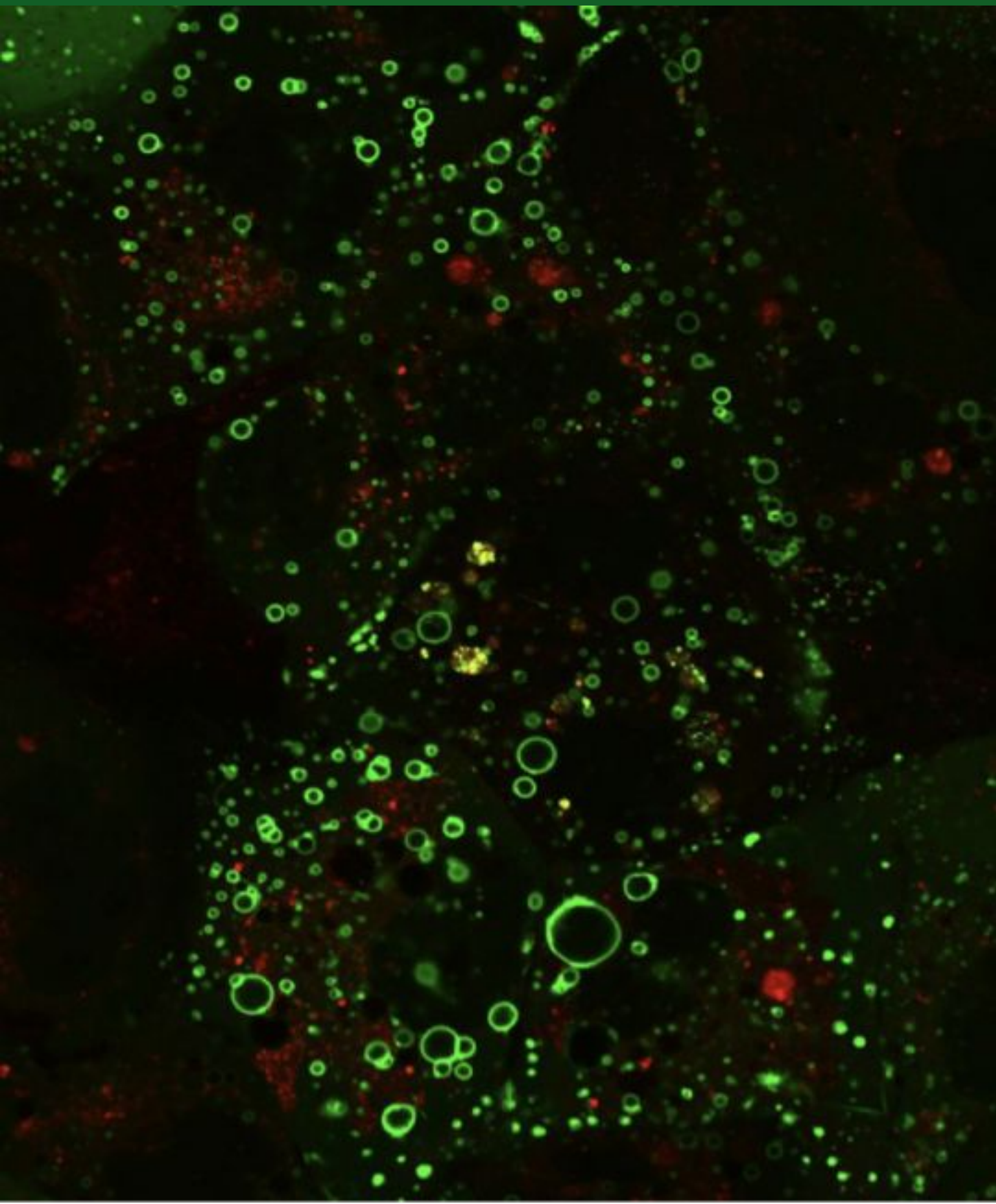
BNMID Book of abstracts

Bridging Nordic Microscopy Infrastructure 2024



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Tuesday 9th - Facility talks and industry presentations

13.00 - 15.00 Arrival and lunch

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17.15 - 17.45 Coffee Break

17.45 - 19.30 Workshop session 1

19.30 - 20.30 Invited speaker

21.00 - Dinner

Wednesday 10th - Super-resolution and Volumeric Imgaing

09.00 - 11.10 Session 1: Super-resolution w/ Invited speaker

11.10 - 11.30 Coffee Break

11.30 - 13.00 Session 2: Volumetric Imaging w/ Invited speaker

13.00 - 15.30 Lunch and activities

15.30 - 17.30 Workshops

17.30 - 18.00 Coffee break

18.00 - 20.00 Welcome drink and Poster session

20.00 - Dinner

Programme

Thursday 11th - Applied microscopy, Electron and Correlative Microscopy

09.00 - 10.00 Session 3: Applied microscopy w/ Invited speaker

11.00 - 11.30 Coffee break

11.30 - 13.00 Session 4: Multimodal microscopy w/ Invited speaker

13.00 - 17.00 Lunch, activities and Aprés-ski

18.00 - 18.45 Invited speaker

19.00 - Dinner and party

Friday 12th - Label free imaging and image analysis

09.00 - 10.00 Session 5: Label free imaging

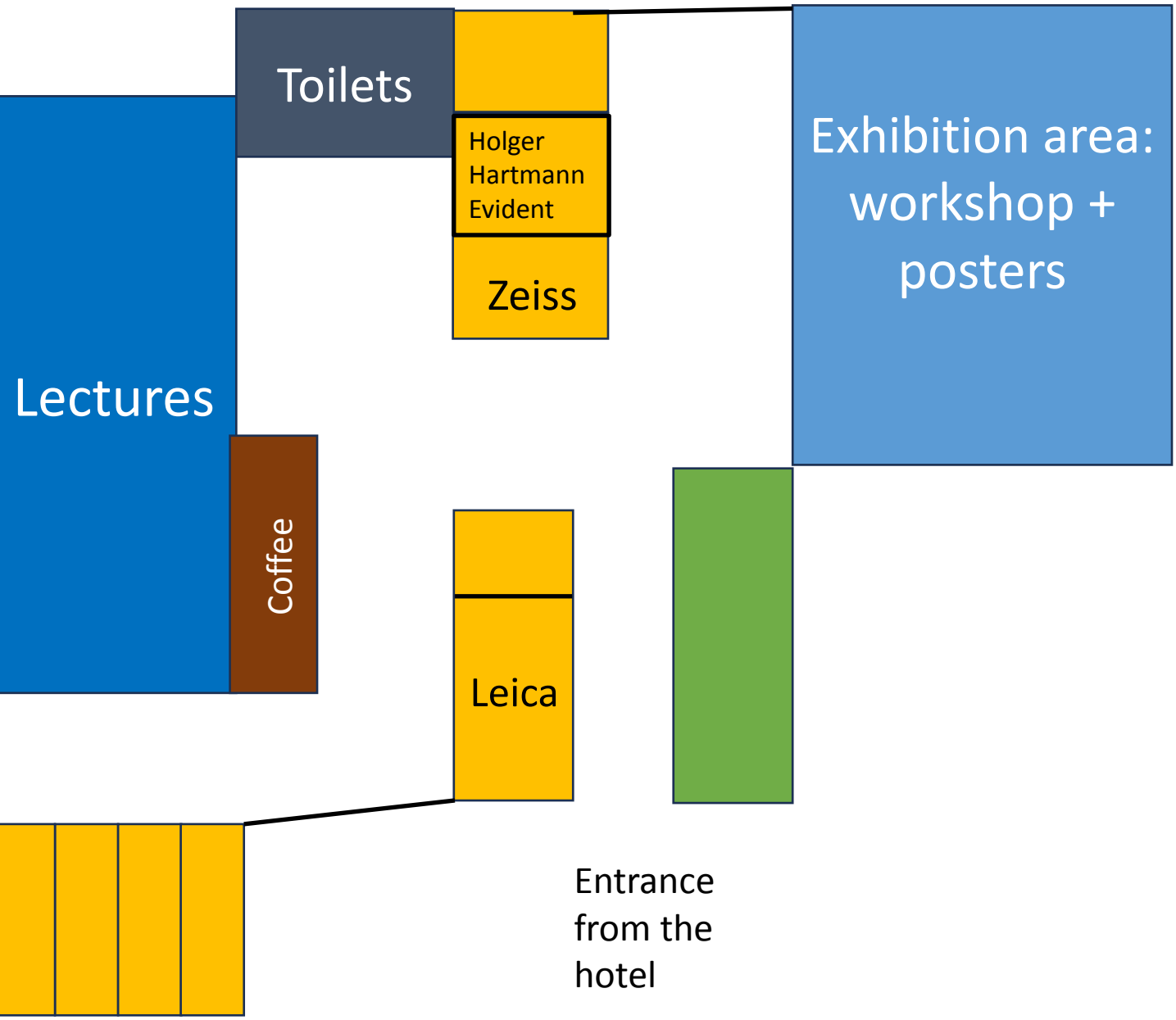
10.00 - 10.30 Coffee break, finalize check out

