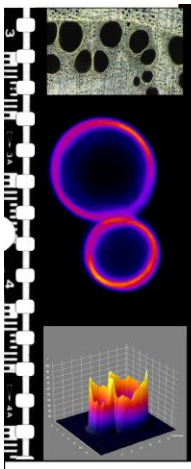
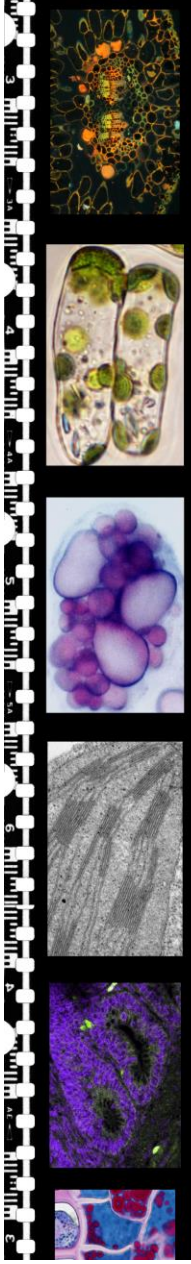


UNIVERSITY OF COPENHAGEN

6th Bioimaging Workshop Copenhagen

22-23 June 2017 with microscope and software exhibition



Rainer Heintzmann

Jena University
Germany

Invited speakers

Peter Friedl

Radboud University
Nijmegen Netherlands

Venue: KU-SCIENCE
Bülowsvej 17 Frederiksberg

http://www.cab.ku.dk

Center for Advanced Bioimaging Denmark

Contents

Welcome

Welcome to the 6th Bioimaging Workshop in Copenhagen! Ten years ago, we used these words to address the audience of the 1st Bioimaging Workshop Copenhagen:

Observing living cells is deeply fascinating, the fact that cells make up the smallest units of life gives the illusion that one can catch a glance of life itself. To visualise life processes makes it easier to understand them; as you will see Bioimaging offers more dimensions than just the four of space and time. To visualise life processes is at the same time a huge challenge, since many cells do need a very specific environment to behave normally which often is incompatible with a microscope stage.

These thoughts still are valid, and it fits well that the enormous development within our methodology over the last decade was highlighted by two Nobel Prizes right in the core of Bioimaging:

for the discovery and development of the green fluorescent protein, GFP, in 2008
to Osamu Shimomura, Martin Chalfie and Roger Y. Tsien, and

for the development of super-resolution fluorescence microscopy in 2014
to Eric Betzig, Stefan W. Hell, and William E. Moerner

We wish in particular to thank our plenary speakers Rainer Heintzmann, Jena University, Germany, and Peter Friedl, Radboud University Nijmegen, The Netherlands for sharing their scientific achievements with us. We are very happy about the contributions, submitted by the participants and are looking forward to discussing them during this workshop. Also this year, the Bioimaging workshop is part of a Summer Course for PhD students on Advanced Live Cell Imaging who will contribute talks and posters.

Finally, in order to give Bioimaging a voice in Denmark and to foster networking within the different biomedical institutions using this methodology, the Danish Bioimaging Network was established and is growing fast (see page 11). The possibility to ensure funding for infrastructure and expertise – microscopes and software as well as image analysis experts – strongly depends on the success of this network. Please consider becoming member of the Danish Bioimaigng Network!

Thanks

We would like to thank everybody who helped setting up the workshop and the Summer School, namely Nynne M. Christensen, Ivana Novak, Michael Lisby, Anne-Marie Heegaard, Helle J. Martens, and Heidi Sachse and all others who will make the organization run smoothly. Last not least we want to thank the University for financing the PhD course and the sponsors, who allow us to keep this workshop free of any charge.

Alexander Schulz for the organizing committee

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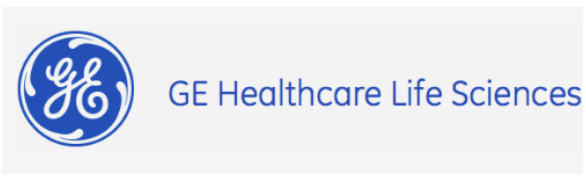
GE Healthcare Life Sciences

Program

Thursday

12:00 Registration opens

13:00 Alexander Schulz: Welcome address

Session I

13:05 Rainer Heintzmann: Lightwedge and Lightsheet-Raman Microscopy

14:00 Rosa Laura Lopez Marques: Flow cytometry vs. Image cytometry for characterization of membrane transporters

14:20 Line Lauritsen: Live cell strategy for detection of curvature-dependent sorting of membrane-associated proteins

14:40 Commercial 1: Bitplane

14:45 Coffee and poster viewing / exhibition

Session 2

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15:15 Louise von Gersdorff Jørgensen: Visualizing infections and immune mechanisms in zebrafish

15:35 Andreas Altenburger: Acquired phototrophy in *Mesodinium rubrum*

15:55 Oliver Quevedo: Unravelling the role of Dpb11 on ultrafine anaphase bridges in budding yeast

16:15 Vasileios Voutsinos: Characterization of large gene expression located at common fragile sites

16:35 Commercial 2: PicoQuant

16:40 Snacks and poster viewing / exhibition

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17:45 Poster presentation and exhibition

18:30 Workshop Dinner

Friday

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09:00 Peter Friedl: Intravital microscopy of cancer invasion, metastasis and therapy response

09:55 Alejandro Mayorca: Imaging the structure of the cancer niche

10:15 Yu Zhang: Elucidating frontotemporal dementia linked to chromosome 3 (FTD3) via isogenic disease modeling

10:35 Commercial 5: Andor Technology

10:40 Coffee and poster viewing / exhibition

Session 5

10:55 Jonas la Cour: The heart arrhythmia associated calmodulin mutant, D129G, changes the phosphorylation and activation profile of CaMKII

11:15 Christopher Henry: Does the structure of the cortical endoplasmic reticulum alter the endoplasmic reticulum Ca2+ homeostasis?

11:35 Robert Hoffmann: A cis-regulatory sequence acts as a repressor in the *Arabidopsis thaliana* sporophyte but as an activator in pollen

11:55 Daniel Pergament Persson: Multi element bioimaging of *Arabidopsis thaliana* mutants with defective root barriers

12:25 Alexander Schulz: Closing remarks

Session 1

Bioimaging methodology

Chaired by Alexander Schulz

Invited lecture: Lightwedge and Lightsheet-Raman Microscopy

Ulrich Leischner, Walter Müller, Michael Schmitt, Jürgen Popp, Rainer Heintzmann

Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller-University and Leibniz Institute of Photonic Technology, Jena, Germany

Two recently developed modes of lightsheet imaging will be presented. Lightwedge microscopy aims at mesoscopic imaging of fixed and optically cleared samples at 1 µm isotropic resolution without the need for sample rotation. The key-idea is to focus a lightsheet at an unusually high NA (thus the name "lightwedge") and still obtain a large field of view due to refocusing of the lightwedge and stitching the multiple small regions of thin illumination back together. This has been simplified by electrical tunable lens technology which has become available recently.

The second mode is hyperspectral Raman imaging in a lightsheet illumination configuration. To recover the spectral information a full-field Fourier-spectroscopic approach has been chosen. The difficulty here is that in a Michelson approach, it would be technically very hard to maintain the angular stability and common path approaches usually tolerate a relatively low product of étendue and maximal optical path difference. We thus developed an optically stable Mach-Zehnder like scheme based on the use of retro-reflecting corner cubes, which is inherently stable. This enabled us to obtain full spectrally-resolved Raman images consisting of over four million spectra in about 10 minutes. Advantages over the conventional Raman imaging are the reduced maximum power on the sample and out of focus heating, the lightsheet-inherent good suppression of crosstalk from the illumination side and the avoidance of glass close to the sample mounting.

