Norwich Single Cell and Spatial Symposium 2025

Earlham Institute

# ABSTRACTS

Keynote speaker

**The tapestry of the human prenatal immune system**

**Muzlifah Haniffa**

Wellcome Sanger Institute

The human immune system is extremely complex, comprised of multiple cell types and states interacting in myriad ways to produce diverse cellular ecosystems.

Muzlifah Haniffa will demonstrate the application of spatially resolved single-cell genomics to decode the human immune system in health and disease. In particular, she will discuss how prenatal immunity is formed and its relevance to understanding neonatal, paediatric and adult diseases.

Detailed understanding of the developing immune system is also relevant to improve stem cell therapy and regenerative medicine.

Invited Speaker

**Unveiling the cellular blueprint for efficient photosynthesis in C4 plants**

**Tina Schreier**[1]

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C4 plants capture CO2 and convert it into energy more efficiently than the more common C3 plants, making them better adapted to hot and dry environments. This efficiency is due to a specialised leaf structure called Kranz anatomy that includes two distinct cell types, mesophyll (M) and bundle sheath (BS) cells that are highly connected and work together to concentrate carbon dioxide around the carbon fixation enzyme, Rubisco. Key to this connection are plasmodesmata, tiny channels that link neighbouring cells for metabolite exchange. However, we still do not fully understand how these connections evolved and how they work in C4 plants. My research aims to discover how plasmodesmata develop and function in C4 plants. We study two independent examples of C4 evolution: Gynandropsis gynandra and the Flaveria genus, the latter including species that show a range of evolutionary steps from C3-to-C4 photosynthesis.

Using 3D electron microscopy techniques, we characterised the formation and structure of plasmodesmata that connect these cells in Gynandropsis gynandra - the closest C4 species compared to Arabidopsis. We discovered that the formation of these plasmodesmata was induced by light, raising an exciting hypothesis that their formation is wired to the induction of photosynthesis. We then created G. gynandra lines where plasmodesmata could be inducibly blocked and provided the first functional evidence that plasmodesmata are crucial for C4 photosynthesis.

We examined plasmodesmata number and distribution in the Flaveria genus - containing many C3-C4 intermediate species, which also requires metabolite transfer between M and BS cells. We found a gradual increase in plasmodesmata numbers at M-BS cell interface along the C3-to-C4 evolutionary gradient. We are currently assessing the distribution and structure of plasmodesmata in these species, to understand how they relate to their function in metabolite exchange and photosynthesis.

We are also elucidating the cell biological mechanisms underpinning plasmodesmata formation and other BS cell traits in C4 plants, using the genetic amenability of G. gynandra, and the evolutionary gradient in Flaveria genus, to understand how cell architecture enables greater photosynthetic efficiency. Ultimately, this knowledge could be applied in engineering C4 photosynthesis into C3 crops to boost their productivity.

Selected Speaker

## A high-resolution spatial transcriptomic map of the developing human intervertebral disc

**Christabel T. Dube**[1], Ellie-May Oldfield[2], Syed Murtuza Baker[3], Michael Buckley[2], Judith A. Hoyland[1], Stephen M. Richardson[1]

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The human intervertebral disc (IVD) consists of a central nucleus pulposus (NP) and an outer annulus fibrosus (AF), and functions as a shock absorber and enables flexibility of the spine. During development, the NP is populated by vacuolated notochordal cells, which are gradually replaced by small chondrocyte-like cells. This transition is linked to disc degeneration, suggesting notochordal cells are vital for disc function. However, studying notochordal cells presents challenges due to their loss during tissue dissociation and difficulty preserving RNA quality during the decalcification of bony spinal vertebrae. To address this, we utilised the 10X Visium HD spatial transcriptomic platform to map the human fetal spine at 5, 7, 8, 12, and 14 weeks post-conception. At 5 weeks, the notochord appeared as an unsegmented rod, with cells expressing high levels of TBXT, CD24, and KRT8. By 7 weeks, we observed heterogeneity within the notochordal population. A peripheral second population of cells expressing CRIP1, TPPP3, TAGLN, TNXB, and CD44 surrounded the central segmenting notochord, suggesting that these cells play a role in regulating segmentation and cell migration. Notably, this peripheral population was absent after segmentation at 9, 12, and 14 weeks. Additionally, we identified LGR6 (Leucine-rich repeat-containing G protein-coupled receptor 6) as a novel marker for developing notochordal cells, with reduced LGR6 expression as development progresses. LGR6 may serve as a potential marker for early notochordal progenitor cells. In summary, our data presents a high-resolution spatial map of the molecular and cellular processes underlying spine formation and IVD development, offering new insights into their behaviour within their natural microenvironment.

