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Poster Presentation Abstracts

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3	Exploring the Molecular Profile and Cellular Diversity of Endometriosis using Spatial Transcriptomics	Antonios Somarakis[1], Jessica Grassmann[1], Paul Gabriel, Martine Seignon, Danielle E. Luciano[2], Bill Flynn[1], Brian S. White[1], Elise Courtois[1]	[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA. [2]Department of Obstetrics and Gynecology, University of Connecticut School of Medicine, Farmington, CT, USA
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			Psychiatry, Psychology & Neuroscience, King's College London, London, UK
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Contributing Author(s)	D. Krijgsman[1,2], S. van Dam[1,5], M. Verdonschot[1,2], W. Blokx[3], K. Suijkerbuijk[4], J. Leusen[2], Y. Vercoulen[1]
Organisation	UMC Utrecht
Email Address	d.krijgsman-4@umcurecht.nl

Poster Title	Myeloid immune spatial landscaping reveals association of early MDSCs and M2 macrophages with immunotherapy resistance in metastatic melanoma
Abstract	Immune checkpoint blockade (ICB) mediated T-cell activation has revolutionized the therapeutic arsenal to treat metastatic melanoma patients. While effective in ~50% of the patients, ICB resistance often occurs, leading to tumor progression. T-cell suppressive immune cells, such as myeloid cells, can contribute to ICB resistance in the tumor microenvironment (TME). In this study, we therefore aimed to unravel the myeloid immune landscape to reveal mechanisms of ICB response in metastatic melanoma. We developed a novel high-plex 40-marker antibody panel focused on detecting myeloid cell subsets using imaging mass cytometry. We employed this panel to characterize the myeloid immune landscape of 14 PD-1 blockade-treated metastatic melanoma patients, including 7 responders and 7 non-responders. We observed an immune hot phenotype in responders, characterized by high expression of T cell markers and immune checkpoints. Moreover, elevated proportions of $CD10^+$ tumor cells, M2 macrophages, and immature $CD33^+$ myeloid-derived suppressor cells (eMDSC) were uncovered in non-responders compared to responders. Importantly, elevated proportions of M2 macrophages and eMDSC were highly associated with poor clinical outcome, indicating a role for these suppressive myeloid cell subsets in therapy resistance. Neighborhood analyses then revealed interactions between $CD8^+$ T cells and M2 macrophages in non-responders, implying macrophage-induced suppression of cytotoxic T cells. Remarkably, no significant cell-cell interactions were observed between eMDSC and other cells, suggesting eMDSC mainly contribute to therapy resistance in a cell-contact independent manner. Together, we revealed an essential role for eMDSC and M2 macrophages in ICB resistance in patients with metastatic melanoma. Our findings warrant further studies of myeloid cell characteristics in the TME and their role in therapy resistance to improve immunotherapy for melanoma patients.

Contributing Author(s)	Florian Wünnemann[1,2]*, Florian Sicklinger[3,4]*, Kresimir Bestak[1,2], Miguel Ibarra[1,2], Margot Chazotte[1,2], Tobias Thiemann[3,4], Florian Leuschner[3,4],+, Denis Schapiro[1,2]+
Organisation	[1]Institute for Computational Biomedicine, Faculty of Medicine, University Hospital Heidelberg and Heidelberg University, Heidelberg, Germany, [2]Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany, [3]Department of Internal Medicine III, University of Heidelberg, Heidelberg, Germany, [4]German Centre for Cardiovascular Research (DZHK), Partner Site Heidelberg, Heidelberg, Germany * These authors contributed equally + Corresponding authors
Email Address	florian.wuennemann@uni-heidelberg.de

Poster Title	Spatial omics analysis of murine acute myocardial infarction reveals novel immune cell infiltration routes
Abstract	Myocardial infarction (MI) – commonly known as heart attack - is one of the most prevalent cardiovascular conditions across Europe and contributes to approximately 20% of deaths. The local inflammation of the site where myocardium dies triggers an intricate chain reaction following an infarct. Recent studies have highlighted the significance of tissue inflammation during the acute phase of MI, which plays a vital role in the healing process that follows, after experiencing a heart attack. Here, we investigated the cellular immune landscape and changing microenvironments during the acute phase of MI using novel spatial transcriptomics (Molecular Cartography by Resolve Bioscience) and highly multiplexed antibody-based imaging (Lunaphore COMET system) technologies. We evaluated several approaches for cell segmentation and established computational pipelines to process and quantify both transcriptomics and antibody-based imaging modalities in cardiac tissue. Our results reveal novel infiltration routes of immune cells (monocytes, macrophages, neutrophils) into the infarct tissue and highlight the role of the changing cellular microenvironment during the acute phase of MI.

Contributing Author(s)	Antonios Somarakis[1], Jessica Grassmann[1], Paul Gabriel, Martine Seignon, Danielle E. Luciano[2], Bill Flynn[1], Brian S. White[1], Elise Courtois[1]
Organisation	[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA. [2]Department of Obstetrics and Gynecology, University of Connecticut School of Medicine, Farmington, CT, USA
Email Address	antonios.somarakis@jax.org

Poster Title	Exploring the Molecular Profile and Cellular Diversity of Endometriosis using Spatial Transcriptomics
Abstract	Endometriosis is a chronic condition where endometrium-like tissue grows outside the uterus, commonly within the pelvic cavity. It affects millions of women of reproductive age, causing chronic and debilitating pelvic pain among many other symptoms, and is associated with infertility [1-4]. The exact cause and mechanisms of endometriosis are unclear, making it hard to develop effective therapeutic treatments and non-invasive diagnostic tools for early detection [1, 3]. Our recent study applied imaging mass cytometry to reveal cell type specificity across lesions and their microenvironments [1]. Here, to more comprehensively characterize transcriptional programs and cellular interactions driving the growth of endometrium - like tissues ectopically, we apply spatial transcriptomics (10xGenomics Visium) to eutopic endometrium (EuE) and ectopic peritoneal (EcP) lesions. We compared the cell types abundance in the different tissue lesions using robust decomposition of cell type mixtures (RCTD) [6]. We used single-cell RNA sequencing profiles from our previous study [1] as a reference dataset for deconvolution. This comparative analysis showed a differentiation of cell type composition according to lesion region. In particular, EcP was enriched for endothelial and myeloid cells, whereas EuE was enriched for epithelial cells PAX8 transcription factor and its downstream target *SLC34A2* is overexpressed in endometriotic tissue [7,8]. Our data has revealed a spatial co-expression of PAX8 and SLC34A2 that is enriched within epithelial cells. Finally, we discerned in EuE lesions that *CD20* (B cells), *CD3E*, *CD3G* (T cells), *HLA-DRA* (dendritic cells) and *PDPN* (lymphoid cells) were co-expressed in the same regions and formed **lymphoid aggregates**. Overall, this study provides insights into the molecular profile and cell types associated with endometriosis in different tissue locations. The findings suggest that tissue origin is linked to enrichment of specific cell types, and that spatial transcriptomics can reveal the spatial distribution of key molecular markers in endometriosis lesions. Our going studies will lead to a better understanding of the importance of molecular profiles of the microenvironment in endometriosis.

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Contributing Author(s)	Zhaoyu Sun, Yaping Wu, Venkatesh Rajamanickam, Wesley Rosales, William L Redmond
Organisation	Earle A Chiles Research Institute, Providence Cancer Institute, Portland OR, USA
Email Address	zhaoyu.sun@providence.org

Poster Title	Characterizing PD-L1 scores and the spatial profile of PD-L1$^+$ cells with T cells within the NSCLC, HNSCC, RCC, and BrCa tumor microenvironment
Abstract	The extent of PD-L1 expression in tumors has been used to select patients for anti-PD-1/L1 checkpoint blockade immunotherapy and to provide insight into the inflammatory milieu of tumors. In the current study, we characterized PD-L1 expression in 4 different types of human tumors collected from primary surgical resections to test the hypothesis that the proportion of PD-L1$^+$ cells within the tumor or stroma and the spatial relationship between PD-L1$^+$ cells and cytotoxic and/or regulatory T cells correlates with clinical outcomes. Using multiplexed immunohistochemistry (mIHC) and immunofluorescence methods developed in our lab, we studied a cohort of patients with head and neck squamous cell carcinoma (HNSCC; n=30), non-small cell lung cancer (NSCLC; n=50), breast cancer (n=30), and renal cell carcinoma (n=30). All samples were evaluated by a board-certificated pathologist using pathological diagnostic criteria. The tumor proportion score (TPS) combined positive score (CPS), proportion of PD-L1$^+$ immune cells (including stromal tumor-infiltrating lymphocytes (TILs), intratumoral TILs, and TILs at the invasive margin), and PD-L1 expression within tertiary lymphoid structures (TLS) were compared cross these four different tumor types. We integrated these data with the spatial localization of cytotoxic CD8$^+$ T cells and regulatory Foxp3$^+$CD3$^+$ T cells to investigate the correlation of PD-L1 and immune cell infiltration in immune inflamed versus immune excluded tumors. Our data revealed differences in PD-L1 expression across different human tumor types and provided more evidence regarding PD-L1 expression patterns and the spatial localization of effector versus regulatory T cells within the tumor microenvironment and their association with clinical outcomes.

