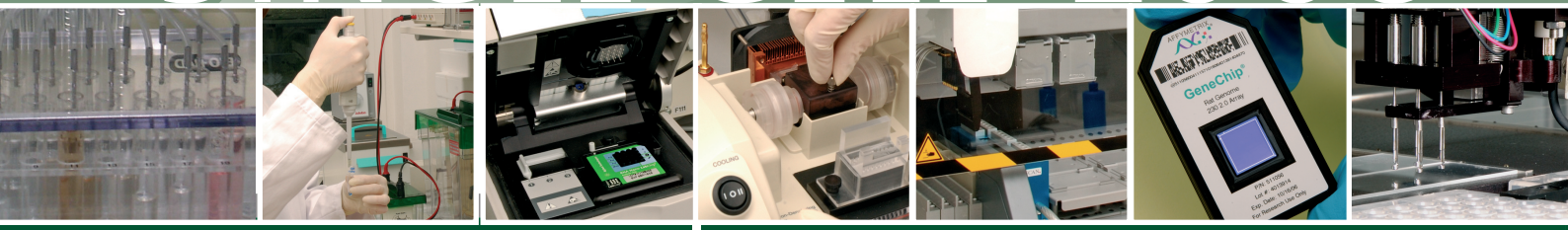
MÜNSTER ZKF

SINGLE CELL 2008



5th Münster Conference on

Single Cell and Molecule Analysis

Progress in Research and Technology

November 24-25, 2008

medizinische fakultät münster. Hohe Schule der Medizin.

medizinische fakultät
Westfälische Wilhelms-Universität Münster

Westfälische Wilhelms-Universität Münster

Under the Auspices

of the Interdisciplinary Center for Clinical Research (IZKF)
„The Chronic Disease“ of the Medical Faculty of the University of Münster

Technology Platform and Core Unit
„Integrated Functional Genomics“ (IFG)

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Program

Monday, November 24

1:00 pm **Stephan Ludwig**
Opening and Introduction

1:05 pm **Alfred Yergey**
Keynote Lecture
Variability in Mass Spectra – How we can get better answers

1:50 pm **Katherina Psathaki**
The cell: An animated introduction

2:15 pm **POSTER & COMPANY EXHIBTION**

2:30 pm **WORKSHOP** - LaVision BioTec - Uwe Schröer
BioAnalyzer Gel - Proteomic imaging system for unstained and stained Gels

3:15 pm **WORKSHOP** - Carl Zeiss - Monika Stich
Laser Capture Microdissection: A new dimension in sample purity

SESSION I **TECHNOLOGY**
CHAIR: **Stephan Ludwig**

4:00 pm **Philip Tinnefeld**
From single molecules to biomolecular interactions and super resolution microscopy

4:25 pm **Andreas Offenhäuser**
Non-invasive monitoring of cellular ion-channel activity using electronic devices

4:50 pm **Uwe Karst**
Quantitative analysis of fluorescently labeled thiols and amines by microchip capillary electrophoresis with wavelength-resolved fluorescence detection

5:15 pm **Johannes Wessels**
Popular Science Lecture
Big Bang & Primordial Soup: The hunt for the Quark-Gluon-Plasma

6:00 pm **GET-TOGETHER**

6:15 pm **MÜNSTER-AT-NIGHT SIGHTSEEING TOUR**

Tuesday, November 25

SESSION II **OPTICAL IMAGING**
CHAIR: **Malcolm Clench**

9:00 am **Christian Lohr**
ATP in the brain: More than an energy currency

9:25 am **Michael Schäfers**
Isotope-based imaging of molecular targets in tissues and living animals

9:50 am **Christoph Bremer**
In vivo cell tracking using magnetic resonance and optical imaging techniques

10:15 am **Klaus Tiemann**
Small animal ultrasound – from functional to molecular imaging

10:40 am **COFFEE BREAK**

SESSION III **MASS SPECTROMETRIC IMAGING**
CHAIR: **Alfred Yergey**

11:00 am **Malcolm Clench**
SPOTS, SMOTS and SLOTS - Shotgun Proteomics, Shotgun Metabolomics and Shotgun Lipidomics on Tissue Samples

11:25 am **Marten Snel**
MALDI MS Imaging coupled with high-efficiency ion mobility separation

11:50 am **LUNCH, SEMINAR & COMPANY EXHIBITION**

1:00 pm **Cell Biosciences -** Andy Higgs
Getting towards protein isoform assays in single cells

1:25 pm **AlphaMetrix** - Udo Schimmel
Gene expression profiling from a minimal number of LCM selected pure cells

Tuesday, November 25

SESSION IV **CELLS**
CHAIR: **Christian Lohr**

1:50 pm **Christian Wilhelm**
Physiological characterization of plant cells by means of single cell analysis

2:15 pm **Heinrich Leonhardt**
Targeting and tracing antigens in living cells

2:40 pm **Christian Korfhage**
Single-cell whole genome amplification: Reliability and limits

3:05 pm **Jürgen Klingauf**
Coupling of exo- and endocytosis: insights from single-vesicle recordings

3:45 pm **END OF CONFERENCE**

KEYNOTE LECTURE

Alfred Yergey
Section on Mass Spectrometry and Metabolism
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Variability in Mass Spectra – How we can get better answers

Mass spectrometry of single cells is not currently possible, but advances in instrumentation may make that possible at some point in the, perhaps not too distant, future. When such a thing becomes possible, there are a number of technical issues that will need to be examined very closely about any results. In fact, we have seen the consequences of these issues already arising in so-called day-to-day experiments in proteomics. The problem lies in the variability of data obtained. There is a fundamental variability in biological materials, but beyond that there exists a variability in the mass spectra themselves that is all too commonly ignored. Issues of experimental variability are discussed and an approach to statistically quantify the variability in mass spectra is presented along with a demonstration of the remarkable improvement in results that it yields.