10.30 - 11.45 Session 6: Image analysis w/ Invited speaker

11.45 - 12.00 Final remarks

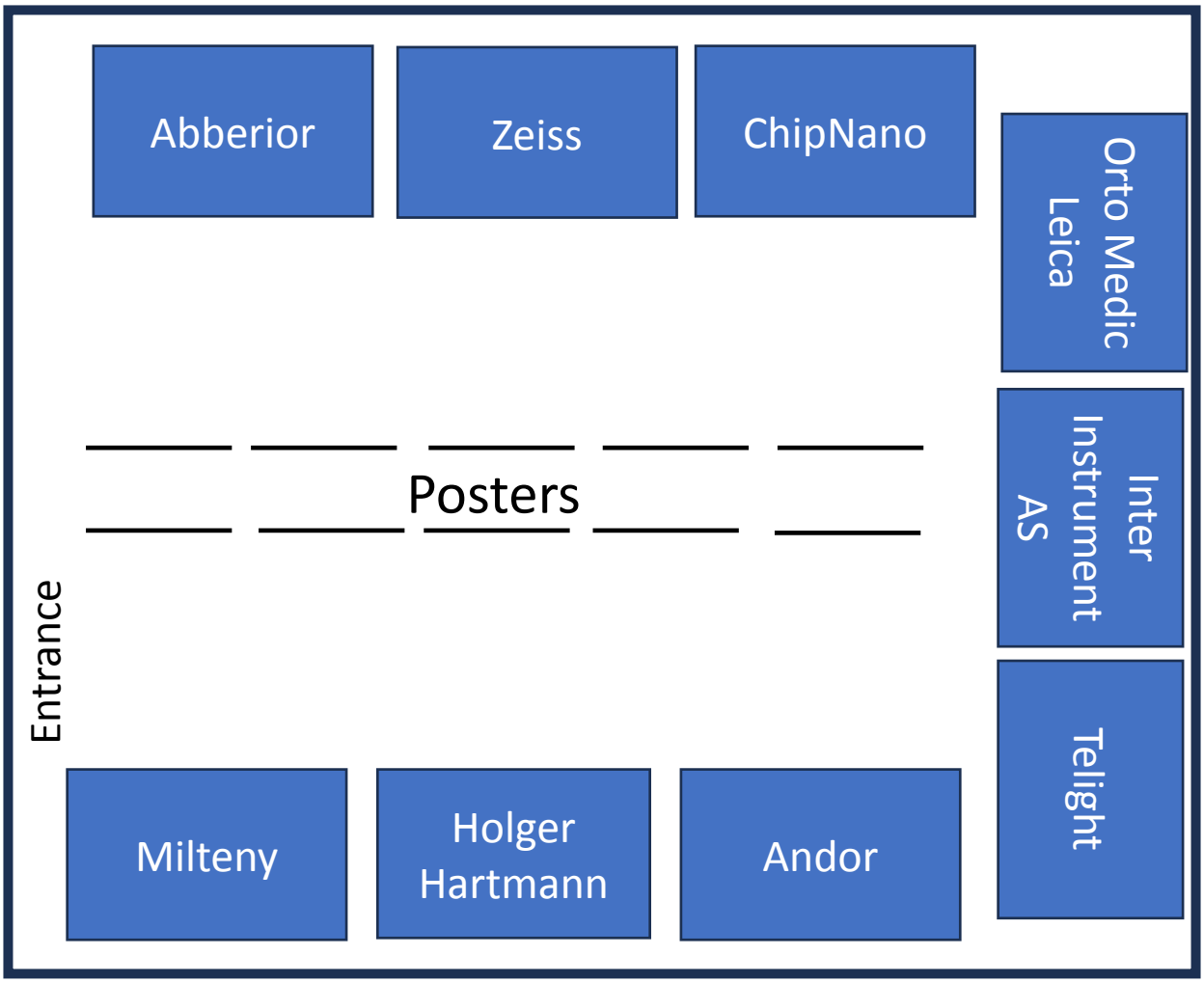
12.00 - 1400 Lunch and departure

Venue overview



Juristen 1: Leica ws
Juristen 4: Zeiss ws
Juristen 5: Holger Hartmann/Evident ws

Venue overview, workshop area



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- Milteny Biotec
- Telight
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- Holger Hartmann
- Zeiss
- Ortemedic/Leica

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Analysis of migratory behaviors in epithelial cell collectives
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EDAM Bioimaging, a program for bioimage informatics, image analysis and imaging
Matúš Kalaš, *Department of Informatics, University of Bergen, and ELIXIR Norway*

Improving light microscopy training routines with proven pedagogical methods

Sylvie Le Guyader
The Live Cell Imaging core facility, Karolinska Institutet

The low reproducibility of published scientific data has been shown to stem from the lack of awareness of researchers of the technologies they use in their research.

Despite the pivotal role of microscopy core facilities in the education of researchers being well documented, facility staff (FS) often learn their trade on the job, without receiving themselves any structured education about the technology they teach others to use. Additionally, despite endorsing an important role at the highest level of education, most FS never receive any training in pedagogy, the field of research on efficient teaching and learning methods.

In a recent article, we argue that the lack of education of training staff in pedagogy leads them to confuse understanding and learning. On the one hand, FS consider that their teaching task is to explain what is needed to produce reliable data. On the other, despite understanding what is being taught, researchers fail to learn the most challenging aspects of microscopy, those involving their judgement and reasoning.

We propose a paradigm shift where training staff at technological core facilities be acknowledged as fullfledged teachers and offered structured education not only in the technology they teach but also in pedagogy.

We also suggest that training routines at facilities be upgraded to follow the principles of the Constructive Alignment pedagogical method. We give an example of how this can easily be applied to existing microscopy training routines.

We believe that upgrading the knowledge of training staff at core facility in both microscopy and pedagogy has a strong potential for improving the reproducibility of published microscopy data.

Faculty talks & industry presentations

Tech Talk Sponsors

Inter Instruments AS - Nikon

Ultra-large field of view using chip-based TIRF microscopy
ChipNano imaging

Chip-based TIRF microscopy provides ultra-large field of view allowing the user to capture the heterogeneity within biological processes, both in super resolution and diffraction limited imaging. Our WaveBeat technology decouples the excitation and emission light path, which allows us to simplify the TIRF illumination and significantly extend the field of view. Illuminate your sample with a well-defined and homogenous TIRF illumination with precise optical sectioning of your sample. Perform super resolution on a millimeter scaled field of view and explore the possibilities of low N.A. TIRF imaging

Abberrior

Milteny Biotec

Telight

Oxford Instruments - Andor

Holger Hartmann

Introducing the new CLSM FV4000 with SilVIR™detectors, achieving much lower noise, higher sensitivity and improved photon resolving capabilities for quantitative and reproducible imaging.

Faculty talks & industry presentations

Tech Talk Sponsors

Lattice SIM 3/5 new approach to Super-resolution Microscopy

Zeiss

ZEISS Lattice SIM 3 is designed to meet the requirements of multicellular samples, such as: developing organisms, organoids, 3D cell cultures, and tissue sections. Optimized for use with objectives from 10× to 40×, ZEISS Lattice SIM 3 exploits the full potential of the SIM Apotome technology: fast optical sectioning at superior quality, large fields of view with access to smaller regions of interest, near-isotropic resolution, and the gentlest super-resolution imaging possible. Additionally, Lattice SIM imaging and SIM² image reconstruction give you super-resolution imaging down to 140 nm. ZEISS Lattice SIM 5 has been optimized for single cell imaging as well as capturing subcellular structures and their dynamics. Powered by the Lattice SIM technology and the SIM² image reconstruction algorithm.

ZEISS Lattice SIM 5 provides you with outstanding super-resolution capabilities down to 60 nm in both living and fixed cells. Additionally, you can choose SIM Apotome imaging mode and a low-magnification objective to achieve fast overview images of your sample before zooming into super-resolution details. Hardware and data-agnostic image analysis software.

Ultra-large field of view using chip-based TIRF microscopy

ChipNano imaging

Chip-based TIRF microscopy provides ultra-large field of view allowing the user to capture the heterogeneity within biological processes, both in super resolution and diffraction limited imaging. Our WaveBeat technology decouples the excitation and emission light path, which allows us to simplify the TIRF illumination and significantly extend the field of view. Illuminate your sample with a well-defined and homogenous TIRF illumination with precise optical sectioning of your sample. Perform super resolution on a millimeter scaled field of view and explore the possibilities of low N.A. TIRF imaging

Faculty talks & industry presentations

Tech Talk Sponsors

TauSTED Xtend - New tools for gentle live imaging at remarkable nanoscale

Leica/Ortomedic

The goal of scientific research is to understand the workings of nature. Given the complex interplay of biomolecules, molecular machines, and higher-order cellular structures, confocal imaging emerged as a fundamental tool owing to the optical sectioning, sensitivity, and the temporal and spatial resolution capabilities.

Imaging intricate cellular structures at nanoscale resolution while characterizing the dynamics of multiple species in the context of live specimens are emerging avenues followed to shed light on biological processes. With the advent of STED (Stimulated Emission Depletion), researchers have realized the visualization of intracellular structures at the nanoscale, unveiling insights into cellular behavior, interactions, and function.

In the talk and showcase, we will introduce our innovation TauSTED Xtend and demonstrate how it enables gentle imaging of live and fixed samples at the nanoscale. We will show how advances in our TauSTED (1) approach to optical nanoscopy deliver cutting-edge resolution and image quality at low light dose, key to accessing fast nanoscale dynamics of cellular processes. We will also show how fluorescence lifetime information can be used for multiplex imaging of different markers, keeping the nanoscopic resolution.

Imaging services: Considerations for installation and operation

Timo Zimmermann

EMBL Heidelberg

Linear - and non-linear super-resolution microscopy, an overview

Rainer Heintzmann
Friedrich-Schiller Univeristy of Jena, Germany

Understanding cardiomyocyte structure: function relationships with super-resolution microscopy

William Louch
University of Oslo / Oslo Univeristy Hospital

Contraction of the heart is reliant on the shortening of individual cardiomyocytes, elicited by calcium release. This process occurs at tiny sub-cellular structures called dyads, where the membranes of t-tubules and the sarcoplasmic reticulum are closely aligned. Despite the fundamental role of dyads in triggering the heartbeat, their precise functional arrangement remains unclear, both in health and disease. My group aims to employ emerging microscopy techniques, including live-cell super-resolution (PALM) imaging, to relate the nanoscale arrangement of key dyadic proteins and membranes to function. PALM imaging in living cardiomyocytes linked fundamental calcium release events called “sparks” to their Ryanodine Receptor (RyR) clusters of origin. This analysis showed that distinct channel clusters can collaborate to generate “travelling” sparks. However, RyR arrangements and function are highly malleable, as prolonged B-adrenergic stimulation leads to dispersal of RyR clusters, and slowed calcium spark kinetics. This phenomenon is particularly relevant during diseases like heart failure with reduced ejection fraction (HFrEF), where B-adrenergic tone is elevated, and dispersed RyR arrangements contribute to dyssynchrony and slowing of calcium release. We have also observed significant plasticity of t-tubules, i.e. the membrane invaginations that carry the electrical signal that triggers RyR opening. Specifically, these structures were found to be dependent on the workload the cell experiences, mediated by the concerted actions of several proteins. Excessively high workload, as occurs in HFrEF, is linked to degradation of t-tubule structure. In conclusion, the nanoscale arrangement of dyads fine-tunes calcium release in health, but derangements on both sides of the dyad critically impair calcium release and contractility in HFrEF.