Light sheet illumination for Raman imaging at few specific wavelengths was previously reported [1, 2]. With a total laser power of 2 W at an illumination wavelength of 577 nm, we obtained images (2048 × 2048 pixels) of polystyrene beads (fig. 1b), zebrafish and a root cap of a snowdrop at a spectral resolution of 4.4 cm-1 with only few minutes of exposure [3]. The olefinic and aliphatic C-H stretching modes, as well as the fingerprint region are clearly visible along with the broad water peak of the embedding medium (fig. 1a).Spectrally resolved spontaneous Raman microscopy therefore promises high-throughput imaging for biomedical research and on-the-fly clinical diagnostics.

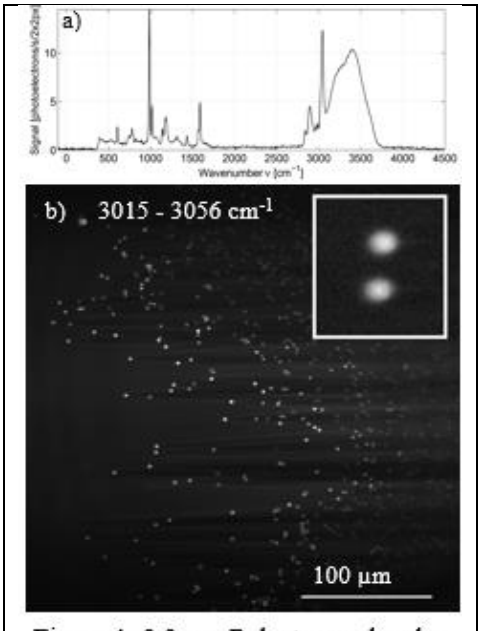

Figure 1: 2.2 µm Polystyrene beads

[1] Oshima et al., "Light sheet-excited spontaneous Raman imaging of a living fish by optical sectioning in a wide field Raman microscope", OPTICS EXPRESS, 20, 16195–16204, 2012.

[2] Barman et al., "Optical sectioning using single-plane-illumination Raman imaging", J. Raman Spectrosc., 41, 1099–1101, 2010.

[3] Mueller et al. "Light sheet Raman micro-spectroscopy", Optica 3, 452-457, 2016.

Flow cytometry vs. Image cytometry for characterization of membrane transporters

Maria S. Jensen[1], Sara R. Costa[1], Lisa Theorin[1], Jan Pravsgaard Christensen[2], Thomas Günther Pomorski[1,3], Rosa L. López-Marqués[1]

[1] Centre for Membrane Pumps in Cells and Disease - PUMPKIN, Department of Plant and Environmental Sciences, [2] Institute for Immunology and Microbiology, University of Copenhagen, Denmark; [3] Faculty of Chemistry and Biochemistry, Department of Molecular Biochemistry, Ruhr University Bochum, Germany

With an increasing number of membrane transporters being identified through genome sequencing, the requirement for high throughput techniques for determination of the specific transported substrate is also increasing. Typically, characterization of new transporters relies on cloning and expression of the desired gene in an easy-to-handle heterologous expression system, most commonly yeast, followed by incubation of the engineered cells with fluorescently labelled substrates. Detection of the fluorescent signal can then be achieved by the use of microscopic or cytometric techniques. While microscopic methods limit the number of samples that can be processed in a reasonable time, cytometric analyses present the advantage of being fast and providing a direct measure of the fluorescent signal, facilitating quantitative comparison among samples. Although flow cytometry is still preferred for detection, recent technological advances have allowed the design of image-based cell counting systems which detection capabilities can compete with those of standard flow cytometers to a much lower cost. These image cytometry systems are often used for mammalian cells, but analysis of yeast cell populations has so far been limited to viability tests, due to technical limitations, making these systems inconvenient for characterization of many heterologously expressed membrane transporters.

Here, we aimed at developing a new protocol to use an automated image-based cell counter to accurately measure uptake of a fluorescent substrate by yeast cells expressing heterologous transporters. As an example, we used lipid flippases, which are ATP-dependent membrane proteins that translocate lipids across biological membranes. The method should be readily adaptable to analyse a variety of other transport systems in yeast, as well as other organisms, provided that a fluorescent analogue substrate is available.

Live Cell Strategy for Detection of Curvature Dependent Sorting of Membrane Associated Proteins"

Line Lauritsen, Ivana Vonkova, Elena Bertseva, Kadla R. Rosholm, and Dimitrios Stamou

Department of Chemistry, University of Copenhagen

The curvature of cellular membranes varies extensively from organelle to organelle and within small subsections of the plasma membrane. A growing amount of evidence suggests that membrane curvature can regulate oligomerization, activity, membrane protein conformation, and recruitment of proteins. We have developed a novel live cell strategy to quantify curvature mediated protein sorting. This fluorescence-based technique takes advantage of a neuronal-derived cell line, which form a high amount of highly curved filopodia of heterogeneous diameters. This system allows us to study correlations between protein densities and membrane curvatures in living cells. Both filopodia diameter and protein density were found to vary along a filopodium. Therefore, we divided each filopodium into several subsegments, and thereby increased the amount of data points per cell. The assay was validated by comparing the membrane localization of the non-curvature sensing, transmembrane protein Aquaporin0 (AQP), and two I-BAR containing proteins, IRSp53 (IRS) and MIM, which interact with negatively curved membranes. As expected our assay revealed a significantly different curvature sensing behavior by Aquaporin0, IRSp53, and MIM. The assay was then used to probe three isoforms of the Ras GTPases (H-Ras, N-Ras, and K-Ras) which revealed a curvature dependent sorting of all three membrane-anchored proteins in living cells.

Session 2

Visualising single-celled organisms, interacting with their environment, and their genomic stability

Chaired by Rosa L. López-Marqués

Status of the Danish Bioimaging Network

Michael Lisby

Department of Biology, University of Copenhagen, Denmark.

Danish BioImaging (DBI) is a national consortium of Danish universities, research institutions and commercial companies with an interest in bioimaging as a tool for life science. It was established to foster research collaboration, share expertise, best practice and bioimaging research infrastructures within the medical and natural sciences, nationally and internationally.

The main goals of DBI are to:

- share knowledge and best practice within the BioImaging community
- promote the development of new bioimaging applications
- facilitate the mobility of students, core facility staff and researchers between bioimaging institutions
- ensure access to state-of-the-art research infrastructures and advanced bioimaging techniques
- represent the Danish bioimaging community nationally and internationally
- connect the Danish bioimaging community and infrastructures with international infrastructures and networks; e.g. the ESFRI EuroBioimaging and European COST action NEUBIAS
- enable strong integration of the ESS and MaxIV facilities with Danish BioImaging facilities
- promote innovation and collaboration with the industry

These goals will be executed by six DBI work groups. For information and registration, please see https://www.danishbioimaging.dk

Visualizing infections and immune mechanisms in zebrafish (Danio rerio)

Louise von Gerstorff Jørgensen, Rozalia Korbut, Foojan Mehrdana, Per Walter Kania, Jacob Günther Schmidt, Maki Ohtani and Kurt Buchmann

Parasitology and Aquatic Pathobiology, University of Copenhagen

The zebrafish has become an important model to study a wide spectrum of vertebrate biological processes. It is used within all major biological sciences and has been especially valuable within developmental biology and genetics. Within the last 20 years the amount of papers describing zebrafish as a model has increased dramatically. It is a valuable model because of several characteristics: it is a vertebrate, it has external embryological development and is fully transparent for the first 24 h post hatching, development is fast; the vertebrate organs can be identified within 48 hours (ears, eyes, brain, internal organs), embryos are simple to genetically manipulate, the genome has been mapped, thousands of transgenic lines exist and a pair of breeders are able to produce up to 300 eggs per week making it a high-throughput but low-cost model organism. Unique tools for non-invasive in vivo imaging has been developed and high quality intravital microscopy can be conducted without comparison. A transparent line has also been developed where transparency is maintained throughout the lifespan of the fish facilitating in vivo visualization of e.g. the spread of fluorescence-tagged cancer cells, immunological reactions during e.g. transplant rejections or the spread and pathogenicity of pathogens. We have, in our laboratory, used the zebrafish as a model for aquacultured fish species and their pathogens. We have 1) visualized antigen uptake in vivo following a bath in a soup containing fluorescent inactivated bacteria, 2) visualized the spread of a bacterium after either a bath or an injection in the peritoneal cavity, and 3) visualized (images + videos) the behaviour of certain immune cells, called neutrophils during a parasite disease. Using adult fish of a transgenic line with GFP-tagged neutrophils, we got an unprecedented view into the interactions between the parasites and the neutrophils."Membrane fusion mediated by optical trapped gold nanoparticles

Acquired phototrophy in Mesodinium rubrum

Andreas Altenburger, Per Juel Hansen, Nina Lundholm

Natural History Museum of Denmark, University of Copenhagen, Denmark

Mesodinium rubrum is a marine ciliate with a cosmopolitan distribution. It is well known for the formation of “red tides” and its ability to retain functional chloroplasts from its ingested algal prey of the cryptophyte genera Teleaulax, Geminigera and Plagioselmis. In order to keep the foreign plastids functional, Mesodinium ‘enslaves’ the prey nucleus. We investigate how the prey nuclei are exchanged when the prey is switched from one species to another using fluorescent in situ hybridization and confocal laser scanning microscopy. We will present preliminary results and challenges from this endeavor.

