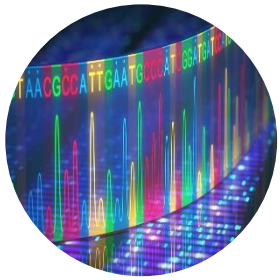
POST EVENT PROCEEDINGS

# Spatial Biology UK 2023

25 - 26 April 2023 | London, UK

Oxford Global were pleased to share with you the 2023 programme for the **2nd Annual Spatial Biology Congress** in London, 25 – 26 April 2023. The spatial biology market is experiencing explosive growth thanks to the latest developments in spatial technologies such as AI, clinical applications and advanced data analysis methods. Challenges though remain pertaining to determining which technologies will advance to the clinic and how we can best integrate various multi omic approaches. This key spatial omics-focused event was full of engaging programme features such as cutting-edge presentations and interactive panel discussions, workshops as well as roundtables.

We are delighted to present you with concise and insightful summaries of presentations delivered by prominent thought leaders in this comprehensive post-event proceedings document.



# CONTENTS

## Future Directions in Spatial Technology Development

### Charles Mein, Centre Manager, Queen Mary University of London

In the presentation titled "Future Directions in Spatial Technology Development" by Charles Mein, he provides an overview of their experiences in the field of spatial genomics. Mein discusses two main technologies: vizeum and Geomax, from 10x Genomics and NanoString, respectively.

He explains that vizeum is based on the 10x genomics platform, which captures RNA from individual cells using beads with specific DNA barcodes. The captured RNA is sequenced, allowing researchers to understand gene expression at the single-cell level. On the other hand, Geomax utilizes UV photo cleavable linkers to release reporter oligonucleotides or antibodies from selected regions of interest on tissue slides. This technology enables the assessment of both the transcriptome and a subset of the proteome.

Mein compares the two technologies, highlighting their differences in spot size and the number of regions that can be analyzed. He presents data from their experiments, demonstrating similarities between vizeum and Geomax at a bulk level, as well as differences in gene detection due to the probe coverage of each platform. He emphasizes that vizeum provides a greater number of positive hits, possibly due to the larger number of individual spots analyzed. Geomax, on the other hand, is better suited for following up on regions of interest where known biology is already established.

Mein concludes that having a portfolio of different platforms allows researchers to choose the appropriate technology based on their specific research goals. The scalability and throughput of both technologies are considered manageable for their university setting. Additionally, the identification of regions of interest is typically driven by the research group, and they collaborate with pathologists to assist in this process.

The presentation provides insights into the use of vizeum and Geomax technologies in spatial genomics research, highlighting their strengths and considerations for their application.

# Day One Track One: Spatial Multi-Omics Techniques & Approaches

## Track Keynote Address: Dissecting Mechanisms Of Metabolic Liver Zonation & Regeneration Using Spatial Tx

Sebastian Bergling, Senior Data Scientist, Novartis Institute for Biomedical Research

Bergling's presented research focuses on the mechanisms of metabolic liver donation and regeneration using spatial transcriptomics. The study involves collaboration between Novartis genomics NGS team and the liver research group at Boston University. The researchers analyze liver tissue samples from mice and humans using spatial transcriptomics, which allows them to study gene expression patterns in specific spatial locations within the liver.

The liver is composed of different regions, each with distinct metabolic functions. Hepatocytes, the main cell type in the liver, have position-dependent tasks along the liver lobule. The researchers observe a gradient of gene expression that is highest near the central vein and decreases towards the portal vein. This gradient helps maintain the functional organization of the liver.

In mice, the researchers observe clear separation between periportal and paracentral hepatocytes based on gene expression patterns. However, in human samples, this separation is not as pronounced. The researchers also investigate the activation of the Wnt signaling pathway and its effect on gene expression. They find that the activation of this pathway leads to changes in gene expression patterns, particularly in the paracentral region.

The study also examines the process of liver regeneration in response to injury. The researchers observe an increase in the number of epcam-positive cells, which are marker cells for the ductal reaction and bile duct cells. They analyze the neighboring cells and investigate potential factors involved in the interaction between different cell types.

