Institute of Hydrochemistry
Department of Chemistry
Technical University of Munich

Annual Report 2020

Institute of Hydrochemistry

Chair of Analytical Chemistry and Water Chemistry

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In Place of a Full Institute Photo in Corona Times:
A (Non-exhaustive) Video Conference Snapshot of Institute of Water Chemistry (IWC) Members in January 2021

Content

Dear colleagues and friends

Research and teaching have been challenging in 2020. In COVID-19 times, we had to navigate with distancing guidelines, home office, working shifts and free masks for everyone. Classes and seminars were online or even recorded, and our Christmas party took place via Zoom. But – knock on wood – as of now no COVID case has occurred among the active institute members. A big Thanks to everyone for their caution and discipline, which helped us all to stay healthy!

At the same time, the COVID crisis was a strong motivation for us to contribute to scientific solutions. The Bioanalytic group's expertise in microarray immunoassays was the basis to start the new CoVRapid project, targeting a rapid differential diagnostic microarray antibody test for SARS-CoV-2 and other infectious diseases. Together with partners from university and industry, and supported by the *Bayerische Forschungsstiftung*, at IWC we are creating an analytical platform to better understand responses to SARS-CoV-2 in relation to immune predispositions.

The Lasers and Microparticles group, in turn, started new activities that center on breath air aerosol, its dispersion and size distribution to assess the lifetime of viruses in air. Funded by the *TUM University Foundation*, different sampling systems were set up for breath air aerosol, including various types of masks and even an aerosol chamber where a person can enter and perform different physical activities inside.

A great recognition for her work at our institute was the Bunsen-Kirchhoff-Award 2020 for Dr. habil. Natalia P. Ivleva awarded "In recognition of her excellent developments in the field of Raman microspectroscopy, in particular stable isotope Raman microspectroscopy." Congratulations, Natascha! As another highlight from the Raman group, the online coupling of Raman Microscopy and Field-Flow Fractionation by optical tweezers took a step towards not only microplastics, but even nanoplastics analysis and made it onto the Front Cover on *Analytical Chemistry*.

Another strong focus at our institute has been the detection of antibiotic resistance. In the Seidel group, the Microarray-based identification of antibiotic resistant pathogens was advanced by a colony-fusion-recombinase polymerase amplification assay that is able to monitor both, antimicrobial resistance genes and their carrying species in surface water. In the Haisch group,

meanwhile successful implementation of isotope labeling allowed for a fast detection of antibiotic resistances of bacteria and even detection of hetero-resistance.

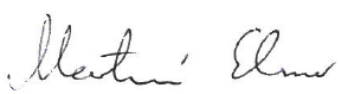
In the Isotope Group, we have been advancing Compound-specific Isotope Analysis not only for contaminated sites, but also for pesticides at low concentrations. Sediment tank experiments within the ERC Consolidator Grant *MicroDegrade* could rule out aqueous diffusion as significant cause of isotope effects, while isotopic evidence revealed that microbial cell walls constitute a significant barrier for biodegradation at low pollutant concentrations. Hydrogeological reactive transport models will thus need to be rewritten in order to account for this hitherto overlooked bottleneck of micropollutant degradation in groundwater.

Finally, big congratulations go Thomas Baumann, group leader of the Hydrogeology group, who was promoted to Apl.-Prof. in the Faculty BioGeoUmwelt when moving into the new labs and joining the Chair of Hydrogeology in Luisenstraße!

Last but not least, the last year has seen a successful new Chemistry class contributed by a team of young lecturers around our institute member Elisabeth von der Esch: an innovative course on Automation and Visualization of Laboratory Processes and Data. Supported by funding of the *Fonds der Chemischen Industrie*, students of chemistry were given the opportunity to learn how to approach automated data evaluation, and to build and program their own laboratory robots. A “Gallery of Awesome” features truly amazing results – Kudos to all participants!

Again, this leads me to thanking all those who have made all our achievements possible in the last year: our Ph.D. students, technicians, secretaries, Postdocs and guest scientists. Thanks to all members of our institute for their unwavering dedication in truly difficult times! And thank you to you - our friends - for your continued support!

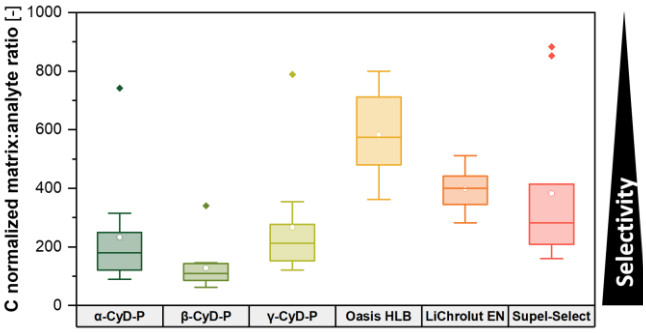
Kind regards,



Crosslinked cyclodextrin polymers for improved selectivity in extraction of micropollutants from surface water - comparison with commercial sorbent phases

Extraction with cyclodextrin-based sorbents can reduce matrix interferences offering an avenue for carbon isotope analysis of water samples with environmental relevant concentrations.

State of the Art Compound-specific isotope analysis (CSIA) has been demonstrated to be highly suitable for evaluating sources and transformation processes of micropollutants in laboratory experiments.[1] However, it remains a major challenge to transfer the method to the field-scale due to low environmental occurrence of micropollutants (sub-µg/L range). To this end, extraction of micropollutants from large volumes (>5 L) becomes inevitable to meet the low mass sensitivity of isotope-ratio mass spectrometry. Commercial sorbents employed for solid-phase extraction (SPE) may process large volumes but lack selectivity to extract micropollutants without co-enrichment of concurrent dissolved organic matter (DOM).



Lower matrix:analyte ratios in samples extracted with crosslinked cyclodextrin polymers indicate higher selectivity compared with commercial SPE sorbents.

Analytical Approach Recently, crosslinked cyclodextrin polymers (CyD-P) have been synthesized with high surface area (263 m^2/g) and fast kinetics.[2] In this work, we explore the feasibility of employing tailor-made P-CDPs for selective extraction of pesticides from surface water for carbon isotope analysis in comparison to HLB-based commercial sorbents. To this end, P-CDPs with different pocket sizes, namely α-, β- and γ-P-CDPs were synthesized and used as SPE sorbents to extract a selection of 11 pesticides from surface water.

Results Among CyD-Ps, β- and γ-CyD-P showed highest mean recoveries (81.7%) followed by α-CyD-P (78.7%) compared to OASIS HLB, LiChrolut© EN and Supel™-Select HLB with 86.8%, 81.0% and 75.2%, respectively. DOC-to-analyte ratios illustrate that sorption onto α- and β-CyD-P is more selective than onto HLB-based sorbents (by factor 2-4 and 2-6, respectively). However, sorption of NOM of different molecular sizes (<1, 1-3, 3-10, >10 kDa) on CyD-Ps did not show selective preference for smaller fractions. Our findings illustrate that the CyD-Ps are promising sorbents for selective extraction of pesticides from surface water contributing thereby to significant improvements of sample preparation for carbon analysis of micropollutants.

David Glöckler, Christopher Wabnitz

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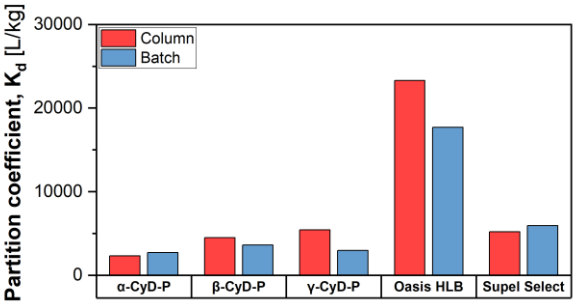
Funding
DFG – CRC 1253: CAMPOS

Cooperation
-

Sorption parameter determination for custom-made and commercially available sorbents using High Performance Liquid Chromatography (HPLC)

Column experiments facilitate a high-throughput determination of thermodynamic partition coefficients of micropollutants for the evaluation of solid phase-extraction materials.

State of the Art A sound study of sorption behavior of new materials for the extraction of micropollutants (e.g. pesticides) from water matrices requires accurate determination of partition coefficients (K_d) of the analytes between sorbent and aqueous phase. Though conceptually simple, conventional batch experiments are tedious and, therefore, do not allow to investigate sorption between a broad suite of analytes and sorbents. This limitation can be overcome by column experiments using an HPLC system.



Partition coefficients of s-metolachlor for cyclodextrin polymers and commercial sorbents (i.e. Oasis HLB, Supel-Select) determined with both column and batch experiment. K_d values are derived from Freundlich isotherm fitting for a theoretical aqueous concentration of 1 mg/L.

Analytical Approach In this approach, the sorbent is packed into an HPLC column allowing repetitive injections of unlimited analytes at different concentrations and under varying conditions (e.g. ionic strength, pH etc.). The relationship between K_d and retardation factor (R_f) is then used to calculate K_d from the obtained breakthrough curve (BTC) if sorption equilibrium is assured by the experimental setup. In this work, we were able to extend this method to custom-made and commercial extraction sorbents by (i) diluting the sorbent with inert silicone carbide prior to packing (1:100 w/w), and (ii) eluting the analytes with a mixture of ultrapure water and ethanol in order to obtain applicable BTCs. To account for the co-solvent effect, apparent K_d values were corrected by means of poly parameter linear free energy relationships (pp-LFER).

Results HPLC columns (1.4 cm length, 0.2 cm diameter) were homogenously packed by gradually increasing the eluent flow rate once the backpressure was maintained for at least 30 min. Mass recovery tests verified complete elution of all investigated pesticides from the columns (mean recovery 102±5%). Comparable to batch experiments, column experiments revealed non-linear sorption behavior as injections at different concentrations resulted in different K_d values. These apparent K_d values could successfully be fitted to the Freundlich sorption model. Finally, K_d values derived from batch and column experiments showed good agreement as exemplarily shown in the figure for s-metolachlor. This method is further used to study the influence of matrix constituents on K_d values among the different sorbents.

David Glöckler, Lucas Hirschberger, Johanna Plansky, Korbinian Geißer

Funding

DFG – CRC 1253: CAMPOS

Cooperation

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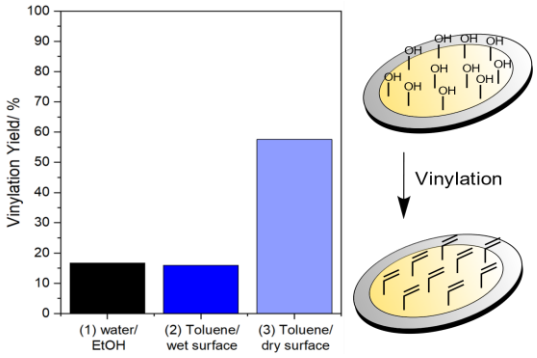
Modification of a Quartz Crystal Microbalance (QCM) sensor in preparation for polymer coating

The use of QCM sensors for online monitoring of analytical online extraction/cleanup processes holds promise to aid in understanding the behavior of constituents, such as natural organic matter (NOM), in complex sample preparation. Modifying the QCM sensor surface is a key step in preparation for its use as online monitoring tool of NOM.

State of the Art Compound-specific isotope analysis (CSIA) often necessitates intensive sample preparation in order to eliminate matrix interferences of real-world samples and to increase sensitivity. Development of selective sorbent phases in combination with automated extraction and clean-up procedures holds promise to make isotope analysis more sensitive, robust, and less time-consuming.[1] Real-time monitoring of matrix load during the automated sample prep procedure can be a key to this development. A Quartz Crystal Microbalance (QCM) sensor approach offers the possibility of a direct mass measurement as a potential solution for real-time monitoring of matrix components like NOM.



Vinylation of silica surface of the QCM sensor under three different conditions using γ-MAPS. Highest surface coverage with vinyl groups is reached using Toluene as solvent under inert/dry atmosphere.

Analytical Approach In order to monitor NOM behavior using a QCM sensor, the silica surface of the sensor needs to be coated by a polymer. In this work, we activated the surface with vinyl groups in preparation for further polymerization using [γ-(Methacryloyloxy)propyl] trimethoxysilane (γ-MAPS). Established procedures were tested, namely (1) *H_2O/ Ethanol* as solvent and 8 % γ-MAPS based on silica weight, (2) dry *Toluene* with a large excess of γ-MAPS under an inert atmosphere and (3) dry *Toluene* with a dry silica surface.[2,3] The surface coverage with vinyl groups upon vinylation was determined by titration with sodium hydroxide under an inert atmosphere.[2]

Results Vinylation of silica particles with different established procedures led to different vinyl yields on the silica surface. Using toluene and an excess of γ-MAPS (procedure 3) led to a three times higher surface coverage compared to using H_2O/Ethanol as solvent and 8 % γ-MAPS (procedure 1). Complete removal of water from the silica surface in a pre-vinylation step proved to be crucial for the vinylation yield. Specifically, adsorbed layers of water on the silica particles in equilibrium with the lab atmosphere (i.e. relative humidity 20%) already reduced the vinylation yield by threefold (procedure 2). The successful modification and activation of the silica surface enables further grafting of polymers onto the QCM-D sensor.

Christopher Wabnitz, David Kostadinov

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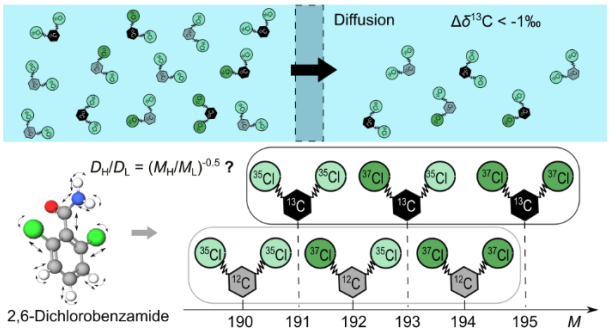
Funding
IWC-TUM

Magnitude of diffusion- and transverse dispersion-induced isotope fractionation of organic compounds in aqueous systems

Isotope fractionation induced by aqueous diffusion and transverse dispersion has been postulated to affect estimates of biodegradation at field sites. This study suggests that the effect is weak to negligible for most organic compounds at natural isotopic abundance. This greatly simplifies the analysis of biodegradation from isotope measurements, in particular for high-resolution profiles in the field.

State of the Art Assessments of natural attenuation at contaminated sites are greatly facilitated by evidence from changes in contaminant isotope values measured by compound-specific stable isotope analysis (CSIA).[1] Whether aqueous diffusion and dispersion may also lead to observable isotope fractionation is important for interpretations. [2]



The mass dependence of aqueous phase diffusion/dispersion induced isotope fractionation of organic compounds at natural isotopic abundance is weak to negligible, because (i) substitution by isotopes of other elements (e.g., ^{37}Cl, ^{15}N in BAM) lends isotopologues a higher molecular mass, such that isotope separation of ^{13}C vs. ^{12}C within the isotopologues is masked (ii) excitation of vibrations or rotations instead of translations may minimize the diffusion mass dependence according to collision theory, and (iii) solute-solvent interactions may further dampen translations.