ORAL PRESENTATION

Katharina Psathaki
Cell and Developmental Biology
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The Cell: A close look

Organisms contain organs, organs are composed of tissues, tissues consist of cells and cells are formed from molecules. All organisms are made of cells and small organisms even consist only of single cells. Cells can survive on their own, but organisms cannot live without cells.

Cells are small and complex, it is difficult to see their structure, hard to discover their molecular composition, and harder still to find out how their various components function and interact. Understanding the structural organization of cells is an essential prerequisite for learning how cells funcion. Using simple light microscopy, individual cells have been identified to be the fundamental unit of life and light microscopy still plays a major role in biological research. An important advantage of optical microscopy is that light is relatively nondestructive. By intrinsically fluorescent proteins tagged on specific cell components, we can watch their movements, dynamics and interactions in living cells. But optical microscopy is limited in resolution by the wavelenght of the visible light and thus limited in the fineness of detail that it can reveal.

By using a beam of electrons instead, electron microscopy can image the macromolecular complexes within the cell at almost atomic resolution. The normal effective resolution for biological objects is 1 nm, which is 200 times better than the resolution of the light microscope. The higher resolution in electron microscopy comes at a cost: specimen preparation is much more complex, cells are fixed and it is difficult to ensure that what we see in the image correspond precisely to the actual structure being examined. However, it is yet possible to use very rapid specimen high pressure freezing methods without using chemical fixatives to prevent artefacts and to preserve the native structures of the cell. Furthermore, a three-dimensional reconstruction of cell structures at the resolution level of electron microscopy (3D electron tomography) is available and will be presented here. Combining light and electron microscopy will strongly impact on our future understanding of cellular structures and dynamics, and thus, will provide much deeper insight into the amazing complexity not only of individual macromolecules but also in their interaction in a living cell.

ORAL PRESENTATION

Philip Tinnefeld
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From single molecules to biomolecular interactions and super-resolution microscopy

In a top down approach, novel microscopic techniques allow the resolution limitations in the far-field to be overcome. In parallel, single-molecule fluorescence techniques are climbing the ladder of complexity from the bottom up and enable problems of increasing diversity to be investigated. These two approaches merge when resolution enhancement in far-field microscopy is achieved by subsequently localizing the position of individual molecules. Many of these impressive approaches require extremely stable fluorophores or even completely new kinds of fluorescent probes such as photoswitchable fluorophores. In this presentation several aspects of these recent developments are discussed. A new approach to control the photophysics of single fluorophores is used to reduce bleaching as well as for a new type of superresolving fluorescence microscopy. Further it will be shown how the scope of single-molecule Fluorescence-Resonance-Energy-Transfer (FRET) measurements is extended to interactions of increasing complexity by involving more than two fluorescent dyes.

ORAL PRESENTATION

Andreas Offenhäuser
Institute for Bio- und Nanosystems-2
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Germany

Non-invasive monitoring of cellular ion-channel activity using electronic devices

Electrophysiological measurement of ion channel activity has been of great importance in areas ranging from fundamental neuroscience research to drug screening and pharmaceutical applications. The conventional patch clamp technique, a high resolution but low efficiency technique, has been established for 25 years. Recent advances in micro- and nanotechnology have opened up new possibilities for non-invasive measurements based on field-effect transistors. Our research activities focus on the functional coupling of biological signal processing and recognition elements with micro- and nanoelectronic semiconductor devices and circuits for the development of future biosensors and molecular diagnostics tools. This talk will describe the concept of directly interfacing genetically modified cells containing G-protein receptors with electronic devices.

ORAL PRESENTATION

Uwe Karst
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Quantitative analysis of fluorescently labeled thiols and amines by microchip capillary electrophoresis with wavelength-resolved fluoresecence detection

In the last few years, various well-established analytical techniques were minaturized to perform their tasks in a lab-on-a-chip. The majority of work focuses on the development and application of such microfluidic devices, but only little work was performed dealing with quantitative analysis. This talk proves that quantification of real samples can reproducibly be realized by chip electrophoresis and illustrates the difficulties and challenges that are associated with miniaturization. On microchips, accuracy and reproducibility are affected by several factors, e.g., electrolysis of the running buffer, capillary clogging, buffer evaporation and unstable voltage switching. Based on two examples this work demonstrates numerous problems that emerge with downscaling of the instrumental dimensions.