Unravelling the role of Dpb11 on ultrafine anaphase bridges in budding yeast

Oliver Quevedo, Xuejiao Yang, and Michael Lisby

Department of Biology, University of Copenhagen, Denmark

Anaphase bridges represent one of the most significant threats to genomic stability. So far, two main types of DNA anaphase bridges have been described: chromatin bridges and ultrafine bridges (UFBs). Contrary to the former, UFBs do not contain histones or chromatin components, and they are not stained by DAPI or similar dyes. UFBs are induced by either replication or topological stress. However, UFBs are also detected with a high frequency in unperturbed cells, being resolved by the cellular machinery during anaphase-telophase. Sgs1/BLM, RPA, PICH and Dpb11/TopBP1 are known to bind UFBs, but the molecular mechanisms of sensing and resolution of UFBs are still unknown. It has been shown by our group that yeast Dpb11 and its ortholog in higher eukaryotes, TopBP1, play a central role in the stabilization of UFBs, suppressing the formation of chromatin bridges and facilitating chromosome segregation by promoting the elongation and resolution of UFBs. Here, we use S. cerevisiae as a model to study UFBs, with the aim of unravelling the role of Dpb11 on UFBs and revealing its interaction partners on UFBs. For this, we used a Bimolecular Fluorescence Complementation assay, in which we have fused Dpb11 to the C terminus of the Venus fluorescence protein. We introduced this construct into a commercial VN-Fusion Library, containing most of the yeast proteins fused to the N terminus of the Venus protein. We screened 5800 proteins for their propensity to interact with Dpb11 under mild replication stress and we identified 155 proteins that interact with Dpb11. We will present the identified hits of the screen and the status of the ongoing validation and characterization of the interactions using FLIM-FRET, CoIP and functional assays.

Characterization of large gene expression located at common fragile sites

Vasileios Voutsinos, Michael Lisby and Vibe H. Oestergaard

Department of Biology, University of Copenhagen, Denmark

Common fragile sites (CFSs) are large genomic regions known for their profound genomic instability that can be found in all individuals. These regions are known to host extremely large genes often spanning more than 1Mb. Transcription of the genes located at CFS often takes more than one cell cycle to complete leading to a high probability of transcription and replication machinery collisions.

Replication inhibition has been shown to increase instability at CFSs. It has been shown that transcriptionally active large genes, including several CFS genes, exhibit high rates of double strand breaks (DSBs) and genomic rearrangements after replication inhibition induction. Genes like that are very commonly expressed in brain cells and have been associated with neurodevelopmental and neuropsychiatric disorders, as well as with cancer development. However, large gene structure is evolutionarily conserved leading to the speculation that they might have a physiological function in the cell and or the organism.

In this study we use DT40 cells and we study the expression of two large genes located at two of the most fragile CFSs of this cell line (PARK2, MACROD2). We have fluorescently labeled the genes with Venus and YFP in tandem and we performed FISH to characterize the CFS expression in the obtained cell lines, which showed a tendency for more fragility with higher PARK2 expression. We want to assess the impact that replication inhibition has in the stability of these CFSs and by fluorescent labeling the genes located, we want to look for changes in their expression levels.

Session 3

Imaging plant particularities

Chaired by Helle J. Martens

Thylakoid membrane plasticity and biogenesis in *Arabidopsis thaliana*

Anurag Sharma[1], Omar Sandoval-Ibáñez[1], Mathias Labs[2], Dario Leister[2], Mathias Pribil[1]

[1] Copenhagen Plant Science Centre, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark.

[2] Plant Molecular Biology (Botany), Department Biology I, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany.

The thylakoid membrane system of higher plant chloroplasts consists of grana, cylindrical discs of membrane stacked over each other, and stroma lamellae, unstacked membranes that interconnect the grana stacks. Thylakoids are highly dynamic and undergo various structural rearrangements upon exposure to different light conditions. Recently, the curvature thylakoid1 (CURT1) protein family has been discovered in *Arabidopsis thaliana* which is involved in grana formation by facilitating membrane bending in the grana margins. CURT1 proteins form oligomeric, high molecular weight complexes among themselves and can facilitate membrane bending in a dosage dependent manner. CURT1A, the major isoform of the CURT1 protein family is sufficient to induce thylakoid membrane bending both *in planta* and *in vitro*. We demonstrate that CURT1 proteins are required for thylakoid plasticity under challenging light conditions. Further, our biochemical and transmission electron microscopy data suggest that CURT1A also influences thylakoid biogenesis.

Transport pathways required for glucosinolate accumulation at the phloem cap of Arabidopsis inflorescence stems

Pascal Hunziker, Deyang Xu, Sebastian Nintemann, Barbara Ann Halkier and Alexander Schulz

DynaMo Center, Department of Plant and Environmental Sciences, University of Copenhagen

The phloem cap of crucifers harbors a glucosinolate (GS)-based defense system to protect vascular tissue. Two components of this system are stored in distinct specialized cell types: S-cells accumulate GS in millimolar range and are located adjacent to myrosinase-containing idioblasts. Upon cell damage, GS and myrosinase mix and react to yield toxic hydrolysis products. To test whether GS are synthesized in S-cells, we expressed the CYP83A1 gene as mVenus-fusion protein driven by its native promoter. Confocal microscopy showed localization to cells adjacent to S-cells. Absence of GS synthesis in S-cells suggests the need for cell-to-cell transport of metabolites.

To define the roles of the membrane transporters, we studied the involvement of known GS transporters (GTRs). Analysis of the S-cell content of transporter knock-out mutants revealed that GTR-mediated GS import is only partially required. Co-localization of biosynthesis and import to cells adjacent to S-cells suggests that active membrane transport takes part in GS retrieval from the apoplast. We hypothesized that sustaining high GS concentrations in these cells fuels symplasmic movement into S-cells. We tested this hypothesis using confocal and transmission electron microscopy. TEM revealed that plasmodesmata (PD) connect S-cells with surrounding cell-types. FLIP and photoactivation of PD-mobile tracer dyes confirmed that these PD are functional and that S-cells are tightly coupled with CYP83A1-positive cells. In conclusion, active transport and de novo biosynthesis lead to accumulation of GS in cells adjacent to S-cells, from where GS reach the S-cells via the symplasm.