Selected Speaker

**Elucidation and targeted engineering of plant nitrate-responsive gene circuits**

**Zhengao Di**[1], Ashleigh Lister[1], Yao-Min Cai[1], Sam Witham[1], Wilfried Haerty[1], Iain Macaulay[1], Nicola Patron[1,5]

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Nitrogen is a critical nutrient for plant growth and yield. While external N has facilitated modern agriculture, over-application of N-containing fertilizers has drastic ecological and environmental consequences. Quantitative traits in crops are typically determined by the actions of suites of genes working combinatorially within complex gene regulatory networks (GRNs), which often contain partially redundant nodes and numerous network motifs that define and finetune network dynamics. In a recent project, we elucidated a regulatory circuit acting upstream of the critical *NIN-LIKE PROTEIN-7 (NLP7)* transcription factor and its conservation and divergence in plant lineages. An outstanding question is how the regulatory networks function across cell types to coordinate changes in root development and nitrogen metabolism. By applying single-cell RNA sequencing, we observed the enrichment of *NLP7*-related sub-networks in specific groups of cells in the root. The resulting network models provide a framework for targeted engineering to increase plant nitrogen use efficiency. To this end, we are applying Cas-based transcriptional activators and repressors with cell-specific promoters to investigate the importance of observed cell-specific expression patterns, and precisely manipulate the N-responsive networks within the most relevant cells.

Invited Speaker

**Scaling of spatial imaging transcriptomics for broad applications using 10x Genomics Xenium**

**Kenny Roberts**[1], Katy Tudor[1], Minal Patel[1], Ian Johnston[1], Andrew Bassett[1]

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The explosion of spatial transcriptomics technologies over the past decade has revolutionised biology, providing an evermore detailed picture of cells and gene expression in biological context. A key challenge for all technologies in this field is applicability to diverse samples in a high-throughput high-reproducibility manner that generates data in a format easily interrogated by researchers and the scientific community. We have built a spatial transcriptomics pipeline around the 10x Genomics Xenium platform in order to serve the broad scientific needs of the Wellcome Sanger Institute including cell atlasing, neurodegeneration, cancer genomics, infectious disease, and evolutionary genomics.

Thus far we have processed over 1700 tissue sections, mapping over 40 billion transcripts across 100 million cells from diverse mammalian tissues, including all major human organ systems across different life stages including healthy and diseased donors, as well as mouse and marmoset.

Applicability to archival (pathology) samples allows retrospective analysis of decades of diseased samples in addition to prospective samples from clinical trials, and on-going adult and developmental tissue cohorts.

Furthermore, we are working to broaden the questions and models that may be addressed using Xenium by diversifying both input sample types and assay targets. We have piloted the application of both mid-plex (480-plex) and high-plex (5000-plex) assays to in vitro models (organoids and cells), and devised a novel protocol for the application of Xenium to PAXgene-fixed paraffin embedded (PFPE) material. We are interested in mapping single nucleotide variants (SNVs) to atlas tissue clonality and perform pooled cell screens, and have generated promising preliminary data mapping SNPs in cell lines. Finally, we have trialled the detection of bacterial transcripts across different sample types with the aim of mapping host-pathogen interactions in the human lung.

Invited Speaker

**Introducing Cell organization and disturbance-mapping using image activated cell-profiling (CODIAC) as a novel tool for high content screening**

**Melanie Krause**[1], Dan Lou[2], Shengdi Li[1], Daniel Kirrmaier[2,3], Krisztina Gubicza[2], Daniel Schraivogel[1], Lars Steinmetz[1*] and Michael Knop[2,3*]

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Protein localization and abundance is a key mechanism to regulate cell homeostasis. Furthermore, diseases are frequently associated with aberrant protein localization. However, high-throughput methods that monitor changes of many proteins at once are missing. Recently, image enabled cell sorting (ICS) has been demonstrated to add spatial and morphological information to classical cell sorting technology. We utilize ICS in combination with improved Cas12a PCR tagging to develop new ways to characterize proteome localization and expression levels within complex cellular pools. Applying ICS to fluorescently tagged protein libraries and machine learning, we have devised a novel way to assess cell organization and to map disturbances using image activated cell-profiling (COPDIAC). Using image-derived measurements from ICS, we group and isolate cells with fluorescently-tagged proteins of similar visual phenotypes. Sorting cell pools in their native state as well as upon chemical perturbation, we are able to identify changes in protein localization and abundance in a pooled fashion at a much faster pace than previously established. We hope for this method to have broad applications in the field of high content screening for the identification of novel drug targets and various medical uses.

Keynote speaker

**Discovering Genomes and Activities of Individual Microbial Cells and Viruses in the Wild**

**Ramunas Stepanauskas**

Bigelow Laboratory for Ocean Sciences

The advent of genomic sequencing of individual microbial cells has profoundly enhanced our comprehension of the coding potential and evolutionary trajectories of uncultured microbes. This technology has also illuminated the extent of genomic variability in natural environments and the complex interactions within microbial communities. This presentation will delve into the recent breakthroughs in this field, with a special focus on the research conducted by my group. I will begin by outlining the fundamental concepts and methodologies employed in microbial single-cell genomic sequencing. I will also provide examples of their application in various microbiology research areas and study environments. The presentation will conclude with a glimpse into the future, with emerging techniques for integrated single-cell genome and phenome analyses and massively parallel sequencing of extracellular genetic elements.

