



In the Biannual Workshop selected speakers from 9 high rank Research Institutes in the fields of Chemistry, Medicine, Life Sciences and Physics, plus 2 start up centres for Companies, introduce their methods and ideas. A perfect opportunity to look for inter-disciplinary cooperation and networking within the campus. Attend and learn more about:



Leibniz-Institut für Altersforschung
Fritz-Lipmann-Institut e.V. (FLI)

- How to perform atmospheric measurements to verify Kyoto CO₂ reduction targets.



Friedrich-Schiller-Universität Jena
seit 1558

- How to look inside a living cell while it is responding to environmental change.

- How to actually watch a plant's immune system at work.



- An efficient method for visualizing autoantibodies in patients' serum.

- How to construct virus vectors for gene transfer.

- The development of a good idea and a small scale method in to production scale at company level.



PROGRAM :

13:00

PD Dr. Jan Kellman (MPI-CE)
Welcome Address & Chair of sessions

Session 1:

13:05 ca.

Dr. Christoph Gerbig (MPI-BGC)
Jan Winderlich and Dr. Rona L. Thomson (MPI-BGC)
The CO₂ budget: methods for estimating CO₂ fluxes from atmospheric observations.



13:35 ca.

PD Dr. Christoph Krafft (IPHT)
Dr. Jürgen Popp (IPHT)
Vibrational spectroscopic imaging in life sciences



14:05 ca.

Rohitt Shroff, IMPRS Fellow (MPI-CE)
Seeing the chemical fingerprint of nature: Mass spectrometric imaging of metabolites.



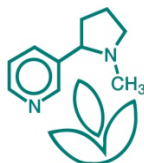
14:35 ca.

Coffee Break

Session 2:

15:00

PD Dr. Christine Skerka (HKI)
Identification of disease-related autoantibodies in patient serum by protein-microarray analysis.



15:30 ca.

PD Dr. Andreas Henke (IVAT, Universitätsklinikum, FSU)
Expression of recombinant proteins by viral vectors: methods and applications.



16:00 ca.

Jürgen Schenk (Jesalis Pharma GmbH) **Dr. Uwe Müller** (Hapila GmbH)
High-efficiency processes for purification of active pharmaceutical ingredients.

The CO₂ budget: methods for estimating CO₂ fluxes from atmospheric observations

Christoph Gerbig, Jan Winderlich and Rona L.Thompson

Max Planck Institute for Biogeochemistry, Jena

e-mail: [cgerbig\[at\]bgc-jena.mpg.de](mailto:cgerbig[at]bgc-jena.mpg.de)

It is important improve estimates of regional CO₂ fluxes for two main reasons. Firstly, the Kyoto Protocol has set binding targets for parties to reduce CO₂ emissions by 5% relative to 1990 levels over the period 2008 – 2012. Under the Protocol, countries' actual emissions have to be monitored to determine if any reduction has occurred and for emissions trading to be effective. Secondly, to understand the natural feedbacks on CO₂ flux from variations in climate.

This talk describes one framework for determining CO₂ fluxes using high precision atmospheric measurements of CO₂ and a regional atmospheric transport model. Atmospheric measurements of the CO₂ mixing ratio over time from ground based stations show significant variability on diurnal, synoptic, seasonal and inter-annual timescales. This variability is caused by changes in atmospheric transport and vertical mixing as well as by changes in CO₂ fluxes. Within the framework, the transport and mixing processes are modelled and with some *a priori* knowledge of the spatial and temporal variation the CO₂ fluxes, this facilitates the estimation of CO₂ fluxes from CO₂ mixing ratios.

However, there are numerous challenges involved; the accuracy of the atmospheric transport model and the measurements, and insufficient *a priori* knowledge of the fluxes all come into play.

Gerbig et. al. (2008): Vertical mixing in atmospheric tracer transport models: error characterization and propagation, *Atmospheric Chemistry and Physics* 8(3): 591-602.

Rödenbeck et. al. (2003): CO₂ flux history 1982–2001 inferred from atmospheric data using a global inversion of atmospheric transport, *Atmospheric Chemistry and Physics* 3: 1919-1964

Vibrational Spectroscopic Imaging in Life Sciences

Christoph Krafft, Jürgen Popp

Institute of Photonic Technology, Jena, e-mail: [Christoph.Krafft\[at\]ipht-jena.de](mailto:Christoph.Krafft[at]ipht-jena.de)

Raman and infrared spectroscopy are the most commonly used techniques in vibrational spectroscopy. They probe molecular vibrations which constitute a highly specific fingerprint of molecules without labels. Due to technical progresses in light sources and detectors, the sensitivity was improved so both methods were successfully applied in life sciences. Combining with lateral information gives powerful imaging techniques. Coherent anti-Stokes Raman spectroscopy (CARS) is an emerging non-linear variant of Raman spectroscopy. It enables the acquisition of images at video-rates. The presentation summarizes previous studies and gives an outlook to diagnose cells and tissues using a combination of vibrational spectroscopic imaging technologies.