In the first study, mercaptoacetic acid and 2-mercaptopropionic acid, were derivatized with ammonium 7-fluoro-2,1,3-benzoxadiazole-4-sulfonate (SBD-F) and then determined in depilatory cream and cold wave suspensions. In the second example, taurine was fluorescently labeled with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazol (NBD-Cl) and then quantified in energy drink samples.

In both studies, the derivatized samples were introduced into the separation channel of a glass microchip by a pinched injection. A self-assembled fluorescence microscope-based instrument was used for detection. This setup features wavelength resolution of the emitted fluorescence light, which reveals additional information about the analyte. The developed methods were compared to reference methods utilizing CE-DAD and HPLC fluorescence.

ORAL PRESENTATION

Johannes Wessels
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Germany

The Hunt for the Quark-Gluon-Plasma

With the advent of the Large Hadron Collider (LHC) at CERN a completely new energy domain will be accessible for nuclear and particle physicists. At these high energies predictions of quantum chromodynamics (QCD), the fundamental theory that describes the role of quarks and gluons in nuclear matter, come into play. In collisions of heavy nuclei the properties of a completely new phase of matter, the so-called quark-gluon-plasma, can be studied. This may illuminate our view of the basic structure of matter on the sub-atomic scale and bears important implications for the development of the universe on the cosmic scale.

In the talk, I shall try to elucidate in very basic terms some of the theoretical concepts as well as the experimental methods employed in modern nuclear and particle physics research. The main focus of the talk will be on the ALICE-Experiment, one of the four large experiments at the LHC.

ORAL PRESENTATION

Christian Lohr
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ATP in the brain: More than an energy currency

Adenosine triphosphate (ATP) is an ubiquitous energy currency molecule in living organisms. In addition, ATP serves as an extracellular messenger that mediates communication between cells. In the nervous system of mammals, e.g., ATP is co-released with classical neurotransmitters such as acetylcholine and norepinephrine at synapses and binds to purinergic receptors of postsynaptic cells, leading to ionic currents and Ca^{2+} signalling. The molecular mechanisms, however, by which ATP is released from neurons are only sparsely investigated. We employed imaging methods to find out whether ATP is released from axons of sensory neurons and how ATP release is accomplished. Olfactory ensheathing cells (OECs), a specialized glial cell type accompaning axon bundles in the olfactory nerve, were used to monitor ATP release from olfactory receptor axons. Electrical stimulation of receptor axons elicited an increase in the intracellular Ca^{2+} concentration in OECs, as measured by confocal Ca^{2+} imaging. The stimulation-induced Ca^{2+} increase was reduced by about 50% by blocking P2Y1 purinergic receptors, and was entirely suppressed by additional blockage of metabotropic glutamate receptors mGluR1, suggesting that both ATP and glutamate mediate communication between receptor axons and OECs. To verify the release of ATP upon electrical stimulation of axons, we measured the ATP-dependent luminescence signal of luciferin/luciferase applied extracellularly. Electrical stimulation of receptor axons resulted in a luminescence signal of luciferin/luciferase, indicating the presence of ATP in the extracellular space upon electrical stimulation. Antibody labelling revealed the presence of the vesicle-associated proteins synaptophysin, bassoon and VGLUT2, and vesicles could be located in axons adjacent to OECs using electron microscopy. To check whether vesicles in receptor axons were functional, we measured fluorescence changes in olfactory receptor axons expressing the fluorescent vesicle fusion marker protein synaptopHluorin. Electrical stimulation of the axons resulted in a synaptopHluorin fluorescence increase, indicative for vesicle fusion with the plasma membrane. In addition, Ca^{2+} signalling in OECs upon receptor axon stimulation could not be induced when vesicular neurotransmitter release was suppressed by bafilomycin A1 and botulinum toxin. In conclusion, our results indicate that both ATP and glutamate are released from olfactory receptor axons via vesicles and stimulate P2Y1 receptors and mGluR1 receptors of OECs, which results in Ca^{2+} signalling.

ORAL PRESENTATION

Michael Schäfers
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Isotope-based imaging of molecular targets in tissues and living animals

Molecular imaging technologies such as positron emission tomography - PET and single photon emission tomography - SPECT are of great preclinical and clinical interest, since these can visualize and quantify molecular targets in living organisms ranging from animals to patients. The uniqueness of these scintigraphic approaches is based on their extraordinary sensitivity: PET and SPECT can assess molecular targets, which are expressed in nano- or picomolar molar concentrations in tissues. A good example is the measurement of cardiac beta-receptors in patients. These are only expressed in picomolar concentrations in the myocardium. The sensitivity is achieved by using isotopes (positron-emitters or gamma-emitters) to label targets. Isotopes can travel long distances through organisms without significant interference with the tissues, the travel process is well described. For PET and SPECT isotopes such as [11C], [18F] or [99mTc] are coupled to a ligand/pharmaceutical which has a high affinity to the respective molecular target (radiopharmaceutical). Upon injection into the blood stream, the distribution of the radiopharmaceutical can be non-invasively traced by the radioactive signal with both high temporal and spatial resolution. Using compartmental modelling algorithms, absolute quantification of target expression and such can be derived from dynamic acquisition of the radioactivity distribution.