Exploring Angiotensin Type 1 Receptor Organization at the Single-Molecule Level: Insights into Actin Networks Using Super-Resolution Fluorescence Microscopy

Yenisleidy de las Mercedes Zulueta Diaz
Univeristy of Southern Denmark, Odense

Angiotensin type 1 receptor, AT1R plays a pivotal role in maintaining salts and fluids homeostasis, cardiac adaptation, and blood pressure regulation within the cell. Consequently, AT1R has emerged as a promising therapeutic target for cardiovascular diseases and drug development. However, AT1R' dynamic nature coupled with its organization occurring at the nanoscale level poses a significant challenge. This requires the use of high-resolution imaging techniques to elucidate its precise localization and interactions with other cellular components. Super-resolution fluorescence microscopy (SRM), such as expansion microscopy (ExM), enables imaging of cellular structures and also locating proteins in a cellular context with nanometer resolution. Expansion microscopy is a method that physically magnifies samples, allowing for the acquisition of super-resolution images by using conventional microscopes. SRM, when combined with image analysis techniques, provides a powerful tool to investigate the complex cellular environment and decipher the mechanisms underlying AT1R organized in the plasma membrane (PM). This study aims to unravel the AT1R organization at the single-molecule level and also explore how their interactions with ligand complexes may influence their distribution within cells. In turn, we plan to study the impact of cytoskeleton dynamics on AT1R´s organization.

To achieve this, we are utilizing HEK cells expressing AT1R tagged with a fluorescent protein (mEos) or Halo Taq-Quantum Dots and an actin filament dye, Phalloidin. ExM coupled with Confocal or Structured Illumination Microscopy, will be employed. For image analysis, we will use Voronoi tessellation and Super-Resolution Radial Fluctuations (SRRF) Microscopy. Our preliminary ExM results have revealed that AT1Rs form nanoclusters at the PM, with the cluster size significantly decreasing after Angiotensin treatment. Additionally, receptor diffusion was observed to be transiently confined within the PM. This study will provide new insights into the role of actin networks in the receptor organization at the nanoscale.

FiberSIM: Revealing cellular nanostructures and dynamics using a highly compact and cost-effective structured illumination microscope

Jakub Pospisil
The Arctic University of Norway, Tromsø

Structured illumination microscopy (SIM) is attractive method for doubling the resolution of a light microscope. This super-resolution (SR) technique is sufficient for examining ~100 nm cellular structures and cell dynamics (Schermelleh, 2019) while using low photon budget, thereby minimizing photoinduced effects that could be detrimental to sample health. Additionally, the SIM technique can image cells over extended periods of time in multiple color channels simultaneously.

Throughout the last decade, several commercial systems offering both 2D and 3D solutions have emerged. However, these microscopes are rather bulky, utilizing relatively expensive opto-electronic components and diffractive optics to perform the structured illumination patterns. This has prompted the development of custom-built systems employing alternative methods to generate striped illumination patterns, such as liquid crystal-based spatial light modulators (Křížek, 2010) or digital micromirror devices (Sandmeyer, 2021). Nevertheless, a significant portion of laser power is lost in these diffraction optics components and electro-optical devices, resulting in longer exposure times, thus, increased phototoxicity.

Here, we introduce fiberSIM, a highly compact and flexible 2D SR-SIM microscope based on all-fiber optics components. A pair of optical fibers focuses two beams at the back focal plane of an objective lens, generating an interference pattern in the sample plane. Fiber-based interferometry significantly reduces power losses of excitation light, enabling faster imaging with shorter acquisition times to minimize phototoxicity and photobleaching. Furthermore, fiberSIM provides a field of view nearly four times wider than commercially available systems. The excitation path includes a telescope unit for easy adjustment of pattern frequency up to total internal reflection fluorescence (TIRF) mode. A custom-built fiber switch performs angle switching and phase shifting of the interference pattern, simplifying the implementation of multicolor SIM illumination.

The extremely flexible and compact design of the SIM illumination unit, together with open-source reconstruction algorithms, makes SR-SIM accessible to any biological laboratory.

Optical Sectioning - The basis for dynamic three-dimensional fluorescence light microscopy

Ernst Stelzer
Goethe-Universität Frankfurt am Main, Germany

Optical sectioning in fluorescence microscopy represents a cornerstone technique that allows scientists to visualize and analyze the three-dimensional architecture of specimens with high resolution. This capability is crucial for advancing our understanding in various fields, particularly in cellular and developmental biology. In this context, several microscopy techniques stand out due to their ability to perform optical sectioning through distinct mechanisms. Here, we discuss and explain in a formal manner how this property is harnessed in confocal, two-photon, confocal theta, and light sheet-based fluorescence microscopy.

Confocal fluorescence microscopy (CFM) achieves optical sectioning through the multiplication of two independent point spread functions (PSFs) – one for fluorophore excitation and one for fluorescence photon detection. This multiplication results from the sequential spatial sampling, where the common focal volume defined by the excitation and detection PSFs moves relative to the specimen. The technique's optical sectioning, or axial resolution, emerges from its dependence on the square of the excitation intensity, making it an incoherent microscope. Despite its advantages, CFM faces challenges like high excitation intensity for each recorded plane, potentially harming live specimens, and decreased axial resolution in parallelized versions like spinning disk confocal microscopy.

Two-photon fluorescence microscopy leverages non-linear effects at high laser intensities, allowing fluorophores to be excited by two lower-energy photons. This method inherently incorporates optical sectioning during the excitation process, as fluorophores outside the focus plane are not excited, and a detection pinhole is unnecessary. While offering distinct advantages, such as reduced photobleaching outside the focus plane, two-photon microscopy suffers from lower resolution compared to single-photon confocal microscopy and requires higher laser powers.

Confocal theta fluorescence microscopy (ϑ-CFM) introduces an innovative approach by employing two lenses arranged at a 90° azimuthal angle. This orthogonal overlap of PSFs significantly enhances axial resolution, surpassing that of traditional confocal microscopy by factors of three to ten. While theta microscopy can dramatically improve resolution, it shares the slow sampling rate limitation with other techniques but offers the potential for massive parallelization without losing optical sectioning capabilities.

Light sheet-based fluorescence microscopy (LSFM) diverges from the epi-fluorescence arrangement by using two independently operated lenses arranged at a 90° angle. This configuration creates a thin light sheet that exclusively illuminates the plane of focus, significantly reducing photobleaching and phototoxicity while eliminating out-of-focus fluorescence. LSFM achieves direct optical sectioning with drastically reduced energy exposure, making it ideal for recording high-resolution, three-dimensional images of live specimens over time. Techniques like cylindrical lens-based static light sheets and galvanometer-driven dynamic light sheets further enhance its versatility.

In summary, these fluorescence microscopy techniques employ unique strategies for optical sectioning, each with specific advantages and challenges. Confocal and two-photon microscopy provide foundational approaches, while theta and light sheet-based microscopy introduce innovative solutions for improved resolution and reduced specimen damage, opening new possibilities for in-depth biological research.

Light sheet microscopy: Opening a window into early mammalian development

Joachim Mossige
Univeristy of Oslo

How does mammalian life evolve, from a fertilized egg to a fully developed organism? This intriguing question has baffled us for centuries, and despite intensive research efforts over the years, it is still far from understood. Especially, we lack a good description of the critical gastrulation stage, where a ball of cells starts to elongate because (a) cells divide preferentially in one direction, (b) because different types of cells divide at different rates, or (c) because cells migrate collectively. We set out to quantify the relative importance of these complementary mechanisms and how they shape early embryos.

To establish a controlled experimental protocol, we use stem cell derived embryo models (so-called gastruloids), and we image them in 3D using our home-built light-sheet microscope (sheet width 6.7 um; x,y,x, voxel size: 0.328x0.328x1.542um^3). By illuminating the cells subsequently with four different wavelengths (375nm, 488nm, 561, 647nm), we can visualize both cell nuclei and membrane, as well as the different germ layers. To enhance the images, we deconvolve them using Generic Deconvolution (developed by Rainer Heitzmann), and we use 3D Slicer, MATLAB, and Huygens to obtain 3d representations from the deconvolved data. Finally, we couple our experiments with numerical simulations to understand how the cells orchestrate their motion to deform the tissue.

The project is funded by the convergence environment ITOM under UiO:LifeScience.

How to choose the right volume EM technique

Eija Jokitalo
Electron Microscopy Unit, Institute of Biotechnology, University of Helsinki

Volume electron microscopy (vEM) includes methodologies, platforms and workflows that generate large three-dimensional volumes of cells or tissues at nanoscale resolutions with a continuous depth greater than one micron. vEM enables 3D visualization, modelling, quantification and characterization of whole cells, their constituents, and organelles in their cellular environment. Organelle shape, size, number, distribution within a cell and their adjacency and relationship to other organelles can now be imaged and analysed in whole cells at resolutions sufficient to resolve individual membranes. Imaging volumes at nanoscales is time-consuming and generates large datasets, thus it is beneficial to optimize resolution and volume to the minimal required level. Apart from platform availability, the primary determining factors for choosing the best vEM technique for a specific biological question are the required x, y, z resolution to visualize and measure the feature of interest; the appropriate volume to reliably capture the event; and the best imaging and fixation/contrasting conditions to efficiently acquire the target structures and data. Additional considerations for selecting a particular vEM imaging modality include ease of instrument use, data processing and image segmentation.

Looking under the hood of cells: from whole cell organelle reconstructions to single molecule dynamics to atomic reconstructions of macromolecules

Jennifer Lippincott-Schwartz
Janelia Research Campus, USA

Powerful new ways to image the internal structures and complex dynamics of cells are revolutionizing cell biology and bio-medical research. In my talk, I will focus on three emerging technologies capable of revealing new properties of cellular organization at scales ranging from nanometer to atomic resolution. Whole cell milling using Focused Ion Beam Electron Microscopy (FIB-SEM) was used to reconstruct the entire cell volume at 4-nm voxel resolution, revealing all membrane-bound organelles and their trafficking intermediates at isotropic resolution. Single particle tracking using Halo dyes revealed unexpected features of mRNA trafficking, including sites where secretory proteins are translated on ER and their regulation by lysosomes. Finally, High Resolution Template Matching (HRTM) of ribosome subunits in cryo-EM images of intact human cells afforded a look at ribosomes at different stages of peptide elongation at the atomic scale. Together, these new tools open-up a plethora of questions related to mechanisms of cell structure/function that can now be studied in intact cells at the nanometric/molecular level.