Due to the limitations of spatial transcriptomics, which provides mixed cell population data without single-cell resolution, the researchers use deconvolution algorithms to estimate the contribution of different cell types. They also explore other techniques such as fluorescence in situ hybridization (FISH) and NanoString analysis for more detailed investigations.

Overall, the research aims to understand the mechanisms of metabolic zonation, regenerative processes, and cell-cell interactions in the liver. The

findings have implications for drug discovery and may provide insights into pathways important for liver regeneration.

## The Complexities of Spatial Genomics – How My Team Have Overcome Some of These Challenges

### Nik Matthews, Head of the Imperial BRC Genomics Facility, Imperial College London

Matthews discusses various challenges and solutions related to spatial genomics. They start by mentioning the difficulty of obtaining live cells for single-cell genomics, particularly with the 10x system. They describe using a filtering method using a molecule that makes dead cells heavy, allowing them to be separated from live cells using a magnet. This method improves cell viability and quality before fixation. They also mention the use of Turbo DNase for removing DNA contamination in bulk RNA sequencing.

They then move on to discuss challenges with the GeoMx system, particularly issues with tissue adherence and staining. They show examples of overlapping tissue on slides and poor adherence, which can lead to loss of tissue during the staining process. They also mention problems with washing off certain stains and difficulties in visualizing nuclei for accurate cell counting. They express interest in collaborating with histology experts to address these challenges.

Next, Matthews talks about their experiences with the 10x system, highlighting issues such as streaks, blotches, and dark spots in the images. They mention the possibility of these problems being related to the distance between their lab and the microscope setup. They express a desire to collaborate with others to find solutions and mention the importance of having their own microscope for better control over the imaging process.

Lastly, Matthews briefly discusses their experiences with the Stereo-Seq and CosMix systems. They mention challenges with autofluorescence and the need for their own microscope for optimal performance. They express interest in the subcellular resolution capabilities of CosMix and the potential of working with larger tissue slides or multiple smaller tissue samples.

Overall, Matthews acknowledges the complexities and challenges in spatial genomics but remains optimistic about finding solutions through collaboration and technology advancements.

## Automated, Multiplexed Co-Detection Of RNA And Protein

### Julia Jones, Scientific Manager, University of Cambridge

Julia Jones gave a presentation on automated multiplex code detection of RNA and protein. She discussed the work done in the histopathology core facility and presented two case studies.

The histopathology core facility at the Cambridge Institute supports various research groups in areas such as cancer, immunology, and drug development. The facility is divided into routine histopathology, immunohistochemistry (IHC), in situ hybridization (ISH), and scanning/image analysis services.

The IHC service is fully automated and offers single and duplex IHC. Over 750 antibodies have been tested, with 400 validated and included in the routine offering. Jody Miller, who runs the IHC service, has also automated the tunnel assay and offers a validation service for new antibodies.

The ISH service primarily uses the RNA scope assays from ACD. They offer various kits, including singleplex, duplex, multiplex, and base scope assays. The service is mostly automated but also offers manual methods for different sample formats. They have recently started using the DNA scope assay from ACD and offer microRNA ISH using dig-labeled probes.

The facility has multiple scanners for scanning and image analysis, including brightfield and fluorescent scanners. The scanning process allows for spectral unmixing to separate autofluorescence and ensure accurate data analysis. They use various image analysis software, including Indica Labs, HALO, and Aperio.

Julia presented two case studies. The first case involved detecting two RNAs with one protein and another with one RNA and one protein using fluorescent output. They used RNA scope multiplex kits combined with CO detection diluent TSA fluorophores and scanned them on a Zeiss Axio scan. The case study demonstrated successful multiplexing and visualization of the targets.

The second case study was a work in progress and aimed to detect three RNAs in combination with two proteins using a fluorescent assay. They combined multiple antibodies and optimized the concentrations to improve the signal. The case study showed some challenges with background noise, but they planned to address it by adding an extra heat-off p-block to the protocol.

