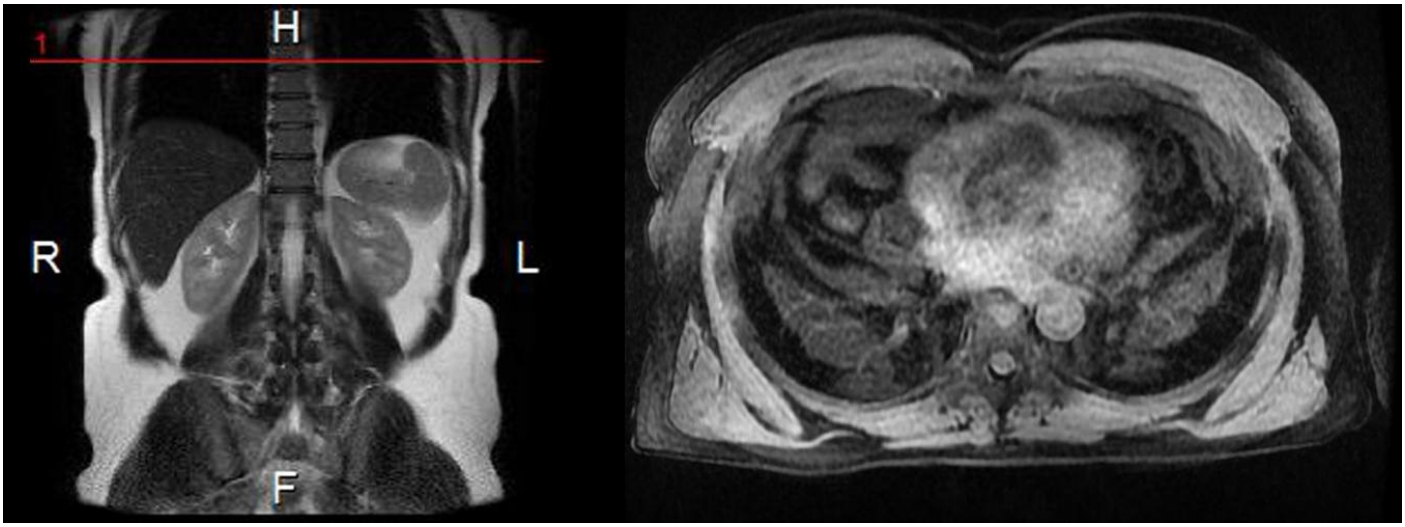
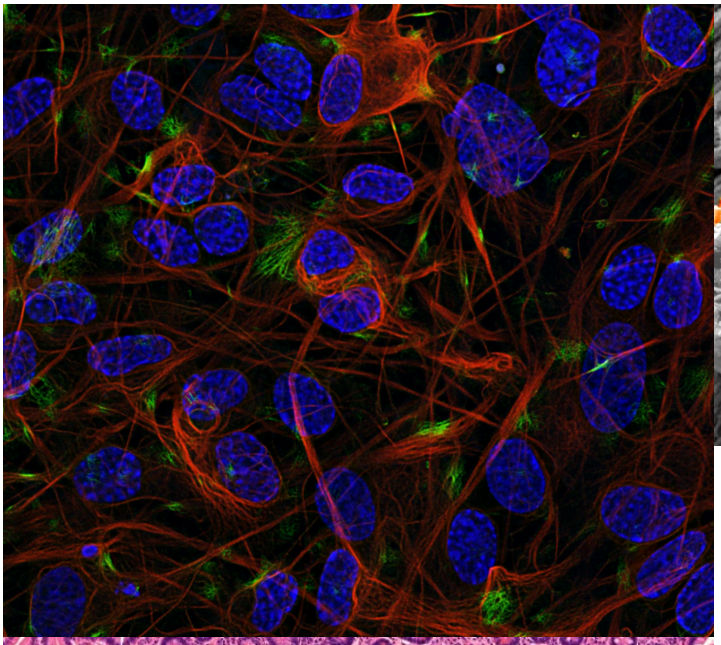
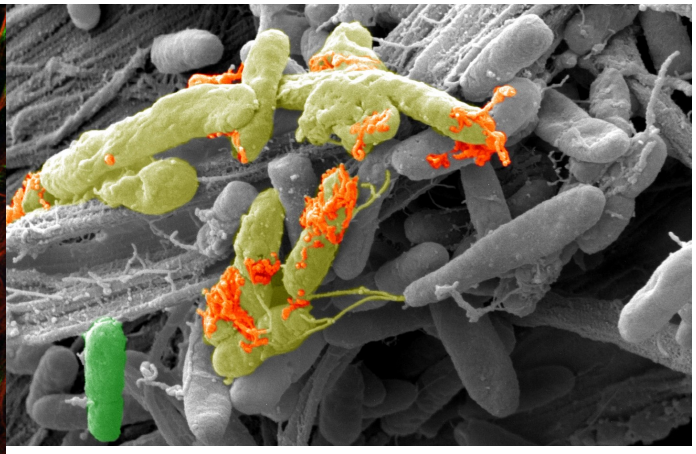


Danish Bioimaging meeting

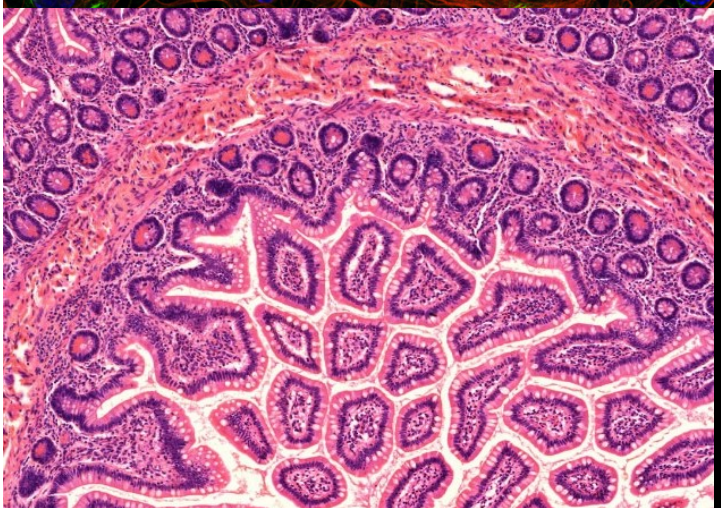
8th of November 2017

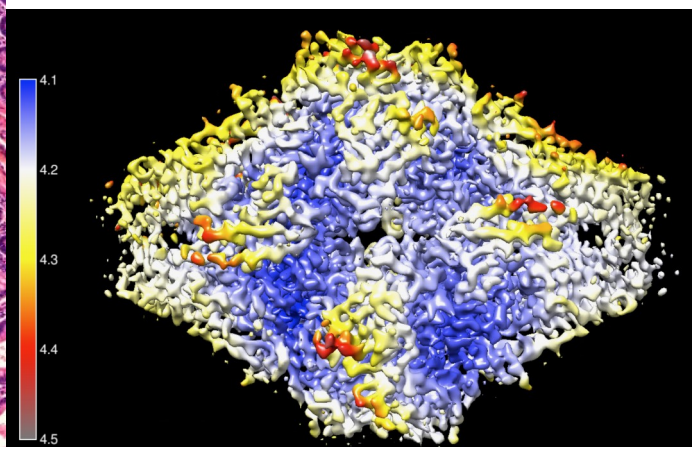
University of Copenhagen





DANISH BIOIMAGING NETWORK





Leica MICROSYSTEMS

GE Healthcare

Holm & Halby TIL LABORATORIER I UDVIKLING

ZEISS

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Sponsors

Location

Haderup auditorium, Building 20,
The Panum Institute, Faculty of Health and Medical Sciences,
University of Copenhagen
Nørre Allé 20,
Copenhagen N

How to get there?