Phosphoinositide switches controlling early macropinocytosis

Kay Oliver Schink
Department of Molecular Medicine, University of Oslo

Macropinocytosis is a non-selective endocytic process by which cells take up large amounts of extracellular fluids into large vesicles known as macropinosomes. This mechanism is used by immune cells to sample the surroundings for antigens and can be exploited by cancer cells for nutrient uptake. Macropinosomes form out of membrane ruffles which can pinch off to form large vesicles. During the transition from membrane ruffles to vesicles, the phosphoinositide composition of the membrane changes - a phosphatase cascade metabolizes PtdIns(3,4,5)P3 by way of PtdIns(3,4)P2 and PtdIns(3)P to PtdIns. At the same time, transient pools of PtdIns4P are generated on the forming vesicle. Phosphoinositides, especially PtdIns3P and PtdIns4P, play a critical role in early steps of macropinocytosis. We find that the protein Phafin2 is recruited transiently to newly formed macropinosomes by coincidence detection of PtdIns3P and PtdIns4P. Phafin2 locally modulates actin reorganization around nascent macropinosomes and is critical for successful macropinocytosis. After internalization, newly-formed vesicles need to gain the correct identity. We have identified a phosphoinositide-driven Rab switch which is critical for identitygeneration on newly formed vesicles. PtdIn3P, generated by the PI3 kinase VPS34, is needed to initially establish endosomal identity on forming macropinosomes. Inhibition of VPS34 completely blocked the establishment of endosomal Rab GTPase RAB5 on newly-formed macropinosomes. Instead, forming macropinosomes under VPS34 inhibition gained a secretory identity, including RAB8A, RAB10, RAB11A, and PtdIns4P. This led to re-fusion of macropinosomes with the plasma membrane. Thus, macropinosome maturation is regulated by a PtdIns3P-controlled switch that balances macropinosome fate between the default, endolysosomal maturation and an alternative, secretory route.

Delivery of therapeutic antibodies across the Blood Brain Barrier

Morten Nielsen
Aarhus University

The Blood Brain Barrier (BBB) is a tight barrier that separates the blood from the brain, and the BBB consist of Brain Endothelial Cells (BEC), astrocytes and pericytes. The BEC's are the cells that forms the physical barrier facing the blood, and these cells are highly polarized with several transporters and receptors which controls the transport of molecules and proteins across the BBB. This transport is very selective which one side protects the brain against toxins, viruses etc., but also limits the access of drugs targeting brain cells.

Using differing imaging modalities and BBB models, we are studying bidirectional transport of transmembrane receptors with aim of utilizing receptor mediated transport for delivery of therapeutic antibodies to the brain. Our data indicates that the endocytic pathway of receptors internalized from respectively the luminal and abluminal membrane differs, and that the Transferrin Receptor is more efficient in mediating transport out of the brain and less efficient in the transport from blood to brain.

Analysis of migratory behaviors in epithelial cell collectives

Anna Lång
Oslo University Hospital

Collective cell behavior plays a crucial role in various biological processes, including development, tissue regeneration, and cancer progression. Our research focuses on understanding the dynamic behavior of epithelial cells, which have the unique ability to form cohesive sheets that function as protective barriers and line internal organs. Under normal conditions (homeostasis), these epithelial layers maintain a relatively static organization, providing structural support and withstanding mechanical stress. However, during episodes of wound healing, these cell sheets transit into highly dynamic entities, exhibiting coordinated movement and flocking. During my presentation, I will highlight some of the microscopy techniques we use to investigate the migratory properties of epithelial cell collectives. I will demonstrate how we employ various live cell microscopy modalities to uncover the dynamic behavior of epithelial cells, as well as mapping of physical forces within epithelial monolayers. Finally, I will present data from our latest study, where we demonstrate how topological defects within the velocity field of active monolayers play a crucial role in orchestrating the coherent collective motion of epithelial cells.

Multi-modal and high-throughput chip-based super-resolution optical microscopy for life sciences

Balpreet S. Ahluwalia
University of Tromsø

3D kidney pathology with AI diagnostic support

Hans Blom
Royal Institute of Technology (KTH), Stockholm, Sweden

Optical 3D Pathology: Combining tissue-clearing preparation with high-resolution fluorescence imaging, we have in the last years developed optical 3D kidney pathology, which with learning-based image analysis allow automatic quantification and kidney disease stratifications. I will in this talk present our development and highlight how our 3D method elevates the possibilities of future pathology investigations and diagnostic support [Deep learning–based segmentation and quantification of podocyte foot process morphology suggests differential patterns of foot process effacement across kidney pathologies.

Optical microscopy beyond diffraction limit through structured illumination

Joby Joseph
Optics and Photonics Centre, IIT Delhi, India

Microscopy is an important tool for studying the structure and properties of materials, and for identifying and characterizing small objects or organisms. However, the resolution we can achieve from optical microscopes is limited by diffraction. However recent years, many new optical techniques have come up with higher resolutions that break this diffraction limit. These microscopy techniques are called super-resolution microscopy or nanoscopy. There are several super-resolution microscopy techniques such as Structured Illumination microscopy (SIM), Stimulated Emission Depletion (STED) microscopy, Single molecule localization microscopy (SMLM) etc.

SIM even though gives a modest resolution enhancement, it stands out from others in many ways due to its practical usages. The biggest limitation of SIM is that even though it is faster than other super resolution microscopy technique, it is still limited to two times resolution enhancement. The talk will focus on the development of a novel Transillumination -SIM [TSIM] that circumvents this two times resolution enhancement limit and has high space bandwidth product. TSIM decouples the imaging and illuminating part by using mirrors. A multi-mirror setup generates illumination patterns with higher spatial frequencies than can be achieved from the imaging objective lens.

Characterizing intracellular traffic using CLEM in combination with tomography

Andreas Brech
Oslo University Hospital

Our lab has been studying intracellular traffic for many years and for that purpose we have taken advantage of using correlative light/electron microscopy (CLEM) in combination with tomography. CLEM is a crucial starting point for most of our experiments allowing us to target cells of interest specifically and performing further EM analysis on them. This is critical when studying rare events such as cytokinesis, focus on cells that express transfected genes or if one wants to follow dynamic events by live cell imaging. Inspection of these samples by electron microscopy can be performed either on thin sections for standard 2d-imaging, but in our hands 3d-imaging is the technique of choice. Understanding the organization of intracellular membrane dynamics and traffic is greatly facilitated by either electron tomography or methods like FIB-SEM. The latter method is not only useful for single cell visualization but can also be used to image much larger volumes, such as brain tissues from a variety of biological systems. However there are severe limitation in terms of resolution, which might not exceed 5 nm/pixel. Electron tomography on the other hand can perform at much higher resolution, depending on sample preparation and imaging mode. We often employ STEM-tomography on plastic embedded samples, a technique that gives useful results in the range of resolution that is possible with that preparation (max. 1 nm). Importantly with STEM tomography sections of up to 1200 nm can be visualized, exceeding by far the possibilities of regular electron tomography (maximum 250-350 nm thickness). Examples of the use of CLEM in combination with STEM-tomography will be presented.

Tracking the deep evolutionary origins of neurons

Pawel Burkhardt
Michael Sars Centre, University of Bergen

Nervous systems evolved around 560 million years ago and allowed animals to rapidly detect environmental cues and to coordinate responses to threats or opportunities. While we now understand much of the underlying molecular mechanisms of how neurons communicate, knowledge about how and when synapses and neurons originated during animal evolution is limited. In the first part of my talk, I will present our recent discoveries on synaptic protein homologs and fast calcium signalling found in choanoflagellates, the closest unicellular relatives of animals with no synapses or neurons and explain how these fascinating protists help us to understand the evolutionary origin of synapses. In the second part of my talk, I will highlight how the study of ctenophores – an ancient animal lineage – has advanced our understanding in reconstructing the evolutionary origin of neurons. Ctenophores possess a rather simple nervous system, a so-called nerve net which spans their entire body surface. Strikingly, using volume electron microscopy and 3D reconstructions we found that their neurons are connected in a completely different way to what was known before. Ctenophore neurons within the nerve net are not connected via synapses instead show a continuous plasma membrane forming a syncytium. Our findings suggest fundamental differences of nerve net architectures between ctenophores and cnidarians or bilaterians and offer an alternative perspective on neural network organization and neurotransmission.

Quantitative phase imaging (QPI) of epithelial monolayer dynamics

Dag Kristian Dysthe
Njord Center, Department of Physics, University of Oslo

QPI is a label-free technique that has been used in bioscience for 60 years. There are a range of optical solutions, but the last 10-15 years have seen an explosion in new computationally based techniques. I will give simple explanations of the physical principles, quickly review historical use and some current instruments and give examples from our recent research.

Life under pressure - developing tools to investigate plant cell mechanics in vivo

Thorsten Hamann
Norwegian University of Science and Technology

Plant cell walls surround all plant cells, provide support during growth and development and form the interface with the environment. In this context they frequently also form the first line of defense against biotic and abiotic stress originating in the environment. The walls consist mainly of different types of polysaccharides such as cellulose, hemicellulose, pectins, proteins and dependent on the specific wall type also lignin. We are interested in understanding the mode of action of the cell wall integrity (CWI) maintenance mechanism, which is monitoring the functional integrity of plant cell walls similar to mechanisms in other organisms such as yeast. Our previous work has shown that changes in the stiffness of cell walls and turgor pressure levels are important during the perception of cell wall damage impairing CWI. To investigate stiffness changes we have built a Brillouin module and connected it to a laser confocal microscope. Brillouin microscopy is based on characterizing the interactions of (laser) light with the sample, which involve picosecond–timescale density fluctuations due to spontaneous molecular motions. Since the interactions couple photons to longitudinal phonons in the sample, variations in the scattering spectra of the laser light can be interpreted as a response of the sample to an infinitesimal, uniaxial compression. This response can be integrated with the refractive index to extract the Brillouin elastic contrast (v"), which can reveal changes in stiffness of the sample examined. Our setup enables us to visualize simultaneously proteins tagged with a fluorophore and characterize the stiffness of cellular structures such as cell walls in a label free manner in vivo in plants. We will present an overview of the system and examples how we apply the equipment.