Julia also mentioned future work, including a five-plex assay with base scope, a chromogenic duplex RNA scope with one protein, and a single-plex RNA scope with a switch to cheaper TSA-opal fluorophores.

Overall, the presentation highlighted the facility's capabilities in automated multiplex code detection of RNA and protein and discussed the challenges and ongoing work in this field.

## Spatial Transcriptomics at the Research and Service Interface

### Saba Asam, Research Fellow, University College London

In the presentation by Saba Asam, she introduces herself as an application specialist at UCL Genomics, a core facility based at the Zed CR. She explains that UCL Genomics offers various services and support for researchers, including extraction, quality control, microarray sequencing, and analysis. They aim to generate data and promote good science by collaborating closely with researchers.

Saba mentions the team members at UCL Genomics and their roles, including Mark Christiansen, the head of UCL Genomics, Dr. Rachel Williams, the head of the sequencing team, and other specialists in single-cell and spatial transcriptomics. They work together to provide expertise and support to researchers.

The focus of the presentation is on spatial transcriptomics, specifically using the 10x Genomics Visium platform. Saba describes how spatial transcriptomics allows researchers to analyze gene expression within tissue sections and combines spatial information with gene expression data. UCL Genomics uses the Visium platform and has acquired the necessary equipment, including the Visium slide scanner, for their service pipeline.

She explains the protocol for spatial transcriptomics, starting with sample preparation, sectioning of tissue, and loading onto Visium slides. Saba emphasizes that the responsibility of loading the slides lies with the collaborators, but UCL Genomics offers support and guidance if needed. Once the slides are loaded, they undergo quality control checks using the slide scanner to ensure proper orientation and loading.

The protocol then involves barcoding, cDNA synthesis, and further quality control checks on the generated libraries before sequencing. UCL Genomics provides data output, including alignment, SFTP transfer, FASTQ files, and Space Ranger HTML output. They also offer bioinformatics support for data analysis.

Saba discusses the workflow of the service at UCL Genomics, starting with initial discussions with potential collaborators about experimental design and cost considerations. Once the project details are settled, the necessary funds are allocated, and the slides are handed over to UCL Genomics for processing.

Saba highlights the personalized and customized approach they take in project design and costing.

She briefly mentions that UCL Genomics also offers single-cell transcriptomics services and encourages researchers to reach out for further discussions or cost estimation for their projects.

Finally, Saba mentions a recent application submitted to the MRC for a centralized spatial hub at the Royal Free Hospital. The costs of the services provided by UCL Genomics depend on factors such as the number of samples and the funding source, and she encourages interested parties to contact them for specific cost estimates.

The presentation concludes with a question-and-answer session, where Saba answers a question about the differences between the Visium platform and other genomics platforms like NanoString, highlighting the resolution and coverage differences between the two and suggesting that the choice depends on the specific research goals and markers of interest.

## Suspension And Imaging Mass Cytometry As A Facility Service

### Machalina Mazurczyk, Facility Manager, University of Oxford

Michalina Mazurczyk discussed the topic of Suspension and Imaging Mass Cytometry as a facility service. The presentation focused on the role of facilities in providing support for researchers using new technologies.

Michalina highlighted the need for facilities to bridge the gap between suppliers of new technologies and researchers. Facilities gather information about available technologies and help researchers make informed decisions about which technology to use for their specific needs.

She provided an overview of the services offered by her facility, including conjugation, staining, data acquisition, and assistance with data analysis. The facility aims to provide technical support throughout the entire process, from panel design to sample optimization and data analysis.

Challenges in the process include optimizing protocols for different tissue samples and selecting appropriate software for data analysis. Michalina mentioned various software options such as Saito Bank, FCS Express, FlowJo, and R, each with its own advantages and licensing requirements.

For imaging data analysis, commercial software like Halo and Visiopharm, as well as in-house solutions like Burmilla Lab's framework, are available. Michalina emphasized the importance of choosing the right software to fit the project requirements and user preferences.