Analytical Approach We performed diffusion experiments with modified Stokes diaphragm cells and transverse-dispersion experiments in quasi-two-dimensional flow-through sediment tank systems to explore isotope fractionation for benzene, toluene, ethylbenzene, 2,6-dichlorobenzamide (BAM), and metolachlor at natural isotopic abundance. Isotope analysis was conducted on a GC-IRMS system.

Results[3] Very weak to negligible diffusion- and transverse-dispersion-induced isotope fractionation was observed, with changes in carbon and nitrogen isotope values within ±0.5‰ and ±1‰, respectively. In comparison to ions, noble gases, and labelled compounds, three aspects stand out. (i) Isotopologue masses of polyatomic molecules may be affected by isotopes of multiple elements leading to smaller element-specific mass dependence. (ii) Collisions do not necessarily lead to translational movement but can excite molecular vibrations or rotations minimizing the mass dependence according to collision theory. (iii) Solute-solvent interactions like H-bonds can further minimize the effect of collisions. Our results suggest that the most important groundwater pollutants - chlorinated solvents and gasoline components - show small to negligible diffusion-induced isotope effects.

Fengchao Sun, Jan Peters

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Funding

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Cooperation

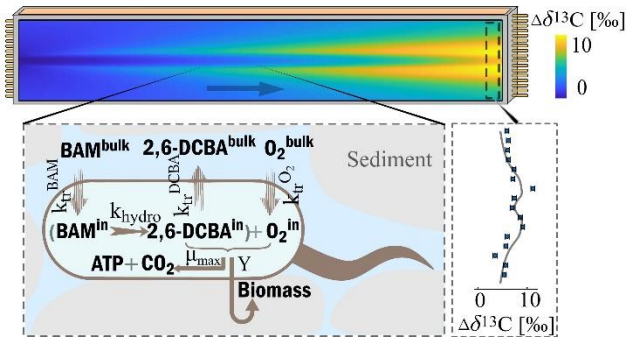
Prof Olaf Cirpka (Tübingen)

Dr. Martin Thullner (UFZ Leipzig)

Mass transfer-limited biodegradation – evidence from reactive transport modeling of isotope profiles in a mesoscale aquifer

Biodegradation is frequently inferred from stable isotope profiles in sediments. This study indicates that neglecting the effect of mass transfer limitations through bacterial cell membranes may significantly bias estimates.

State of the Art Recent studies observed reduced isotope fractionation of organic contaminants due to mass-transfer limitation through bacterial cell membrane at low concentrations in biodegradation experiments using mixed reactors.[1,2] However, the question remains whether an onset of mass-transfer limitation on biodegradation and isotope fractionation is observed under realistic *in situ* conditions in porous media.



We monitored concentrations of BAM and the intermediate product 2,6-dichlorobenzoic acid (2,6-DCBA), carbon and nitrogen isotope values of BAM, and suspended bacterial cells at the tank outlet ports. The decline of carbon isotope fractionations, at the BAM concentrations below the experimental threshold value of 920±440 µg/L indicated that the mass-transfer process between the bulk solution and the bacterial cell interior was the bottleneck for further biodegradation of organic contaminants at low concentrations. The experimental observations could be well reproduced by the model accounting for mass-transfer limitations.

Analytical Approach We studied aerobic degradation of 2,6-dichlorobenzamide (BAM) with compound-specific isotope analysis (CSIA) in a quasi two-dimensional flow-through sediment tank system inoculated with the bacterial strain *Aminobacter* sp. MSH1. An anoxic BAM solution was fed through the center port, yielding a system with strong transverse concentration cross-gradients of BAM. We simulated concentration and isotope ratio profies of the contaminant plume with a reactive transport model accounting for the combined effects of transverse dispersion and mass transfer through the bacterial cell membrane.

Results [3] Reduced carbon and nitrogen isotope fractionations was observed at low BAM concentrations in the upper and lower regions of the tank. Experimental observations could only be reproduced by the model when accounting for mass-transfer limitations. Specifically, the simulated apparent isotope enrichment factor *ε decreased strongly below a simulated threshold concentration of about 0.6 mg/L BAM. These results suggest that mass-transfer on the cellular level may become generally limiting for biodegradation when solute concentrations are very low.

Fengchao Sun, Adrian Mellage, Mehdi Gharasoo

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(2) Kundu, K.; Marozava, S.; Ehrl, B.; Merl-Pham, J.; Griebler, C.; Elsner, M. Defining lower limits of biodegradation: atrazine degradation regulated by mass transfer and maintenance demand in Arthrobacter aurescens TC1. ISME J. 2019, 13 (9), 2236-2251.

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Funding

European Research Council Consolidator Grant - Grant number 616861

Cooperation

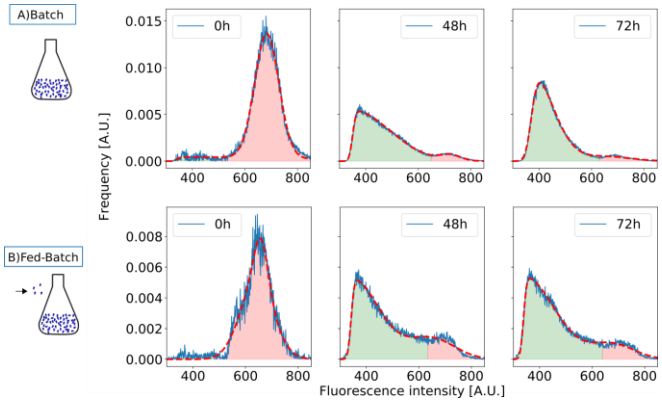
Prof Olaf Cirpka (Tübingen)

Prof. Christian Griebler (Vienna)

Dr. Martin Thullner (UFZ Leipzig)

Phenotypic heterogeneity as key factor for growth and survival under oligotrophic conditions

How do microorganisms adapt at population level under constant low energy fluxes? Under catabolic energy limitation, an isogenic population was discovered to diversify into multiple phenotypes. Modelling shows that the capability to switch between different phenotypes supports growth and survival under harsh conditions of energy limitation.



Diversification into non-growing cells and growing cells. Non-growing cells are recognizable, because they maintain their high intensity of fluorescence staining (to the right, red), whereas growing cells are recognized, because they maintain the intensity of staining due to cell division (to the left, red).

State of the Art Productivity-poor oligotrophic environments are plentiful on earth. Yet it is not well understood how organisms maintain population sizes under these extreme conditions. Most scenarios consider the adaptation of a single microorganism (isogenic) at the cellular level, which increases their fitness in such an environment. The adaptation of microorganisms at population level – that is, the ability of living cells to differentiate into subtypes with specialized attributes leading to a coexistence of different phenotypes in isogenic populations – remains a little-explored area under oligotrophic conditions.

Analytical Approach *Arthrobacter aurescens* TC1 was grown on atrazine as limiting nutrient. Changes from batch experiments (high energy flux) to medium energy flux (fed-batch experiments) were monitored with respect to heterogeneity (growing and non-growing cells) in the population after each cell division. Chemostat experiments (low energy flux) studied population adaptation by observing the difference in nucleic acid content of the cells. Observations were matched by predictions in a new modeling framework.

Results Fluorescence cytometry and turnover rates revealed that sub-populations differed in their nucleic acid content and metabolic activity. A mechanistic modeling framework for the dynamic adaptation of microorganisms with the consideration of their ability to switch between different phenotypes was experimentally calibrated and validated. Simulation of hypothetical scenarios suggests that responsive diversification upon changes in energy availability offers a competitive advantage over homogenous adaptation for maintaining viability and metabolic activity over time.

Kankana Kundu

References

K. Kundu, Nina Weber, Ch. Griebler, M. Elsner, Phenotypic heterogeneity as key factor for growth and survival under oligotrophic conditions, Environmental Microbiology 22 (2020), pp. 3339-3356

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European Research Council Consolidator Grant - Grant number 616861

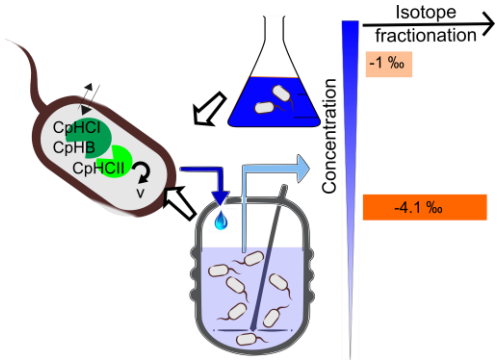
Cooperation

Prof Christian Griebler (University of Vienna)

Linking increased isotope fractionation at low concentrations to enzyme activity regulation: 4-Cl phenol degradation by *A. chlorophenolicus* A6

Recent biodegradation studies observed drastically decreased isotope fractionation specifically at low concentrations and provided evidence of mass transfer limitation. Here, a study with *Arthrobacter chlorophenolicus* A6 gave unexpected evidence on the contrary.

State of the Art Recent atrazine biodegradation studies observed drastically decreased isotope fractionation when concentrations fell below the Monod constant of microbial growth. [1, 2] Hence, once enzyme kinetics were no longer saturated, biochemical turnover became rapid relative to cell wall permeation masking isotope effects and revealing uptake as bottleneck of micropollutant degradation at µg L^{-1} concentrations.



4-Chlorophenol degradation by *Arthrobacter chlorophenolicus* A6 showed increasing isotope fractionation at low concentrations demonstrating enzyme activity regulation rather than mass transfer as dominating rate control at low concentrations.

Analytical Approach This study investigated degradation of the pollutant 4-chlorophenol by *Arthrobacter chlorophenolicus* A6, which may adapt its activity to concentrations. Isotope fractionation was measured by GC-IRMS to explore limitations by mass transfer in high concentration (mg L-1) cultivation in batch and chemostat, and at low concentrations (µg L-1) in chemostat. To probe for physiological adaptation, comparative label-free proteomics observed changes in protein expression and cell membrane fatty acid analysis tested for membrane fluidity

Results Cell-specific turnover in chemostat first increased with dilution rates, then decreased through substrate inhibition at high (200 mg L^{-1}) concentrations. Contrary to expectations, isotope fractionation of 4-chlorophenol *in*creased at lower concentrations, from $\varepsilon(C) = -1.0‰ \pm 0.5‰$ in chemostat ($D = 0.090\ h^{-1}$, 88 mg L^{-1}) and $\varepsilon(C) = -2.1‰ \pm 0.5‰$ in batch (c_0 = 220 mg L^{-1}) to $\varepsilon(C) = -4.1‰ \pm 0.2‰$ in chemostat at 90 µg L^{-1}. Surprisingly, fatty acid composition also indicated *in*creased cell wall permeability at high concentrations while catabolic enzymes (CphCI and CphCII) were differentially expressed at $D = 0.090\ h^{-1}$. These observations emphasize regulation on the enzyme activity level – through either a metabolic shift between catabolic pathways, or decreased enzymatic turnover at low concentrations.

Kankana Kundu, Aileen Melsbach, Benjamin Heckel, Sviatlana Marozava

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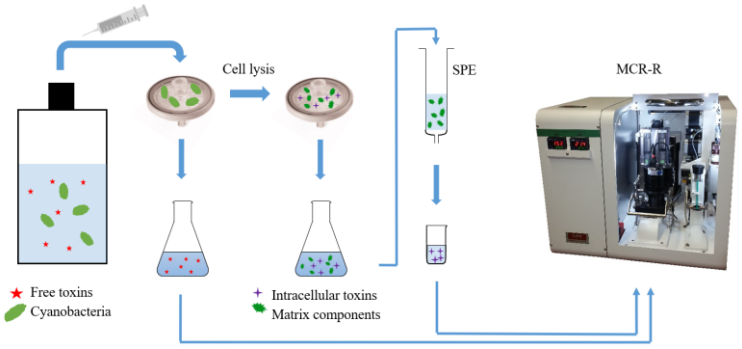
Cooperation

Dr. Juliane Merl-Pham, (Helmholtz Zentrum München)

Development of an early warning system for increased algal growth and release of algal toxins in surface waters by means of online parameter determination

The increased growth of cyanobacteria is favored by climate change as well as the eutrophication of water bodies and have been observed more frequently in recent years. Often, potential dangers from algal blooms are not discovered until accidents have occurred. To prevent this, it is essential to develop an early warning system for increased algal growth and the release of algal toxins.

State of the Art Currently, hazard assessment is based on experience, visual inspection of water bodies, and discrete sampling.[1] Contrary to these efforts, accidents continue to occur, as at Lake Mandicho in 2019 or at Lake Constance in 2020. To prevent accidents, comprehensive monitoring of potentially endangered waters is urgently needed. Therefore, an online monitoring system with cloud-based data processing is to be developed in cooperation with A.U.G Signals Ltd., Toronto and Hydroisotop GmbH, Schweitenkirchen, as part of an AIF-ZIM project in order to be able to predict increased cyanobacterial growth and algal blooms at an early stage.



Schematic representation of sample preparation with sampling, separation of free toxins and cyanobacteria, cell lysis, purification of toxins exemplary with SPE and MCR-R as analysis platform.

Analytical Approach The online monitoring system is designed to collect information on certain parameters that can lead to excessive growth of cyanobacteria and to evaluate them online using an algorithm. These include nutrient concentrations of nitrate, phosphate and oxygen, as well as pH, solids content and redox potential. In addition, stress factors such as S- and Se-organic compounds that could induce algal blooms will be monitored. In addition to these parameters, it should also be possible to monitor cyanotoxins like microcystin LR, which are free in water or intracellular. For this purpose, an automated analysis platform is required, which, in addition to the online determination of cyanotoxins, also has an automated sample preparation, which includes the separation of the free and intracellular toxins, the lysis of the cyanobacteria, enrichment and lavation of the toxins.

Andreas Auernhammer

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Funding

AIF-ZIM

Cooperation

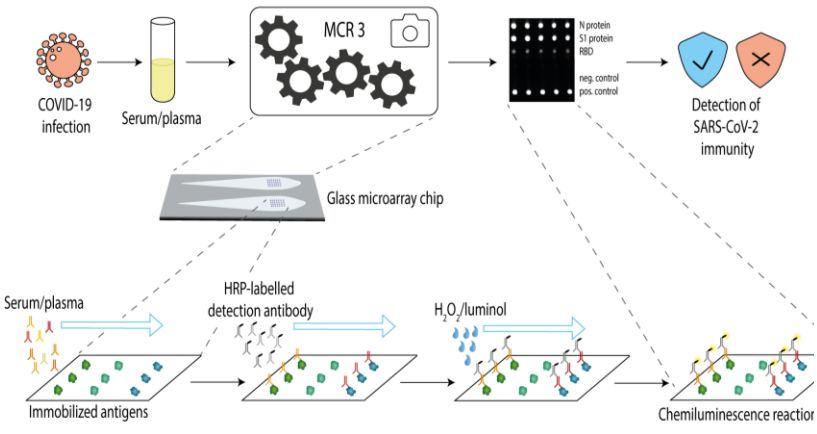
A.U.G. Signals Ltd.