By car

It is not possible to park your car at the Panum institute if you do not work at the Faculty

By bike

A sheltered bike parking can be accessed from Blegdamsvej 3B and Nørre Allé 20.

By train

The closest train station is Nørreport (25min walking distance).

By bus

The closest bus stop is Nørre campus.

From Nørreport station

- Bus 6A (direction Emdrup Torv),
- Bus 184 (direction Holte St.),
- Bus 15E (direction Søhuset, Forskerparken),
- Bus 42 (direction Emdrup Torv),
- Bus 150 S (direction Gl. Holte).

Programme overview

10:50—11:05	Welcome address and Network Updates Clara Prats & Morten Schallburg Nielsen
11:05—11:35	Morning session Chair: Michael Lisby and Christoffel Dinant
12:35—13:15	Lunch Sponsored by Leica
13:15—14:35	Mid-session Chair Casper Hempel & Jonathan Brewer
14:35—15:00	Coffee break Sponsored by GE Healthcare
15:00—16:30	Afternoon session chair Jon Sporring and Rasmus Reinhold Paulsen
16:30—17:45	Poster session and networking Sponsored by Holm & Halby
17:45—18:00	Image and Poster prizes Sponsored by Zeiss

Morning session Chair: Michael Lisby and Christoffel Dinant	
11:05—11:35	Keynote: Force Dependent regulation of adhesive contacts. Soichiro Yamada, Biomedical Engineering Department, University of California, Davis, USA.
11:35—11:55	Overview of in vivo imaging in animal experiments Henrik Lauridsen, Clinical Medicine Department, Aarhus University Hospital
11:55—12:15	Whole tissue imaging of macrophage subsets in a mouse model of ovarian cancer Anders Etzerodt, Biomedical Department, Aarhus University
12:15—12:35	High-Throughput Screening – Challenges & Opportunities Jutta Maria Bulkescher, Center for Protein Research and Danish Stem Cell Center, Copenhagen Univ.

Mid-session Chair: Casper Hempel & Jonathan Brewer	
13:15—13:35	Aquaporin-5 and the effects on cell adhesion Lene N. Nejsum, Clinical Medicine Department, Aarhus University
13:35—13:55	Membrane damage and repair Jesper Nylandsted, Membrane Integrity Group, Cell Death and Metabolism, Danish Cancer Society Research Center
13:55—14:15	Linear- and nonlinear optical microscopy combined with force measurements for the characterization of spider silk. Irina Iachina, University of Southern Denmark
14:15—14:35	Electron microscopy characterization of nanomaterials; ex-situ, in-situ and in-vivo. Poul Kempen, DTU NANOTECH, Department of Micro- and Nanotechnology, Technical Univ. of Denmark

Afternoon session chair: Jon Sporring and Rasmus Reinhold Paulsen	
15:00—15:20	Serial block face, segmentation and 3D rendering with Amira Emilie Tresse-Gommeaux, BRIC, Copenhagen Univ.
15:20—15:40	Application of deep learning for tissue-based cancer screening and research Jeppe Thagaard, Visiopharm and DTU compute
15:40—16:00	Measuring sub-cellular structures in neurons from electron microscopy Hans J.T. Stephensen, The Image group, Computer Science Department, Copenhagen Univ.
16:00—16:10	How to fit in? Mechanics of epithelial cell renewal in vivo. Jakub Sedzinski, DanStem, University of Copenhagen
16:10—16:20	TIRF microscopy for single-molecule FRET investigations of tandem G-quadruplex structures Emil Laust Kristoffersen, Aarhus University
16:20—16:30	Combined X-ray microscopy study of the malaria parasites in human red blood cells Sergey Kapishnikov, University of Copenhagen
16:30—17:45	Poster session and networking
17:45—18:00	Image and Poster prizes

Force-dependent regulation of adhesive contacts

Soichiro Yamada

University of California Davis

The unique, albeit devastating, ability of cancer cells to migrate out of their home tissue and metastasize is facilitated by an altered form of cell adhesion. To propel cell body forward, cell adhesion of invasive cells is critical for the application of traction force onto extracellular matrix and neighboring cells. The regulation and mechanics of cell adhesion mediated traction force, however, remain elusive. This is partly due to the limited availability of experimental tools for simultaneous analysis of mechanical forces and protein interactions at adhesive contacts. Using micron-sized cellular force probes with analysis of protein dynamics, we demonstrate highly transient nature of the protein interactions at force-bearing adhesive contacts. In addition, by applying forces to live cells, we can track force-sensitive proteins such as zyxin. Together with biochemical analysis of force-sensitive proteins, these imaging techniques reveal molecular insights into force-dependent regulation of adhesive contacts. By precisely defining how forces are regulated in normal epithelial cells and cancer cells, our experimental platforms will help understand how epithelial cells migrate during tissue developemnt and repair, and cancer cells invade and metastasize.

Overview of in vivo imaging in animal experiments

Henrik Lauridsen

Comparative Medicine Lab, Department of Clinical Medicine, Aarhus University

In vivo imaging is the process of acquiring visual representations of the interior of a living organism to answer questions related to e.g. anatomy, morphology, physiology, metabolism, and function. Current in vivo imaging technologies span several orders of magnitude in terms of spatiotemporal resolution and field-of-view size, ranging from technologies that provide microscale resolution of small objects such as micro computed tomography, optical coherence tomography, and light sheet fluorescence microscopy to techniques allowing for larger sample sizes at the cost of lower resolution such as ultrasound, magnetic resonance, and positron emission tomography imaging. Some techniques rely on innate contrast properties of different cell and tissue types under study, whereas others require the use of contrast agents to visualize the structures or phenomenon of interest.

One of the most important pillars in the wise design of biomedical experiments was formulated by the Danish Nobel laureate, August Krogh, stating that “For a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied” or simply that among the diversity of animal species there will be one or a few ideally suited as an experimental model for any biomedical problem. The same can be said about selecting the most appropriate imaging technology to study a given problem and provide answers to a biomedical question.

In this talk I will touch upon a number of different in vivo imaging technologies and discuss how these can be applied with reference to Krogh’s principle both in terms of the choice of animal model and the choice of imaging technology.

Whole Tissue Imaging of Macrophage Subsets in a mouse model of ovarian cancer

Anders Etzerodt[1,2], Morgane Moulin[2], Søren K. Moestrup[1,3] and Toby Lawrence[2]

1) Department of Biomedicine, University of Aarhus, Aarhus, Denmark
2) Centre d'Immunologie de Marseille-Luminy, Marseille, France
3) Department of Inflammation Research, South Danish University, Odense, Denmark

There is now a wealth of clinical and experimental evidence that strongly links tumour-associated macrophages (TAM) with tumor progression, invasion and metastasis (Noy and Pollard, 2014).

Until recently, it was thought that tissue macrophages, with the exception of the brain microglia and Langerhans' cells in the skin, were maintained from bone marrow-derived monocyte precursors (Geissmann et al., 2010) and the same has generally been assumed for TAM (Lahmar et al., 2016).