With the increasing interest in imaging surgical or transgenic mouse model of human disease, PET and SPECT technologies were developed which are now suited for animal imaging. Beside miniaturisation of existing human devices, special techniques have been developed with both optimized spatial resolution and field-of-view. For PET and SPECT resolution was brought down to values well below 1 mm. These high-resolution approaches are complemented by new digital autoradiographic techniques, where excised cryo-fixated tissues can be assessed for radioactivity distribution in a resolution of 40 micron or better.

This talk covers principles of the scintigraphic techniques, state-of-art small animal equipment and examples of applications in preclinical research.

ORAL PRESENTATION

Christoph Bremer
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In vivo cell tracking using magnetic resonance and optical imaging techniques

Efficient cell labeling using e.g. superparamagnetic iron oxide particles (SPIO) have successfully been established which allow a sensitive detection of labeled cell populations by MRI. SPIOs are typically stored in the cytoplasm with little to no effect on cellular function or viability respectively. Prolonged label retention allows for follow up studies over several days depending on the doubling time of the cells. MRI offers exquisite anatomical resolution and whole body coverage even in large animal models or humans respectively. More recently imaging sequences have been refined in order to accurately quantify the amount of labeled cells in a given volume. Moreover imaging techniques including positive contrast and T2(*) relaxometry are currently underway which improve discrimination of tagged cells from other (i.e. non cell bound) iron deposits.

Compared to MRI Optical (OI) Imaging has molecular (single cell) sensitivity, which is equal to that of conventional nuclear imaging and several orders of magnitude greater then MRI. OI moreover can exploit fluorescent markers known from e.g. fluorescence microscopy and can thus be considered a translation from in vitro to in vivo studies. In vivo Optical Imaging encompasses various techniques such as fluorescence reflectance imaging (FRI), fluorescence mediated tomography (FMT) and bioluminescence (BLI). Particularly in the near infrared range depth penetration in live animals is considerable so that whole body imaging studies can be performed in small rodents. However compared to MRI anatomical resolution is poor due to scattering and absorption in the tissues. Thus hybrid imaging techniques (e.g. MR/FMT) are currently under development.

This talk intends to provide a brief overview of MRI and OI techniques to study cell populations non-invasively in vivo.

ORAL PRESENTATION

Klaus Tiemann
Medical Clinic C - Cardiology and Angiology
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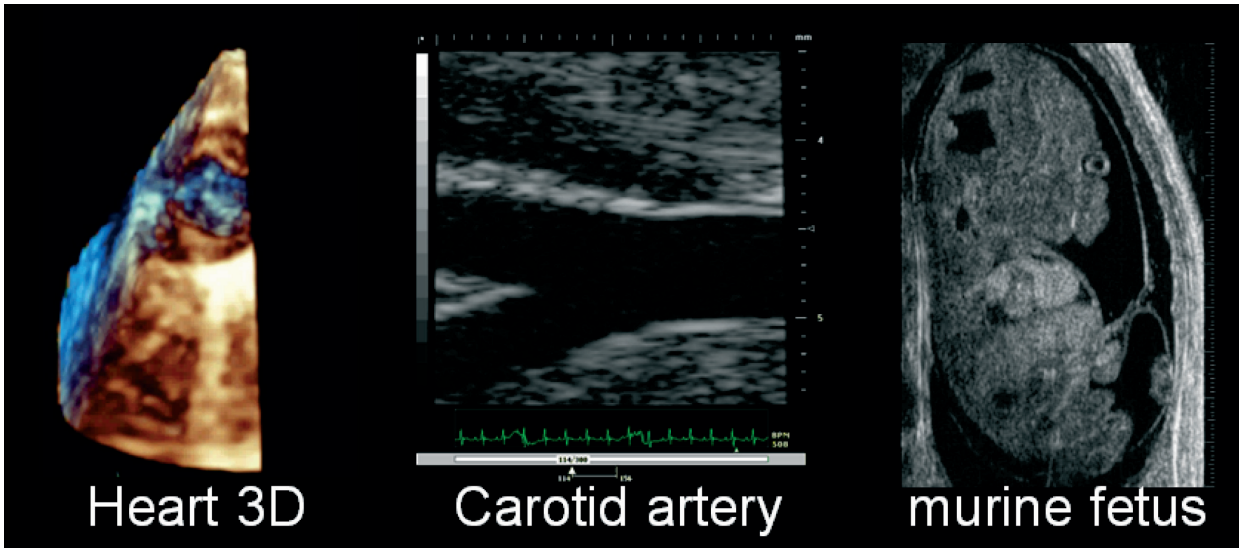
Small animal ultrasound – from functional to molecular imaging

The increasing number of transgene and complex experimental murine models demands high-qualitative and high-resolutional imaging techniques. Pioneering work using clinical Ultrasound (US) equipment has demonstrated the feasibility to study morphology and function, tissue perfusion and to perform targeted imaging in small animal models and might thereby be ideally suited to play an important translational role in small animal imaging. The technology has matured into a robust multi-modality imaging platform which provides a wide range of tools to assess morphology, function and even molecular targets. The spatial and temporal resolution of most recent imaging platforms allow even in-vivo imaging of the murine embryo. Labeling of cells by ultrasound contrast agents and nanoparticles allows for imaging of inflammation, cell tracking and –trafficking. In addition to phenotypisation, morphological and functional imaging US offers a wide range of therapeutic options. Particularly for cell-therapy high-resolutional real-time imaging can be used for in-vivo transplantation of cells by computer assisted injection technology. New US-devices allow even for drug- and gene-delivery. In summary, small animal ultrasound allows for high-resolutional real-time imaging in phenotypisation, morphological-, functional- and molecular-imaging.



