes the chemical composition of the aerosol gas- and particle phase and leads to new particle formation and particle growth by gas phase to particulate phase conversion. Many studies suggest that the occurring oxidation and photolysis reactions can cause an alteration or increase in the toxicity of the aerosol. The investigation of the impact of aerosol aging processes onto the biological and health effect represents a main objective of the Helmholtz International Lab aeroHEALTH. Decisive for the planned studies are laboratory simulations of aging processes that are as realistic as possible, allowing the "production" of sufficient quantities of reproducibly aged aerosols for *in-vivo* and *in-vitro* aerosol toxicological investigations. Smog chambers, essentially consisting of a Teflon bag with a volume of a few to hundreds of m^3, are regarded as a very realistic model to describe atmospheric aging in the laboratory. However, they fail to generate aerosols with an equivalent photochemical age of more than two days because wall losses start to prevail and cannot produce sufficient material of the same aging states over a longer time, as required for many *in-vivo* and *in-vitro* toxicological studies. In order to depict aerosols with an equivalent photochemical age of equal to the atmospheric particle lifetime of about two weeks, oxidation flow reactors (OFR) and the concept of "potential aerosol mass" (PAM) were developed and extensively assessed for their benefits and limitations, in particular atmospherically relevant concentration ranges and oxidant ratios (Peng and Jimenez, 2020). Despite its meanwhile frequent use in atmospheric science, OFRs have been rarely used in exposure studies because of the insufficient aerosol mass provision. Here, we describe a novel OFR, developed at the aeroHEALTH-associated partner institute, University of Eastern Finland, suitable for parallel comprehensive aerosol analytics and exposure studies.

Setup of the Photochemical Emission Aging flow tube Reactor (PEAR): The *Photochemical Emission Aging flow tube Reactor* (PEAR) (Ihalainen *et al.*, 2019) was developed during the HICE project at the UEF and consists of a stainless steel tube with a volume of 139 L and is equipped with four UV lamps inside and portable to be rapidly installed in other laboratories. The low inner surface of only 2.28 m^2 leads to a surface-to-volume ratio of 164 m^{-1}, which is lower than for other OFR of similar working principle and dimensions, and thus minimizes wall losses of (charged) particles and vapors. First, the aerosol is swirled (*i.e.* with a high Reynolds number) in the inlet of the PEAR with minimized backflow probability before it enters the cylindrical reactor part of 120 cm length and 36 cm inner diameter, containing four UV lamps close to the inner wall of the reactor. In this part, the aerosol follows a laminar flow profile (*i.e.* low Reynolds number) for reproducible aging conditions. Covering the UV lamps, quartz tubes are continuously flushed with nitrogen to avoid heating of the aerosol by the UV lamps. The emission peak at 254 nm decomposes externally fed ozone (1), which has been premixed with water vapor and the aerosol sample before, and initiate atmospheric radical chemistry according to the reactions:

$$O_3 + h\nu\,(254\text{ nm}) \rightarrow O(^1D) + O_2 \qquad (1)$$

$$O(^1D) + H_2O \rightarrow 2\,OH \qquad (2)$$

The product of reaction (2) refers to two moles of hydroxyl radicals, which denotes the main atmospheric oxidant at daylight conditions. By changing the intensity of the UV

lamps, the equivalent photochemical age of the aerosol can be varied from less than one day up to the average lifetime of an ambient particle of two weeks. Finally, the aged aerosol reaches the outlet, which is divided into two concentric areas: the ring flow, removing the near-wall portion of the volume flow, and the main sample flow. In this manner, particle losses are further reduced, which is also supported by numerical modeling with Computational Fluid Dynamics (CFD).

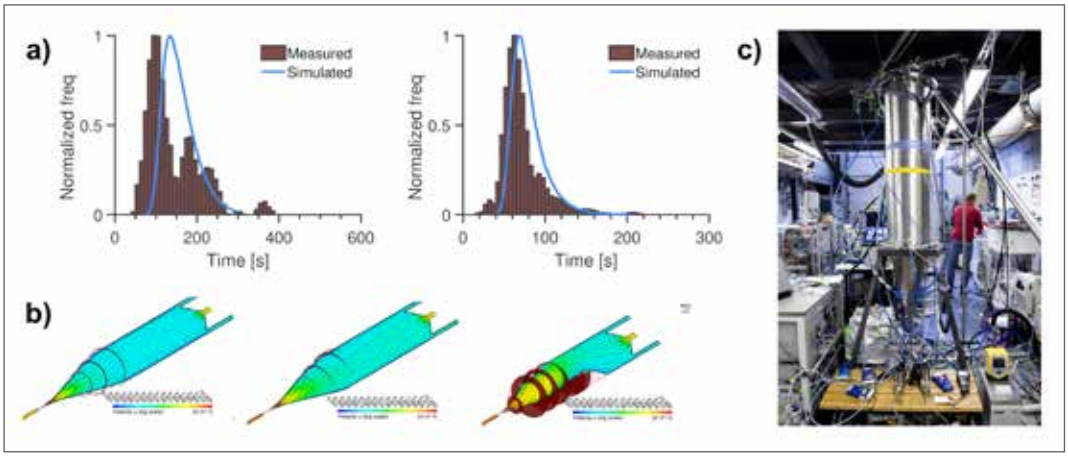


Figure 1: Residence time distribution inside the PEAR at a) 50 L min-1 (left) and 100 L min-1 (right) with b) associated flow profiles modeled by CFD. The flow profile in b) on the right shows an example of an unfavorable design for comparison purposes. c) Operation of the PEAR in a measurement campaign in ILMARI facility of the aeroHEALTH-associated partner, the University of Eastern Finland.

Designed for exposure studies: The PEAR was intentionally designed to conduct exposure studies with cells and animals for toxicological effects of aged aerosols. OFRs described in the literature may not provide sufficient flow for both *in-vitro* exposure in air-liquid-interface technology and *in-vivo* exposure on the one hand and comprehensive chemical analytics of filter samples by advanced analytical instrumentation, such as ultra-high resolution mass spectrometry or comprehensive two-dimensional gas chromatography, on the other one. We may operate the PEAR with flow rates from 50 to 100 L min^{-1} (even up to 200 L min^{--1} are possible), causing mean residence times for aerosol processing from 2 to 1 min (figure 1).

Comparison of PEAR-aged aerosol with ambient measurements by aerosol mass spectrometry: We conducted a set of aging experiments with several combustion emissions, including batch-wise combustion emissions of spruce logwood and sparkignition engine emissions under constant (120 km h^{-1}, 80 km h^{-1}) or dynamic mode of operation (equivalent to New European Driving Cycle NEDC), as well as toluene as representative anthropogenic precursor of secondary organic aerosols. The equivalent photochemical age of the aerosol was adjusted by using different levels of ozone (0-9 ppm) and UV lamp power. All experiments were performed at room temperature and relative humidity of 50%.

From numerous ambient measurements by aerosol mass spectrometry, it was found that the vast majority of the particles appear in a triangular space, spanned by the relative contribution of m/z 43 (f_{43}) and m/z 44 (f_{44}). Because f_{43} is mainly associated with the fragment ions $C_3H_7^+$ and $C_2H_3O^+$, it shows a higher contribution for primary aerosol. Atmospheric aging increases the oxygen content of the aerosol, leading to a higher contribution of f44, which is linked with the fragment ion CO_2^+. Consequently, data points from aerosol measurements move from the bottom to the top of the triangle with ongoing atmospheric aging. The aged aerosol processed with the PEAR appears perfectly within this triangular space (figure 2). Aging of toluene denotes the most extreme case with aging along the hypotenuse of the triangle and the highest contribution of f_{43} compared to other aerosols. Wood combustion aerosol is already partly oxidized due to the substantial content of oxygen in the wood polymers cellulose, hemicellulose, and lignin. Therefore, it reasonably appears in the upper part of the triangle, whereas engine emissions from gasoline combustion mainly consist of hydrocarbons

and consequently lead to a low fraction of f_{44}. Both wood combustion and engine gasoline combustion aerosols of the highest equivalent photochemical age between 8 and 9 days are located close to the vertex of the triangle, giving evidence for the atmospheric relevance of aging conditions inside the PEAR.

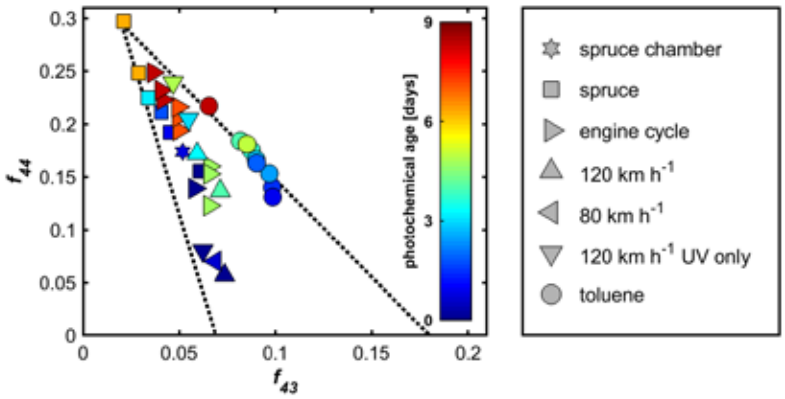


Figure 2: *Triangular space (dotted lines) of the ambient aerosol obtained from numerous aerosol mass spectrometry analyses in the Northern Hemisphere (Ng et al., 2010) and PEAR-aged aerosols from wood combustion emissions, engine gasoline combustion emissions and toluene.*

Significance of the PEAR OFR approach in the framework of aeroHEALTH: The development of the PEAR is a very important pre-work for the aeroHEALTH research program, involving researchers from UEF, HMGU, FZJ, and UR. It enables studying systematically the toxicity of secondary aerosol from model systems over the influence of NOx on particle formation and properties to the aging of already complex combustion aerosols in the laboratory, for example, wood combustion aerosols (Miersch *et al.*, 2019). Additionally, the design of the PEAR provides sufficient aerosol mass to flank *in-vitro* and *in-vivo* exposure studies (*i.e.* for aerosol toxicology) by comprehensive chemical and physical aerosol characterization down to the single-particle level for pioneering analysis of the exposome (Schade *et al.*, 2019; Passig *et al.*, 2020) and begin a deeper analysis of biological endpoints based on recent pre-work and results by aeroHEALTH consortium members (Chowdhury *et al.*, 2019; Li *et al.*, 2020; Ihantola *et al.*, 2020; Pardo *et al.*, 2020). Finally, these experiments are crucial to bridge the gap between laboratory-generated and ambient aerosols to unravel cause-effect relationships of aerosol-induced biological mechanisms (Jiang *et al.*, 2019).

* H. Czech performed a postdoctoral study at UEF
§ FMI: Finnish meteorological Institute, Finland

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(aeroHEALTH 2) Combustion aerosols of different wood species result in mild but aerosol specific *in-vitro* and *in-vivo* effects

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In the framework of the Helmholtz Virtual Institute HICE – Aerosol and Health, the permanent newHICE-prolongation at HMGU as well as prework for the Helmholtz International Lab aeroHEALTH, the health effects of wood combustion emissions were investigated. Outdoor and indoor pollutants from household combustion of solid fuels, such as wood and coal, are rapidly increasing, causing important public health concerns. For instance, longterm exposure to fine particulate matter (PM2.5) from residential and commercial sectors caused up to 4.2 million deaths worldwide (Cohen *et al.*, 2017). This report summarizes the findings of a recently published publication (Ihantola *et al.*, 2020) on the physico-chemical characteristics of spruce and pine smoke and their toxicological responses in exposed cell lines and mice and discusses how aerosol composition affected *in-vitro* and *in-vivo* outcomes.

Wood combustion emissions have been studied previously either by *in-vitro* or *in-vivo* models using collected particles to perform submerse cell exposure, thus mostly neglecting gaseous compounds. Aimed to gain a more accurate and holistic view of toxicological profiles of combustion aerosols, an extensive experimental campaign was organized by HMGU, UR, and UEF in Kuopio (Finland), to conduct parallel *in-vitro* and *in-vivo* studies by using direct aerosols exposure methods instead of collected particles. For this scope, modern exposure techniques such as Air-Liquid Interface (ALI) automated exposure systems and whole-body mouse inhalation systems, enabling a more physiological assessment of the toxicity of the applied aerosols than the previous state-of-the-art submerged cell exposure techniques, were used to depict the different toxicological profile of spruce and pine smoke by concurrent *in-vitro* and *in-vivo* exposures.

Emission aerosols were produced and characterized by their chemical and physical properties using online and offline gold standard mass spectrometry-based methods. Murine macrophages (RAW264.7), human alveolar epithelial (A549) cells, and healthy C57BL/6J mice were used to investigate potentially induced cytotoxicity, genotoxicity, and inflammatory markers while a systems biology approach was used to focus on the complex interactions within generated transcriptome and proteome data as shown by a schematic overview in figure 1. Finally, a computer model approach was used to determine both *in-vitro* and regional lung PM deposition on exposed mice.

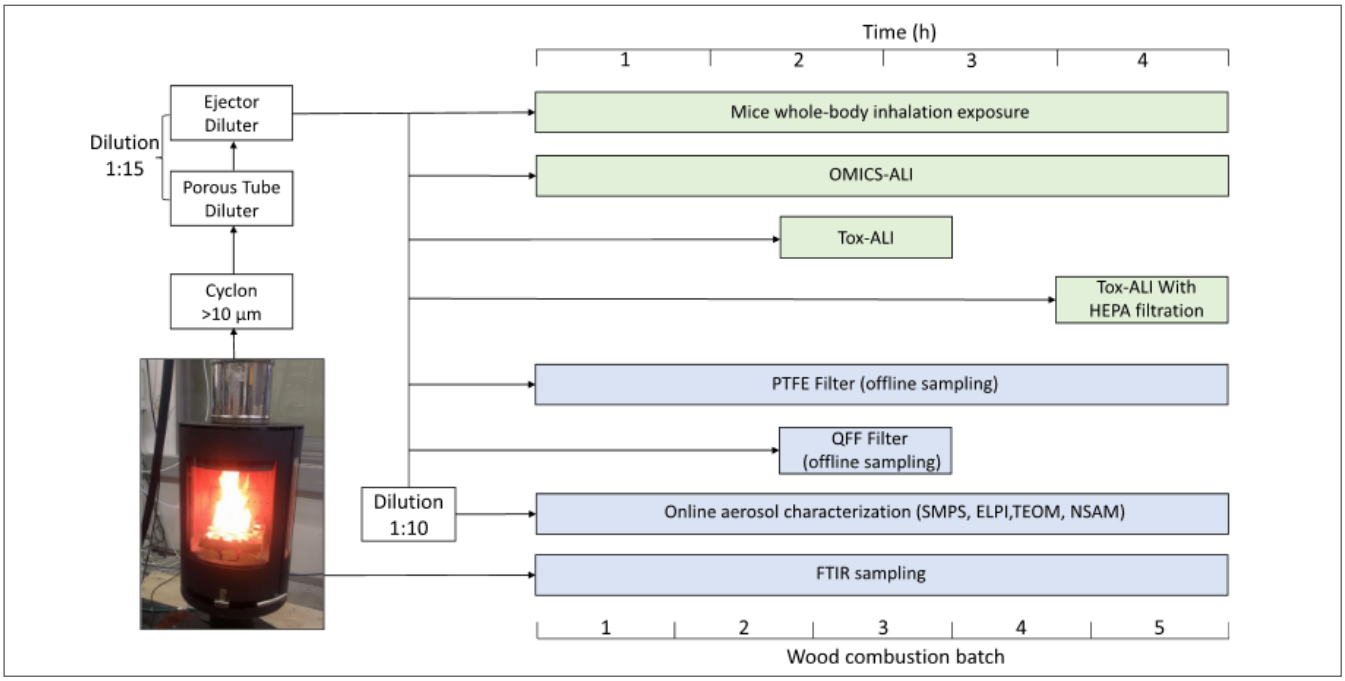

Figure 1: *Schematic overview of in-vivo and in-vitro spruce and pine combustion emission exposures. Combustion aerosols emitted from the stove are led to a diluting sampling setup. 1:15 diluted emissions for in-vitro and in-vivo exposures, indicated in green, were directed into two Vitrocell automated systems (OMICS-ALI), a thermophoretic ALI (Tox-ALI), and a whole-body mice inhalation chamber. Diluted emissions, indicated in blue, were directed to a gaseous online emission analyzer with a dilution ratio of 1:50 (FTIR), and to offline filter samplers as well as to different online characterization instruments (Ihantola et al., 2020).*

Combustion characteristics of spruce and pine exposure emissions differed in total mass, deposited surface area, and particle number concentration. For instance, the average of fine PM1 (diameter below 1 µm) mass emissions showed that the aerosol from pine combustion contained almost 1.5 times higher fine PM1 than spruce smoke. The geometric mean diameter of pine combustion particles was slightly larger (110 nm) than that of spruce (91 nm).

Pine emissions showed two- to three times higher concentrations of PAH and oxidized PAH than spruce combustion. For instance, the well-known carcinogenic benzo[a]pyrene (BaP) was measured as 6.6 µg m^{-3} in pine emission against the 2.1 µg m^{-3} of spruce. The sum of the PAH toxic equivalent value (PAH-TEQ, calculated equivalent value with respect to BaP) for spruce resulted in 14.8 µg TEQ m^{-3} while pine emission showed a much higher PAH-TEQ value corresponding to a sum of 40.4 µg TEQ m^{-3}. The amount of carbonyls such as formaldehyde, acetaldehyde, and acrolein was similar between the two combustion aerosols as well as the concentrations of anhydrosugars, while the resin acids like dehydroabietic acid (methyl ester) and abietic acid were more abundant in pine than in spruce aerosols. However, spruce combustion emission was richer in inorganic elements like K, S, Zn, Cu, Fe, Pb than pine which showed a higher content of Mg.