Hydroisotop GmbH

Rapid, multiplex detection of SARS-CoV-2 antibodies by an automated, flow based chemiluminescence microarray immunoassay (CoVRapid CL-MIA)

The COVID-19 pandemic has kept the world in suspense for all of 2020. Thorough serosurveillance might be key in the way back to normality. Therefore, we developed an immunoassay for the rapid automated detection of antibodies to SARS-CoV-2.

State of the Art With increasing numbers of infections and more than 1,700,000 deaths so far, the world is still threatened by the COVID-19 pandemic. High hopes are placed in the vaccination, promising immunity and a return to normality. Hence, the monitoring of immunity by antibody tests is highly relevant but commercial tests like ELISAs are often time-consuming, laborious and sometimes exhibit insufficient sensitivity as only one antigen can be considered.



Test principle of the CoVRapid CL-MIA.

Analytical Approach We made use of the long-established microarray glass chips for the automated analysis platform MCR 3[1] and developed an indirect, non-competitive microarray immunoassay that allows for the simultaneous detection of IgG antibodies to SARS-CoV-2 receptor binding domain, spike (S1) and nucleocapsid protein within eight minutes. To allow for a qualitative classification of serum and plasma samples, receiver operating characteristic (ROC) curves were used to determine optimal cut-off values.

Results With a sample set of 65 serum and plasma samples (32 SARS-CoV-2 serology negative, 33 positive) a diagnostic sensitivity and specificity of 100% was obtained for the CoVRapid CL-MIA. With this result, it outcompeted two commercial comparison tests in diagnostic performance and additionally had the advantages of rapid, fully automated multiplex analysis within as few as eight minutes. The information about patient samples gained with this test can be of high value in SARS-CoV-2 serosurveillance tasks. Future research efforts in the field will be put into the quantitative application of the test that has already been proven to be possible in general.

Julia Klüpfel

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ISAR Bioscience GmbH

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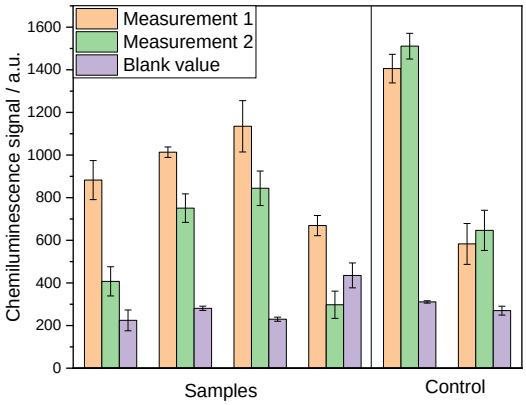
Flow-based chemiluminescence microarrays as screening platform for affinity ligands against bacteria platform

For the affinity filtration of pathogens in body fluids suitable ligands are needed. On our flow-based microarray and using biotinylated bacteria we are investigating these ligands. The proof of this concept was performed with the ligand Polymyxin B and *Escherichia coli*.

State of the Art Infection of body fluids is a severe illness, which can lead to death. As the concentration of pathogens are often very low, culture-based detection methods, the current gold standard, come to their limits. In order to overcome this problem, enrichment of the pathogens is necessary, e.g. by filtration.



Results for CL measurements of biotinylated *E. coli* in tap water pH 4. Measurement 1 was performed before and Measurement 2 after desorption. Control was without desorption.

Analytical Approach The ligands later used for monolithic affinity filtration of the pathogens need to be identified first. For this, a flow-based chemiluminescence microarray was established. A polycarbonate (PC) surface gets coated with succinylated Jeffamine® ED-2003, (sJeff) a diamino-PEG/PPG triblock copolymer. After immobilization of the ligands the PC gets assembled to a flow-through microarray chip. The measurements get carried out on the MCR-R (microarray chip reader – research). First, biotinylated bacteria are flown over the microarray chip, via a stopped-flow incubation, then the streptavidin conjugated horseradish peroxidase is injected. The chemiluminescence signal produced by luminol and hydrogen peroxide is recorded by a CCD-camera.

Results As a first ligand Polymyxin B (PmB) was used. It was already confirmed as a suitable ligand for affinity filtration of *E. coli*.[1] First it was shown by chemiluminescence detection of PmB on the chip surface by a monoclonal antibody that PmB was successfully immobilized. Afterwards the biotinylated *E. coli* were flown over the chip. In tap water at pH 4 a signal was obtained. However, in tap water (pH 7) the signal was very low which proofs that immobilized PmB binds to *E. coli* only at low pH. Desorption experiments were conducted by using carbonate buffer (pH 8.2). Lower chemiluminescence signals were observed afterwards. Summarizing, it was possible to use this setup for testing affinity ligands for bacteria. Now suitable ligands for body fluids (protein rich, pH 7) must be identified.

Julia Neumair

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Cooperation

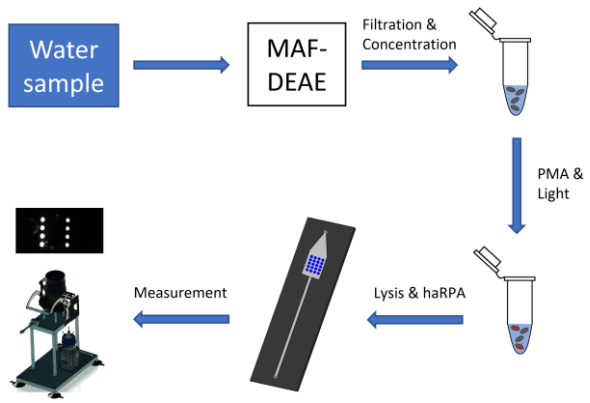
Experimental Orthopedics, Klinikum Rechts der Isar (TUM)

Klinik und Poliklinik II, Klinikum Rechts der Isar (TUM)

Rapid quantification of viable *Legionella spp.* by isothermal nucleic amplification test on chemiluminescence DNA microarrays

In the future viable *Legionella* can be detected fast without the risk of under- or overestimation. Additionally, the differentiation of viable and dead cells allows a good monitoring of biocide effects in all kind of freshwater systems.

State of the Art. Due to increasing number of legionellosis outbreaks in the last years and new guidelines (eg. 42. BImSchV), the importance of a reliable *Legionella* detection method for e.g. cooling towers, evaporative cooling systems and air filter systems is becoming more and more important. The current gold standard for detection is the culturing method. It takes long 7-10 days and has a high risk of underestimation because of viable but not culturable cells. That is why culture-independent molecular biological methods like qPCR and isothermal nucleic acid amplification methods are becoming increasingly popular. For this kind of detection, studies shown that a differentiation of viable and dead cells is important to avoid overestimation.



Schematic workflow of the viability haRPA with real samples.

Analytical Approach. Extracted *Legionella* spp. DNA from concentrated water samples is amplified by the isothermal heterogenous asymmetric recombinase polymerase amplification (haRPA) using genomic sequences of the 16S rRNA on a DNA microarray. The amplification can be quantified by a visible chemiluminescence reaction detected with a CCD camera. To distinguish between viable and dead cells a DNA-intercalating dye propionium monazide (PMA) is added. It can pass the cell membrane of dead cells and inhibit the amplification of the haRPA.

Results. With several changes of the already established assay (1) including optimizing the primer concentration, incubation time and decreasing the flow channel by half of the volume the LoD and linear working range could be drastically improved. Also, the microarray surface was changed from glass to polycarbonate for a faster and cheaper production. With an LoD of 0.0019 CFU/mL and a linear working range from 0.911 – 3.2 x 10^4 CFU/mL this assay can now compete with similar qPCR approaches and can be test for real water samples from cooling towers, evaporative cooling systems or air filter systems. Additionally, the decreased volume of the flow cell, reduce the RPA-reagent cost by half and showed in calibration measurements a decreased intra-microarray-standard deviation.

Gerhard Schwaiger

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Funding

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FKZ 28N-2-002-02

Cooperation

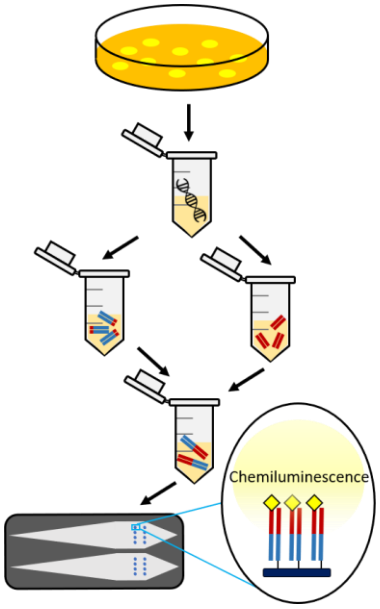
Thünen-Institut für Agrartechnologie

A chip-based colony-fusion-recombinase polymerase amplification assay for monitoring of antimicrobial resistance genes and their carrying species in surface water

The monitoring of antibiotic-resistant bacteria in the environment is a big challenge. Unfortunately, numbers of these pathogens in important matrices such as water or food are rising and therefore reliable methods for their identification are needed.

State of the Art In order to enable accurate risk assessment regarding antibiotic-resistant bacteria, knowledge about the species and its pathogenicity is needed. Mostly, molecular biological methods only look for the latter, namely the resistance gene. Sometimes, the species is also identified by analysis of specific genes or culture methods. Especially for analyzing potential danger to human health and to monitor the spread of genes, both aspects are needed. In this project we aimed answer both questions with one method.

Analytical Approach The aim of this work is the generation and detection of DNA fusion-products carrying an antibiotic resistance- and a species-specific gene at the same time. For the generation of these fusion products, an isothermal recombinase polymerase amplification (RPA) was used to amplify the antibiotic resistance gene and species-specific gene. Afterwards, both gene amplicons are fused together to a single strand using an additional RPA. The fusion-product was detected on a chemiluminescence based microarray chip[1] by a further RPA amplification directly on the chip surface.



Workflow for the generation of fusion products and their detection.

Results For a final method, an easy 3-step workflow for colony- fusion-haRPA was established. The assay was developed for *E. coli* carrying CTX-M cluster 1 resistance genes and was confirmed to be specific for the latter. A cut off value for positive samples could be set without the generation of false-negative or false-positive results. The method was successfully used on river samples in first experiments.

Katharina Sollweck and Philipp Streich

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Funding

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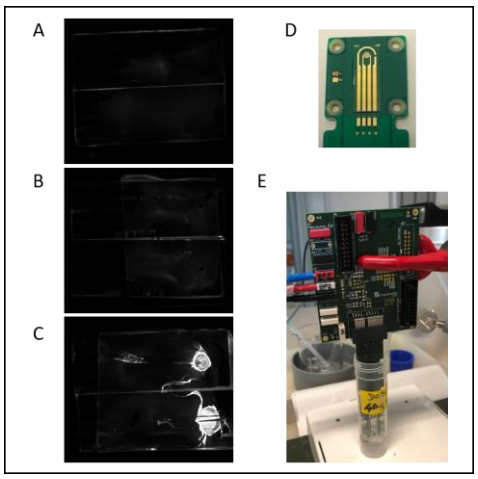
Functionalization of gold electrodes for impedance spectroscopy-based immunosensors

Using impedance spectroscopy for bio-sensing is very valuable as it is a technique which offers high detection sensitivity, is simple, label-free and portable. It is very suited for immunological analysis of samples, as antibodies can be immobilized on the electrode surfaces. In this project, an immobilization strategy for anti-cortisol antibodies on gold electrodes was developed.

State of the Art Many different methods exist for the covalent modification of gold surfaces. Almost all of them rely on the interaction between gold and thiol groups. While chemicals such as mercaptoundecanoic acid can be used for this, it can be useful to placer a linker between the thiol group and the active group. This can lead to higher binding capacity due to less steric hindrance.



Gold electrodes were activated with SH-PEG-COOH (A), activated and EDC and S-NHS crosslinking agents were added (B) and activated and antibodies together with EDC and S-NHS as crosslinkers were added. After washing, blocking and incubation with secondary antibody, chemiluminescence signal was recorded. Electrodes were wire-bonded (D) and attached to an electronic circuit for impedance measurements.

Analytical Approach In this work, the amino-groups of anti-cortisol antibodies were immobilized on gold electrodes via activation of the gold surfaces with Thiol-PEG-COOH and subsequent crosslinking of the antibodies via EDC/S-NHS. The electrodes were washed, blocked and incubated with a secondary antibody coupled to a horseradish peroxidase targeting the anti-cortisol antibody. After addition of hydrogen peroxide and luminol the electrodes were placed under a CCD camera to record chemiluminescence.

Results As can be seen in the figure, when only SH-PEG-COOH is present on the surface, no signal can be recorded (A). When SH-PEG-COOH is present together with the activation reagents EDC and S-NHS no signal can be observed as well (B). Only when antibody is added to EDC and S-NHS after activation with SH-PEG-COOH, can a chemiluminescence signal be observed (C). This means that the antibody successfully bound to the gold electrode. In the future, impedance spectroscopy measurements will show if specific binding of cortisol can be recorded.

Katharina Sollweck and Jamila Boudaden[1]

Funding

Fraunhofer EMFT

Cooperation

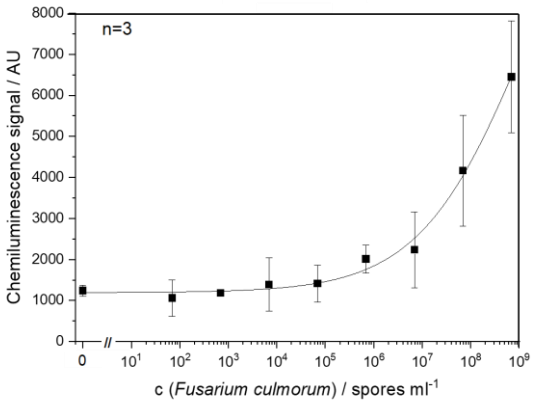
Fraunhofer EMFT

A chemiluminescence-based heterogeneous asymmetric recombinase polymerase amplification assay for the molecular detection of mycotoxin producers

In industrialized countries, a big part of people's life is spent indoors. Indoor mold as a cause for allergies and infections is long known but hardly dealt with. Also, the fact that indoor molds can produce very harmful mycotoxins is largely undervalued. Mycotoxins are small volatile secondary metabolites produced by fungi, which can cause disease and even death in humans. Little is known about the quantity and occurrence of these in indoor scenarios. In this project, we developed a method for quantification of mycotoxin producers by haRPA on a flow-based chip system.