However, recent advances in molecular techniques that allows the fate mapping of macrophages in vivo have revealed that the majority of tissue macrophages, at least in steady-state, develop from embryonic precursors and are maintained by local proliferation with little input from bone-marrow derived monocytes (Schulz et al., 2012). Subsequent studies have shown that monocyte-derived cells gradually replace some embryonic tissue-resident macrophages although the numbers vary and depend on the specific tissue (Ginhoux and Guilliams, 2016). However, the functional implications of these distinct developmental origins and certainly their respective contributions to tumor progression have not yet been explored.

In present work, we have used several imaging techniques to characterize the ontogeny, homeostasis and tissue localization of different TAM subsets in a mouse model of epithelial ovarian cancer (EOC). The omentum (OM), an apron of visceral white adipose tissue in the abdomen, is a frequent site for metastasis of EOC cells (Ben Arie et al., 2013). Specifically, we have defined a subset of tissue-resident macrophages in the OM that are maintained independently of bone marrow-derived monocytes and we show that the selective depletion of the tissue resident cells but not monocyte-derived TAM had a major impact on tumor progression and development.

Noy, R., and J.W. Pollard. 2014. Immunity 41:49-61.
Geissmann, et al. 2010. Science 327:656-661.
Lahmar, Q. et al. Biochim Biophys Acta 1865:23-34.
Schulz, C., et al. 2012. Science 336:86-90.
Ginhoux, F., and M. Guilliams. 2016. Immunity 44:439-449.
Ben Arie, A, et al. 2013. Gynecol Oncol 131:780-783.

High-throughput Screening-Challenges & Opportunities

Jutta Bulkescher

Center for Protein Research/Danish Stem Cell Center, UNiversity of Copenhagen

Microscopy-based high-throughput and high-content screening is used to measure a variety of parameters in cellular processes. Advances in fluorescent probes in recent years have increased the ability to visualize multiple features in phenotypes as they allow to label virtually any cellular structures.

The complexity of those visual phenotypes is an important factor in classifying genes into certain biological groups and pathways based on phenotypic similarities. Combined with perturbations, such as gene silencing, drugs and mutations such screens are a powerful method for gaining insights into biological processes. Over the past years, technological advances have enabled automated image acquisition and allowed large scale screens from small to genome-wide libraries.

Establishing an assay design in automation-friendly microtiter plates provides the advantage of unbiased, low cost, high speed experiment performance. Those advantages can at the same time become the biggest challenges when performing the first experiments in the pilot screen and need to be addressed carefully.

In such a screening setup, the availability of standardized software workflows simplifies extracting quantitative data and finally provides biological meaning.

Aquaporin-5 and the effects on cell adhesion

Frédéric H. Login[1], Helene H. Jensen[1,2], Jeanette J. Morgen[1,2], Mikkel R. Holst[1], Gitte A. Pedersen[1,4], Jennifer S. Koffman[3], Ute Hahn[4], Eva B.V. Jensen[4], Johan Palmfeldt[1], Peter Bross[1], Maddy Parsons[5], Tae-Hwan Kwon[6], and Lene N. Nejsum[1,3]

1) Dpt of Clinical Medicine, Aarhus University, Aarhus, Denmark;
2) Dpt of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark;
3) Interdisciplinary Nanoscience Center, Aarhus University, Aarhus, Denmark;
4) Dpt of Mathematics – Centre for Stochastic Geometry and Advanced Bioimaging (CSGB), Aarhus University, Aarhus, Denmark;
5) King's College, London, United Kingdom;
6) Dpt of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Taegu, Republic of Korea

Aquaporins (AQPs) are water channels that facilitate transport of water across plasma membranes. Moreover, increasing evidence indicates involvement of some AQPs in carcinogenesis, especially AQP5. AQP5 is overexpressed in several cancers, including breast cancer, where overexpression correlates with poor prognosis. So far, little is known regarding the mechanisms of how AQP5 contributes to cancer in vivo.

We report that AQP5 overexpression in normal epithelial cells promoted actin stress fiber formation and lamellipodia dynamics. Moreover, a population based single cell spreading analysis revealed reduced single cell circularity and suppression of cell spreading. AQP5 overexpression downregulated junctional proteins in cell cultures and correlated with low levels of the junctional protein plakoglobin (γ-catenin) in breast cancer biopsies. AQP5 interacted with junctional proteins β-catenin, plakoglobin and ZO1 and AQP5 expression negatively affected both mRNA and protein levels. Surprisingly, the AQP5S156A mutant that is impaired in phosphorylation by PKA also promoted low levels of adhesion proteins, indicating a Ras independent pathway.

The Wnt pathway feedback inhibitor Axin2, a β-catenin/TCF-regulated gene, was also down-regulated in AQP5 expressing cells. Moreover, inhibition of glycogen synthase kinase-3β (GSK-3β) with LiCl partially restored β-catenin levels in AQP5 expressing cells. This indicates that AQP5 expression may interfere with the Wnt signaling pathway by activating or preventing inhibition of GSK-3β.

Degradation of adhesion proteins is a key event in epithelial-to-mesenchymal transition, which increases cancer cell invasion. Thus, AQP5 may contribute to carcinogenesis by degradation of adhesion proteins.

These findings highlight AQP5 as a target for intervention strategies in cancers exhibiting high AQP5 expression levels.

Membrane Damage and Repair

Jesper Nylandsted

Danish Cancer Society Research Center

Injuries to the cell membrane of cancer cells, which are caused from invasive behavior, enhanced membrane dynamic and metabolic stress pose lethal threats to cells. Cancer cells cope by activating their plasma membrane repair system, which depends on annexin proteins and includes mechanisms to remove damaged membrane by excision, internalization by endocytosis or reorganization of actin around the hole to seal the wound. Annexin proteins characterized by their Ca2+-dependent binding to anionic phospholipids and ability to aggregate membranes appear to have specific functions in the repair machinery. Besides their ability to fuse adjacent membranes they have specific roles in repair by regulating membrane excision, shedding, and induction of membrane curvature. Here, novel aspects of plasma membrane repair mechanisms implicating annexins will be presented revealed by laser induced membrane wounding and live-cell imaging.

Linear- and nonlinear optical microscopy combined with force measurements for the characterization of spider silk

Irina Iachina, Jacek Fiutowski, Serguei Chiriaev, and Jonathan Brewer

University of Southern Denmark

Spider silk has many properties that may be of industrial use as the tensile strength of spider silk is comparable to that of alloy steel and the silk is about half as strong as for example Kevlar but has the advantage of being spun at room temperature. The aim of this project is to characterize spider silk from the orb web weaving spider Nephila Madagascariensis by determining the nano- and microscopic structures within the silk and couple these to the macroscopic properties such as tensile strength and elasticity.
In order to image the nanoscopic structures within the silk a technique used is Scanning He ion Microscopy. With this technique it is possible to resolve structures of about 1 nm without treating/altering the sample and to mill through the sample in order to visualize the internal structures of the silk fiber.

Using fluorescence microscopy the lipids and proteins of the fiber are visualized showing the overall structure of the fiber. Force and stress-strain measurements are performed while imaging the silk using multiphoton- and widefield microscopy in order to monitor the structural changes of the fiber during stress.

Electron microscopy characterization of nanomaterials; ex-situ, in-situ and in-vivo

Paul Kempen

Denmarks Technical University

As the use of nanomaterials for biological applications grows, questions regarding the uptake and ultimately the fate of these nanoparticles will continue to arise. Characterizing the uptake and localization of these nanomaterials both at the cellular and subcellular level is crucial to understand how they interact with the cell and can be used to help guide the development of novel targeting strategies. Due to their size and composition, electron microscopy is ideally suited to examine these nanomaterials ex-situ, in-situ and in-vivo.