Living cells are preferably investigated by Raman spectroscopy using 785 nm excitation lasers because this wavelength has been shown to be non-destructive even at intensities in the milliwatt range. The lateral resolution of Raman microscopes is limited by diffraction to ca. 500 nm and enables to resolve subcellular details such as the nucleus and vesicles. Glyoxal-induced cell stress was recently studied using Raman imaging and demonstrated that both morphological and biochemical changes could be probed simultaneously¹.

Fourier transform infrared (FTIR) imaging spectrometers with focal plane array (FPA) detectors offer shorter acquisition times than Raman imaging. Hundreds of individual cells can be investigated within minutes by FTIR microscopic imaging. However, the lateral resolution is decreased to 5 to 10 μm due to the longer wavelength of mid-IR radiation from 2.5 to 25 μm . Unstimulated and stimulated mesenchymal stem cells were distinguished with and without expression of glycogen and hydroxyapatite, respectively, using FTIR imaging and supervised data classification².

Coupling of Raman spectrometers to fiber optic probes increases the flexibility of data acquisition. Miniaturized probes can be inserted into the working channel of endoscopes for minimal invasive applications. The methodology was developed using murine brain metastases under ex vivo conditions³ and transferred to in vivo situations through a cranial window.

1) C. Krafft et al. Anal. Chem (2006) 78: 4424-4429

2) C. Krafft et al. Analyst (2007) 132: 647-653

3) C. Krafft et al. Anal. Bioanal. Chem (2007) 389: 1133-1142

Seeing the chemical fingerprint of nature: Mass spectrometric imaging of metabolites

Rohit Shroff

IMPRS Fellow; Mass Spectrometry Research Group, Max Planck Institute for Chemical Ecology, Jena. e-mail: rshroff@ice.mpg.de

The precise location of various metabolites in biological systems may have significant biological importance. Conventional analytical approaches involve whole tissue or organ level pooling of information, whereby all the information on spatial distribution within a single tissue or cell is entirely lost. Hence high resolution imaging technologies are required that could provide spatial information. Here we present a mass spectrometry base label free imaging method¹ to study the distribution of secondary plant metabolites in *Arabidopsis thaliana* leaves and its relevance as an intricate plant defence system against chewing herbivores.

The generalist lepidopteran larvae, *Helicoverpa armigera* (the cotton bollworm), avoided the midvein and periphery of *Arabidopsis thaliana* rosette leaves and fed almost exclusively on the inner lamina. This feeding pattern was attributed to glucosinolates since it was not evident in a myrosinase mutant that lacks the ability to activate glucosinolate defences by hydrolysis. To measure the spatial distribution of glucosinolates in *A. thaliana* leaves at a fine scale, we constructed ion intensity maps from MALDI-TOF (matrix assisted laser desorption ionization-time of flight) mass spectra. The major glucosinolates were found to be more abundant in tissues of the midvein and the periphery of the leaf than the inner lamina, patterns that were validated by HPLC analyses of dissected leaves. In addition, there were differences in the proportions of the three major glucosinolates in different leaf regions. Hence, the distribution of glucosinolates within the leaf appears to control the feeding preference of *H. armigera* larvae. The preferential allocation of glucosinolates to the periphery may play a key role in the defence of leaves by creating a barrier to the feeding of chewing herbivores which frequently approach leaves from the edge².

1) Caprioli, R.M., Farmer, T.B. & Gile, J. Molecular imaging of biological samples: Localization of peptides and proteins using MALDI-TOF MS. *Anal. Chem.* 23, 4751-4760 (1997).

2) Shroff, R., *et al.* Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. *Proc. Nat. Acad. Sc.* 105 (16), 6196-6201 (2008).

Identification of disease related autoantibodies in patient serum by protein-microarray analysis

Christine Skerka

Leibniz-Institute for Natural Product Research and Infection Biology

e-mail: [christine.skerka\[at\]hki-jena.de](mailto:christine.skerka@hki-jena.de)

Diagnostic tools in medicine are a fast developing research area. The goal is to develop a test or assays which are cheap, fast and simple and which generate significant information about specific parameters of a disease. These key informations are essential for diagnosis and therapy of a disease.