Keynote speaker

**Integrating single cell and spatial transcriptomics in healthy and pathological lungs**

**Pascal Barbry**

CNRS/Inserm/Université Côte d'Azur, IHU Respirera – 3IA Côte d'Azur – IPMC, Sophia Antipolis, France

The Human Lung Cell Atlas defines the healthy respiratory system as a complex cellular ecosystem composed of nearly 80 different cell types. Chronic respiratory diseases often alter the cellular composition of tissues, which can be characterised by multiple spatial and single-cell transcriptomic technologies. I will describe the construction of different atlases of pathological human lungs after collection of biopsies from patients and matched healthy controls, in which altered composition and different gene expression can be documented, with emphasis on the essential quality of initial cell annotation. Merscope and Xenium spatial transcriptomics were used to delineate precise cellular interactions after transferring single-cell information with scMusketeers, a home-made automatic annotation tool optimised for detecting rare cell types. A comprehensive analysis framework has also been developed to analyse RNA splicing at single-cell resolution, based on sequencing data generated by Nanopore sequencing. These different aspects illustrate how different artificial intelligence techniques can contribute to a better understanding of normal and pathological lung function.

Invited Speaker

**Single-cell multiomics of healthy pediatric bone marrow reveals age-related differences in lineage differentiation linked to stromal signaling**

**Mirjam Belderbos**

Princess Máxima Center Utrecht

Childhood represents a critical window for hematopoietic development and the onset of hematologic diseases. However, comprehensive single-cell maps of healthy pediatric bone marrow (BM) remain limited. To characterize hematopoietic changes from infancy through adolescence, we generated an extensive multi-modal single-cell atlas capturing mRNA and surface protein expression of 90.710 BM cells, including >20.000 hematopoietic stem/progenitor cells and >500 mesenchymal stromal cells, from seven healthy children and two young adults. We demonstrate that pediatric BM undergoes dynamic developmental shifts, transitioning from B-lineage dominance in early childhood to a myeloid- and T-lineage bias in adolescence and young adulthood (AYA). These transitions were validated using spatial transcriptomics of BM biopsies. We identifytwo distinct lymphoid progenitors (LyPs) subsets driving this shift: CD127+ LyPs with B-lineage output, enriched in early childhood, and CD127- LyPs with lymphoid and myeloid features, more prevalent in AYAs. Importantly, age-dependent changes in stromal signaling, mediated by IL-7 and TGF-β, correspond with these this shift, indicative of cell-extrinsic regulation of HSPC fate decisions during development. This study provides a high-resolution resource for understanding normal hematopoietic development and potential early-life perturbations underlying pediatric hematologic diseases.

At the conference, I will present our findings on healthy pediatric bone marrow and discuss ongoing studies investigating HSPC–niche crosstalk in the context of specific developmental and disease-related perturbations.

Invited Speaker

**Unravelling RNA Structure in Space: Imaging and Spatial Transcriptomic Innovations**

**Susan Duncan,** Zongyun Yan, Eva Wegel and Yiliang Ding

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Messenger RNA structure represents a critical layer of gene regulation, contributing to transcript stability and translation efficiency. Although recent advances have expanded our ability to probe RNA structure, two key challenges remain: capturing this information in a spatially resolved, cell type–specific manner, and achieving the subcellular resolution necessary to understand its role in RNA localisation. This talk will cover two complementary methods that aim to address these challenges in plants. The first is a sequencing-based spatial transcriptomic approach designed to reveal cell type–specific RNA structures across complex tissue architectures. The second is a super-resolution imaging method that reveals RNA concentration across subcellular compartments. Together, these approaches can help reveal the spatial principles by which RNA structure modulates gene expression across cells and tissues.

Selected Speaker

## Predicting Antibody Affinity using gene expression markers

**Michele Chirichella**[1^], Matthew Ratcliff[1], Shuang Gu[1], Ricardo Miragaia[2], Massimo Sammito[3], Valentina Cutano[4], Suzanne Cohen[5], Davide Angeletti[6^], Xavier Romero-Ros[5^†] and Darren Schofield[1^†].

