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The effects of acute and chronic inflammation on aortic function in Sprague-Dawley rats

Adalayne Ramsamy, University of the Witwatersrand

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Inflammation is a driving factor of arterial remodelling, a major contributor to cardiovascular disease. Despite this, the mechanisms involved in inflammation-induced arterial remodelling are unclear. This study investigated the molecular pathways involved in aortic vessel remodelling in rat models of acute and chronic inflammation. The expression of molecular markers of inflammation and vascular function was determined in aortic vascular cells. Stiffness parameters of the localised vessel wall were determined by transabdominal echo-tracking ultrasonography. Acute bouts of inflammation caused a significant increase in the expression of markers of inflammation and endothelial activation. Rats terminated one week after a single LPS administration showed active remodelling and a decrease in vessel compliance. Chronic inflammation caused increases in molecular markers of inflammation and stiffness parameters. These results suggest that arterial remodelling is initiated in the early stages of inflammation and could ultimately result in substantial, long-term functional changes of the aortic vessel.

KEYWORDS
Inflammation, vascular remodelling, aortic stiffness

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BioImage Informatics, SciLifeLab, Sweden

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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 unit of the National Bioinformatics Infrastructure Sweden NBIS, with funding from SciLifeLab, National Microscopy Infrastructure NMI (VR-RFI 2019-00217), and the Chan-Zuckerberg Initiative. 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.

KEYWORDS
Bioimage analysis, Facility, spatial transcriptomics, microscopy

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A topographic lung cell atlas reveals regional variation in cell-type specific gene programs and identifies healthy and diseased cellular neighborhoods

Alexandra Firsova, Stockholm University

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Single cell mRNA sequencing data from millions of cells revealed a high diversity of cell types in the human lung. In this complex air-exposed organ, it is crucial to understand the influence of tissue topography on gene expression in each cell type. Here, we applied three spatial transcriptomics approaches, to: (i) localize the majority of lung cell types, including rare epithelial cells, (ii) describe consistent anatomical and regional variability in gene expression within and across cell types, and (iii) reveal distinct cellular neighborhoods. We thus provide a spatially resolving tissue reference atlas including cell type composition and gene expression variations in three representative regions of the healthy human lung. We further demonstrate its utility by defining previously unknown imbalances of cell type compositions and cellular neighborhoods in tissues from GOLD stage II COPD patients. Our topographic atlas enables description of regional cellular responses upon experimental perturbations or during disease progression.

KEYWORDS
Human lung cell atlas, SCRINSHOT, HybISS, spatial transcriptomics

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Strained Cyclooctynes with Photocages for Subcellular 3D Photolithographic Barcoding

Alfred Larsson, Uppsala University

Larsson Alfred, Uppsala University, Sweden. Odell Luke, Uppsala University, Sweden. Fürth Daniel, Uppsala University, Sweden.

We evaluate various synthetic routes for strained cyclooctynes functionalized with cyclopropenone photocages. These photocaged cyclooctynes are designed for high-resolution (~700 nm) subcellular 3D labeling in both fixed and living cells through photochemical decarbonylation triggered by multiphoton irradiation. We compared three approaches: two for strain-promoted azide-alkyne cycloaddition (SPAAC) and one for inverse electron-demand Diels-Alder (iEDDA) reactions.

For SPAAC, photo-ODIBO (Oxocin-Dihydro-Dibenzo) was optimized to eliminate hazardous precursors, while photo-TAMA (Tetramethoxy Azocin Monocarboxylic Acid) demonstrated significant promise due to its high yields and the use of commercially available, inexpensive methoxy-substituted aromatic compounds as precursors. Notably, photo-TAMA provides regioselective SPAAC reactions and maintains high single-nucleotide fidelity. In contrast, photo-DMBO for iEDDA, though promising, suffers from stability issues and a lengthy, low-yield synthesis. Given these challenges, photocaged tetrazines may offer a better alternative for iEDDA reactions.

Overall, photo-TAMA emerges as the preferred functional moiety for oligonucleotide barcoding applications moving forward.

KEYWORDS
click chemistry, organic synthesis, photochemistry

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Molecular Pixelation: Spatial proteomics of single-cells by next generation sequencing

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Authors: Alvaro Martinez Barrio, Johan Dahlberg, Max Karlsson, Ludvig Larsson, Pouria Tajvar, Vincent van Hoef, Stefan Petkov; Affiliation: Pixelgen Technologies, Sweden

Molecular Pixelation (MPX) is a novel single cell spatial proteomics panel able to simultaneously quantify protein abundance, the spatial distribution, and colocalization of targeted cell surface proteins on up to 1,000 individual cells.

MPX creates 3D spatial maps of cells by imprinting spatial information on antibody oligonucleotide conjugates using a DNA reagent we call DNA-pixels. These DNA-pixels form over 1,000 connected spatial zones per single cell producing graphs that reconstruct the cell surface in silico; forming a single cell surface map of 80 proteins. By applying spatial statistics on these cell surface graph representations, we uncover both known and novel patterns of protein spatial polarization and co-localization associated with vital immune processes such as intercellular communication and mobility.

Pixelator together with our data processing pipeline, nf-core/pixelator, eases the downstream data analysis that can happen in both Python and R. Our tools are all open source and free to use. Herein, we explain several of the computational methods that deliver the different hallmarks of MPX: abundance, polarity, colocalization and graph layout.

With high throughput and multiplex, MPX is a powerful tool for investigating the intricate spatial organization of proteins in single cells, paving the way for new discoveries in immune system research. Pixelator facilitates the data processing and analysis of MPX data.

KEYWORDS
MPX, nf-core pipeline, single-cell proteomics, graph layout, spatial statistics

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A Comprehensive Approach for Sequential MALDI-MSI Analysis of Lipids, N-Glycans, and Peptides in Rodent and Alzheimer's Brain Tissues

Yea Rin Olivia Lee, Uppsala University

Yea-Rin Lee, Ibrahim Kaya, Elin Wik, Sooraj Baijnath, Henrik Lodén, Anna Nilsson, Dag Sehlin, Stina Syvänen, Per E. Andrén

Multimodal molecular imaging of single tissue sections using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) provides comprehensive molecular insights. However, optimizing tissue sample preparation for MALDI-MSI to achieve high sensitivity and reproducibility for biomolecules such as lipids, N-glycans, and tryptic peptides within the same tissue section presents a significant challenge. This study introduces a reproducible protocol for the comprehensive sequential analysis of these molecules using MALDI-MSI in fresh-frozen rodent brain tissue samples. The protocol's effectiveness was validated across various rodent brain tissues and applied to Alzheimer’s disease (AD) tgArcSwe mouse models as a proof of concept. Significant molecular alterations were observed in sphingolipids and biantennary core-fucosylated N-glycans in the cerebral cortex, linked to amyloid-beta plaque accumulation. These findings may shed light on mechanisms underlying cognitive and memory impairments in AD. This standardized methodology offers valuable insights into neurodegenerative diseases and holds promise for developing biomarkers and targeted therapies for AD.

KEYWORDS
MALDI-MSI, Multi-omics, Method development, Alzheimer’s disease, Fresh-frozen brain tissue

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Spatial Transcriptomics @ NGI

Anja Mezger, KTH Royal Institute of Technology

KEYWORDS
Spatial Transcriptomics, Visium service

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NBIS - SciLifeLab Bioinformatics Platform

Anna Johansson, Uppsala University

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NBIS (National Bioinformatics Infrastructure Sweden) provides bioinformatics and data science support to the Swedish life science research community. 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.

KEYWORDS

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Spatial metabolomics of Parkinson's Disease Dementia and Dementia with Lewy Bodies

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Parkinson's Disease (PD), with and without dementia, and Lewy Body Dementia (LBD) are common progressive neurodegenerative disorders. Distinguishing between these diseases and other atypical parkinsonian syndromes, is challenging because diagnosis relies solely on clinical symptoms and signs. Currently, no specific chemical diagnostic tests are available for these conditions.
Here, we performed MALDI mass spectrometry imaging analyses focusing on neurotransmitters and lipids in dual polarity on human post-mortem tissue (parietal cortex) from controls, PD patients without and with dementia, and LBD patients (n=16/group). Neurotransmitter analysis utilized the derivatizing matrix FMP-10 to target metabolites containing primary amines or hydroxy phenols, while norharmane was employed for dual polarity lipid analysis. Multivariate classification, specifically partial least-squares discriminant analysis (PLS-DA), was used to identify significant metabolic differences in the targeted neurotransmitter analysis between the different PD patient groups and control subjects. Metabolites such as serotonin, 3-O-methyldopa, and putrescine were found to be significantly regulated.