Contributing Author(s)	Su Wang, Toon Verheyen, Amanda Janesick, Robert Shelansky, Albert Kim, Janine Hensel, Francesca Meschi, Navid Farahani, Vijay Kumar, Xiaoyan Qian, Sarah Taylor
Organisation	10xGenomics
Email Address	

Poster Title	High resolution mapping of human breast ductal carcinoma using Xenium In Situ platform
Abstract	The novel Xenium In Situ analysis platform enables targeted gene expression analysis at subcellular resolution with high sensitivity, high specificity and high throughput. One key benefit of Xenium compared to other in situ platforms is that the expression data can be integrated with classical histological stainings, such as H&E and standard immunofluorescence (IF) on the same tissue section. This is particularly useful in dense heterogeneous tissue compartments and tumor microenvironments, maximizing data that can be obtained from the same tissue section. Xenium uses a microscopy based read-out to identify spatial patterns of expression in tissue sections, which allows us to map ductal carcinoma in situ (DCIS) at single cell level. Using a pre-validated gene panel for human breast, we localized 203 genes with subcellular resolution in a DCIS FFPE sample to identify cell types and clusters. This is extremely useful to reveal RNA expression patterns and to classify cell types, reveal key gene players in cancer progression to metastasis. Differential expression analysis reveals distinct markers associated with tumor progression. The single cell in situ gene expression profiles complement the pathological assessment of the tissue but provide an additional level of information that is not accessible using traditional methodologies. Here, we were able to identify tumor regions with unique molecular signatures indicative of the progression from DCIS to invasive cancer. Using the Xenium workflow in breast DCIS cancer samples, we show that we can accurately identify cell types and discern different tumor regions based on high resolution spatial gene expression signatures relevant to understanding cancer heterogeneity, which will ultimately inform prognostic outcomes in oncology research.

Contributing Author(s)	Yahui Long, Kok Siong Ang, Sha Liao, Raman Sethi, Yang Heng, Chengwei Zhong, Hang Xu, Nazihah Husna, Min Jian, Lai Guan Ng, Ao Chen, Nicholas RJ Gascoigne, Xun Xu, Jinmiao Chen
Organisation	Singapore Immunology Network, A * STAR, Singapore
Email Address	chen_jinmiao@immunol.a-star.edu.sg

Poster Title	Deciphering spatial domains from spatial multi-omics with SpatialGlue
Abstract	Spatial transcriptomics has emerged as a powerful tool for studying tissue heterogeneity while preserving its spatial context. Over the past year, spatial technologies have been expanding the scope to encompass spatial multi-omics, enabling simultaneous measurement of multiple omics (e.g., protein and RNA, chromatin accessibility and RNA) on the same tissue slice while retaining the spatial context. To exploit the richness of the data generated by such techniques, analytical methods need to accomplish two inter-related tasks, integrating multiple data modalities (i.e., multiple omics) and integrating omics data with spatial information. However, currently available algorithms target either spatial single omics or non-spatial multi-omics data, and thus are inadequate for handing spatial multi-omics data. To address this gap, we present SpatialGlue, a novel spatial multi-omics data analysis tool that performs cross-omics integration in a spatially aware manner. It leverages a graph neural network architecture with dual attention to perform within-modality integration of expression data with spatial information and between-modality integration of different omics in an adaptive manner. In addition, it learns and outputs the contributions of different modalities to each spatial spot, which is highly relevant as the information content of different modalities can be complementary and vary in contribution at different spots. In our examples, we demonstrated SpatialGlue's performance on the latest available spatial multi-omics datasets acquired with different technological platforms, as well as its computational efficiency. SpatialGlue was able to accurately aggregate cell types into spatial domains at a higher resolution on different tissue types and technology platforms, as well as gain insights into cross-modality spatial correlations. (https://www.researchsquare.com/article/rs-2921471/v1, Nature Methods under review)

Contributing Author(s)	Putignano A[1], Cortese N[1], Barbagallo M[1], Grizzi F[1], Pasqualini F[1], Basso G[2], Marchini S[2], Spaggiari P[4], Di Tommaso L[3,4], Marchesi F[1,5]
Organisation	[1]Department of Immunology and Inflammation, IRCCS Humanitas Clinical and Research Hospital, Rozzano, Italy, [2]Genomic Unit, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy, [3]Department of Biomedical Sciences, Humanitas University, Rozzano, Italy, [4]Pathology Unit, IRCCS Humanitas Clinical and Research Hospital, Rozzano, Italy, [5]Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy
Email Address	anna.putignano@humanitasresearch.it

Poster Title	Spatial transcriptomics on cancer tissue
Abstract	Recent approaches of spatial transcriptomic are changing our capability to investigate the complexity of the Tumor microenviroment (TME), allowing to detect the expression of multiple genes while preserving the spatial information of the tissue. Here, we tested two different spatial transcriptomics technologies on tumor tissue specimens (FFPE sections). We performed Nanostring GeoMx Digital Spatial Profiling (DSP) on 2 FFPE tissue slides from Pancreatic Ductal Adenocarcinoma (PDAC) primary tumors and 10X Genomics Visium assay on 2 FFPE tissue samples from patients with colorectal liver metastases (CLM). The DSP approach was deployed to sections of 35.3 mm by 14.1 mm placed onto a glass slide and stained with fluorescently labeled antibodies recognizing the morphology markers CD45/PanCK/αSMA/DNA. The assay contained in situ hybridization probes conjugated to unique indexing-oligonucleotides (DSP barcodes) via a UV-photocleavable linker targeting over 18,000 protein-coding human genes (WTA). After selecting at least 6 regions of interest (ROIs) for each slide, including tumoral, stromal and immune components, the selected ROIs were UV illuminated and the UV-cleaved oligos collected. The DSP barcodes are tagged with the ROI location, then sequenced on an Illumina sequencer. After a step of quality control based on the metrics reported in the DCC files, we generated the count matrix at the gene level for each illuminated CD45+ and CD45- segment within each ROI per slide. After quality control, filtering and normalization steps, we used the GeoMx SpatialDecon algorithm to estimate, for each segment, the abundance of cell types, by generating a custom cell profile matrix from PDAC single cell data. For the Visium assay, four-µm FFPE tissue sections were stained with hematoxylinand eosin (H&E) to select the appropriate tissue regions (inclusive of tumor, TU and Invasive Margin, IM) to be placed in the capture area on the Visium slide. Each area contains ~5000 barcoded spots to capture the gene expression levels at the spatial resolution. In brief, probes targeting the whole transcriptome are added to the tissue and hybridize to the target gene. The ligated probes are released from the tissue and bind to spatially barcoded probes on the capture area. Libraries prepared from ligated and barcoded probes were sequenced on Illumina sequencer. The count matrices generated from each tissue slide with the Space Ranger pipeline (10X Genomics) were processed and normalized with Scanpy python package. Features' expression projection onto spatial coordinates was used to evaluate the consistency of the expression patterns of known adjacent liver (APOC3), stromal (COL1A2), and tumor (EPCAM) marker genes in spatial transcriptomics data with the H&E images. To infer the cell types present within each spot, we performed cell label transfer by applying the CytoSPACE framework. We first generated a single cell RNA-seq reference representative of the cell types present in the CLM tissue and then performed deconvolution taking into account the number of cells present within each spot estimated by a cell segmentation step of the tissue. DSP and Visium technologies use two different approaches to spatially locate the RNA expression levels on the tissue. Both spatial transcriptomics technologies are suitable for analyzing the degraded RNA extracted from FFPE tissues since they use a panel of spatially barcoded probes targeting the whole transcriptome. Nanostring DSP assay performs very well in terms of single population analysis enabling the gene profiling of specific immune cell types infiltrating the tumor tissue in user-defined regions of interest. On the other hand, 10X Visium assay, even though it is not at single cell resolution, gives a broader picture of the transcriptome of the entire tissue area allowing the dissection of the TME heterogeneity.