ORAL PRESENTATION

Malcolm Clench
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SPOTS, SMOTS and SLOTS - Shotgun Proteomics, Shotgun Metabolomics and Shotgun Lipidomics on Tissue Samples

Malcolm R. Clench, Simona Francese, Sally J. Atkinson, Caroline Earnshaw, David Anderson, Tasneem Muharib, Paul Trim and Marie-Claude Djidja, Biomedical Research Centre, Sheffield Hallam University, Sheffield S1 1WB

Matrix assisted laser desorption ionisation mass spectrometry imaging (MALDI-MSI) is a technique developed in the USA by the group of Richard Caprioli1. In the most common embodiment of this technique the sample is imaged by moving it by set increments under a stationary laser. At each position the laser is fired for a pre-selected time or number of shots and a mass spectrum acquired. Images are obtained subsequently by plotting the spatial dimensions of x and y versus the abundance of a selected ion or ions, which is represented as a grey or colour scale.

In this presentation strategies for the „on-tissue“ examination of protein, lipid and metabolite distribution are discussed and the use of normal scan, accurate mass, tandem mass spectrometry and ion-mobility separations in conjunction with MALDI-MSI described. Examples from the analysis of formalin fixed paraffin embedded (FFPE) and fresh frozen tumour tissue2, brain tissue3, whole body animal sections4 and plant sections5 are given. Particular emphasis will be given to strategies combining multivariant statistics and bioinformatics approaches for the identification of analytes following MALDI-MSI or MALDI profiling experiments.

1. Caprioli R.M, Farmer T.B and Gile J. Anal. Chem. 1997; 69, 4751.
2. Lemaire R et al. J. Prot Res. 2007; 6, 1295.
3. Trim P.J et al Rapid Commun. Mass Spectrom 2008; 22, 1503.
4. Rohner T.C. et al Mech. Ageing and Develop. 2005, 126, 177-185.
5. Burrell M et al J. Exp Bot. 2007, 58, 757.

ORAL PRESENTATION

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MALDI MS Imaging coupled with high-efficiency ion mobility separation

Introduction: Imaging Mass spectrometry is an emerging tool in proteomics, lipidomics and metabolomics. Biomolecules (i.e. proteins, lipids and drugs) are analysed directly from a tissue section, providing spatial information. It can provide complementary information to traditional costly and time consuming techniques, such as autoradiography. The two main instrumental challenges for the mass spectrometric analysis of tissue samples are sensitivity and specificity, i.e. how well the compound of interest can be distinguished from background ions. A means of increasing the separating power of a MALDI imaging experiment is the use of high efficiency ion mobility separation (IMS), coupled with time-of-flight mass spectrometry which offers a new dimension of separation. Using this technique it is possible to separate different compound classes.

Methods: The samples studied were thin sections of animal tissue. Sections of 12µm thickness were produced using a cryotome and deposited onto a sample support, such as thick aluminium foil or microscope slides. Several coats of α-cyano-4-hydroxycinnamic acid matrix were evenly deposited onto the samples using an airbrush or an automated matrix spraying/spotting device, and the samples were subsequently mounted onto MALDI target plates. The tissue areas were selected and imaged by MALDI IMS-MS. All data were acquired on a MALDI hybrid orthogonal acceleration time-of-flight mass spectrometer. After acquisition IMS-MS data were evaluated in software to export regions of drift time vs m/z. Data were converted into Analyze file format and subsequently analysed using BioMap (Novartis, CH).

Results: It is desirable to increase the specificity of the imaging experiment. Typically, this would be achieved by adding additional dimensions of separation, but, unlike with complex samples in the liquid phase, where a number of additional separation and clean-up techniques such as liquid chromatography, affinity based depletion etc. are well developed, for tissue samples only a few clean-up protocols are so far available. Here we show how ion mobility separation can be used to provide a dimension of separation that can be used post ionisation and hence can be utilised in a MALDI imaging experiment. The feasibility of this approach has been shown previously[1], we further develop this method through the use of a high efficiency ion mobility separation device.

We will show data demonstrating that different compound classes, such as peptides and lipids can be separated, as well as examples where the intensity contribution of MALDI matrix ions could be eliminated from an ion intensity image. Furthermore we will show examples of ion mobility separation of isobaric peptides generated by on tissue digestion of formalin fixed paraffin embedded samples.