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Single-cell technologies help identifying the enormous B-cell repertoire arising from immunisation, dramatically increasing the number of antibodies available to develop new drugs. However, selecting the highest affinity clones from thousands of sequences is not a straightforward feat. Here we hypothesise mechanisms governing affinity maturation in germinal centres might be crucial to predict antibody affinity. To address this, we leveraged seven independent single-cell datasets of antigen-specific B-cells from both in-house and public sources. These included mouse spleen-derived antibodies and human B-cells from PBMCs of COVID-19 patients. By integrating BCR sequences and gene expression (GEX) data with affinity measurements from hundreds of clones, we identified and validated a novel transcriptomic affinity signature, High Signature, with predictive power for both mouse and human antibodies. High Signature was derived using differential GEX methods and machine learning approaches, such as Topic Model Analysis and Multinomial Logistic Regression. High Signature enriched clones with sub-nanomolar affinities in *de novo* immunization campaigns up to three times with respect to randomly picked antibodies and phylogenetic tree clustering, bypassing the need for sequence analysis. Mechanistically we identified RUVBL2, an AAA+ ATPase involved in cell cycle, DNA repair and transcription, as the main driver of High Signature, suggesting a possible role in the positive selection of higher-affinity B-cells for its targets. Besides immediate applications in antibody discovery, our work has the potential to provide a novel tool to study germinal centre-dependent autoimmunity and identify the most autoreactive clones involved in autoantibody production.

Link to manuscript pre-print: https://www.biorxiv.org/content/10.1101/2025.01.15.633143v1

Selected Speaker

**Expression quantitative trait locus mapping in recombinant gametes using single nucleus RNA sequencing**

**Matthew T. Parker**, Samija Amar and Korbinian Schneeberger

Dept. of Chromosome Biology, Max Planck Institute for Plant Breeding Research, Cologne, Germany

Phenotypic differences between individuals of a species are often caused by differences in gene expression, which are in turn caused by genetic variation. Expression quantitative trait locus (eQTL) analysis is a methodology by which we can identify such causal variants. Scaling eQTL analysis is costly due to the expense of generating mapping populations, and the collection of matched transcriptomic and genomic information. We developed a rapid eQTL analysis approach using single-cell/nucleus RNA sequencing of gametes from a small number of heterozygous individuals. Patterns of inherited polymorphisms are used to infer the recombinant genomes of thousands of individual gametes and identify how different haplotypes correlate with variation in gene expression. Applied to Arabidopsis pollen nuclei, our approach uncovers both *cis*- and *trans*-eQTLs, ultimately mapping variation in master regulators of pollen development that affect the expression of hundreds of genes. This establishes snRNA-sequencing as a powerful, cost-effective method for the mapping of meiotic recombination, addressing the scalability challenges of eQTL analysis and enabling eQTL mapping in specific cell-types.

Selected Speaker

**Analysis of single-cell somatic copy number variations in the brains of Parkinson's disease patients and healthy controls**

**Zeliha Gozde Turan*,** Ester Kalef-Ezra*, Dominic Horner, Caoimhe Morley, Diego-Perez Rodriguez, Vanda Knitlhoffer, Ben Thompson, Alexandra Pancikova, Lucia Friscioni, Andy Goldson, Iain Macaulay, Thierry Voet, Zane Jaunmuktane, Fritz Sedlazeck, Jonas Demeulemeester, Christos Proukakis

* Equal contributions

Parkinson's disease (PD) is the most common neurodegenerative movement disorder, yet its etiology remains incompletely understood. While the heritability of PD is estimated at ~30%, this has prompted investigations into additional contributing factors, including somatic copy number variations (CNVs). To explore the role of somatic CNVs in PD, we performed low-coverage singlecell whole genome sequencing after whole-genome amplification with PicoPLEX. We analyzed 11 PD and 10 control post-mortem brains. Using fluorescence-activated nuclei sorting, we isolated neuronal and non-neuronal nuclei from the cingulate cortex (CC), and both oligodendrocytes and other cells—including neurons—from the substantia nigra (SN), the most affected region. We sequenced ~4,500 single nuclei, with 3,768 (85%) passing quality control. CNVs ranging from 500 kb to whole-chromosome were detected using Ginkgo with extensive filtering. Although not statistically significant, the overall proportion of CNV-bearing cells was higher in controls compared to PD (48.68% vs. 44.33%; p = 0.35). In the SN, neurons with gains were slightly more frequent in PD than in controls (57.51% vs. 47.06%; p = 0.3), whereas oligodendrocytes with gains were significantly less frequent in PD (56.14% vs. 34.26%; p = 0.029). Key PD genes were only occasionally affected. Ongoing analyses include quantification of CNV burden (size and number per cell) across cell types and disease groups, and gene set enrichment analysis to identify pathways disrupted in PD. This study provides novel insights into the somatic genomic landscape of PD and highlights cell type-specific alterations, contributing to a more nuanced understanding of the disease's complex pathology.

Grants: This research was funded in whole by Aligning Science Across Parkinson's [564669] through Michael J. Fox Foundation for Parkinson's Research.

Selected Speaker

**Transcriptional Profiling of Embryonic Diapause in Turquoise Killifish: Characterizing Diapause Stages, Stress Response, and Development Resumption**

**Haoyu Wang**[1], Jürg Bähler[2]

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Diapause II in the turquoise killifish (*Nothobranchius furzeri*) is a reversible arrest of embryonic development, yet its cellular architecture and molecular hallmarks remain poorly defined. Here, we construct an integrative transcriptomic atlas by profiling ~36,000 cells with Parse Biosciences single-cell RNA-seq and performing bulk RNA-seq on embryos in five conditions: normal development, early diapause, long-term diapause, heat-stressed diapause, and timed exit from diapause.

