Poster No: 1

Title:
A multi-modal and spatially resolved human breast cancer atlas

Name:
Leire Alonso Galicia

Affiliation:
KTH Royal Institute of Technology

Abstract:
Human breast cancer is a complex and heterogeneous disease. Within the evolving field of spatially resolved transcriptomics (SRT) technologies our objective is to develop a comprehensive, multi-modal, and spatially detailed map to piece together the intricate aspects of breast cancer. We applied Visium CytAssist methodology to generate whole-transcriptome wide spatially resolved data from 200 breast cancer patients. Within this project we have also generated high resolution H&E images for pathology annotation. In addition, we obtained patient-matched single-cell RNA sequencing data, whole genome sequencing data and patient metadata to create a multidimensional human breast cancer atlas. The transition between in situ breast cancer to invasive disease remains to be explored. Our findings support the hypothesis of interactions between cancer cells and other cells in the tumor microenvironment, such as immune cells. Additionally, early copy number aberrations can be detected in tumor clones from the spatial transcriptomics data. In conclusion, our spatially resolved breast cancer atlas project has generated extensive data that can be used to deepen the knowledge in the tumor microenvironment and invasive behaviour of human breast cancer, ultimately guiding treatment.

Poster No: 2

Title:
TissUUmaps: interactive visualization, exploration, and quality assessment of large-scale spatial omics data

Name:
Christophe Avenel

Affiliation:
Uppsala University, ScilifeLab

Abstract:
Spatially resolved techniques for exploring the molecular landscape of tissue samples, such as spatial transcriptomics, often result in millions of data points and images too large to view on a regular desktop computer, limiting the possibilities in visual interactive data exploration. TissUUmaps is a free, open-source browser-based tool for GPU-accelerated visualization and interactive exploration of 107+ data points overlaying tissue samples. TissUUmaps 3 provides instant multiresolution image viewing and can be customized, shared, and also integrated into Jupyter Notebooks. TissUUmaps introduces new modules where users can visualize markers and regions, explore spatial statistics, perform quantitative analyses of tissue morphology, and assess the quality of decoding in situ transcriptomics data. Thanks to targeted optimizations the time and cost associated with interactive data exploration were reduced, TissUUmaps 3 enables to handle the scale of today's spatial transcriptomics methods

Poster No: 3

Title:
MolBoolean: Unlock the secrets of protein-protein interactions

Name:
Nancy Dekki

Affiliation:
Atlas antibodies

Abstract:
The dynamic mechanisms of protein-protein interactions (PPIs) in cells and tissue are heavily dependent on the spatial location and the abundance of the respective interacting partners. Therefore, it is essential to not only understand the number of interaction events, but also the total number of ingoing protein components.

MolBoolean:tm: is a novel in situ proximity technology developed by Atlas Antibodies in collaboration with Ola Söderberg, Professor at the Department of Pharmaceutical Biosciences, Uppsala university. Unlike traditional in situ proximity ligation assays, MolBoolean enables the simultaneous detection of both free and interacting fractions for two endogenously expressed protein targets in fixed cells and tissue.

MolBoolean:tm: relies on dual target recognition with proximity probes and rolling circle amplification (RCA) as a mean to amplify signal. A series of molecular steps incorporates information into the amplified products indicating antibody target engagement of individual versus interacting proteins. This information is converted to fluorescent signals by the binding of detection reporters, allowing visualization on a conventional fluorescent microscope. An image analysis software is provided to segment and differentiate fluorescent signals, enabling the relative quantification of free versus interacting fractions for the two analyzed protein targets in the sample.

The more comprehensive extraction of information provided by MolBoolean enables normalization of your interaction data to total target protein levels. Therefore, MolBoolean will add more confidence to the interpretation of your protein proximity data.

Here, we showcase application data for several protein interaction partners in both cells and tissue using the MolBoolean technique. We also show how to enable the image analysis software which enables normalization of the protein interaction to ensure accurate interpretation of protein proximity data.

Poster No: 4

Title:
SciLifeLab OMERO – Advancing Image Data Management in Sweden

Name:
Daniela A. Garcia-Soriano

Affiliation:
Chalmers

Abstract:
In an era where biological imaging generates ever-growing datasets, efficient image data management and visualization are crucial for life sciences research. SciLifeLab OMERO is a national service designed to provide researchers in Sweden with a powerful, accessible platform for handling large-scale biological imaging data. Our poster will introduce the underlying OMERO software, an open-source image management system, describe how it will be accessible at a national level for various use cases, and outline the significance of the resulting national service for Swedish life science researchers and imaging facilities.

Poster No: 5

Title:
cryoSPHERE: Single-Particle HEterogeneous REconstruction from cryo EM

Name:
Lukas Grunewald

Affiliation:
Uppsala University

Abstract:
The three-dimensional structure of proteins plays a crucial role in determining their function. Protein structure prediction methods, like AlphaFold, offer rapid access to a protein's structure. However, large protein complexes cannot be reliably predicted, and proteins are dynamic, making it important to resolve their full conformational distribution. Single-particle cryo-electron microscopy (cryo-EM) is a powerful tool for determining the structures of large protein complexes. Importantly, the numerous images of a given protein contain underutilized information about conformational heterogeneity. These images are very noisy projections of the protein, and traditional methods for cryo-EM reconstruction are limited to recovering only one or a few consensus conformations.