The estimation of deposited masses of PM1 and total suspended particles, and lung deposited surface area, during *in-vitro* and *in-vivo* exposures, generally showed higher deposition of pine with respect to spruce combustion emissions both *in-vitro* and *in-vivo* while in the mouse lungs the aerosols were estimated to deposit at a much lower amount with respect to *in-vitro* exposures. The use of an electrical field between aerosol inlets and *in-vitro* cell culture transwells of both macrophages and epithelial cells resulted in a deposition enhancement by a factor of ca. 4.

Despite the complex diverse physico-chemical characteristics of wood smokes, the different emissions showed no impact on cell viability following *in-vitro* normal deposition exposures while significant cytotoxic effects were observed for both wood combustions after electrostatic enhanced particle deposition. However, both aerosols induced DNA damage as assessed by Comet assay and slightly increased IL-8 secretion with respect to clean air controls without enhanced deposition. Spruce emission was slightly more effective than pine in inducing DNA damage, with the complete aerosols more potent than HEPA-filtered aerosols (gas phase only exposure), while the HEPA-filtered aerosols were slightly more effective than the non-filtered aerosols in increasing IL-8 release without significant differences between spruce and pine exposures.

In mouse experiments, however, a clear decrease in viability was observed in lung tissue homogenate single-cell suspension that was not observed in bronchoalveolar lavage fluid (BALF) cells. Pine combustion exposure was more cytotoxic than spruce exposure in lung tissue cell suspension with a 39% lower cell viability than untreated mice while spruce was reducing cell viability up to 26%. As for *in-vitro* studies, the emission effects on single and double DNA strand breaks were more pronounced than cytotoxic effects with pine smoke significantly more effective than spruce in inducing DNA damage both in BALF and in lung cell suspensions (figure 2).

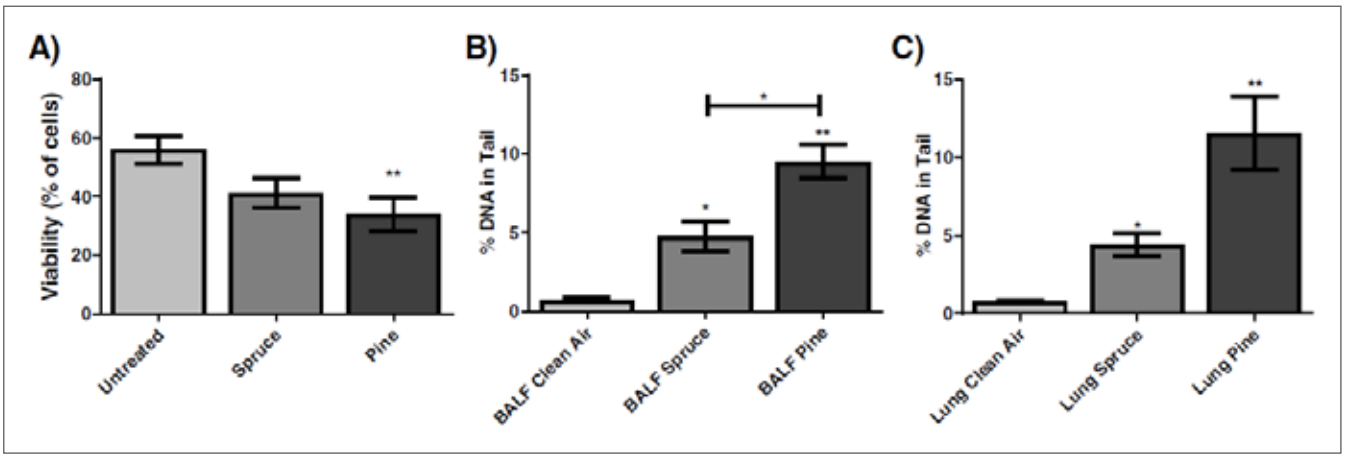

Figure 2: *Lung cell viability (A) and DNA damage in BALF (B) and Lung (C) cells after in-vivo healthy C57BL/6J mice exposures to spruce and pine combustion emissions (Ihantola et al., 2020).*

Combustion emissions differentiated also the induced inflammatory responses in BALF cells. In fact, pine combustion aerosol exposure increased the total cell number in BALF compared to both untreated and spruce treaded mice, mainly due to an increased macrophages count. However, spruce combustion emission exposures induced a neutrophil influx which can explain the observed increase of cytokine levels like for IL-4, IL-5, IL-6, TNFα, and KC (the murine IL-8 homolog) that were induced by spruce exposure to a higher extent with respect to pine aerosol.

Systems biology approaches, with both transcriptome and proteome studies, supported the findings and a number of pathways were significantly regulated both at transcriptome and proteome levels like for oxidative and cellular stress responses as well as the immunomodulation of the respiratory system and DNA damage (figure 3). Specific canonical pathways were also differentially induced by spruce and pine emission exposures. For instance, spruce smoke mostly induced acute *in-vivo* inflammatory responses, *e.g.* TREM1 signaling, alkaloid, and polyphenol compound metabolic pathways as well as activation of glutathione detoxification mechanisms. Following pine aerosol exposures, the most *in-vivo* regulated pathways refer to immune responses, either in form of the coordination of agranulocyte infiltration in inflammation sites or in terms of crosstalk between the innate and adaptive immune systems.

Our findings indicate that concurrent *in-vitro* and *in-vivo* exposures exhibit different outcomes and highlight new aspects in the interactions of combustion aerosols in different exposure systems. Pine combustion emissions are more effective *in-vitro*, but not *in-vivo*, in activating the Nrf2 pathway with respect to spruce exposed cells, thus pine exposures are able to induce a better control of redox homeostasis and related DNA repair processes than spruce exposures. The different amounts of antioxidants such as phenolic compounds in spruce and pine smoke could explain the reduced carcinogenic effects of PAHs as supported by a previous HICE study on masonry and pellet heater (Kanashova *et al.*, 2018). Our study did not measure the antioxidant capabilities of the PM and the gases but our findings strongly suggest to assess the different amounts of phenolic compounds in future studies.

In conclusion, our study shows mild effects of wood combustion but major differences in the biological responses upon exposure to logwood stove aerosols can be observed for different wood species, as examined by *in-vivo* and *in-vitro* approaches. This finding is in line with other studies within HICE, which are concluding that the fuel type (see *e.g.* Öder *et al.*, 2015) and composition are important for the adverse biological effects of the respective aerosol emissions.

Significance of the HICE approach for the aeroHEALTH Helmholtz International Lab: With the Helmholtz Virtual Institute HICE, many concepts important for the aeroHEALTH research program were developed. Within the 4 large HICE measurement campaigns and smaller activities, *e.g.*, the mobile ALI exposure station approach, concepts for the combination of omics and toxicological endpoints with detailed chemical and physical aerosol exposure information as well as the strategy to combine *in-vitro* and *in-vivo* exposure concepts were developed and applied.

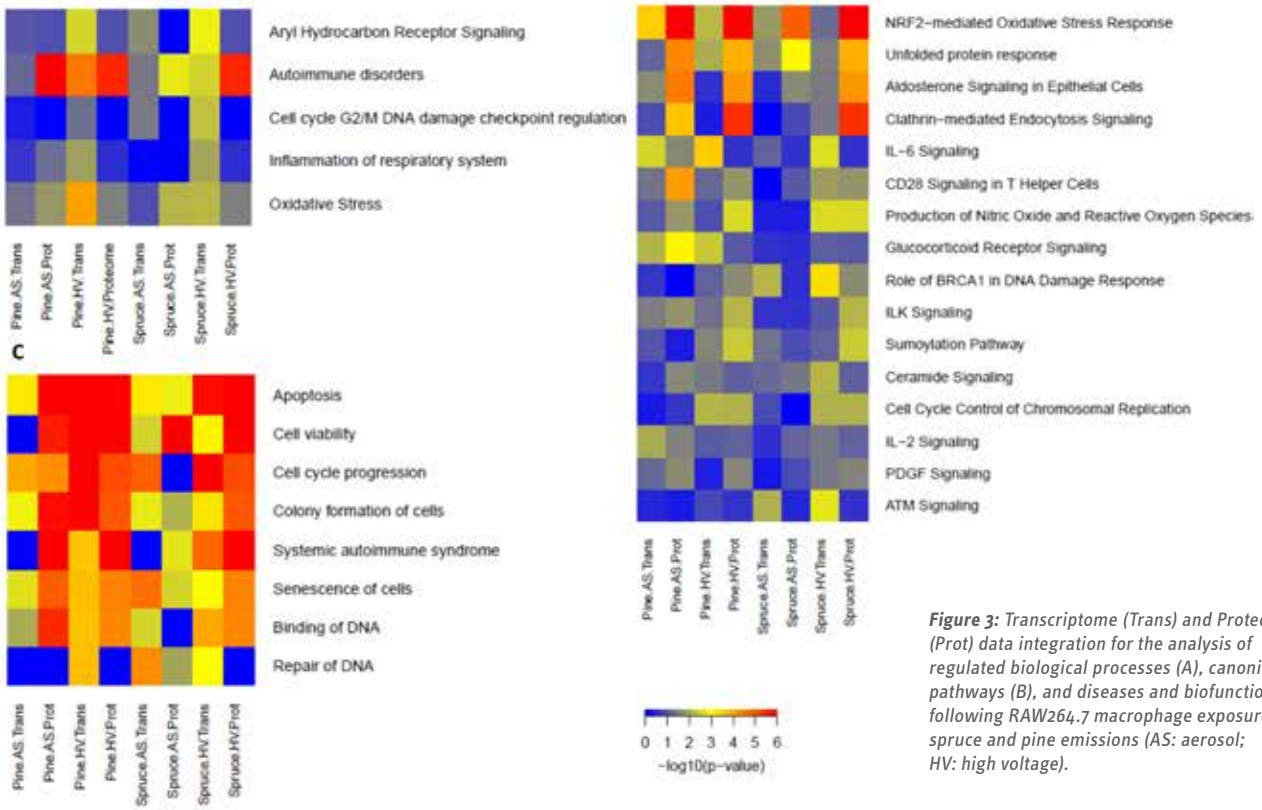

Figure 3: *Transcriptome (Trans) and Proteome (Prot) data integration for the analysis of regulated biological processes (A), canonical pathways (B), and diseases and biofunctions (C) following RAW264.7 macrophage exposure to spruce and pine emissions (AS: aerosol; HV: high voltage).*

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(aeroHEALTH 3) Studies on the composition and lung toxicity of biomass burning aerosols and their components: wood tar aerosols (tarballs)

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In order to assess the health effects of secondary organic aerosols (SOA) and components, the impact of primary biomass burning and wood combustion particles must also be studied by aeroHEALTH. In this context, the Weizmann Institute and the JMSC groups conducted specific experiments. Under smoldering conditions, tar from biomass pyrolysis is distilled from the solid fuel and forms condensation particles, so-called "tarballs". In wood combustion, wood tar aerosols can represent a substantial fraction of carbonaceous emissions. However, the chemical properties, the atmospheric behavior, the health impacts, and the toxicity of the tarball aerosols are still not well known. The properties and the composition of the fresh and photochemically aged tarball model aerosols were recently investigated (Li C. *et al.*, 2019, Pardo *et al.*, 2020a) in the framework of aeroHEALTH. The morphology of the tarball particles is perfectly spherical (fig. 1). Following wood pyrolysis, tarball aerosols were generated in the laboratory from wood tar separated into polar and nonpolar phases. The chemical composition of polar and nonpolar fresh tarballs was studied using high-resolution time-of-flight aerosol mass spectrometer (HR-ToF-AMS) and single-particle laser desorption/resonance enhanced multiphoton ionization mass spectrometry (SP-LD-REMPI-MS).

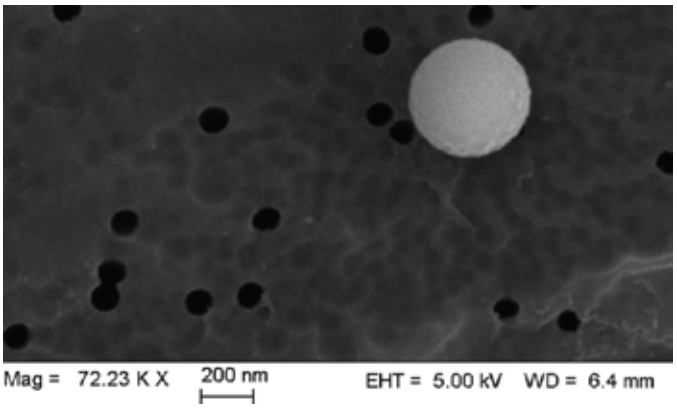
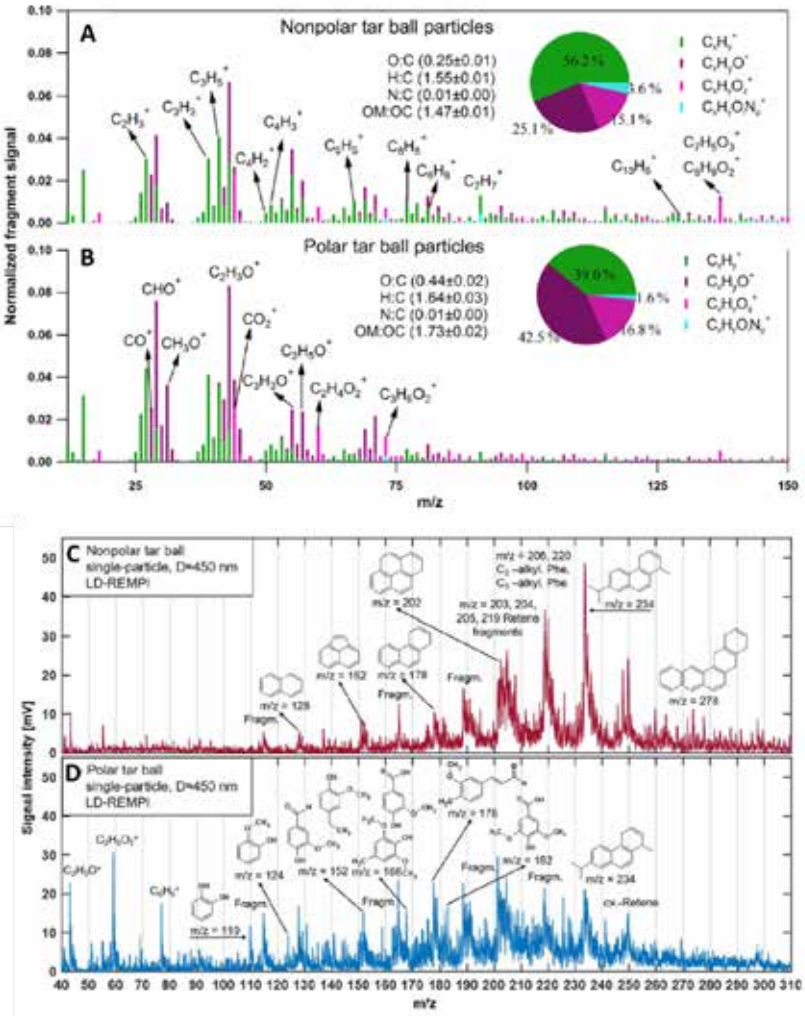


Figure 1: *Scanning electronic microscopy (SEM) image of a fresh tarball particle generated from polar phase tarry solution. The morphology of the particles is perfectly spherical and amorphous in internal composition. Modified after Li et al., 2019.*

Figure 2 shows online recorded mass spectra of nonpolar and polar tarball particles. The upper two mass spectra in figure 2 show on-line recorded high-resolution mass spectra measured by an Aerodyne aerosol mass spectrometer (AMS) of fresh nonpolar and polar tarball particles. Four ion groups are grouped for clarity as CxHy (green), CxHyO (purple), CxHyOz (z>1) (violet), and CxHyOiNp (light blue). The mass fractions of the four fragment groups are presented by pie charts. The nonpolar fraction is less oxidized than the polar fraction. The lower two mass spectra show single particle mass spectra (SPMS) using a Laser Desorption – Resonance Enhanced Multiphoton Ionization (LD-REMPI). The technique allows profiling of aromatic molecular structures from individual single tarball particles. Some of the observed peaks were identified and labeled. C) The nonpolar tarball spectrum shows predominantly alkyl-substituted and unsubstituted PAHs. D) The polar tarball spectrum reveals many oxidized aromatics, *e.g.*, methoxy-phenol and benzenediol. Note the softwood combustion marker retene at m/z =

Figure 2: *Upper two mass spectra: On-line recorded high-resolution AMS mass spectra of fresh A) nonpolar tarball particles and B) polar tarball particles. The polar tarballs are more oxidized. Lower two mass spectra: Single particle mass spectra (SPMS) using a Laser desorption – Resonance enhance multiphoton ionization (LD-REMPI) of C) a nonpolar tarball particle, showing predominantly PAH derivatives and D) of a polar tarball particle, showing predominately phenolic compound. Modified after Li et al., 2019, for details see text.*



234, its characteristic fragments (m/z = 203, 204, 205, 219), and retene derivatives (m/z = 248, 250). Distinct differences in the chemical composition of the fresh polar and nonpolar tar aerosols were identified by unique mass spectrometric techniques used in the aero-HEALTH project. Nonpolar tar particles contain predominantly high molecular weight unsubstituted and alkyl-substituted polycyclic aromatic hydrocarbons (PAHs), while polar tar particles consist of a high number of oxidized aromatic substances (*e.g.*, methoxy-phenols, benzenediol) with higher O:C ratios and carbon oxidation states. Treatment by UV light (254 nm) photolysis, without strong oxidants, slightly increased the oxidation state of the tarballs. Oxidation under varying OH exposure levels and in the absence of NOx further increased the OC ratio of the tarballs, while oxidation through nitrate radicals increased O:C and also added nitrogen-conatining functional groups $-ONO_2$ and $-NO_2$ (Li *et al.*, 2020). Note that the chemical composition of real tarballs is an internal mixture of the compounds detected in polar and nonpolar model tarball particles (*i.e.* the solution in a solvent is necessary for the aerosolization process). From the toxicity point of view phenolic and polyphenolic compounds, are on one hand strong antioxidants, and on the other hand, may exhibit antagonistic biological effects (Kanashova *et al.*, 2018). After inhalation water insoluble tarball particles are taken up by lung cells and macrophages (*e.g.* by phagocytosis) while soluble fractions of the tarballs dissolve in the lung lining fluids and reach the cells in the dissolved state. For the latter case, a water-soluble fraction is a good model for the testing of the biological activity. The toxicity of the water-soluble fraction of pyrolyzed wood tar particles in exposed mice and lung epithelial cells was investigated (Pardo *et al.*, 2020a). Mice exposed to water-soluble wood tar particles showed increased inflammatory and oxidative stress responses. Human bronchial epithelial cells (BEAS-2B) exposed to the same water-soluble wood tar particles showed increased cell death with apoptotic characteristics in a flow cytometric analysis (figure 3).