State of the Art To date the commonly used methods to test for fungal contamination in indoor air are culture-based or microscopy methods which are relatively inaccurate. They often estimate wrong numbers due to spores aggregating or not being culturable. Also, these methods mostly do not give information about possible mycotoxin production.

Analytical Approach The aim of this work is to provide a fast and reliable molecular biological method to specifically detect mycotoxin producers. For this, an isothermal recombinase polymerase amplification assay on a microarray chip was developed. A mycotoxin biosynthesis gene is amplified on the chip surface where the amplicon can directly be detected via chemiluminescence. In the future, many different genes are planned to be detected.



Calibration curve for the detection of mycotoxin producers. Spore extracts were generated and lysed and the resulting DNA was used as template in haRPA reactions. Thereby a correlation between spore numbers and chemiluminescence signal could be obtained. The amplified gene is the polyketide synthase 4.

Results For the establishment of this method, glass and polycarbonate chip surfaces were compared and due to their low cost and good performance the latter were used for method development. We were able to develop this assay for zearalenone producers by amplification of a zearalenone biosynthesis gene from *Fusarium culmorum* on the DNA-chip. The method was tested for specificity and was calibrated. A quantitative detection of zearalenone producers is now possible. Successful preliminary experiments were performed also for trichothecene producers.

Katharina Sollweck and Gerhard Schwaiger

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AIF-ZIM

Cooperation

Domatec GmbH

Optimization and validation of the CL-SMIA for culture-independent screening and detection of *Legionella pneumophila* in artificial water systems

Cooling towers, evaporation coolers and wet separators can be responsible for Legionellosis outbreaks by releasing contaminated bioaerosols over several kilometers. Therefore, the combination of monolithic adsorption filtration (MAF) with chemiluminescence sandwich microarray immunoassay (CL-SMIA) can extend the assay to a culture-independent screening and detection method to describe the current status of such water systems.

State of the Art For the determination of *Legionella* contamination in cooling towers, evaporation coolers and wet separators, the industry is searching for culture-independent screening and detection methods. As the gold standard, the cultivation method needs 7 - 10 days with only unspecific detection of *Legionella spp.* Furthermore, the slow growth rate supports overgrowing of accompanying flora and cannot describe the status of such artificial water systems.

Analytical Approach The CL-SMIA uses antibody spotted polycarbonate chips for the detection of *L. pneumophila* serogroup 1 subgroups. The whole system was already evaluated for culture-depended characterization of *Legionella* bacteria concentrations over 10^5 CFU/mL. For culture-independent utilization, the sensitivity of the assay must be improved by combining the CL-SMIA with MAF as a suitable enrichment method for *Legionella.*

The device Microarray-Chip-Reader-Research (MCR-R) for the CL-SMIA performs a multiplexed serotyping and detection of *L. pneumophila* Sg 1 within 34 min.

Results For rapid quantification of *L. pneumophila* in surface water the MAF technology was already used with OH functionalized filters, which worked best at an acidic pH-value.[1] In cooling tower samples, the pH-value is 8 – 9 wherefore other functionalized filters would be more promising. For this, the diethyl amino ethanol (DEAE) filters seem to ensure a higher binding affinity without pre-treatment or acidifying of the sample. The final aim will be a factor 1000 enrichment from a sample volume of 1.0 L which can be detected and analyzed via the CL-SMIA.

Philipp Streich and Catharina Kober

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Bavarian Health and Food Safety Authority, Association of German Engineers, GWK Präzisionstechnik GmbH, Institute of Medical Microbiology and Hygiene, Institute of Virology, Medical Faculty “C. G. Carus”, Technical University of Dresden

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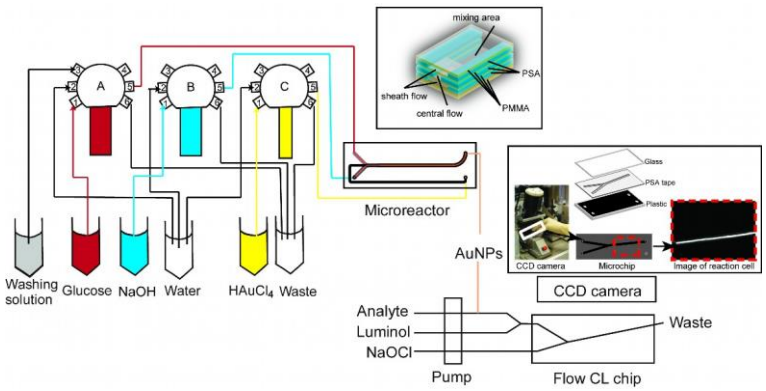
Synthesis of gold nanoparticles in a 3D hydrodynamic focused microreactor and their application for online chemiluminescence detection

A new method was developed for online synthesis of gold nanoparticles (AuNPs) with a 3D hydrodynamic focusing microreactor and directly coupled with a CCD camera for recording chemiluminescence (CL) signals.

State of the Art AuNPs have drawn big attention in CL system for biosensing application. The catalytic activity of AuNPs depends on size, shape, and surface charge property.[1] The conventional batch synthesis of gold nanoparticles is well known, however it was shown, that microfluidic reactors are able to synthesize AuNPs with narrower size distributions and faster reaction rates.[2]



Scheme of online synthesis and CL measurement system

Analytical Approach A 3D microreactor with layered PMMA sheets and pressure sensitive adhesive (PSA) tape was used to automatically synthesize AuNPs through a single-phase reaction in room temperature. The complete fabrication process from design concept to working device can be completed in minutes without the need of expensive equipment. The synthesis was coupled directly with CCD camera for recording CL signals. All operations were performed in an automatic way.

Results Fouling was prevented by utilizing 3D hydrodynamic focusing flow. The property of AuNPs was easily controlled by tuning concentration of reagents during synthesis. The synthesized AuNPs were used as catalyst for luminol-NaOCl CL system, and with optimized parameters of synthesis, the CL signal was enhanced hundreds of times. By adding some salt, AuNPs aggregated and the catalytic activity was greatly enhanced. Glutathione was detected as an example and the CL signal was inhibited which was related to the concentration of glutathione. For further application, synthesized AuNPs can be bind with antibody or aptamer for specific detection of analytes.

Yanwei Wang

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Funding

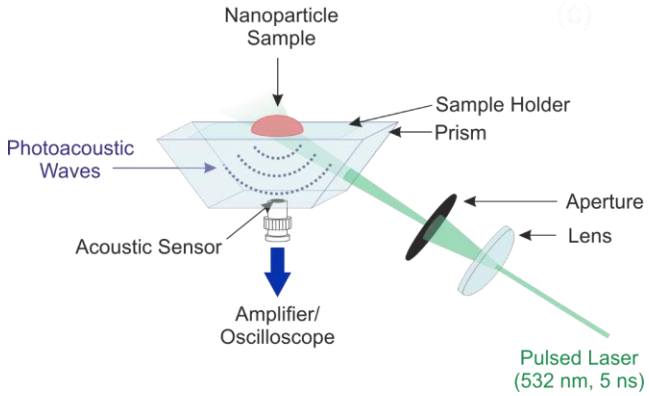
China Scholarship Council

Photoacoustic signal generation by coated gold nanoparticles

Gold nanoparticles (GNPs) have been shown to offer many advantages in biomedical photoacoustic (PA) imaging, perhaps most notably as exogenous contrast agents. Although GNPs have been used in previous biomedical PA imaging studies, more in-depth fundamental research can lead to new imaging methods.

State of the Art Photoacoustic imaging is an emerging biomedical imaging modality based on the formation of sound following the absorption of light. Nanoparticles are often used as molecular-targeting contrast agents in photoacoustic imaging. However, explicit photoacoustic detection of nanoparticles against the background absorption by biological tissue remains a challenge, limiting the potential of nanoparticles for deep dynamic monitoring of molecular targets.

Analytical Approach We investigate experimentally the PA signal generated in different colloidal suspensions of GNPs using our in-house custom-built PA scanner and compare our experimental results to theoretical predictions from our own developed theoretical model. The effect of particle coatings on the photoacoustic signal were investigated by coating a silica shell onto the GNPs.



Custom photoacoustic scanner for collecting signals from gold nanoparticle samples.

Results Our results clearly show for particle diameters of 100 nm and larger the photoacoustic signal is quadratic with excitation fluence, in contrast to most absorbers, which show a linear signal with respect to fluence. This nonlinearity was exploited into a novel method of discriminating nanoparticle contrast agents in photoacoustic imaging, entitled “Serial 3D PA Tomography”. We also discovered that the nonlinear photoacoustic signal from large gold nanoparticles can be quenched by coating the particle with silica. This newly discovered effect has the potential to be developed into a photoacoustic-based biochemical sensor.

Genny Pang

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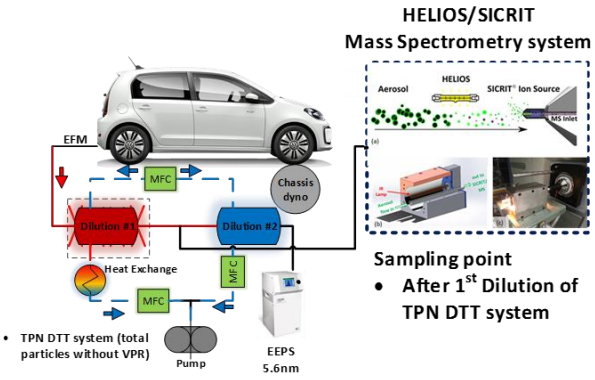
IWC-TUM

Cooperation
Institut für Physik Martin-Luther-Universität Halle-Wittenberg. Univ. Grenoble Alpes CNRS LIPhy

Down to Ten – characterization of combustion engine exhaust particles in the size range from 10 to 23 nm

Nanoparticles from combustion engine exhaust are a significant health issue. Current legislation limits particle concentration and size, but chemical composition information is important.



Our HELIOS/SICRIT/Mass Spectrometry system deployed in field measurements

State of the Art Current legislations typically characterize systems of aerosols, such as from vehicle exhaust, primarily by number concentration and size distributions. The chemical composition of particles, including the volatile and semi-volatile components adsorbed onto nonvolatile particle cores present at roadside and urban settings is important in understanding the impact of exhaust particles on health. To date, the only tools suitable for an online in-depth chemical aerosol characterization are aerosol mass spectrometers composed of complex and cost-intensive instrumentation. One focus of the DownToTen project is the development of analytical methods for the detection and characterization of sub-23 nm exhaust particles.[1]

Analytical Approach We present a new analytical system, which combines a novel inexpensive infrared-radiation-based evaporation system (HELIOS) with a commercially available highly efficient atmospheric ionization source (SICRIT) connected to a rather low-price ion-trap mass spectrometer. Our inexpensive, robust, and mobile aerosol characterization system enables highly sensitive chemical analysis of particle-associated volatile substances. We validated our HELIOS/SICRIT/Mass Spectrometry system in laboratory experiments and then employed our system to analyze real-world vehicle engine exhaust aerosol.

Results Our system was employed at the Aristotle University of Thessaloniki to monitor vehicle exhaust from a VW Up with different fuels, alkylate petrol and petrol. Measurements of real-world engine exhaust with the HELIOS/SICRIT/Mass Spectrometry system show that different chemical species types can be correlated with different size aerosol particles in the exhaust gas. Our work demonstrates that online chemical composition analysis is feasible with an appropriate aerosol characterization system, and this could potentially be implemented in future legislation on vehicle exhaust particle emission.

Genny Pang

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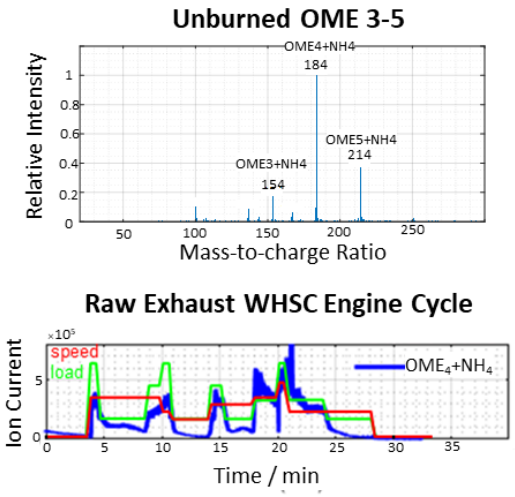
Sub-zero-emissions Diesel engine

The use of the soot-free burning fuel oxymethylene ether (OME) opens up completely new possibilities and potentials, which are to be taken up and innovatively implemented with the goal of sub-zero emissions. However, it has to be ensured that this approach does not result in the emission of other harmful contaminants.

State of the Art The oxygen-containing fuel oxymethylene ether (OME) is able to significantly reduce the raw emissions of diesel engines. Due to its largely soot-free combustion, particle emissions are reliably avoided in real, dynamic vehicle operation, which creates new degrees of freedom for reducing NOx, CO, and organic pollutant components. There is thus the prospect of moving vehicles with OME combustion engines locally emission-free. The aim of the project is to demonstrate emissions at a sub-zero level in real engine operation by avoiding emissions inside the engine with the OME fuel in combination with a specially adapted exhaust gas treatment. This highly ambitious goal requires extensive non-target screening for potential new contaminants.

Analytical Approach To investigate the products of OME combustion, we deployed our in-house developed aerosol characterization system based on a newly invented atmospheric ionization source (SICRIT) combined with a special infrared-based evaporation system (HELIOS) coupled with an ion-trap mass spectrometer to measurements of exhaust gas from a full motor at MAN Truck & Bus. We also performed controlled laboratory experiments in a heated flow reactor to investigate the storage and oxidation of OME in a diesel oxidation catalyst (DOC). The products exiting the DOC were analyzed with a mass spectrometer, a Fourier-transform infrared spectrometer, and a Scanning Mobility Particle Sizer.



Representative mass spectra of unburned OME fuel mixture (top) and representative mass trace of a single OME component in the raw exhaust from a test motor (bottom).

Results Mass spectra of unburnt OME fuel show that the SICRIT ionizes the OME components to form adducts with NH4. Measurements from the test site at MAN show that unburned OME is present in the raw exhaust gas, and the amount of unburned OME in the exhaust rises with the speed and load of the engine. Mass spectra of the exhaust exiting the after-treatment system show that not only is the unburned fuel in the exhaust removed but also that there are no other unidentified chemical substances with significant concentration present in the tailpipe exhaust. The flow reactor experiments show that the DOC oxidizes all OME, even in the absence of oxygen. At room temperature, the DOC has a high propensity for OME storage.