Here, we introduce cryoSPHERE, which is a deep learning method that uses a nominal protein structure (e.g., from AlphaFold) as input, learns how to divide it into segments, and moves these segments as approximately rigid bodies to fit the different conformations present in the cryo-EM dataset. This approach provides enough constraints to enable meaningful reconstructions of single protein structural ensembles. We show that cryoSPHERE is very resilient to the high levels of noise typically encountered in experiments, where we see consistent improvements over the current state-of-the-art for heterogeneous reconstruction.

Poster No: 6

Title:
Modeling ligands in cryo-EM with generative AI and density-guided simulations

Name:
Nandan Haloi

Affiliation:
KTH Royal Institute of Technology

Abstract:
Visualizing protein-ligand interactions in atomic detail is key to understanding how small molecules regulate macromolecular function. Although recent breakthroughs in cryogenic electron microscopy (cryo-EM) have enabled high-quality reconstruction of numerous complex biomolecules, the resolution of bound ligands is often relatively poor. Furthermore, automated methods for building and refining molecular models into cryo-EM maps have largely focused on proteins, and may not be optimized for the diverse properties of small-molecule ligands. Here, we present a pipeline integrating generative artificial intelligence (AI) with cryo-EM density-guided simulations to fit ligands into experimental maps. Using three inputs: 1) a protein amino-acid sequence, 2) ligand specification, and 3) an experimental cryo-EM map, we validated our approach on a set of biomedically relevant protein-ligand complexes including kinases, GPCRs, and solute transporters, none of which were present in the AI training data. In cases for which generative AI was not sufficient to predict experimental poses outright, integration of flexible fitting in GROMACS improved ligand fits from 40–71\% to 82–95\% accuracy relative to the deposited structure. This work offers a straightforward template for integrating generative AI and density-guided simulations to automate determination of cryo-EM structures of ligand-protein complexes, with potential applications to efficiently characterizing and designing novel modulators and drugs.

Poster No: 7

Title:
Synergistic Anticancer Effects of Onalespib and X-Ray Combination Therapy in Glioblastoma

Name:
Mehran Hariri

Affiliation:
Uppsala University

Abstract:
Glioblastoma (GBM) is the most aggressive type of brain cancer affecting both adults and children, characterized by extremely high morbidity and mortality rates despite standard treatments, including surgery, radiation therapy, and chemotherapy. Consequently, there is a critical need for novel therapeutic approaches to improve patient survival and minimize treatment side effects.

In this study, we examined the impact of HSP90 inhibition combined with radiotherapy in both established and patient-derived glioblastoma cell lines. The potential radiosensitizing effects of the HSP90 inhibitor Onalespib were evaluated using XTT and clonogenic survival assays, along with tumor-mimicking multicellular spheroid models. Additionally, migration capacity and protein expression changes following Onalespib and radiation exposure were analyzed using the Proximity Extension Assay.

Our results demonstrated that HSP90 inhibition with Onalespib significantly enhanced the radiosensitivity of glioblastoma cells in both 2D and 3D models, leading to increased cell death, reduced migration, and activation of apoptotic signaling pathways. Proteomic analysis of treated glioblastoma cells revealed substantial changes in protein expression related to growth signaling, immune modulation, and angiogenesis. Furthermore, the combination treatment appeared to promote cell cycle arrest and apoptosis, highlighting its potential as an effective anti-tumor strategy.

These findings suggest that HSP90 inhibition could be a valuable approach to improving radiotherapy outcomes for GBM, offering a promising path for enhancing treatment efficacy in this highly aggressive cancer.

Poster No: 8

Title:
Human intestinal organoids as an advanced 3D model for drug absorption and nanoparticle uptake

Name:
Dinh Son Vo

Affiliation:
Uppsala University

Abstract:
Nano-sized formulations for targeted oral delivery of new drug modalities such as nucleic acid therapeutics show promise in treating local gastrointestinal diseases (e.g., inflammatory bowel disease), offering a patient-friendly alternative to predominantly parenteral treatments. However, assessing their efficacy requires more in vivo-like intestinal models. Bridging the gap between conventional 2D cell-based systems and in vivo models, 3D intestinal organoids (IOs) are able to partially recapitulate a cellular phenotype closer to the normal human intestinal epithelium. In this project, IOs derived from stem cell-containing crypts of human intestinal tissue were initially cultured as basal-out structures within an extracellular matrix. The polarity of these organoids was then reversed to an apical-out orientation, allowing direct access to the apical surface of the epithelium. Here, immunostaining of actin filaments and tight junction proteins confirmed successful polarity reversal, with the apical membrane facing the suspension environment. In contrast to basal-out organoids, apical-out organoids take up fatty acid fluorescent analog C1-BODIPY-C12 through the fatty acid transporters (FATP4) located on the apical surfaces of epithelial cells, confirming reversed polarity as well as FATP4 functionality. Electron microscopy demonstrated apical microvilli, typical tight junction structure as well as presence of glycocalyx in developed IOs. Barrier integrity was further demonstrated by exclusion of a fluorescent hydrophilic marker using live-cell microscopy. Especially, the presence of key proteins involved in cell uptake as well as intracellular trafficking were revealed from the organoid proteome, presenting an opportunity to utilize the IO model as a screening tool for nano-sized delivery systems.