Poster presentations

and Sponsor exhibition

Session 4

Imaging with medical focus

Chaired by Ivana Novak

Invited Lecture:
Intravital microscopy of cancer invasion, metastasis and therapy response

Peter Friedl[1,2]

[1] Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands and
[2] The University of Texas, MD Anderson Cancer Center, Houston, TX, USA

Cancer cell migration is a plastic and adaptive process integrating cytoskeletal dynamics, cell–extracellular matrix and cell–cell adhesion, as well as tissue remodeling. In response to molecular and physical microenvironmental cues during metastatic dissemination, cancer cells exploit a versatile repertoire of invasion and dissemination strategies, including collective and single-cell migration programs. This diversity generates molecular and physical heterogeneity of migration mechanisms and metastatic routes, and provides a basis for adaptation in response to microenvironmental and therapeutic challenge. Time-resolved intravital microscopy has greatly advanced the understanding of how tumor cells adapt to microenvironmental cues. With tissue confinement, invading cancer cells undergo a jamming transition towards collective migration and circulate as multicellular clusters for collective organ colonization. Conversely, adoptive cytotoxic T cell (CTL) therapy results in preferential CTL accumulation in the invasion niche and effective tumor eradication, whereas other regions of the lesions survive. Dissecting the steps underlying individual-to-collective adaptation, and its cross-talk to leukocyte trafficking routes will enhance to derive “antimigration” therapies and combat metastatic transitions.

Imaging the structure of the cancer niche

Alejandro Mayorca-Guiliani, Chris Madsen, Thomas R Cox, Freja A Venning, Edward Horton, Sebastian R Nielsen, Raphael Reuten, Janine T Erler.

Biotech Research Innovation Centre. University of Copenhagen

The extracellular matrix (ECM) is a master regulator of cell behaviour in the cancer niche. Here we present a method to decellularise tumours and obtain cancer-associated ECM scaffolds with their architecture intact. These scaffolds can be interrogated using mass spectrometry and imaged to produce three dimensional maps of the cancer associated ECM in sub-micron scale. Using mouse metastasis models we quantified protein composition of primary and secondary tumours, revealing the heterogeneity of the primary and metastatic niches. Using a combination of multiphoton and confocal imaging we outlined the anatomy of the cancer associated extracellular matrix in tumours and their metastases, including submicron imaging of vascular basement membranes during angiogenesis. To our knowledge, we are the first to offer detailed imaging of the cancer associated ECM, basement membrane disruption in metastatic progression and ECM remodeling preceding tumour invasion.

Elucidating Frontotemporal Dementia Linked to Chromosome 3 (FTD3) via Isogenic Disease Modeling

Yu Zhang, Poul Hyttel and Kristine K. Freude

Department of Veterinary Clinical Sciences, University of Copenhagen, Denmark

One gene affected in familial frontotemporal dementia (FTD) is the charged multivesicular body protein 2B (CHMP2B) located on chromosome 3 (FTD3). Patients display global cortical and central brain atrophies, with no apparent amyloid plaque formation or conclusive hyper-phosphorylated tau aggregates. To study the cellular and molecular events of FTD3, we have previously established a well characterized human induced pluripotent stem cells (iPSCs) disease model from patients carrying the heterozygous 31449G>C mutation in CHMP2B and isogenic gene-corrected controls generated via the CRISPR/Cas9 system with subsequent in vitro neuronal differentiation. In order to systematically decode the pathogenesis, we further integrated high-throughput RNA sequencing, mass spectrometry-based proteomic studies and metabolic assays. Intriguingly we identified several candidate genes and pathways mis-regulated, which are important in celluar organization, subcellular component transportation and neuronal development including neural transmitter transportation. In order to directly decipher impaired protein protein interactions resulting from truncated CHMP2B, homozygous inserted mutant hiPSCs were generated. We subsequently performed affinity-purification mass spectrometry analyses, revealing a list of key proteins, which lost their binding capacities due to the truncation of CHMP2B. These key proteins enabled us to directly align cellular phenotypes with molecular events, indicating that such integrated omics analysis is providing a comprehensive tool for interpreting the role of mutant CHMP2B in FTD3 pathogenesis. Strikingly, we found that several dysregulated genes and pathways, which are also, affected in other neurodegenerative disorders. These findings open up for possibilities to develop pharmaceutics targeting several distinct neurodegenerative diseases at once.

Session 5

Imaging calcium-upake related processes

Chaired by Michael Lisby

The heart arrhythmia associated calmodulin mutant, D129G, changes the phosphorylation and activation profile of CaMKII

Jonas M. la Cour

Department of Biology, University of Copenhagen, Denmark

How can an omnipresent simple ion like Ca^{2+} control a huge number of highly diverse biological processes? To answer this question we have studied the Ca^{2+}-binding proteins and their down-stream targets using various microscopy methods. Mutations in one of these proteins, Calmodulin (CaM), are associated with heart arrhythmias. Using a FRET based sensor we found that the Ca^{2+}/CaM-dependent kinase II (CaMKII) could not be fully activated by a CaM mutation, D129G, connected to LQTS. This is likely due to premature phosphorylation of the inhibitory phosphorylation sites T305/T306 of CaMKII. In addition, the phosphorylation pattern of eight functionally naïve CaMKII autophosphorylation sites were altered following D129G CaM stimulation. Mutation of these sites showed a marked change in Ca^{2+}/CaM sensitivity of the CaMKII-sensor. These results provide a possible explanation for the dominant phenotype of the arrhythmogenic CaM mutation D129G and introduce a novel regulatory level of CaMKII signaling through phosphorylation of the linker region between the catalytic and the hub domains of CaMKII.

Does the structure of the cortical endoplasmic reticulum alter the endoplasmic reticulum Ca^{2+} homeostasis?

Christopher Henry, Nicolas Demaurex

Department of Cellular Physiology and Metabolism, University of Geneva, Switzerland

Store-Operated Calcium Entry (SOCE) is a signalling mechanism allowing Ca^{2+} entry from outside the cell to the cytoplasm, that is mediated by Ca^{2+} Endoplasmic Reticulum (ER) depletion. Membrane Contact Sites (MCS) are close appositions of cortical ER (cER) with the Plasma Membrane (PM) that are crucial for this process. The lateral dimensions of MCS or the distance between cER and PM are shown to be dynamic, however their impact on ER Ca^{2+} is unknown. This project aims to study this impact by remodelling cER architecture through molecular tools, and concurrently monitoring $[Ca^{2+}]ER$ using total internal reflection microscopy (TIRFm).

A cis-Regulatory Sequence Acts as a Repressor in the Arabidopsis thaliana Sporophyte but as an Activator in Pollen

Robert D. Hoffmann, Lene I. Olsen, Jeppe O. Husum, Jesper S. Nicolet, Jens F.B. Thøfner, Anders P. Wätjen, Chukwuebuka V. Ezike, Michael Palmgren

Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

Flowering plants undergo a life cycle in which the diploid sporophyte constitutes the predominant generation, and the haploid male and female gametophytes are reduced to only a few but highly specialized cells. To achieve expression restricted to certain cell types, genes are not only actively expressed in these cells, they might also be repressed elsewhere. Here, we report that deleting an 88-base-pair promoter sequence of the pollen-specific gene ACA7 redirects its expression to the sporophyte. Our study thus identified a bifunctional cis -regulatory module that functions as a repressor in a plant sporophyte and as an activator in the male gametophyte.

Multi element bioimaging of *Arabidopsis thaliana* mutants with defective root barriers

Anle Chen[1], Søren Husted[1], Jan Schjørring[1], David Salt[2] and Daniel P. Persson[1]

[1] Department of Plant and Environmental Sciences, Copenhagen University, Denmark
[2] University of Nottingham

We have developed a novel LA-ICP-MS method for multi element bioimaging of Arabidopsis thaliana roots. The method is based on encapsulation of fresh Arabidopsis roots in paraffin so that they subsequently can be sectioned, freeze-dried and analysed without changing the native tissue compartmentation of element ions [1].

With this method we have tested the applicability of the method on various Arabidopsis mutants with defective or altered root barriers in the endodermis. Our combined results from shoots, xylem sap and element bioimaging indicate that especially calcium (Ca) and magnesium (Mg) experience depressed transport over the endodermis upon strong suberization. This was tested using the esb 1-1 mutant (enhanced suberin mutant 1-1) with a documented strong suberization of the root. In the same mutant, potassium (K) and zinc (Zn) had higher concentrations in the vascular tissues, xylem sap and in the shoots than the corresponding wild-type, indicating that for these nutrients suberin helps maintaining a high concentration in the vascular tissues, possibly by reducing leak out of the stele. The opposite results for different elements may indicate different pathways for these elements, where elements that mainly travel through the symplastic pathway are less affected by suberization than elements that preferentially travel by the apoplastic or transmembrane pathways. This novel method allow the molecular and genetic approaches available in Arabidopsis to be fully exploited with respect to gaining a mechanistic understanding of how root barriers affect nutrient transport processes.