She also introduced their in-house pipeline for analyzing mass cytometry imaging data, called Spatial Omics Oxford Pipeline (SPOX). The pipeline includes cell segmentation, dimensional analysis, clustering, and spatial analysis, providing interactive visualizations and spatial statistics.

In conclusion, Michalina acknowledged the people and funding that supported her facility and welcomed any questions from the audience.

Overall, the presentation discussed the role of facility services in supporting researchers using Suspension and Imaging Mass Cytometry technologies, providing technical expertise, and assisting with data analysis.

# Day One Track Two: Applications Of Spatial Research & Technologies In Biology

## Track Keynote Address: Spatial Analysis of Multiplex Immunofluorescence To Predict Disease Outcomes

Martin Fergie, Lecturer in Healthcare Sciences, University of Manchester

In his presentation, Martin Fergie discussed two projects related to multiplex immunofluorescence analysis. The first project focused on follicular lymphoma, a common type of non-Hodgkin lymphoma. The aim of the study was to use multiplex immunofluorescence as a biomarker tool for risk stratification in follicular lymphoma patients. The research team developed a panel of immune cell markers to analyze the tissue samples and quantified the interactions between different cell types using a thresholding methodology. They found that the diversity of interactions within the tumor microenvironment was associated with survival outcomes. Specifically, patients with lower diversity of interactions had worse overall survival and progression-free survival.

The second project involved analyzing spatial effects in Hodgkin lymphoma. The team used different analysis strategies to study cell interactions within tissue sections. They employed techniques such as measuring nearest neighbour distances and distance ratios to assess spatial relationships between cell types. These methods allowed them to identify interactions that favoured engagement or avoidance between cells. The team validated their findings through visual inspection of the tissue samples.

Throughout the presentation, Martin emphasized the importance of careful strategy selection in spatial analysis and the need for collaboration between pathology, computer science, and cancer immunology disciplines. He also mentioned ongoing projects in other tissue types, including melanoma and acute myeloid leukemia, as well as future steps for further validation and assay development.

Overall, Martin's presentation highlighted the potential of spatial analysis in predicting disease outcomes and understanding the tumor microenvironment.

## Multi-Omic Spatial Profiling Reveals the Unique Virus-Driven Immune Landscape Of COVID-19 Placentitis

Kelly Hunter, Honorary in Molecular Histology, University of Birmingham

Kelly Hunter, a Scientific Officer at the CRO ProPath, gave a presentation on the unique immune landscape of COVID-19 placentitis. The study was conducted by a research team headed by Matthew PTMS of the University of Birmingham. The study aimed to understand the immune pathology of COVID-19-positive mothers with pregnancy complications.

The presentation discussed the use of various spatial platforms to analyze the samples. The researchers observed that COVID-19-associated placentitis is characterized by immune infiltration, fibrin deposition, and macrophage activity in the placenta. They found that macrophages were tightly associated with the trophoblast layer, separating the mother and fetal blood supplies. COVID-19 infection was detected in the trophoblast layer, and the virus was found inside trophoblast cells.

The study employed several technologies, including bulk RNA sequencing and spatial platforms like NanoString genomics, multiplex RNA scope in situ hybridization, and the Luna for Coment platform. These platforms allowed the researchers to analyze gene expression, immune cell populations, and immune cell interactions.

The findings revealed that COVID-19-positive placentitis exhibited an immunosuppressive environment characterized by the presence of specific macrophage populations and T cells expressing immunosuppressive markers. The virus also blunted the interferon response, contributing to the breakdown of the placental barrier.

The study highlighted the importance of using high-throughput genomic approaches to investigate the immune pathology of diseases. By combining different platforms, the researchers were able to validate their findings and gain a comprehensive understanding of the disease. The presentation concluded by acknowledging the research team and emphasizing the potential for collaboration and services in the field.

During the question-and-answer session, topics such as data handling between the platforms and the variation in gene expression within morphological features were discussed. The researchers found that both the genomics and spatial platforms provided valuable insights into the degraded samples, and there was generally stable gene expression within similar morphologies.