Poster No: 9

Title:
Somatostatin triggers local cAMP and Ca2+ signaling in primary cilia to modulate β-cell function

Name:
Ceren Incedal Nilsson

Affiliation:
Uppsala University

Abstract:
Background and aims

Primary cilia, despite being continuous with the plasma membrane and cytosol, function as cellular antennae for sensory input and signal integration. Enriched with GPCRs, they provide alternative pathways for signal processing alongside canonical receptor signaling. The hedgehog (Hh) pathway, vital for pancreas development and islet cell function, relies on functional cilia. Hh stimulation leads to cilia-specific, Gi-dependent cAMP lowering and GLI transcription factor activation. Somatostatin receptor type-3 (SSTR3) is also Gi-coupled and localized to primary cilia, but whether these receptors cross-talk with the Hh pathway is not known. This study aimed to elucidate the role of primary cilia in somatostatin action in islet β-cells.

Material and methods

The spatial relationship between β-cell cilia and somatostatin-secreting δ-cells in mouse islets was determined using FIB-SEM and immunofluorescence confocal microscopy. A cilia-targeted somatostatin sensor and confocal microscopy were used to measure somatostatin release onto cilia in intact mouse islets. TIRF microscopy of intact mouse islets and MIN6 pseudo-islets expressing cilia-targeted cAMP and Ca2+ sensors were used to record somatostatin-induced ciliary signal transduction. Functional evaluation of ciliary somatostatin signaling was performed in MIN6 pseudo-islets following the shRNA-mediated knockdown of SSTR3.

Results

FIB-SEM images from different axial positions of a mouse islet revealed islet δ-cells that were in direct contact with β-cell primary cilia. Similar observations were made in transgenic mouse islets with δ-cell-specific expression of td-tomato or wild-type islets immunostained against somatostatin, with each δ-cell making contact with on average 3.2 cilia, compared to 1.8 cilia for non-δ-cells (n=35; N=7; P=1.0E-5). To test if somatostatin is released onto primary cilia, we expressed a somatostatin sensor (SST1.0) based on SSTR5. The sensor localized to both the plasma membrane and primary cilia, and stimulation with 10 mM glucose or 100 nM ghrelin

triggered robust increases in SST1.0 fluorescence that were most prominent in the primary cilia. This indicates that ciliary SSTRs can sense endogenously released somatostatin. Using cAMP sensors, we next showed that 100 nM somatostatin induced cAMP lowering in both cytosol and primary cilia, but only the lowering in the cilium was prevented in MIN6 pseudo islets with reduced SSTR3 expression. Prolonged exposure to somatostatin (18h) caused a slight shortening of primary cilia (9±0.08%, n=214, N=4, P<0.001) and induced the translocation of GLI2 from the primary cilium to the nucleus. The magnitude of the translocation was similar to that induced by the Hh agonist SAG and was prevented by SSTR3 knockdown (WT-control: 25%, WT-SST: 56%, WT-SAG: 52%, KD-control: 43%, KD-SST: 42%; N=3, n=250-300 cells). These results indicate functional cross-talk between somatostatin and the Hh pathway. Consistent with the long-term effects of somatostatin on β-cell function, we find that 18h somatostatin or SAG exposure both lowers resting cAMP levels and strongly attenuates GLP-1-induced (10 nM) cAMP elevations in β-cells (resting cAMP: PSAG< 0.0001, PSST= 0.005; 10 nM GLP-1: PSAG <0.001, PSST< 0.0001; n=140; N=3).

Discussion

Our findings highlight the primary cilium as an alternative target of somatostatin action in islet cells and suggest roles of somatostatin beyond acute inhibition of hormone release.

Poster No: 10

Title:
NBIS - SciLifelab Bioinformatics Platform

Name:
Anna Johansson

Affiliation:
Uppsala university

Abstract:
NBIS (National Bioinformatics Infrastructure Sweden) provides bioinformatics and data science support to the Swedish life science research community since 2008. We provide a wide spectrum of services, including advanced bioinformatics analysis, bioimage informatics, data management, imaging AI support, systems and tools development, and support to national compute resources.

NBIS is a national research infrastructure which comprises ~120 experts in different fields, distributed across six sites in Sweden: Lund, Gothenburg, Linköping, Stockholm, Uppsala and Umeå. NBIS constitutes the SciLifeLab Bioinformatics platform and forms the Swedish node in ELIXIR (the European infrastructure for biological information).

In addition to hands-on assistance during research projects and systems development, NBIS also provides advanced training, offering a large catalogue of bioinformatics and data science courses, as well as a mentor programme for PhD Students.

To provide state-of-the-art bioinformatics services to the Swedish life science researchers community, NBIS is supported by the Swedish Research Council (Vetenskapsrådet), Science for Life Laboratory, major Swedish universities, the Knut and Alice Wallenberg Foundation, NordForsk, and the European Commission.