Transmission electron microscopy is a powerful tool to accurately determine the location of nanomaterials within the cell to better understand the mechanisms responsible for nanoparticle uptake or lack thereof. For successful imaging of nanomaterials in-situ and in-vivo¬ however, it is necessary to be able to clearly distinguish the nanomaterial from the surrounding matrix. For inorganic nanoparticles this is relatively straightforward due to mass and diffraction contrast, but for organic nanoparticles this is more of a challenge. Through the use of energy dispersive x-ray spectroscopy in conjunction with TEM one can definitively locate and identify nanomaterials with unique elemental compositions. Thus, by loading organic nanoparticles with a unique elemental marker, it is possible to locate and characterize even organic nanoparticles in-situ and in-vivo.

Use of Serial block-face electron microscopy to determine mitochondria shape modifications in a mouse model of Parkinson Disease

Emilie Tresse-Gommeaux, Guinevere Sew and Shohreh Issazadeh-Navikas

Neuroinflammation Unit, Biotech Research & Innovation Centre; University of Copenhagen; Ole Maaløes Vej 5, 2200 Copenhagen N, Denmark

Parkinson disease (PD) is the most common progressive neurodegenerative movement disorder. Two prominent pathological features are associated with the neurological lesions: the appearance of cytoplasmic inclusion bodies called Lewy bodies (LB), and defective mitochondria. We wanted to determine whether mitochondrial homeostasis was modified in a model of sporadic PD.

We wanted to assess mitochondrial shape using confocal microscopy in cultured cortical neurons. However, the resolution obtained was a strong limiting factor, as mitochondrial accumulation was confined to particular location in neurons. Additionally, it was not easy to get conclusive results utilizing brain tissues. To circumvent these limitations, we used serial block-face Scanning Electron Microscopy (SEM), a technique combining in situ sectioning and imaging. Images were then process using Amira 3D software to segmentate whole mitochondria from different part of neurons. This allowed us to reconstitute in 3D the shape of mitochondria in the thalamus of one-year-old mice and to quantify numbers, volumes and branching. We found that individual mitochondria size was increased in our PD model. We also observed an increased proportion of branched mitochondria. We further demonstrated that this was due to impairment of mitochondrial fission, a process allowing the creation of new mitochondria, the removal of defective ones and controlling oxidative stress in postmitotic neurons.

In conclusion, serial block-face SEM is a valuable tool to image and analyse organelles at the best resolution within tissue samples.

Application of deep learning for tissue-based cancer screening and research

Jeppe Thagaard[1], Anders B. Dahl[2], Søren Hauberg[2], Thomas Ebstrup[2]

1) Visipharm, DTU;
2) DTU

Deep learning is probably the hottest keywords of 2017 within the healthcare research community, strongly driven by amazing state-of-the-art results in computer vision and natural language processing combined with popular media interest. It could seem like a perfect tool for novel applications in digital pathology by unlocking large and high-dimensional datasets in an end-to-end algorithmic learning approach. In this talk, Jeppe will present research conducted during his master thesis on application of deep learning for tissue-based breast cancer research. Here, he will share his experiences from participating in the CAMELYON17 Grand Challenge of IEEE International Symposium on Biomedical Imaging for automated detection and classification of breast cancer metastases in whole-slide images (WSIs) of histological lymph node sections. In this international challenge, he was ranked 5th of 23 qualifying teams of international research groups and commercial teams, with a marginal score difference to the winner of the CAMELYON17 competition. Finally, he will also give his insights to some of the challenges of applying deep learning to gigapixel images and the essentials, that need to be covered for this technology to excel. Here, he will present his current research conducted at Visiopharm on methods to automatically obtain better and more objective ground truth annotations for the development of deep learning models in digital pathology.

Distribution of organelles using shortest path metrics on the intracellular space of the neuron active zone

Hans JT Stephensen, Jon Sporring

University of Copenhagen

Measuring distances inside of animal cells is usually done without regard for obstructing structures or organelles, but as a straight line in euclidean space between two points of interest. Often, this does not accurately represent the distances inside cells such as nervous system cells, as they bend through the tissue and contain a numerous amount of obstructing organelles. By developing a framework for segmentation and annotation of organelles, and for generating distance functions based on the annotations, we are able to take into account the obstructions in the cell. With this, we can measure distance as the shortest path between two sets of points inside the cell without having to traverse organelles or the exterior of the cell. As a result, we get a different view on the intracellular space of the neuron as we examine the resulting distribution of organelles.

How to fit in? Mechanics of epithelial cell renewal in vivo.

Jakub Sedzinski[1], Edouard Hannezo[2], Fan Tu[3], Maté Biro[4], John B. Wallingford[3]

1) DanStem, University of Copenhagen;
2) University of Cambridge, UK,
3) University of Texas, Austin, USA,
4) EMBL, Sydney, Australia

Epithelial sheets are crucial components of all metazoan animals, enclosing organs and protecting the animal from its environment. Epithelial homeostasis poses unique challenges, as addition of new cells and loss of old cells must be achieved without disrupting the fluid-tight barrier and apicobasal polarity of the epithelium. Several studies have identified cell biological mechanisms underlying extrusion of cells from epithelia, but far less is known of the converse mechanism by which new cells are added. Here, we combine molecular, pharmacological, and laser-dissection experiments with theoretical modeling to characterize forces driving emergence of an apical surface as single nascent cells are added to a vertebrate epithelium in vivo. We find that this process involves the interplay between cell-autonomous actin-generated pushing forces in the emerging cell and mechanical properties of neighboring cells. Our findings define the forces driving this cell behavior, contributing to a more comprehensive understanding of epithelial homeostasis.

TIRF microscopy for single-molecule FRET investigations of tandem G-quadruplex structures

Emil Laust Kristoffersen, Victoria Birkedal

Interdisciplinary nanoscience center (iNANO), Aarhus University, Gustav Wieds Vej 14, 8000 Aarhus, Denmark

Consecutive DNA G-quadruplexes, which are non-canonical nucleic acids structures, can form from long guanine-rich sequences in the genome. One such region is the single-stranded human telomeric overhang, which contains the 5'-TTAGGG-3' motif in multiple repeats. G-quadruplex formation has been shown to inhibit the essential elongation of telomeres in cancer cells and is considered as a promising drug target for cancer treatment. Single telomeric G-quadruplex structures are highly polymorphic, which can influence ligand G-quadruplex binding. Here, we investigate folding and polymorphism of DNA sequences capable of forming two consecutive G-quadruplexes.

We investigate the structure and dynamics of immobilized fluorophore labeled telomeric DNA sequences able to form two G-quadruplexes using single-molecule TIRF microscopy for Föster Resonance Energy Transfer (FRET) measurements and photo spectroscopic methods including circular dichroism and UV. Our data show that various structures form under different cation conditions (K+, Na+ or Li+). In the presence of Li+, we primarily observe a low FRET peak as G-quadruplex folding is not favored in these conditions. G-quadruplex folding occurs in the presence of both Na+ and K+, where we observe high FRET peaks. Our data suggest formation of multiple different G-quadruplex structures under cellular like salt conditions (100 mM KCl).