Overall, the presentation shed light on the unique immune landscape of COVID-19 placentitis and demonstrated the importance of employing multiple platforms to comprehensively study complex diseases.

## From Spatial Single Cell Patterns To Cell Migration Dynamics And Cancer: A Developmental Genetics Perspective In The Age Of Crowd-Sourcing

### Georgy Koentges, Professor, University of Warwick

In his talk, Georgy Koentges discussed his work on imaging and analyzing complex cellular behaviors in cranial facial development. He highlighted the challenges of predicting outcomes based on cellular behaviors and the need to understand how genetic systems work in specific contexts.

Koentges described his early work in spatial transcriptomics, where he captured gene expression profiles from specific locations in the developing brain. He emphasized the importance of dimensionality reduction to prioritize genes and pathways for further analysis. He discussed the advancements in single-cell profiling techniques and the ability to recover lineage time using dimensionality reduction and velocity analysis.

He presented different spatial patterns of cell migration dynamics, including radial growth, limited migration leading to self-organization, and long-distance migration filling voids. Each pattern requires a different approach for analysis and potential intervention. Koentges explained the importance of defining time windows and understanding the behaviour of cells during development.

He introduced the use of recombination-mediated lineage mapping to make the system genetically trackable and genetically accessible. By applying this technique, he was able to study cell behaviour and create maps of cellular movements using multiple colors to define cell populations. He also discussed the use of crowdsource discovery, where a large number of trained individuals tracked and analyzed cells computationally, leading to the identification of behaviours and phases of development.

Koentges highlighted the challenges of analyzing the vast amount of data generated and the need for direct spatial mapping of gene expression profiles. He discussed the potential applications of his research in understanding metastasis and glioblastomas and expressed his interest in collaborating with companies and exploring the use of developmental genetics in tackling these diseases.

During the Q&A session, Koentges addressed questions about inter-individual variation in behaviour, the smallest possible timescale for observing

migrations, and the comparison between tracking mRNA splicing and his method of tracking cell behaviour.

Overall, the presentation focused on the importance of understanding cellular behaviours, spatial patterns, and genetic systems to gain insights into development, disease progression, and potential intervention strategies.

## From Mouse To Man: Spatial Transcriptomics To Understand The Effect Of Plaques On Microglial Gene Expression

### Frances Edwards, Professor of Neurodegeneration, University College London

Frances Edwards' presentation focused on a study that aimed to uncover how plaques, a hallmark of Alzheimer's disease, influence the gene expression of microglial cells, which play a crucial role in the brain's immune response. The research utilized spatial transcriptomics, a technique that allows scientists to analyze gene expression within specific regions of brain tissue, in both mouse models and human brain samples.

The presenter highlighted the intricate nature of Alzheimer's disease, where changes start occurring decades before clinical symptoms become apparent. Plaques, composed of a protein called amyloid beta, develop in the brain, followed by the formation of tau tangles within neurons, and ultimately leading to brain tissue loss and cognitive decline. Mouse models are widely used to study Alzheimer's, but the speaker acknowledged that these models may not fully capture the complex disease progression seen in humans.

The study used mouse models that gradually developed plaques, resembling a more realistic disease timeline in humans compared to previous transgenic models. By examining gene expression changes in microglial cells near, on, and away from plaques, the researchers aimed to understand how microglia respond to these pathological features. The presentation highlighted how these changes are much more subtle in slowly developing plaque models, making them harder to detect through conventional methods like RNA sequencing (RNA-seq).

Spatial transcriptomics allowed the researchers to delve deeper into the intricate changes occurring within microglia. The study revealed significant differences in gene expression profiles when microglia were in close proximity to plaques, suggesting a direct interaction between plaques and microglia. Notably, the expression of genes associated with immune responses, synapse function, and lysosomal activity was altered, shedding light on potential mechanisms underlying the disease.

The presentation also touched on the challenges of working with human brain tissue. The complexities of tissue preservation, degradation, and gene expression analysis were discussed, emphasizing the need for advanced techniques like RNA scope and fluorescence lifetime imaging microscopy to assess gene expression in human samples accurately.