Contributing Author(s)	Sanem Sariyar[1,2], Alexandros Sountoulidis[1,3], Sergio Marco Salas[1,4], Jan Niklas Hansen[1,2], Mariya Mardamshina[1,2], Anna Martinez Casals[1,2], Frederic Ballllosera Navarro[1,2], Sergio Marco Salas[1,4], Emelie Braun[5], Zaneta Andrusivova[1,6], Xiaofei Li[7], Paulo Czarnewski[1,6], Joakim Lundeberg[1,6], Erik Sundström[7], Mats Nilsson[1,4], Sten Linnarsson[5], Christos Samakovlis[1,3,8], Burcu Ayoglu[1,2,*,#], Emma Lundberg[1,2,10,11,*,#]
Organisation	[1]Science for Life Laboratory, Solna, Sweden; [2]Department of Protein Science, KTH - Royal Institute of Technology, Stockholm, Sweden; [3]Department of Molecular Biosciences, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden; [4]Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden; [5]Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden; [6]Department of Gene Technology, KTH - Royal Institute of Technology, Stockholm, Sweden; [7]Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden; [8]Molecular Pneumology, Cardiopulmonary Institute, Justus Liebig University, Giessen, Germany; [10]Department of Pathology, Stanford University, Stanford, CA, USA; [11]Department of Bioengineering, Stanford University, Stanford, CA, USA
Email Address	sanem.sariyar@scilifelab.se

Poster Title	High Parametric protein maps reveal the spatial organization in early developing human lung
Abstract	The respiratory system, consisting of the lungs, trachea and the associated vasculature, is essential for terrestrial life. Recent studies on lung development have described the origins of the different cell lineages in the lung, and the molecular pathways regulating them. This also provided new insight into congenital lung diseases, lung abnormalities and acquired lung diseases. The recently initiated Human Developmental Cell Atlas (HDCA) project is one of the focus areas of the international Human Cell Atlas project, and it aims to create a comprehensive molecular and spatial atlas of human prenatal development across anatomical space and developmental time, including the lung. This study, within the HDCA framework, focuses on molecular and spatial characterization of lung cells and their interactions during the first trimester of human lung development. We performed highly multiplexed tissue imaging on 6-13 post-conception week (pcw) old human lungs using a 30-plex antibody panel. We analyzed around 1 million single lung cells at five distinct developmental timepoints. We characterized the spatio-temporal organization and development of the cell types in the early embryonic lung. Our data provide a new fundamental basis to understand the developmental origins of health and disease in the human lung.

Contributing Author(s)	Albert Pla-Planas, Het Barot, Mercè Coll, Mena Kamel, Sunaal Mathew, Cindy Qin, Amrut Sarangi, Pavel Senin, Sergio Villordo, Seqian Wang, Ziv Bar-Joseph
Organisation	Sanofi Digital
Email Address	

Poster Title	Spatial Omics Analysis at Scale
Abstract	Spatial Omics technologies provide key advances into disease mechanisms and therapeutic target discovery. Nonetheless, the varying resolutions and limitations of different spatial technologies difficult the standardization of downstream analysis, preventing the replication of analysis across technologies. The fact that even different versions or experimental set ups of the same technology may have differentiated constrains poses a significant challenge for the processing of spatial experiments at scale, in a coherent, semi-automated, and reproducible manner. To address these challenges, we are developing a cloud-based framework for processing and analyzing spatial data at scale. Our approach involves breaking down the analysis into two blocks: data processing and downstream analysis. This allows us to abstract common elements of the analysis that may require different approaches depending on the experiment setup and the selected technology. The results of the data processing stage are stored in a core data model that defines regions and cells in the tissue. Consequently, the downstream analysis becomes independent of the technology and can be reused across different experiments and research teams. The data processing stage includes cell segmentation, cell deconvolution, cell morphological characterization and clustering, and cell assignment. The downstream analysis focuses on cell distribution and neighbourhood analysis, gene/protein expression analysis, cell infiltration, co-occurrence pattern analysis, and comparative analysis between samples or regions. Each element of the pipelines is implemented as an independent service, so it can be reused in different scenarios and set ups. This approach empowers scientists to autonomously compose their processing and downstream analysis based on their specific needs and technological constraints. Currently, our project has successfully adapted the framework to accommodate Visium and we are currently focussed on integrating Phenocycler fusion technologies. Our framework has been successfully used for the analysis of lung, kidney, esophagus, and colon samples from both mice and humans.

Contributing Author(s)	Bernarda Majc, Anamarija Habič, Marta Malavolta, Mateja Mlinar, Jernej Mlakar, Andrej Porčnik, Marija Skoblar Vidmar, Aleksander Sadikov, Barbara Breznik, Metka Novak
Organisation	National Institute of Biology
Email Address	barbara.breznik@nib.si; metka.novak@nib.si

Poster Title	Personalized in vitro glioblastoma models for evaluation of therapy response and resistance
Abstract	Glioblastoma remains one of the most lethal malignancies due to tumour heterogeneity and resistance to standard therapies. Personalised tumour models that mimic the unique genetic background and microenvironment of the patient's tumour are critical for exploring glioblastoma progression and novel therapeutic options. We constructed glioblastoma organoids from fresh tumour biopsies that recapitulated the cellular composition and gene expression pattern of the parental tumour tissue. The organoids preserved a wide variety of secreted growth factors, cytokines, and chemokines, as well as the presence of noncancerous cells in the tumour microenvironment, such as endothelial cells, T lymphocytes, macrophages, and microglia, for at least four weeks. The organoids were resistant to radiation and the chemotherapeutic agent temozolomide and activated p53-related signalling pathways after treatment. Spatial technologies at the gene and protein levels are now being used to decipher molecular and cellular changes in the tumour microenvironment after treatment and to identify molecular mechanisms and biomarkers for treatment resistance. Our in vitro models allow us to explore the pathobiology of glioblastoma under clinically relevant conditions, and initial results are providing insight into potential mechanisms underlying glioblastoma treatment resistance.

Contributing Author(s)	Henning Hvid, Jette Mandelbaum, Heidi S. Nielsen, Jeanette Juul
Organisation	Novo Nordisk A/S
Email Address	

Poster Title	Exploration of the feasibility of performing spatial transcriptomics on human pancreas samples from healthy donors and donors with type 2 diabetes
Abstract	The islets of Langerhans in the pancreas are of key importance in diabetes research and are often studied for identification of novel drug targets. Spatial transcriptomics is a valuable new technology, which potentially can be applied to characterize global gene expression in tissue compartments like the pancreatic islets. However, the pancreas can be a challenging tissue to work with. It is not uncommon that human pancreas samples available from biobanks have been preserved under suboptimal conditions, and the abundant content of enzymes in the pancreas can lead to rapid onset of autolysis. Hypothetically, it could therefore be a challenge to generate high-quality spatial transcriptomics data from human pancreas. The aim of the present study was to explore the feasibility of generating spatial transcriptomics data from surgically excised samples of human pancreas, from healthy and type 2 diabetic (T2D) donors. Formalin fixed and paraffin embedded (FFPE)samples of human pancreas from healthy as well as T2D donors were first characterized by performing in situ hybridization for GLP1R and PPIB. Samples with high mRNA levels were then used for spatial transcriptomics using the Visium FFPE v1 assay from 10X Genomics and the software Partek Flow software was used for exploration of differentially expressed genes (DEG) in in islets of Langerhans between healthy and T2D donors. DEG were finally compared to previous studies, where single-cell RNAseq had been used to explore differentially expressed genes in the endocrine pancreas between healthy and T2D donors. Visium spatial transcriptomics yielded acceptable numbers of detected genes per spot in the FFPE samples of human pancreas. Expression signals of highly abundant transcripts INS and GCG were not restricted to spots covering islets of Langerhans, but displayed some diffusion to spots covering exocrine pancreas. However, a graph-based cluster specific for islets could be identified, and DEG in this cluster included genes which had also been identified as DEG in islets from donors with T2D in previous studies using scRNAseq. In conclusion, Visium spatial transcriptomics on FFPE samples from human pancreas is feasible and can be used to explore gene expression in tissue compartment of interest like islets of Langerhans in the pancreas.