[1] Persson, D.P., Chen, A., Aarts, M.G.M., Salt, D.E., Schjoerring, J.K., and Husted, S. (2016). Multi-Element Bioimaging of Arabidopsis thaliana Roots. Plant Physiol **172,** 835-847.

Posters

In alphabetical order

Poster 1

Free Granzyme A in sepsis patients with absence of influence on acute kidney injury

Andreja Figurek[1], Ludwig Wagner[2]

[1]Medical Faculty Banja Luka, University of Banja Luka, Republic of Srpska, Bosnia and Herzegovina
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Background: Cytotoxic lymphocytes possess cytoplasmic granules containing cytolytic molecules. A group of these granular proteins are proteases named granzymes and the most abundant granzyme present in cytotoxic T-cell granules is granzyme A.

Sepsis patients frequently start suffering from capillary leakage and loos fluid into the interstitial tissue. As it presents our constant goal to identify factors which initiate sepsis induced acute kidney injury (AKI), we looked at one of the lymphocyte derived proteases granzyme A in sepsis patients.

Material and methods: Patients fulfilling sepsis criteria at the time-point of admission or during their hospital treatment periods were enrolled in this study. 60 patients (21 female, 39 male) with a mean age of 66.20±16.52 years who had given consent participated in the study. Blood sample analysis was performed in retrospect on frozen samples. Microbial isolates were obtained either from blood culture, cutaneous wound swoops and BAL fluid. Data were analyzed in SPSS 22.0 and GraphPad Prism programs. A p value of <0.05 was considered statistically significant.

Results: Out of the 60 patients included, ten patients showed at least at one time point during the six-day study period a level of more than 35 ng/ml. Patients undergoing AKI stage 1 or 2 showed lower survival rate during the study period ($p<0.0001$) and higher mean CRP and leucocyte count, but no significantly increased levels of granzyme A.

Conclusion: These data provide no evidence that elevated levels of immunologically detectable levels of GRA would contribute to incidence of kidney injury and that level measured by ELISA does not reflect absolute levels of plasma granzyme A.

Poster 2

High-frequency firing interneurons: What makes their metabolism so efficient?

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Abnormal development and/or function of cortical GABAergic interneurons are believed to play a major role in neurodevelopmental disorders like schizophrenia, autism, and epilepsy. Parvalbumin (PV) interneurons are the main subtype of GABAergic interneurons affected in these disorders. PV interneurons are fast spiking neurons with high metabolic requirements. Since 90% of neuronal ATP production occurs in the mitochondria through oxidative phosphorylation, we seek to investigate the impact of cytochrome c oxidase (COX) depletion on PV interneurons activity. COX is the IV complex of the electron transport chain and it couples the transfer of electrons from reduced cytochrome c to molecular oxygen with the translocation of protons across the mitochondrial inner membrane. This creates a proton gradient that is used by the ATP synthase to produce ATP. Thus, the main aim of my project is to investigate possible effects of COX depletion on the metabolism (i.e. ATP production), physiology, morphology, and connectivity of PV interneurons. We also seek to analyze differences in the gene expression profile of cortical wild-type and COX ablated PV interneurons to determine how impairment of metabolism could affect the transcriptome of PV interneurons.

Poster 3

Role of annexins in plasma membrane dynamics

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Interactions between membranes and proteins are of great importance when studying cell dynamics and functions. The plasma membrane consist of several different types of lipids and is a very dynamic environment containing binding sites for many different proteins.

From a biophysical point of view, one of the very interesting protein families is the Annexin protein family. This 12 member large (in humans) family is involved with a multitude of functions and processes such as membrane repair, vesicular transport, endo-and exocytosis, anti-inflammatory responses and many others.

However, many questions have presented themselves as more knowledge has been obtained: How are they recruited? Do they interact with other proteins? Do they interact with other membranes than the plasma membrane?

Two of the key characteristics of the family is the slightly bend structure of the proteins and the fact that they interact with the membrane in a Ca^{2+} dependent manner.

Hence, we are looking into if there is any physical features - such as the shape of the protein - that help it up-regulate in certain areas.

On a longer timescale, the photothermal effect will be used for investigation of the mechanism behind the protein repair complex.

Poster 4

Characterization of yeast-yeast interactions during alcoholic fermentation

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The aim of this research is to gain new knowledge on the interactions occurring between the wine yeast, *Saccharomyces cerevisiae*, and the non-Saccharomyces yeast, *Kluyveromyces thermos-tolerans*, during wine fermentation by using metabolomics analysis. Once, we have established a better understanding of the metabolic profile during yeast-yeast interactions, the research aims to further investigate outcomes in the metabolites of interest. Potential outcomes include development of non-Saccharomyces yeast strains that can survive during the course of fermentation.

Poster 5

Metaviromes in whey samples produced over 12 years using TK5 starter culture.

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TK5 is a mixed strain Cheddar starter culture that was used every day for Cheddar production over a 12 year period (1982-1994) in a Danish dairy "Øster Tørslev" [E.W. Nielsen (1998) Int. Dairy J. 8, 1003-1009]. Whey samples were obtained approx. twice a year and they have been stored at -80 C since arrival.

The bacteriophages in the whey samples were amplified twice on the TK5 culture. Metaviromes were isolated from 21 whey samples obtained over the 12 years as well from 3 controls (TK5 starter culture without added whey).

The metaviromes were sequenced using Illumina MiSeq 2x300 bp paired-end technology. The metaviromes were analysed using custom database containing > 200 lactococcal phage genomes from all 10 recognized lactococcal phage species.

The results showed that the whey metaviromes all contained sequences lactococcal 936 phage species or the lactococcal P335 phage quasi-species (92-99 % of reads). In most wheys the 936 phage species was dominating but in some P335 phages were dominating. Sequences mapping to c2 phage species, KSY1 species, and 949 species were found in low (<0.1 to 5 %) levels in some whey metavirome samples. Metaviromes from control samples were dominated by sequences mapping to the 936 phage species, showing that the TK5 starter is phage carrying.

Bacteriophages infecting starter cultures have been claimed as one of the most important elements of dairy fermentation failure. Before the bacteriophages inject their DNA into the target organism, they need to bind to specific receptors on the target organism using their receptor-binding protein (RBP). Therefore, analyses of the RBP of phages could provide a way for detection of bacteriophages related to their host-range. Here, we present the development of real time PCR (qPCR) based system to detect different groups of lactococcal bacteriophages by their RBP. We compared the DNA sequences of RBP genes from a large number of recently sequenced phages belonging to the 936-species and correlated sequences with host-range. A number of host-range related groups were identified. For most of the groups a pair of qPCR specific primers was designed targeting the region of RBP genes. Quantification of fluctuation between RBP-types in whey samples will be presented.

Poster 6

A targeted next-generation sequencing method for identifying clinically relevant mutation profiles in lung adenocarcinoma

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Molecular profiling of lung cancer has become essential for prediction of an individual's response to targeted therapies. Next-generation sequencing (NGS) is a promising technique for routine diagnostics, but has not been sufficiently evaluated in terms of feasibility, reliability, cost and capacity with routine diagnostic formalin-fixed, paraffin-embedded (FFPE) materials. Here, we report the validation and application of a test based on Ion Proton technology for the rapid characterisation of single nucleotide variations (SNVs), short insertions and deletions (InDels), copy number variations (CNVs), and gene rearrangements in 145 genes with FFPE clinical specimens. The validation study, using 61 previously profiled clinical tumour samples, showed a concordance rate of 100% between results obtained by NGS and conventional test platforms. Analysis of tumour cell lines indicated reliable mutation detection in samples with 5% tumour content. Furthermore, application of the panel to 58 clinical cases, identified at least one actionable mutation in 43 cases, 1.4 times the number of actionable alterations detected by current diagnostic tests. We demonstrated that targeted NGS is a cost-effective and rapid platform to detect multiple mutations simultaneously in various genes with high reproducibility and sensitivity.