In conclusion, the presentation highlighted the importance of understanding the molecular changes that occur in microglia in response to plaques, as this knowledge could uncover potential therapeutic targets for Alzheimer's disease. The findings have implications not only for improving our understanding of the disease but also for developing interventions to slow its progression.

## Spatial Transcriptomics At The UK DRI: Challenges & Needs Of The Academic Research Community

### Sam Jackson, Tools and Technology Platform Manager, UK Dementia Research Institute

In the presentation delivered by Sam Jackson, the main focus was directed towards shedding light on the challenges that the academic research community encounters when applying spatial transcriptomics to the study of neurodegenerative diseases. The speaker began by emphasizing the historical importance of spatial observation in neuroscience, tracing back to ancient times and highlighting its significance in understanding the intricate functioning of the brain.

Introducing the UK Dementia Research Institute (UK DRI), the presentation highlighted its collaborative nature, spanning various universities and centers across the United Kingdom. The UK DRI's core objective is to advance the understanding of neurodegenerative diseases through research, and the speaker touched on the distributed nature of the institute's labs and centers, each with its unique focus and expertise.

The discussion then shifted towards the establishment of the UK DRI's spatial transcriptomics platform at University College London (UCL). This platform aims to not only provide services but also foster learning and collaboration among researchers in the field. The speaker highlighted the platform's early stages and the technologies it plans to utilize, including genomics, mass spectrometry, and imaging mass spectrometry.

Three key challenges were underscored during the presentation. Firstly, the need to define the value of various techniques in a competitive field was discussed. This involves highlighting specific use cases, niches where techniques excel, and producing example data to showcase their effectiveness. Additionally, the challenge of handling the substantial volumes of data

generated was addressed, along with the importance of data storage, retrieval, and sharing. The presentation further highlighted the imperative of extracting meaningful biological insights from complex datasets, stressing the role of data analysis and visualization in this process.

The concept of building networks and resources to support academics in the realm of spatial biology emerged as a crucial theme. The speaker discussed the idea of creating local communities within institutes and universities, which could facilitate sharing of information and resources among researchers. Moreover, the need for a broader international network focused on standardization, data repositories, and collaboration was emphasized. The presentation concluded by emphasizing the importance of bridging the gap between raw data and meaningful insights, making data analysis accessible to biologists without extensive bioinformatics expertise.

In summary, the presentation highlighted the challenges faced by the academic research community in leveraging spatial transcriptomics for neurodegenerative disease research. It underlined the collaborative efforts of the UK DRI, the significance of defining technique value, managing data, and extracting insights, and the importance of networks and resources to collectively address these challenges and advance our understanding of neurodegenerative diseases.

## Space and Time in Peanut Allergy Immunotherapy – Learning How To Explore An Evolving Immune Response

### Victor Turcanu, Senior Lecturer in Allergy, King's College London

Turcanu begins by highlighting the severity of peanut allergies and the increasing prevalence of this condition. He mentions that peanut allergies are often lifelong and can lead to severe reactions, making it important to find effective treatments.

Turcanu emphasizes the environmental factors contributing to the rise in allergies and autoimmune diseases in developed countries. He presents examples from Germany, Finland, and Australia to demonstrate the differences in allergy rates across regions. He also suggests that the microbiome plays a role in the development of allergies, citing the higher prevalence of nut and peanut allergies in Australian children with East Asian-born parents.

The focus then shifts to the role of T cells in peanut allergies. Turcanu explains that T cells in the skin trigger allergic responses, while T cells in the gut prevent allergies. He provides evidence from mouse models and human transplants to support this dual allergy exposure hypothesis.

The presentation highlights a prospective randomized control trial that investigated the impact of early peanut consumption on peanut allergy prevention. The trial demonstrated that introducing peanuts early in life significantly reduced the risk of developing peanut allergies.

Turcanu acknowledges the challenges in studying peanut allergies, including variability in human samples and the presence of batch effects. He suggests leveraging core facilities and collaborating with experts to overcome these challenges and optimize research techniques.