Using large language model based cross-species annotation against zebrafish embryonic markers, together with deep-learning–based heterogeneous graph neural networks, we refine cell-type identities and delineate the principal embryonic systems: (1) central nervous system, (2) epidermal and placodal system, (3) hematoendothelial-circulatory system, (4) mesenchymal and neural-crest system, (5) musculoskeletal (somites), and (6) notochord and axial system.

Although cellular composition remained largely conserved, long-term diapause decreased the proportions of Central Nervous System and Epidermal & Placodal System cells while increasing the Hematoendothelial Circulatory System population. Our findings reveal that cell-type-specific gene expression signatures predominate over condition-specific signatures, demonstrating cellular identity stability even during dormancy and heat stress. We identified robust upregulation of autophagy-related genes and Polycomb repressive complex components (particularly EZH1), coupled with decreased TOR signalling, with highest expression levels in neural, mesenchymal, and notochordal cells. Tissue-specific bulk RNA-seq analysis revealed that head tissue mounted a weaker yet more variable transcriptional response during long-term diapause compared to early diapause.

Collectively, our findings illustrate how scalable single-cell genomics and cross-species computational approaches can unravel the cellular complexity of vertebrate diapause, providing a comprehensive framework for studying dormancy.

Invited Speaker

## Exploring Wheat Grain Development with Single-Nucleus Sequencing and Spatial Transcriptomics

**Chen Ji**[1*], Emilie Knight[1], Samuel Burrows[1], Holly Kitley[1], Rosa Whittingham[1], Andrew Goldson[2], Vanda Knitlhoffer[2], Katie Long[1], James Simmonds[1], Carlo de Oliveira Martins[1], Rob Ellis[1], Iain Macaulay[2], Burkhard Steuernagel[1], Gerhard Saalbach[1], Cristobal Uauy[1], Philippa Borrill[1]

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Wheat (*Triticum aestivum*) grain filling is coordinated with cell expansion that significantly enlarges the grain size. During this stage, the accumulation of starch and protein relies on the efficient transport of nutrients and signals between tissue compartments. Although different tissues (e.g. pericarp, endosperm) have been identified in the grain, we have limited knowledge about the diversity of cell types within these tissues and how they function.

In this study, we use spatial transcriptomics and single-nucleus RNA sequencing to examine cell expansion, endosperm development, and nutrient accumulation in wheat grains at 12 and 18 days post-flowering. Using Stereo-seq spatial transcriptomics, we pioneered the profiling of gene expression patterns and identified 12 functional cell clusters distributed within the grain, including the aleurone layer, modified aleurone, and embryo-adjacent endosperm (EAS) which are key transport interfaces during grain filling. Using our spatial gene expression data we constructed a comprehensive model describing sucrose transport, as well as starch storage dynamics. Also we provide an online resource that allows visualization of gene expression across the entire grain section using gene IDs.

We present a case study demonstrating the utility of our spatial transcriptome approach in quickly identifying functionally relevant members from a gene family. We identified that sucrose transporter 1 (*SUT1*) exhibited specific expression in the aleurone, modified aleurone and scutellar epithelium and was the only sucrose transporter gene with this unique expression pattern. TILLING mutants in *SUT1* exhibited disrupted sucrose transport and grain filling defects, leading to abnormal grain development and a significant reduction in kernel weight. These findings illustrate the power of resolving gene expression at the single-cell level in wheat grains and provide insights into improving wheat grain nutrient content.

Keynote speaker

**Molecular trajectories underlying quiescence exit and re-entry among adult neural stem cells**

**Tanya Foley,** Jules Samaran, Isabelle Foucher, Gaëlle Letort, David Morizet, Nicolas Dray, Laura Cantini, Laure Bally-Cuif

Adult neurogenesis among vertebrates is a continuous process sustained by the activity of neural stem cells (NSCs). Adult NSCs retain the capacity to proliferate and differentiate, generating neural progenitors (NPs) that further differentiate to neurons. To preserve NSCs over time, NSC divisions are followed by phases of quiescence (G0), a dynamic and reversible state of cell cycle arrest.

To understand the molecular dynamics of quiescence among NSCs, a major research aim of our laboratory, we use the adult pallium (dorsal telencephalon) of the zebrafish brain as a model, where NSCs are identical to their mouse counterparts but more numerous. Quiescent pallial NSCs are transcriptionally heterogeneous and vary in quiescence depth, or resistance to activation cues, suggesting complex cellular trajectories during quiescence. Using scRNA-seq, we recently described a continuum of transcriptional substates, named q1-q5, thought to differ in quiescence depth and lineage progression. Now, we aim to explore the cellular trajectories between these transcriptional substates.