Combined X-ray microscopy study of the malaria parasites in human red blood cells

Sergey Kapishnikov[1], Daniel Grolimund[2], Gerd Schneider[3], Leslie Leiserowitz[4], Eva Pereiro[5], Yang Yang[6], Peter Cloetens[6], James G. McNally[7], Jens Als-Nielsen[1]

1) University of Copenhagen;
2) Paul Scherrer Institute;
3) Humboldt University,
4) Weizmann Institute of Science,
5) ALBA Synchrotron Light Source,
6) ESRF,
7) Helmholtz Zentrum Berlin

Alarming signs of the malaria parasite resistance to current drug treatments highlight the need for identification of efficient targets to improve present antimalarial treatment strategies. A key drug target for malaria has been the crystallization pathway of the small iron-containing molecule heme, a byproduct of the digestion of hemoglobin, the malaria parasite's food source. Sequestration of heme into hemozoin crystals is the parasite's main mechanism of heme detoxification. How heme is crystallized remains uncertain, but current models predict very different rates of crystallization. We have developed a correlative X-ray transmission and X-ray fluorescence microscopy approach to enable estimation of the in-vivo rate of heme crystallization in the malaria parasite by mapping the distribution and concentration of heme relative to the 3D ultrastructure of the parasite subject only to cryo-preservation. Our measurements are consistent with in-vitro rates of hemozoin crystallization via the heme detoxification protein. Our measurements also demonstrate the presence of considerable amounts of non-crystalline heme in the digestive vacuole, which we show is most likely contained in hemoglobin. Together these results suggest an assembly line process of heme monomer release from digestion of hemoglobin, followed by catalyzed dimerization of heme via the heme detoxification protein and, finally, hemozoin crystallization. The rates of heme monomer release, heme dimerization and hemozoin formation must closely match one another, and a feedback mechanism between hemoglobin digestion and heme dimerization must exist. Two targets for drug development become apparent. The first target is the inhibition of the heme detoxification protein which would lead to accumulation of toxic heme monomers. The second target is inhibition of the feedback mechanism, which would result in uncontrolled release of heme from hemoglobin ingested by the parasite causing its destruction. More generally, our correlative X-ray method establishes a new approach for the measurement of element-specific concentrations within cellular ultrastructure.

Poster 1

Danish Molecular Biomedical Imaging Center (DaMBIC)

Vita Solovyeva and Jonathan Brewer

University of Southern Denmark

The DaMBIC core facility provides access to state-of-the-art microscopy equipment, training and assistance required for the whole range of light microscopy for imaging specimens ranging from single molecules to cells and tissue. Some of the available imaging techniques at DaMBIC are:

- Confocal laser scanning microscopy
- STED (STimulated Emission Depletion microscopy)
- CARS (Coherent anti-Stokes Raman microscopy)
- Spinning disk microscopy (live cell imaging)
- TIRF (Total Internal Reflection Fluorescence microscopy)
- FLIM (Fluorescence Lifetime IMaging)
- FCS (Fluorescence Correlation Spectroscopy)
- Two-photon microscopy.

These techniques are used to study a wide variety of samples and questions including, live cell imaging, diffusion in tissues, cancer diagnostics, super-resolution imaging and biomaterials and food analysis. At DaMBIC we offer individual training, assisted use of the microscopes and individual experimental protocol optimization. For more information see http://www.dambic.dk/

Poster 2

Center for Advanced Bioimaging (CAB)

Nynne M. Christensen[1], Michael Hansen[2], Anne-Marie Heegaard[3], Ivana Novak[1], Michael Lisby[1], Poul Hyttel[4] and Alexander Schulz[2]

1) Department of Biology;
2) Department of Plant and Environmental Sciences;
3) Department of Pharmacology and Pharmacotherapy,
4) Department of Veterinary and Animal Sciences, Copenhagen University, DK

The ability to tag and image proteins within cells and tissues have had paramount importance for our understanding of cellular processes. Research in life science relies extensively on imaging techniques to visualize location, processes, interactions, movement and kinetics of cellular components, often in living cells and tissues. Advanced microscopes and the knowledge on how to use them is therefore a great asset. The Center for Advanced Bioimaging (CAB) Denmark was funded by the programme for National Research Infrastructure in 2010, based on a proposal which gathered more than 50 research groups.

CAB's instrument park comprises wide-field, confocal and 2-photon microscopes, a dedicated fluorescence life time (FLIM), a robot high-content screening spinning disk robot system and a super-resolution microscope, a BioStation for long term observations and a whole animal imager. Moreover, a laser micro dissection device, a laser ablation ICP-MS, a class S2 cell culture lab and a histology lab allow for tissue preparation and cell -specific analyses. Access is by a browser-based booking system that also does the invoicing. Time fees cover service contracts, drift costs, replacement of lasers and other parts, software licenses, training and conferences of the CAB team.

Beside the microscopes, CAB also offers master and ph.d. courses in Advanced Microscopy and Biological Imaging. Should you get interested in trying one of the microscopes, want to get more information or have someone holding a seminar on possibilities within microscopy please contact Nynne Christensen (nmchristensen@bio.ku.dk) or Michael Hansen (mh@plen.ku.dk). You can find out more about CAB on our homepage: http://cab.ku.dk/

Poster 3

MALDI and DESI mass spectrometry imaging at University of Copenhagen

Christian Janfelt

University of Copenhagen

Mass spectrometry imaging (MSI) comprises a group of recently developed techniques, which utilize the information richness and molecular specificity of mass spectrometry in the creation of molecular images. By scanning a tissue sample below the ionization spot of a mass spectrometer, molecule-specific images may be created for all detectable compounds present in a sample. In addition to monitoring drugs and their metabolites in an organism, these techniques may be used to map and characterize endogenous compounds that serve as tumor biomarkers, enabling accurate discrimination between for example cancerous and healthy tissue.

At the Department of Pharmacy, University of Copenhagen, we have worked with DESI-MSI since 2010, and in 2015 we obtained a new system for high-resolution MALDI MS imaging. The system is based on an Orbitrap mass analyser enabling accurate mass identification of imaged compounds, and has a laser which can be focused to 5 µm spot size, enabling MS imaging with a spatial resolution of 3-5 µm (in contrast to the approx. 20 µm offered by most commercial systems).

The presentation will contain an introduction to MSI, followed by examples of previous MSI studies from our research group. Such examples include whole-body imaging of drugs and metabolites in mice, imaging of secondary metabolites in plant tissue, imaging of lipid biomarkers in ischemic mouse brains and in testis samples (MSI based histology) as well as MSI in drug delivery studies in skin and other tissue barriers.

Poster 4

3D Craniofacial Image Research Laboratory

Tron A. Darvann, Nuno V. Hermann, Sven Kreiborg

3D Craniofacial Image Research Laboratory

The 3D Craniofacial Image Research Laboratory is an interdisciplinary research unit jointly sponsored by:

- School of Dentistry, Faculty of Health Sciences, University of Copenhagen;
- The Centre of Head and Orthopaedics, Copenhagen University Hospital Rigshospitalet; and
- Department of Applied Mathematics and Computer Science , Technical University of Denmark.

The unit carries out research in the interface between medicine/dentistry and engineering. In particular, its mission is to develop state-of-the-art medical image analysis methodologies and apply them to craniofacial research. Topics include morphology and growth in congenital and acquired craniofacial deformities and cleft lip and palate, surgical treatment planning and evaluation, outcome prediction and genotype-phenotype correlation.