The discussion also touches on the use of multi-omics approaches in studying peanut allergies. Turcanu mentions the need to identify the most relevant markers and discusses the challenges of integrating different data sets from various omics techniques.

Overall, the presentation provides insights into the prevalence of peanut allergies, the role of T cells, and the potential for early peanut consumption to prevent peanut allergies. It highlights the importance of further research and collaboration to better understand and address this health issue.

# Day Two Track One: Spatial Biology In Pharma & Translational Drug Research

## Track Keynote Address: Spatially- Varying Characteristics As Biomarkers In Drug Development: A Roadmap for Clinical Translation And Qualification

John Waterton, Professor of Translational Medicine, University of Manchester

John Waterton's presentation focused on the discovery, translation, and validation of imaging biomarkers in living subjects using modalities such as MRI, PET, or CT. He emphasized the importance of imaging biomarkers in oncology, atherosclerosis, cardiac liver, lung, and arthritis, with the goal of getting these biomarkers accepted in drug development by regulatory authorities.

John highlighted that biomarkers are characteristics, not just molecules, and can include molecular, histologic, and radiographic characteristics. He argued that many challenges faced in developing imaging biomarkers are similar to those encountered in emerging spatial biology, and experiences from medical imaging biomarkers could be applied to spatial biology.

The presentation discussed the gap between basic research and biomarkers trusted in clinical trials and patient care. Drug developers often prefer quantifiable continuous scales, while healthcare providers may rely on binary cutoffs. John emphasized the importance of understanding spatial biomarkers and the decisions involved in their analysis, such as averaging across organs, measuring hotspots, and dealing with tumor shrinkage. He provided examples from oncology trials, where imaging biomarkers were used successfully to set doses and understand pharmacodynamics.

John also mentioned the FDA Biomarker Qualification initiative, which aims to scrutinize biomarkers used in drug development to gain regulatory acceptance. He listed challenges in qualifying imaging biomarkers, including varying acquisition techniques, software approaches, and measurement methods.

In conclusion, John provided a roadmap for taking imaging biomarkers through qualification and validation, stressing the need for continuous assessment, consensus guidelines, and attention to reporting to ensure reliable and accepted biomarkers for drug development decisions.

## What Can We Learn From Spatial Proteomics

### Charlotte Stadler, Co-Director of Spatial and Single Cell Biology Platform and Head of Spatial Proteomics, SciLifeLab and KTH – Royal Institute of Technology

In Charlotte Stadler's presentation, she delved into the field of spatial proteomics and its relevance in clinical practice. Her work at Salaff, a national hub in Sweden for life science research, focuses on integrating different omics types and implementing multiplex imaging solutions to gain a comprehensive understanding of tissue sample biology.

The Spatial Biology platform at Salaff encompasses a range of cutting-edge spatial methods used to study tissue sections. These methods include systems from 10x, accoya platforms, comments, and MALDI Imaging, which help extract diverse information from tissue samples, spanning from global maps to specific single molecule analysis.

Charlotte's background involves significant contributions to the Human Protein Atlas program, where they generated numerous antibodies and utilized immunohistochemistry and immunofluorescence to analyze the localization of proteins across various tissues and cells. This work has resulted in a vast dataset of more than 70,000 proteins profiled with immunohistochemistry and over 12,000 proteins with immunofluorescence, providing valuable insights into protein expression and localization patterns.

She highlighted some key findings from the Human Protein Atlas program, such as the discovery that approximately 50% of genes are expressed everywhere (housekeeping genes) and around 20% of proteins show single-cell variation independent of cell cycle. This vast amount of data underscores the importance of capturing information at single-cell resolution.

Charlotte's lab primarily works with the Phenoptix platform, allowing them to combine large antibody panels for highly multiplexed imaging. This technology has been applied to several research projects, with a focus on immuno-oncology, liver regeneration, human development, inflammation, and wound healing. Their aim is to bring these highly multiplex imaging methods closer to clinical practice, as there is still a considerable gap between current clinical imaging approaches and the capabilities offered by research techniques.