NBIS is working in close collaboration with other SciLifeLab platforms and other national infrastructures, the SciLifeLab Data Centre, the National Academic Infrastructure for Supercomputing in Sweden (NAISS), and many other stakeholders and collaborators to create synergies and provide the most optimal services to the research community.

Poster No: 11

Title:
High-Content Cell Painting for Morphological Profiling in Progeria Fibroblasts: A Platform for Large-Scale Drug Screening and Therapeutic Efficacy Evaluation

Name:
Martin Johansson

Affiliation:
Uppsala University

Abstract:
Progeria is a rare genetic disorder characterized by accelerated aging resulting from LMNA gene mutations that lead to the production of the toxic protein progerin. Leveraging advanced high-content imaging, particularly the cell painting assay, we perform comprehensive morphological profiling to capture the intricate cellular phenotypes associated with the disease. By integrating these imaging techniques with quantitative, data-driven analysis, our work demonstrates that cell painting of fibroblast cells from a proband and their parents provides a robust platform for large-scale drug screening. Proof-of-concept experiments using Lonafarnib (a farnesyltransferase inhibitor) and Baricitinib (a JAK inhibitor) underscore the method’s potential to detect therapeutic efficacy and modulate disease phenotypes. This study exemplifies how imaging-based approaches can bridge basic research with clinical applications, fostering collaboration across academia and healthcare to push the frontiers of cell and molecular biology in a data-driven era.

Poster No: 12

Title:
BioImage Informatics, SciLifeLab, Sweden

Name:
Anna Klemm

Affiliation:
Uppsala University, SciLifeLab

Abstract:
The BioImage Informatics unit (BIIF) develops new computational technologies and provides access to expertise and state-of-the art software for processing and quantitative analysis of all kinds of microscopy image data, primarily for applications in the life sciences. BIIF is a SciLifelab unit, and part of the National Bioinformatics Infrastructure Sweden NBIS. We are active within the GloBIAS and EuroBioImaging networks.

Services

* Advice on best-practice and guidance on overall experimental design for research involving microscopy imaging and quantitative data analysis.

* Guidance on image analysis assay development, including image processing algorithm development and software engineering to address challenging project goals.

* Advice on best-practice and guidance on high throughput/large-scale image processing using computing clusters, including data transfer and storage during the activity of the project.

* Guidance on large-scale data analysis and visualization.

* Dissemination of bioimage analysis knowledge in courses and workshops.

Poster No: 13

Title:
Norepinephrine Reduces T Cell Motility in Pancreatic Islets During Late Stage Islet Infiltration During Type 1 Diabetes

Name:
Robin S. Lindsay

Affiliation:
Uppsala University

Abstract:
Neuroimmune interactions have been shown to extensively regulate immune responses in a variety of disorders, including autoimmune diseases. However, under different conditions it has been shown to activate or inhibit the adaptive immune system including T cells. Norepinephrine has been shown to have immunomodulatory effects on a variety of immune cells, including T cells. Our lab has discovered that norepinephrine levels are higher within the islets than the exocrine pancreas, but that this level is not uniform throughout all islets. As T cells in all stages of activation express the receptor for norepinephrine it is possible norepinephrine may directly alter T cell motility and function in the pancreatic islets. To test if norepinephrine had any effect on T cell function, we examine the effect on individual T cell motility using fluorescent microscopy. Reduced T cell motility and interactions have been correlated to T cell effector function and β-cell destruction.

Our results showed that activated CD8+ T cells in vitro had reduced motility following stimulation with 10-6 M norepinephrine, but not lower levels (10-8 M) of norepinephrine stimulation. To test if the also occurred within the pancreatic islets, we induced type 1 diabetes in mice using the Ins2-ova mouse model, which express ovalbumin only in β-cells. Ovalbumin specific CD8+ T cells (OT-I) expressing fluorescent reporters were transferred into Ins2-ova mice and disease was initiated by immunization with ovalbumin peptide. During early stages of islet infiltration (day 6 of disease induction) T cells have lower levels of motility and increased activation. No effect on T cell motility was observed following norepinephrine stimulation under these conditions. In later stages of islet infiltration (Day 8) T cells have increased motility due to partial tolerance induction in the islet environment. Interestingly, in later stages of islet infiltration norepinephrine stimulation significantly reduced T cell motility and increased arrest. Overall these results suggest that increased levels of norepinephrine in pancreatic islets may contribute to breaking T cell tolerance in tissues and the pathogenesis of type 1 diabetes.

Poster No: 14

Title:
X-ray ultrafast biological imaging without crystals

Name:
Filipe Maia

Affiliation:
Uppsala University

Abstract:
The quest to image single molecules played a crucial role in the development of X-ray free-electron lasers (XFELs). The ultra-bright, ultrafast pulses of XFELs enable the recording of a particle's diffraction pattern before radiation damage destroys it—a concept first proposed by Neutze et al. (Nature, 2000) and experimentally demonstrated at FLASH over a decade ago (Chapman et al., Nature Physics, 2006). Since then, flash X-ray imaging (FXI) has been successfully applied to imaging live cells (van der Schot et al., Nature Communications, 2015), cellular organelles (Hantke et al., Nature Photonics, 2014), and viruses in both two (Seibert et al., Nature, 2011) and three dimensions (Ekeberg et al., PRL, 2015).