Genny Pang

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Bayerische Forschungsstiftung

Cooperation

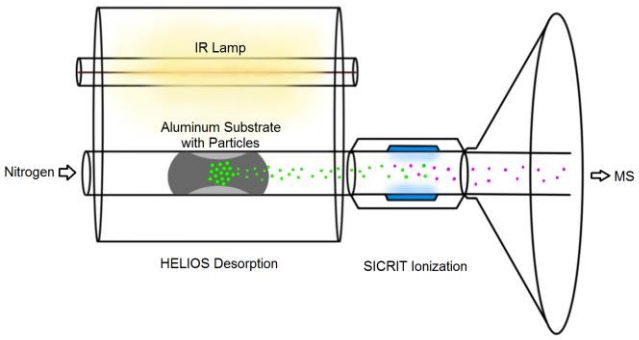
TUM Chair of Internal Combustion Engines, MAN Truck & Bus AG, Continental Emitec GmbH, ASG Analytik-Service-Gesellschaft mbH

Chemical characterization of atmospheric ultrafine particles

Ultrafine particles (UFPs) are known to impact climate and health, yet no method for daily monitoring of atmospheric UFPs exists. In this project, different methods are investigated to fill that gap.

State of the Art Many different techniques for the chemical analysis of atmospheric particles have been developed in the last decades.[1,2] However, no field-applicable method for day-to-day monitoring of ultrafine particles exists.

Analytical Approach Atmospheric particles were collected with an electrical low-pressure impactor (ELPI) on aluminum substrates. For the chemical analysis, different desorption methods coupled with atmospheric pressure ionization mass spectrometry were investigated. Laser desorption was realized with a 450 nm, 3 W continuous-wave laser. For thermal desorption, two different approaches were tested. One, using a heating cartridge and one using the HELIOS IR-heating device, previously developed within the group. As an ionization source, the SICRIT from Plasmion, the start-up founded by alumni J.-C. Wolf was used. The Desorption/SICRIT/MS methods were evaluated with test substances before investigating real samples. The collected particles were also analyzed with scanning electron microscopy and energy-dispersive x-ray spectroscopy (SEM-EDX).



Schematic drawing of the HELIOS/SICRIT/MS-setup. The particles collected on an aluminum substrate are thermally desorbed by IR-heating. A nitrogen flow carries them to the SICRIT, where ionization occurs and further into the mass spectrometer.

Results The applicability of laser desorption and thermal desorption for the investigation of different substance classes was shown. For the most promising approach using the HELIOS IR-heating device, a detection capability in the range of nanograms was found with pyrene as a test substance. Measurements of the collected particles with subsequent cluster analysis showed a similarity of the chemical composition of neighboring impaction stages of the ELPI. SEM-EDX measurements showed a shift in the composition of the particles from mainly carbonaceous species to inorganic species with increasing size.

Kevin Maier, Nina Weidlein, Karin Wieland

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Funding

Bayerisches Landesamt für Umwelt (LfU), IWC

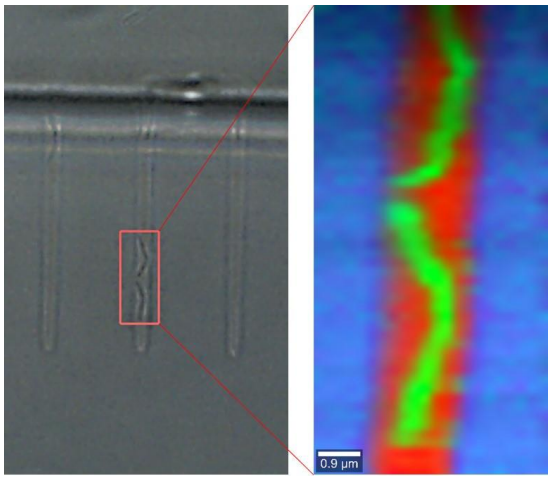
Cooperation

Bayerisches Landesamt für Umwelt (LfU)

Theoretical and experimental studies on microfluidic systems for bacteria cultivation

The mother machine, a microfluidic device, allows monitoring the growth and metabolism of single cells in a non-fixed approach. Through the adaption of a suitable design for *M. smegmatis*, it is possible to monitor single-cell metabolism by the combination of Raman spectroscopy and stable isotope labeling.

State of the Art The so-called mother machine (MM) is a microfluidic lab-on-chip device, which enables investigations on a single-cell level over hundreds of generations. It generally consists of several hundreds of dead-end growth channels, enabling observation of a high number of cells. These are connected to a broad main channel, which ensures continuous nutrient supply through diffusion-based mass transport.[1] Up to now, the MM is mostly used for the investigation of *E. coli* and there are no studies of *M. smegmatis*. This could be of great interest because *M. smegmatis* serves as a model organism for *M. tuberculosis*, which causes tuberculosis, the deadliest infectious disease worldwide.[2]



Left: Image of a 2 µm wide side channel filled with *M. smegmatis* (marked by a red square); right: color-coded image of Raman scan of the marked area: blue area: PDMS; red area: buffer inside the side channel; green area: *M. smegmatis* with incorporated deuterium.

Analytical Approach The system of a MM is adapted for *M. smegmatis* regarding cell dimensions. After the work with a MM is established in the lab and the side channels can be successfully filled with single cells, the metabolic activity of bacteria is investigated through the combination of Raman spectroscopy and stable isotope labeling.

Results A MM device was designed and fabricated using PDMS. With this, it was possible to fill 2 µm wide side channels with *M. smegmatis*. The process was observed by optical microscopy. The successful growth of bacteria in side channels after incubation of the MM overnight at 37 °C was monitored by the incorporation of a medium containing 50% deuterated water. Thus, a C-H/C-D exchange in single cells could be measured by Raman spectroscopy after flushing with a non-deuterated buffer (see figure).

Eva Krois

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Funding
IWC-TUM

Cooperation

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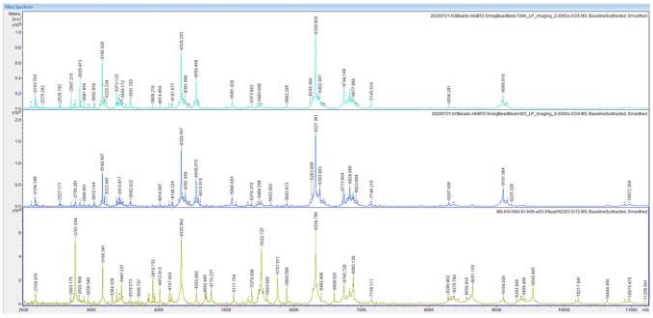
Altpeter, P. & Leu, C., Department of Physics, LMU, München

Magnetic bead-based isolation of DprE1 from Mycobacteria

BTZ043 is currently being tested in Phase II clinical trials as an antibiotic for the treatment of tuberculosis. Its target is the cell wall-integrated enzyme DprE1, which is irreversibly inhibited by the formation of a covalent bond. The isolation of pure DprE1 is very important for further research on DprE1 and as a control substance for the development of further antimycobacterial agents.



MALDI-TOF MS measurement of bead-alkynylated-BTZ043 conjugates after incubation from a cell lysate. Two dilutions of alkynylated BTZ043 of 1 ng/µL (Lane1) and 1 µg/µL (Lane 2) were applied.This measurement was compared to a protein spectrum from M. smegmatis. MALDI-TOF MS using positive ion linear mode with a mass range of 2-12 kDa.

State of the Art While the situation around the disease tuberculosis is worsening due to the increase of multidrug-resistant (MDR) and extreme drug-resistant (XDR) tuberculosis infections, research groups are working on a new group of antibiotics for the treatment of tuberculosis called 8-nitrobenzothiazinones. BTZ043 is currently being tested in Phase II clinical trials as an antibiotic for the treatment of tuberculosis. Its target is the cell wall-integrated enzyme DprE1, which is irreversibly inhibited by the formation of a covalent bond. The isolation of pure DprE1 is very important for further research on DprE1 and as a control substance for the development of further antimycobacterial agents.

Analytical Approach The isolation of DprE1 using magnetic beads coupled to BTZ043 derivatives pursued by MALDI-TOF MS did not lead to the desired result of pure isolated DprE1. Up to now, the transforming of *Mycobacterium smegmatis* towards an increased DprE1 expression and therefore higher chances in isolating DprE1 did not lead to clones. The MALDI-TOF MS measurement of the enzyme acetyl-/propionyl-coenzyme A carboxylase alpha chain indicates protein-protein interactions, which have to be considered during the analysis of the results. Based on the measurement of the carboxylase it is estimated that the experiment using a bead-BTZ043-conjugate, i.e. first bioconjugation then isolation of DprE1, to incubate it with a cell lysate could be important to prevent modifications of e.g. BTZ043 before the experiment started. Otherwise, the biotinylation of BTZ043 could be altered and the experiment could not be performed optimally. Consequently, the development of a detection method revealing if BTZ043 is bound to the beads or not would help to improve the bead-based isolation of DprE1.

Results The quality of the individual steps in the production of transformed *M. smgmatis* cells was evaluated by PCR, gel electrophoresis, and sequencing. Ligation of the plasmid caused difficulties under the tested conditions and could not be performed successfully.

Amelie Hohensee, Jessica Beyerl

Funding

IWC-TUM

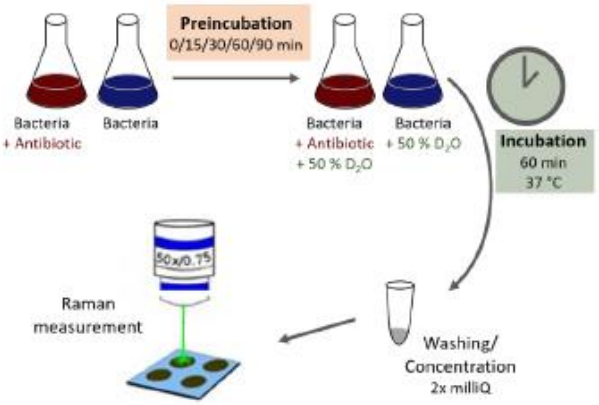
Cooperation

PD Dr. Wieser A. & Dr. Neumann-Cip, A. C., Max von Pettenkofer Institute, LMU, München

Antibiotic resistance and heteroresistance revealed by an extended Raman-based antibiotic susceptibility test (AST)

Worldwide, multiresistant bacterial strains are emerging at unprecedented rates. This development seriously threatens the ability of humanity to treat even common infections, resulting in disability and death. Evaluating metabolic activity of bacteria upon treatment by various antibiotics by Raman microscopy quickly reveals antibiotic resistance resp. heteroresistance.

State of the Art Antibiotics are one of the most effective ways of fighting bacterial infections, but the number of resistant germs is increasing, so it is even more important at this time to develop reliable and fast AST methods. However, the drastic increase of antibiotic-resistant bacteria is a fatal consequence of the wrong and careless use of the drugs. According to the World Health Organization (WHO), antimicrobial resistance constitutes a critical public health issue. One important way to tackle the spread of resistant bacteria is the rapid diagnosis of antibiotic susceptibility with analytical tools that help to monitor and understand the mechanisms of resistance.



Workflow of our preincubation strategy, which accounts for the time required until the antibiotic becomes effective.

Analytical Approach Raman spectroscopy as a non-destructive technique providing chemical-specific information is employed to monitor the deuterium uptake of metabolically active bacteria during antibiotic treatment, enabling fast and reliable AST. For this purpose, a bulk sample-preparation method was developed, allowing a high-throughput analysis of a significant number of cells.

Results A protocol was developed for Gram-positive (*E. faecalis*) and Gram-negative (*E. coli*) reference strains and tested on 51 clinical isolates with well-characterized resistance phenotypes against ampicillin, ciprofloxacin, meropenem, and vancomycin. Borderline resistant and heteroresistant phenotypes were observed and further investigated. This is of critical importance as the sensitive detection of low-frequency heteroresistance in bacterial populations is a huge challenge. Such isolates seem susceptible but are resistant to treatment in vivo. Automatable analysis detects strong phenotypes within 3h. Based on experimental and modeled data, heteroresistance is estimated to be detectable down to frequencies of 10^{-6} and investigated on clinical isolates as a proof of concept study; however, requiring longer incubation times.

David Bauer, Dr. Karin Wieland, Li Qiu

Funding

Deutsche Forschungsgemeinschaft DFG

Cooperation

Division of Infectious Diseases and Tropical Medicine, Department of Urology

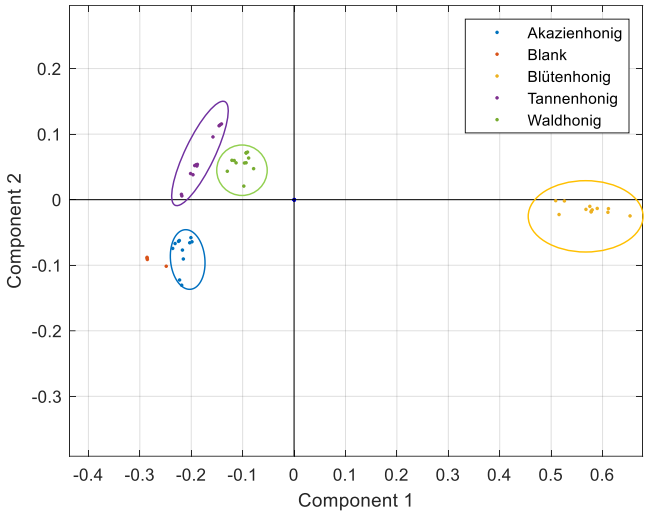
University Hospital

Ludwig-Maximilians-University Munich, Germany

SICRIT-MS: fast food classification and quality control

The SICRIT (“Soft Ionization by Chemical Reaction In Transfer”) ionization source is a versatile ambient ionization source capable of direct measurements as well as coupling to gas chromatography (GC). In combination with an autosampler, it enables a wide variety of automated measurements including aroma profiling matching a human sensory panel, classification of origin, non-targeted screening, and detailed quantitative analysis.

State of the Art Food fraud causes major financial losses and can be hazardous for human health. One example is the dilution of honey with corn sirup. Quality control in the food industry still relies heavily on human sensory panels for quality control and aroma profiling. This comes with the drawback of high costs, poor reproducibility, and a lack of quantitative information. Other instrumental techniques for quality control are often expensive and time-consuming, as for instance chromatographic techniques or NMR, which are currently used for honey analysis.



Principal component analysis of different types of honey. Based on the principal components different machine learning algorithms can be applied for classification.

Analytical Approach The SICRIT ionization source was coupled with a PAL autosampler and a triple quadrupole MS. This combination allows for direct headspace measurements of a wide variety of food samples such as honey, chocolate, or alcoholic beverages without further sample preparation or chromatography. Coupling to a high-resolution mass spectrometer can be used to increase selectivity. Different algorithms such as principal component analysis (PCA), linear discriminant analysis (LDA), gaussian naïve Bayes, or hierarchical clustering have been employed to classify samples or match measurements with a human sensory panel.

Results SICRIT was tested for a wide variety of classification tasks on food samples. Amongst others, classification of five different types of honey (fir, rap, acacia, forest, and flower) based on headspace measurements and identification of characteristic masses and corresponding components. In another study, unroasted coffee beans were classified based on their origin and the change of aroma compounds during the roasting process was monitored. Typical measurement times for the classification of headspace samples are below five minutes.