One specific project she presented involves improving the prediction of patient responses to immune checkpoint inhibition therapy in muscle-invasive bladder cancer. By combining proximity ligation assays to visualize protein-protein interactions within the tumor microenvironment, they aim to gain insights into the interactions involving PD-1 and PDL-1 proteins, which play critical roles in immune regulation.

Moreover, Charlotte's lab is working on the simultaneous detection of genetic alterations and proteins. By combining DNA probe detection with multiplexed protein imaging on the Phenoptix platform, they can gain a more comprehensive understanding of clonal heterogeneity and identify specific genetic alterations driving cancer progression.

Overall, Charlotte Stadler's presentation emphasized the significance of spatial proteomics in advancing our understanding of tissue biology, providing potential applications in clinical diagnostics, and improving the prediction of patient responses to various therapies. The integration of different omics data and advanced imaging techniques holds promise for the future of precision medicine and personalized treatments.

# Day Two Track Two: Spatial Bioinformatics, Data Analysis, & Interpretation

## B- Cell Expansion Hinders the Stroma – Epithelium Regenerative Crosstalk During Mucosal Healing

### Paulo Czarnewski, Senior Bioinformatician, SciLifeLab

Paulo Czarnewski's presentation focused on the integration of vision data with metabolomics, proteomics, and gene expression to study disease development and identify potential therapeutic targets. He discussed their research on longitudinal differential gene expression time series to understand pathways that are upregulated during different stages of the disease. They explored the role of B cells in tissue repair and found that B cells dominate the mucosa during inflammation and promote tissue regeneration and repair.

Using single-cell analysis and RNA sequencing, they identified genes related to tissue repair, the TGF beta pathway, and the epithelial-mesenchymal transition. Spatial transcriptomics was also employed to study cell-cell interactions without measuring distance. The results showed enhanced interactions between epithelial cells and fibroblasts in the absence of B cells.

Additionally, Paulo discussed their efforts in creating a single-cell omics community to deliver high-quality training and standardization of tools and data analysis in the field of vision data. They showcased a multimodal analysis of transcriptome and metabolites, which allowed them to map the spatial coordinates of metabolites to gene expression data. He also mentioned benchmarking efforts on various spatial domain identification and cell segmentation tools.

The presentation concluded with Paulo highlighting future research directions, collaborations, and opportunities for using vision data in diagnostics and therapeutic developments.

Overall, the presentation showcased the importance of combining vision data with other omics technologies and employing advanced analytical tools for a comprehensive understanding of disease processes and potential therapeutic interventions.

## Computational Methods for Integration of Spatial Metabolomics With Other Modalities

### Alex Dexter, Senior Research Scientist – NPL

Alex Dexter's presentation focused on the integration of spatial metabolomics with other modalities, particularly mass spectrometry imaging (MSI). The presentation highlighted the use of MSI as an unlabeled molecular imaging method to study tissues, acquiring mass spectra at different pixel locations. This generates high-dimensional data that can provide insights into various molecules.

Various computational methods were discussed for analyzing spatial metabolomics data, including T-SNE, clustering, PCA, data pre-processing, and visualization. Open-source software tools were also developed for data analysis.

Integration with other modalities, such as histology and immunohistochemistry, was explored. Combining morphological information with MSI data allowed researchers to gain a deeper understanding of the relationship between metabolic and structural changes.

Data fusion approaches aimed to enhance spatial resolution, sensitivity, and specificity. Challenges in data fusion were highlighted, given the differences in data types, experimental nuances, and spatial disparities between omics data.

Validation and molecular identification were emphasized as important steps in mass spectrometry imaging. Molecular assignment remains a challenge, and validation through LCMS and isotopic labeling was recommended to confirm molecular identities and differentiate metabolically distinct regions.

Co-registration and integration with transcriptomics were discussed, as well as the challenges associated with aligning different methodologies and data types.

The presentation underscored the need to carefully evaluate different image registration methods and emphasized finding meaningful scenarios for multi-omics integration to advance disease research and diagnostics.