The advent of the European XFEL ushered in an era of high-intensity, high-repetition-rate, and high-data-rate XFELs, demonstrating that FXI could fully leverage these advancements. However, the ultimate goal of achieving sub-nanometer, high-resolution imaging remains unfulfilled.

In this talk, I will present the first three-dimensional X-ray images of isolated proteins. Additionally, I will discuss microsecond time-resolved measurements of protein dynamics in solution, obtained via SAXS/WAXS at the European XFEL (Konold et al., Nature Methods, 2024). I will explore how combining the enhanced signal-to-noise ratio of brighter sources with powerful machine-learning-based constraints can provide new insights into molecular dynamics. Finally, I will share preliminary results on pushing X-ray solution scattering to the limit of one particle per shot, FXI in solution, highlighting how ultrathin liquid sheets, coupled with advancements in X-ray optics, hold great promise for ultrafast, nanoscale imaging of biological macromolecules in solution at room temperature.

Poster No: 15

Title:
Towards single cell metabolomics in neurooncology and neurodegeneration

Name:
Wojciech Michno

Affiliation:
Uppsala University

Abstract:

Poster No: 16

Title:
Characterization of single-domain FusB homologs

Name:
Maryam Paryar

Affiliation:
Uppsala University

Abstract:
Fusidic acid (FA) is an antibiotic used clinically against Staphylococcus aureus infections. FA inhibits protein synthesis by binding to elongation factor G (EF-G) and preventing its release from the ribosome. In Staphylococcus aureus, the most common resistance mechanism to FA is through the resistance protein FusB, which binds to the FA-locked EF-G and releases it from the ribosome. Through bioinformatic analysis, we identified single-domain FusB homologs (cFusBs) in several Gram-positive bacteria. These are often encoded in operons where the cFusB gene is surrounded by genes for a TetR-family repressor, and a lipase. We hypothesize that these are uncharacterized FA-resistance operons and set out to characterize one such operon from Streptomyces canus. We have previously solved the crystal structure of S. canus cFusB. In addition, we showed that the S. canus TetR homolog binds to an operator sequence between the cFusB gene and its own gene, and that its DNA binding is regulated by FA .

Now we aim to characterize the function of the predicted S. canus lipase. Differential scanning fluorimetry experiments show that the denaturation temperature increases in presence of FA, suggesting that the lipase binds FA and may mediate FA resistance.

Future experiments will focus on solving the structures of the TetR and the lipase bound to FA, as well as evaluating the possible catalytic activity of the lipase on FA.

Poster No: 17

Title:
Linking genetic modifications to cell biomechanics in plants using spatial chemical imaging

Name:
Edouard Pesquet

Affiliation:
Stockholm University

Abstract:
Spatial chemical imaging has enabled to reach unprecedented quantification of specific molecules at the cellular to subcellular levels in pluricellular organisms. These methods massively reduced the averaging errors in biopolymer characterization due to bulk biochemical analyses. Although promising, spatial chemical imaging methods need to be established and validated. We thus developed a range of spatial chemical imaging methods specifically for plant extracellular polymers. We focused on lignins, biosphere second most abundant biopolymer that represents more than 30% of the biological carbon on Earth. We used both light and electron microspectroscopic methods to determine changes in lignin concentration, in composition and in polymer structure at the subcellular level with below micrometer resolutions in millimeter-wide plant biopsies. We validated our methods using both synthetic chemistry, to assemble lignin polymers with known chemistry, and genetic engineering, to establish collections of loss-of-function mutants, to quantitatively determine changes in lignin concentration, chemistry, distribution and structure. We extended the capacity of our spatial chemical imaging analyses to also determine changes in cell biomechanical properties. Altogether, our breakthrough spatial imaging methods for chemistry and biomechanics established the relationship between specific changes in lignin chemistry/structure and variation in cell biomechanics.

Poster No: 18

Title:
Subcellular protein architecture of human sperm

Name:
Emmie Pohjanen

Affiliation:
KTH Royal Institute of Technology

Abstract:
From ejaculation to fertilization, the human sperm cell needs to travel a long distance, navigate in a highly-complex environment, and mature to be capable to ultimately penetrate the egg vestments and fertilize the egg. These processes rely on the expression, functionality and localization of a large set of proteins, many of which are unique to sperm and poorly characterized. How proteins are spatially organized in the sperm cell is poorly understood. In this study, we applied antibody-based spatial proteomics coupled to high-resolution confocal microscopy to systematically map the subcellular spatial distribution of a wide range of proteins in single human sperm cells. We identified 654 proteins and annotated their localization to 11 different subcellular structures, including 73 proteins not previously detected in human sperm. We observe that roughly 55% of the sperm proteome varies in expression levels and/or spatial distribution between individual cells. Our spatial map of the human sperm proteome is available through the Human Protein Atlas, making it broadly accessible as a resource for advancing research on the molecular architecture and mechanisms of the sperm cell and male infertility phenotypes.

Poster No: 19

Title:
GPCRs Signaling in the Primary Cilium

Name:
Gonzalo Sanchez

Affiliation:
Uppsala University

Abstract:
The primary cilium is a rod shaped organelle with a length of about 5μm and a diameter of 250nm. The enrichment with distinct ciliary receptors and downstream partners is cell type dependent and dynamic. We study the molecular steps involved in ciliary transduction in pancreatic β-cells and neurons employing a variety of imaging methodologies.