[1]*McLean JA, Ridenour WB, Caprioli RM. Profiling and imaging of tissues by imaging ion mobility-mass spectrometry. Journal of Mass Spectrometry. 2007, 42 (8): 1099-1105.*

ORAL PRESENTATION

Alan G. Cox
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Towards Cellular Imaging – An elemental perspective

A.G. Cox, J. Seuma, J. Bunch, C.W. McLeod

Mass Spectrometry imaging via laser ablation (LA-ICP-MS) provides new opportunities for imaging of biological structures. The presentation in the context of metallodrug development will review the current state of the art with particular reference to La and Sr based formulations. In particular we will consider spatially resolved measurement of biopsy/autopsy samples utilising a 4µm laser diameter (NdYag, 266 nm). A further opportunity for imaging relates to exploiting immunohistochemical elemental tagged antibodies in order to map protein biomarker distribution in sections. This aspect will be elaborated with reference to fine scale imaging (2µm) of a key breast cancer biomarker, MUC1.

ORAL PRESENTATION

Andy Higgs
Cell Biosciences
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Palo Alto, CA 94304
USA

Getting towards protein isoform assays in single cells

Development of methods for analyzing proteins have lagged behind those of DNA and transcribed RNA. The most widely used method for analyzing proteins is Western blotting, which has remained largley unchanged in the 26 years since development. The amount of material required for a Western blot prevents analysis of small samples or single cells. Analysis of Western blots for different proteins and protein isoforms is also cumbersome.

Assessment of biologic endpoints is increasingly important in developing molecularly targeted therapeutics. Ideally, tumours would be serially sampled during treatment to document that the biologic endpoint has been reached. However, current approaches for solid tumour sampling are severely limited by the invasiveness of procedures required to acquire adequate number of cells for investigation.

We have developed a capillary-based immunoassay that is functionally equivalent to Western blotting while providing enormously better sensitivity. This provides a tool with the ability to quickly assess the levels of a variety of proteins and their post-translational modifications from exceedingly small samples and offer the possibility of monitoring tumour response to targeted therapies. We will provide an example of the application of blotless nano-Western technology where its ability to analyze limited samples is well utilized.

ORAL PRESENTATION

Udo Schimmel
AlphaMetrix Biotech GmbH
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Gene expression profiling from a minimal number of LCM selected pure cells

Accurate gene expression analysis requires the analysis of specific cell types without interference from surrounding cells. Starting with these pure cell populations often means working with small samples. Special technologies are needed to overcome the challenges of handling these precious samples. The combination of Arcturus LCM, RNA amplification, and microarray analysis reveals differential gene expression between cell types.

Microarrays are valuable tools for studying normal and induced variations in gene expression. Microgram amounts of total RNA are required for target preparation for most microarray platforms. Consequently, whole tissue biopsies are typically used for these studies. However, distinct differences have been shown between gene expression data obtained from whole tissue biopsies, which are essentially mixed cell populations, and that obtained from homogenous populations of few cells.

In this presentation we will show how microdissection, combined with RNA amplification to produce the amounts of aRNA needed for microarray analysis, have allowed us to generate expression profiles in specific cell populations obtained from biopsy samples. These highly reproducible expression profiles have been used to generate molecular signatures for different stages of breast cancer using frozen biopsy tissues and microarray analysis.

We will focus the discussions on the one- source solution for isolation, amplification, labeling and analysis of RNA from both frozen and formalin fixed tissue samples to obtain the profiling of native expression levels of thousands of genes, in a few selected pure cells.

ORAL PRESENTATION

Christian Wilhelm
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Physiological characterization of plant cells by means of single cell analysis

The physiological characterisation of single cell level is a clue technique to study taxon resolved proliferation in natural populations of complex biodiversity. For many ecological and hygienic applications the measurement of species specific growth rates is a big challenge e.g. in the context of drinking water supply. We show a recently developed system which combines flow cytometry based cell sorting with other bio-optical methods to determine physiological activity. The optical features which can be determined on the single cell level are absorption, auto-fluorescence emission and fluorescence quantum yield. In addition sorted cells can be transferred to single cell FTIR spectroscopy which allows the measurement of protein to lipid or protein to carbohydrate ratios. Finally, in-situ hybridisation was established and the fluorescence signal in flow cytometry was compared to quantitative RT-PCR. The data show that calibrated in-situ hybidization yields quantitative results on gene expression of selected marker genes. Together with the results from bio-optics the system delivers a data set which characterises the cells on the basis of activities, gene expression and the macromolecular composition with a high taxonomic resolution. The approach is open to include other physiological data which can be measured by fluorescent dyes to complete the data set sufficient to predict growth or survival rates.

ORAL PRESENTATION

Heinrich Leonhardt
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Targeting and tracing antigens in living cells

Antibodies can detect antigens but not their mobility, while fluorescent fusion proteins reveal dynamic changes but do not cover endogenous antigens and posttranslational modifications. We generated fluorescent, antigen-binding proteins, termed chromobodies, that combine the epitope-recognizing fragment of single-chain antibodies from Camelidae with a fluorescent protein. With chromobodies against GFP fusions and endogenous proteins like cytokeratin and lamin we demonstrated that chromobodies can be expressed in mammalian cells and recognize antigens in different subcellular compartments. Even antigens from central parts of the replication machinery or deeply embedded in chromatin could be traced throughout S phase and mitosis demonstrating the suitability of chromobodies for live cell studies. Based on this technology we now engineered a nanotrap for green fluorescent proteins. This GFP-nanotrap can easily be produced in bacteria and coupled to a monovalent matrix and allows a fast and efficient isolation of GFP fusion proteins and their interacting factors for biochemical analyses. Most importantly, the GFP-nanotrap can be fused with cellular proteins to ectopically recruit or deplete fusion proteins allowing targeted manipulation of cellular structures and processes in living cells. This versatile nanotrap enables a unique combination of microscopic, biochemical and functional analyses with one and the same protein.