Contributing Author(s)	Xinyu Hu, Bob van Sluijs, Óscar García Blay, Yury Stepanov, Koen Rietrae, Wilhelm T.S. Huck, Maike M. K. Hansen
Organisation	Radboud University
Email Address	xinyu.hu@ru.nl

Poster Title	Simultaneous Spatial Profiling of RNAs and Proteins at the Same Resolution
Abstract	Spatial multi-omics technologies have revolutionized our ability to quantitatively and spatially analyze molecules at the single-cell level in their microenvironments. The fields of spatial transcriptomics and proteomics have emerged, offering a range of experimental and computational methods to comprehensively map diverse cell types. Despite the exponential growth of spatial multi-omics, current technologies come with distinct advantages and disadvantages. Notably, the visualization of mRNAs and proteins at the same resolution remains a challenge. The resolution of detected mRNAs and proteins in existing methods differs significantly, with mRNA resolution being much higher. To address this limitation, we have developed a multiplexed spatial detection method called Antibody-RNA Targeting Sequential Fluorescent *In Situ* Hybridization (ARTseq-FISH), which enables simultaneous detection and quantification of mRNAs and proteins in single cells at sub-micrometer resolution. Our approach utilizes DNA-conjugated antibodies to target proteins of interest, along with target-specific padlock probes that bind both cellular mRNAs and the unique DNA barcode on each antibody. The padlock probes are circularized through a ligase reaction, and rolling circle amplification is performed to generate long repeat sequences for each padlock probe. Subsequently, we visualize the targets by introducing bridge probes and readout probes. The bridge probes contain a fragment corresponding to a specific sequence on each padlock probe and four overhanging sequences corresponding to four different readout probes. Through sequential removal and rehybridization of readout probes, we successfully multiplexed the detection of mRNAs and (phospho-)proteins, achieving up to 67 targets within five rounds of hybridization. The integration of mRNA and protein detection provided by ARTseq-FISH not only overcomes the challenges associated with data integration but also has the potential to simultaneously detect thousands of targets.

Contributing Author(s)	Rebekka Wehner[1,2,3], Ioana Plesca[1], Iva Benešová[1], Susanne Doms[1], Ulrich Sommer[4], Luise Müller[1], Max Heiduk[2,3,5], Daniela Aust[2,3,4], Gustavo Baretton[2,3,4], Jürgen Weitz[2,3,5], Lena Seifert[2,3,5], Adrian M Seifert[2,3,5], and Marc Schmitz[1,2,3]
Organisation	[1]Institute of Immunology, Faculty of Medicine Carl Gustav Carus, TU Dresden, Fetscherstraße 74, 01307 Dresden, Germany, [2]National Center for Tumor Diseases (NCT), University Hospital Carl Gustav Carus, TU Dresden, Fetscherstraße 74, 01307 Dresden, Germany, [3]German Cancer Consortium (DKTK), partner site Dresden, and German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany, [4]Institute of Pathology, University Hospital Carl Gustav Carus, TU Dresden, Fetscherstraße 74, 01307 Dresden, Germany, [5]Department of Visceral, Thoracic and Vascular Surgery, University Hospital Carl Gustav Carus, TU Dresden, Fetscherstraße 74, 01307 Dresden, Germany, [6]Tumor Immunology, University Cancer Center (UCC), University Hospital Carl Gustav Carus, TU Dresden, Fetscherstraße 74, 01307 Dresden, Germany
Email Address	

Poster Title	Detection of native human dendritic cell subsets in the tumor microenvironment
Abstract	As professional antigen-presenting cells, dendritic cells (DC) play an essential role in the activation and maintenance of antitumor immune responses. They stimulate NK cells, bridge innate and adaptive immune system, facilitate T-cell priming. Therefore, they are associated with improved clinical outcomes in various cancers. Nevertheless, DCs show high plasticity and can develop a tumor-induced tolerogenic phenotype. These DCs promote the development of an immunosuppressive milieu and are associated with poor patient survival. Multi-parameter fluorescence microscopy imaging provides the opportunity to dissect the role of DCs and their relationship to other cell types in the tumor microenvironment. This poster presents our work in the DC research field since quantification and spatial organization of native human dendritic cell subsets may support the identification of pathways as well as mechanisms, which promote cancer elimination and therapy response.

Contributing Author(s)	Bhardwaj, Archana ; Tran, Florian; Gilbert, Felix; Sommer, Felix; Rosenstiel Philip
Organisation	Institute of Clinical Molecular Biology (IKMB)
Email Address	a.bhardwaj@ikmb.uni-kiel.de

Poster Title	Spatial transcriptome of mouse small intestine under malnutrition
Abstract	Malnutrition is a major issue that affects millions of people worldwide. It can affect immune systems and cause various changes in the intestine, such as alteration in villi length and crypt depth. Therefore, it is crucial to understand how malnutrition affects gene expression at a spatial level. Spatial transcriptomics (ST) is an emerging area in genomics that could provide gene expression patterns within multiple tissue regions. To explore this, we collected tissue samples from the small intestine of control and protein deficient diet mice (PEM) for a 10x visium ST experiment. We implemented quality control (QC) on the raw sequencing data by eliminating low-quality spots and performed normalization. To analyze processed ST data, there are various analytical approaches available. One of these methods involves utilizing gene expression space without spatial location. To achieve this, we merged the processed gene expression matrices of PEM and control mice and prepared an integrated object to identify distinct clusters. Selected approach revealed high concordance between identified clusters and tissue morphology. We also found differential genes that shows unique spatial expressions covering villi and crypts. Functional analysis of differential genes resulted in many significant biological process that were associated to stress, protein transport and metabolism. Our analytical workflow used for the analysis of ST data revealed several potential targets that might be useful to develop effective treatments for malnutrition.

Contributing Author(s)	Assya Legrini, Colin Wood, Holly Leslie, Rachel Pennie, Silvia Martinelli, Fiona Ballantyne, Leah Officer-Jones, Ian Powley, John Le Quesne, Nigel Jamieson
Organisation	University of Glasgow
Email Address	a.legrini.1@research.ac.uk

Poster Title	Spatial Multi-omic characterisation of Human Pancreatic ductal adenocarcinoma in naïve vs Neoadjuvant treated patients
Abstract	Pancreatic Ductal Adenocarcinoma (PDAC) long-term outcomes following surgical resection remain poor, with only 20% of patients surviving 5 years post pancreatectomy. With its immune-privileged nature, it appears to easily evade the antitumor immune response. Although there is rationale for targeting PDAC immune pathways, little benefit has been observed to date. The study's aim was to interrogate the protein and RNA immune landscape utilising Akoya Biosciences Phenoptics and Nanostring GeoMx Digital Spatial Profiler (DSP) respectively. Combining these technologies allows for multi-layered, in-depth characterisation whilst maintaining tumour microenvironment topographical features. We assessed tumour samples from ~60 treatment-naive and neoadjuvant treated (NAT) PDAC cases using an FFPE tissue microarray (TMA) for which extensive IHC, molecular, genomic characterisation and clinicopathological data is available. A multiplex immunofluorescent (mIF) panel was developed for PanCk, aSMA, CD3, CD8, CD68 and FOXP3 to determine the base immune landscape. The GeoMx Whole transcriptome assay (WTA) was used in a serial section, selecting for different tissue compartments defined by immunofluorescence (tumour (PanCk+), Fibroblasts (aSMA+) and Immune regions). Multiplex protein analysis demonstrated prognostic power with cellular density analysis and different spatial analysis (e.g., high density of CD3+ ($p=0.0035$)). These were included as comparison groups in the WTA analysis. In WTA unsupervised analysis of mIF CD3 groups, we found immune regions in CD3 high patients had significant upregulation of T cell and B cell related genes/pathways compared to CD3 low patients e.g., Translocation of zap-70 to immunological synapse ($P<0.001$), and IGKC (Log2: 2.8, $P<0.001$)). This study shows the potential of using complimentary technologies to identify spatially informed biomarkers with prognostic relevance and explore the underlying biology of characterized patients. Relevant candidate immune predictors identified in the spatial context are undergoing validation in a larger independent cohort. Ongoing work is taking place to add in novel single cell spatial assays to these cohorts.