KEYWORDS
Spatial Mass Spectrometry, Parkinsson's Disease, metabolomics

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Enabling high-content phenotyping in pooled CRISPR screens by in situ guide RNA readout

Bernhard Schmierer, Karolinska Institutet

Alexander Lindberg, Miriam Selle, Chika Yokota, Marco Grillo, Mats Nilsson, Bernhard Schmierer

Classical pooled CRISPR screens are limited to cell phenotypes that can be physically separated from each other, such as live-dead screens, or sortable cell phenotypes. We are developing a method using in situ guide RNA sequencing directly on a microscope slide, allowing complex phenotyping of cells followed by determination of the gene perturbation they harbour. Briefly, cells containing a pooled CRISPR library (one guide copy per cell) are grown on a slide, imaged, phenotyped, and their position on the slide is recorded. In a second step, the identity of the single CRISPR guide RNA present in each individual cell is determined directly on the microscope slide by barcoded padlock ligation and cycles of hybridization, imaging and stripping. Thus, both phenotype and genetic perturbation of the individual cells in a population can be assessed microscopically. The method combines the advantages of pooled CRISPR screening (scalability and low cost) with those of arrayed screens (complex phenotypic readouts), and allows CRISPR screens in 3D (organoids or tissue sections).

KEYWORDS
CRISPR screening, in situ sequencing, functional genomics

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Investigating the impact of inter-organelle interactions on axonal transport in motor neuron models of neurodegeneration

Chloe Williams, Umeå University

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Disruption of axonal transport is a common feature across neurodegenerative diseases and a key early pathogenic event in amyotrophic lateral sclerosis (ALS). Subcellular organelles undergo highly dynamic and organised interactions to maintain cellular functions. The role of organelle interactions within motor neurons axons is not fully understood.

In this work we have developed a custom analysis pipeline that integrates established tracking programs, interactive machine learning software and novel MATLAB scripts to define 'contact events' between axonal organelles. We have analysed the changes in shape, speed and directionality of organelles prior to, during and subsequent to contact. We have shown that upon contact with endosomes, mitochondria become elongated and continue to grow in length following contact. We demonstrate that interaction with mitochondria induces lysosomes and endosomes to mirror the mitochondrial trafficking behaviour. We are currently exploring how axonal organelle interactions are changed in neurodegenerative disease using ALS-patient derived iPSC motor neurons.

KEYWORDS
Machine learning, live microscopy, axon biology, neurodegeneration.

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Metabolomics Platform

Christine Wegler, Umeå University

Metabolomics Platform

NA

KEYWORDS
Metabolomics Platform

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

Christophe Avenel, Uppsala University

Christophe Avenel, Nicolas Pielawski, Axel Andersson, Andrea Behanova, Eduard Chelebian, Anna Klemm, Fredrik Nysjö, Leslie Solorzano, Carolina Wählby, Department of Information Technology and SciLifeLab BioImage Informatics Facility, Uppsala University, Uppsala, Sweden.

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.

KEYWORDS
Spatial Omics, Spatial Transcriptomics, Web based, Quality control

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SciLifeLab Proteomics Platform

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The Proteomics platform provides technologies and expertise in mass spectrometry (MS), and affinity proteomics for advanced protein analyses in body fluids, cells and tissue biopsies. We support clinical and basic research projects. Our services include (i) phenotyping of humoral and cellular immune response; (ii) in-depth protein profiling with semi and absolute quantification; (iii) protein structural characterization and subtyping to support design of new vaccines and drug targets and (iv) analysis of self-sampled specimens to facilitate biomarkers monitoring and populations health surveillance studies

KEYWORDS
proteomics mass-spectrometry affinity microsampling

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Quantifying the Spatial Coherence of DNA Barcode Networks

David Fernandez Bonet, KTH Royal Institute of Technology

David Fernandez Bonet, Johanna I. Blumenthal, Shuai Lang, Simon K Dahlberg, and Ian T. Hoffecker (all KTH Gene Technology, Sweden)

Sequencing-based microscopy is a novel, optics-free method for imaging molecules in biological samples using DNA barcodes, spatial networks, and sequencing technologies. Despite its promise, the principles governing how these networks preserve spatial information are not fully understood. Current validation methods, which rely on comparing reconstructed positions to expected results, would benefit from a deeper understanding of these principles. Here, we introduce the concept of spatial coherence— a set of fundamental properties of spatial networks that quantifies the alignment between topological relationships and Euclidean geometry. Our findings show that spatial coherence is an effective method for evaluating a network's capacity to maintain spatial fidelity and identify distortions, independent of prior information and purely computational. This framework provides a cost-effective validation tool for sequencing-based microscopy by leveraging the fundamental properties of spatial networks in nanoscale systems.

KEYWORDS
spatial biology, dna networks, graph theory, sequencing-based microscopy

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FunCoup 6: advancing functional networks across species with directed links and improved user experience

Davide Buzzao, Stockholm University

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FunCoup 6 (https://funcoup.org/) represents a significant advancement in global functional association networks, offering researchers a comprehensive view of the functional coupling interactome. This update introduces novel methodologies and integrated tools for improved network inference and analysis. Key developments include vastly expanded gene regulatory link coverage, a new framework for bin-free Bayesian training, and a redesigned website. Around half a million directed links between 838 transcription factors and 14,989 target genes were added in the human network, enhancing understanding of transcriptional regulation. FunCoup 6 also integrates a new tool for disease and drug target module identification using the TOPAS algorithm and incorporates KEGG pathway enrichment analysis with ANUBIX, BinoX, and EASE. Bin-free training was applied to 22 primary species, networks for 618 additional species were generated via a new ortholog-based method. FunCoup 6 demonstrates superior benchmark performance, representing a pivotal step forward in functional genomics research.

KEYWORDS

Systems Biology; Functional Association Network; Gene Regulatory Network, Bayesian integration; Comparative Interactomics

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Spatial Proteomics Unit

Eleanor O'Roberts, KTH Royal Institute of Technology

O'Roberts Eleanor, Oses Carolina, Bäckström Anna, Panshikar Pranauti, Ullman Tony, Thorsson Johan, Stadler Charlotte, KTH, Sweden

Spatially resolved omics technologies have emerged in recent years and have undoubtedly changed the way we understand the spatial organization of complex multicellular biological systems. The aim of the Spatial Proteomics unit is to do full-service multiplexed immunofluorescence projects covering discovery, translational and diagnostic research questions.
In the unit we offer two main technologies: the Phenocycler-Fusion from Akoya Biosciences uses conjugated antibodies detected in cycles by addition of fluorescent reporters; and the COMET from Lunaphore uses off-the-shelf antibodies in sequential rounds of immunofluorescence. Both these methodologies allow us to run targeted spatial proteomics analysis of up to 40 markers at single cell level in tissue sections.
Furthermore, the unit also works on method development and implementation of new services such as isPLA and multiomics analysis.

KEYWORDS
spatial, proteomics, immunofluorescence, immunology

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Optimizing sample substrates for single-cell metabolomics

Eszter Szombati, Uppsala University

Eszter Szombati (Uppsala University BMC - Department of chemistry), Ingela Lanekoff (Uppsala University BMC - Department of chemistry)

Cells are inherently heterogeneous, making it crucial to study them individually, especially since metabolic changes can signal disease progression. Single-cell analysis techniques, such as microfluidics and microfabrication, are often combined with single-cell mass spectrometry. Here, we present a novel approach to sample single INS-1 cells using tapered PA nano-DESI on six microarray substrates: silanized glass slides, wet-etched glass microarrays with three microwell dimensions, and 3D-printed microarrays with two microwell dimensions. Cells were sorted using the CellenOne robot and sampled by PA nano-DESI with fused silica capillaries, detecting analytes at 240k resolution.
Substrates were compared based on ease of use, sampling speed, and lipid extraction profiles. Wet-etched glass microarrays showed the highest sampling efficiency and more intense lipid detection, while 3D-printed arrays showed false positives due to background ions. Glass microarrays were cost-effective, robust, and reusable, making them the optimal choice for single-cell metabolomics analysis.