Acoustic wave-induced stroboscopic optical mechanotyping of adherent cells

Thomas Combriat
University of Oslo, Hybrid Technology Hub, Institute of Basic Medical Sciences
University of Oslo, Department of Physics, Njord Centre

I will present a novel, high content technique which, by using a combination of an innovative acoustic transducer, stroboscopic imaging and homodyne detection allows to subject cells to oscillatory stresses at low ultrasonic frequencies and image and measure precisely their induced motion.

By analyzing these micro-oscillations and the strains that resulted on the cells, we recently demonstrated (https://doi.org/10.1002/advs.202307929 - in press) the capacity of this method to simultaneously measurequantitative mechanical properties (dynamic shear modulus) of whole populations of cells with sub-cellular resolution at a throughput and frequencies not attainable but other mechanotyping methods (AFM notably). This technique was used to discriminate the cell type of a mixed population of cells using their mechanical properties.

As this method provides a way to precisely input and measure mechanical strains caused by ultrasonic waves, and is compatible with fluorescent imaging, it is also a useful tool for studying mechanotransduction. During and following a mechanical input, which can be stress or acoustical pressure or a combination, the biological response can be studied using fluorescence imaging. We applied this to study the biological responses to low-intensity pulsed ultrasound (LIPUS) using calcium imaging as a proxy for the biological output.

By studying the correlations between the strains cells are subjected to and the opening of their calcium channels, we preliminary show that cells' response is associated with motion but not with acoustic pressure. This has the potential to resolve the huge variability observed in the effects of LIPUS on biological material in the literature as it suggests that the good observable for mechanical effect is the induced stress but not necessarily the acoustic pressure.

Label free imaging and image analysis

Studying cancer cell metastases in the era of deep learning for microscopy

Guillaume Jacquemet
Åbo Akademi University, Finland

To disseminate, cancer cells use the vascular system in which they must survive and escape by attaching to and crossing the vascular wall. However, how cancer cells arrest and cross the vasculature remains poorly understood.

We set up a microfluidic system to perfuse human pancreatic cancer cells (PDAC) on top of human endothelial cells. This system enabled us to capture the dynamics of cancer cell extravasation with unprecedented detail through live imaging. To analyze these complex datasets, we developed and utilized a suite of open-source image analysis software. Fast4DReg, a Fiji plugin, corrects time-lapse imaging drift. ZeroCostDL4Mic and DL4MicEverywhere harness deep learning for artificial labeling and segmentation. TrackMate v7 offers enhanced cell tracking capabilities by incorporating advanced segmentation algorithms. CellTracksColab facilitates the compilation and analysis of tracking data, providing insights into cell behavior during extravasation.

We report that PDAC cells arrest nearby endothelial cell-cell junctions. PDAC cells then use long filopodia protrusions to probe for gaps. When gaps are found, the extravasation process starts, and PDAC cells progressively traverse the endothelial layer between endothelial cells. We are currently targeting filopodia regulators to assess their contribution to the extravasation process. By combining advanced imaging strategies and image analysis software development, we are advancing our understanding of cancer cell extravasation.

Deep Learning in Microscopy: A Multi-faceted Approach to Intestinal Cancer Detection

Alberto Diez
CMIC-NTNU

EDAM Bioimaging, a program for bioimage informatics, image analysis and imaging

Matúš Kalaš
Department of Informatics, University of Bergen, and ELIXIR Norway

EDAM Bioimaging is an ontology dedicated to bioimage informatics, image analysis, and imaging in general. It has been developed in collaboration between the ELIXIR research infrastructure, the NEUBIAS and COMULIS COST Actions, the Euro-BioImaging research infrastructure and the Global BioImaging network.

EDAM Bioimaging contains an inter-related hierarchy of concepts including bioimage analysis and related operations, bioimaging topics and technologies, and image data and their formats. A major part is formed by concepts related to artificial intelligence and especially machine learning (AI/ML).

EDAM Bioimaging is crucial for FAIR (findable, accessible, interoperable, reusable) image data, imaging services, software, publications, data, workflows, and training materials. Enabling findability and provenance of all such kinds of resources related to bioimaging, EDAM fosters open science and "reproducible" bioimage analysis.

EDAM Bioimaging continues being under active development, with a growing and diversifying community of contributors. It is used in BIII.eu, the registry of bioimage analysis tools, workflows, and training materials, and it forms an essential part of REMBI (Recommended Metadata for Biological Images), the global standard for FAIR image data.

The live development version of EDAM Bioimaging can be viewed and commented on WebProtégé (free registration required). New contributors are warmly welcome!

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1. Using liquid-jet X-ray sources to investigate nanoparticle uptake *in vitro* and *in vivo*

Komang Arsana
Department of Applied Physics, Biomedical and X-ray Physics, KTH Royal Institute of Technology, Stockholm, Sweden

X-ray technology has emerged as an important method for biological studies due to its high spatial resolution, deep penetration depth, and non-invasive nature. This holds true for both soft X-ray microscopy (SXM) and X-ray fluorescence imaging (XFI). SXM offers high resolution and high contrast for cellular biology under unperturbed conditions. Its short wavelengths enable nanometer-scale imaging of thick samples, eliminating the need for labelling, chemical fixation and staining. On the other hand, XFI allows the detection of specific contrast agents based on their characteristic X-ray emissions. This spectral specificity facilitates the analysis of bio-distribution in preclinical studies, allowing for longitudinal tracking and multiplexed imaging.

Traditionally, these techniques operate in synchrotron facilities, limiting accessibility. However, the development of laboratory-based X-ray sources, particularly liquid-jet sources, offers comparable capabilities with increased accessibility. In this study, we developed a methodology using liquid-jet X-ray sources to investigate nanoparticle (NP) uptake and interaction both in vitro and in vivo. By employing liquid nitrogen and liquid-metal jet sources for cellular and preclinical imaging respectively, we demonstrated the ability to observe NPs within cells and assess their bio-distribution in living organisms. Our findings highlight the robustness of liquid-jet X-ray imaging in studying bio-nano interactions, offering insights at both cellular and whole-body levels. This synergistic approach between SXM and XFI holds great promise in advancing our understanding of NP interactions in biological systems.

2. The Finnish Advanced Microscopy Node

Irina Beleia
Turku BioImaging, Åbo Akademi University and University of Turku, Turku, Finland

The Finnish Advanced Microscopy Node (FiAM, https://eurobioimaging.fi/FiAM/) is a service organization of Euro-BioImaging ERIC, providing open access to cutting-edge imaging technologies, user training, and expertise in image acquisition, image data management, and analysis. FiAM is a multimodal and multisited research infrastructure and consists of eight imaging facilities in three cities: Helsinki, Oulu, and Turku. FiAM has been operational since 2016 and specializes in biological imaging and microscopy service provision. It is the second most popular Node in Euro-BioImaging.

In December 2023, FiAM received its largest national funding to date from the Research Council of Finland, 7,2 M€ for the 3D-BioImage project, to develop FiAM´s services and instruments with a continuum of stateof-the-art 3D imaging approaches. In the 3D-BioImage project, FiAM will develop and implement an extensive set of new high-resolution 3D imaging services based on the latest microscopy and image data analysis developments. The goal is to make instrument upgrades on all scales, from “nano” to “micro”: AFM, volume EM, super resolution light microscopy, and high throughput light microscopy. Considering that quantitative image analysis is becoming more and more complex and requires special expertise, the 3DBioImage project will also develop 3D/4D image analysis services related to the new instruments, focusing on AI-solutions. To support the increasing amount of image data from 3D-BioImage´s new services, FiAM will also increase its OMERO server capacity, allowing better shareability of the data. In addition, the remote access service model of the different FiAM Node sites will be improved to make it more usable. 3DBioImage is designed to promote digitalization and greener operations of FiAM on a broad scale. Overall, 3D-BioImage is expected to bring FiAM’s capabilities to a completely new level, allowing both national and Euro-BioImaging users from both academia and industry to increase the quality and impact of their science.

3. Nexilin: A novel regulator of endolysosomal dynamics

Marie Bergundhaugen
Department of Biosciences, University of Oslo

The interactions between the actin cytoskeleton and endolysosomes are important for endolysosome positioning, movement and exocytosis. Apart from this, the dynamics between actin and the endolysosomes are unclear. To further unravel the role of the actin cytoskeleton for endolysosome function, we performed an siRNA screen targeting actin-regulating proteins. From the screen, we identified Nexilin, an actin-interactor protein, as a novel regulator of endolysosomal dynamics and transport.

Nexilin was first identified as an actin-interactor protein localised to adherens junctions. Since then, it has been mainly characterised in muscle cells, where it has been identified as a Z-disc related protein, and is also shown to affect calcium release in muscle. Additionally, it is described to affect migration and adhesion in cancer cells.

Using microscopy and molecular biology techniques, we investigated the function of Nexilin in intracellular transport, focusing on endolysosomes. Our results indicate that Nexilin bridges late endosomes to actin cytoskeleton and that this interaction is important for correct cargo fission and transport.