Contributing Author(s)	Iva Sutevski[1,2], Ellen Appleton[3], Niclas Branzell[3], Anna Nilsson[4,5], Luke Odell[6], Lukas Käll[1,7], Mats Nilsson[1,8], Emma Lundberg[1,2,9,10], Per Andrén[4,5], Per Svenningsson[3,11], Burcu Ayoglu[1,2]
Organisation	[1]Science for Life Laboratory, Solna, Sweden, [2]Department of Protein Science, KTH – Royal Institute of Technology, Stockholm, Sweden, [3]Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden, [4]Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, [5]Science for Life Laboratory, Uppsala, Sweden, [6]Department of Medicinal Chemistry, Uppsala University, Uppsala, Sweden, [7]Department of Gene Technology, KTH – Royal Institute of Technology, Stockholm, Sweden, [8]Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden, [9]Department of Pathology, Stanford University, Stanford, CA, USA, [10]Department of Bioengineering, Stanford University, Stanford, CA, USA, [11]Department of Basic and Clinical Neurosciences, The Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK
Email Address	iva.sutevski@scilifelab.se

Poster Title	Spatial proteomic studies of Lewy Body diseases
Abstract	Neurodegenerative diseases (NDDs) are a heterogeneous group of disorders characterized by the accumulation of misfolded and aggregated proteins, impaired synaptic and neuronal functions, deregulated proteostasis, cytoskeletal defects, mitochondrial dysfunction, DNA and RNA damage, inflammation, and neuronal death. As a consequence of these biological processes, NDD patients exhibit impaired cognitive, behavioural, and locomotor functions. Parkinson´s disease (PD) is the second most common NDD. It´s neuropathological presentation includes accumulation of alpha-synuclein aggregates, called Lewy Bodies, and loss of dopaminergic neurons in the substantia nigra. Between 30 to 70 % of PD patients also develop dementia, defined as Parkinson´s disease dementia (PDD). PDD shares many clinical and pathological features with Lewy Body dementia (LBD), yet the relationship between these two disorders, and whether they are the same or distinct diseases, has been an ongoing debate over the years. Based on current international consensus, PDD or LBD are diagnosed depending on the onset of cognitive impairment. However, cellular and molecular factors governing these diseases (PD, PDD and LBD) are not entirely understood. Through high-parametric spatial protein mapping in healthy and diseased human brains, our study aims 1) to shed light on the cellular features causing and/or involved in PD, PDD and LBD, and 2) to discover potential biomarkers of these NDDs. Our cohort consists of 68 post-mortem patient samples, including 15 PD, 5 PDD, 23 LBD and 25 age-matched controls, covering several brain regions including hippocampus, amygdala, prefrontal cortex, parietal cortex, cingulate cortex, substantia nigra, and caudate. To explore and describe the spatial brain architecture in these diseases, an antibody-based, high-parametric spatial phenotyping system is used offering single-cell resolution analysis of ca. 50 disease- and cell type-specific protein markers. Further integration of our spatial protein maps with spatial transcriptomics and spatial metabolomics maps will unravel the pathophysiological mechanisms to understand and differentiate these parkinsonian conditions.

Contributing Author(s)	Madhavi Dipak Andhari[1], Daniela Massi[2], Asier Antoranz[1], Anna Szumera-Ciećkiewicz[3,4] , Francesca Bosisio[1,5]
Organisation	[1]Lab of Translational Cell and Tissue Research, KU Leuven, Belgium, [2]Division of Pathological Anatomy, Department of Surgery and Translational Medicine, University of Florence, Italy, [3]Department of Pathology, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland, [4]Diagnostic Hematology Department, Institute of Hematology and Transfusion Medicine, Warsaw, Poland, [5]UZ Leuven, Belgium
Email Address	madhavidipak.andhari@kuleuven.be

Poster Title	Spatial, single cell landscaping of the immune microenvironment and the melanoma cell heterogenicity in metastasizing and non-metastasizing thin melanomas.
Abstract	Patients with thin melanoma (Breslow thickness <1) at early stages are typically not closely monitored due to their low risk of developing metastatic disease. However, a small percentage (3-8%) of thin melanoma cases do metastasize, warranting the need for identifying these cases and potentially providing more intensive follow-up and neoadjuvant therapy. Unfortunately, the small size of early-stage lesions and the lack of fresh samples for single-cell dissociation have hindered comprehensive characterization of these tumors until the development of spatial single-cell technologies. In this study, we employed state-of-the-art single-cell and spatial technologies to deeply characterize the immune microenvironment of metastasizing (M+) and non-metastasizing (M-) thin melanoma samples, laying the groundwork for biomarker discovery in this patient population. In collaboration with the Pathology subcommittee of the Melanoma group of the EORTC, we collected 29 M+ and 36 M- thin melanoma patient samples, matched for Breslow thickness, age, gender, location of occurrence, ulceration, and mitosis. Using the MILAN (Multiple iterative labeling by antibody neo deposition) method, we investigated the cellular and functional landscape of these samples by measuring 35 protein markers spatially at the single-cell level. By comparing recurrent cell communities in metastatic and non-metastatic patient samples, we found that M+ cases were enriched in cell communities comprising PD-L1+ M1 and M2 macrophages and cytotoxic T cells. These results suggest a potential role of the immune microenvironment in facilitating metastasis in patients with thin melanoma. Our study provides valuable insights into the cellular composition and functional differences between metastasizing and non-metastasizing thin melanoma samples.

Contributing Author(s)	Caoimbhe Burke[1], Chowdhury Jahangir[1], Claudia Aura[1], Clodagh Murphy[1], Grace Gormley[1], Christine McCaffrey[1], Fiona Lanigan[1], Adrian Bracken[2], Björn Nodin[3], Karin Jirström[3], Arman Rahman[1], and William M. Gallagher[1]
Organisation	[1]Cancer Biology and Therapeutics Laboratory, UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Dublin, Ireland; [2]Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland; [3]Department of Clinical Sciences, Division of Oncology and Pathology, Lund University, Lund, Sweden
Email Address	caoimbhe.burke@ucdconnect.ie

Poster Title	Multiplex *In Situ* Hybridisation for the Detection and Visualisation of OncoMasTR Target Gene Expression in Breast Cancer Tissue
Abstract	We have previously identified a novel prognostic gene panel named OncoMasTR, comprising of a set of master transcriptional regulators (MTRs), which when assessed with RT-qPCR outperformed other commercial prognostic signatures in successfully stratifying early-stage breast cancer patients into high and low risk categories for distant recurrence. Most RT-qPCR based assays do not relay the spatial context of gene expression, as samples are homogenised prior to running the assay. Moreover, due to variations in quality of antibody reagents, ensuring specificity and reproducibility is always a concern in protein-based immunohistochemistry assays. Recent advancements in RNA *in situ* hybridisation (ISH) offer digital pathology-based quantification of gene expression within histological samples while displaying the spatial and temporal distribution of the RNA transcripts. Along with total expression, the spatial distribution of a marker across tumour tissue can also carry important prognostic information. We have previously used the RNAscopeV2 assay to successfully detect the OncoMasTR target gene, UHRF1, in a breast cancer tissue microarray (TMA) cohort (n = 498). UHRF1 mRNA expression as determined by ISH showed moderate correlation with protein expression measured by IHC ($R = 0.55$, $p<2.2e-16$) and mRNA expression of bulk tissues quantified by RT-qPCR ($R = 0.23$, $p = 0.0015$). Survival analysis with the preliminary data showed high expression of UHRF1 was associated with reduced distant recurrence-free survival ($HR = 2.03$, $p = 0.001$), aligning with existing IHC and RT-qPCR data. Here, we have established a duplex ISH staining and image analysis workflow combining two OncoMasTR target genes; UHRF1 and ZNF367, which has been tested on overexpression cell line controls and full-face tissue sections with TMA staining to follow. Data generated will then be used to conduct survival analysis to elucidate the prognostic power of marker mRNA expression within a spatial context.