Using multiplexed single-molecule RNA fluorescence in situ hybridization (smRNA-FISH) in the whole mount pallium, a 12-gene transcriptomic code was used to transfer substate identities q1-q5, derived from scRNA-seq, onto cells measured in situ. We combined this with BrdU-labelling of proliferating cells to trace dividing NSCs and their progeny at varying chase times post-division, allowing for quiescence durations post-division to be estimated and for cellular trajectories associated with quiescence re-entry to be deduced. Our findings suggest that immediately post-division, NSCs returning to quiescence enter a transient state of deep quiescence in which they become highly resistant to activation. Resistance to activation resolves over time as quiescent NSCs gradually transition to shallower quiescence substates, undergoing morphometric changes associated with increased likelihood for self-renewal upon division. This work exemplifies how transitioning between single-cell-omics and smRNA-FISH, combined with BrdU-based time-stamping in situ, can be used to read time in situ and reconstruct transcriptomic trajectories in the adult brain while maintaining kin and spatial relationships. Our observations reveal previously unknown molecular trajectories between transcriptional substates among quiescent NSCs and highlight the importance of dynamic regulation of quiescence depth to balance neurogenesis with self-renewal over time.

Poster

**MobiuSCOPE: A novel single-cell RNAseq library construction method to achieve full-length RNA Information with short-read sequencing**

**Marisa L. Amato, PhD**[1]

[1] Singleron Biotechnologies

Accurate and comprehensive RNA sequencing at the single-cell level is crucial for understanding cellular heterogeneity, gene regulation, and the complexities of disease mechanisms. Traditional short-read methods capture either the 5’ or 3’ end of transcripts, missing critical information such as alternative splicing, gene fusion, and allele-specific expression. This limitation obscures cellular transcriptome complexity, hindering biomarker and therapeutic discoveries.

MobiuSCOPE overcomes these challenges by enabling a full-length RNA sequencing solution that captures the entire mRNA transcript at the single-cell level. Using the SCOPE-Chip, thousands of cells are partitioned into microwells, where mRNA from each single cell is captured and barcoded. An optimised reverse transcriptase (RT) generates full-length 3’ barcoded cDNA, each carrying unique cell barcodes and UMIs. A circularisation step combined with subsequent reverse PCR brings the 5’ sequence closer to the cell barcode and UMI, allowing the construction of 5’ barcoded cDNA. Sequencing libraries are constructed by random fragmentation of both 3’ and 5’ cDNA pools and ligating the fragments to Illumina sequencing adaptors. A proprietary bioinformatics pipeline then reconstructs full-length transcripts with the same cell barcode and UMI, yielding full-length transcript information.

By combining advanced molecular barcoding with a highly sensitive workflow, MobiuSCOPE minimizes technical biases while ensuring high precision and reproducibility. Its scalable approach enables the analysis of hundreds to thousands of single cells, making it ideal for applications such as detecting splicing isoforms, fusion genes, expressed mutations, and SNPs under diverse physiological conditions.

Keywords: MobiuSCOPE, single-cell RNA sequencing, full-length transcriptome, alternative splicing, molecular barcoding, cellular heterogeneity, transcriptomic profiling.

Poster

**Towards dual individual and cell-type identification in complex mixtures using a single-cell multi-omic approach**

**Diya Balloo**[1], Vanda Knitlhoffer, Andrew Goldson, Lesley Ives, David Wright, Michelle Grey, Nick Dawnay, Katherine Brown, George Zouganelis, Ardhendu Behera, Michael Chen, Lorna Dawson and Iain Macaulay

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Conventional forensics DNA profiling uses bulk DNA extraction, resulting in the destruction of cells and therefore any information about the cell-of-origin. Crucial information about the cell-types retrieved could help to indicate the nature of transfer of the DNA. Bulk approaches make deconvoluting DNA mixtures from multiple contributing individuals challenging. This could lead to a perpetrator's DNA signature remaining undetected and suspects being falsely implicated. Cell imaging and RNA-seq data may help to link the identity of the contributor to the cell-type.

Our aim is to demonstrate proof-of-principle that a single-cell multi-omics approach, Genome and Transcriptome Sequencing, can be applied to identify individuals from mixed cell populations using genomic data and to identify cell-types from RNA-seq data.

Single cells from a mixture of PBMCs from 6 individuals were sorted via spectral flow cytometry into a 96-well plate. Genomic DNA and mRNA from the cells were separated and then amplified and sequenced in parallel.

DNA data and RNA (whole transcriptome) can be retrieved from a single cell, with many thousands of transcripts detected with RNA data, enabling cell-type identification. Due to stochastic events, only partial genomic data profiles were recovered, rendering complete deconvolution of the mixture challenging.

We demonstrate feasibility of a multi-omic assay in a mock forensic setting. Improvements in DNA and allelic recovery from each cell will enhance the capability to deconvolute mixtures. We aim to test the approach on more complex mixtures of cell-types — for example, different epithelial cell sources and sperm cells.