Poster 7

rapidFLIM: The New Innovative Method for Ultra-fast Imaging of Biological Processes

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Imaging the dynamics of life requires high spatial and temporal resolution. Therefore, expanding the combination of excellent time resolution from Fluorescence Lifetime Imaging (FLIM) based on Time-Correlated Single Photon Counting (TCSPC) with high spatial resolution towards ultrafast image acquisition times is most desirable. Speeding up FLIM enables to study processes and changes occurring on short time scales, such as signal transduction pathways in cells or fast moving subcellular structures.

In contrast to other conventional FLIM methods, our rapidFLIM approach allows combining high optical resolution of confocal laser scanning microscopes with acquisition speeds of more than 15 frames per second, while retaining the high temporal resolution of TCSPC and offering good lifetime contrast even for samples with multiple fluorescent species. Thus, rapidFLIM is essential to monitor even smallest changes in the fluorescence behavior of living or mobile samples.

The method exploits recent hardware developments such as the TimeHarp 260 Nano TCSPC card with virtually no dead time allowing to record huge photon numbers without loss. This, in combination with our extreme low dead time detector, enables to achieve significantly higher count rates (up to 40 MHz) within a specific time frame than with a classic setup and to speed up FLIM image acquisition by more than a factor of 100.

Even complex fluorescence decay data can be efficiently and quickly analyzed with our easy-to-use pattern matching method. Now, complete turn-key systems such as stand-alone units or upgrade kits enable to study fast and dynamic processes such as protein interactions involved in endosome trafficking.

Poster 8

Detection of balanced chromosomal rearrangement by whole-genome low-coverage sequencing

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Balanced chromosomal rearrangement (BCA) is a common chromosomal structural variation. Next-generation sequencing has been reported to detect BCA-associated breakpoints with the aid of karyotyping. However, the complications associated with this approach and the requirement for cytogenetics information has limited its application. Here, we provide a whole-genome low-coverage sequencing approach to detect BCA events independent of knowing the affected regions and with low false positives. First, six samples containing BCAs were used to establish a detection protocol and assess the efficacy of different library construction approaches. By clustering anomalous read pairs and filtering out the false-positive results with a control cohort and the concomitant mapping information, we could directly detect BCA events for each sample. This approach was further validated using another 13 samples that contained BCAs. Our approach advances the application of high-throughput whole-genome low-coverage analysis for robust BCA detection—especially for clinical samples—without the need for karyotyping.

Poster 9

Tumor genome sequencing of laser-microdissected cell clusters and single nuclei

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Breast cancer is a heterogeneous neoplasm with a high degree of diversity between and within tumors as well as among cancer-bearing individuals. Invasive micropapillary breast carcinoma（IMPC） is a very aggressive form of breast cancer, with a very high rate of lymph node metastasis, which represents approximately 2%-6% of all breast cancers. The molecular mechanism of highly metastasis is still unclear. To investigate its metastasis mechanism, we conducted a genomics study of IMPC. Large-scale genomics studies revealed the cancerous genomic signature and mutation profiling. However, bulk tissue sequencing swamped intra-tumor heterogeneity. Hence, we developed pathological microdissection based tumor genome sequencing. In our study, Cell clusters and single nuclei were isolated from formalin fixed and paraffin embedded (FFPE) primary tumor tissue or lymph node metastasis sections by laser capture microdissection. Pathological type of each cluster was confirmed by experienced pathologist. After whole genome amplification and library preparation, genomics data of these tumor cell clusters and single nuclei generated by next generation sequencing. Finally, we acquired genomic mutation profiling of cell clusters and single nuclei with different pathological type of primary tumor IMPCs, metastatic IMPCs, and invasive ductal carcinoma from the same patient. With parallel comparison of different pathological clusters and single nuclei, we could explore underlying cause of intra-tumor heterogeneity and metastasis, which would help the clinical diagnosis and treatment for IMPC.

Poster 10

Giant plasma membrane vesicles to study membrane properties

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The biophysics of membranes and membrane proteins has been explored extensively in synthetic membrane vesicles and in living cells. However, both synthetic membrane vesicles with simple lipid compositions and living cells with an underlying actin skeleton might not adequately reflect the dynamics of the plasma membrane. Here, we show how the membrane can be extracted from cells, to form Giant Plasma Membrane Vesicles (GPMVs) which can be used to study membrane properties, including the effect of membrane curvature, by pulling out membrane tubes using an optical trap. These tethers, have an unexplored potential to study dynamic processes in native cellular membranes.

Poster 11

Role of STIM calcium sensors in the brain of zebrafish

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Store-Operated Calcium Entry (SOCE) is a major calcium influx mechanism in non-excitable cells, however, there is increasing evidence indicating its significance in neurons. STIM proteins, that are calcium sensors localized in the endoplasmic reticulum membrane, are crucial for this process. When calcium level is decreased in the ER, STIMs aggregate and interact with channels such as Orai located in the plasma membrane to activate calcium influx.

Dysregulated calcium homeostasis is a feature of many neurodegenerative disorders. Since zebrafish is a promising model of many human diseases we analyzed its SOCE components to make models of some brain pathologies in the future. The aim of this study was to determine the expression pattern of stims and orais in zebrafish at different stages of development. Both stim2 isoforms (a and b) dominate over stim1 in the skeletal muscles of adult zebrafish, while in rodents Stim2 was shown to be expressed mainly in the brain. Nevertheless, both stim2 isoforms are present in the zebrafish brain and their level is relatively high. Thus, Stim2 seems to have a role in zebrafish brain. Interestingly, stim1b and stim2b as well as orai1a and orai1b expression significantly increases with aging.

We also plan to investigate Stim2 functions in neurons. Using CRISPR/Cas9 technology stim2b-/- zebrafish line was generated and then crossed with Tg(HuC:GCaMP5G) line. Using Light-Sheet microscope we are conducting imaging of calcium signaling in vivo in the brain of zebrafish stim2b-/- larvae expressing GCaMP5G calcium probe in neurons. In the future we plan to investigate Stim2b sensitivity to different factors by measuring calcium response to various kinds of stimuli using Genetically Encoded Calcium Indicators in vivo or in vitro: GCaMP6 (cytosolic), GEM-CEPIA1er (ER), CaMPARI (permanent marker of Ca^{2+} entry event), and Fluo4 (enabling to measure Ca^{2+} response dynamics). Thus, imaging will be a crucial method in my studies.

Poster 12

Endothelial aquaporins: involvement in cardiovascular disease

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Aquaporins (AQPs) facilitate water (classical aquaporins) and glycerol (aquaglyceroporins – AQP3, 7, 9 and 10) permeation through membranes being crucial for water and energy homeostasis in humans. In endothelium, the tissue that drives the distribution of blood components to all body, AQP1 was reported to be highly expressed and important for water permeation; however, less is known about aquaglyceroporins expression and function. Human umbilical vein endothelial cells (HUVECs) were used to investigate which aquaglyceroporins are expressed in endothelium. In addition, due the involvement of aquaglyceroporins in several metabolic-related dysfunctions, their expression pattern and function were evaluated in a model of endothelial dysfunction.

Our data confirmed a high expression level of AQP1 and revealed, for the first time, that AQP3 is highly expressed in HUVEC; AQP7 and AQP10 were also detected, whereas AQP9 was absent. In disease condition, the expression of most representative aquaporin (AQP1) and aquaglyceroporin (AQP3) was evaluated. Our results show that the mRNA and protein expression levels of AQP1 were decreased; however, levels of AQP3 were not affected. Biophysical assessment of endothelial aquaporins showed a 20% impaired water permeability in disease condition suggesting that AQP1 is important in endothelial function and its expression and function are affected by disease. On the other hand, glycerol permeability was not affected, suggesting that AQP3 is not implicated in endothelial dysfunction.