Contributing Author(s)	Chowdhury Jahangir[1], Clodagh Murphy[1] (**Nominated Presenter**), Joseph McGinley[1], Claudia Aura[1], Caoimbhe Burke[1], Amelie V. Pulsawatdi[2], Victoria Bingham[2], Fiona Lanigan[1], Adrian Bracken[3], Björn Nodin[4], Karin Jirström[4], Matt Humphries[2], Manuel Salto-Tellez[2], Arman Rahman[1], and William M. Gallagher[1,5]
Organisation	[1]Cancer Biology and Therapeutics Laboratory, UCD School of Biomolecular and Biomedical Sciences, UCD Conway Institute, University College Dublin, Dublin, Ireland; [2]School of Medicine, Dentistry and Biomedical Sciences, Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Ireland; [3]Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland; [4]Department of Clinical Sciences, Division of Oncology and Pathology, Lund University, Lund, Sweden, [5]OncoAssure Ltd, NovaUCD, Dublin, Ireland.
Email Address	clodagh.murphy@ucd.ie

Poster Title	Exploration of the Prognostic Efficacy of a Combined OncoMasTR and Immune Marker Signature in Early Stage Breast Cancer Using Multiplex Immunofluorescence
Abstract	We previously identified OncoMasTR, a prognostic gene panel of master transcriptional regulators that predicted recurrence risk for lymph node-negative breast cancer (BC) patients. Validation of OncoMasTR at the protein level in two independent BC tissue microarray cohorts using chromogenic Immunohistochemistry has shown promising results. Moreover, various immune markers have been proven to have significant prognostic utility in BC; therefore, we hypothesise that the integration of immune status information will improve the prognostic capacity of OncoMasTR. Using one of these cohorts (n=498), we have profiled three selected markers from the OncoMasTR panel (UHRF1, ATD2, and TCF-19), and three T-cell markers (CD4, CD8, and FoxP3) using multiplex immunofluorescence(mIF). Survival analysis of OncoMasTR markers showed high expression of UHRF1 (HR=2.0, p=0.032) and ATAD2 (HR=1.7, p=0.078), and low TCF19 expression (HR=0.47, p= 0.01), are individually correlated with reduced distant recurrence-free survival (DMFS). A double-positive population (ATAD2+ UHRF1+) with significant prognostic association (HR=1.9, p=0.031) was also identified. In immune marker survival analysis, high intratumoural (HR=0.47, p=0.046), stromal(HR=0.56, p=0.042), and whole core(HR=0.34, p=0.001) CD8+ cell density demonstrated positive association with DMFS. High intratumoural CD4+ (HR=0.48, p=0.007) and Treg cell (CD4+FOXP3+) density (HR=0.26, p=0.042) were associated with better DMFS. Furthermore, the close proximity of CD4+ cells and tumour cells was found to have significant association (HR=1.8, p=0.021) with improved DMFS. A combined signature from immune, OncoMasTR and clinical markers (tumour size, node involvement) was identified. It showed significantly higher (HR=7, p<0.001) prognostic efficacy than models containing OncoMasTR and clinical markers only (HR=3.7, p=0.007). This demonstrates the promise of a multiplex IHC biomarker panel containing OncoMasTR and immune markers, potentially offering superior prognostic prediction for early-stage BC. Recently, we have developed a multiplex panel (6-plex) of immune markers to characterise B-cell, macrophage and T-cell populations in multiple independent BC cohorts. Additionally, a high plex 42 marker panel is under development for deep profiling of immune contexure in BC using CODEX technology.

Contributing Author(s)	Anna Gerdtsson, Lavanya Lokhande, Daniel Nilsson, Wilma Olsson, Tobias Westholm, Artur Mezheyeuski, Patrick Micke, Sara Ek
Organisation	Lund University
Email Address	anna.sandstrom_gerdtsson@immun.lth.se

Poster Title	Spatial immune infiltration niches in non-small cell lung cancer
Abstract	The immune microenvironment of non-small cell lung cancer (NSCLC) is heterogeneous, which impedes the prediction of response to immune checkpoint inhibitors. We have mapped the expression of 49 proteins to spatial immune niches in 33 NSCLC tumors using GeoMx Digital Spatial Profiling, and report key differences in phenotype and function associated with the spatial context of immune infiltration. A cyclic immunofluorescence (cycIF) protocol was adapted for compatibility with the GeoMx protocol, allowing for single-cell resolution, spatial mapping of multiple immune markers, in addition to the standard 3-color morphology analysis and digital protein profiles generated by the GeoMx analysis. Tumor-infiltrating leukocytes (TIL), identified in 42% of tumors, had a similar proportion of lymphocyte antigens compared to stromal leukocytes (SL) but displayed significantly higher levels of functional, mainly immune suppressive, markers including PD-L1, PD-L2, CTLA-4, B7-H3, OX40L, and IDO1. In contrast, SL expressed higher levels of the targetable T-cell activation marker CD27, which increased with a longer distance to the tumor. Correlation analysis confirmed that metabolic-driven immune regulatory mechanisms, including ARG1 and IDO1, are present in TIL. Tertiary lymphoid structures (TLS) were identified in 30% of patients, based on visual scoring of CD45 intensity and density of lymphocytic clusters. The cycIF analysis showed that the majority of regions identified as TLS had high presence of B- and T-cells, and a subset expressed CD23, which indicates TLS maturity. In general, TLS showed significantly higher levels of lymphocyte activation markers, dendritic cells, and antigen presentation compared to other immune niches. The apparent discrimination in functional profiles of distinct immune niches, independent of the overall level of leukocytes, illustrates the importance of spatial profiling to deconvolute how the immune microenvironment can dictate a therapeutic response and to identify biomarkers in the context of immunomodulatory treatment.

Contributing Author(s)	Karin D. Prummel[1*], Kevin Woods[2,3*], Maksim Kholmatov[1*], Marie Schneider[4], Michael Cross[4], Sinem K. Saka[1], Uwe Platzbecker[4], Judith B. Zaugg[1], Borhane Guezguez[2,3]
Organisation	[1] European Molecular Biology Laboratory (EMBL), Heidelberg, [2] German Cancer Research Institute (DKFZ), DKTK Frankfurt/Mainz, Heidelberg, [3] University Medical Center Mainz, Mainz [4] University Medical Center Leipzig, Department of Hematology and Cell Therapy, Leipzig *equal contribution
Email Address	karin.prummel@embl.de

Poster Title	Harmony Disrupted: Dysregulated Bone Marrow Stromal Cells and the Inflammatory-Stress Induced T Cell Landscape in Clonal Hematopoiesis and Related Myelodysplasia
Abstract	The hematopoietic system relies on cues from bone marrow (BM) niche, guiding hematopoietic stem/progenitor cell (HSPC) differentiation as well as immune responses. Through single-cell studies in mice, key niche subpopulations are identified, including endothelium, smooth muscles, multipotent stromal cells (MSCs), and their derived osteoblasts, fibroblasts, and adipocytes. Nevertheless, the hierarchical and functional heterogeneity of the BM niche in healthy individuals, clonal hematopoiesis (CH), and its progression to myelodysplastic neoplasms (MDS), remain unknown. Furthermore, the influence of CH's and MDS's sustained chronic inflammatory state on the T cell landscape is still unclear. Here, we employed scRNA-seq, proteomics, and imaging to examine the pathological interactions between HSPCs, stroma, and T cells. We focused on the $CD34^+$ HSPC, ($CD271^+$) MSC, endothelial, and $CD3^+$ T cell populations from a curated cohort of matching BM liquid and trephine biopsies of a prospective longitudinal CH/MDS study. Through high-resolution imaging, we found significant BM remodeling in CH (n=9) compared to healthy non-mutation carriers (n=10), which was dependent on the clonal size of mutant HSCs and accompanied with emergence of inflammatory-biased MSCs and expansion of sinusoidal endothelium. These stromal changes were exacerbated in MDS (n=16), in addition to an accumulation of exhausted regulatory T cells. scRNA-seq (n=10) not only supported our observations but also identified stress-induced MSCs in CH and enrichment of inflammatory IFN-responsive cytotoxic T cells in MDS. Additionally, proteomics of an MSC-MDS co-culture model confirmed various inflammatory and pro-angiogenic processes were affected. We are currently further investigating the spatial organization of the stromal and T cell subpopulations by setting up multiplexed imaging (immuno-SABER) and uncovering the transcriptome of sensitive and rare stromal cell types, including endothelium, through targeted spatial transcriptomics (10X Xenium, Light-Seq). Altogether, these discoveries serve as the basis for mapping BM niche alterations in CH and MDS, which is critical for understanding the pathological interplay between HSPCs/MSCs/T cells toward potential therapeutic interventions.