Poster

**Meta analysis of published scRNA-Seq data to provide a more complete understanding of aortic ageing**

**Alice Bradford** and Derek Warren

School of Chemistry, Pharmacy, and Pharmacology, University of East Anglia

Aortic compliance, the ability to change shape in response to changes in blood pressure, is essential for healthy cardiac output and peripheral blood perfusion. Wall stiffness and elasticity are key determinants of aortic compliance. As we age, compliance decreases, leading to increased cardiovascular disease risk. Aortic single cell sequencing (scRNASeq) has vastly increased our understanding of cellular behaviour during aortic ageing. Vascular Smooth Muscle cells (VSMCs) are the major cellular constituent of the aortic wall. Previously thought to exist in two distinct phenotypes (quiescent and synthetic), we now know that these cells are progenitor-like and can differentiate along several different cellular lineages. We set out to access and compare published aortic scRNA-Seq datasets to identify potential genes of interest to support our in vitro studies. This proved a more complex task than originally considered. We found major inconsistencies between cell identification, cell number, and cellular content. In order to compare these studies, we created a standardized methodology that included assigning cell type based on a robust gene list by including shared genetic markers between multiple published datasets. We also found potential inconsistencies in cell isolation steps, as determined by a lack of vsmcs in some data sets. Our analysis revealed consistent cell type identification in healthy and diseased samples across species. We found across studies very little overlap in enriched gene expression. However, with GO/KEGG pathway analysis we identified robust pathways present in different vsmc populations across species both in health and disease.

Poster

**Single-Cell Transcriptomic Profiling Reveals Cell-Type-Specific Consequences of TDP43 proteinopathy in ALS.**

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons, with multiple cell types involved in the disease onset and progression. However, the cell-type specific molecular changes remain poorly understood. To address this, our lab developed a human iPSC-derived neuronal model that recapitulates TDP43 proteinopathy, a hallmark of ALS. In this model, the mislocalization of endogenous TDP43 from the nucleus to the cytoplasm was induced without overexpression or any external stressors. This model captures key pathological features including cytoplasmic aggregation, splicing alterations and microRNA dysregulation – providing a relevant model to understand cell-type specific molecular mechanisms at a single-cell level.

We used single cell sequencing to profile transcriptomic changes across neuronal cell types upon TDP43 mislocalization. Following quality control, we performed clustering and annotated distinct cell types using canonical markers. Furthermore, differential expression analysis revealed cell-type specific transcriptomic changes in pathways involved in RNA processing, cytoskeletal organization, and stress responses, with motor neurons specifically showing impaired axonal maintenance and actin regulation.

We performed Weighted Gene Co-expression Network Analysis and functional enrichment analysis, identifying disease relevant co-expression network modules. Notably, modules enriched for RNA-binding proteins and mitochondrial function were disrupted in ALS-affected motor neurons and V1 interneurons. Together, our findings underscore the ability of single-cell transcriptomics in uncovering nuanced, cell-type-specific responses to TDP43 proteinopathy.

By understanding disease-associated transcriptional networks at cellular resolution, this study provides novel insights into ALS pathogenesis and highlights potential molecular pathways for therapeutic targeting.

Poster

**in-situ sequencing in 3D**

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We aim to elucidate three-dimensional aspects of the human skin organoids as models for disease, developmental and differentiation processes. These have three-dimensional structures and a variety of cell types: ectoderm, mesoderm, hair follicles, vasculature, a rudimentary nervous system organization etc. The visualization of these structures is critical for their understanding in a spatial context.

Exploration of tissue to single-cell resolution is currently achieved by using commercial methods (e.g. the 10x Genomics Xenium platform). This is limited to thin, essentially 2D 10 µm sections, which are processed individually, with the output information stacked bio-informatically to reconstruct the 3D makeup of the tissue. This method allows for medium throughput, at considerable cost.. Organoids can grow to millimeter-size, and only a limited number of sections can be explored for cost reasons. In addition, 3D reconstructions of 2D sections will never be exact due to losses in sectioning, and distortions of shape.

Our method of direct RNA in situ sequencing is instrument-free, non-commercial, and open source. Its flexibility is advantageous for protocol development, in this case to increase the thickness of input sections to reach increasing levels of 3D. It utilizes a low number of specifically chosen gene transcripts, and detects a limited number of transcript-specific amplification products, e.g. 20 per cell. While this qualitative detection method can’t be used for quantifying transcript abundance, it can be supplemented with whole-transcript sequencing of a proportion of the tissue. Eventually, we envisage a degree of automation to increase the throughput of the process and reduce hands-on time.

Poster

**From shape to sequence: Exploring diversity in bone marrow megakaryocytes with single-cell morpho-transcriptomics**

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Megakaryocytes (MKs) are highly polyploid, bone marrow and lung resident cells, producing more than 1 million platelets every second. They are a very diverse population of 2n-128n polyploid cells. To date, very few studies have investigated the cellular and molecular features associated with the *in vivo* polyploidisation process in mammals. Therefore, single-cell resolution analysis is key to provide biological insights into the cellular heterogeneity of MKs when studying the intrinsic and extrinsic factors determining their function.