Poster No: 20

Title:
Spatial biology at KIGene core facility

Name:
Lars Selander

Affiliation:
Karolinska Institutet

Abstract:
KIGene genetic analyses and spatial biology is a core facility at Karolinska Institutet. We provide the GeoMx and CosMx platforms from nanoString (recently acquired by Bruker) for academic as well as non-academic customers.

GeoMx Digital Spatial Profiler (DSP) provides morphological context in spatial transcriptomics and spatial proteomics experiments from a regular tissue section, e.g., fresh frozen, fixed frozen or FFPE. GeoMx is the only spatial biology platform that non-destructively profiles expression of RNA and protein from distinct tissue compartments and cell populations with an automated and scalable workflow that integrates with standard histology staining. Spatially profile the whole transcriptome and 570+ protein targets separately or simultaneously from your choice of sample inputs: whole tissue sections, tissue microarrays (TMAs), or organoids.

CosMx SMI is the first high-plex in situ analysis platform to provide spatial multiomics with FFPE and fresh or fixed frozen tissue samples at cellular and subcellular resolution. CosMx SMI enables rapid quantification and visualization of up to 6,000 RNA and 64 validated protein analytes. It is the flexible, spatial single-cell imaging platform that will drive deeper insights for cell atlasing, tissue phenotyping, cell-cell interactions, cellular processes, and biomarker discovery.

AtoMx:tm: Spatial Informatics Platform (SIP) is a cloud-based, fully-integrated informatics platform for spatial biology. No coding experience is required to use AtoMx SIP. Researchers can analyze and visualize spatial multiomics data with ease while utilizing NanoString-configured analysis modules and pipelines. For researchers with computational experience, custom analysis modules and pipelines can be created and executed all while leveraging the compute power of the cloud.

For project planning information or other inquires please contact:

kigene-spatialbiology@mmk.ki.se

Poster No: 21

Title:
Looking for the short-lived and rare with structural biology - Structural mechanism of FusB-mediated antibiotic resistance

Name:
Maria Selmer

Affiliation:
Uppsala Universitet

Abstract:
Single-particle cryo-EM can be used to visualize macromolecular complexes at high resolution, and data can be sorted to obtain structures of complexes of different composition or in different conformational states. Still, the study of functionally important states that are rare or short-lived remains challenging. We have developed a simple mix-on-grid methodology for time-resolved cryo-EM to be able to catch such states using standard equipment for grid vitrification.

The bacterial ribosome is a target of approximately half of all clinically used antibiotics. One of those is fusidic acid (FA), that acts by locking elongation factor G (EF-G) to the ribosome.

Here, we aimed to clarify the structural mechanism of how the clinically prevalent resistance protein FusB mediates rescue of FA-locked ribosomes. Through time-resolved cryo-EM, we have obtained multiple structures of the different stages of rescue at two different time points after adding FusB. The structure of the short-lived rescue complex shows how FusB wedges its N-terminal domain between two domains of EF-G, breaking its contacts with the ribosome, and promoting EF-G release, revealing the molecular mechanism of FA resistance.

Poster No: 23

Title:
Nanoparticle diffusion through native and artificial porcine colonic mucus model studied by diffusional fingerprinting - A machine learning framework

Name:
Marco Tjakra

Affiliation:
Department of Pharmacy, Uppsala University

Abstract:
The mucus layer in the gastrointestinal (GI) tract is a critical barrier for nanoparticle delivery, especially in the colonic region where the mucus is thicker than other GI tract regions. This study investigated the diffusion behavior of fluorescently labeled polystyrene nanoparticles, varying in size (100-1000 nm) and charge, through native and two artificial mucus models, with the objective to assess how well the artificial models mimic the native environment. The artificial mucus models were made with the addition of gelling polymers, polyacrylic acid (PAA) and hydroxyethyl cellulose (HEC), due to the lack of gelling properties of purified mucin. By recording nanoparticle movement and employing particle tracking (PT) to analyze their motion, we investigated the complexities of nanoparticle diffusion through the porous and highly viscous mucus structure. Given the inherent complexity of nanoparticle diffusion through this hydrogel, a method known as diffusional fingerprinting was employed to process, analyze, and classify the SPT data. Through diffusional fingerprinting, 20 descriptive features were extracted from the trajectories of the nanoparticles, capturing their diffusional behavior in detail. These features were analyzed using logistic regression to classify the identity of the diffusing particles and the type of mucus model. Highest accuracy (86%) was achieved for negatively charged 1000 nm nanoparticles, with linear discriminant analysis further revealing the key diffusional features that differentiated the mucus models. Notably, the artificial mucus model composed with HEC closely mirrored the native mucus features, highlighting the potential of diffusional fingerprinting for studying drug transport in the mucus lining of importance to drug delivery and intestinal absorption.