4. Application of Coherent Anti-Stokes Raman Microscopy to study crystalline calcium phosphate and lipids in mineralized hydrogels, cell monolayers and cone sections

Astrid Bjørkøy
Department of Physics, Norwegian University of Science and Technology, Trondheim, Norway

Coherent Anti-Stokes Raman Scattering Microscopy (CARS) is a multiphoton microscopy technique that uses vibrational signatures of molecules to create high-resolution, label-free images of the sample. CARS signal is sensitive to vibrations of chemical bonds, and it can be orders of magnitude stronger than the Raman scattering signal used in traditional Raman microscopy. CARS is a third-order nonlinear optical process. We used this microscopy technique to study crystalline calcium phosphates (CaP) and lipids in mineralized hydrogels, cell monolayers, and bone sections. The ability to detect mineralization with a labelfree, chemically specific and accurate method would contribute to a better understanding of range of processes important for bone biology and tissue engineering research fields. CaP minerals have a welldefined Raman spectrum and CaP in bone has been investigated extensively using Raman spectroscopy and microscopy. Some examples of CARS microscopy applied to bone cells or bone samples exist in the literature, however, this technique has not been used extensively. We use a custom-build CARS microscope equipped with an integrated dual pulsed 2ps laser with wavelength 1031 nm for the Stokes beam and a tunable output for the probe beam. The microscope has an upright geometry, and the signal is detected in the transmitted direction with appropriate filters for the vibrational mode of interest. In addition, the samples can be studied and images acquired in brightfield and multiphoton fluorescence mode. We image vibrations associated with CaP as well as vibrations model associated with lipids. Our results show that CARS microscopy can be used for label-free imaging of samples containing calcium phosphate with high spatial resolution and chemical sensitivity

5. MINFLUX microscopy - 10 nm cellular 2D & 3D resolution

Hans Blom
Royal Institute of Technology (KTH), Science for Life laboratory

The recently introduced MINFLUX (minimal photon fluxes) single molecule targeting image concept pushes the resolution of fluorescence microscopy to molecular dimensions. Since a few months we can support users with the technique in our advanced light microscopy facility in Stockholm, Sweden. The poster will highlight the workflow of MINFLUX imaging and some initial results of achieving cellular few nanometer precision visualisations.

6. Segmentation-free measurement of cell motion from quantitative phase imaging

Silja Borring Låstad
Department of Physics, University of Oslo

Quantitative phase imaging (QPI) is becoming more and more common in biomedicine. 2D quantitative phase images differ from most other images in that the intensity value in every pixel can be interpreted as the "optical thickness" of the object at that point. For cells, this optical thickness can be directly linked to the non-aqueous mass thickness of the cell, as the refractive index of a cell is proportional to its molecular density. On short timescales the non-aqueous mass inside the cell membrane is constant.

Established methods for quantifying cell motion, flow, and deformation from image sequences typically have cellular resolution, thus subcellular variations are lost. To overcome this limitation, we combine optical flow (OF) and direct image correlation (DIC) while making use of intensity conservation from QPI. Here, OF applies the continuity equation to predict intensity shifts, using displacements computed with DIC as constraints. By doing so, we can analyze cell layer velocity fields with pixel size resolution, giving a more nuanced picture of collective cell movement.

The method is validated on synthetic data before we apply it to images of confluent MDCK monolayers in the absence of external flow. We study the scales of variations in cell velocity and demonstrate the potential for deeper insights in the dynamics of confluent cell layers.

7. Jonathan Brewer

University of Southern Denmark / DaMBIC

8. Enhancing intestinal Organoid Analysis with YOLOv8 Deep Learning in Brightfield Microscopy

Alberto Diez
CMIC-NTNU

In the realm of microscopic imaging, the analysis of complex biological structures such as intestinal organoids poses significant challenges. This conference presentation introduces a custom-trained YOLOv8 deep-learning model for the precise segmentation and detection of various classes within brightfield images of intestinal organoids.

Traditional methods, including thresholding and pixel classifiers, have limitations in accurately delineating organoid boundaries and distinguishing between different cell types. Our study showcases the superior performance of YOLOv8 in overcoming these challenges.

The deep learning model is trained on a diverse dataset of brightfield images, allowing it to generalize well across different experimental conditions and organoid morphologies. This adaptability ensures robust performance, even in the presence of variations in imaging parameters (i.e. illumination and focus).

The advantages of our YOLOv8-based approach extend beyond accuracy, as it significantly reduces the need for manual intervention and parameter tuning. This enables researchers to streamline their workflow, saving valuable time and resources while maintaining high-quality results.

9. Extended live-cell fluorescence microscopy of novel intrinsically fluorescent cholesterol analogues with improved photophysical properties

Senjuti Halder, Max Lehmann, Peter Reinholdt, Mohammad Bashawat, Holger A. Scheidt, Duccio di Prima, Jacob Kongsted*, Peter Müller*, Pablo Wessig*, and Daniel Wüstner*
CMIC-NTNU

Fluorescence microscopy is an interesting alternative to understand the sterol-driven physiological processes and heavily relies on the functional relevance of fluorescent sterol analogs. This results into the development of a wide variety of luminescent cholesterol mimics to visualize the trafficking of cellular cholesterol. However, existing fluorescent analogs have their limited physico-chemical properties compared to cholesterol and so far, none of them seems to be ideal in all aspects. Here, we introduce novel intrinsically fluorescent sterols containing four conjugated double bonds in the ring system and either a 3'- hydroxy group (1) or a 3'-keto group (2). Both sterol probes exhibit significant red-shift in their excitation and emission spectra and can be easily taken up by cultured human control fibroblasts and diseased fibroblasts from albumin complexes, which together with a much-improved photostability allows for their convenient live-cell imaging on conventional wide field and confocal microscopes with a DAPI/filipin filtercube. The imaging potential of the both polyene sterol probes is further evaluated by studying their photobleaching kinetics and monitoring fluorescence recovery after photobleaching (FRAP) in living cells to have information on non-vesicular sterol transport kinetics and exchange dynamics between lysosomes and cytosol. Thus, our observations demonstrate that the cholesterol-mimicking probe 1 has comparable physicochemical and membrane properties as its endogenous counterpart and validate the fact that the strategy of extending the conjugated double bond in sterol ring system is worthwhile to achieve improved optical properties which offers many future applications in live-cell imaging including multiphoton, spectral and lifetime imaging on conventional microscope systems, thereby providing novel insights into cellular cholesterol trafficking under normal and disease-related conditions

10. Positive feedback loops between α-synuclein aggregation and mitochondrial dysfunction

Jialing Hu
University of Southern Denmark

Parkinson's Disease (PD) is a highly prevalent neurodegenerative disease among older adults. Protein aggregation, mitochondrial dysfunction and neuroinflammation are pathognomonic features of PD. Moreover, the existence of positive feedback loops between these pathological components, which accelerate, and sometimes make irreversible, the neurodegenerative process, is apparent. However, the mechanisms underlying the dynamic evolution of PD due to these positive feedback loops remain unclear. In our previous study, we investigated the interaction between α-synuclein oligomers and organelle membranes at atomic resolution in live cells and how this interaction affected mitochondrial structures. Isotope-labeled α-synuclein oligomers were introduced into SH-SY5Y cells by electroporation and the obtained NMR spectra indicated that α-synuclein oligomers interacted strongly with the cells. Furthermore, cellular interactions and localization of α-synuclein oligomers were elucidated using confocal and STED microscopy. The results revealed that α-synuclein oligomers interacted with various cellular components, including uptake via the endo-lysosomal pathway and subsequent localization of some oligomers at mitochondrial membrane. Notably, these interactions with organelle membranes eventually leaded to mitochondrial fragmentation. To study the positive feedback loops between α-synuclein aggregation and mitochondrial dysfunction, we further evaluate how mitochondrial dysfunction, especially mitochondrial DNA damage, reversely affects α-synuclein aggregation in the cells. Rotenone, a potent inhibitor of mitochondrial complex I, is used to impair mitochondrial functions. The extrusion of damaged mitochondrial DNA into cytoplasm and α-synuclein aggregation are observed in SH-SY5Y cells using immunofluorescence microscopy. These results shed light on developing multifunctional compounds with the capacity to attack several of the key components of neurodegenerative processes to slow down the progression of neurodegenerative diseases.

11. Contour Learning Localization Provides Super Resolution Tracking of Endosome Maturation in Extended Field of Depth Microscopy

Xian Hu 1,4, Duarte Mateus 1,3, Anna Vik Rødseth 1, Vinodha Manovaseegaran 1, Xiaochun Xu 2, Felix Margadant 3*& Oddmund Bakke 1*

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2. Mechanobiology Institute, National University of Singapore, Singapore, Singapore
3. Radium Hospital, OUS, Oslo, Norway
4. NCMM, University of Oslo, Oslo, Norway

It is a great challenge to study the rapid dynamics of intracellular membrane trafficking events in live cells. Multiple intracellular membrane trafficking pathways co-exist and interlace with each other in close proximation in time and space. On top of that, many vesicles undergo fusion and fission events and change in size, shape and even identity as they move more or less rapidly in the cytoplasm along cytoskeletal paths driven by molecular motors. All these events make tracking and analysing their behaviour using modern microscopy technology very challenging. Here we present a new imaging and tracking method tailored for intracellular traffickingvesicle tracking and how we use this method to study the coating protein dynamics during the endosome maturation process. Live cell image series obtained by the superresolution microscopy techniques(Airyscan and Live-SR) of maturing endosomes are tracked by our new ImageJ based seedgrowing polygon detection algorithm. The intensities of coat proteins labelled with either the green fluorophore(GFP) or the red fluorophore(RFP/mCherry/mApple) are automatically recorded and analysed. The method was first tested with the well-studied Rab5 and Rab7 switch during endosome maturation and then applied to study the dynamics of other proteins that have been reported to interact with either Rab5 or Rab7 (EEA1, Rab9, Rab 4, Rab8, etc)during the Rab switch phase of the endosome maturation process. We found that some of the proteins are merely visiting the endosomes during the process(e.g. Rab11); but the presence of several others are in phase with the Rab switch(e.g. Rab4, Rab8 and Rab9), indicating that they may play an role in regulating the endosome maturation process.