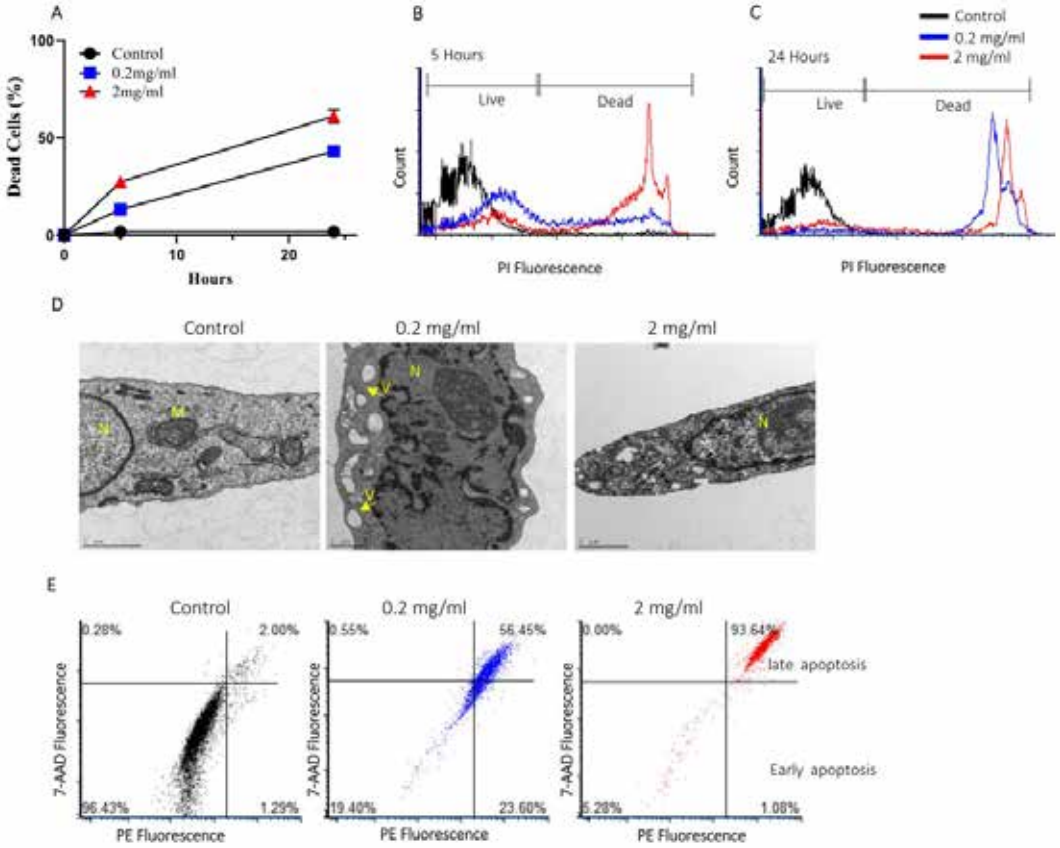


Figure 3: *Cell toxicity after exposure to wood tar extract. BEAS-2B Cells were exposed to water-soluble extracts of a wood tar solution with a concentration of 0.2 mg/ml or 2 mg/ml for 5 or 24 h prior to the analysis, as specified. A) PI-positive cells were detected by flow cytometry (ZE5 Cell Analyzer, Bio-Rad) as a measurement of cell viability. B) Flow cytometry histogram after 5 h of exposure. C) Flow cytometry histogram after 24 h of exposure. The data are expressed as the mean ± SD. Means marked with different letters are significantly different from each other at $p < 0.05$. D) TEM images of control (blank-treated) cells, 0.2 mg ml^{-1} wood tar extract-treated cells, and 2 mg ml^{-1} wood tar extract-treated cells after 5 h of exposure. M, mitochondria; N, nucleus; V, vacuoles. E) Flow cytometry histogram of apoptosis stages determined after 5 h of exposure using the Guava Nexin Reagent (Pardo M. et al., 2020a).*

With increasing wood tar concentrations, the proportion of cells in the stage of late apoptosis increased. The high concentrations of wood tar also induce changes in the appearance of cellular organelles, in particular of the mitochondria (see transmission electron microscopy images in figure 3). Alterations in oxidative status, including changes in reactive oxygen species (ROS) levels and reductions in the expression of antioxidant genes related to the transcription factor Nrf2, were observed and were confirmed by increased levels of MDA, a lipid peroxidation adduct. The damage to mitochondria was observed as an early event and may trigger other adverse effects (figure 3). The mitochondrial functions of the wood tar extract-exposed BEAS-2B cells were evaluated by analyzing the extracellular acidification rate (ECAR, a measure of glycolysis) and the mitochondrial oxygen consumption rate (OCR, a measure of mitochondrial respiration) by a Seahorse analyzer (figure 4).

As shown in figure 4 exposure of BEAS-2B cells to wood tar extracts resulted in complete inhibition of the oxygen consumption rate (OCR, red and blue lines) for both concentrations tested (figure 4c). This observation further supports the notion that wood tar extracts induce toxicity via mitochondria-related mechanisms. Also, the glycolysis rate (CAR) after exposure was significantly reduced after 5 h of exposure, indicating that water-soluble wood tar compound impairs cellular bioenergetics (figure 4d). The toxicity and health effect-related mechanisms of water-soluble wood tar were investigated for the first time in the context of biomass burning. Wood tar particles may account for major responses such as cell death, oxidative stress, suppression of protection mechanisms, and mitochondrial damaged caused by exposure to biomass burning aerosols. In a recent review article, Pardo et al (2020b) summarize the main findings about the activation of antioxidation pathways and mitochondrial dysfunction, as revealed by aeroHEALTH and other studies. These studies are highly multidisciplinary and involve the state of the art toxicological study combined with sophisticated analytical chemistry capabilities in order to decipher the possible health effects of biomass burning aerosols. They were conducted through close collaboration between aerosHealth's partners.

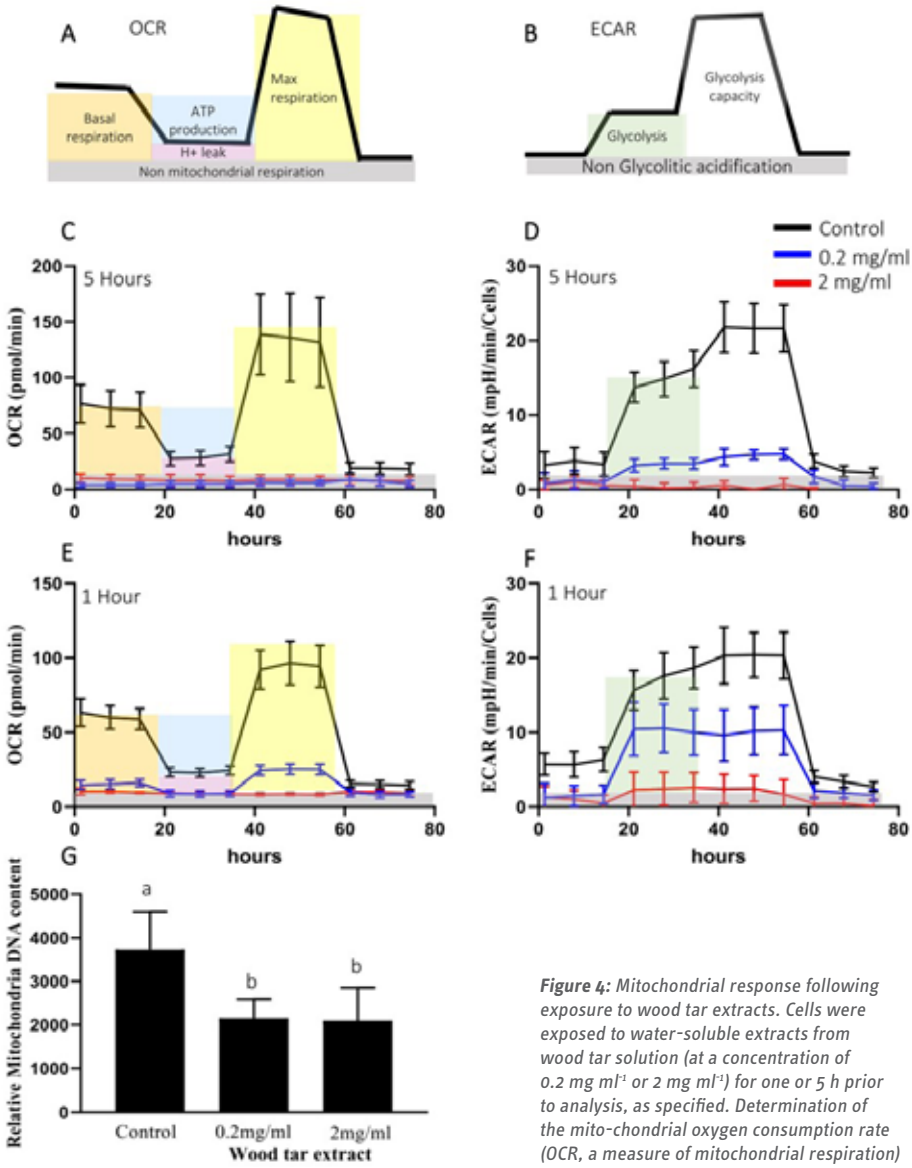


Figure 4: Mitochondrial response following exposure to wood tar extracts. Cells were exposed to water-soluble extracts from wood tar solution (at a concentration of 0.2 mg ml^{-1} or 2 mg ml^{-1}) for one or 5 h prior to analysis, as specified. Determination of the mito-chondrial oxygen consumption rate (OCR, a measure of mitochondrial respiration) and extracellular acidification rate (ECAR, a measure of glycolysis) was performed with a Seahorse analyzer. A) Description of the mito-chondrial respiration (mito-chondrial stress) and B) Seahorse glycolysis assays. Selected results showing C) the basal and mean OCR and D) the basal and mean ECAR following injection of inhibitors and substances after 5 h of exposure are shown. E) The OCRs after 1 h of exposure and F) the ECARs after 1 h of exposure are shown. G) MtDNAcn. The data represent the mean ± SD. These experiments were performed in triplicate and repeated twice. (Pardo M. et al. 2020a)

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(aeroHEALTH 4) The naphthalene/ß-pinene-SOA Experiment: Generation of secondary organic aerosol from anthropogenic and biogenic precursors seeded by soot particles

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The aim of the first joint aeroHEALTH campaign, of which the experimental phase was performed between November 28th, 2019, and March 12th, 2020 at CMA in Munich, is the investigation of the biological response of secondary organic aerosol (SOA) on different cell cultures. For the simulation of SOA formation and atmospheric oxidation (aging) processes, naphthalene and ß-pinene were chosen as volatile model compounds, representing anthropogenic and biogenic precursors for SOA. The SOA was produced in an oxidation flow tube reactor (*i.e.* the so-called "potential aerosol mass", PAM, reactor), seeded on soot aerosol and guided to a battery of online and offline instruments for comprehensive physical and chemical analysis and guided to the "air liquid interface" (ALI) for cell exposure after appropriate dilution. The campaign was split into two main parts: In-depth physicochemical investigations of the aerosol formation process of our model systems were performed by one-hour experiments with constant conditions were conducted with varying aging times (2–9 days) and a relative humidity of 40 and 70%, respectively. These tests were accompanied by comprehensive aerosol characterization measures. A critical aspect for all tests was the reliable and controlled generation of fresh and aged model aerosols. The soot aerosol that was used as seed was produced by a CAST-Burner (CAST = Combustion Aerosol Standard, Fa. Jing, Switzerland) diluted to a final concentration of 0.25 and 1 mg/m³ for the aging experiments. Naphthalene and ß-pinene were evaporated at a defined temperature to maintain a constant final precursor concentration of 1 and 4 mg/m³. The precursor/seed mixtures were humidified and aged in the PAM. Aging was controlled by setting the intensity of the VUV-lamps (185nm) of the PAM to maintain defined OH-radical and O3 concentrations and simulating different atmospheric aging times (Bruns *et al.*, 2015; Kang *et al.*, 2007). During aging, SOA (Secondary Organic Aerosol) was produced from the gaseous precursors and condensed on the added seed particles. Furthermore, a new formation of SOA particles by nucleation could be observed for some conditions.

For the main part, evaluating biological effects of secondary organic aerosol (SOA) on human lung cells, four-hour lasting biological exposures of different human lung cell models were conducted at fixed and constant conditions: SOA of naphthalene, as well as β-pinene (mixed with soot as condensation nuclei), were produced at a relative humidity of 40% and a simulated atmospheric aging time of about 3 days. After applying an appropriate dilution (undiluted, 1:3, 1:10, 1:30, and 1:100) the aerosol was guided to the two air-liquid interfaces (ALI). Similarly, pre-cleaned air, pure soot, and aged soot were used as references. All experiments were done at least with three replicates (for the first results from the biological experiments within this campaign see reports aeroHEALTH 7, and 8). The figures 1A-D give an impression of the complex aerosol generation and characterization setup.

In addition to the stability of the aerosol formation, the physico-chemical characterization of the secondary organic aerosols is of utmost importance. Particle number concentration, particle size distribution, black carbon (BC), and brown carbon (BrC) concentration, as well as ozone, were determined. Condensation particle counter (CPC), two scanning mobility analyzers (SMPS+CPC), an Aethaometer, and a "tapered oscillation microbalance" (TEOM) for online particle mass determination were applied. Atmospheric aging time was estimated by the decay of deuterated butanol, added at a low ppb and quantified by a proton-transfer reaction mass spectrometer (PTR-MS), whereas ozone, formed by the VUV-radiation was quantified using a ozone-monitor. Gaseous components were analyzed by online "single photonionization mass spectrometry" (SPI-TOFMS) and "high resolution proton-transfer-reaction mass spectrometry" (HR-PTR-MS), whereas an "aerosol mass spectrometer" (AMS, see reports aeroHEALTH 5–6) and an

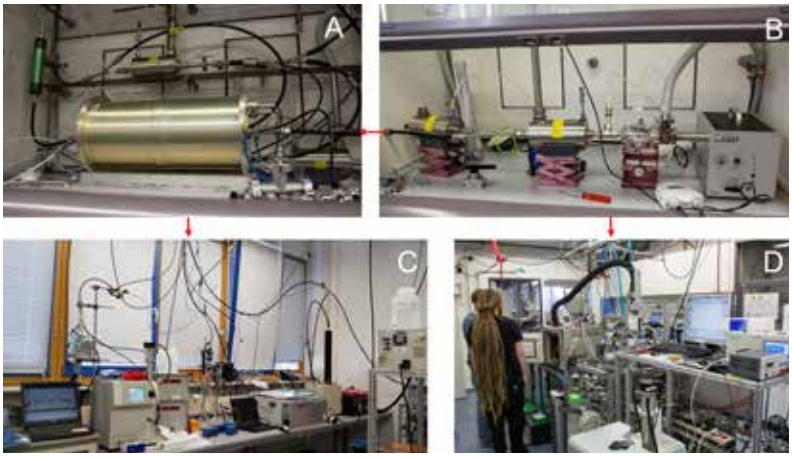

Figure 1: *Images from the SOA generation and characterization set up at the CMA in Munich for the first aeroHEALTH campaign. A) In the oxidation flow tube reactor (Potential Aerosol Mass reactor, PAM) secondary organic aerosol (SOA) is formed from VOC (i.e. naphthalene or ß-pinene); B) combustion soot particles generated by a CAST-burner are used as seed; C) Physical characterization of the exposure aerosol is performed by state-of-the-art instruments (SMPS, CPC, and TEOM; for explanation see text); D) Online chemical characterization of organic aerosol compounds is performed by advanced on-line mass spectrometric methods (AMS, SPI-TOFMS, and HR-PTR-MS; for explanation see text) in addition to the detailed off-line analysis of collected filters.*