Poster 13

Viability prediction of invasive species of microplankton in ship's treated ballast water

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Viability prediction of invasive species of microplankton in ship's treated ballast water: The spreading of aquatic invasive species in ship's ballast water has huge environmental and health-related consequences and is causing socio-economic losses around the world in the order of US$100 billion per year. Regulations now require all large ships to have an approved ballast water treatment system (BWTS) on-board for cleaning of the ballast water to avoid further spreading of invasive species. To ensure BWTS compliance with discharge standards water samples need to be verified for the number of viable organisms in different size classes. The current standard method for assessing organism viability in the 10-50 µm range utilizes fluorescence of CMFDA/FDA stains. The stains are activated by enzyme activity present only in the viable cells with intact cell membrane which are then quantified using labour intensive direct manual microscope counting. Different concerns have been raised using this method. A special challenge is the verification UV based BWTS. UV causes damage to DNA, but leaves the cell membrane unaffected. DNA damage can either result in later death or in survival due to DNA repair. Current staining methods may therefore produce false positives as dead or dying organisms are recorded as viable. Effects on DNA cannot be measured by the methods currently prescribed. Comparative studies will be carried out to evaluate a number of novel markers and staining methods for more efficient and reliable viability assessments. Especially with focus on markers for DNA damages. The project will likewise test the efficiency of multi-labelling. Also, the potential complementary use of advanced microscopy and image analysis techniques for a more automated and robust quantitative assessment will be explored. The aim is that the established methods and the science behind will support the development of more efficient BWMS to avoid any further spreading of invasive species.

Poster 14

In vivo imaging of RNA-mediated regulation

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The paradigm of localization- and interaction-related function is highly accepted and has been intensely studied for proteins. In contrast, the localization of functional RNAs and especially the interaction of RNAs and proteins remains difficult.

We have set out to establish a method for in vivo localization of RNA-protein interaction using Pumilio homology domain polypeptides (PUF). The design of genetically encoded RNA binding probes fused to split YFP reporters enables specific RNA tagging of two short, closely adjacent sequences of free choice. In addition, CFP fusions of known RNA-interacting proteins can be used to visualize the interaction of protein and RNA in vivo by FRET. We proof this method on the known interaction between the RNA binding protein AtGRP7 and the long non-coding (lnc) RNA COOLAIR, an important regulator of flowering time in Arabidopsis thaliana.

In addition, the interaction and localization of the AtGRP7 protein and the RNA of AtAOP2, a key component of glucosinolate metabolism and function as lncRNA in flowering time will be performed. The in vivo localization of these RNA-protein interactions will improve our understanding of the molecular functions of lncRNAs and help to decipher the complex regulatory networks coordinating metabolism and developmental transitions.

Poster 15

Optical H_2O_2 Sensors for Studying Immune Responses and Bacterial Infections

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H_2O_2 is involved in vital and deadly processes in cells and is a key compound in the immune response to bacterial and fungal infection; this makes it an important analyte for clinical diagnostics. Due to its reactivity, quantitative measurements in biological samples are very challenging and most optical measurement principles are based on irreversible reactions, which do not enable continuous sensing of H_2O_2 dynamics. In the present PhD project, we aim to alleviate these methodological limitations by developing novel fiber-optic H_2O_2 sensors, as well as by modifying of a commercially available flow-injection system, to allow measurements of H_2O_2 in e.g. highly viscous sputum, or other complex matrices as blood or tissue.

Poster 16

Development of a novel in-vitro FRET-FLIM method to screen polymerase mutants

Ou Wang

Department of Biology

To characterize the dynamic properties of enzymes, lots of ways and experiments have been developed to measure the kinetics of the enzyme action. Although those biochemical approaches are indispensable for identifying enzyme-substrate interactions, they seldom provide single-molecule level resolution of that reaction. However Förster resonance energy transfer (FRET) together with fluorescence lifetime imaging microscopy (FLIM) can easily provide the distance and affinity of two different molecules interaction at single molecule resolution, and truly reflect the enzyme activity in certain environments. Thus lots of studies have been carried out to utilize the FRET/FLIM to characterize the enzyme/protein activities. But only characterization is not enough, to enable high speed catalyzing and tolerate non-standard substrate, enzyme mutant screening is also very critical for new enzyme development. So in this study we proposed a new method, which utilizes the in vitro FLIM FRET technology to screen polymerase mutants. We compared the kinetics of different combination of mutants and substrates, and further extended the throughput of this assay by taking advantages of microfluidic devices.

Poster 17

The regulation of the store operated calcium entry (SOCE) process by Sigma-1R

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The Sigma1R is a 223 amino acid long chaperone protein targeted to the membrane of the endoplasmic reticulum (ER). It is primarily located at mitochondria-associated ER membranes (MAM), but can also translocate to the periphery of the cell and localize to ER-plasma membrane (PM) junctions. Loss of activity of the Sigma1R is associated with Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis (ALS). As a chaperon, it exerts a protective effect on neurons in the central nervous system and in cancer cells, where it is highly overexpressed. Underlying its protective effect is its ability to regulate calcium (Ca^{2+}) homeostasis. At the MAM it is thought to promote Ca^{2+} transfer to mitochondria and at ER-PM junctions we recently showed that it inhibits SOCE. Increased expression of Sigma1R in HEK293 cells via baculoviral infection profoundly inhibited SOCE mediated via STIM1 and Orai1. Interestingly, overexpression of STIM1 could reverse the inhibition of SOCE by Sigma1R whereas overexpression of its homolog STIM2 could not. We also recently reported that STIM1 recruits the Sigma1R in sub-PM puncta. We used total internal reflection fluorescence (TIRF) microscopy to visualize translocation of mCh-STIM1 and Sigma1R-EGFP in response to thapsigargin. Currently, we are applying TIRF microscopy to investigate translocation of mCh-STIM2 in the presence of Sigma1R-EGFP.

Poster 18

Activation of transcription factors by oncogenic Orai proteins

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The store-operated Ca^{2+} channel components STIM1 and Orai1 are important for T cell activation but pathophysiological STIM1/Orai1 Ca^{2+} signaling has been shown to contribute to critical steps in malignant cancer cell development. Here we analyzed Orai1 point mutants, determined from large genome wide cancer studies, for constitutive calcium influx and the activation of calcium-regulated transcription factors. We analyzed these Orai1 cancer mutants upon overexpression in HEK cells for constitutive Ca^{2+} influx and activation of the nuclear factor of activated T-cells (NFAT), transcription factor EB (TFEB) and microphthalmia-associated transcription factor (MITF). We found that five Orai1 cancer mutants induced significant increased MITF translocation to the nucleus and one Orai1 mutant which lead to increased TFEB activation in the absence of STIM1 and store-depletion. In confocal fluorescence microscopy, we studied the time-dependent translocation from the cytosol to the nucleus for NFAT in comparison to MITF and TFEB upon co-expression of STIM1 and Orai1 by thapsigargin. Only NFAT translocated quickly upon store-depletion in contrast to the other transcription factors, suggesting different Ca^{2+} dependent processes. NFAT translocation mediated by constitutively active Orai1 mutants is induced by activation of the Ca^{2+} dependent phosphatase calcineurin. Further, we will discuss a potential role of calcineurin in the activation process of MITF and TFEB.

Poster 19

Regulation of DNA repair proteins by the PP2A-B56 phosphatase.