Markus Weber

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Funding
Plasmion GmbH
Cooperation
Plasmion GmbH

Raman spectroscopy for continuous reaction monitoring in microflow reactors

For the nitration of aromatic substrates in microflow reactors, Raman spectroscopy is employed and evaluated to monitor continuous flow reactions in line.

State of the Art The monitoring of nitration reaction of aromatics with nitrating acid in microflow reactors is typically carried out offline by GC-MS. Time-consuming sample preparation is necessary for this offline measurement. A faster alternative for reaction monitoring is the in-line Raman spectroscopy, which allows real-time and in-situ analysis. Based on the spectral fingerprint and elaborate data analysis, several parameters of interest such as the consumption of reactants, the formation of products, and the monitoring of hazardous species can be performed simultaneously. Compared to off-line GC-MS techniques, online Raman spectroscopy measurements are faster and do not require any sample preparation.

Figure: Microflow reactor system for nitration reactions with the Raman probe adapter for in-line Raman spectroscopy.

Analytical Approach An adapter fixing the Raman probe head to the tubing of the microflow reactor was constructed in a way that the focus of the Raman laser lays directly inside the process stream. Raman spectra were recorded in 60s intervals through the tube walls. The spectra are processed by a MATLAB (R2020b) script to monitor the temporal evolution of educt and product concentrations. A CW laser (532 nm; 100 mW) is used as an excitation source. The investigated reaction was the nitration of 4-Chloro-1H-pyrazole with nitrating acid to 4-Chloro-3,5-dinitropyrazole in a mixture of concentrated sulfuric and nitric acid.

Results The custom-made adapter for the Raman probe head was optimized for the highest Raman signal intensity of educt and product during monitoring in a PFA tube while minimizing background effects such as spectral interference of the PFA bands. It was possible to detect the educt and nitro compounds in the flow with our in-line Raman spectroscopy set-up. Product formation was determined indirectly using a calibration curve of the educt and considering the stoichiometry of the reaction. The intensities of a prominent band of educt were selected for this calibration.

Lucas Hirschberger, Karin Wieland

Funding

TUM

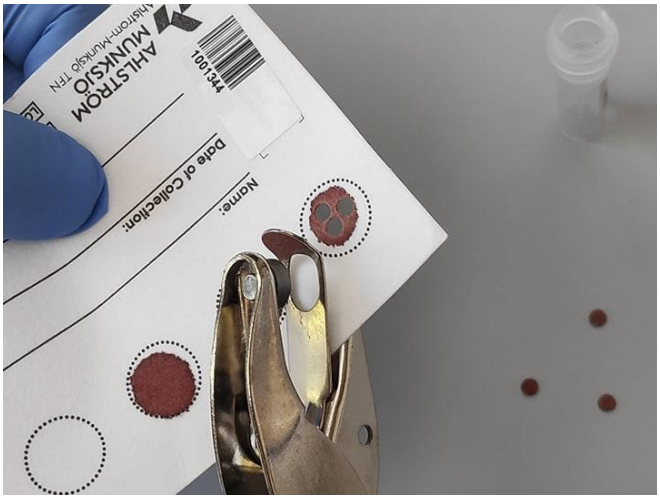
Cooperation

Department of Chemistry, Energetic Materials Research, Ludwig-Maximilian University of Munich

Dried blood spot protocol (DSP) for high throughput analysis of SARS-CoV-2 anti-N serology

SARS-CoV-2 is the well-known causative agent of the current pandemic. Serological tests detect antibodies that are formed after an immune reaction to the pathogen has taken place. We have developed a protocol to use self-sampled DBS for serological control and have tested this in a representative cohort in Munich.

State of the Art Seroprevalence studies are a valuable epidemiological tool that serves to refine a reasonable measure of exposure and spread within the population (R) moreover, they may also reveal key information on immune responses which could identify the potential donors for convalescent plasma (CP) therapy.



Dried blood spots and an extraction tool on special home-sampling card.

Analytical Approach In the past, Dried Blood Spots (DBS) based specimen collection have been extensively used in large-scale population screening programs where the specimens also reported showing less infectivity and increased target analyte stability for large epidemiological surveillance studies. DBS is preferred to the conventional venipuncture method for obvious reasons as it is less invasive and requires minimal storage conditions where samples can be shipped to the testing facility using regular mail services. Our current study aims to take advantage of the DBS sampling strategy to identify anti-N serological signatures of SARS-CoV-2 infection using Roche anti-N assay - which has sensitive and robust performance characteristics as evidenced by large-scale studies.

Results The assay combines high sensitivity with specificity (sensitivity of 99·20% and specificity of 98·65%). It is fast and the automated workflow allows for high throughput analysis. Roche Elecsys infrastructure is widely distributed globally and should be available at least in centralized laboratories in almost very country in the field of clinical chemistry. Combined with the use of globally widely available neonatal screening cards, this combination allows also for the transfer of the protocol to resource-limited settings. In many regions of the world, DBS sampling is often the only possible way to obtain large amounts of samples, making such a protocol especially valuable.

Jessica Beyerl, Raquel Rubio-Acero, Noemi Castelletti

Funding

LMU

Cooperation

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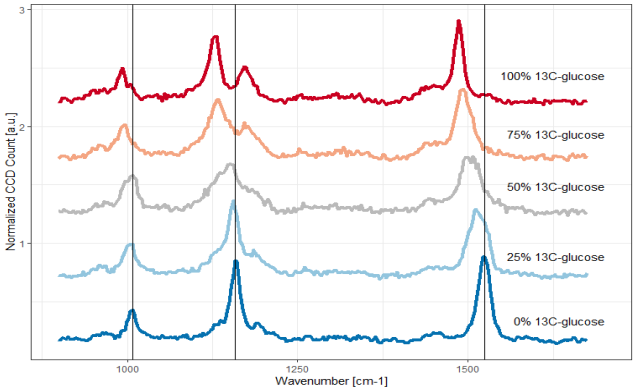
Ludwig-Maximilians-University Munich, Germany

Stable isotope Raman microspectroscopy for the analysis of plastic biodegradation

Plastics are considered as one of the most widespread environmental contaminants. However, the fate especially of micro- and nano-sized plastics remains mostly unclear. It is known that microbial digestion takes up a key role in relevant transformation pathways. Therefore, we apply Stable isotope Raman microspectroscopy (SIRM) to obtain further insights into microbial plastic degradation.

State of the Art Plastic biodegradation is often assessed by indirect observations like plastic mass loss or microbial growth. However, these methods lack a direct relation to the plastic's carbon conversion and are therefore not suitable as stand-alone approaches. Unequivocal information can solely be gained from observing metabolic degradation products (CO_2, CH_4) or the conversion of carbon from plastic into biomass. The latter can be achieved by stable isotope labeling techniques.[1,2]

Analytical Approach Inter alia Stable isotope Raman microspectroscopy is applied as a technique capable of both, to screen the metabolic activity (via Raman band redshifts at presence of stable carbon isotope ^{13}C) as well as to perform spatially resolved analysis of single cells.[2] *Sphingomonas koreensis* has been isolated from aged suspensions of polylactide (PLA) microparticles and serves as a model bacterium. Since ^{13}C-isotope labeled polymers are costly, a reverse labeling approach is applied initially to elucidate the potential of the system: bacteria are i) labeled with easier accessible ^{13}C-substance (redshift of bands) and then ii) incubated with unlabeled polymers (blueshift in case of carbon conversion).



Mean single cell Raman spectra (n ~ 50) of *S. koreensis* cultivated with different ratios of ^{13}C-glucose / ^{12}C-glucose (532 nm laser, 2 s integration time, 0.5 mW).

Results *S. koreensis* cells provide resonance Raman spectra related to β-carotene, an orange-colored pigment expressed by the bacterium. Cultivation with ^{13}C-glucose was successfully established, leading to ^{13}C-labeling of these pigments. A gradual redshift of bands was observed for increasing ratios of ^{13}C-glucose / ^{12}C-glucose provided in the culture medium. In a second step, labeled cells will be incubated with unlabeled polymer (PLA).

Julian Weng

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(Project IV 110/2-2)

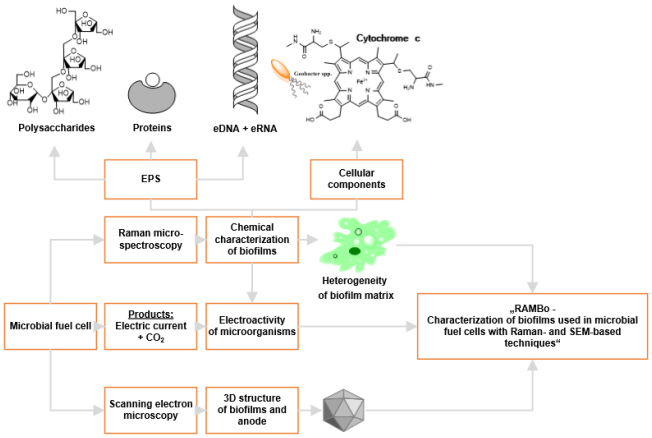
Cooperation

Dr. Michael Sander,

ETH Zürich

Characterization of biofilms used in microbial fuel cells with Raman- and SEM-based techniques / RAMBo

Exploring the potential of Raman microspectroscopy (RM) and scanning electron microscopy (SEM) for the *in situ* characterization of biofilms from microbial fuel cells (MFC)



RAMBo – an overview chart

State of the Art Electroactive biofilms (EAB) can produce free electrons through the process of metabolizing organic substrates and release those electrons into the surrounding environment via different shuttle mechanisms (e.g. *Cytochrome c* (*Cytc*) or conductive nanowires) (1). This phenomenon can be used to generate electricity with a simultaneous conversion of wastewater in MFC. Characterization of EAB represents one of the key elements to the longevity and stability of MFC-systems.

Analytical Approach The objective of this study is the *in situ* characterization of biofilms using Raman microspectroscopy accompanied by SEM analysis. Both techniques create a basis for information gathering and visualization of multispecies communities embedded in a matrix of extracellular polymeric substances (EPS) with the aim of determining the composition, structure, thickness and heterogeneity of electroactive biofilms.

Results In order to accelerate the initial bacterial adhesion onto graphite anodes different surface treatment approaches were considered. Surface modifications regarding porosity resulting from chemical (nitric acid, potassium hydroxide, acetone and sulfuric acid), heat- and ultrasonic treatments were monitored with SEM. Nitric acid and the combination of nitric acid and acetone revealed structures with pronounced pores of varying diameter and depth sizes. RM experiments were conducted for the targeted enrichment of electroactive microorganisms in fed-batch reactors by using activated sewage sludge. Bacterial *Cytc* was found in the liquid phase of the reactor vessel. The analysis of EPS composition of biofilms deposited on the electrode surface is in progress. Additional mass spectrometry results revealed *Citrobacter freundii* sp. as a potential electroactive microorganism.

Irina Beer

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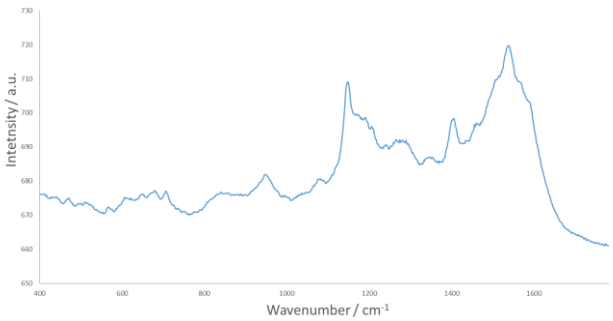
Cooperation

Prof. Dr. Thomas Hofmann, Dr. Karl Glas (Water System Engineering, Chair of Food Chemistry and Molecular Sensory Science, TUM)

SERS analysis of the active bacterial cells coupled with the modified silver nanoparticles

Atrazine biodegrader *Arthrobacter aurescens* TC1 cells were incubated with artificial amino acids (AHA) and coupled with alkyne-modified nanoparticles. SEM analysis and SERS were applied to detect distribution of nanoparticles on cell surface and to determine specific features of active bacterial cells.

State of the Art Mostly, cell consortia contain living, dead and dormant cells. Up to now efficient identification and characterization of active cells responsible for eco-physiological functions is on demand. Classical screening (e.g., gene sequencing, gene fingerprinting) describes bacteria which are present in a studied sample but does not disclose who is really metabolically active and growing. Therefore, efficient analytical approach for characterization of the active cells is required.



Average SERS spectrum of active *Arthrobacter aurescens* TC1, n = 16

Analytical Approach Surface-enhancement Raman scattering (SERS) is based on Raman microspectroscopy and provides highly sensitive signals allowing for detailed characterization of cells. Noble metal nanoparticles (NPs) are commonly used to significantly increase Raman signal. SERS combines the ability of Raman microspectroscopy to deliver information about chemical structure at high spatial resolution with the advantage of specific detection of cells in direct proximity to nanoparticles. The analysis is conducted in a rapid and non-destructive way. SERS can be combined with bioorthogonal non-canonical amino acid tagging (BONCAT) via modified NPs. BONCAT relies on the *in vivo* incorporation of amino acids analogues into cells and is used to study individual cell response to external signals *in situ*. Atrazine degrader *Arthrobacter aurescens* TC1 cells were used as a model.

Results Alkyne-modified NPs were synthesized and coupled to *Arthrobacter aurescens* TC1 cells. Specific distribution of NPs on the bacterial cell surface was determined via scanning electron microscopy (SEM). SERS signals were obtained on the active cells coupled with alkyne-modified silver NPs.

Oleksii Morgaienko

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Funding
IWC-TUM

Nanoplastic analysis by online coupling of Raman microscopy and Field-Flow Fractionation enabled by Optical Tweezers

An online coupling of Raman microscopy (RM) to field-flow fractionation (FFF) has been developed by a flow-cell that utilizes a 2D optical trap. Here, the separated particles in the size range of 200 nm – 5 µm are focused for spectra acquisition.

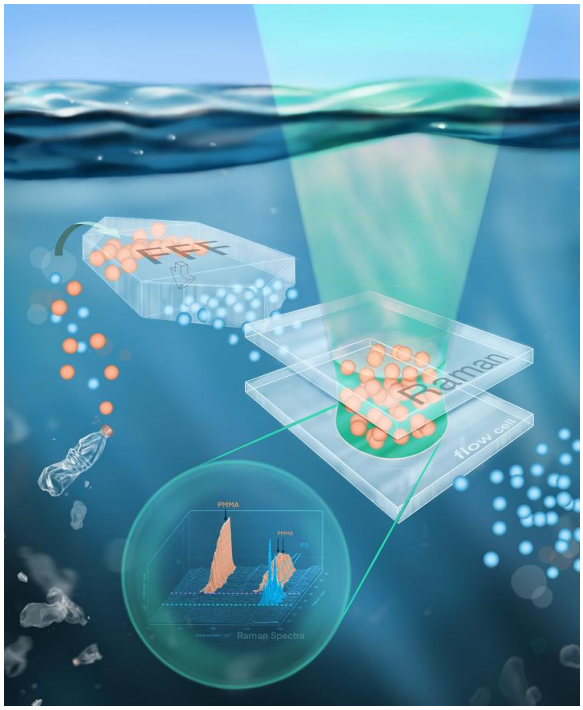

Illustration on the online coupling of RM and FFF, where particles are separated in the FFF channel and then chemically identified in the RM flow-cell providing time-resolved spectral information.[1] (Copyright 2020 ACS)

State of the Art Nanoplastic analysis poses new challenges compared to microplastic. Due to its smaller size, established methods are not sufficient. Flow-cells for Raman spectroscopy typically suffer a sensitivity problem that requires either concentrated samples or an enrichment.