Contributing Author(s)	Nathalie Agudelo-Dueñas[1], Julia Lyudchik[1], Caroline Kreuzinger[1], Mojtaba R. Tavakoli[1], Giulio Abagnale[2], Julia M. Michalska[3], Christoph Sommer[1], Johann G. Danzl[1]
Organisation	[1] Institute of Science and Technology Austria (ISTA), [2] St. Anna Children's Cancer Research Institute (CCRI), [3] E11 Bio
Email Address	nathalie.agudelo@ist.ac.at

Poster Title	Visualizing the transcriptional landscape with tissue context
Abstract	Biological systems are intrinsically heterogeneous, from the level of molecular arrangements and interactions to whole tissue organization. To understand the complexity of these systems, it is fundamental to study them in their native context, which requires assessing their intricate structure and function in a spatially informed manner. Over the last decade, there has been a rapid advancement in the field of *spatial omics*, especially at the transcript level measuring gene expression, which has been instrumental in understanding how mRNA distribution and abundance define cell identity and function. This project aims to develop a highly multiplexed and modular methodology for integrated structural and multi-molecular characterization, as a means to visualize the spatial arrangement of the transcriptome with subcellular to tissue context. Given the importance of the compartmentalized organization of mRNAs (*local transcriptome*) in neurons, we apply a 242-gene panel to target neuron-specific transcripts in mouse brain tissue via Multiplexed Error Robust FISH (MERFISH). We also combine the transcriptional information with a morphological readout based on labeling the extracellular domain, which provides us with richer contextual information and allows us to locate mRNAs within distinct neuronal compartments at subcellular resolution. We envision that this technology will enable a more accurate characterization of the local transcriptome, to achieve a better understanding of how neurons respond to their functional demands in both health and disease.

Contributing Author(s)	Daniel Gyllborg, Michelli F Oliveira*, Karen Lai, Syrus Mohabbat, Juan Pablo Romero, Luigi Alvarado, Robert Shelansky, Sara Taylor
Organisation	10x Genomics, Pleasanton, CA, USA
Email Address	

Poster Title	Evaluating Alzheimer's disease-associated gene expression with Xenium in situ analysis
Abstract	Development of effective therapeutic strategies for neurodegenerative diseases such as Alzheimer's disease (AD) relies on a thorough understanding of their underlying cellular and molecular states. While next-generation sequencing technologies like single cell RNA sequencing have contributed significantly to the AD field, the lack of spatial context can limit biological interpretations, particularly in light of AD's strong association with amyloid-beta (Aβ) deposition in the brain. Here, we evaluated AD-associated gene expression in the TgCRND8 mouse model and its wild-type (WT) counterpart, using the Xenium In Situ platform. We designed a mouse brain panel including 248 genes covering major brain cell types, and a 99 gene add-on panel including additional cellular state markers (e.g., activated and disease-responsive microglia, reactive astrocytes), plaque-associated genes, and AD-risk genes curated from the literature. We selected three age-matched timepoints for WT and TgCRND8 mice, corresponding to sparse, moderate, and severe Aβ plaque burden. Coronal FFPE brain sections (5 µm) were prepared on the Xenium slides, then gene-specific padlock probes were hybridized to the target mRNA. Following a rolling circle amplification step, the gene-specific amplification products were decoded and analyzed on the Xenium instrument. With Xenium, we accurately mapped canonical neuronal layer markers previously described in the literature and annotated major brain cell types. Our results showed differences in cell proportions between WT and TgCRND8 mice over time. TgCRND8 mice showed a decrease in neuronal cell populations and an increase in astrocyte and microglia populations, particularly in reactive glial cells. Moreover, we found an enrichment of activated glial cells in TgCRND8 mice compared to WT mice at 13+ months of age. By overlaying Xenium data with an Aβ immunofluorescence-stained image of a serial adjacent section, we mapped plaque-associated genes in the vicinity of Aβ plaques in TgCRND8 mice, which were downregulated in the WT mice. Our results demonstrate that Xenium is a powerful tool to resolve AD-associated gene expression at both single cell and spatial resolution.

Contributing Author(s)	Sebastian G. Gilbert, Prof. Iain Styles, Christopher Bagnall, Arthur Lewis, Dr Fabian Spill
Organisation	Centre for Doctoral Training in Topological Design, University of Birmingham and AstraZeneca
Email Address	sgg897@student.bham.ac.uk

Poster Title	Quantifying cellular interactions with topological biomarkers
Abstract	Evidence shows that, throughout their life, a person will develop tumours which are identified and eradicated by normal immunological mechanisms, without notice or consequence by the individual. However, in many cases when 'normal' immunological responses fail, we observe the detrimental results as cancer. Multiplex immuno-fluorescence imaging identifies the proximity of many different immune cell types within the tumour microenvironment. Designing mathematical and computational methods to interpret spatially-resolved ex-vivo datasets can lead to crucial ways in understanding and predicting disease progression. We introduce a branch of mathematics, called topology, to measure colocalization and identify communities within these multi-layered cellular networks. Topology is a branch of mathematics that studies the properties of objects preserved under continuous transformations, such as networks, shapes, spaces, and the relationships between them. We apply techniques from Topological Data Analysis to the coordinates of multiple cell types, which benefits from methods robust to noise, stability between heterogeneous samples, and an intuitive reduction of multi-dimensionality; providing innate metrics to identify cellular interactions associated with tumour growth and progression. In one such dataset, of colorectal carcinoma surgical resections, we have determined patients whose tumours proceeded into remission had pronounced colocalization between M2 phenotype macrophages and tumorous epithelial cells, when both presenting the HLA-DR antigen. In addition, we accurately identify tertiary lymphoid structures and quantify analogous immuno-aggregations, known to relate to positive patient outcome. These and similar results propose insights into biological mechanisms beyond current prognostic tools such as grading, multi-satellite stability/instability, and Immunoscore®.

Contributing Author(s)	Jiaji George Chen, Joselyn Chávez, Matthew O'Brien, Irzam Sarfraz, Eddie Ruiz, Pratishtha Guckhool, Guo-Cheng Yuan, Ruben Dries
Organisation	[1]Boston University School of Medicine, MA, US, [2]Icahn School of Medicine at Mount Sinai, NY, US
Email Address	rdries@bu.edu

Poster Title	Giotto Suite: a multi-scale and technology-agnostic spatial omics analysis framework
Abstract	New and emerging spatial omics technologies continue to advance the study of the role of tissue architecture and morphology in specific biological processes, such as the establishment of cellular phenotypes or crosstalk between cells. With recent commercialization efforts and academic advancements in platforms that provide spatial imaging or sequencing based solutions, the range of spatial resolutions and information has widened significantly. From genome-wide information in coarse grained arrays down to the acquisition of individual transcript coordinates at subcellular resolution and unprecedented sensitivity. In addition, the number of different molecular analytes that can be profiled keeps increasing, and includes RNA, but also protein, chromatin accessibility, and DNA. Integrating these modalities which represent different, but interconnected, layers of the cell regulatory network is often key in understanding intricate biological processes. Together, these technologies and methods promise to offer unprecedented insights that can lead to unparalleled opportunities in both discovery and clinical research. However, handling multiple modalities or different scales of spatial information, including tissue architecture, niches, or cell morphology, in a non-trivial and scalable manner is currently a substantial challenge. Here, we present Giotto Suite an open-source project that overhauls our previous efforts with Giotto to create a completely technology agnostic framework that can represent virtually any type of spatial dataset and provide scalable and extendible solutions from raw data processing to visualization. Giotto Suite disentangles morphology, spatial and feature information to create a responsive and flexible framework to analyze spatial data at multiple resolutions. By building interoperability interfaces and creating data structures that overlap the established fields of genomics and spatial data science in R Giotto Suite aims to create an immersive ecosystem of spatial data analysis and tool development. We demonstrate the flexibility and use of Giotto Suite on several state-of-the-art spatial technologies and illustrate the unique features of our framework and how it can be easily extended to create custom engineering solutions for spatial data analysis.