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Poster 5

Bioimaging of teeth and their surrounding tissues and biofilm

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At the Department of Dentistry and Oral Health, bioimaging is a central part of our research of dental tissues and diseases in the oral cavity. We conduct research in the understanding, preventing, and treating of such diseases and there has been a strategic focus on the image-based investigation of clinical problems. For example, because of the etiological role of biofilms in many diseases including dental caries and periodontitis, we have investigated biofilm ecology combining newer molecular techniques such as Confocal Laser Scanning Microscopy (CLSM) and fluorescence techniques. These methods offer new opportunities for analyzing bacterial population dynamics and dental biofilm architecture. Recently, we have developed a novel pH-ratiometric-method using CLSM together with a fluorescent dye which allows us to define ecological micro-niches within intact biofilms. Radiographic imaging methods are also used within Dentistry. Especial attention is given to those methods allowing the three-dimensional visualization of the mineralized structures of the maxillo-facial region, being the most relevant cone-beam CT (for in vivo imaging) and micro-CT (for ex vivo and in vitro imaging). For in vivo investigations, to keep the radiation dose to the lowest achievable, images with submillimeter resolution are a reality. For those investigations in which the radiation dose is not relevant, the achievable resolution is higher. Although advances, has been made, within the field of radiographic imaging, basic histology is still the gold standard for study of the detailed anatomy of hard and soft tissue. We investigate outcome in both calcified and decalcified tissue, using saw microtome, sliding POLYCUT microtome and rotary microtome. Imaging of tissue sections have been greatly improved by new technologies allowing rapid creation of high-resolution panoramic images that allow digital analysis. Several of the methods used can be used in research fields outside the dental field, and interdisciplinary collaborations are welcome to strengthen such possibility.

Poster 6

CFIM – The Core Facility for Integrated Microscopy

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CFIM, Faculty of Health and Medical Sciences, University of Copenhagen

The Core Facility for Integrated Microscopy (CFIM – www.cfim.ku.dk) is a technology platform aiming at democratizing the access to microscopy equipment and techniques within the scientific community. Located at the Faculty of Health and Medical Sciences, University of Copenhagen, CFIM offers access to a wide range of state of the art light and electron microscopes, from conventional, yet essential, instruments, such as widefield microscopes and classic SEM/TEM to specialized, high-end systems including super resolution or single particle cryo-TEM. Housing both light and electron microscopy under the same roof allows for interaction and correlation between the different disciplines. Besides hosting equipment, CFIM provides expertise, training, and support. CFIM is committed to education; we are offering several courses:

- A two-week PhD course on Microscopy Principles and Fluorescence and Confocal Microscopy, organized twice a year;
- A one-week PhD course on Electron Microscopy, organized once a year;
- A one-week course in Image Analysis, organized once a year;
- A two-day course in light microscopy open to all, organized once or twice a year.

CFIM is open to all. Check our website for becoming a user: www.cfim.ku.dk

Poster 7

NEUBIAS, Network of European Bioimage Analysts

Neubias community[1], Laure Plantard[1,2], Clara Prats[1,2], Julien Colombelli[1,3]

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Bioimage analysis is the quantitative measurement of biological systems by objectively processing image data, independently of human observation. NEUBIAS is a unique network aiming at strengthening the bridge between the scientists (life scientists, computer scientists, microscopists…) involved in bioimage analysis and boosting the productivity of bioimaging-based research in Europe .

NEUBIAS organizes various activities and actions that can benefit biologists working with images.

1. NEUBIAS courses: twice a year, Neubias organizes courses in image analysis in Europe. There are three levels of competences covered, from beginner to expert.
2. Neubias conference: once a year, Neubias organizes a conference regrouping all actors in the field. In addition to classic presentations, the conference features
    - an open source software lounge: demonstration of newly developed software packages allowing image analysis,
    - a call-4-help session where scientists present their analysis problems to the audience of experts and get concrete feedback and suggestions
3. Neubias Short Term Scientific Missions: fellowships awarded for short visits to other labs to solve image analysis problems
4. BISE: a searchable repository of bioimage analysis tools: http://biii.eu/
5. Open publication of textbooks in Bioimage analysis. The first edition of bioimage data Anaysis is already available.

NEUBIAS (Network of EUropean BioImage AnalystS) is an action fully funded by COST (CA15124). For more information, visit our webpage: http://eubias.org/NEUBIAS/

Poster 8

Rendering a real time beating Zebrafish heart model from synchronized images from a stack

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In order to look for arrhythmias in Zebrafish larvae hearts expressing a Ca2+ sensitive GFP, we scanned a live fish larvae heart in a Yokogawa spinning disk system. We soon faced the problem that the spinning disk is not nearly fast enough to capture a full 3D heart with a pulse of app. 2 Hz. Inspired by a talk on the problem of reconstructing a beating fly's wing with a too slow setup, we instead did a full 3D stack while the heart was beating. We moved the piezo table only 30 nm in Z between frames. The resulting stack of 4000 images covering 652 contractions was afterwards processed to isolate images from different phases of the contraction, resulting in 6 different time points of the heart's contraction cycle. With these time points six 3D stacks could be assembled and joint together to form a 3D movie showing the full contraction cycle of the Zebrafish larvae heart in real time. Come by and see the resulting movie, discuss the method and the usefulness of the resulting 3D heart in the search for arrhythmias.

Poster 9

Visualizing nanoparticles in histological tissue samples using enhanced darkfield hyperspectral microscopy

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The fate of nanoparticles (NPs) in an organism is a key aspect of nanotoxicology. Here, we show the applicability of enhanced darkfield hyperspectral imaging of unlabeled NPs in tissue. Metal oxide NP aggregates in lung and liver show intense light scattering in enhanced darkfield. This enables visualization of cells and biological barriers involved in accumulation, translocation and clearance of NPs, which is valuable for clarifying mechanisms of toxicity. However, in secondary organs where NP content is low, it requires expert opinion to distinguish NPs from light-scattering artefacts and endogenous material. Hyperspectral imaging gives a spectral fingerprint of each pixel in a sample. However, many NPs are not spectrally unique and the spectral features are complicated by the variability and complexity in histological samples. Thus, using spectral classification algorithms for specific identification of NPs in tissue requires optimization of sensitivity and specificity.

Poster 10

Using lanthanide emission to create infinite contrast in fluorescence microscopy

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Fluorescence microscopy relies on the ability to detect the difference between a signal emitted from a probe and the background. The main issue is that the background rarely is truly dark, and image analysis is needed to enhance the fluorescent signal that is ascribed to the selected feature. This process is facilitated by using bright probes, strong illumination, and high concentration of probes, thus increasing the fluorescent signal more than the corresponding background signal. However, all these approaches are of limited scope.

Here, we introduce a method for completely removing the background signal in spectrally resolved fluorescence microscopy without time-gating, thereby allowing the origin of the detected fluorescent signal to be assigned with full confidence; the detected photons arise only from emission of a specific molecular probe. The method is applicable for all probes with narrow and well-defined emission bands (FWHM < 20 nm), here we use lanthanide(III) based probes.

We demonstrate a data treatment method that analyzes spectrally resolved imaging data by exploiting the narrow emission bands arising in the f-f transitions of europium(III) and terbium(III) ions. The method uniquely identifies the sharp emission lines arising from lanthanide centered emission, and automatically subtracts the background signal. Therefore, the origin of the photons used to generate an image can be assigned with absolute certainty. We have used a home-build microscopy set-up, but most high-end modern microscopes are capable of spectral imaging with sufficient resolution to implement the presented data treatment method.