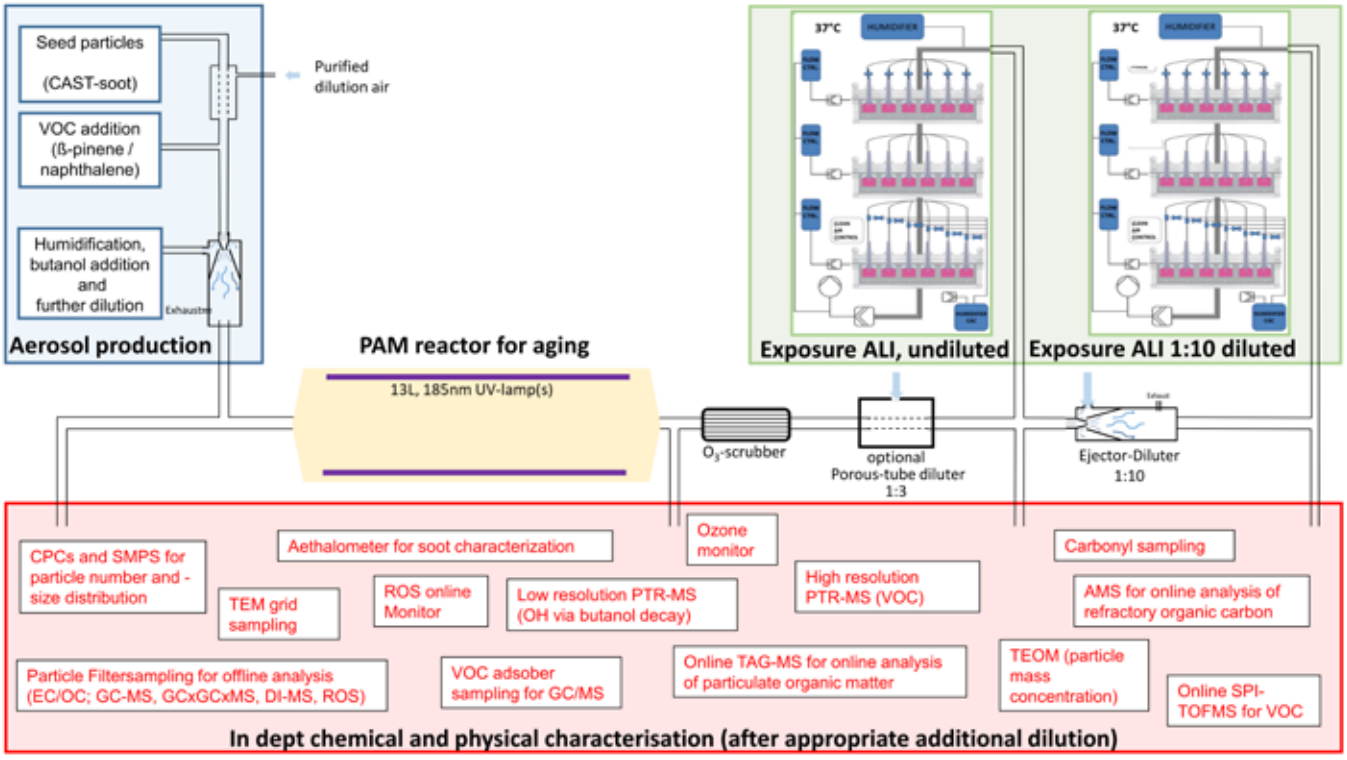

Figure 2: A simplified experimental scheme of the aerosol generation, aging and SOA formation (PAM), aerosol characterization (see also figure 1), and Air-Liquid-Interface (ALI) cell exposure setup (see reports aeroHEALTH 5–8 for more details).

online "thermodesorption aerosol gas chromatography mass spectrometer" (TAG) monitored the organic components in the particulate phase. Additionally, (cooperation with Prof. Kalberer, University of Basel) a "ROS – online monitor" was operated to trace the oxidative potential. For later in-depth chemical and morphological analysis, several hundreds of filter samples of particulate matter were taken and their EC/OC content, as well as organic components, were determined by two dimensional gas chromatography mass spectrometry (GCxGC-MS) and high resolution direct insertion probe mass spectrometry (DIP-HRTOFMS), see report aeroHEALTH 6. For some experiments, samples were collected for later analysis by transmission electron microscopy (TEM), done in cooperation with the University of Eastern Finland. Analysis of semivolatile organic vapors and carbonylic components was conducted after sampling on appropriate adsorbent-materials by GC-MS and liquid chromatography. Figure 2 summarizes the complete aerosol generation and characterization setup.

Preliminary results: Due to ultrapure dilution gas (without any significant contaminations), added via several mass flow controllers, a very constant source of aged aerosol with good reproducibility of seed and SOA production could be realized during all experiments. Especially BC and particle number concentration proved as fast online and very robust indicator for the constant seed preparation during the exposures (fig. 3A). Exemplarily for BC, figure 3B shows the high reproducibility of all experiments during the whole campaign as well.

Figure 4 demonstrates exemplarily the influence of the aging regime (OH-radical reactivity) on the formation of SOA. Depending on equivalent atmospheric aging time and humidity about 30–60% of the added gaseous naphthalene and ß-pinene were converted into particle phase SOA. Whereas the number count concentration of SOA from ß-pinene increased with longer aging, mass concentration decreased due to photochemical oxidation to volatile components or even CO_2. Comparing four days of aging with non-aged conditions, the equivalent particle density rises by at least a factor of 2.

The change of particle density and morphology by aging of the soot + VOC mixtures is illustrated by figure 5 A compared to B and C. The soot cores partly collapse and glue together with ß-pinene resp. naphthalene SOA and the shape changes dramatically from amorphous fragile structures to condensed more or less spherical particles. The average effective density (calculated by the aid of the TEOM mass concentration) raised from about 0.5 g cm³ to 1.2 – 1.3 g cm³, whereas the equivalent mean mobility diameter of about 100nm did not change considerably.

Akknowledgement: We thank William H. Brune (Pennsylvania State University, USA) for providing the PAM reactor.

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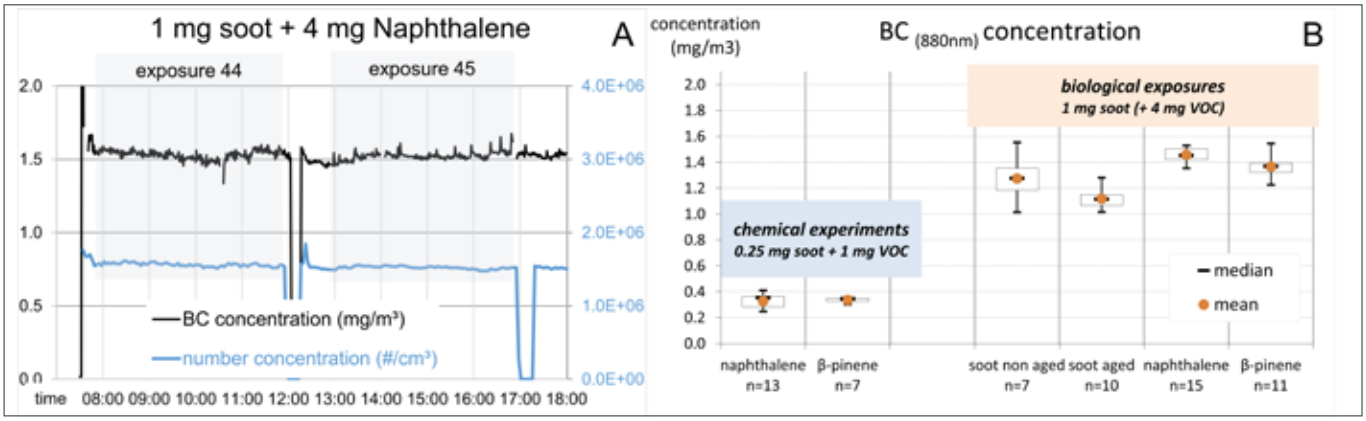


Figure 3: Short-term and overall reproducibility of seed particles production. A) Real-time data of black carbon and particle number concentration during two 4-hour exposure. B) Boxplot of BC concentration of all chemical experiments (0.25mg/m³ soot) and biological exposures (1mg/m³ soot).

Figure 4: Particle number and mass concentrations with different equivalent atmospheric OH aging times. Mass concentrations were determined online by a TEOM. As the TEOM needs at least 30 minutes for stabilization after changed conditions, only the values at the end of the individual period are valid for mass determination.

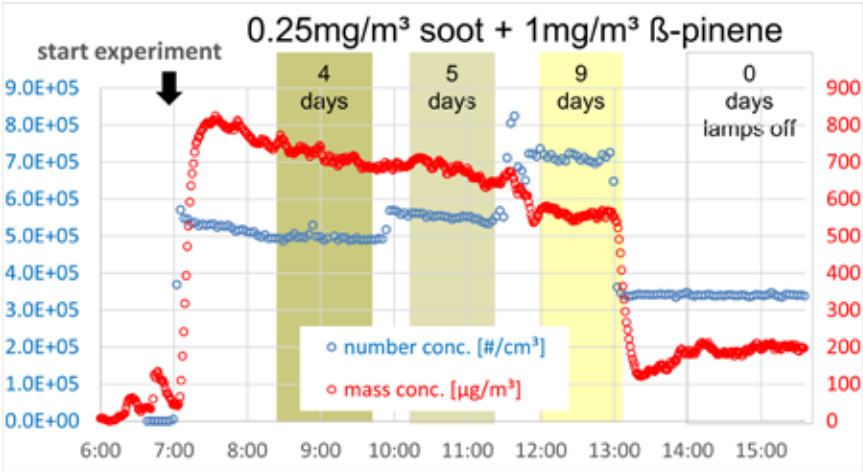
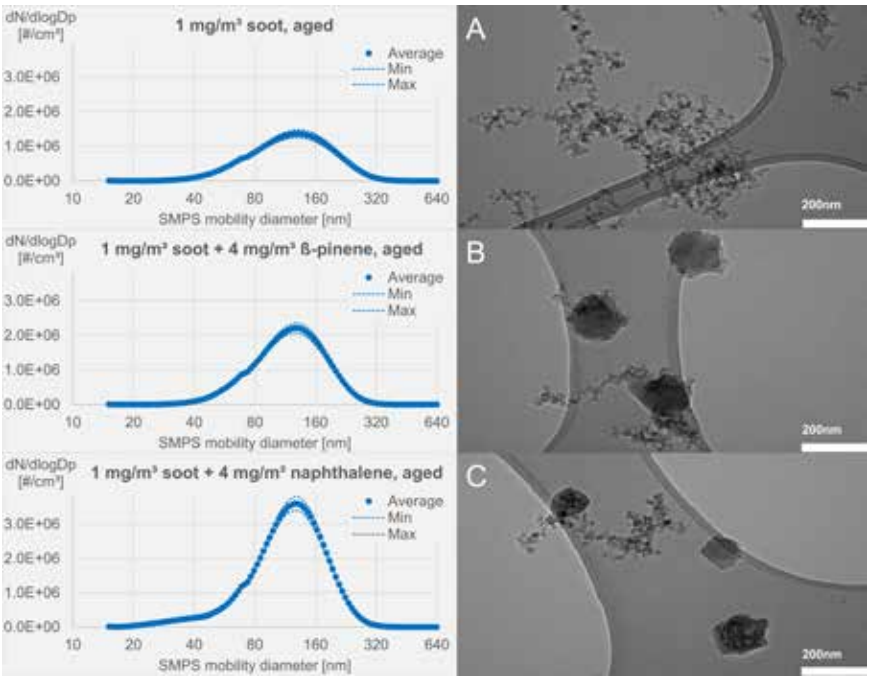


Figure 5: Particle mobility size distribution and transmission electron microscopy pictures of aged pure soot at 1 mg/m^{-3} (A), aged soot + ß-pinene (B) and aged soot + naphthalene (C) at 1 mg/m^{-3} + 4 mg/m^{-3}, resp. and an atmospheric aging time of about 3 days (calculated according to Barmet et al., 2012, from the decay of added deuterated butanol and an estimated OH-radical concentration of 10^{6} molec cm^{-3}).



(aeroHEALTH 5) Chemical characterization of secondary organic aerosols (SOA) from an oxidation flow tube reactor by on-line aerosol mass spectrometry

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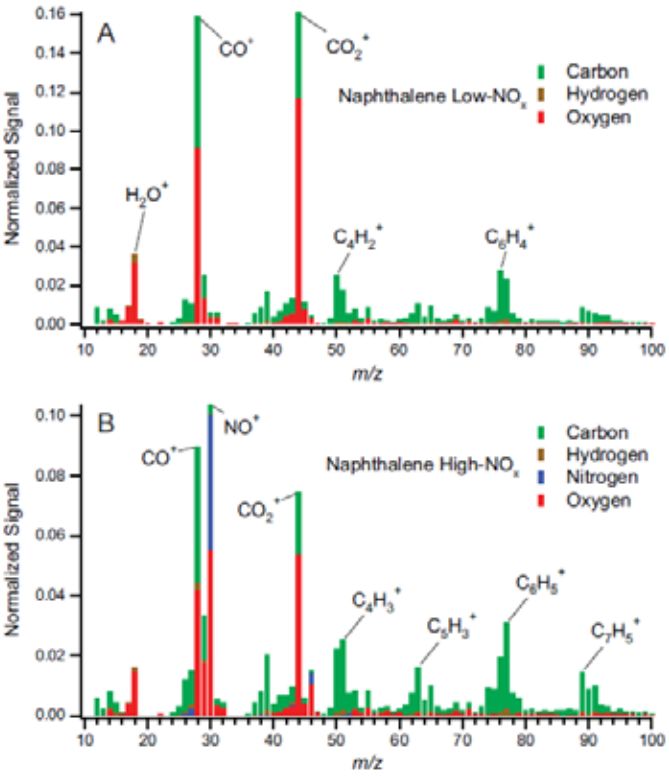
During the first international aeroHEALTH campaign at the HMGU/CMA in München November 2019 to March 2020, cell culture aerosol exposure experiments, as well as a set of experiments to better understand the aerosol aging and to better characterize the aerosols used for the exposure experiments, were conducted. During all experiments of the aeroHEALTH campaign, particles were produced and aged in an oxidation flow reactor (OFR, see aeroHEALTH reports 1 and 4). OFRs allow for continuous and stable production of secondary organic aerosol (SOA) when keeping flow inside the OFR, precursor concentration inflow, and exposure to OH radicals and O3 stable. Aging of the precursors and the formation of SOA for different experiments was achieved by increasing O3 and OH exposure, allowing for aerosol aging regimes stretching from a few days to more than a week. A second important parameter that was varied was the relative humidity (RH) in the OFR to examine its effect on product distribution in the SOA. The SOA precursors used during the campaign were naphthalene and ß-pinene.

Precursors concentrations were high compared to atmospheric conditions and were either 4 mg m^{-3} (exposure studies) or 1 mg m^{-3} (more in-depth chemical characterization studies). For all experiments, soot from a CAST burner using propane as fuel was used to produce seed soot particles at a concentration of 1:4 compared to the precursor's concentration (mass basis). Online Aerosol Mass Spectrometer (AMS) and the Thermal Desorption Gas Chromatograph (TAG) were used to determine the composition of the SOA in these studies. Considering the high SOA mass concentration compared to atmospheric



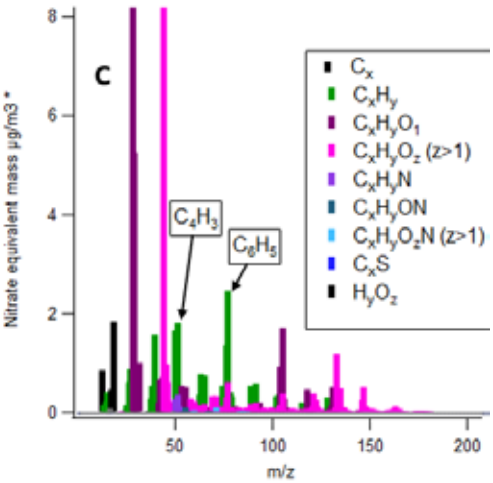


Figure 1: *A and B are AMS mass spectra published in Chhabra et al., 2010. In A a result from a low NOx experiment is shown, 1B shows a result from a high NOx experiment, both at low precursor concentrations. Figure 1C shows a result of naphthalene aging from the aeroHEALTH campaign, with an equivalent aging of 2.4 days at 40% RH.*

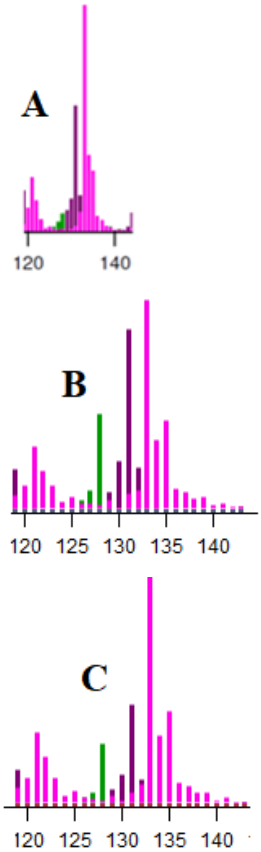


Figure 2: Small part of the naphthalene mass spectrum centered around the 128 m/z peak which is indicated in green on all 3 spectra, peak 128m/z is known to be naphthalene. Green shows the CH group, purple indicates the CHO group, and pink indicates the CxHyOz group with Z> 1.

A) Literature spectrum (McWhinney et al., 2013), B) result from the aeroHEALTH campaign with a naphthalene concentration of 4 mg m^{-3} and C) from the aeroHEALTH campaign with a naphthalene concentration of 1 mg m^{-3}.

conditions one goal was to determine how representative in composition the produced SOA was at the different stages of aging and relative humidity.