KEYWORDS
metabolomics, single-cell, microarray

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Clinical Genomics platform

Eva Berglund, Uppsala University

Eva Berglund, Marcela Davila, Markus Heidenblad, Linda Köhn, Malgorzata Lysiak, Malin Melin, Bianca Stenmark, Valtteri Wirta

The aim of the Clinical Genomics platform is to support translational and clinical research, and to develop tomorrow's diagnostics within healthcare.

KEYWORDS

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A Tailored Data Handling Workflow for Metabolite Profiling of Single Cells

Felix Friedrich, Uppsala University

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Cell-to-cell heterogeneity in single-cell metabolomics reveals distinct subpopulations and cellular responses. High-resolution mass spectrometry allows for the analysis of numerous single cells, but an effective data-handling workflow is essential for complex direct infusion data. Herein, a tailored method for profiling hundreds of single cells arranged spatially in an array is presented. Using an Orbitrap:tm: IQ-X:tm: Tribrid:tm: and various extraction conditions, data from 100 IMR-90 cells are acquired with a tapered pneumatically assisted nanospray desorption electrospray ionization probe (PA nano-DESI). The MATLAB-based pipeline manages data processing from cell detection, feature extraction, quantitation, and background removal. Preliminary results from 97 cells quantified 86 species and annotated 644 features, revealing diverse metabolite distributions and subpopulations. This workflow effectively handles spatially distributed single-cell data, enhancing cellular profiling and understanding metabolic variations.

KEYWORDS
Single-Cell Metabolomics, Spatially Distributed Single-Cells, Mass Spectrometry, Data Processing Pipeline, Cellular Heterogeneity

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A spatio-temporal atlas of human spermatogenesis based on single-cell transcriptomics and multiplex antibody imaging

Feria Hikmet, Uppsala University

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We constructed a high-resolution spatial map of the adult human testis by integrating single-cell transcriptomics with multiplex immunohistochemistry (mIHC) and image analysis. Single-cell transcriptomic analysis identified 12 germ cell states, including subsets of spermatogonia, spermatocytes, and spermatids. Utilizing a large-scale mIHC pipeline, we mapped approximately 500 proteins across different germ cell states, and this enabled us to cluster proteins into expression groups based on state-specific patterns, forming a foundation for functional analysis. We identified 18 cell-type clusters, linking proteins to germ cell-specific functions related to sperm differentiation and cell cycle regulation during spermatogenesis. Many poorly defined proteins previously not associated with sperm development were uncovered. By examining mRNA and protein correlation dynamics, we highlighted the complex spatio-temporal landscape of the testis. The data are available as open-access in the HPA (www.proteinatlas.org), with the potential to contribute to the understanding of male reproductive biology and associated disorders.

KEYWORDS
multiplex imaging, spermatogonial stem cells, male germ cell differentiation

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a high-resolution, large-scale method to study the subcellular location of proteins in ciliated cells

Filippa Bertilsson, Uppsala University

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Motile cilia keep us alive through a wide range of functions, such as removing pathogens from the lungs, and pushing the oocyte from ovary to uterus for fertilization, thus understanding their molecular constituents is at utmost importance. To address this, we developed a multiplex immunofluorescence panel of five marker proteins targeting the axoneme, transition zone, basal body, cytoplasm and nucleus of the ciliated cells, respectively. This allows us to spatially visualize the subcellular location of proteins of interest, gaining more insight into protein expression in cilia. To obtain quantitative data, an automated image analysis method was developed for high-throughput analysis of the multiplex stainings, estimating a spatial location for proteins within the frame of the panel using pixel values to determine their expression across five human tissues.

KEYWORDS
Motile cilia, subcellular location, automated image analysis

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

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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.

KEYWORDS
mass spectrometry imaging, neurodegenerative diseases, lipidomics

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The Cellular Molecular Imaging platform

Hans Blom, KTH Royal Institute of Technology

CMI platform staff

The CMI platform makes visualization of biological systems, from the molecular to the tissue level possible. Cryo-EM enables the reconstruction of near-atomic structures of molecular machines, super-resolution microscopy makes it possible to produce images with fluorescence at the cellular nanoscale, and with clearing and lightsheet microscopy it is possible to reconstruct small organisms or even entire organs. FIB-SEM (focused ion beam-scanning electron microscopy) and CAT (correlative array tomography), bridging electron and light microscopy, allow visualization of sub-cellular structures in EM volumes, which can be correlate with light microscopy to study network of interactions within and between cells.

KEYWORDS
Imaging across scales

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Hydrazide-based Reactive Matrices for the Sensitive Detection of Aldehydes and Ketones by MALDI MSI

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A one-step, on-tissue selective chemical derivatization method for improved detectability of aldehydes and ketones by MALDI Mass Spectrometry Imaging (MSI) is presented.

The performance of in-house developed reactive matrices, containing a UV-chromophore, ionizable moiety and hydrazide group, was evaluated by MALDI FTICR MSI using three model compounds (i.e., budesonide, fluticasone propionate and progesterone). Improved or equal signal intensities of the model compounds were obtained versus the more established Girard's reagent P (Gir P), especially for progesterone, even without DHB on top of the reactive matrices.

Further confirmation of the ionization efficiency of the reactive matrices was obtained from MSI of endogenous compounds (e.g., 17-hydroxypregnanolone, pregnenolone and pregnanolone) in adrenal tissues. In the brain, e.g., sugars (pentose and hexose), DOPAL (3,4-dihydroxyphenylacetaldehyde) and fatty aldehydes (decanals) were imaged, without the need of an additional co-matrix, which is a requirement for achieving sufficient ionization with Gir P.

KEYWORDS
MALDI-MSI; Derivatization; Aldehydes; Ketones

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Integrative proteo-transcriptomic characterization of advanced fibrosis in chronic liver disease across etiologies

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Various causes of chronic hepatic injury and inflammation can lead to fibrosis and cirrhosis, potentially predisposing individuals to hepatocellular carcinoma. Despite extensive research, the molecular mechanisms underlying liver fibrosis and its associated progression to cancer remain incompletely understood. In this study, we employed an integrated proteo-transcriptomics approach to characterize the molecular pathophysiology of liver fibrosis in both liver and plasma samples from 330 individuals. This cohort included 40 healthy subjects and 290 patients with histologically characterized fibrosis due to chronic viral infection, alcohol consumption, or metabolic-dysfunction associated steatotic liver disease. We demonstrated that pathways related to extracellular matrix alterations, immune response, inflammation, and metabolism are dysregulated in advanced hepatic fibrosis, regardless of the underlying cause. Additionally, our analysis of peritumoral hepatic tissues revealed transcription signatures linked to cell proliferation, survival, and inflammation in hepatocellular carcinoma. Furthermore, we observed extensive remodeling of the plasma proteome linked with severe fibrosis and identified 132 circulating proteomic signatures associated with advanced fibrosis by integrative analysis of plasma proteomics with hepatic transcriptomics. We finally developed predictive models using machine learning to facilitate the non-invasive detection of advanced fibrosis and cirrhosis.

KEYWORDS
Chronic liver disease; Liver fibrosis; Multi-omics; Systems biology; Non-invasive

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Mass spectrometry imaging reveals region-specific alterations of brain lipids induced by parkinsonism and L-DOPA-induced dyskinesia

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We employed matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) to comprehensively map different molecular species (spatial omics), such as neurotransmitters, neuropeptides, and lipids in specific brain regions. Using brain samples from an experimental Parkinson's disease (PD) model (MPTP, Macaca mulatta) with L-DOPA-induced dyskinesia (LID), we previously observed abnormal elevations of L-DOPA and its metabolite, 3-O-methyldopa, in the whole brain of LID animals. This resulted in increased dopamine and downstream metabolites in all brain regions, except putamen and caudate. Furthermore, we found that the abundance of selected neuropeptides was associated with L-DOPA concentrations in the putamen, emphasizing their sensitivity to L-DOPA.