Protein micro array systems allow to address specific questions regarding the presence and concentration of certain proteins in samples such as human plasma, cell culture supernatants or cell lysates of different strains of microorganisms. In all cases specific antibodies can be used for the detection of the proteins and the quality of the antibodies mostly determines the sensitivity and quality of the assay. The detection of autoantibodies and immune complexes is an additional important aspect of application of the array systems.

Autoimmune diseases are a heterogeneous group of disorders which represent a major challenge for diagnosis and adequate therapy. One of these autoimmune diseases is 'atypical hemolytic uremic syndrome (aHUS)'. aHUS is a severe kidney disease and can effect children as well as adults. A short overview of this disease and the use of microarray technique for the diagnosis of a subgroup of aHUS patients will be presented.

1) Józsi et al. Blood 111:1512-1514 (2008).

Expression of recombinant proteins by viral vectors: methods and applications

Andreas Henke

Institute of Virology and Antiviral Therapy, Clinical Center, Friedrich Schiller University Jena. e-mail: [i6hean\[at\]rz.uni-jena.de](mailto:i6hean[at]rz.uni-jena.de)

During the last decades, several different procedures have been developed to deliver essential genes safely, to the right organ, and turn them on and off at will. The original idea of these methods was focused on treatments of certain hereditary diseases.

Today, gene delivery systems are also being developed to prevent or treat cancer, heart disease, or a variety of infectious diseases. All of them are based on the expression of a specific gene to produce a desired protein when and where it is needed. Basically, viruses are very simple organized parasites, but their ability to invade cells and reorganize the cellular machinery to express proteins has won them an important part in gene delivery experiments.

A number of reports have been published demonstrating the potential to use different picornaviruses as gene transfer vehicles. Infectious full length cDNA copies of these viruses exist, which allows manipulating the viral RNA. Experimental data indicate that approximately 700-750 additional nucleotides can be inserted into picornaviral genomes and enables a stable expression of foreign proteins. Recombinant proteins are not incorporated into mature virus particles; the virus structure, antigenicity, and host range are not altered. The site of gene material insertion into the viral RNA is different and seems to be important for expression efficacy. So far four different principles have been established to construct recombinant picornavirus vectors to express small peptides or even biological active proteins. A short review of these methods to establish such expression systems and data about their experimental application are presented⁽¹⁻³⁾.

1) Henke et al., Expert Rev. Vaccine, 2003, 2(6), 805-15.

2) Jarasch et al., Apoptosis, 2007, 12, 1633-43.

3) Henke et al., Expert Rev. Vaccine, 2008, in press.

High-efficiency processes for purification of active pharmaceutical ingredients

Jürgen Schenk Jesalis Pharma GmbH, Jena

Uwe Müller HAPILA GmbH, Gera. mailcontact: [mail\[at\]biocentiv.com](mailto:mail[at]biocentiv.com)

Jesalis Pharma and HAPILA are companies founded in 2006/2007, both engaged in the pharmaceutical business. The main focus of HAPILA's activities is the development and manufacturing of active pharmaceutical ingredients, including chemical syntheses, purification and particle design. The customers are R&D-based pharmaceutical companies as well as biotech, generic and fine chemical companies worldwide.

Jesalis Pharma is orientated to drug product development with main focus on analytical issues. With its own products Jesalis Pharma supplies international markets with focus on South East Asia, China and Middle East.

Both companies built up and promote a close co-operation network to regional partners like University of Jena, University of Applied Sciences Jena, Hermsdorf Institute for Technical Ceramics, chemical network 4chiral and others. With their know-how and experience, both companies cover a wide range of the value chain of pharmaceutical drug substances and drug products. HAPILA and Jesalis Pharma successfully started cooperation projects like highly efficient processes for the synthesis and purification of active pharmaceutical ingredients including analytics:

Nowadays the requirements a pharmaceutical active substance has to comply with are very high. For unspecified impurities a content of below 0.1 % or 0.05 % is specified in ICH Q3A¹. To reach this purity level different purification principles are used, mainly simple crystallisation, chromatography and membrane technology. Often, these processes are expensive with low yield and therefore they contribute significantly to the costs of production.

The team has developed a new continuous counter current crystallisation process, which is characterized by

- achievement of high purity combined with a high yield,
- cost-effective process design,
- good scalability from lab to production scale,
- a qualified process, meeting the GMP regulations and appropriate for handling high active substances safely.
- A patent applied for by HAPILA is pending.

13.30¹ ICH Harmonised Tripartite Guideline: Impurities in New Drug Substances Q3A(R1), 07.02.2002