The figures 1A and 1B, display a comparison of naphthalene mass spectra measured by Chhabra *et al.*, 2010 in the Caltech dual chamber with low NOx (1A) and high NOx (1B). Finally, figure 1C displays a naphthalene SOA mass spectrum measured at the aeroHEALTH campaign after the OFR with an equivalent aging of 2.4 days at 40% RH. The differences between figure 1A and 1B come from the different chemistry that occurs in high and low NOx reaction schemes. Under high NOx conditions, the NO allows for more ring-opening reactions and thus very different chemistry compared to the low NOx where oxidation also occurs but with ring retaining reactions dominating (Kautzman *et al.*, 2010). Comparing 1C to 1A and 1B it can be seen that below 50 m/z the spectrum from the OFR (1C) is similar to the low NOx spectrum(1A). However, spectrum (1C) above 50 m/z resembles the high NOx experiment (1B). This could indicate that there was a small amount of NOx in the OFR which could facilitate more pathways for ring-opening reactions as described by Kautzman *et al.*, 2010. As NOx was not directly measured during the experiments this cannot be strictly confirmed. Another possible explanation is that due to the high naphthalene concentration in the OFR, the ring-opening ROO + ROO radical reaction suggested by Kautzman *et al.*, 2010, could be more dominant than at atmospheric concentrations. The ring-opening reaction in the low NOx setting usually occurs from the reaction of ROO + OH and normally dominates the ROO+ROO reaction due to the much higher concentration of OH. This should still be the case in the OFR, but the results could suggest that regardless of which of these reactions are causing it, the ring-opening reactions are being favored.

A key concern, when it comes to the high precursor concentrations in the OFR, is forcing more of the precursor into the particle phase. Thus, to explore this we investigate how 3 different concentrations of naphthalene impact the 128 m/z peak signal strength. The 128 m/z peak is chosen as it is most likely the peak for pure naphthalene. Figure 2A shows a result from a low concentration experiment from literature (McWhinney *et al.*, 2013), it can be seen that there is a small CxHy peak (indicated in green) at 128m/z. The experiment shown in figure 2B was performed with a concentration of 4 mg m^{-3} and the experiment shown in figure 2C used a naphthalene concentration of 1mg m^{-3}. Comparing the green 128 peak to its adjacent peaks, it is clear that the peak is larger in figure 2B (naphthalene 4 mg m^{-3}) compared to figure 2c (naphthalene 1 mg m^{-3}) and figure 2C (low naphthalene case). Albeit the effect is relatively small with respect to the amount of oxidized compounds, this suggests that there is a tendency that increased precursor concentration forces more naphthalene into the particle phase. However, as naphthalene, even at low precursor concentrations, is present in the particle phase and the overall concentration remains low, the effects are supposedly not important for the toxicological effect analysis.

A common method used to interpret the aging process of the aerosol is through a triangle plot as shown by Ng *et al.* (2011). Ambient aerosols usually fall within the triangle shown in figure 3, where more aged particles will be found towards the upper left and fresh hydrocarbon like aerosols can be found in the bottom center and right side

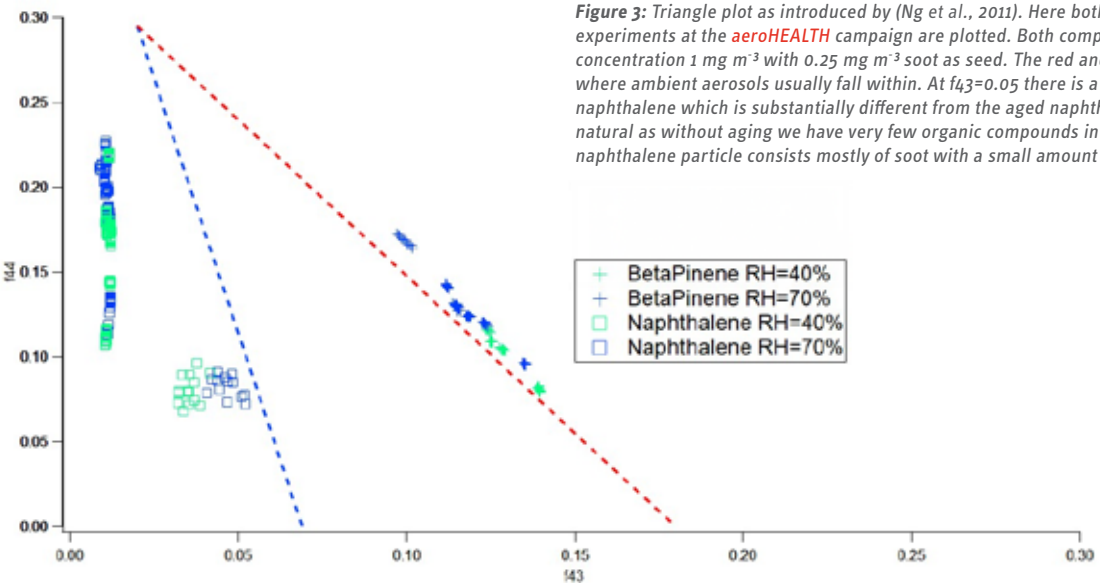


Figure 3: Triangle plot as introduced by (Ng et al., 2011). Here both ß-pinene and naphthalene experiments at the aeroHEALTH campaign are plotted. Both compounds were done at precursor concentration 1 mg m^{-3} with 0.25 mg m^{-3} soot as seed. The red and blue dotted lines indicate where ambient aerosols usually fall within. At f43=0.05 there is a noticeable cluster of unaged naphthalene which is substantially different from the aged naphthalene at f43=0.01. This is natural as without aging we have very few organic compounds in the particle; thus, the unaged naphthalene particle consists mostly of soot with a small amount of pure naphthalene.

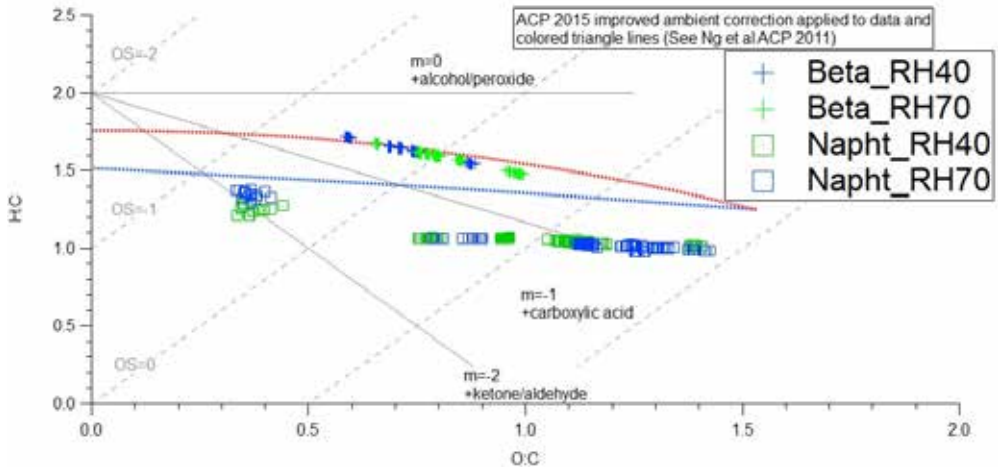


Figure 4: *Here the O:C is plotted against H:C, this type of plot is known as a Van Krevelen plot (Ng et al., 2011). Results from ß-pinene and naphthalene experiments at the aeroHEALTH campaign are plotted. Both compounds were aged at precursor concentration 1 mg m ³ with 0.25 mg m ³ soot as seed. The red and blue dotted lines indicate where ambient aerosols usually fall within.*

of the plot. In figure 3 it can be seen that SOA from naphthalene oxidation experiments in this study does not fit within the triangle, and as it is aged it increases only in f44 whilst there is no substantial change to the f43 value. A similar trend for naphthalene has been found in multiple papers such as Chhabra *et al.*, 2011 and Lambe *et al.*, 2011. For ß-pinene we see that it falls just outside the triangle, but in general, follows the trend as expected for ambient aerosols. It has been found that lab measurements usually fall slightly outside the triangle (Ng *et al.*, 2011). In this metric, the system with high concentrations is not notably different from the low concertation experiments, as found for both naphthalene and ß-pinene.

Furthermore, Lambe *et al.*, 2011's experiments were similar to ours during the aeroHEALTH campaign using an OFR at different equivalent aging times at around 40% RH making the only major difference between, Lambe *et al.*, 2011's experiments our experiments the concentration of precursor and the type of seed particle. Comparing the aging of the two systems in a Van Krevelen plot as done in figure 4, we see that the ratios of O:C found are similar ranging from 0.7 to 1.5 and that the H:C ratio is stable. One major difference is however the H:C as here it has been measured to about 1 for all experiments, Lambe *et al.*, 2011 found H:C to be 0.8. It should be noted, however, that the method of calculating the O:C and H:C ratios is different, as Lambe *et al.*, 2011)was written before Canagaratna *et al.*, 2015 who improved the method described by Aiken *et al.*, 2007. The difference between the old and new method of calculating O:C and H:C ratio could be up to 10%. Furthermore, figure 4 also shows the ß-pinene fitting within what would be expected from ambient aerosols. Additionally, for both compounds there are no sudden changes to the curve, suggesting that the oxidation pathway doesn't significantly change when the effective oxidation time is increased.

In summary, the SOA formed by the OFR setup during the aeroHEALTH campaign was investigated by on-line AMS aerosol mass spectrometry. The AMS-results show that the chemical fingerprint pattern of the obtained OFR SOA aerosol is located within or near the indication spaces for ambient aerosols at the Van Krevelen and m/z triangle plots. This is an indication that the chemical space obtained by the different precursors is at least similar to the ones observed under real atmospheric conditions. For deeper molecular information on the SOA aerosol formed from the naphthalene and ß-pinene precursors by the OFR see also aeroHEALTH report 4.

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(aeroHEALTH 6) Investigation of the complementarity of different mass spectrometric techniques for the determination of secondary organic aerosols under laboratory conditions

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Photochemical aging can rapidly alter the physicochemical composition of aerosols and thus supposedly affects their impact on human health. It is commonly related to the generation of highly complex organic mixtures and the introduction of heteroatoms, such as oxygen and nitrogen (Hallquist *et al.*, 2009), leading to unpredictable effects on the health and environment. A crucial step to evaluate these effects is the exploration of the chemical nature of such aerosols. In the framework of the aeroHEALTH measurement campaign (see report aeroHEALTH 4 and 7), innovative mass spectrometric (MS) approaches in combination with a thermal or high resolution gas chromatographic separation were used to investigate the complex SOA mixtures. The secondary organic aerosols (SOA) were derived from photochemical aging of naphthalene and ß-pinene by the means of a potential aerosol mass (PAM) oxidation flow reactor (Kang *et al.*, 2007). Among others, particulate matter was directly investigated by time-of-flight – aerosol mass spectrometry (TOF-AMS) and in parallel collected on quartz fiber filters. For the off-line chemical characterization of deposited SOA on those filters, we applied two complementary MS techniques: A) Direct insertion probe – high-resolution time-of-flight mass spectrometry (DIP-HRTOFMS) and B) comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS). The HRTOFMS enables exact mass measurements with mass resolutions in the range of 50.000 at very high mass spectral acquisition rates of >50 Hz. Both techniques can be classified in the field of thermal analyses by thermally desorbing analytes from filters and use electron ionization (EI) as an interface for mass spectrometric detection of the thermally desorbable particulate phase. With DIP-HRTOFMS, short analysis times of less than 10 min per sample were possible, whereas GC×GC TOFMS analysis times ranged between 2 to 3 h per sample. Conversely, the on-line TOF-AMS allowed the analysis of aerosols in real-time and indicated an increase of the degree of oxidation with increased photochemical aging (see aeroHEALTH report 5). Compared to the gradual thermal desorption gradient of 2 °C sec 1 for DIP-HRTOFMS and GC×GC-TOFMS, the TOF-AMS applies flash vaporization at a temperature of 600 °C, which on the one hand results in limited chemical information on thermally fragile organics due to extensive thermal fragmentation (pyrolysis), but on the other hand extends the application range to high boiling, non-refractory compounds. However, results may depend largely on empirically determined correction factors, which may not reflect the properties of a complex experimental design.

With DIP-HRTOFMS, the applied filter sample is heated and thermally desorbed directly in the ion source of a multireflectron TOFMS. The reduced pressure enables mild evaporation of compounds at lower temperatures. Therefore, thermally labile compounds can be evaporated and thermal degradation is suppressed. EI is applied as an ionization technique and leads to reproducible and defined ions. Major structural building blocks of analytes could be identified also with high ionization energies (70 eV) and without chromatographic pre-separation. Moreover, the high mass resolution allows discrimination of isobaric oxidized species and reliable assignment to elemental compositions (figure 1). Short analysis times and automated data processing, enables DIP-HRTOFMS as a suitable at-line technique for fast evaluation of chemical characteristics and fingerprinting approaches. However, as a direct mass spectrometric technique, the identification of compounds as well as isomeric separation is very limited and only partly compensated due to evaporation characteristics (Käfer *et al.*, 2019).

The complementary application of GC×GC-TOFMS added a molecular basis for interpreting the results and thus allowed a more comprehensive analysis of the aerosols. Compounds

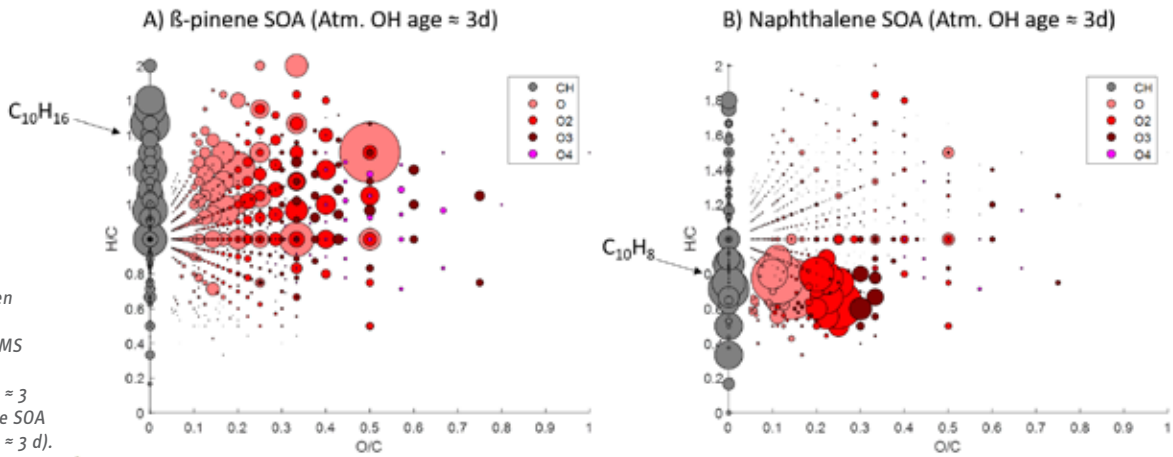

Figure 1: *Van-Krevelen plot of oxidized ions found by DIP- HRTOFMS for A) ß-pinene SOA (Atmospheric OH age ≈ 3 d) and B) naphthalene SOA (Atmospheric OH age ≈ 3 d).*

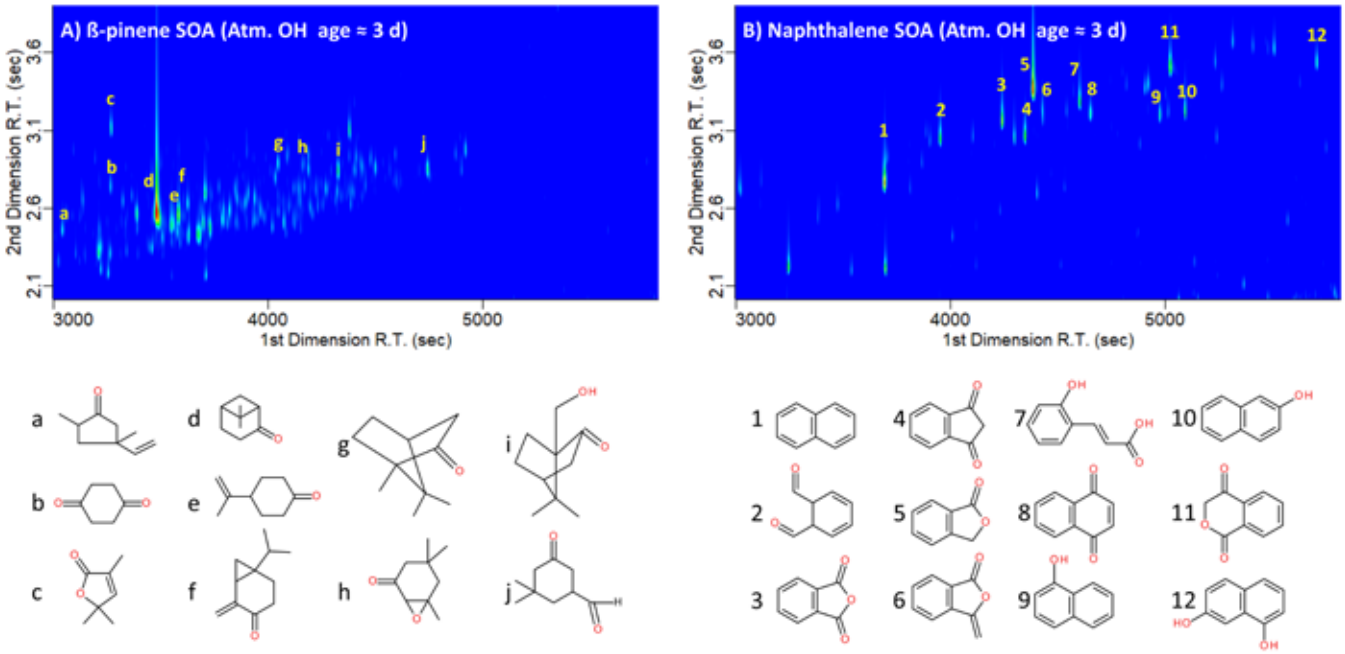

Figure 2: GC×GC-TOFMS contour plots for A) ß-pinene SOA (Atmospheric OH age ≈ 3 d) and B) naphthalene SOA (Atmospheric OH age ≈ 3 d), which show clear differences in the chemical composition of the produced SOA compounds depending on the precursor; Assignment of most abundant peaks to their molecular formulas via true peak spectrum (excluding inconclusive MS specta): ß-pinene SOA chromatogram with 10 peaks (a-j) out of 362 peaks in total and naphthalene SOA chromatogram with 12 peaks (1-12) out of 190 peaks in total.

could be chromatographically separated up to their isomeric composition, which supports a reliable assignment of the elemental composition of both, naphthalene and ß-pinene SOA (figure 2) and further allows a toxicological interpretation of individual compounds and classes. However, higher molecular weight or thermolabile compounds, which exceed the application range of GC due to their low volatility or stability, preferably need to be investigated by DIP-HRTOFMS. Further investigation by hybrid DIP/GC×GC-HRMS is planned such as reported in (Käfer et al, 2019). Note, that both approaches can be performed with the same HRMS platform without any adaptation effort. Due to the technical differences in the vaporization of the analytes, the larger chemical specificity (*i.e.* high mass resolution or chromatographic resolution), and the concomitant different application ranges, off-line methods as DIP-HRTOFMS and GC×GC-TOFMS complement the on-line TOF AMS for a thorough and comprehensive investigation of SOA. The analysis of the aeroHEALTH campaign samples will be accompanied by other ultrahigh resolution mass spectrometry approaches, such as Fourier Transform Ion Cyclotron Resonance MS (FT-ICRMS).