In the present study, we conducted extensive imaging of various lipid species, enabling the detection of specific distributions of hydroxylated and non-hydroxylated sulfatide lipids within the same individual brains. Hydroxylated sulfatides were abundant within several basal ganglia brain regions, whereas long-chain hydroxylated sulfatides were depleted in motor-related regions, with non-hydroxylated showing an elevation. When comparing individuals with LID to those without dyskinesia, we observed a decrease in plasmalogen phosphatidylcholines and an increase in polyunsaturated fatty acid-containing glycerophospholipids specifically in the internal segment of globus pallidus. These changes were significantly correlated with the LID scores, L-DOPA and dopamine levels within the specific brain regions of the animals. This MALDI-MSI study provides valuable insights into signaling system dynamics during PD and its treatment.

KEYWORDS
MALDI-FTICR-MSI, Lipidomics, Parkinson's disease, L-DOPA-induced dyskinesia

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Mass Spectrometry Imaging Provides Insights Into The Fate Of Carnitine And Acylcarnitines In Ischemic Mouse Brain

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Leonidas Mavroudakis, Ingela Lanekoff - Dept. of Chemistry-BMC, Uppsala University, Sweden

The function of carnitine is to transport long-chain fatty acids in the mitochondria for subsequent β-oxidation. Therefore, carnitine and acylcarnitines are important molecules involved in the energy metabolism. Here, we have used nanospray desorption electrospray ionization (nano-DESI) mass spectrometry imaging (MSI) to study the distribution of carnitine and acylcarnitines in mouse model for ischemic stroke. Quantitation of acylcarnitines was achieved by using internal standards doped in the solvent. Results show that acylcarnitine was depleted and that medium and long-chain acylcarnitines were accumulated in the ischemic area of the brain. Collectively, we show that nano-DESI MSI of thin brain tissue sections from ischemic model provides unique insights into the energy metabolism of brain during ischemia that promotes further understanding on the biochemical processes of stroke.

KEYWORDS
mass spectrometry imaging, stroke, acylcarnitines

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Untangling the Complexity of Lewy Body Disorders Using Spatial Proteomics

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Parkinson´s disease (PD), characterized by an accumulation of alpha-synuclein aggregates (Lewy Bodies), and loss of dopaminergic neurons in the substantia nigra, is the second most common neurodegenerative disease. Around half of PD patients develop dementia, defined as PD Dementia (PDD). PDD shares many clinical and pathological features with Lewy Body dementia (LBD), making proper diagnosis challenging. Yet, the cellular and molecular factors that govern these diseases (PD, PDD, and LBD) are poorly understood.
By applying high-parametric spatial protein mapping on post-mortem human brain in a cohort of 68 patients (15 PD, 5 PDD, 23 LBD and 25 controls), our study aims to 1) shed light on the cellular features involved in PD, PDD and LBD, and 2) discover potential clinical biomarkers.
Further, by integrating our spatial protein maps with spatial transcriptomics, metabolomics and lipidomics data, we aim to unravel the pathophysiological mechanisms, and better understand and differentiate these Parkinsonian conditions.

KEYWORDS
neurodegeneration, Lewy Body disorders, spatial proteomics

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Training Hub Mission and Vision

Jill Jaworski, Stockholm university

The SciLifeLab Training Hub is dedicated to fostering a diverse and inclusive community of instructors and learners, where we can all gain new skills and benefit from the minds of many. The Training Hub was established at SciLifeLab in 2023 to support, consolidate, and enable access to the Research Infrastructure's knowledge, skills and expertise. We are building a training portal where the life science community in Sweden can find opportunities to gain relevant skills, and experts can find support in creating training that captures their knowledge.

KEYWORDS
Training, Education, Skills, Knowledge Transfer

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InfraLife

Josefin Lundgren Gawell, SciLifeLab

Claire Lyons, Josefin Lundgren Gawell

The InfraLife project aim to increasing knowledge and facilitating access to large-scale research infrastructures in Sweden, stimulating cross-sectorial collaborations and advancing science.

KEYWORDS
SciLifeLab, ESS, MAX IV, synchrotron, imaging

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Integrated RNA and protein profiling in the same tissue section: deciphering tumor microenvironment dynamics via automated spatial multiomics analysis

Justyna Zaborowska, Lunaphore

Alix Faillétaz1, Alice Comberlato1, Arec Manoukian1, Paula Juričć1, Pino Bordignon1,Justyna Zaborowska1, Anushka Dikshit2, Rose Delvillar2, Steve Zhou2, Li-Chong Wang2, Saska Brajkovic1 1Lunaphore Technologies, Tolochenaz, Switzerland, 2Advanced Cell Diagnostics, Inc., Newark, California, USA

Spatial biology techniques have revolutionized our understanding of the tumor microenvironment (TME) and its intricate cellular interactions. Multiplex immunofluorescence (mIF) methods enable precise profiling of immune cells and other key players within the TME, revealing their spatial distribution and interactions (PMID: 38012408). In situ hybridization (ISH) technologies complement protein profiling by mapping cytokine- and chemokine-expressing cells, crucial for deciphering signaling networks and immune activation.

In this context, a new multiomics method that integrates RNAscope:tm: and sequential immunofluorescence (seqIF:tm:) protocols is presented, allowing co-detection of RNAs and proteins within the TME by using COMET:tm:, an automated tissue imaging and staining platform. The integrated protocol allows up to 12 RNA and 24 protein biomarker detection cycles, generating a 12-plex RNA and 24-plex protein panel without human intervention.

We validated the assay sensitivity and specificity and developed a panel of 12 probes to target essential RNA biomarkers of tumor-infiltrating lymphocytes and their activation states in human FFPE tumor tissues. Simultaneously, we employed a 24-antibody panel to detect protein biomarkers to enable single-cell identification within the TME. Applying this approach to human FFPE tumor tissues, we demonstrated that co-detection of RNA and protein biomarkers in the same section enhances our understanding of key cellular components involved in tumor progression and immune response. This integrated approach promises deeper insights into cancer biology.

Our findings underscore the promise of deeper insights into tissue biology with spatial multiomics technologies. By fully automating these technologies on platforms like COMET:tm:, we improve the throughput of spatial multiomics assay, reduce user interventions, and increase the robustness of assays. This progress has important implications for creating predictive markers, refining cancer diagnoses, and tailoring personalized therapies.

KEYWORDS

1.Spatial multiomics 2.Tumor microenvironment 3.Multiplex immunofluorescence 4.Spatial biology technique 5.Immuno-oncology

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Spatial Transcriptomics As A Complete Service At The Scilifelab National Genomics Infrastructure – From Tissue To Gene Counts

Jörg Bachmann, Stockholm University

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The emerging field of spatially resolved transcriptomics has already given insight into cellular identity, location and function in health and disease. NGI, the SciLifeLabs National Genomics Infrastructure processes thousands of samples every month with a large degree of automation and quality control in both the lab and bioinformatics analysis. To offer 10x Genomics Visium Spatial Transcriptomics as a service, we have optimized sectioning of a wide range of tissue types (such as mouse, human or insects), implemented automation in the Visium Fresh Frozen workflow and incorporation of the CytAssist machine, allowing us to accept already sectioned samples on slide (fresh frozen or FFPE) and offering a complete service from tissue samples / sections to basic analysis of spatial transcriptomic data. Most recently we can also offer protein analysis together with gene expression on Visium CytAssist, as well as Visium HD, with spatial resolution bellow 8 µm.