12. Insights into Intestinal Inflammation and Cell Barrier Integrity in Caco-2 Cells Treated with the Natural Compound 4-Methylsculetin

Gilia Cristine Marques Ruiz
University of Southern Denmark

Coumarins belong to a group of promising natural substances from a biotechnological perspective. They exhibit a broad spectrum of biological activities, including anti-inflammatory, antioxidant, and antimicrobial effects, with potential applications as theranostic drugs. These compounds are found in various plant species, especially in aromatic, food, and medicinal plants. Among the over 1300 identified coumarins, the derivative known as 4-methylsculetin (4-ME) has shown significant results in in vivo models of intestinal inflammation, suggesting its potential use in the treatment of inflammatory bowel diseases. In this study, we employed an in vitro model comprising Caco-2 cells to mimic both healthy and controlled inflamed states of the intestine, aiming to investigate the effect of 4-ME in maintenance of the cell barrier integrity. To induce an inflamed state, the Caco-2 cells were stimulated with LPS, both in the absence and presence of 4-ME, and submitted to immunocytochemical staining. Additionally, cytotoxic experiments were conducted to assess the potential toxicity of 4-ME on the cells. The highest concentration of 4-ME (100 μM/mL) exhibited compatibility with over 85% cell viability. Microscopic analysis confirmed the integrity of tight junction (TJ) membranes in non-treated Caco-2 cells, as evidenced by staining for nuclei (DAPI), cytoskeleton (Phalloidin), and the TJprotein zonula occludens (ZO)-1. In the inflamed culture, the TJ network and cytoskeleton appeared irregular and less organized compared the control. Moreover, nuclear fragmentation was observed. The intestinal anti-inflammatory activity of 4-ME was confirmed by its ability to reduce such damages in the morphological aspects of Caco-2 cells.

13. Delivery of therapeutic antibodies across the Blood Brain Barrier

Morten Nielsen
Aarhus University

The Blood Brain Barrier (BBB) is a tight barrier that separates the blood from the brain, and the BBB consist of Brain Endothelial Cells (BEC), astrocytes and pericytes. The BEC's are the cells that forms the physical barrier facing the blood, and these cells are highly polarized with several transporters and receptors which controls the transport of molecules and proteins across the BBB. This transport is very selective which one side protects the brain against toxins, viruses etc., but also limits the access of drugs targeting brain cells.

Using differing imaging modalities and BBB models, we are studying bidirectional transport of transmembrane receptors with aim of utilizing receptor mediated transport for delivery of therapeutic antibodies to the brain. Our data indicates that the endocytic pathway of receptors internalized from respectively the luminal and abluminal membrane differs, and that the Transferrin Receptor is more efficient in mediating transport out of the brain and less efficient in the transport from blood to brain.

14. Optical super-resolution microscopy on a flexible research platform

Sven zur Oven-Krockhaus
University of Tübingen, Center for Plant Molecular Biology, Tübingen

Recent decades have seen significant advancements in microscopy techniques that have greatly benefited the life sciences. A major breakthrough in this field has been the development of super-resolution methods, which provide novel ways of observing molecular mechanisms in living cells. These methods often require advanced hardware and sophisticated data management and analysis. As a sub-project of the Microscopy Core Facility within the collaborative research center (CRC1101) of the Center for Plant Molecular Biology, a flexible research microscope has been developed. This microscope adapts and implements modern spectromicroscopy techniques for various projects within the CRC, including spectral and time-resolved confocal measurements such as FLIM, FCS, and fluorescence spectroscopy. In recent years, singlemolecule localization superresolution techniques have become increasingly important, and many current projects use sptPALM, in which individual, fluorescence-labeled molecules in the plasma membrane of plants can be tracked to analyze, e.g., the spatiotemporal properties of proteins. Since this technique is still relatively new in the field of plant research, we not only focus on the technical implementation and training of researchers in the use of this technique, but also on methods to analyze the resulting data. To this end, the OneFlowTraX software was developed, which provides an intuitive and user-friendly pipeline for the main steps of sptPALM analysis, including the localization and tracking of single molecules, mobility and cluster analysis. Additionally, a comprehensive batch processing function enables the analysis of large data sets, storing metadata and all user-defined fitting parameters to ensure that all results can be traced back to the raw data. While OneFlowTraX was originally based on the needs of plant researchers to analyze spatiotemporal protein dynamics in the plasma membrane, it is an adaptable and open-source tool that can be beneficial for other research areas, including the mammalian field.

15. MolMed Imaging Platform (MIP)

Laura Rodriguez de la Ballina
Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, Norway

MIP starts as a bottom-up initiative to coordinate scattered microscopes individually managed and to professionalise imaging at the Institute of Basic Medical Sciences (IMB) , its goal is to create a professional imaging infrastructure at the Department of Molecular Medicine and make it accessible to users.

16. Core Facility for Advanced Light Microscopy, Radium Hospital

Vigdis Sørensen
Oslo University Hospital, Institute for Cancer Research, Radiumhospitalet, Dept. Core Facilities

The Core Facility for Advanced Light Microscopy at The Radium Hospital provides services within a range of light microscopy imaging techniques. We provide access to state-of-the-art light microscopes for researchers for performing a variety of imaging techniques including live-cell imaging, widefield imaging, confocal microscopy (point scanning or spinning disk), and also high content imaging and a range of superresolution techniques such as 3D-SIM, Airyscan, SoRa, STORM and TIRF. CLEM can be performed in collaboration with the Advanced Electron Microscopy Core Facility. Furthermore, we provide access to advanced image analysis software and training or assistance in microscopy as well as in image analysis. The imaging technologies and methodologies developed at the Core Facility are being used to study various model systems including cultured cells, organoids, tissue, and model organisms such as fruitflies and zebrafish, and are routinely applied in studies of intracellular structures and processes (e.g. endocytosis, autophagy, mitochondria) and in studies of cell behavior (e.g. migration, division, cell-cellinteractions).

17. Correlative imaging of altered lipid transport and mobilization in yeast as a model for neurodegenerative diseases

Katja Thaysen
University of Southern Denmark

Yeast is a suitable model organism for studying human diseases, but its use in correlative microscopy of disease mechanisms is at its infancy. Here, we combine fluorescence microscopy and soft X-ray tomography (SXT) with quantitative image analysis to study lipid transport in a yeast model of Niemann Pick type C disease.

Niemann-Pick type C1 (NPC1, NCR1 in yeast) and C2 (NPC2) are sterol transporter proteins localized in lysosomes (vacuole in yeast). Previously, we have shown that sterols and LDs accumulate in the vacuole of yeast lacking one of these proteins upon starvation. Cells depend highly upon their ability to adapt in periods of nutrient depletion. Among these metabolic regulations is lipophagy, the uptake and consumption of lipid droplets (LD) by the vacuole. We used correlative imaging to observe how the vacuole and LDs of the cells are affected by lacking NCR1 or NPC2 during starvation. Our results suggest that the LDs are engulfed by the vacuole independent of NCR1 and NPC2. However, in the mutants, we segmented larger LDs compared to the WT in the vacuole, which indicates a slightly impaired hydrolysis of LDs. Interestingly, studies have shown that yeast can survive significantly longer when acutely depleted of glucose compared to gradually depleted. In this case, fatty acids are mobilized by catabolizing LDs and used for β-oxidation thereby maintaining energy levels. Quantifying the intensity of the LD marker, BODIPY, shows that acute glucose-starved cells have significantly less BODIPY than the gradually starved cells, indicating upregulation in LD consumption. Since this is also observed for the NPC mutants, we suggest that the LDs are hydrolyzed in the cytosol as observed in other cell types.

18. Danish Bioimaging Infrastructure Image Analysis Core Facility (DBI-INFRA IACF)

Sébastien Tosi, Tricia Loo Yi Jun, Julia Mertesdorf
DBI-INFRA IACF

The Danish Bioimaging Infrastructure Image Analysis Core Facility (DBI-INFRA IACF) runs an open service in image analysis to help life scientists visualize, analyze, and extract quantitative information from their bioimaging datasets. Our experts can handle virtually any image analysis task with sound scientific foundations for imaging modalities ranging from light and electron microscopy to (pre-)clinical imaging.

Our team is dedicated to discuss your image analysis needs and help you to further refine them, guide and train you in the most suited software solutions and, when needed, develop a whole image analysis workflow customized to your research project. All services, including project meetings, training, booking and access to our image analysis workstations, software and storage are provided either on premise or remotely.

You can contact us through the IACF webpage to take part in a free Call4Help consultation or to submit an image analysis project. Projects involving software or methodological development must be submitted online and are bound to fees estimated during a free quotation prior to a project start. Software training and support is provided on demand on our workstations and at a fixed hourly cost. We additionally plan training sessions on how to efficiently share and preserve scientific images and associated analysis software, and we are actively involved in teaching bioimage analysis during workshops and training schools.

The facility received funding from a national infrastructure grant (DBI-INFRA 2022-2027, Danish Ministry of Higher Education and Science) and it aims at actively promoting open science and enforcing high sharing and reproducibility standards through good practices, dedicated data and software repositories, and a community discussion forum. To this end, we will systematically try to reach agreements with the researchers to define a timeline for sharing the assets developed in the facility, especially for those with high re-usability potential. The facility also aims to establish a new open service model embracing professional project management practices, fair service billing, and an optimal utilization of existing local resources and expertise.

19. A Photonic Chip-based Microscopy Platform For Super-Resolution Histology of FFPE Tissue Sections

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While modern optical microscopes allow for fast imaging of commonly available formalin-fixed paraffinembedded (FFPE) tissue sections at the subcellular scale, the resolution capabilities of these instruments fall short for the study of many ultrastructural features that are fundamental both for research and clinical diagnosis.

Traditionally, the ultrastructural observation of tissues has required the resolving capabilities of electron microscopy, at the expense of slow and sophisticated sample preparation, low imaging throughput, and high operational costs. Over the last two decades, however, the advent of fluorescence-based optical superresolution microscopy (SRM) techniques has facilitated nanoscale visualizations of the biological machinery in living organisms, contributing to significant breakthroughs in life sciences. Particularly in histology, the SRM methods have given us a glimpse of their potential benefits for the study of standard FFPE samples, allowing the identification of ultrastructural changes associated with various pathologies. Nevertheless, multiple barriers defer the practical adoption of the existing SRM methods in standard histological laboratories. These include complex and expensive equipment, the need for highly specialized operators along with specialized sample preparations, and insufficient data throughput for routine analyses. Hence, a microscopy platform capable of high-throughput and high-resolution imaging while compatible with standard processing workflows would prove advantageous for the adoption of SRM in histology.