Poster No: 24

Title:
Multimodal imaging of oxidized cholesterol in multiple sclerosis lesions of human brain tissue

Name:
Gabor Toth

Affiliation:
Uppsala University

Abstract:
In multiple sclerosis, the myelin sheath of nerve cells is deteriorated, resulting in lesion formation throughout the central nervous system. To understand the mechanism of disease progression, deciphering the spatial distribution of neuroinflammatory lipid mediators and myelin lipids around lesions is of utmost importance. Here, we show multimodal imaging using immunohistology and ion images from silver-doped pneumatically assisted nanospray desorption electrospray ionization mass spectrometry imaging to characterize cryosectioned tissue sections from 5 multiple sclerosis and 3 control subjects. Our results suggest that multiple sclerosis pathology is linked with several lipid oxidation pathways. In particular, the spatial distribution of cholesterol and its oxidized metabolites was uniform in control samples, while significantly altered in multiple sclerosis tissues. All lesions displayed cholesterol depletion and oxysterol accumulation. Specifically, oxysterols originating from 27-hydroxycholesterol showed significant correlation with myelin degradation. In conclusion, our results indicate disrupted cholesterol metabolism and elevated oxidation levels in multiple sclerosis.

Poster No: 25

Title:
Mitosis in Motion: Live Imaging Reveals Non-Transcriptional Roles of POU/OCT Factors in Mitosis

Name:
Vasilios Tsarouhas

Affiliation:
Stockholm University, Molecular Biosciences, The Wenner-Gren Institute, (MBW)

Abstract:
Mitosis is a highly dynamic and precisely coordinated process in which chromosomes are captured by the mitotic spindle and physically segregated to ensure faithful genetic transmission. Live-cell fluorescence microscopy enables real-time analysis of these dynamics under various conditions. Our research explores the transcription-independent roles of POU/OCT transcription factors during mitosis. The POU family regulates genes involved in cell proliferation and lineage specification, yet their roles in mitosis remain largely unexplored. We developed an efficient imaging method to study mitosis in live Drosophila embryos during the syncytial stage, when transcription is absent. This approach revealed that Nub/Pdm1 is essential for mitotic progression. Specifically, Nub/Pdm1 plays a key role in maintaining spindle integrity and regulating the speed of spindle elongation during the rapid syncytial nuclear divisions, in a transcriptionally independent manner. Furthermore, real-time imaging provided direct evidence that Nub/Pdm1 and human Oct1 cooperate with Kinesin-5 to regulate chromosome separation velocity. Moving forward, we aim to expand our methodology for large-scale transcription factor analysis and implement automated systems to quantify mitotic division velocities.

Poster No: 26

Title:
Functional characterization of human 3D intestinal organoids as models for drug absorption

Name:
Foteini Tzioufa

Affiliation:
Uppsala University

Abstract:
Oral drug delivery is favored for its ease, patient compliance, and cost-effectiveness, posing critical questions in drug development regarding drug behavior post-oral administration. Traditional 2D cell lines like Caco-2 and HT-29 are commonly used for in vitro drug absorption assessment in pre-clinical drug development, but often lack physiological relevance, especially in transporter expression. Conversely, 3D intestinal organoids (IOs) offer a cutting-edge model for predictive pharmacokinetic research, closely mimicking intestinal tissue structure and function in vivo. They can be everted to generate apical-out IOs based on study needs. As such, the aim of this project was to evaluate human IOs derived from jejunal tissue as models for predicting drug absorption, by assessing their barrier integrity and the activity of influx and efflux transporters. Healthy jejunal crypt-derived IOs were initially grown in Matrigel:tm: in standard basal-out polarity (BO). IOs were differentiated in suspension to apical-out (AO) polarity (Matrigel:tm:-free) or BO polarity for 6 days. Functional studies were performed to evaluate IO barrier properties as wells as influx and efflux potential. The human intestinal epithelium is expected to maintain an intact physical barrier. AO and BO IOs were incubated in suspension with hydrophilic marker lucifer yellow (LY) and dye accumulation was monitored over time using time-resolved fluorescence microscopy. Results showed that the marker did not diffuse paracellularly in either AO or BO IOs, confirming barrier integrity. Influx was assessed by measuring the activity of fatty acid transport protein 4 (FATP4) using the fluorescent substrate C1-BODIPY-C12. In AO IOs, the fatty acid fluorescent analogue C1-BODIPY-C12 was taken up through apically located FATP4 transporters and accumulated in lipid droplets, demonstrating FATP4 functionality. Efflux was studied using rhodamine 123 (Rho123), a substrate of P-glycoprotein (P-gp), which is an intestinal membrane efflux pump. In BO IOs, Rho123 accumulated in the lumen, indicating functional apical P-glycoprotein (P-gp) transporters. In summary, IOs were successfully developed and grown in two configurations: AO with an exposed lumen, and BO with a contained lumen, in suspension culture. Our results show that both AO and BO IOs form an intact epithelial barrier and exhibit active influx (FATP4) and efflux (P-gp) transporters, making them promising models for studying drug absorption.