KEYWORDS
Spatial Transcriptomics, Core Unit, Service

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Implementation of spatial transcriptomics to study the murine enteric nervous system

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The gastrointestinal system performs a diverse range of functions which allow the ingestion and digestion of food products and expulsion of waste material. Such functions rely on defined spatial organisation in multiple organs and the coordinated activity of diverse cell types, including the enteric nervous system (ENS) which innervates the gastrointestinal tract. Deficiencies in the ENS contribute to many gastrointestinal disorders which currently lack satisfactory treatments. Cell-based replacement therapies are putative strategies for these conditions, however their success will require a greater understanding of ENS composition and patterning to ensure appropriate regeneration of affected neuronal circuitry. Although spatial transcriptomics has been previously applied to the murine colon, no published studies to date have utilised spatial technology to characterise the ENS across multiple regions of the gastrointestinal tract at single cell resolution. Here, we present our recent efforts to profile the embryonic and juvenile murine ENS using MERSCOPE.

KEYWORDS
MERSCOPE, neuroscience, enteric nervous system, regeneration, development

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Single Cell Map Of The Human Ovarian Cortex: Unravelling Molecular Dynamics From Birth To Reproductive Maturity And Across Treatment Exposures

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The human ovary is vital for both endocrine and reproductive systems, but its structural changes throughout life remain unclear, limiting advancements in fertility treatments. This study constructs a spatiotemporal map of the ovarian cortex, focusing on chemotherapy's impact. Using transcriptomic and proteomic technologies, we analyzed ovarian tissue from 17 patients: 12 pediatric patients undergoing fertility preservation and five adults (gender reassignment surgery or caesarean section). Single-cell analysis of over 52,000 cells revealed over ten distinct cell types, including novel populations like theca and Schwann cells. High-parameter multiplex immunohistochemistry identified five key cell types and provided insights into ovarian spatial organization across different age and therapy groups. Our findings enhance understanding of ovarian biology, with potential applications in fertility preservation and personalized medicine.

KEYWORDS
Multiplex immunohistochemistry, Ovary, Chemotherapy

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Novel quantitative mass spectrometry imaging strategy using the method of standard addition

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Davidová, Lucie, Uppsala University, Sweden; Lanekoff, Ingela, Uppsala University, Sweden

Pneumatically assisted nanospray desorption electrospray ionization (PA nano-DESI) MSI allows direct quantification of endogenous analytes by spiking internal standards into the extraction solvent. To account for matrix effects, isotopically labelled internal standards (IS) are commonly used but can be costly or unavailable. We propose an alternative quantification approach using the method of standard addition (SA). SA was evaluated by spiking amino acid standards into PA nano-DESI solvents and analyzing mouse brain cortex. Recorded SA curves showed good linearity, with concentrations closely matching those obtained using IS. Alternating line scans with solvents containing no, low, or high SA standards enabled the generation of quantitative ion images, yielding comparable results for SA and IS quantification across different regions of interest. Additionally, endogenous molecules from rat brain tissue were successfully used for untargeted metabolite quantification. This study provides proof-of-concept for SA in MSI, offering a cost-effective and accessible alternative to IS quantification.

KEYWORDS
quantitative MSI, nano-DESI, matrix effects, method development

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Computational inference reveals cancer lineages and identifies early markers of tumor invasion

Marcel Tarbier, Karolinska Institutet

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Assigning single cell transcriptomes to cellular lineage trees by lineage tracing has transformed our understanding of differentiation during development, regeneration, and disease. However, lineage tracing is technically demanding and most scRNA-seq datasets are devoid of lineage information. Here we introduce Gene Expression Memory-based Lineage Inference (GEMLI), a computational tool allowing to robustly determine cell lineages solely from scRNA-seq datasets. GEMLI allows to study heritable gene expression, to discriminate symmetric and asymmetric cell fate decisions and to reconstruct individual multicellular structures from pooled scRNA-seq datasets. In human breast cancer biopsies, GEMLI allows to zoom in on phenotype switches and reveals previously unknown gene expression changes at the onset of cancer invasiveness. Now, we combine GEMLI with spatially resolved in situ sequencing data to examine it's utility and the spatial aspect of phenotype switches.

KEYWORDS
bioinformatics, cell lineage tracing, single cell, in situ sequencing, cancer phenotypes

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Mapping the effect of fludarabine in leukemia cells using single-cell spatial proteomics

Maria Globisch, Uppsala University

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Recent advancements in high-throughput ex vivo drug profiling have spurred interest in functional precision medicine, aiming to personalize cancer treatment based on an individual's cellular characteristics. This proof-of-concept study utilizes Molecular Pixelation (MPX), a single-cell spatial proteomics platform, for quantifying protein abundance, spatial distribution, and co-localization of targeted proteins in an ex vivo drug response model system. Using glucocorticoid-resistant acute lymphoblastic leukemia (ALL) cells, we investigated fludarabine, a drug used prior to chimeric antigen receptor T-cell therapy. Fludarabine treatment induced changes in both protein abundance (n=25) and polarity (n=25). Additionally, MPX identified 138 protein pairs that changed in co-localization after treatment. Notably, MPX revealed increased abundance, polarization, and co-localization of CD82 and CD53, suggesting a potential therapeutic scaffold. This study offers new insights into the spatial organization of the cell-surface proteome in leukemia cells during therapy, enhancing our understanding of fludarabine treatment in ALL at the single-cell level.

KEYWORDS
Leukemia, single-cell, spatial-proteomics, fludarabine, MPX

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The Swedish National Genomics Infrastructure (NGI)

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The National Genomics Infrastructure (NGI), hosted by Science for Life Laboratory (SciLifeLab), is a pioneering genomics resource in Sweden (https://ngisweden.scilifelab.se). With over 25 years of experience, NGI offers high-throughput genomics services to both academic and industrial users. NGI offers a unique combination of consultative support, biospecimen processing, and expert data handling via state-of-the art data processing, quality control, and delivery. NGI provides comprehensive services for a wide-range of -omics capabilities crucial for the development and implementation of precision medicine. NGI's services span SNP genotyping, short- and long-read sequencing, single-cell analysis, spatial transcriptomics, and high-throughput proteomics assays. In addition to providing access to cutting-edge services for genomics, a major part of NGI's operation is devoted to evaluating and introducing new methods, instruments, and protocols in collaboration with researchers and companies, or through internal development. NGI also provides comprehensive scientific support to its users and education and outreach in the form of seminars, workshops, and webinars for the Swedish research community, and participates in higher education courses. NGI is a partner in many national and international collaborative projects, and is a key part of the biomolecular research and precision medicine ecosystem in Sweden.

KEYWORDS
SciLifeLab Genomics Platform NGI

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Spatially mapping lipid mediator heterogeneity in pulmonary tissue using targeted mass spectrometry imaging

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Asthma is characterised by chronic inflammation in the lung that is mediated by immune and structural cells. Lipid mediators (e.g., eicosanoids) are oxygenated metabolites of fatty acids that exert a pivotal role in asthma pathogenesis. Traditionally, chromatography-based mass spectrometry has been used to investigate these compounds, while spatially mapping their distributions using mass spectrometry imaging (MSI) has been limited due to low endogenous concentrations.
We employed advances in desorption electrospray ionisation (DESI) combined with targeted multiple-reaction-monitoring (MRM) to successfully image lipid mediators in the airways. These efforts required development of custom software to analyse this new datatype. We demonstrate the application of this workflow in guinea pig lung following house dust mite (HDM) exposure. DESI MRM-based MSI can map the intratissue heterogeneity of lipid mediators, providing a step change in our understanding of asthma at the molecule level and offering opportunities to image low abundant metabolites in multiple tissue types.

KEYWORDS
Mass spectrometry imaging; Desorption electrospray ionization (DESI); Lipid mediators; Eicosanoids; Asthma

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Sensitivity validation to DSB signatures in XRCC4 and DNA-PKcs deficient colorectal cancer cells

Mehran Hariri, Uppsala University

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DSB is the most critical types of DNA damage. If DSB is not repaired precisely it can lead to genome instability, mutation, cell death, and cancer. NHEJ is the significant DSB repair pathway in mammalian cells. DNA-PKcs and XRCC4 are two critical components of NHEJ pathway. However, DSB generation and repair after XRCC4 and DNA-PKcs deficiency is not fully understood. In this study we investigated the effect of different DNA damage inducers (X-rays, phleomycin, etoposide, temozolomide, and calicheamicin) on the DSB induction and DSB repair of XRCC4 and DNA-PKcs knockout cell lines of human colorectal cancer cells (HCT116). To pursue this, we measured prompt DSB induction repair, non-DSB heat-labile sites generation, DSB chromosomal biomarker and clonogenicity using PFGE, 53BP1 immunohistochemical staining, and clonogenic assay, respectively.