Recently, photonic chip-based microscopy has been proposed as a tool for bioimaging applications, enabling SRM over large fields of view in live and fixed cells, as well as in tissue cryosections. In this work, we propose chip-based microscopy as a multimodal imaging platform for histological assessment of FFPE tissue sections. By following standard preparation protocols on diverse paraffin-embedded specimens, we demonstrate the suitability of this technique for high-resolution and high-contrast microscopy imaging over large fields of view across diverse microscopy modalities including waveguide-based total internal reflection fluorescence, intensity fluctuation-based analysis, and correlative light-electron microscopy.

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Imaging protein complexes at molecular scale has been on the wish list of researchers in the life sciences for decades

Abberior

In MINFLUX single fluorophores are localized by reading out the fluorescence signal at pre-defined positions in its vicinity. Using a donut-shaped excitation focus allows to determine the localization of the fluorophores with a minimal number of photons and consequently within a spatio-temporal regime exceeding alternative techniques [1, 2]. Until now, microscopy techniques with such a performance were unfortunately only usable by experts in the field of optics.

Here, we report on a MINFLUX microscope based on a common fluorescence microscope stand. This microscope allows to combine highest localization precisions with standard workflows allowing non-experts to apply this technique.
Imaging of Nuclear Pore Complex samples in the 2D MINFLUX mode was possible with localization precisions below 2 nm. The 3D MINFLUX imaging mode allowed to achieve isotropic localization precisions below 2.5 nm (Fig. 1). In turn, this microscope was used to image several samples from the life sciences. Together with the option to do multi-color imaging it facilitates colocalization analysis in the nanometer range and will allow to address numerous biomedical and biophysical questions on the molecular scale.

Spinning Disk Confocal Microscopy in Life Science Research

Andor

Life Science Research is accelerating at an unprecedented pace. In this talk we will present the strength of spinning disk confocal microscopy to aid the complex research questions in fixed and living model systems. We present our Benchtop Confocal Microscope, BC43, a super-compact unit that is rich in features and benefits. With no requirement for a dark room, you can now access spinning disk confocal microscopy on the same work bench as your samples. This increases the precision when setting up live cell imaging experiments and enables fast 2D and 3D acquisitions.

Ultra-large field of view super-resolution microscopy

ChipNano Imaging AS

Chip NanoImaging provides a complete TIRF-based microscope system, along with image acquisition software. The chip-based TIRF illumination is achieved by multimode waveguide technology which ensure tight light confinement with fine optical section of your sample. Our WaveBeat technology ensures high intensity and improved image contrast. During the workshop, we will demonstrate that the ultra-large field of view chip-based TIRF is ideal for both diffraction limited as well as superresolution microscopy using single molecule localization microscopy (SMLM). We will also demonstrate how the large SMLM datas.

Introducing the new CLSM FV4000 with SilVIR™detectors

Holger Hartmann - Evident Europe

The new Olympus FV4000 has quickly become a favorite among researchers with its game-changing dynamic range, signal efficiency covering 400-900nm and photon counting-abilities for quantitative imaging. Olympus FV4000 offers up to ten laser lines in parallel, up to 6 spectral detectors, redesigned high-speed, high-resolution scanners and a new laser power monitor system for reproducible imaging. But don’t just believe the facts, see it for yourself, and feel free to bring your own samples.

Eclipse Ji: Nikon's first benchtop inverted microscope

Inter Instrument AS - Nikon

With the Eclipse Ji a research grade microscope becomes an easy-to-operate laboratory instrument. Building on the expertise from market leading Ti2, the concept of the Eclipse Ji is following the needs of customers for more approachable hardware. Teaching and learning times are reduced. Novice and sample-centric users will immediately be able to conduct an experiment. The inbuilt camera and neural networks make an autonomous microscope possible: one that self-aligns, adjusts acquisition conditions and performs the analysis on behalf of the researcher. Eliminating the error-prone adjustments of a regular microscope and ensuring consistent data generation.

The Ji's Smart Experiment mode offers more than a dozen fully automated assays ranging from transfection efficiency to phagocytosis, complete with detailed protocols and materials lists. All assays are customizable to adjust to the user's needs. The list of available assays is continuously expanded and custom assays are available on request.
In Research mode, the Ji supports high-end imaging using all Nikon CFI60 objectives, including oil and silicone oil immersion and automated water-immersion. The free side port is available to mount an additional camera or confocal scan head such as Nikon's AX series point scanning confocal.

Visit our booth for a live demo of the Ji!

STELLARIS: From Power HyD enhanced photon detection to molecular interactions with TauSense

Leica-Ortemedic

Tuesday: Confocal microscopy has become a staple for fluorescence imaging in the life sciences, owing to the optical sectioning, sensitivity, and the temporal and spatial resolution capabilities. With STELLARIS, we have introduced key innovations such as the Power HyD family of detectors (1), the next generation of white light lasers (WLL), and the TauSense technology (2).

The fully digital and photon counting proprietary approach, Power Counting, allows straightforward and robust quantification of fluorescence signals in STELLARIS. Together with the WLL excitation flexibility, STELLARIS delivers extended near infrared capabilities for expanding the palette of fluorophores in every experiment., In addition, TauSense opens the door to functional imaging insights thanks to straightforward access to fluorescence lifetime-based information.

In this workshop we will learn how all the technological innovation in STELLARIS facilitate fluorescence quantifications . We will also highlight a new TauSense tool built to evaluate molecular interactions. Join us for a first-hand view of what STELLARIS can bring to functional imaging.

TauSTED Xtend - New tools for gentle live imaging at remarkable nanoscale

Leica-Ortemedic

Wednesday: The goal of scientific research is to understand the workings of nature. Given the complex interplay of biomolecules, molecular machines, and higher-order cellular structures, confocal imaging emerged as a fundamental tool owing to the optical sectioning, sensitivity, and the temporal and spatial resolution capabilities.

Imaging intricate cellular structures at nanoscale resolution while characterizing the dynamics of multiple species in the context of live specimens are emerging avenues followed to shed light on biological processes. With the advent of STED (Stimulated Emission Depletion), researchers have realized the visualization of intracellular structures at the nanoscale, unveiling insights into cellular behavior, interactions, and function.

In the talk and showcase, we will introduce our innovation TauSTED Xtend and demonstrate how it enables gentle imaging of live and fixed samples at the nanoscale. We will show how advances in our TauSTED (1) approach to optical nanoscopy deliver cutting-edge resolution and image quality at low light dose, key to accessing fast nanoscale dynamics of cellular processes. We will also show how fluorescence lifetime information can be used for multiplex imaging of different markers, keeping the nanoscopic resolution.

Emerging technologies in Spatial Biology

Milteny Biotec

Spatial biology is an emerging field that studies the spatial organization of cells and molecules within tissue to better understand complex biological processes. Currently available methods may only provide information from thin tissue sections, limiting analysis to rather roughly selected areas.

We constantly develop our workflows for both 3D imaging with light sheet fluorescence microscopy (LSFM) of whole mount tissues and for multiplex spatial analysis of tissue section. To get an overview of complex large samples, identify target structures within them, LSFM is proven unmatched for many applications. The complexity from sample preparation, long acquisitions, and data handling has been a limiting factor. Our recent speed and workflow improvements through unique solutions with reagents, 3D-immunofluorescence (3D-IF) antibodies, UltraMicroscope Blaze™ hardware and software will uncovered.

To further analyze carefully selected tissue regions in depth with multiple markers– all on the same valuable specimen we provide a Multiomics solution for same section RNA and protein analysis. Tissue sections (or adherent/suspension cells) can be analyzed with MACSima™ Imaging Cyclic Staining (MICS), providing expression levels of up to hundreds of protein markers from individual cells on a single sample. A fully automated workflow ensures ease of use, high precision and data quality, and we provide our tailor made first-in-class software solution to analyze complex spatial imaging data.

By combining these two techniques we show the ability to obtain both, a comprehensive 3D context, and detailed information about cellular diversity in a spatial context from a single sample makes our workflow game changing in spatial biology

LiveCodim and Q-Phase: Live cell solutions for gentle super-resolution and quantitative label-free imaging

Telight

The ability to visualize the dynamics and structure of living samples has marked a revolutionary achievement of light microscopy in the life sciences. It has paved the way for major breakthroughs in biomedical research as researchers can image and track interactions of subcellular structures within and around cells. Fundamentally, the versatility and relative simplicity of confocal laser-scanning microscopy has allowed it to enjoy its continued status as a major workhorse tool within biology. Unfortunately, in order to visualize finer details within cells and in the context of light-sensitive samples, the diffraction limit and phototoxicity are persisting challenges with confocal microscopy.

To address the first challenge of seeing beyond the diffraction limit (200-250 nm), many super-resolution microscopy solutions have successfully circumnavigated this and can visualize structures down to just a few nanometers in scale. However, many of these techniques invite multiple drawbacks including fluorophore restrictions, long integration times and, in particular, higher levels of photo-damage. There remains a great need for simple, versatile, and gentle super-resolution imaging methods.

Here, we present how super-resolution can be achieved without intense inhibition lasers or through photophysical manipulation of the sample. Using conical diffraction, this can be done quickly, passively, and achromatically for any visible wavelength of light, making it possible to produce structured illumination light patterns inherent to a scanned beam of light. Such patterns can have features sharper than the diffraction limit, thereby directly optimizing the point-spread function of the system. We present how conical diffraction is used as a beam-shaping tool that when integrated as an add-on to a microscope, can serve as a highly-sensitive, gentle imaging system with little-to-no photobleaching or phototoxicity on any sample.

Walkthrough Lattice SIM 5

Zeiss

The ZEISS arivis Pro analysis strategy and iterative approach allow processing and segmentation of a small field of view, a 3D/4D subset, or the entire dataset. Analysis results can be viewed in synchronized split windows in 2D and 3D views simultaneously, which is particularly useful for densely packed structures and tracking experiments. The software's integrated Machine Learning/Deep Learning functionality (available as local training option or in the ZEISS arivis Cloud) allows for setting up an image segmentation and analysis pipeline with ease and without extensive knowledge of AI analysis methods.

Arivis post processing software

Zeiss

ZEISS Lattice SIM 5 provides you with outstanding super-resolution capabilities down to 60 nm in both living and fixed cells. Additionally, you can choose SIM Apotome imaging mode and a low-magnification objective to achieve fast overview images of your sample before zooming into super-resolution details. Hardware and data-agnostic image analysis software.

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