As the lanthanide(III) probe, we used a model system, where Eu(III) or Tb(III) ions were doped inside the pores and cavities of Linde Type A zeolites.9 The doped zeolites were mixed with common fluorophores (fluorescein) in 3% (w/v) polyvinyl alcohol and spin-coated to form a polymer thin film.

Poster 11

Synaptic membrane contacts surrounded with perineuronal nets – quantitative analysis of the complex microstructure

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Perineuronal net is a highly structured subtype of the CNS extracellular matrix colocalized with the interface of the presynaptic, postsynaptic and astrocyte membranes in the brain and spinal cord. Perineuronal nets play important role in synaptic plasticity, neuronal excitability and CNS pathologies including epilepsy and regeneration failure. While physiological roles and molecular content of perineuronal nets have been extensively studied within last decade, the microstructure of the complex of perineuronal net with the presynaptic and postsynaptic membranes remains largely unstudied. We previously demonstrated that perineuronal nets form spatial patterns and distinct clusters at the surface of the neuronal cell body plasma membrane (Arnst et al., Brain Research, 2016). Those spatial structures suggest additional mechanism for compartmentation of the neuronal cell surface at the contact sites of presynaptic and postsynaptic plasma membranes.

Here we propose a method for the quantitative image analysis of perineuronal nets stained with Wisteria floribunda agglutinin colocalized with the postsynaptic membrane-associated scaffolding protein gephyrin. The method allows autothresholding segmentation of the gephyrin signal and quantitative studies of the postsynaptic marker location in relation to the perineuronal net. We then further extend the proposed approach to include combination of the presynaptic and postsynaptic markers together with the extracellular matrix staining. We suggest that the present method can be widely used for a range of presynaptic, postsynaptic and astrocytic membrane-associated proteins for the studies of synaptic structure and function.

Poster 12

SorCS2 in postnatal development of GABAergic interneurons in the hippocampus

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Neuronal differentiation during development of the hippocampus is critical for the functionality of the adult brain. Here, activity dependent release of Brain-derived Neurotrophic Factor (BDNF) is critical for changes in gene expression as well as morphological changes of GABAergic interneurons.

SorCS2, a member of the Sortilins family, has been shown to be important for BDNF mediated signaling in the adult brain. During the prenatal period, as the hippocampus is formed, SorCS2 expression was not found in the migrating interneurons and in concert with this, no differences were found in the number of interneurons in mice lacking SorCS2 expression (SorCS2-/-). However, during postnatal development, high level of SorCS2 expression was seen. Here, in vitro studies showed that SorCS2 is important for GABAergic differentiation. Reconstruction of hippocampal networks from brain slices revealed a reduced connectivity between GABAergic interneurons in SorCS2-/- mice. Altered expression of GABAA receptor subunits was also seen in SorCS2-/- mice where especially GABAA receptor subunit α2 was heavily reduced. As a possible consequence of this, SorCS2-/- mice injected with Pentylenetetrazole (PTZ), a GABAA receptor antagonist, showed increased susceptibility to developing epileptic seizures. This indicates that SorCS2 plays an important role for differentiation of the GABAergic system during postnatal development.

Poster 13

Selective activation of the nigrostriatal dopaminergic pathway induces pathway specific changes in dopamine and glutamate levels.

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Objectives: The dorsal striatum is a region which receive glutamatergic and dopaminergic inputs and regulate our motor response to stimuli. The aim of the study was to measure the neurochemical effects in striatum following selective stimulation of the nigrostriatal dopaminergic pathway. DREADDs (Designer receptors activated exclusively by designer drugs) were inserted in striatum of tyrosine hydroxylase (TH) Cre-positive rats using a Cre dependent and mainly retrogradely transported viral vector. Such an activation is hypothesized to cause burst firing of dopaminergic neurons modulating dopamine release and behavioral responses.

Methods: Female TH:Cre+ (Tg(TH:Cre 3.1)Deis) animals were injected bilaterally in the dorsomedial striatum with a primarily retrogradely transfecting Gq coupled DREADD receptor virus (rAAV6-hSYN-DIO-hM3Dq-mCherry). A viral vector without the DREADD sequence were used in control animals. Subsequent assessments of neurometabolic (1H-Nuclear Magnetic Resonance Spectroscopy) and dopaminergic ([18F]Fallypride Positron Emission Tomography) levels as well as locomotor activity were performed at baseline and after administration of the DREADD selective ligand Clozapine-N-oxide (CNO). Results: Administration of 0.5 mg/kg CNO to TH:Cre+ hM3Dq DREADD animals increase the total distance moved between 30-60 minutes post administration, cause increase the concentration of glutamate (Glu) and glutamate and glutamine (Glu+Gln) and results in a decrease of [18F]Fallypride binding potential solely within the dorsomedial striatum. The administration of 0.5 mg/kg did not result in detectable neurometabolic changes in control animals.

Conclusions: We selectively transfected the nigrostriatal dopaminergic pathway with DREADDs and elicited behavioral hyperactivity as well as in vivo neurochemical changes suggesting successful modulation of selective dopaminergic signaling.

Poster 14

Vesicular transport in blood-brain barrier models

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The blood-brain barrier -formed by microvascular brain endothelial- cells is the most important interface for molecular fluxes between blood and neurones. Therefore in vitro blood-brain barrier models are useful tools to study drug uptake and transcytosis from blood toward the brain. Given the growing number of neurological patient and the low success rate of drug candidates, it is necessary to investigate the intracellular trafficking pathways which are responsible for the translocation of drugs. These pathways use complex subcellular vesicular system which has not been investigated in details, yet. Therefor our aim was to investigate the intracellular vesicles in different in vitro blood-brain barrier models. For our investigation we have chosen three widely-used models; the bEnd.3 mouse, the hCMEC/D3 human brain endothelial cell line and the primary porcine blood-brain barrier model. To describe the endo-lysosomal system, specific vesicular markers for early, recycling, late endosomes, lysosomes and the retrograde transport rout were marked by immunocytochemistry with specific markers. High content screening of images was performed using Scan^R image and data analysis software for Life Science (Münster, Germany). Number, distribution, shape and area of the different type of vesicles were compared among these in vitro blood-brain barrier models and remarkable differences were observed. Since the primary porcine model reflects closer the human in vivo condition, our data raise the question; Are the immortalized brain endothelial cell lines sufficient blood-brain barrier models for drug transport studies? Data from our investigation could be potentially used to optimize drug delivery to the brain more efficiently.

Poster 15

The endothelial glycocalyx and malaria

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The luminal side of all blood vessels is covered by a negatively charged layer of complex carbohydrates termed the endothelial glycocalyx. The endothelial glycocalyx is involved in vascular function and often lost upon damage to the vasculature. Malaria leads to a systemic inflammatory response due to the rupture of infected red blood cells as well as local stimulation of the vasculature upon binding of parasitized red blood cells to the vessel wall. Our results show significant loss of endothelial glycocalyx in murine models of malaria. This has been assessed by transmission electron microscopy. Image analysis showed a less dense and narrower zone of glycocalyx in response to the parasitic infection. These findings are supported by detection of glycocalyx components in plasma. In humans, markers of glycocalyx loss are also significantly increased. By performing non-invasive imaging of the buccal cavity in human patients one can assess the microcirculation. Qualitative changes include leaky and occluded vessels. Image analyses of red blood cell movements demonstrate a more disordered pattern of movement suggesting glycocalyx loss. In conclusion these data suggest loss of endothelial glycocalyx to be part of malaria pathogenesis, which might be relevant to target for adjunctive therapy.