KEYWORDS
DNA damaging agents, NHEJ, DSB repair, Clonogenicity, XRCC4, DNA-PKcs

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Integration of Spatial Transcriptomics and Function in Pancreatic Disease Progression

Mercedes Aline Joy Petenera Dalman, University of Gothenburg

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The spatial organization of the pancreatic microenvironment is key to health maintenance, with tissue disruptions driving pathologies like Type 1 and Type 2 diabetes and obesity. These conditions involve significant cellular changes, such as β-cell loss, immune infiltration, and altered cell interactions within the islets of Langerhans. Spatial transcriptomics offers a powerful tool to study these alterations while preserving spatial context.
In this project, we apply Slide-seq to generate high-resolution spatial transcriptomic profiles of pancreatic tissue from healthy, diabetic, and obese donors. Integrating additional data types, including immunohistochemistry and calcium imaging, allow us to explore transcriptional and functional profiles. This comprehensive dataset enables for a deeper understanding on critical cell-cell interactions and regulatory networks disrupted in disease.
Leveraging advanced analytical tools, we aim to uncover key molecular pathways involved in β-cell dysfunction and tissue remodeling, offering new insights into disease mechanisms and potential therapeutic strategies for pancreatic diseases.

KEYWORDS
Spatial transcriptomics, diabetes, obesity, immunohistochemistry, calcium imaging

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Host-pathogen interactions in the Plasmodium-infected mouse liver at spatial and single-cell resolution

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Upon infecting its vertebrate host, the malaria parasite initially invades the liver, where it undergoes massive replication while remaining clinically silent. The coordination of host responses within the liver during malaria infection remains unexplored. Here, we perform spatial transcriptomics and single-nuclei RNA sequencing over multiple time points to delineate host-pathogen interactions across Plasmodium berghei-infected liver tissues. Our data reveals significant changes in spatial gene expression, particularly related to lipid metabolism near sites of Plasmodium infection, as well as distinct inflammation programs between lobular zones, and regions with enrichment of different inflammatory cells, which we term ‘inflammatory hotspots’. This study provides insights into transcriptomic changes during host-parasite interactions, offering a valuable tool for developing new interventions targeting malaria liver stage infection.

KEYWORDS
Malaria, Plasmodium, Infection, transcriptomics

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The transcription factor LHX2 mediates and enhances BMP signaling in Medulloblastoma

Mohamad Ali, Uppsala University

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Bone morphogenesis proteins (BMPs) signaling cascade contributes to normal cerebellar development and has been linked to the progression of medulloblastoma disease, which is the most common brain malignancy in young children. Clinically, medulloblastoma tumors are classified into four molecular subtypes: sonic hedgehog (SHH) group, WNT group, Group 3 and Group 4 medulloblastoma. Our research investigates specifically the role of BMP signaling in the Group 4 subtype. We performed multiplexed immunofluorescence and spatial whole transcriptome in situ analysis of medulloblastoma tissues which confirmed the higher activity of BMP signaling and identified the LHX2 transcription factor as a mediator of BMP oncogenic activity. The modulation of LHX2 expression significantly altered the cancer stem cell frequency in vitro and affected the tumor initiation capacity and metastasis in vivo. Collectively, our research demonstrates the oncogenic activity of BMP signaling in medulloblastoma and indicates the role of LHX2 in maintaining cancer stem cell populations.

KEYWORDS
Medulloblastoma, Spatial transcriptomics, BMP

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

Patrick Van Houts, 10x Genomics

Patrick van Houts, Morgane Rouault, Hiroshi Sasaki, Jordan Sicherman, Vijay Kumar, Francesca Meshi, Patrick Marks

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.

KEYWORDS
Spatial Transcriptomics, 5000 plex gene expression, FFPE tissue.

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Met-ID: An Open-Source Software for Comprehensive Annotation of Multiple On-Tissue Chemical Modifications in MALDI-MSI

Patrik Bjärterot, Uppsala University

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Met-ID enables the annotation of m/z features from MALDI-MSI experiments, utilizing either derivatizing or traditional matrices. It employs structural information from target molecules to generate a narrowed list of identification candidates by matching m/z values within a specified range. Met-ID enables users to define custom derivatizing matrix settings and to support multiple derivatization sites of the same molecule within the software. It facilitates mass matching for MS1 data, with the database settings being limited to only the ions potentially derivatized by a specific matrix. Furthermore, Met-ID includes a database featuring MS2 spectra of numerous chemical standards, all accessible directly through the software. The MS2 spectral database supports user-uploaded spectra from any derivatization type and facilitates the comparison of these spectra with user-provided tissue MS2 spectra for similarity assessment. Developed in alignment with the FAIR Guiding Principles for scientific software, Met-ID is freely available as an open-source tool on GitHub.

KEYWORDS
Software, MALDI-MSI, Derivatization, Metabolomics, FAIR

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In Situ Sequencing Facility. Targeted Spatial Transcriptomics

Rapolas Spalinskas, Stockholm University

Tiklova Katarina, Yokota Chika, Spalinskas Rapolas (Stockholm University, Sweden, for all authors)

The advancements in single-cell spatial imaging analysis offered by In Situ Sequencing Facility empower scientists to examine their samples at sub-cellular resolution, profiling thousands of targets while maintaining the spatial context of each. This analytical approach enables researchers to not only pinpoint and classify cells within their biological environments but also explore cell-cell communication, characterize cellular microenvironments, and detect rare cell infiltration.

KEYWORDS
Service Provider

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Quantitative Mass Spectrometry Imaging of neurotransmitters using standard addition methods

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Reza Shariatgorji, Michael Niehues, Tina Angerer, Anna Nilsson, Per Svenningsson, Per E. Andren

Mass spectrometry imaging (MSI) is a powerful label-free imaging technique for molecular mapping in biological tissue sections. Despite its potential for spatial quantitative analysis, MSI faces challenges from heterogeneous structures and varying matrix effects. To address these issues, we propose standard addition protocols to quantitate neurotransmitters. A robotic sprayer was used to apply standard solutions. For all methods, consecutive rat brain tissue sections were placed on a conductive glass slide. In the standard addition method (Method A), a labelled reference compound was sprayed over all tissues as an internal standard. Subsequently, standard solutions containing the analyte(s) at different concentrations were applied to the tissue sections, followed by spraying the matrix. Method B was similar to Method A, except that a second labelled compound at different concentrations was used as a calibration standard. Several neurotransmitters were quantified using these methods and compared with data acquired by LC-EC.

KEYWORDS
Mass spectrometry imaging, Quantitation, Neurotransmitters

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The neurophysiological effects of CBD on the regional expression of inflammatory markers and oxidative properties in a healthy rodent model

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Cannabidiol (CBD), a pharmacologically active constituent of the Cannabis sp., has been widely suggested to have anti-inflammatory and antioxidative properties. However, these effects have not yet been studied in the spatial context of the brain. This study investigated the acute and chronic region-specific anti-inflammatory and antioxidant effects of CBD in the brain using mass spectrometry imaging and inflammasome expression analysis. AP-MALDI analysis revealed the region-specific increase in antioxidant amino acids levels following CBD administration. Acute CBD treatment reduced IL-1β expression, often a marker of neurodegeneration in all brain regions, except the midbrain. Interestingly, chronic CBD administration significantly decreased IL-6 expression in most brain regions, but not in the cortex. Additionally, acute CBD administration led to differential expression in NLRP3, IL-18 and IL-6 expression in the brain. Our findings indicate that CBD may exert region-specific anti-inflammatory antioxidant effects in the brain, which may be beneficial in managing neurodegenerative disorders

KEYWORDS
Neurosciences, inflammation, cannabidiol

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Nuclear export-based RNA velocity models to explore cellular dynamics in situ

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Sergio Marco Salas, Ömer Çağatay Talikacı, Mats Nilsson, Fabian Theis

RNA velocity is a computational method that predicts future transcriptional states by analyzing the ratio of unspliced to spliced mRNA, offering insights into cellular dynamics. Although successful in single-cell transcriptomics, RNA velocity lacks spatial context due to the dissociation-based nature of scRNA-seq data. Spatial transcriptomics (SRT) technologies have emerged to capture transcriptomic profiles with spatial resolution, yet the application of RNA velocity in situ remains largely unexplored. To overcome this, in this study we explore the applicability of RNA velocity algorithms to characterize the nuclear export dynamics using spatial transcriptomics data. We further investigate the impact of the most common spatial artifacts in the performance of nuclear-export-based RNA velocity.