In conclusion, it can be clearly seen from the chemical profiling data that the investigated SOA types result in totally different chemical species with respect to molecular structures, aromaticity, and oxidation state. While naphthalene SOA is dominated by oxidized species which are exhibiting one or two aromatic rings, ß-pinene SOA is characterized by aliphatic oxidized structures. The van-Krevelen data from the DIP-MS analysis suggests that more higher-oxidized compounds are present in ß-pinene SOA. These compounds, however, likely are not measurable by GC-based approaches. The chemical fingerprints of naphthalene and ß-pinene SOA, in general, match the expectations and biogenic SOA. The following detailed bioinformatic (see aeroHEALTH report 9) and chemometric analysis of the chemical profile in respect to the transcriptome and functional toxicological data will help to explain the differences in toxicity (see aeroHEALTH report 7 and 8) observed for the SOA-types in the ALI lung cell model exposure experiments.

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(aeroHEALTH 7) Investigation of toxicity and health effects of atmospherically aged anthropogenic and biogenic emissions: A human airway barrier model for Air-Liquid Interface exposurebased aerosol toxicology

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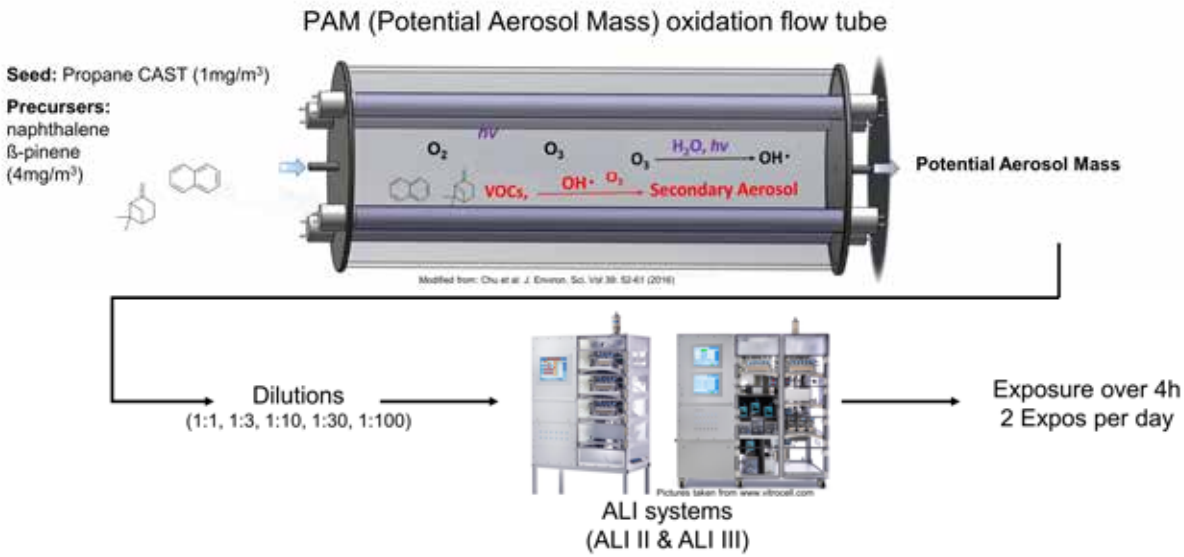
Already in the 1990s, several epidemiological cohort studies have shown an association between pollution with ambient particulate matter (PM) and substantial effects on human health (Hallquist *et al.*,2009; Shakya and Griffin, 2010; Goldstein and Galbally, 2007). The Global Burden of Disease (GBD) study estimated that in 2015 pollution with airborne PM was responsible for 4.2 million deaths worldwide (Jia 2010). Especially fine PM with an aerodynamic diameter smaller than 2.5 µm (PM2.5) has been correlated with adverse effects in several organ systems such as pulmonary and cardiovascular diseases (Guenther *et al.*, 2012; IARC 2010). Over the past decades, in particular, the impact of atmospheric aerosols on global disease burden gained more and more attention. Albeit there is strong evidence that atmospheric aging has an impact on the toxicity and health impact of aerosols (Chowdhury *et al.*, 2018; Chowdhury *et al.*, 2019; Miersch *et al.*, 2019), it is still largely unknown how atmospheric aging and the formed secondary organic aerosol compounds (SOA) is affecting the potency to induce biological responses (Fuzzi *et al.*, 2015). Within the aeroHEALTH measurement campaign, performed for 4 months around the turn of the year 2019/2020, the question on the biological activity SOA was addressed.

The sources contributing to the global PM load are due to primary anthropogenic and natural primary particle emissions, including industrial processes, residential heating and wildfire burnings (Ihantola *et al.*, 2020) as well as due to secondary aerosols. A major contributor to the secondary aerosols is SOA formed from anthropogenic and natural volatile and semi-volatile organic ((S)VOC) precursors. In-depth, the analysis found naphthalene and β-pinene as significant contributors and model compounds to the global anthropogenic (Jia and Batterman, 2010) and biogenic (Guenther *et al.*, 2012) SVOC SOA-precursor emission, respectively. Naphthalene, the smallest polycyclic aromatic hydrocarbon (PAH), can be formed during combustion processes, amongst others, through the chemical and primary metals industry, coal, gasoline and oil combustion, or tobacco smoking (Jia and Batterman, 2010). Due to its high vapor pressure, naphthalene is susceptible to undergo gas phase reactions in the atmosphere, which can result in a lower vapor pressure reaction products accompanied by conversion into the particle phase and thus generating secondary organic aerosols (SOA) (Williams *et al.*, 2010). On the other hand, β-pinene belongs to the class of monoterpene and, together with α-pinene, is one of the main semivolatile compounds released by vegetation. Its oxidation leads to the formation of low-volatile organic compounds, which then are able to condense on aerosol surfaces to generate SOA (Ehn *et al.*, 2014). Those SOA can be further oxidized by atmospheric oxidants (*i.e.* ozone, hydroxyl, or nitrate radicals) resulting in aged aerosols.

Within the aeroHEALTH measurement campaign 2019/2020 we operated a potential aerosol mass (PAM) oxidation flow tube using propane CAST soot (1 mg/m^{-3}) as seed aerosol together with either naphthalene (4 mg/m^{-3}) or β-pinene (4 mg/m^{-3}) as SOA precursors (for details of the experimental design see also report aeroHEALTH 4). After photo-chemically aging, the aerosol was diluted with sterile clean air in ratios of 1:1, 1:3, 1:10, and 1:30 and for naphthalene, an additional dilution of 1:100 was created before directing through the air-liquid interface (ALI) exposure system (fig. 1).

Figure 1: *Experimental set-up of the first aeroHEALTH measurement campaign (see also report aeroHEALTH 4). Seed together with different precursors were photochemically aged in a potential aerosol mass (PAM) oxidation flow tube. The resulting potential aerosol mass was applied to several dilutions before being supplied to the ALI systems in which the different cell systems were exposed over a time period of 4 h.*



Chemical and physical properties of the aged aerosols were comprehensively characterized using state-of-the-art, online and real-time techniques as well as off-line filter sample analysis (see reports aeroHEALTH 4, 5 and 6). In parallel with the aerosol characterization, three different cell model systems (monoculture of human epithelial lung cell line A549, human SV40-immortalized bronchial epithelial cells BEAS-2B, and a co-culture model of A549 cells together with a human umbilical vein cell line EA.hy926) were exposed to the diluted aged aerosols for 4 h at the ALI. The fact that epithelial cells have direct contact with inhaled aerosols makes them a useful model for studying toxicological effects after aerosol inhalation. By choosing three different cell model systems, we would like to enlarge our knowledge of cell-specific responses. Especially the usage of a co-culture cell model consisting of epithelial and endothelial cells is of relevance due to its greater closeness to the human physiology. We were able to conclude the exposure part of the experiment just before the Covid-19 lockdown in Germany.

In order to investigate the toxicological response of the different cell models upon aerosol exposure with focus on cell model and aerosol type specific effects, we developed an adverse outcome pathway (AOP) (fig. 2).

The idea of an AOP is to simplify complex toxicological processes in a linear format, starting with a molecular initiating event (MIE) that is connected with key events (KE) to the final adverse outcome (AO) (Halappanavar *et al.*, 2020). During this first aeroHEALTH campaign, we were interested in the interaction of aged aerosols with the cell membrane as MIE. To investigate this, we examined the concentration of intracellular lactate dehydrogenase (LDH) in the culture medium after exposure. The LDH assay is a meaningful method to determine the cytotoxic potential of nanomaterials. As KE, we elucidated the role of pro-inflammatory cytokines, oxidative stress, and genotoxicity. Here the focus was set on the detection of the pro-inflammatory and angiogenetic cytokine IL-8 in the cell culture medium after the exposure. In addition, the cell culture medium was probed for the oxidative stress marker malondialdehyde (MDA), one of the final products of the lipid peroxidase reaction, by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). After the exposure, cells were also sampled and were assessed for comet assay to identify DNA breaks, concluding the grade of genotoxicity triggered by the aerosol. For the final AO, we checked for the viability of the cell systems after exposure by Alamar Blue assay and tested the angiogenetic potential of the collected media on the human umbilical vein cell line EA.hy926. Transcriptome analysis of the sampled cells is underway to confirm the already gained results and to unveil new pathways, networks, and upstream regulators induced by the examined aged aerosols. Figure 3 is summing up the used cell culture model systems and methods.

All experiments were performed in more than three independent exposures with non-aged seed aerosol as reference, aged soot, β-pinene, and naphthalene, respectively. First, the optimal dose for cell exposure and omics analysis was tested in increasing aerosol dilution. The reaction of the cell systems was monitored using the LDH and Alamar Blue Assay. No significant decrease in cell viability was observed with aerosol concentrations of 1 mg/m^{-3} and 2 mg/m^{-3}. Also, the results of the LDH assay supported the obtained conclusion from the Alamar Blue Assay. Based on those results, the decision of taking an aerosol concentration of 4 mg/m^{-3} was made. This was the starting point of the measuring campaign with implementing all the assays described above (see also fig. 3). Over an experimental period of four weeks, we determined the cellular toxicity of the four different aerosols by the week. After every week the tubes to the ALI system were washed and cleaned so that no overlay of the previous aerosol occurred before changing to the next tested aerosol. In daily meetings with all colleagues involved in the campaign, results and problems were discussed and possible reworks were decided. The gained physical, chemical, and biological results are now evaluated and will be summarized in a first

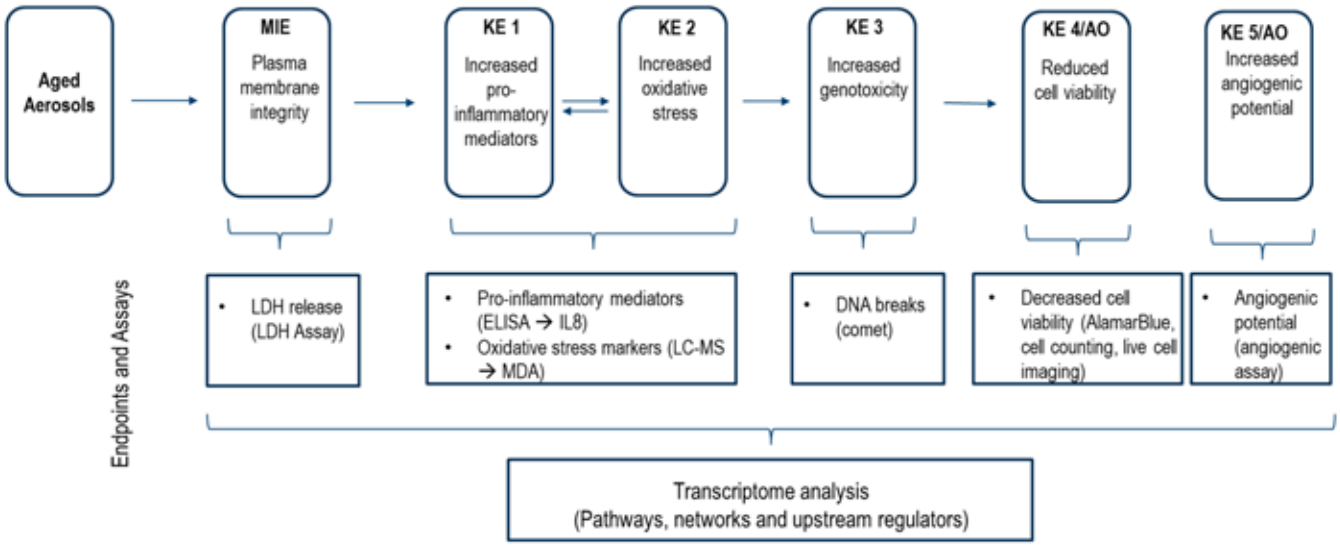


Figure 2: *Workflow of assessing the toxicological responses of the cellular models after aerosol exposure for the first and future aeroHEALTH campaigns addressing molecular initiating event (MIE) that is connected with key events (KE) to the final adverse outcome (AO).*

publication. Preliminary results are indicating higher toxic effects of naphthalene SOA compared to β-pinene SOA in all tested cell culture systems. We were able to show an increased release of LDH, IL-8, and MDA, as well as, increased accumulation of DNA damage and decreased cell viability after the exposure to naphthalene. Moreover, we observe a dose-dependent trend in all so far analyzed assays. This was true for naphthalene and β-pinene SOA. However, more obvious in naphthalene SOA experiments due to the higher toxic effects in all dilutions. Almost no effect was detected after the exposure with non-aged soot (seed aerosol). The evaluation of omics results and remaining assays are expected to unveil the underlying pathways and regulators for the observed different reaction of cell culture systems to the exposed aged aerosols.

To conclude, we assessed human lung cell response to aged aerosols. A unique combination of comprehensive chemical and physical aerosol characterization (see aeroHEALTH reports 4, 5 and 6) and multiple toxicological tests were used to generate a broad overview of cellular mechanisms affected by aged aerosols. Transcriptomic analyses (RNA-Seq) have been performed at the Weizmann institute and are under analysis by the aeroHEALTH consortium. By using different cell culture model systems, we aim to identify cell-specific toxicological responses and to converge closer to the human physiology by abstaining from *in-vivo* experiments. The planning of the next aeroHEALTH experimental campaign has already started and is preliminarily scheduled for the months November and December 2021 in Kuopio (delayed due to the Covid-19 pandemic). In this campaign, we will not only focus on further aerosols, but also on advanced cell culture models. For this purpose, we are already establishing a triple-culture model system with differentiated THP-1, A549, and fibroblasts (MRC5) or endothelial cells (EA.hy926) (fig. 4). This would give us the possibility to additionally involve the response of immune cells in our cell culture model, which is known to play an important role in the response to nanomaterial in the lungs.

In the following aeroHEALTH report 8 the initial results for mono and co-culture models exposed to naphthalene and ß-pinene SOA obtained during the first aeroHEALTH measurement campaign are presented.