Poster 16

Nanoscopy, a tool for understanding transdermal drug delivery

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The penetration properties of substances across biological barriers and membranes are vital for many areas of research. The barrier in human skin is primarily found in the stratum corneum and consists of protein-enriched cells surrounded by a lipid membrane -enriched intercellular spaces. Characterization of the structural and dynamical processes occurring across the skin barrier is essential for understanding healthy and diseased skin and for designing successful transdermal drug delivery strategies. In this study we use Stimulated emission depletion (STED), two photon excited STED and Förster Resonance Energy Transfer (FRET) microscopy to probe the structure of human skin and a combination of super resolution optical microscopy and a multiphoton excitation based fluorescence fluctuation spectroscopy method, namely raster image correlation spectroscopy (RICS), to study the mechanism of action for liposomes as a transdermal drug delivery system in excised human skin. STED microscopy enables resolving structures in the skin below 60 nm allowing visualization of the stratum corneum intercellular lipid matrix and individual liposomes. To further probe the nanoscopic structure of the intercellular lipids and the nanoscopic diffusion routes of hydrophilic and hydrophobic particles through the skin barrier we use FRET measurements of lipophilic and hydrophilic dye pairs.

Our results suggest that the liposomes do not act as carriers that transport their cargo directly through the skin barrier, but in fact mostly burst and fuse with the lipid layers of the stratum corneum. It was also found that the flexible liposomes showed a greater delivery of the fluorophore into the stratum corneum, indicating that they functioned as permeability enhancers.

Poster 17

Bioimaging of dermal liposomal drug delivery by CARS

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Melanoma cancer is an increasing world vide problem. Melanoma is a malignant tumor where even small tumors can create metastasis and become a very deadly cancer. The treatment for localized melanoma cancer is a surgical procedure, while radiation and chemotherapy is only included in its metastasis form.

The skins barrier properties limit the dermal penetration of drugs, complicating localized chemo therapy. Different penetration enhancing methods can be used to increase the penetration, enhancing methods such as deformable liposomes, the use of derma roller or laser ablation, which has shown to increase the dermal absorption. By using fluorophore, the dermal penetration can be observed by the use of bioimaging methods such as coherent anti-stokes raman scattering.

Poster 18

Coherent anti-Stokes Raman scattering microscopic evaluation of skin perforation by laser ablation and microneedle treatment

Suzanna C. F. Frederiksen, Judith Kuntsche, Jonathan R. Brewer

Memphys, SDU

The skin represents an interesting route for drug delivery due to its large surface and ease of drug administration. However, the uppermost layer of the skin, the stratum corneum, represents a tight barrier against drug penetration. To overcome this barrier, various chemical and physical approaches are used such as penetration enhancers, laser ablation and microneedles. Microneedles can be incorporated into a device such as the Dermaroller, which is intended to be rolled on the skin surface creating micron-scale pores. Another approach, laser ablation, is breaking down the skin barrier with a laser. This method has emerged over the past decade and it has been studied as a strategy for drug delivery in various medical conditions e.g. non-melanoma skin cancer.

Skin structure is viewed and skin perforation is compared by microneedle and laser treatment applying imaging methods such as laser scanning confocal microscopy and coherent anti-Stokes Raman scattering. Furthermore, the effect of skin perforation on the penetration and permeation of selected compounds such as nanobeads is assessed by using Franz diffusion cells together with imaging techniques.

Poster 19

Influence of liposomal formulations on the lipid organization in human stratum corneum

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Liposomes have the potential to influence the drug delivery of both hydro- and lipophilic drugs after dermal administration. However, how different liposome formulations enhance the drug penetration and how liposomes may influence the lipid packing of the stratum corneum lipids is still not fully understood.

The aim with this master project is to investigate the influence of different liposome formulations on the stratum corneum lipid packing and the penetration depth into human skin in vitro. For this, liposome formulations differing in in membrane elasticity, will be prepared and characterized. The effect of the formulations in SC lipid packing will be investigated with excised human skin. Analysis will be performed with fluorescence microscopic methods using Laurdan as a marker to ascertain disruption in the lipid packing. DMSO, a known compound disrupting the SC lipid packing in skin at higher concentrations, as well as the influence of increased temperature will be studied for comparison.

Poster 20

Uptake and Response of Falcarindiol Lipid Nanoparticles in Stem Cells as Drug Delivery Systems for Cancer Therapy

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Dietary polyacetylenes such as Falcarindiol have increased relevance as possible anticancer compounds as well as other health promoting effects such as anti inflammatory and anti-bacterial activity. Falcarindiol has been found to prevent and reduce the number of neoplastic lesions in the colon and to increase the number of cholesteryl-ester lipid droplets in treated cells, an effect also seen when using other anticancer drugs. With Raman Spectroscopy we have showed that Falcarindiol leads to an increased number of lipid droplets in treated hMSC. In this study, the examination of its effect in hMSC with white light and fluorescence microscopy confirmed those results as more BODIPY stained lipid droplets could be visualized in free Falcarindiol treated cells, and a statistical test was performed to prove significant differences between the treated hMSC and the control group.

An alternative delivery system for Falcarindiol wanted to be assessed in order to stabilize the administration of the drug. Nanoparticles have an increased interest regarding their function as drug delivery systems for cancer therapy. Specially lipid nanoparticles coated with LDL, since cancer cells have an increased need for lipids and cholesterol to proliferate, and this has been exploited as a mechanism for delivering anticancer drugs to tumor cells. Therefore, in this study nanoparticles with a PEGylated monolayer of the phospholipids DSPC (Distearoyl-sn-glycero-3-phosphocholine) and cholesterol with a DiI label were designed.

The cellular uptake of the designed nanoparticles was examined in hMSC cells using fluorescence and confocal microscopy. Live-cell microscopy was performed as well in order to visualize the nanoparticles uptake process in a real-time video and to estimate the duration of the uptake. Nanoparticles successfully entered the cells, so they could be stable drug carrier for Falcarindiol, and the uptake happened in less than 30 minutes.

Poster 21

Antibody Conjugated Nanoparticles as a Drug Delivery System for Cancer Therapy by Active Targeting

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In the last two decades, nanoparticles have experienced an increased interest both in terms of diagnostics but also in relation to therapy as drug delivery for various diseases including cancer therapy. The aim of this study was to synthesize antibody conjugated solid lipid nanoparticles (SLNs) in the sub-500 nm range to function as an active and targeted drug delivery system in cancer therapy. The SLNs were formulated using a one-step solvent injection method where the organic phase, containing the lipid, and a reagent, was injected into an aqueous solution containing albumin and antibodies (anti-EGFR) resulting in the formation of SLNs where both albumin and antibodies were covalently bound to the surface. In this study, we used this method to make SLNs with diameter down to 89 nm and a zeta potential of -29 mV. We further did in vitro cellular uptake of the SLNs and examined the uptake in different cell lines using confocal microscopy and flow cytometry. The results indicated that (i) SLNs were internalized by the cells, (ii) by incorporating hydrophobic molecules into the SLNs the unspecific uptake of SLNs could be reduced and, (iii) conjugation of the antibody (anti-EGFR) initiated an elevated cellular uptake. Based on the findings, we therefore conclude that the SLNs produced in this study may function as an active targeted drug carrier for hydrophobic therapeutics in cancer therapy. Additionally, due to the properties of the SLNs, developed in this study we plan to study their potential as cancer drug carriers.

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