KEYWORDS
RNA velocity, image-based spatial transcriptomics, cellular dynamics

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Hidden network preserved in Slide-tags data allows reference-free spatial reconstruction

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We reanalyzed data from the Slide-tags method developed by Russell et al. and discovered a hidden, spatially informative network formed during the transfer of spatial tags to nuclei. The structure of this network conveys sufficient information to infer cell locations entirely without ground truth from spatial indexing, placing Slide-tags among a new generation of optics-free, network-based imaging-by-sequencing approaches, a fundamental departure from classical spatial sequencing technologies based on pre-indexed arrays.

KEYWORDS
Spatial transcriptomics, sequencing-based microscopy, DNA microscopy, molecular networks, DNA barcodes

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Mass spectrometry imaging identifies AAS-specific changes in monoamine neurotransmitter signaling and metabolism, together with alterations in the brain lipidome of the male rat brain

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The use of anabolic androgenic steroids (AAS) to enhance physical strength, performance, and appearance is associated with physical and psychological adverse effects. Long-term AAS-use is demonstrated to cause behavioral and cognitive impairments. However, the underlying mechanism to AAS-induced psychological problems is not fully elucidated. The present study applied ultra-high mass resolution Fourier-transform ion cyclotron resonance (FTICR) matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) to map the monoaminergic neurotransmitter metabolic pathways and perform a comprehensive lipidomics analysis in the male rat brain following treatment with the decanoate-esters of testosterone, nandrolone, and trenbolone. Results demonstrated AAS-specific effects on the levels of monoamine neurotransmitters, their precursors, and metabolites. Additionally, AAS altered the brain lipidome, particularly affecting phosphatidylethanolamines and ether-linked phosphatidyletanolamines across the brain. Lipid alterations corresponded with increased levels of dopamine and serotonin, which implies AAS to dysregulate the brain lipidome and subsequently impact neurotransmitter transmission, potentially leading to behavioral changes.

KEYWORDS
Anabolic androgenic steroids, mass spectrometry imaging, neurotransmitters, lipids, brain

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Molecular Pixelation: Spatial proteomics of single cells by next generation sequencing

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The spatial distribution of cell surface proteins governs vital processes of the immune system such as inter-cell communication and mobility. However, previous technologies have limited scalability in the multiplexing and throughput needed to drive spatial proteomics discoveries at subcellular level.

Molecular Pixelation (MPX) is an optics-free DNA-sequence based method for spatial proteomics of single cells, able to simultaneously quantify protein abundance, the spatial distribution, and co-localization of targeted cell surface proteins of thousands of individual cells in parallel. MPX creates three-dimensional spatial maps of cells by imprinting spatial information on antibody oligonucleotide conjugates bound to protein on the cell surface, using DNA-based nanometer sized molecular pixels. These DNA-pixels form over 1,000 connected spatial zones per single cell, producing graphs that reconstruct the cell surface in-silico; forming a single cell surface map of membrane proteins, without the involvement of any light source or specific instrumentation.

In our recent study, we show how MPX can be used to monitor constellations of proteins on the cell surface after the effects of treatment or stimulation. By applying spatial statistics on these cell surface graph representations, we uncover both known and novel patterns of protein spatial polarization and co-localization associated with vital immune processes such as intercellular communication, antibody dependent cellular cytotoxicity and mobility.

KEYWORDS
Molecular Pixelation, DNA microscopy, Spatial proteomics, Single cell, DNA microscopy, NGS

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Development of an Ultra High-Plex Antibody Panel for Spatial Phenotyping of Murine Cancer Models

Stuart Logan, Akoya Biosciences Inc.

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Single cell spatial phenotyping of protein biomarkers is an established tool for cancer researchers aiming to understand the biology of the tumor microenvironment (TME). The technology has been developed around human tissues, but Mouse models are also widely used for immuno-oncology (IO) research. In collaboration with Cell Signaling Technology Inc. (CST), Akoya Biosciences Inc. (AKYA) developed a 32-plex murine IO antibody panel that covers immune cell lineages, immune
checkpoints and cell state markers, as well as proliferation and structural markers. The antibody panel that was developed as part of this study can be readily deployed for discovery and translational research and will engender future comparative mouse and human spatial phenotyping studies that further our understanding of cancer biology.

KEYWORDS
spatial phneotyping, IO, mouse models, high-plex proteomics

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Origin, structure, and composition of the spider major ampullate silk fiber revealed by genomics, proteomics, and single-cell and spatial transcriptomics

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Spider silk, a sustainable and high-performance biomaterial, offers a promising alternative to synthetic fibers. Despite its potential, the underlying biological processes of silk production remain poorly understood. This study investigates the major ampullate silk gland, responsible for producing the strongest type of spider silk. Utilizing single-cell RNA sequencing and spatial transcriptomics, we identified six distinct cell types within the gland, each expressing specific sets of silk proteins. These cell types are organized into three glandular zones, suggesting a compartmentalized silk production process. Image analysis of histological sections showed that the secretions from the three zones do not mix, and proteomic analysis revealed that these secretions form layers in the final fiber. Our multi-omics approach provides valuable insights into the structure and function of the major ampullate silk gland, as well as the architecture and composition of the silk fiber it produces.

KEYWORDS
spatial transcriptomics, bulk RNAseq, single cell RNAseq, histology, sample preparation

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The Live Cell Imaging core facility at KI Flemingsberg campus

Sylvie Le Guyader, Karolinska Institutet

Sylvie Le Guyader

The Live Cell Imaging core facility on the Flemingsberg campus at Karolinska Institute provides access to perfectly maintained, fully automated light microscopes to all researchers in the region. Our offer includes widefield, light sheet, point confocals, spinning disk confocals and super resolution SIM microscopy, as well as powerful remote servers for image analysis.
Additionally, at the LCI core facility, you can enjoy high quality training, in depth consultancy and Imaging-by-staff services.

KEYWORDS
Microscopy core facility

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Spatial profiling of RNA of insulin-producing beta cells on human biopsies and pancreatic islets

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Pancreatic islets are clusters of endocrine cells scattered through the pancreas, accounting for 1-2% of the pancreas volume. Isolated human pancreatic islets are extensively used to investigate the physiological function of endocrine cells, unravel phenotypic changes occurring in diabetic conditions, and most importantly, treat diabetic patients through transplantation procedures. Human islets are isolated from brain-dead organ donors through a process in which the islets are detached from their vasculature and exposed to both cold- and warm ischemia. The impact of this process on islet biology has been however largely ignored. In this project, we aim to uncover the role of cold ischemia on gene expression and to compare the transcriptome of endocrine cells from peri-operative biopsies collected from living patients with minimal exposure to cold-ischemia to pancreatic biopsies and isolated islets. To this end, we used GeoMX DSP to perform the RNA profiling of the insulin-producing beta cells. Preliminary GeoMX data support the view that there are a large number of differences in beta cell gene expression when comparing peri-operative biopsies with biopsies of brain-dead organ donors or isolated islets.