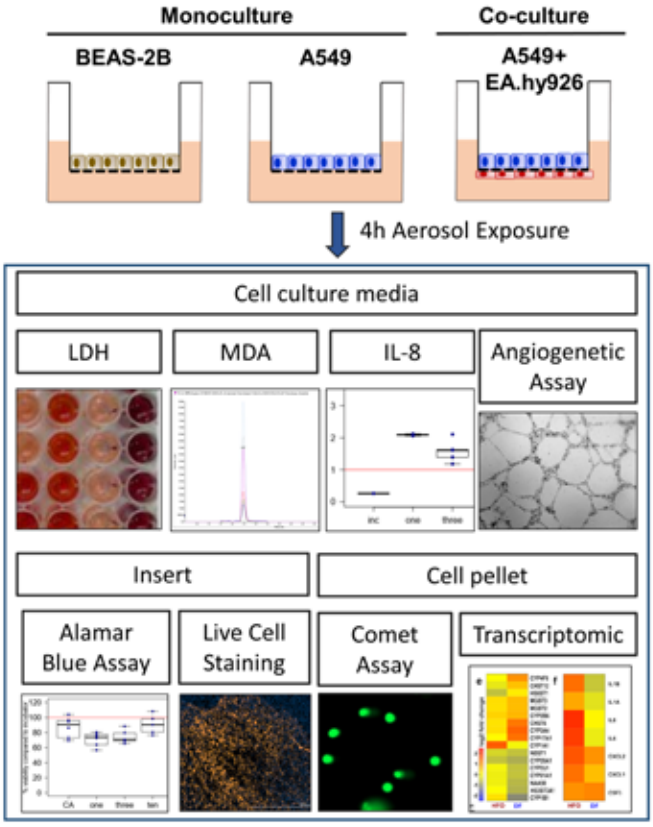


Figure 3: *Summary of the used cell culture models and executed analysis in the first aeroHEALTH campaign.*

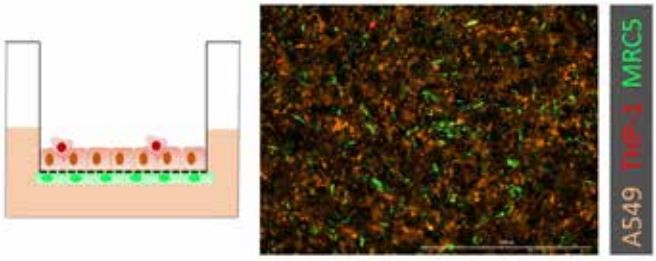


Figure 4: *Immunofluorescence images of the triple-culture (A549-orange, THP-1-red, MRC5-green).*

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(aeroHEALTH 8) Emission soot particles covered with anthropogenic SOA are more effective than biogenic SOA in inducing adverse health-related effects in lung cell models at the air-liquid interface

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Secondary organic aerosols (SOA) are known to represent a large proportion of ambient fine particulate matter (PM2.5), which in turn have been correlated to several adverse health outcomes (*e.g.*, respiratory and cardiovascular diseases, and a risk factor for increased morbidity and mortality). However, a direct link between single aerosol compounds and their health hazards remains largely unknown (Burkholder *et al.*, 2017; Park *et al.*, 2018; Wyzga and Rohr, 2015). In the first aeroHEALTH measurement campaign, we were interested in differentiating the toxicological effects of combustion-derived soot particles, from now on referred to as non-aged soot, from the effects induced by the soot photochemically aged together with SOA from a biogenic (β-pinene) or an anthropogenic (naphthalene) semi-volatile organic compound. Three different cell culture models were used, namely i) human alveolar basal epithelial cells A549, ii) human bronchial epithelial cells BEAS-2B, and iii) A549 and human endothelial cells EA.hy926 co-cultured on the opposite side of an insert membrane. All cell exposures were performed at the Air-Liquid Interface (ALI) by exposing cells to different dilutions of generated aerosols (see aeroHEALTH reports 4 and 7). Figure 1 shows the work at the two ALI Exposure stations during the aeroHEALTH measurement campaign.

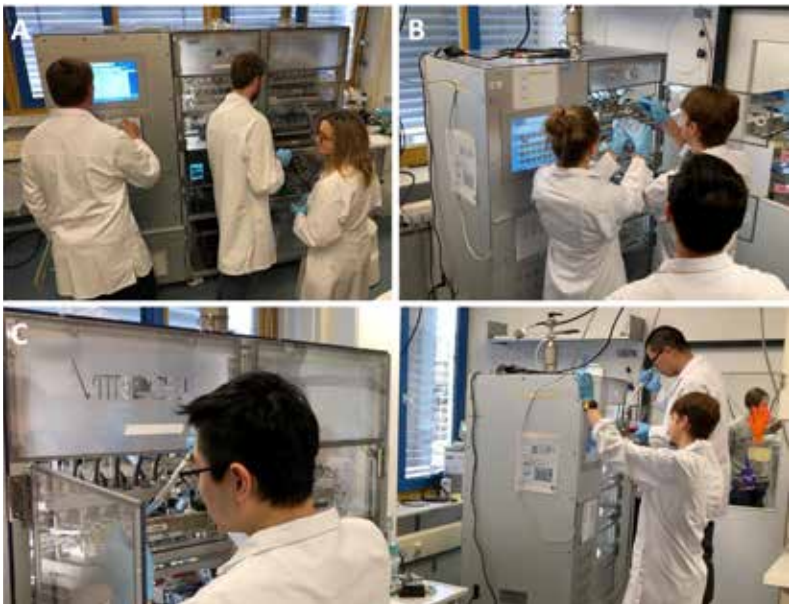

Figure 1: *During the first aeroHEALTH measurement campaign two automated ALI exposure stations developed during the HICE projects and during a project of UR to elucidate the toxicity of carbon fiber reinforced concrete (C^3-project, see third party projects (31)) were applied. A) The C^3 projects automated ALI exposure station system B) The HICE project automated ALI exposure station system. C) The two systems were operated in parallel during the aeroHEALTH exposures with a difference in dilution of the exposure aerosol by a factor of 10.*

To explore how non-aged soot, soot containing β-pinene SOA, or soot containing naphthalene SOA are affecting the used cell culture models, as well as in order to define the exposure concentration range, the percentage of cell metabolic activity as an indicator of cell viability was investigated by using the redox-sensitive dye AlamarBlue™. The exposure of A549 and BEAS-2B cell mono-cultures with non-aged soot and β-pinene SOA revealed no obvious effect on the cell viability in all dilutions. In contrast, the exposure with naphthalene SOA showed a dose-dependent reduction on the cell viability with slightly greater effects in the A549 model (1:1 dilution, 40 % cell viability) compared to the BEAS-2B model (1:1 dilution, 50 % cell viability) (figure 2A). This was also corroborated by live-cell imaging relying on the differential cell membrane permeability of live (blue, Hoechst dye) and dead (orange, propidium iodide dye) cells (figure 2B). No AlamarBlue™ assay was performed in the co-culture model due to space limitation of the Vitrocell automated ALI systems.

In addition to cell viability, further possible implications of tested aerosols on the cytotoxicity of our cell models were investigated. The release of lactate dehydrogenase (LDH) in the basolateral cell culture medium was used as an indicator of plasma membrane damage. An approximately 4-fold increase of LDH release compared to the clean air (CA) control in all dilutions and both cell culture models (A549 and co-culture) after the exposure to non-aged soot indicated no statistically significant aerosol effects. Also following both A549 and co-culture exposures to β-pinene SOA, no significant effects in the dilutions 1:30, 1:10, and 1:3 were observed. Beside, up to a 6-fold enhanced LDH release was

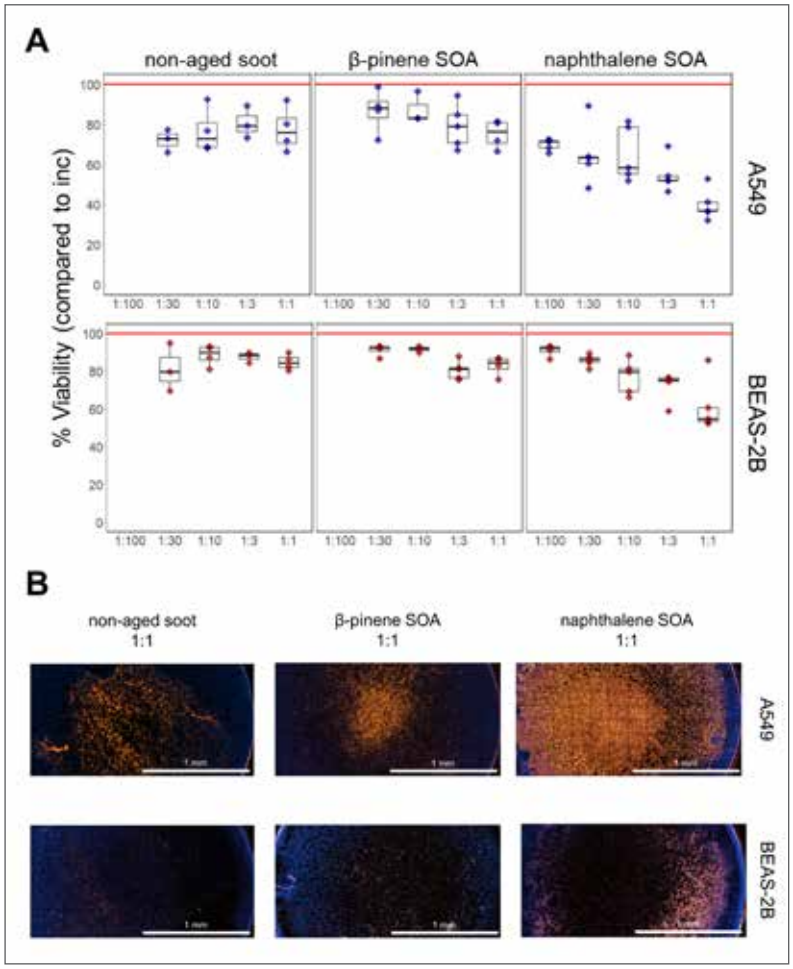

Figure 2: Exposure to naphthalene SOA compared to β-pinene SOA in the framework of the aeroHEALTH campaign resulted in a greater loss of cell viability in A549 and BEAS-2B cell culture models. Especially the naphthalene results suggest a dose-dependent effect of the applied dilutions. No effects were detected after the exposure to non-aged soot.

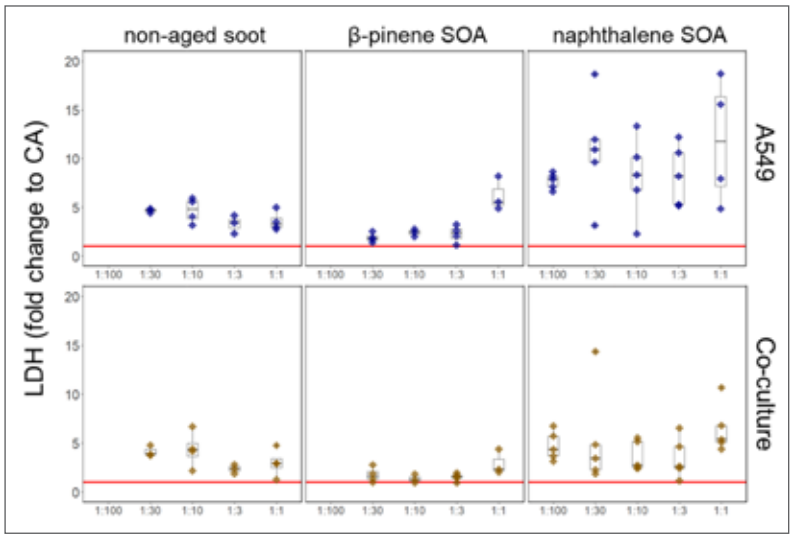

Figure 3: Increased release of LDH after the exposure to naphthalene SOA with greater detection in the mono-culture (A549) compared to the co-culture (A549/EA.hy926).

observed after the exposure of undiluted (1:1) β-pinene SOA in mono-culture treatments but not following co-culture exposures. Supporting the data gained by the AlamarBlue™ assay, a greater detection of LDH after A549 exposure to naphthalene SOA was shown in all dilutions with a peak (11-fold) following undiluted aerosol exposures. Interestingly, the co-culture released less LDH compared to the mono-culture in all settings (figure 3). No LDH was detected in the BEAS-2B model system probably due to the collagen-coated inserts.

To reveal the functional mechanism possibly leading to the observed cytotoxicity, the release in the basolateral cell culture media of malondialdehyde (MDA), generated by the peroxidation of cell membrane polyunsaturated fatty acids, was measured by LC-MS/MS and used as an indicator of oxidative stress. The analysis of MDA release showed no effects after the exposure to non-aged soot in all cell models (figure 4A). A slightly enhanced release after the exposure to β-pinene SOA in the dilutions 1:3 and 1:1 with the greatest detection in the A549 model (approx. 3-5 fold), followed by the co-culture (approx. 2-4 fold) and by the BEAS-2B model (approx. 2 fold), was observed. A significant increased and dose-dependent release of MDA was observed following exposures to naphthalene SOA with a peak in the A549 undiluted (1:1) aerosol treatments of 11-fold, and 7-fold in the co-culture and 5-fold following BEAS-2B exposures (figure 4A). Considering the fact, that intracellular oxidative stress can trigger genotoxicity (Møller *et al.* 2014), we next investigated the potentially induced DNA strand breaks by Comet assay (figure 4B). Indeed, the exposure to non-aged soot induced a significant increase of DNA breaks only following the highest exposure concentration in all tested cell model systems while greater effects of β-pinene SOA and naphthalene SOA were observed starting from 1:10 and 1:30 dilution exposures, respectively, in all cell models containing A549 cells but not following BEAS-2B exposure. A possible explanation for those similar effects may be due to the different metabolic competence of A549 and BEAS-2B cells as well as to their different DNA repair ability (Biola-Clier *et al.*, 2017). Perhaps, similar trends were observed in the induced intracellular oxidative stress levels (figure 4A) indicating a strong association between oxidative stress and DNA damage.

For instance, significant DNA damage effects were observed following 1:1 β-pinene SOA treatments with 11 % damaged DNA in A549 and 12 % in the co-culture (figure 4B). However, higher DNA damage effects were observed following naphthalene SOA 1:3 and 1:1 dilution

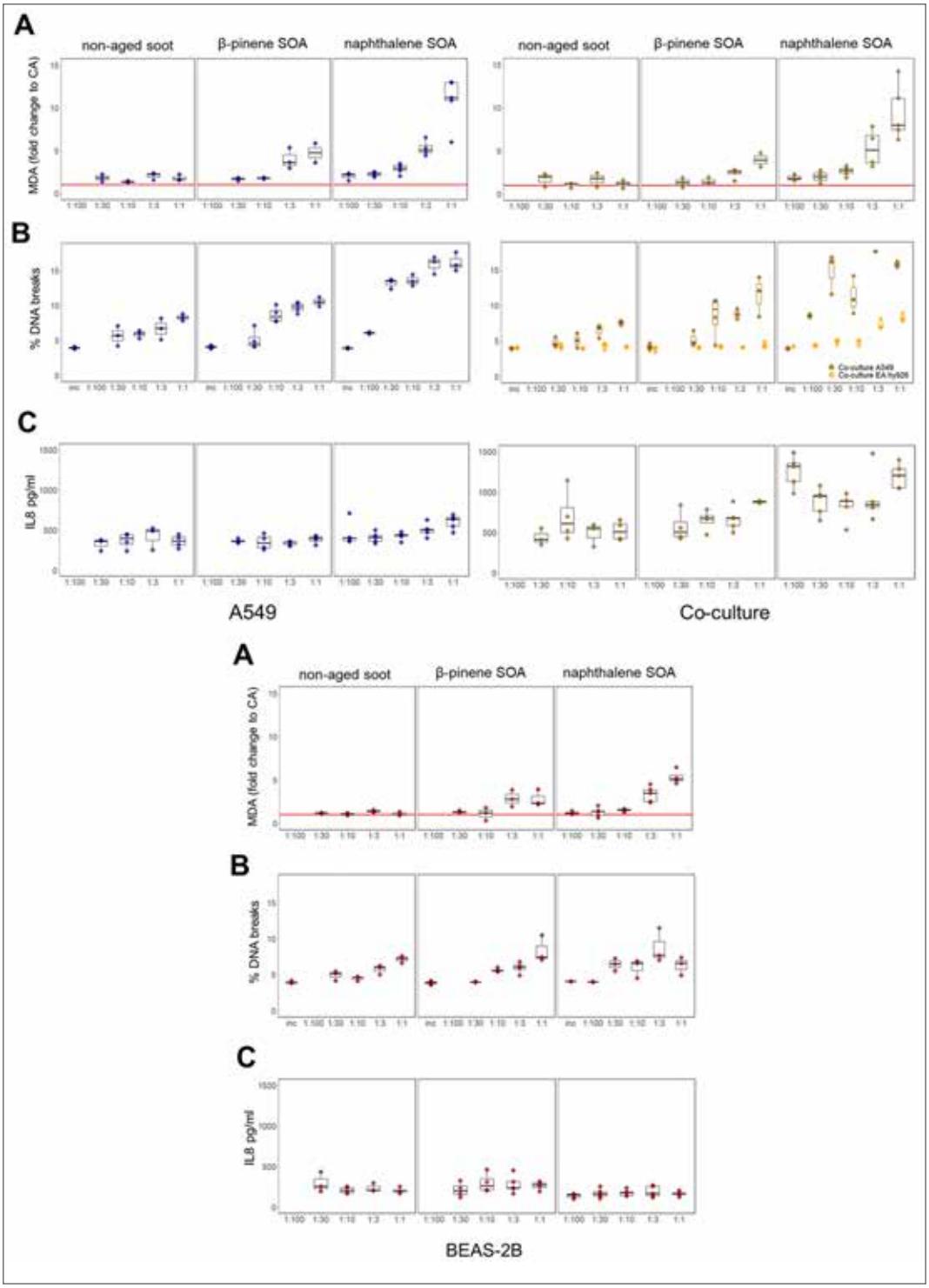

Figure 4: Exposure to naphthalene SOA revealed greater intracellular oxidative stress (A), genotoxicity (B), and pro-inflammatory IL8 release (C). In several assays, a dose-dependency was observed. No significant effects were observed after the exposure to non-aged soot.