Contributing Author(s)	Verena M. Prade, Miljenka Vuko, Andrea Storti, Mari Heininen-Brown, Helen Angell, Tze Heng Tan, Günter Schmidt, Andreas Spitzmüller
Organisation	AstraZeneca Computational Pathology GmbH, Munich, Germany
Email Address	

Poster Title	Border correction is crucial for calculating spatial readouts in whole slide tissue samples
Abstract	Spatial statistics allows for an examination of spatial patterns and dependencies within biological samples, aiding in understanding tissue structure and organization. However, bias and distortion can arise when analyzing spatial data near the boundaries of a study area. Examples of such boundaries are the borders of tissue sections or image artifacts. It is possible to exclude regions affected by border effects, especially if the remaining data is sufficient for analysis. However, when dealing with small sample areas heavily impacted by border effects, like biopsies or fine needle aspirations, correcting border effects rather than removing affected data is crucial. An example of a metric affected by border effects is the spatial proximity score calculated to account for the effect radius of antibody drug conjugates (ADCs) around receptor positive cells. ADCs target antigen expressing cells, get internalized where their cytotoxic drug payload is cleaved off, at which point the payload can relocate and affect surrounding cells regardless of their expression. Here we first demonstrate the impact of border effects and isotropic correction on a spatial proximity score calculated entirely on random simulated data. Correcting the spatial proximity scores leads to a median 72% improvement compared to the ground truth. We finally demonstrate the applicability of our method on spatial data from 20 PD-L1 expressing non-small cell lung cancer (NSCLC) whole slide tissue sections from the Tumor Immune Cell Atlas (TICA). Our results suggest that while border effects can have a large impact on spatial data, methods for border correction can compensate for them and should routinely be considered when performing spatial tissue analysis.

Contributing Author(s)	Sebastiano Cultrera di Montesano, Ondrej Draganov
Organisation	Institute of Science and Technology Austria
Email Address	sebastiano.cultrera@ist.ac.at; ondrej.draganov@ist.ac.at

Poster Title	Capturing spatial patterns with topological methods
Abstract	Given a collection of cells of various biological types with known spatial coordinates, how can we quantitively describe their intertwined spatial arrangements? Can certain spatial patterns in, e.g., a tumor microenvironment be algorithmically summarised? We will present a new topological descriptor that can be used to extract spatial features from such systems. The method builds on techniques from the mathematical field of topological data analysis.

Contributing Author(s)	François Rivest[1], Victor de Gautard[1], Vytautas Navikas[1], Nadine Nelson[2], Bastian Nicolai[1], Erica Geneletti[1], Joanna Kowal[1], Saska Brajkovic
Organisation	[1]Lunaphore Technologies SA, Tolochenaz, Switzerland, [2]Abcam, Cambridge, United Kingdom
Email Address	

Poster Title	Sequential Immunofluorescence protocol provides solution for tumor stroma analysis
Abstract	**Background:** The tumor microenvironment (TME) consists of malignant cells and supporting non-malignant cellular and non-cellular components that form the tumor stroma. The tumor stroma plays an important role in tumor progression and has emerged as a modulator of anti-tumor immunity (Salmon et al., Nat Rev Cancer 2019) and responses to therapy (Hirata and Sahai, Cold Spring Harb Perspect Med 2017). As such, several therapeutic approaches have recently been developed to target stromal cells as anti-cancer treatments (Valkenburg et al., Nat Rev Clin Oncol 2018, Bejarano et al., Cancer Discov 2021). In addition, the composition of the TME has been recognized as a prognostic factor for survival in cancer patients (Pagès et al., Oncogene 2011). Current protein-based approaches to characterize and better understand the cell composition of the tumor stroma face many limitations such as reagent availability and lengthy protocols. In this study, we identified a list of 22 markers to characterize non-tumoral immune cells, fibroblasts and endothelial cells in the TME, in a single tissue slide. We propose an approach that overcomes reagent incompatibility and opens new avenues of research of tumor stroma. **Method:** Multiorgan Tumor Microarray (TMA) was interrogated with a sequential immunofluorescence (seqIF™) panel encompassing protein markers enabling characterization of TME. A 22-plex panel was created based on expanding an already established 13-plex panel (CD3, CD4, CD8, CD11c, CD20, CD45, CD56, CD68, aSMA, FoxP3, Ki67, PD1, PD-L1) by adding 9 additional antibodies (CD11b, CD14, CD31, CD47, CK, FAP, LaminB1, SIRPα, Vimentin). Hyperplex immunofluorescent staining was performed using automated staining-imaging COMET™ platform generating ome-tiff images containing 25 layers: DAPI, 2 autofluorescent and 22 marker channels. Postprocessing of images was done with HORIZON™ image analysis software. **Results:** We established a panel of 22 markers that can be analyzed simultaneously on a single tissue slide despite limited variability in primary antibody species. Using seqIF™ protocol allowed the study of colocalization and co-expression of markers not compatible to study simultaneously in the traditional immunofluorescence approach. Our hyperplex data revealed distinct composition of the stromal compartment between different tumor types, highlighting high heterogeneity in the tissue composition and stromal architecture. **Conclusion:** COMET™ platform enabled studying in detail TME components and highlighted heterogeneity of tumor stroma across different tissue types. SeqIF™ protocol lifted the limitation imposed by same species antibodies and allowed simultaneous interrogation of markers' expression preserving their spatial relationship.

Contributing Author(s)	Amir Zaki Abdullah Zubir, Daniel Lambert, Syed Ali Khurram
Organisation	University of Sheffield
Email Address	a.abdullahzubir@sheffield.ac.uk

Poster Title	Spatial transcriptomics of oral squamous cell carcinoma reveals heterogeneity in the tumour and its microenvironment.
Abstract	**Background:** Head and neck cancer is the 8th most common cancer in the UK and 6th worldwide. The number of cases has not seen any reduction over the past decades in the UK and project to rise by 3%. Oral squamous cell carcinoma (OSCC) is the most prevalent head and neck cancer that is characterised by aggressive local invasion and metastasis. Here, we explore the spatial transcriptomics capabilities explore signalling patterns in normal oral mucosa (NOM) and OSCC cancer. **Methods:** FFPE block was retrieved from the OSCC samples archive with normal (n=1) and OSCC cases (n=2) (Charles Clifford Dental Hospital, Sheffield) and were profiled using the 10x Genomics Visium Spatial Gene Expression platform. The tumour and stromal region were annotated by pathologist. Spatial differential gene expression (DGE) analysis was performed using Loupe browser with secondary analysis by using the Seurat package in R. **Results:** OSCC displayed unique transcriptional and signalling profiles in each OSCC patient samples by spatial gene clustering visualised by the Loupe browser. The genotype mapping showing heterogeneity expression in OSCC cluster within the tissue when compared to NOM. DEG analysis for top 10 features of specific OSCC region reveals different profile of upregulated genes signatures in each OSCC tissue with a log2FC > 0.58 and adjusted p-value < 0.01. Highlighted spatial gene features across the OSCC tissue were COL1A1, COL1A2, and COL3A1, where this is only being expressed by the stromal cells in NOM. **Conclusions:** This is the study characterise the spatial gene expression distribution in NOM and OSCCs using 10X Visium technology elucidating heterogeneity of gene expression. Future matched genomic and protein data can better support the transcriptomics evidence. Further investigation of the therapeutic potential of identified genes and their signalling pathways may improve our understanding of OSCC and its outcomes.

Contributing Author(s)	Shani Perera, Anna Wilbrey-Clark, Katy Tudor, Ilaria Mulas, Monika Dabrowska, Sophie Pritchard, James Cranley, Kazumasa Kanemaru, Veronica Kedlian, Nadav Yayon, SGP platform, Kerstin Meyer, Sarah Teichmann
Organisation	Wellcome Sanger Institute
Email Address	jp31@sanger.ac.uk

Poster Title	Spatial Transcriptomics for Human Cell Atlas (HCA)
Abstract	The International Human Cell Atlas (HCA) initiative aims to create comprehensive reference maps of all human cells to further understand health and disease. Our team's research focuses on five complementary study areas: Transcriptional regulation and gene expression, Single-cell genomics, Immunology, Protein complexes and Spatial transcriptomics. Our wet lab support team and Spatial Genomic Platform (SGP) combine expertise from histology and molecular backgrounds to support the rapidly evolving field of Spatial Transcriptomics. Spatial Genomic Technologies such as 10x Visium, Visium CytAssist, Curio Seeker, RareCyte Orion for multiplex protein staining, RNA scope, Xenium technologies are described in the poster which is currently in use at Wellcome Sanger Institute.