KEYWORDS
pancreatic biopsies, peri-operative biopsies, isolated human islets, pancreatic beta cells, RNA profiling

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Activation of the NLRP3 inflammasome drives dysregulation of the heart-brain axis in chronic, systemic inflammation

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The heart-brain axis, particularly the neuroinflammatory link between mood disorders and cardiac dysfunction, remains poorly understood in chronic systemic inflammatory conditions. The collagen-induced arthritis (CIA) rat model was used to investigate the molecular mechanisms of this relationship. Mass spectrometry imaging was used to determine brain region-specific expression of neurotransmitters, revealing dysregulation of key markers implicated in mood disturbances in specific brain regions. Additionally, structural and functional cardiac changes were observed, this was coupled with NLRP3 inflammasome activation and subsequent cardiac cell death. These findings suggest a mechanism by which chronic inflammation from autoimmune disorders may drive the localised activation of the NLRP3 inflammasome driving both cardiac dysfunction and dysfunctional neurotransmission. The use of spatial mass spectrometry highlights its potential in elucidating the complex interplay within the heart-brain axis, offering insights into how systemic inflammation influences both brain and heart health.

KEYWORDS
Heart-brain axis, spatial mass spectrometry, cardiac dysfunction, neurotransmitter dysregulation

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Unraveling the molecular mechanisms of spider glue production: Insights from single-cell RNA sequencing and spatial transcriptomics

Tobias Fietze, Karolinska Institutet

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Orb-weaving spiders produce up to seven distinct types of silk, one of which is aggregate silk - a sticky glue rather than a fiber. This glue aids in prey capture and holds potential for industrial applications such as adhesives. However, the molecular mechanisms behind its formation remain unclear. Using single-cell RNA sequencing and spatial transcriptomics, we identified several distinct cell types in the aggregate glands of Larinoides sclopetarius. A subset of these cell clusters showed high expression of silk proteins called aggregate spidroins (AgSp). Comparing single-cell data of fiber-producing ampullate glands with glue-producing aggregate glands revealed unique gene expression profiles in the gland epithelium. Interestingly, duct cells were found to share similarities with ampullate duct, while the aggregate gland's overall expression profile was unique, highlighting its specialized role in glue production. These findings offer new insights into the biology of aggregate glands and provide important knowledge for developing novel biomaterials.

KEYWORDS
Aggregate glands, spider glue, spatial transcriptomics, single-cell RNA-seq

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Integrating in situ Proximity Ligation Assay for PD1-PDL1 interaction with multiplexed immunofluorescence imaging

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PD1 and its ligand PD-L1 are crucial components of the immune checkpoint machinery, playing a significant role in regulating immune responses in cancer. Their interaction is a major therapeutic target, yet immunotherapies like pembrolizumab show only a modest response (~30%), likely due to limited molecular insights at the time of clinical diagnosis. The recent development of in situ Proximity Ligation Assay (isPLA) represents a significant advancement, allowing for the visualization of direct protein interactions within tissues, which provides important information beyond what traditional multiplexed immunofluorescence (IF) offers. In this study, PD1-PD-L1 interactions in FFPE tonsil and bladder cancer tissues were detected using the NaveniFlex Tissue Atto647N kit, followed by multiplexed IF on the Phenocycler platform with a 14-antibody panel. We successfully established an automated isPLA workflow and integrated it with multiplexed IF, demonstrating its effectiveness in enhancing our understanding of the tumor microenvironment. This method may improve patient stratification for immune checkpoint therapies, with clinical significance to be assessed in a retrospective cohort study.

KEYWORDS
spatial proteomics, multiplex immunofluorescence, bladder cancer, immunotherapy, pembroluzimab

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Parallel data acquisition for multiplexed mass spectrometry imaging enables isobaric and isomeric resolution

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Varun V. Sharma, Gabor Toth, Johan Lillja, and Ingela Lanekoff

A long-standing challenge in mass spectrometry imaging (MSI) is the accurate identification of isobaric and isomeric biomolecules for structure-specific analysis. Herein, we present a unique pipeline that enables image acquisition of high-resolution FTMS in parallel with up to 28 ITMS2. We show that ITMS data acquired parallel to FTMS conserves both experimental time and spatial resolution and that the parallel strategy vastly increases the number of annotated analytes to the isomeric level in one imaging experiment. Furthermore, annotation of unknown endogenous analytes was confirmed by correlating MS1 and MS2 ion images.
Finally, we apply the developed method to map and annotate more than 50 isobaric and isomeric lipid species in the mouse brain in one imaging experiment. Our method holds the potential to revolutionize imaging and annotation of metabolites with isomeric specificity.

KEYWORDS
mass spectrometry imaging, isomers, isobers, parallel data acquisition

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Spatial Transcriptomics in non-model organisms at NGI

Xesus Abalo, KTH Royal Institute of Technology

Xesús Abalo, Jörg Bachmann, Annelie Mollbrink, Jun Wang and Anja Mezger. NGI

Allowing visualisation of the dynamics of transcription directly on top of tissue morphology gives unprecedented insight into developmental and pathological processes. To achieve this, a combination of histology and transcriptome profiling of the same tissue section was first published in 2016 as a method under the name of Spatially Resolved Transcriptomics (SRT) (Ståhl et al., 2016).

The National Genomics Infrastructure (NGI) of Sweden is one of the largest sequencing facilities in Europe. NGI's purpose is to provide state-of-the-art sequencing services to the research community, sequencing samples from a broad variety of organisms and using different library preparations, depending on the starting material.

As part of this effort, 10X Genomics Visium was incorporated into NGI´s catalogue in 2020 to perform SRT which, in combination with the Spatial Proteomics, Spatial Mass Spectrometry and In Situ Sequencing facilities, makes up a platform for a complete spatial analysis of histological samples. The scope is very broad, and NGI has taken projects to analyse the transcriptome of human tumor samples but also of different tissues from mice, zebra finches (Taeniopygia guttata), spiders (Trichonephila clavipes) or sponges (Chondrosia reniformis).

KEYWORDS
Visum, coordination, unbiased

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Detection of protein-protein interactions by bio-orthogonal fluorogenic proximity probes

Andreas Torell, Uppsala University

Torell, Andreas Uppsala University, Larsson, Alfred N., Uppsala University, Phillipson, Storm, Uppsala University, Odell, Luke R., Uppsala University, Fürth, Daniel, Uppsala University

Detecting protein-protein interactions within cells is challenging. Transgenic approaches risk altering protein function via fluorescent tagging, while in situ methods lack in vivo compatibility. Here, we introduce fluorogenic probes with dual-tetrazine pegylated branched arms linked to xanthene dye. Activation requires both tetrazine arms to interact simultaneously with target proteins, enabling dual-substrate recognition. We applied our method to detect protein-protein interactions in both fixed and living cells, utilizing antibody conjugation for fixed cells and genetic code expansion for real-time detection in living cells. Our strategy ensures versatile applicability and seamless transition between fixed and living systems.

KEYWORDS
Click-chemistry, Protein-Protein Interaction, in vivo imaging, Fluorescence

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Chemoselective primer extension for sequencing in fixed and living cells

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Sequencing by enzymatic primer extension, such as sequencing by synthesis (SBS) or sequencing by ligation (SBL), faces limitations in situ due to off-target events on endogenous nucleic acids, especially in fixed genomic DNA. We introduce a bio-orthogonal, non-enzymatic, sequencing chemistry for paired-end primer extension, allowing multiple rounds without primer buildup on fixed nucleic acids. Using templated diene-dienophile and strain-promoted azide-alkyne ligation, a single primer can be extended in both directions with high fidelity, achieving single-nucleotide resolution. This method is as fast as polymerase-based extension but avoids extending endogenous DNA. Additionally, we applied this approach in living cells, successfully tracking single molecules of beta-actin mRNA in embryonic fibroblasts. This chemoselective sequencing tool is versatile across various tissues and organisms, offering a foundation for future direct sequencing applications in fixed and living cells.

KEYWORDS

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Spatial omics analysis reveals immune diversity and landscape

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Recently, cutting-edge technologies enables us to reveal the profiling of transcriptome and proteome in spatial context. Although it is still under development and has some limitations, it is expected to bring novel insight, especially in the field of oncology, neuroscience, and development. These technology enables us to reveal the association of each cell during biological processing. Here, we show some example datasets analyzed by spatial omics technologies. Our results suggested that potential of spatial analysis to reveal the immune diversity and landscape.

KEYWORDS
spatial omics, PBMCs, MPX, proteome, immune diversity