exposures with 16 % (A549) and 15-17 % (co-culture) DNA strand breaks. Remarkably, the induction of DNA breaks was shown in the endothelial EA.hy926 cells of the co-culture system, which were not directly exposed to the aerosols. In fact, following the 4h exposure to naphthalene SOA a significantly high level of DNA strand breaks, up to 8 % in 1:1 dilution exposure, was observed in endothelial cells indicating a possible secondary genotoxicity mechanism (figure 4B). Besides inducing DNA breaks, oxidative stress can trigger inflammatory responses (Møller *et al.*, 2014). Especially IL8 is known to be abundant in people suffering from various chronic lung diseases, including chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS) and asthma (Aggarwal *et al.*, 2000; McGarvey *et al.*, 2002). Supporting the already gained findings, we observed no effect of non-aged soot on the increased release of IL8 in all cell culture models. Only in the co-culture systems, the exposure to β-pinene SOA leads to an enhanced release of IL8. Naphthalene SOA induced significant effects on the IL8 response in the 1:1 dilution of the A549 model (600 pg/ml^{-1}) and in all dilutions of the co-culture model (1000 pg/ml^{-1} – 1500 pg/ml^{-1}) with greater magnitude than in monocultures (figure

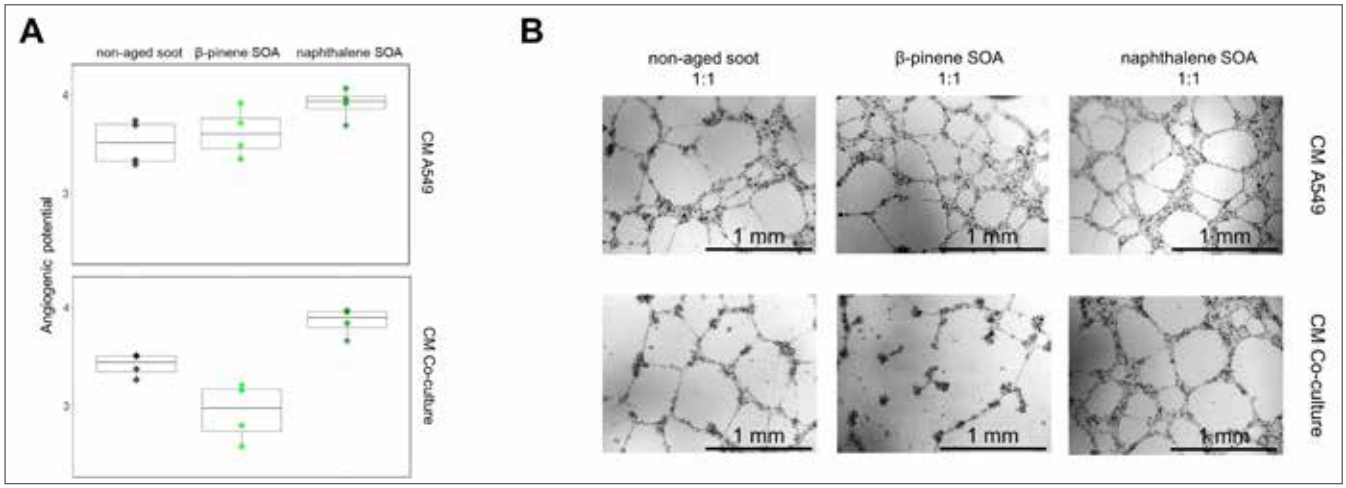

Figure 5: Conditioned media (CM) of naphthalene SOA exposures triggered an enhanced angiogenic potential of EA.hy926 endothelial cells.

4C). These results further support the observed secondary genotoxic effects since cytokines may promote genomic instability.

The increased release of IL8 in the A549 and co-culture model after the exposure to the tested SOA aerosols intrigued for studies on a possible aerosol induced activation of endothelial cells. For this purpose, we performed an angiogenic assay on freshly thawed EA.hy926 by exposing endothelial cells to the collected conditioned media (CM) after the aerosol exposures (figure 5). Compared to the angiogenic potential caused by non-aged soot and β-pinene SOA, naphthalene SOA exposure triggered an enhanced angiogenic potential of endothelial cells (figure 5A). This was observed after the treatment with the CM of both the A549 and the co-culture model. Interestingly, the exposure to β-pinene SOA lead to a comparable angiogenic potential with respect to non-aged soot in the A549 model. However, a lower proangiogenic effect was detected after the treatment with the co-culture CM with respect to the mono-culture CM (figure 5A).

In-depth chemical characterizations are ongoing to better understand these different outcomes and depict the role of chemical species in the observed effects (see also aeroHEALTH reports 4,5,6). Furthermore, the necessity of using several cell culture models to gain a more comprehensive assessment and understanding of toxic effects was demonstrated. RNA-Seq analysis, currently under evaluation, will help to further dig into the molecular mechanisms and the transcriptome of gene expression pathways, mechanistic networks, and causal analysis of induced health-related effects in different in vitro lung cell model systems.

In conclusion, these results indicate the importance of the chemical identity of SOA on soot surface in inducing adverse health-related effects, here shown in several toxicological assays representing molecular and cellular events depicted in an adverse outcome pathway framework. Greater toxicological effects were observed following exposure to naphthalene SOA on soot (reflecting anthropogenic SOA precursors) compared to β-pinene SOA on soot (reflecting biogenic SOA precursors). Both SOA were increasing the adverse biological effects on the exposed lung cell models in comparison to the exposure with fresh combustion-derived soot without aging.

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(aeroHEALTH 9) Knowledge guided multi-modal network inference approach to reveal health effect of polluted air in the framework of aeroHEALTH

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An important aspect in the aeroHEALTH research program is the development of new data analysis strategies for the complex exposome and biological response data sets. In the following two of the strategies and concepts are described.

Linear Mixed Models to predict cytotoxicity effects of aerosols on biological systems: To delineate the viability and cytotoxicity effects during the aeroHEALTH campaign 2019/2020 of four different aerosol exposures, naphthalene SOA, β-pinene SOA, aged, and non-aged soot on cultured cells A549, BEAS-2B, and A549 together with EA.hy926 co-culture, we applied a linear mixed model (LMM).

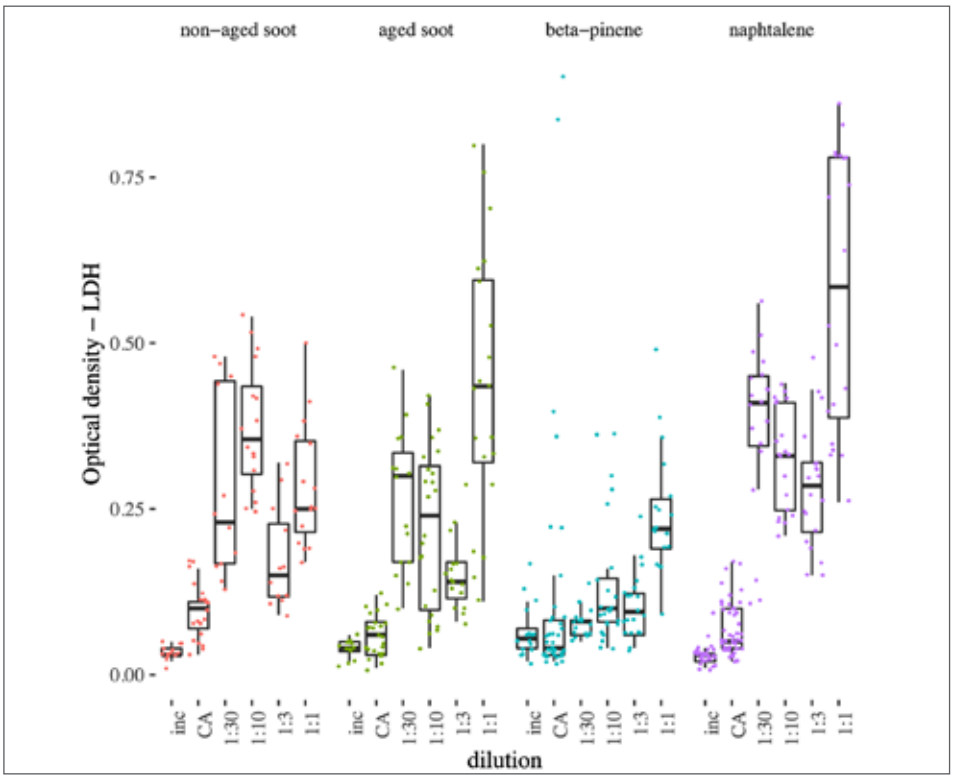
In a LMM, the response is modeled in dependence of fixed and random effects, and thus, provides the flexibility to assess both the mean as well as the variance and covariance of the input data. Fixed effects are thought to be variables that should explain the bulk of the variance in the data and are usually the effects of the treatment or condition of interest. We modeled as fixed factors the quality (non-aged and aged soot, naphthalene SOA, β-pinene SOA) and quantity (1:1, 1:3, 1:10, 1:30 dilution with clean air) of aerosols in viability and cytotoxicity assays.

Random factors come into play when data points are not truly independent of one another and an underlying correlation structure can be assumed. This applied to the experimental design for the campaign, as it is often not possible to guarantee the same conditions in experimental setups, *i.e.* the air-liquid interface (ALI) exposure system. This could lead to a bias towards higher or lower levels for certain well positions within and between the systems. Thus, the ALI exposure system and the well position within the ALI systems were taken into account as random factors to assess the effects of aerosols on cell cultures by a range of response variables, such as measurements from Lactate Dehydrogenase and Presto Blue assay. A negative trend in viability and positive in cytotoxicity for increased dose was observed for aerosols (see figure 1).

Multi-modal network inference using KiMONo: Cytotoxicity and viability assay gave a good impression of the deleterious effects of aerosols on cultured cells. Further, within the first aeroHEALTH campaign, a transcriptomic study on RNA-Seq level consisting of the transcriptome and secretome sequencing was planned and performed. It is hypothesized that this omic information carries the key information about the cellular molecular responses to the aerosol exposures. For the sequencing experiments, only BEAS-2B cells immortalized from non-tumorigenic bronchial epithelium were quantified in their responses to aged soot, ß-pinene SOA, and naphthalene SOA in 1:3 and 1:30 dilution, each with controls of clean air.

A further strength of the aeroHEALTH campaign design is the wide range of physical and chemical characteristics that are quantified during the exposures, some with high time resolution. These include measurements of physical aerosol parameters such as *e.g.* the particle distribution (by a SMPS), the content of black carbon (by an aethalometer), the content of elemental and organic carbon (by an EC/OC-analyzer), the particle number (by a CPC) or the particle mass (by a TEOM) as discussed in aeroHEALTH report 4. The chemical information,

Figure 1: *Dose dependent cytotoxicity (Optical density of LDH assay) of the test aerosols tested at the first aeroHEALTH measurement campaign: non-aged soot, aged soot, β-pinene SOA, and naphthalene SOA on A549 cell culture. The incubator (inc) and clean (CA) represent control samples. Aerosols were diluted with clean air (1:1, 1:3, 1:10, 1:30).*



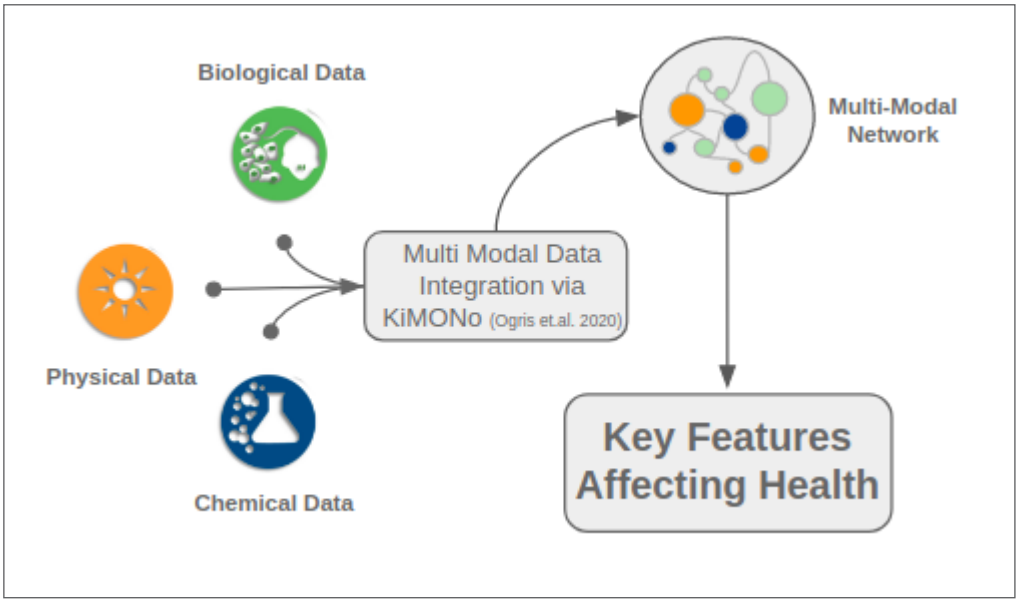


Figure 2: *Data integration using the knowledge guided multi-omic Network inference approach KiMONO. The inferred network will consist of nodes, representing the features and measured properties of the aeroHEALTH input data, while links represent statistically identified associations between them. Key features affecting health will be represented by densely connected nodes which can be further identified via network statistics like node degree or betweenness.*

e.g. on the oxidation state and rough composition of the aerosols (by AMS), the equivalent OH radical exposure age (by gas-phase PTR-MS), the qualitative and quantitative chemical analysis of aerosols on the molecular level (GCxGC-TOFMS, DIP-HRTOFMS, and GC-MS), see aeroHEALTH reports 4 and 6, is comprehensively acquired as well. To understand the effects of certain treatments or conditions on multi-omic levels, many multi-omic methods until now focus on the analysis of the different information layers separately and only in the end combining all significant hits. The interplay of 'cross-omic' might however be overlooked (Guala *et al.*, 2014; Ogris *et al.*, 2017). Thus, we hope to integrate the sequencing information with the physical and chemical characteristics in a holistic way using a network approach (see figure 2). This way direct links between genes that interact during exposure, as well as the physical and chemical characteristics that are likely to drive the expression of some genes or the interaction of genes can be identified.

The method that we have developed for this purpose is called KiMONo (freely available as R package https://github.com/cellmapslab/kimono), which infers a multi-omic multi-modal network of different information sources, based on prior available information (Ogris *et al.* 2020).

For every gene of interest, first, features are preselected using a prior *e.g.* from previous experimental validated interactions, such that only genes and secreted proteins that are known to interact with the gene of interest are included. This alleviates the computational burden and allows us to focus on relevant biomarkers. Further, all physical and chemical information is incorporated in the input matrix. Then, to determine which features explain the expression of the gene of interest, a sparse group lasso algorithm is applied that accounts for predefined group structures of the features while performing feature selection on the individual feature level within groups. This way, only features that are linked to the gene of interest are maintained in a multivariate model. The steps of preselecting features and calculating sparse group lasso models are then iteratively done for all of the available genes. In the last step, all gene models are combined to generate a multi-omic multi-modal network. In this network, an edge represents a feature that has been selected in the regularized model.

The resulting network can then be utilized in different ways to study links and interactions between genes and proteins, and their links to physical and chemical properties. Another strength of this method is that the resulting network can be employed to infer important nodes, such as key gene regulators, by prioritization using network statistics such as betweenness and connectedness. This approach allows for a holistic analysis of the effects of aerosol exposure, delineating the effects of physical characteristics of aerosols and cellular responses, in particular, it will pave the way to understand how the chemical composition and physical properties of aerosols influence gene expression at different levels.

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4.3 aeroHEALTH-related publications

2021 (Period covered until editorial deadline on 31.01.2021)

22. Bauer, S. *et al.*: In vivo and in vitro toxicity of emissions from a non-road diesel engine. (in process of submission)
21. Binder, S. *et al.*: In vitro genotoxicity of dibutyl phthalate on A549 lung cells at air-liquid interface in exposure concentrations relevant at workplaces (in process of submission)
20. Cao, X. *et al.*: Adenine derivatization for LC-MS/MS epigenetic DNA modifications studies on monocytic THP-1 cells exposed to reference particulate matter, Anal. Biochem. https://doi.org/10.1016/j.ab.2021.114127 (2021)
19. Di Bucchianico, S. *et al.*: System analysis of respiratory response to wood pellet combustion reveals inflammatory effect. (in process of submission)
18. Gat, D. *et al.*: The biogeography of dust in the Eastern Mediterranean, Communications Earth & Environment (submitted)
17. Gehm, C. *et al.*: Advanced VOC speciation in headspace and on-line analysis by combining hyper-fast gas chromatography and single-photon ionisation time-of-flight mass spectrometry with integrated electric modulator-based sampling, Analyst (submitted)
16. Momenimovahed, A. *et al.*: Comparison of black carbon (BC) measurement techniques for marine engine emissions using three marine fuel types, Atmospheric Environment (submitted)
15. Nguyen, D. L. *et al.* Carbonaceous aerosol composition in air masses influenced by large-scale biomass burning: a case-study in Northwestern Vietnam. Atmos. Chem. Phys. Discuss., Doi.org/10.5194/acp-2020-1027, in review (2020)
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13. Pardo, M. *et al.*: Toxicity of water- and organic-soluble wood tar fractions from biomass burning in lung epithelial cells, Chemical Research in Toxicology (submitted)
12. Passig, J., *et al.*: Detection of Ship Plumes from Residual Fuel Operation in Emission Control Areas using Single-Particle Mass Spectrometry, Atmos. Meas. Tech. Disc., (submitted) DOI: 10.5194/amt-2020-482 (2021)
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Imprint

Publisher

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Printing

Druckerei: kelly-druck GmbH

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