ORAL PRESENTATION

Christian Korfhage
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Single-cell whole genome amplification: Relaibility and limits

Genetic analyses often require large amounts of genomic DNA. Since the availability of DNA from a single cell is limited, accurate replication of genomic DNA is required. This replicated DNA must be identical to the original genomic DNA template to allow precise genetic testing. Ideally, replication of DNA should be possible directly from a single cell comprising the individual genome of interest.

Here we describe the reliability and limits of single-cell whole genome amplification by focusing on the cellular material, sample preparation and the amplification process. For our analysis, we used QIAGEN´s REPLI-g Kit utilizing multiple displacement amplification (MDA). This technique is capable of accurate in vitro DNA replication of whole genomes, without sequence bias, yielding DNA suitable for most common genetic analysis techniques, including SNP genotyping, STR analysis, and DNA sequencing. In contrast to genome-fragment amplification based on PCR, genomic DNA amplified by REPLI-g is suitable for techniques requiring high-molecular-weight DNA including Southern.

ORAL PRESENTATION

Jürgen Klingauf
Institut of Medical Physiks and Biophysiks
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Coupling of exo- and endocytosis: insights from single-vesicle recordings

During synaptic transmission small synaptic vesicles filled with neurotransmitter fuse with the plasma membrane to release their content. For maintaining synaptic transmission the exocytosed vesicle proteins have to be retrieved thereafter by compensatory endocytosis. What is the fate of synaptic vesicle proteins post fusion? Do they stay together in a raft-like structure, that can be retrieved efficiently in toto or do they disperse in the plasma membrane and have to be resorted and reclustered for retrieval? While it was recently shown that synaptic vesicles exocytosed and retrieved by compensatory endocytosis are non-identical with respect to their protein complement, this does not necessarily imply dispersion of vesicle proteins after fusion. By optically recording single fusion events with high-resolution scanning microscopy we show for four different transmembrane vesicle proteins, synaptobrevin 2, synaptotagmin 1, VGlut1, and synaptophysin, fast dispersion post fusion. Proteins diffused within the synaptic bouton membrane with diffusion constants around 0.25 µm2/s, but only 10 % were lost into the axonal membrane. This suggests a mechanism by which vesicle proteins are rapidly cleared from the release site to allow for the next docking and priming event, but can be efficiently recaptured outside the active zone.

WORKSHOP

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BioAnalyzer Gel - Proteomic imaging system for unstained and stained Gels

Since 30 years (Klose, O´Farrel) gel electrophoresis was a widely used standardized analytical technology to separate complex protein mixtures. Simple staining methods (Silver, Coomassie) allow imaging of the results, and specific labeling of proteins with fluorescent dyes increased sensitivity and specificity.

LaVision BioTec now presents a fast gel imaging system, which scans stained as well as unstained proteins via broadband ultraviolet and visible fluorescence excitation.

Visualization of proteins is usually accomplished by the application of dyes (Coomassie, Silver staining, SYPRO-Ruby…). However, different dyes have limitations in linearity, sensitivity and affordability. The BioAnalyzer Gel offers new perspectives, as no dyes are required to make the protein spots visible.

The BioAnalyzer Gel utilizes native fluorescence of amino acids (tryptophan, tyrosine…) to visualize the proteins within the gel. The outstanding advantage is of course time and cost reduction. Neither are lengthy diffusion based staining processes, nor are covalent modifications necessary. In addition the native fluorescence is highly quantitative. After imaging the gel can be directly processed by subsequent methods. Because of unstained proteins no purifying process is required.

WORKSHOP

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Laser Capture Microdissection from Carl Zeiss: A new dimension in sample purity

Pure sample preparation is an essential precondition for convincing reliable results in molecular biomedical research. Amongst various options to achieve homogeneous material, only non-contact LCM (Laser Capture Microdissection) offers high-resolution control of sample composition by selecting or rejecting individual cells.

Tissue preparation and extraction protocols allow the utilization of microsamples for qualitative and quantitative molecular and proteomic analyses like, e.g., PCR and RT-PCR amplification and microarray analysis.

The PALM MicroBeam from Carl Zeiss combines laser technology with high quality robotic tools for precise microdissection of specimens, whilst the patented method of lifting up against gravity allows for non-contact collection with no impairment to the recovery of DNA, RNA or protein. The integration of image analysis platforms into the microscope fully automates screening, identification and finally subsequent high-throughput sample handling.

Especially in the field of single cell research PALM MicroBeam offers new approaches for LCM and downstream analysis: in combination with the AmpliGrid technology from Advalytix it is possible to perform a PCR on-chip in an extremely low volume reaction format. Single cells can be selected, lifted up by LCM and collected in 48 discrete reaction sites and serve as templates for a subsequent DNA amplification.