Sara Marie Ambjørn
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Reversible phosphorylation is a key modulator of protein function, regulating a wide variety of biological processes in the cell, including several DNA repair pathways that are vital to maintaining genome integrity. Recently, Hertz, Kruse et al. (2016) identified a conserved binding motif for the B56 regulatory subunit of the protein phosphatase 2A (PP2A). This consensus motif, termed the LxxIxE motif, is found in a variety of central DNA repair proteins including the breast cancer type 2 susceptibility protein (BRCA2) and the Fanconi anemia group M protein (FANCM). BRCA2 and FANCM are both phosphorylated at numerous sites, but the mechanisms and functional roles of dephosphorylation remain largely uncharacterized. We hypothesize that the PP2A-B56 phosphatase binds specifically to the LxxIxE motifs in BRCA2 and FANCM and mediates dephosphorylation to control the phosphorylation states of the proteins. As a consequence, we suggest that abrogation of B56 binding will lead to deregulation of the phosphorylation state, which in turn may disturb the DNA repair pathways BRCA2 and FANCM are part of. To investigate our hypothesis, we have developed a set of tools in both human cells and the avian cell line DT40. In DT40, we have generated both BRCA2 and FANCM knockout cells stably complemented with fluorescently-tagged human cDNA containing a set of mutations that alter the affinity for B56. Using human cell lines, we are able to downregulate BRCA2 using siRNA-mediated knockdown and complement with siRNA-resistant WT or mutant BRCA2 cDNAs. Using these setups, we will determine whether DNA repair pathways depending on BRCA2 or FANCM functionality are impaired or deregulated.

Poster 20

Cellular localization of cerebellar degeneration-related proteins using super resolution microscopy

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BACKGROUND AND AIMS: The cerebellar degeneration-related (CDR) proteins are associated with autoimmune-mediated paraneoplastic cerebellar degeneration (PCD) where they are targeted by auto-antibodies. The auto-antibodies are typically initiated by gynecological-associated cancers. Previously, we have shown that binding of auto-antibody Yo to the CDR proteins CDR2 and CDR2L in the cerebellum affects the calcium homeostasis of Purkinje neurons with subsequent excitotoxicity. To improve PCD therapies with aspect to neuroprotective capacity we have to identify the cellular and molecular mechanisms driven by the CDR proteins. Therefore, we have studied the subcellular location of CDR2L, the major PCD-associated protein, in cancer cell lines and Purkinje neurons.

MATERIAL AND METHODS: We have immunocytochemically stained primary rat cerebellar neuron cultures and two relevant human gynecological cancer cell lines (BT474 and OvCar3), which express the CDR2L protein endogenously, to visualize the location of CDR2L compared to well-known cell organelles such as the Golgi, endoplasmic reticulum and mitochondria. All three organelles are associated with cell calcium homeostasis regulation. The visualization was performed with Leica SP8 STED 3X super resolution microscope.

RESULTS: We showed that rabbit-CDR2L antibody and anti-Yo patient cerebrospinal fluid are colocalizing in Purkinje neurons. First data indicate that there is no colocalization of anti-Yo or anti-CDR2L with GM130 (Golgi), TOMM20 (mitochondria) or SEC61A (rough endoplasmic reticulum) in the cancer cell lines or in the Purkinje neurons.

CONCLUSION: Mainly CDR2 has previously been associated with anti-Yo PCD, however we found that also CDR2L showed a major reaction with anti-Yo. Further investigations will evaluate other organelle-specific proteins to determine the precise location and function of CDR2L.

Poster 21

Extracellular ATP as a survival factor in cancer progression

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Extracellular ATP is involved in different responses such as chemotaxis, cytokine release and cell growth. It has been shown that ATP in TME is in the hundreds micromolar range while in healthy tissues the extracellular space is very low in ATP. In order to gain insight into the relationship between intracellular and extracellular ATP levels we measured extracellular ATP in B16F10 mouse melanoma cells cultured in presence and absence of serum. Cell growth in serum starved B16F10 cells was reduced to about 20% of controls and extracellular ATP levels were much higher in serum-starved than in control cells while intracellular ATP was much higher in control cells. To confirm this observation we measured extracellular or intracellular ATP levels with the luciferase enzyme engineered to be targeted to the plasma membrane and to the cytosol using IVIS bioluminescence imaging system. Also with this probe extracellular ATP was higher in serum-starved cells than in control cells. Accumulation of ATP into the extracellular space of serum-starved cells was paralleled by a strong inhibition of P2X7R-dependent responses and by a depletion of intracellular Ca^{2+} stores. P2X7R is a main plasma membrane receptor mediating the effects of extracellular ATP and, depending on the level of activation, it can support cell proliferation or cell death, especially in the absence of serum. Conceivably, inhibition of P2X7R responses is a safeguard mechanism to protect cells against the excessive extracellular ATP level while preserving the growth-promoting activity. To test this hypothesis, we added apyrase which a reduction of cells growth. These findings suggest that cancer cells can modulate the extracellular ATP concentration depending on their growth conditions.

Poster 22

The Role of Fanconi Anemia Pathway in Transcription-Coupled Genomic Instability

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Flavopiridol is a CDK inhibitor that inhibits transcription by preventing CDK9-mediated phosphorylation of RNA polymerase II, thereby blocking the transition from transcription initiation to elongation. Flavopiridol is currently assessed as an anticancer compound in a number of clinical trials, but the actual mechanism behind its anticancer effect is not known (1).

We have characterized the response of FANCD2 to flavopiridol. FANCD2 is a protein involved in repair of DNA interstrand crosslinks and DNA damage caused by aldehydes. In response to DNA damage, FANCD2 is monoubiquitylated, which in turn leads to chromatin-association of FANCD2 [2].

Here we find that flavopiridol results in an increase in FANCD2 focus formation at early time points after treatment, whereas the ATR activator TopBP1 does not respond with increased focus formation. In line with this, flavopiridol does not trigger ATR activation. Surprisingly, FANCD2 monoubiquitylation is not increased by flavopiridol treatment. Nevertheless, colony formation assay reveals that FANCD2 is highly important for cell survival after a short treatment with flavopiridol.

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Thursday

12:00 Registration opens

13:00 Alexander Schulz: Welcome address

Session I

13:05 Rainer Heintzmann: Lightwedge and Lightsheet-Raman Microscopy

14:00 Rosa Laura Lopez Marques: Flow cytometry vs. Image cytometry for characterization of membrane transporters

14:20 Line Lauritsen: Live cell strategy for detection of curvature-dependent sorting of membrane-associated proteins

14:40 Commercial 1: Bitplane

14:45 Coffee and poster viewing / exhibition

Session 2

15:05 Michael Lisby: Introduction to the Danish Bioimaging network

15:15 Louise von Gersdorff Jørgensen: Visualizing infections and immune mechanisms in zebrafish

15:35 Andreas Altenburger: Acquired phototrophy in *Mesodinium rubrum*

15:55 Oliver Quevedo: Unravelling the role of Dpb11 on ultrafine anaphase bridges in budding yeast

16:15 Vasileios Voutsinos: Characterization of large gene expression located at common fragile sites

16:35 Commercial 2: PicoQuant

16:40 Snacks and poster viewing / exhibition

Session 3

16:55 Anurag Sharma: Thylakoid membrane plasticity and biogenesis

17:15 Pascal Hunziker: Transport pathways required for glucosinolate accumulation at the phloem cap of Arabidopsis inflorescence stems

17:35 Commercial 3: Leica Microsystems

17:40 Commercial 4: PerkinElmer

17:45 Poster presentation and exhibition

18:30 Workshop Dinner

Friday

Session 4

09:00 Peter Friedl: Intravital microscopy of cancer invasion, metastasis and therapy response

09:55 Alejandro Mayorca: Imaging the structure of the cancer niche

10:15 Yu Zhang: Elucidating frontotemporal dementia linked to chromosome 3 (FTD3) via isogenic disease modeling

10:35 Commercial 5: Andor Technology

10:40 Coffee and poster viewing / exhibition

Session 5

10:55 Jonas la Cour: The heart arrhythmia associated calmodulin mutant, D129G, changes the phosphorylation and activation profile of CaMKII

11:15 Christopher Henry: Does the structure of the cortical endoplasmic reticulum alter the endoplasmic reticulum Ca2+ homeostasis?

11:35 Robert Hoffmann: A cis-regulatory sequence acts as a repressor in the *Arabidopsis thaliana* sporophyte but as an activator in pollen

11:55 Daniel Pergament Persson: Multi element bioimaging of *Arabidopsis thaliana* mutants with defective root barriers

12:25 Alexander Schulz: Closing remarks