Analytical Approach RM provides unambiguous identification of (plastic) particles by fingerprint spectra. This chemical characterization is complemented by the physical characterization that is offered by FFF, which, specifically, gives a particle size distribution by static light scattering and further detectors bring the potential for quantification.

Results The Raman flow-cell offers an inert bottom surface onto which particles are focused by the optical tweezers. Here, the particles experience a force equilibrium of the optical gradient force, optical scattering force, and shear force, which determine the required laser power to stabilize the trap (usually 20 mW). A periodic release preserves the separation information since the focusing could trap the particles indefinitely. The online coupling has been shown to work in the size range of 200 nm – 5 µm and in particle concentrations or around of 10^9 particles/L^{-1}. Further, two variants of FFF have both been demonstrated to be applicable: Asymmetrical flow FFF and Centrifugal FFF. Therefore, depending on the analysis scenario and the required information, the appropriate technique can be chosen. Thus, this setup offers the potential to close the methodological gap in nanoplastic analysis and possibly different fields, as well.

Christian Schwaferts

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Cooperation

Prof. Dr. Drewes, Chair of Urban Water Systems Engineering, TUM;

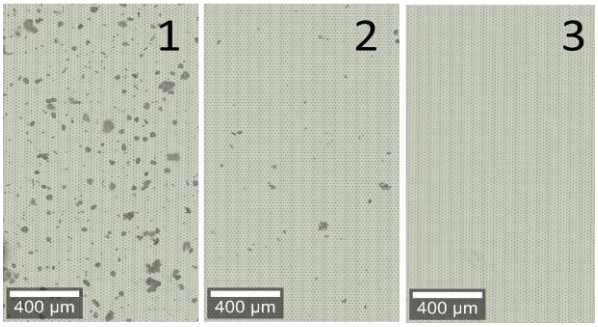
Postnova Analytics GmbH

Microplastic sample preparation avoiding contamination from sample arrival to measurement

Abstract: From sample arrival to measurement, how to avoid contamination for microplastic samples and deliver reliable results.

State of the Art Microplastic (MP) particles are polymer fragments (1 µm – 1 mm) that are increasingly found in a multitude of matrices. To determine the number of MP particles in a sample it is very important to avoid contamination during sampling, sample preparation, and measurement to deliver an accurate result.

Analytical Approach Different steps in the sample preparation were investigated for MP contamination, e.g., virgin PC filters and MilliQ water used for sample dilution and rinsing. For reusable Si filters, the aptitude of the RCA cleaning process was evaluated.[1] Additionally, we participated in a ring trial by the JRC to determine the accuracy of our measurements.



Surface of a Si filter: Efficiency of a modified RCA cleaning process[1]: Initial state (after filtration of PLA standard particles) (1), after five minutes of ultrasonic cleaning (2), after conducting the whole cleaning procedure (3).

Results Microplastic contaminations and the ring trial samples were analyzed by automated single-particle Raman microspectroscopy (RM) using *TUM-ParticleTyper*.[2-4] The analysis ruled out a contamination of virgin PC filters prior to their use for microplastic samples. The RCA clean method was identified as a promising method for the regeneration of Si filters after their use. The main contamination source determined by this approach was the MilliQ water used for sample dilution. Extreme variations in contamination levels were observed (seven measurements, 50−1300 PS particles/L, 5000−14000 particles/L overall). To ensure the purity of the MilliQ water an additional filtration step (cellulose acetate filters, 0.45 µm pore size) was added. It removed 100% of PS contamination and decreased the overall number of particles by about three quarters. The improved sample preparation procedure together with the established analysis method for automated single-particle RM was applied to ring trial samples. The achieved results were within the specification of the reference material provided for the trial, verifying a successful quantification of microplastic.

Elisabeth von der Esch and Oliver Jacob

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Funding

Bayerische Forschungs-stiftung (Project MiPAq)

BMBF (Project HOTMIC)

Cooperation

Prof. Dr. Jürgen Geist

Dr. Karl Glas, TUM;

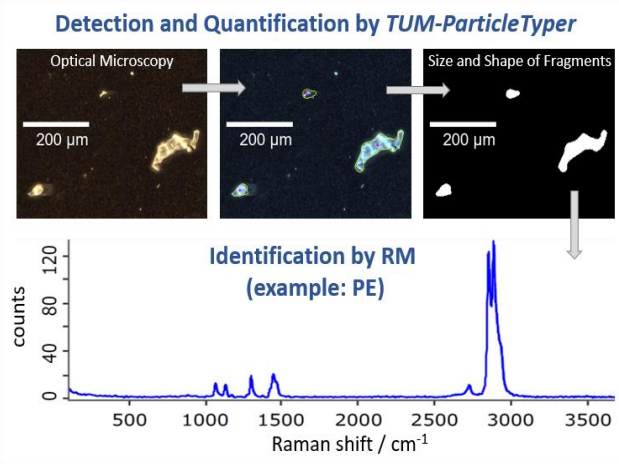
Dr. Aaron Beck,

GEOMAR, Kiel, Germany

Raman microspectroscopic analysis of small micro-plastic particles

Further development of a method for quantification of microplastic particles in aqueous matrices. The aim is a reliable quantification of particles smaller than 10 µm.

State of the Art The JPIOceans project HOTMIC (horizontal and vertical oceanic distribution, transport, and impact of microplastics) investigates the fate of plastic waste. This includes the need for the detection of small microplastic particles (<10 µm). A reliable method for the analysis of particles down to 10 µm (maximum Feret's diameter) has already been developed by E. von der Esch and A. J. Kohles et al. [1]. With this method, the recognition of all particles with respect to the entire filter surface is done automatically employing the software *TUM-ParticleTyper*. Then, up to 7000 randomly selected particles are measured by Raman microspectroscopy.



Schematic representation of the three steps involved in the microplastic analysis: 1) taking an optical stitched image of the filter surface, 2) detecting number, size, and shape of fragments and 3) identifying the material of individual fragments with single point measurement (Raman microspectroscopy).[1]

Analytical Approach Firstly, improved image processing can help to reduce the minimum number of pixels per particle needed for reliable recognition. The avoidance of false negatives is most important in that context. This is combined with the use of higher optical magnification. Accordingly, a large increase in the time required must be avoided. A statistical approach for particle detection may be a solution, so only parts of the filter surface are then measured (C. Schwaferts and P. Schwaferts et al., in preparation).

Results An additional opening filter as a part of image processing reduces the minimum number of pixels per particle needed to approx. 35 pixels. In combination with 50× magnification (instead of 20×) and the statistical approach mentioned above, the reliable detection of particles down to 3 µm might be achieved. These improvements correspond to the further development of *TUM-ParticleTyper*.

Oliver Jacob

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(1) von der Esch, E.; Kohles, A. J.; Anger, P. M.; Hoppe, R.; Niessner, R.; Elsner, M.; Ivleva, N. P., Simple generation of suspensible secondary microplastic reference particles via ultrasound treatment. PLOS ONE, 2020, 15, e0234766. DOI: 10.1371/journal.pone.0234766.

Funding

Bundesministerium für Bildung und Forschung, BMBF (Project HOTMIC)

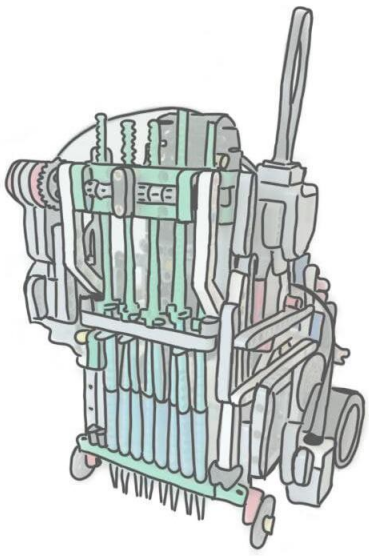
Cooperation

Dr. Aaron Beck, Prof. Eric Achterberg (GEOMAR, Kiel, Germany)

New seminar: automation and visualization of laboratory processes and data

In the chemical industry, laboratory processes are often automated, yet in our research labs, we still find many manual steps. However, chemistry students are not yet equipped to automate their research. Therefore, we created a course to teach students how to design pipetting, sorting, and transport robots, as well as programming automated data analysis scripts. Under pandemic conditions, the course also works at a distance.

State of the Art Research experiments are often unique. They must be tailored precisely to the research question at hand. To maintain flexibility, it is therefore often easier to run experiments manually. However, this comes at the expense of efficiency, accuracy, and, frankly spoken, the researcher's patience. Many research laboratories have fully automated state-of-the-art measuring equipment. However, repetitive manual steps are often required from sample preparation to data analysis, which most chemistry students lack the knowledge to automate.



Approach Our goal was to show chemists how to automate their experiments using simple methods so that they can spend their valuable time interpreting the results and developing new ideas. Experimental progress can thus potentially be accelerated enormously, and at the same time, the reproducibility of experiments and data analysis can be increased.

Results Our course on "Automation and Visualization of Laboratory Processes and Data", addresses this gap by teaching students 1) the basics of programming in Python, statistics, and robotics to work on 2) chemometrics projects, and 3) LEGO robotics projects. The overall goals in designing all three parts of this 5 ECTS (150 h) course were to stimulate creativity and encourage a high and continuous level of student involvement and participation. The results of our first seminar (summer term 2020) can be viewed on our YouTube channel.

Trailer: youtu.be/VGvVTpfsBKc

Video on chemometrics: youtu.be/F7PxeptH1Tk

Laboratory robots in action: youtu.be/gezAQ6Zll64

Elisabeth von der Esch, Alexander J. Kohles, Beatriz von der Esch

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Funding

Fond der Chemischen Industrie

Fakultät Chemie, TUM

Cooperation

TUMlab im Deutschen Museum: Mike Kramler and Miriam Voss

Publications

Amininejad, S.; Ivleva, N. P.; Baumann, T., Characterization of coatings on metallic nanoparticles by surface-enhanced Raman scattering (SERS) for environmental purposes. Vadose Zone Journal 2020. doi.org/10.1002/vzj2.20076

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Qiu, L.; Pang, G. A.; Zheng, G.; Bauer, D.; Wieland, K.; Haisch, C. Kinetic and mechanistic investigation of the photocatalyzed surface reduction of 4-nitrothiophenol observed on a silver plasmonic film via surface-enhanced Raman scattering. ACS Applied Materials & Interfaces 2020 doi:10.1021/acsami.0c05977

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Conference Presentations

Oral Presentations

Göpfert L., von der Esch, Schwaferts, C.; Koros, R.; E.; Elsner M., Ivleva N.P., Seidel, M., Seidel, M., Characterization of bacterial microplastic deg-radation via Raman microspectroscopy. SETAC 2020 SciCon, 3.-7.5.2020, virtual

Schwaferts, C.; Sogne, V.; Drexel, R.; Meier, F.; Klein, T.; Niessner, R.; Elsner, M.; Ivleva, N. P., On-line coupling of Raman microscopy and field-flow fractiona-tion enabled by optical tweezers for nanoplastic analysis. 20th International Symposium on Field- and Flow-Based Separations (FFF2020), 23.-27.2.2020, Vienna, Austria.

Schwaferts, C.; Sogne, V.; Welz, R.; Meier, F.; Klein, T.; Niessner, R.; Elsner, M.; Ivleva, N. P., Subμ-Plastik-Charakterisierung mittels einer Online-Kopplung von Raman-Mikrospektroskopie und Feldflussfraktionierung, Wasser 2020, Short presentations. July 2020. The Gesellschaft Deutscher Chemiker (GDCh), Germany.

Poster Presentations

Glöckler, D.; Bakkour, R.; Müller, M.; Werneburg, M.; Zwiener, C.; Elsner, M., Occurrence of organic micropollutants in the Schönbrunnen sub-catchment and its implication for higher order streams. CAMPOS International Confer-ence 2020, 23-25.03.2020, virtual.

Glöckler, D.; Bakkour, R.; Jiménez Fernández, Ó.; Schwientek, M.; Osenbrück, K.; Elsner, M., Occurrence of organic micropollutants in the Schönbrunnen 2.Development of rapid and sensitive isotope analysis of δ15N and δ18O to decipher sources and turnover of nitrate in the Ammer Catchment. CAM-POS International Conference 2020, 23-25.03.2020, virtual.

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Morgaienko, O.; Weng, J.; Elsner, M.; Ivleva, N. P., Raman microspectroscopy analysis of atrazine biodegraders under different physiological conditions. Wasser 2020, Short presentations. July 2020. The Gesellschaft Deutscher Chemiker (GDCh), Germany.

Schwaferts. C.; Sogne, V.; Welz, R.; Meier, F.; Klein, T.; Niessner, R.; Elsner, M.; Ivleva, N. P., Nanoplastic analysis by Raman microscopy on line coupled to field flow fractionation via optical tweezers. SETAC 2020 SciCon, 3.-7.5.2020, virtual.

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von der Esch, Lanzinger, M.; Kohles, A. J.; Schwaferts. C.; Weisser, J.; Hofmann, T.; Glas, K.; Elsner M., Ivleva N.P., Simple generation of suspensible secondary microplastic reference particles via ultrasound treatment. SETAC 2020 SciCon, 3.-7.5.2020, virtual.

von der Esch, E.; Kohles, A. J.; Elsner, M.; Ivleva, N. P., „Wie viele Mikroplastik-Partikel haben wir in unserer Probe?“ Wie können wir diese Frage in einem akzeptablen Zeitrahmen beantworten? Wasser 2020, Short presentations. July 2020. The Gesellschaft Deutscher Chemiker (GDCh), Germany.

Invited Lectures

Elsner, M. Herausforderungen, Möglichkeiten und Entwicklungen der Wasseranalytik, *Abschiedskolloquium der DVGW Landesgruppengeschäftsstelle Bayern für Herrn Möller und Herrn Traue*, 11.02.2020, Munich, Germany

Haisch, C.; Bauer, D.; Qiu, L.; Wieland, K.; Magistro, G.; Stief, C.; Neumann-Cip, A.-C.; Wieser, A. Raman plus isotopes for antibiotic susceptibility and heteroresistance testing Analytica Conference 2020, 18.11.202020, Munich, Germany, virtual.