Identification, isolation and analysis of individual single cells are possible from various sources, such as tissue sections, cell cultures, cytospin preparations and cell smears. For example in forensic medicine there is a great demand on isolation of specific single cells like spermatozoa or epithelial cells for genotyping. With the technology of LCM an improvement in the generation of pure homogenous samples will be received.

POSTER PRESENTATION

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LDI mass spectrometric imaging on a single cell level of Hypericum species for studying the distribution of hypericins and biflavonoids.

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Strong variations in molecular content of entire cell populations within diverse organs demand the application of single-cell based analytical methods to avoid the pooling of data that are averaged over an entire sample size. Hence techniques are needed allowing sub-cellular scale resolution. MALDI mass spectrometric imaging was recently shown to obviate such sensitive and selective demands and represents highly sensitive detection methods for metabolites.

Hypericum perforatum L., frequently known as Common St. John´s wort is one of the best-selling herbal medicinal plants worldwide. In the case of *Hypericum* a high degree of functional differentiation is exemplified by certain multi-cellular, globular- or tunnel-shaped aggregates, separated from the neighbouring tissues by one or a double layer of flattened cells containing secondary metabolites. These areas are easily visible under magnification and show intense fluorescence. Thus compounds in these highly localized areas are presumably the biologically active napthodianthrones hypericin, protohypericin, pseudohypericin and protopseudohypericin containing highly aromatized skeleton. Furthermore the biflavonoids biapigenin and amentoflavone show an even higher grade of localization.

The poster reports for the first time on a matrix-free laser desorption/ionisation mass spectrometric imaging (LDI-MSI) of highly localized phytochemical contents of members of the plant genus Hypericum. Naphthodianthrones like hypericin and pseudohypericin are traceable in secretory cavities, the placenta, the stamina, and the styli. Additionally, biflavonoids in pollen of this important phytomedical plant were detected. In all cases high degree of spatial resolution (~ 15 µm) was achieved using smartbeamTM laser on Ultraflex III (Bruker) MALDI instrument. Furthermore, a combination of different techniques like laser microdissection microscopy and LDI MS has been proven to be a powerful tandem arrangement to get information of the phytochemical profile of specialized plant areas. This technical advance could be applied to other tissues if their constituents show strong UV absorption.

POSTER PRESENTATION

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GABA transport-mediated calcium signaling in olfactory bulb astrocytes.

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We studied the mechanism of GABA-induced signaling in astrocytes of olfactory bulb slices using confocal Ca^{2+} imaging and 2-photon Na^{+} imaging. GABA evoked Ca^{2+} transients and Na^{+} transients in astrocytes that persisted in the presence of GABAA and GABAB receptor antagonists, but were greatly reduced by inhibition of GABA uptake by SNAP 5114. We hypothesize that GABA uptake-mediated Na^{+} rises reduce Na^{+}/Ca^{2+} exchange, thereby leading to intracellular Ca^{2+} transients. To test the effect of reduced Na^{+}/Ca^{2+} exchange on Ca^{2+} signaling, we used the Na^{+}/Ca^{2+} exchange inhibitor KB-R7943. Application of KB-R7943 mimicked GABA-induced Ca^{2+} signaling. Withdrawal of external Ca^{2+} entirely suppressed GABA-induced Ca^{2+} transients, and depletion of intracellular Ca^{2+} stores with cyclopiazonic acid reduced the Ca^{2+} transients by approximately 90%. This indicates that the Ca^{2+} transients depend on external Ca^{2+}, but are mainly mediated by intracellular Ca^{2+} release, in line with Ca^{2+}-induced Ca^{2+} release. Neither activation nor inhibition of ryanodine receptors affected basal Ca^{2+} or GABA-induced Ca^{2+} transients, whereas the InsP3 receptor blocker 2-APB inhibited the Ca^{2+} transients. The results suggest a novel mechanism of GABAergic signaling, composed of GABA uptake-mediated Na+ rises that reduce Na^{+}/Ca^{2+} exchange efficacy, thereby leading to a small Ca^{2+} increase sufficient to trigger Ca^{2+}-induced Ca^{2+} release via InsP3 receptors.

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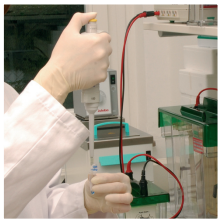
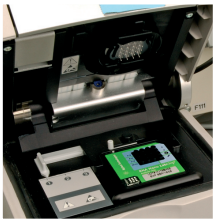
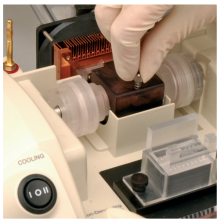
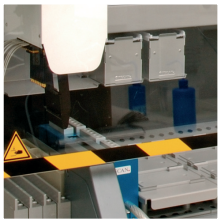
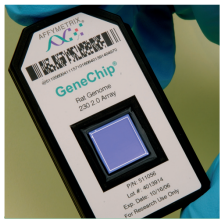

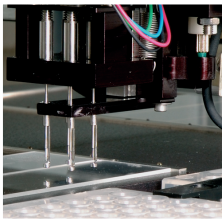

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