Poster No: 27

Title:
AI-Driven Live-Cell Imaging for Large-Scale Data Generation in Generative Modeling

Name:
Hanzhao Zhang

Affiliation:
KTH

Abstract:
Modeling human cells in silico remains a grand challenge due to their complexity and dynamic nature. Achieving this goal requires data-driven approaches, particularly advanced generative AI models, which are inherently data-hungry. A key obstacle is acquiring large-scale, high-quality imaging data, ideally from living cells. Here we present a high-throughput imaging approach for generative modeling that integrates live-cell fluorescence microscopy with AI-based analysis to map protein localization and cellular processes. Using the U2OS FUCCI cell line, which enables visualization of cell cycle progression, we will introduce an additional layer of protein staining through a DNA-encoded intrabody library. Complementary markers for cellular compartments like endoplasmic reticulum (ER) or mitochondria will further enhance spatial resolution, optimizing the use of available fluorescence channels. This approach is designed for adaptability, allowing the study of various biological processes, including cellular senescence. Our automated laboratory workflow ensures large-scale data acquisition, while AI-driven analysis will be trained to correlate protein localization with specific cellular compartments and processes.

Poster No: 28

Title:
Multi-omic profiling of healthy and diseased brains with high-plex single-cell spatial molecular imaging

Name:
Rudy van Eijsden

Affiliation:
Bruker Spatial Biology

Abstract:
Single cell transcriptomics and proteomics can provide complementary information about the form and function of neurons and glia throughout the brain. However, most high-plex spatial analyses to date have primarily utilized one of these two modalities to interrogate cell activity and cell-to-cell communication. Here, we simultaneously leveraged the detection of 68 proteins and over 6,000 RNA targets on the same FFPE human brain sections to perform extended segmentation of neural processes and integrated analyses of protein and RNA expression. The protein targets are well-suited for dissecting neurodegenerative disease pathology (e.g. amyloid beta variants). Moreover, they cover major neural cell types and enable robust cell typing, alongside 4,900 neuroscience-related genes.

Poster No: 29

Title:
The Cellpainting Assay as a tool for studying p53-mutations in colorectal cancer

Name:
Amelie Susanna Wenz

Affiliation:
Uppsala University

Abstract:
Colorectal cancer is one of the most common cancer types in Western countries, with increasing incidence numbers. A considerable subset of colorectal cancer patients exhibit p53 mutations, which are connected to a poorer disease outcomes. This study aims to establish the cell painting assay as a potential tool for differentiating between different p53 statuses of the human colon cancer cell line HCT116. The morphological profiles of HCT116 wildtype cells (WT) and HCT 116 p53 knockout cells (KO) were analysed and compared both in absence of any treatment as well as after exposure to relevant compounds. The results showed that even in an untreated state, HCT116 WT and KO cells were morphologically distinguishable. Furthermore, the two cell lines responded differently to different treatments.These findings suggest that the cell painting assay could be a valuable tool for studying the p53 status of colorectal cancer cells.

Poster No: 30

Title:
In situ detection and subcellular localization of 5,000 genes using Xenium Analyzer in cancer tissue samples

Name:
Patrick van Houts

Affiliation:
10x Genomics

Abstract:
Spatial transcriptomics enables the exploration of cell types, interactions, and states within intact tissues by mapping gene expression while preserving morphological context. The Xenium Analyzer offers a comprehensive solution with automated multiplexed decoding and analysis, detecting up to 5,000 genes simultaneously in FFPE or Frozen tissue. This allows for detailed in situ mRNA gene expression analysis across various tissues, including healthy and diseased states. A 5,000-plex gene panel facilitates pan-tissue cell typing, analysis of cell signalling pathways, and identification of cancer-associated genes. Fully automated enhanced cell segmentation techniques improve cell boundary definition and cell type identification accuracy. The Xenium platform's flexibility accommodates smaller plex panels for targeted studies and larger panels for discovery research, advancing our understanding of cancer biology. Extra customization to add genes of interest is possible.

Poster No: 31

Title:
Image-Based Morphological Profiling for Antiviral Drug Discovery

Name:
Jonne Rietdijk

Affiliation:
Uppsala University

Abstract:
The continuous evolution and emergence of new human pathogenic viruses underscores the need for new approaches to study viral infections and to identify effective antiviral therapeutics. Small-molecule drugs can significantly reduce morbidity and mortality caused by viral infections by targeting various stages of the viral life cycle. However, many traditional antiviral screening approaches focus primarily on viral replication, often overlooking the impact on the host-cell. Here, we present an image-based screening approach focusing on the host cell to identify antiviral compounds. We modified the Cell Painting assay, a widely used method for morphological cell profiling, by substituting the mitochondrial stain with a virus-specific antibody and developed a high-throughput image analysis pipeline using Cellpose and CellProfiler. This pipeline segments individual cells and extracts thousands of features at the single-cell level, generating rich morphological profiles. We show that infected cells form a distinct morphological cluster, reflecting virus-induced changes in host-cell morphology. We leverage this signature to identify compounds that reverse the phenotype toward a healthy state. Using PLS-DA, a supervised classification method, we measure the distance between compound-treated cells and the uninfected cluster to identify potential antiviral candidates. We demonstrate this approach through a drug screening experiment of over 5,000 repurposed drugs tested on SARS-CoV-2-infected cells. This data-driven approach provides an unbiased view of host-cell morphology, yielding insights into viral replication, host-cell health, and the mechanisms of antiviral compounds. This adaptable and scalable platform can be easily modified for different viruses and host cell lines, offering a powerful host-centric strategy for antiviral drug discovery.