Ivleva, N. P., Bunsen-Kirchhoff Award Lecture: Raman Microscopy for Environ-mental Analysis. Analytica Conference 2020, 20.11.2020, Munich, Germany.

Seidel, M., Modern analytical methods for pathogens and antibiotic resistant bacteria.

Scientific Committees & Memberships

Elsner, M., Young Academy of Europe, YAE (Member)

Elsner, M., Wasserchemische Gesellschaft, Fachgruppe der GDCh (Board Member)

Elsner, M., Environmental Science & Technology (Member of the Editorial Advisory Board)

Elsner, M., ACS ES&T Water (Member of the Editorial Advisory Board)

Elsner, M., Journal of Isotopes in Environmental and Health Studies (Member of the Editorial Advisory Board)

Elsner, M., Evaluation Panel Member of the Swiss National Science Foundation

Ivleva, N. P, DIN-Normenausschuss NA 054-01-06 AA „Kunststoffe und Umweltaspekte“)

Ivleva, N. P, NA 057 DIN-Normenausschuss Lebensmittel und landwirtschaftliche Produkte (NAL)

NA 057-08-05 AA Arbeitsausschuss Bestimmung von Mikroplastik in Lebensmitteln

Ivleva, N. P, ISO/TC 61/SC 14 "Plastics and Environment" / WG 4 „Microplastics“ (DIN Expert)

Seidel, M., Member of the Scientific Committees at the European BioSensor Symposium

Seidel, M., Member of the working group „Messen und Bewerten von Legionellen“ (NA 134-03-07-09 UA) bei der Kommission Reinhaltung der Luft im VDI und DIN

Seidel, M., Member of the working group „Bioaerosole und biologische Agenzien – Luftgetragene Mikroorganismen und Viren“ (NA 134-03-07-04 UA) bei der Kommission Reinhaltung der Luft im VDI und DIN

Seidel, M., Member of the working group CEN/TC 264/WG 28 “Microorganisms in ambient air” (NA 134-03-07-03-01 AK)

Seidel, M. Chairman of the expert committee at the Wasserchemische Gesellschaft: Pathogens and antibiotic resistant bacteria in the water cycle

Theses

PhD Theses

MSc Chem. Philipp Anger: Strategien zur Analyse von Mikroplastik mittels RAMAN-Mikrospektroskopie

MSc Chem. Elisabeth von der Esch: The Automation and Validation of a Morphological and Chemical Quantification Procedure for Microplastic Fragments using Raman Microspectroscopy

MSc Chem. Catharina Kober: Entwicklung von flussbasierten Chemielumineszenz-Mikroarrays zur schnellen Quantifizierung, Lebensfähigkeitsuntersuchung und Serotypisierung von *Legionella spp.* und *Legionella pneumophila* in Kultur-, Wasser- und Urinproben

MSc Chem. Christina Lihl: Deciphering Chlorohydrocarbon Transformation Mechanisms by Advancing δ13C/δ37Cl Compound-Specific Isotope Analysis

MSc Chem. Li Qiu: Applications of D_2O Labelling Combined with Raman Microscopy for Mycobacteria Analysis and Application of SERS for the Investigation of Photocatalytic Reaction

MSc Geol. Marina Spona-Friedl: Substrate Dependent Heterotrophic CO_2-Fixation as Indicator for Metabolic Phenotypes

MSc MBT Katharina Zirngibl: Generierung und Charakterisierung eines monoklonalen Antikörpers für die Detektion von Retronecin-basierten Pyrrolizidinalkaloiden und seine Verwendung für die Entwicklung eines Immunoassays

M.Sc. Theses

BSc Andreas Auernhammer: Development of a Residue Analysis Microarray Chip for Milk

BSc Michael Becker: Development of an Extraction Method for Non-Targed Screening in River Sediment and Suspended Particulate Matter

BSc Carolin Feyerabend: Particle Meets Pollutant – Investigating the Sorption of Organic Trace Substances on Microplastics via TD-Pyr-GC/MS

BSc Leonhard Prechtl: Development of an Effect Orientated Analytical Method by Coupling of a Neurotoxicity Assay with LC-MS

BSc Sonja Hoffmann: Investigation of Functionalized Porous Aluminium Oxide as Sensitive Layer for Electronical Gas Detection

BSc Amelie Hohensee: Production of DprE1 by Genetic Transformation into pGFPHYG2 and Magnetic Bead-based Isolation of the Protein from Mycobacteria

BSc Oliver Jacob: Characterisation of Microplastics by Means of Raman Microspectroscopy and TUM-Particle Typer

BSc Eva Krois: Theoretical and Experimental Studies on Microfluidic Systems for Bacteria Cultivation

BSc Philipp Streich: Development of a Chip-based Detection System for Rapid Identification of Antibiotic Resistance Genes in *Klebsiella Pneumoniae* and *Escherichia Coli* via Molecular Fusion

B.Sc. Theses

Raffaela Geier: On-site Phage-mediated Detection of Bacteria using Colorimetry

Rosa Carolina Koros: Analysis of Different Surface Activation Strategies in the Development of a SARS-CoV-2 Microarray Immunoassay

Charlotte Heinritz: Characterization of pH Dependence for Concentration of Pseudomonas Aeruginosa with Monolithic Adsorption Filtration

Nur Atiqah Binte Bedin: Synthesis of Gold Nanoparticles in a 3D Flow-based Microreactor and their Application in Analytics

Teaching

Winter Semester 2019/2020

Analytische Chemie I, Instrumentelle Analytik

240242322 Geo-Umwelt LMU (BSc Geo.) M. Elsner

Chemische Analytik II - Organische Spurenanalytik für Geowissenschaftler

820486258 Geo-Umwelt LMU (BSc Geo.) M. Elsner

Wasserchemie 1

820005191 Geo-Umwelt LMU (BSc Geo.) M. Elsner

Angewandte Wasserchemie

0000005206 Chemistry (MSc Hydrogeo.) M. Elsner, R. Bakour

Chemische Analytik II – Organische Spurenanalytik für Geowissenschaftler

820486258Geo-Umwelt LMU (BSc Geo.) M. Elsner

Current Research in the Instrumental Analysis of Trace Components 1 (Practica)

0000001973 Chemistry (MSc Chem.) M. Elsner, C. Haisch, N. P. Ivleva, M. Seidel

Current Research in the Instrumental Analysis of Trace Components 1 (Lecture)

0000002469 Chemistry (MSc Chem.) M. Elsner, C. Haisch, N. P. Ivleva, M. Seidel

Environmental Chemistry

0000001972 Chemistry (MSc Env. Eng.) M. Elsner, R. Bakour

Fortgeschrittene analytische Verfahren

0000004763 Chemistry (BSc Chem.) M. Elsner, C. Haisch, N. P. Ivleva, M. Seidel

Hydrochemisches Praktikum

820678299 Hydrology (MSc) R. Bakour, C. Haisch

Hydrochemisches Praktikum für Geologen

0000003397 Hydrology (MSc Geo.) R. Bakour, C. Haisch

Hydrogeologisches, hydrochemisches und umweltanalytisches Seminar

240037914 Chemistry M. Elsner, C. Haisch, M. Seidel

Instrumentelle Methoden der Anorganischen Chemie

(CH3000b) 0000002336 (MSc Chem.) M. Elsner, N. P. Ivleva…

Lab Rotation Analytical Chemistry 1 (CH3124)

0000002910 Chemistry (MSc Chem.) M. Elsner, C. Haisch, N. P. Ivleva, M. Seidel

Lab Rotation Analytical Chemistry 2 (CH3125)

0000002932 Chemistry (MSc Chem.) M. Elsner, C. Haisch, N. P. Ivleva, M. Seidel

Seminar Institut für Wasserchemie

0000004167 Chemistry M. Elsner, C. Haisch, M. Seidel

Summer Semester 2020

Automatisierung und Visualisierung von Laborprozessen und Daten

0000004577 Chemistry (MSc Chem.) M. Elsner, N. P. Ivleva, E. v. d. Esch

Biochemische Analytik

0000001651 Weihenstephan (BSc Bio.) M. Seidel

Biochemische und molekularbiologische Verfahren in der Umweltanalytik II – Enzymatische Verfahren, DNA Sonden

820032502 M. Seidel

Case Studies in Analytical and Environmental Chemistry

0000002532 Chemistry (MSc Chem.) M. Elsner, R. Bakour

Aerosole: Bedeutung, Vorkommen und deren Charakterisierung

0000005602 Chemistry C. Haisch, R. Nießner

Hydrogeologisches, hydrochemisches und umweltanalytisches Seminar

240037914 Chemistry M. Elsner, C. Haisch, M. Seidel

Instrumentelle Methoden der Anorganischen Chemie (CH3000b)

0000002336 (MSc Chem.) M. Elsner, N. P. Ivleva

Lab Rotation Analytical Chemistry 1 (CH3124)

0000002910 Chemistry (MSc Chem.) M. Elsner, C. Haisch, N. P. Ivleva, M. Seidel

Lab Rotation Analytical Chemistry 2 (CH3125)

0000002932 Chemistry (MSc Chem.) M. Elsner, C. Haisch, N. P. Ivleva, M. Seidel

Physikalisch-chemische Aerosolcharakterisierung

0500003556 Chemistry C. Haisch

Physikalisch-chemische Aerosolcharakterisierung Blockpraktikum

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Student Assistants

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Korbinian Geißer
Alexander Kohles
Gerhard Schwaiger

Equipment

Hydrogeology

Two pilot-scale tanks with flow lengths of up to 10 m allow transport experiments in a controlled environment while preserving almost natural conditions. Apart from studies on the transport behavior of contaminants and colloids, these facilities are used for testing sensor prototypes and serve as a testbed for numerical models

Großhadern Unsaturated Zone field laboratory (10 m deep)

Aerosol Research

1 Aerosol chamber (1 m^3)

1 Aerosol flow tube (10 L)

1 Ozone analyzer (UV absorption)

1 NO/NO_2 analyser (Chemiluminescence)

1 Aerodynamic particle sizers (0.5-25 µm)

1 Laser Aerosol Spectrometer (size range 90 nm -7.5 µm)

1 Berner impactor (9 stages, 50 nm - 16 µm)

1 Electrical low-pressure impactor (12 stages, 30 nm – 10 µm)

2 Low-volume filter samplers (PM 10, PM2.5)

1 High-volume filter sampler (PM 2.5)

3 Differential mobility particle sizer systems (10-1000 nm)

2 Diffusion batteries (5-300 nm)

5 Condensation nucleus counters

3 Electrostatic classifiers (10-1000 nm)

2 Spark-discharge soot aerosol generators (polydisperse ultrafine carbon aerosol)

1 Berglund-Liu aerosol generator (monodisperse aerosols, 0.8-50 µm)

1 Floating bed aerosol generator (powder dispersion)

1 Rotating brush aerosol generator (powder dispersion)

1 Tube furnace

1 Cyclone impinger (Coriolis µ, Berlin)

1 AVL Micro Soot Sensor with dilution unit

2 FT/IR gas analyzers

Bioseparation

1 Crossflow-ultrafiltration unit (6 m^2 hollow fibre module, Inge-AG)

1 Munich Microorganism Concentrator (MMC 3)

1 Monolithic Affinity Filtration Unit

Microarray Technology

2 Chemiluminescence Microarray Reader (Immunomat, IWC)

4 Chemiluminescence Microarray Reader (MCR 3, GWK GmbH)

1 Ink-Jet Microdispenser (SciFlexarrayer 31, scienion)

2 Contact Microarrayer (BioOdyssee Caligrapher, BioRad)

2 Cutting Plotter (Graphtec CE6000-40)

Microbiology

1 Flow Cytometer (CyFlow Cube 6, Sysmex Partec GmbH)

1 Water Microbiology (Colilert-18 and Quanti-Tray 2000, IDEXX)

3 Clean benches

1 Microbiological Incubator (BD 53, Binder)

1 Autoclave (Century 2100, Prestige Medical)

1 Autoclave (SHP Steriltechnik)

Standard Lab Equipment

1 Lyophilizer (Alpha 1-4 LSC, Christ)

1 Washer Disinfector (DS 500 Lab, International Steel CO.SPA)

1 Ultrapure Water System (Direct-Q 3 UV, Millipore)

1 Cooled Centrifuge (Universal 320R, Hettich)

1 Centrifuge (Eppendorf 5804 R)

1 Climatic chamber (Memmert HCP 108)

2 Fluorescence reader systems, time-resolving

3 Photometric reader systems

1 384-channel washer, Biotek

1 Turbidometer (WTW GmbH)

1 Nanophotometer (Implen GmbH)

Chromatography, Mass Spectrometry and Particle Separation

2 GC-IRMS (Isotope Ratio Mass Spectrometer) Instruments

2 GCs with FID and ECD

1 Orbitrap-based benchtop MS, Exactive/HCD-System, Thermo Fischer

1 MS, Thermo Fisher LTQ

1 Asymmetrical Field-flow-fractionation system, Postnova

2 Concentrators for dynamic headspace analysis

4 HPLC, UV/VIS array detector, programmable fluorescence detector

1 Capillary electrophoresis system

1 Ion chromatograph, Dionex

1 LC system, ECONO

1 Preparative HPLC

Elemental Analysis

1 Flame-Photometer, BWB Technologies

1 ICP-MS, Perkin -Elmer Nexion 350D

Laser

2 He/Ne-laser

5 Nd-YAG -laser, pulsed

1 Nd-YAG Laser 2 W cw, 532 nm narrow band

3 Nd-YAG-laser, cw

several diode lasers (600-1670 nm; up to 2 W CW)

several Quantum Cascade Laser systems

3 Optical parameter oscillator (410 nm-2.1 µm)

Optoelectronics/Spectrometer

3 Echelle spectrometer

1 FTIR-Spectrometer, Thermo Scientific Nicolet 6700

1 Fluorescence spectrometer, Perkin Elmer LS-50

1 Fluorescence spectrometer, Shimadzu RF 6000

1 UV/VIS spectrometer, analytic jena Specord 250 plus

1 UV/VIS spectrometer, analytic jena Spekol 1500

4 Digital storage oscilloscopes (400 MHz, 500 MHz)

1 Wavemeter

Microscopy

3 Laser Raman microscope, WITec alpha300R (532/633 nm)

1 Laser Raman microscope, Renishaw 2000 (514/633/785 nm)

1 Laser Raman microscope, Horiba LabRam HR (532/633/785 nm)

1 Temperature controlled stage (-196 °C – 600 °C, Linkam THMS 600)

1 SEM/EDX system, Zeiss Gemini

Sum Parameters

2 Coulostat for C quantification, Coulomat 702

1 DOC analyser, UNOR 6 N

1 TOC analyser, Shimadzu TOC-L