Current single MK isolation protocols do not record information about cell morphology, limiting the discrimination of MKs containing/attached to other blood cells and platelets decorating these other non-MK cells. We established a robust MK isolation method using high-throughput image-based flow cytometry sorting that, combined with the AI-based tools we are designing and optimising, will allow us to integrate morphological and ploidy information with transcriptomic analysis (morpho-transcriptomics).

We are applying this multiomic approach to study *in vivo* the effect of stressors like acute inflammation and dietary interventions on megakaryocyte ploidy, morphology and gene expression. This will provide an unprecedented understanding of the transcription regulation in polyploid genomes and consequences for function in health and disease, with the long-term goal of contributing to develop diagnostic, preventive and/or therapeutic tools.

Poster

**Whole Genome Single-Cell Sequencing for Recombination Mapping in Arabidopsis Thaliana**

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Meiotic recombination is a fundamental process driving genetic diversity through crossovers, reciprocal exchanges of genetic information between homologous chromosomes. Crossovers are crucial in plant breeding, as they can break up linkages and allow the creation of novel allele combinations. Breeders are therefore highly invested in identifying and controlling the mechanisms underlying crossover formation and distribution in plant genomes. Traditional approaches to studying recombination patterns require extensive backcross populations, making them time-consuming and resource-intensive. Here, we adapt a single-cell whole genome sequencing platform to work with Arabidopsis thaliana and map meiotic recombination events in individual pollen nuclei with high resolution. This approach reduces the number of plants needed for recombination studies and eliminates the need for multiple generations of crosses. By analyzing individual meiotic products, our method provides direct insight into recombination mechanisms and their regulation. The pipeline performs doublet detection and makes use of a specialized hidden Markov model to accurately identify crossover events, resulting in high-resolution recombination maps that match the recombination landscapes obtained through previous approaches. This single-cell approach allows the analysis of thousands of individual meiotic products, opening up new possibilities to rapidly screen meiotic mutants and characterize genetic factors influencing recombination.

Poster

**Spatial Transcriptomic analysis of Ovine Pulmonary Adenocarcinoma.**

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Ovine pulmonary adenocarcinoma (OPA) is an infectious tumour caused by Jaagsiekte Sheep Retrovirus (JSRV), which infects type II pneumocytes within the lung alveoli and transforms them into tumour cells. OPA is a chronic disease, which typical remains subclinical until tumours are advanced, resulting in major welfare and economic issues for the sheep industry. OPA tumours have previously been characterized by immunohistochemical and bulk RNA-sequencing experiments. In addition to producing valuable information for OPA for veterinary research purposes, this bulk transcriptomic data also highlighted the potential for OPA as a model for human lung adenocarcinoma. Combined, this immunohistochemical and transcriptomic data aided in the design of a custom Nanostring GeoMx probe set, enabling the first spatial transcriptomic analysis of lung samples containing OPA. Using antibodies that label the JSRV envelope protein (anti-SU) and type II pneumocytes (anti-DC-LAMP) to visualize the tissue, regions of interest were selected. The target RNA was collected for differential expression analysis of cells within and adjacent to OPA tumour, from healthy tissue distal to the tumour, and from healthy controls. The Nanostring analysis identified differentially expressed genes (DEGs) that mirrored the bulk RNA-seq analysis and confirmed spatial aspects of previously described histopathology, validating the characterization of OPA so far. Importantly, significant DEGs were identified between SU+ and SU- cells within OPA tumour foci, and between tumour foci and histologically unaffected regions of the lung. This study provides new information on the tumour microenvironment of OPA, which will inform the design of future disease control strategies.

Poster

**Optimisation of Long read single nuclei RNA-seq in TRACERx**

**Lacey Luke,** Jones Thomas, Ward Sophie, Mcgranahan Nicholas

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Cancer development is an evolutionary process, where genetic and non-genetic alterations generate diverse cellular states and intra-tumour heterogeneity (ITH). Whilst research has traditionally focussed on genomic alterations, more recent work has highlighted the significance of non-genetic factors in shaping tumour evolution. However, mapping these events to the clonal architecture of tumours remains a significant challenge. Here we evaluate, identify, and describe a streamlined workflow for single-nuclei, long-read RNA-seq (LR-snRNAseq) from snap frozen tissue. In brief, nuclei are isolated from snap frozen tissue using either the Frankenstein protocol and FACS, or the Miltenyi GentleMACS OCTO dissociator and anti-nucleus beads. Nuclei from two different patient samples are pooled together and cDNA is generated using the 10x Chromium GEM-X Single Cell 3' kit. The cDNA libraries undergo a biotinylated-streptavidin bead pull down protocol and amplification before entering SQK-LSK114. Libraries are subsequently sequenced on a PromethION flow cell for 110hrs. This works enables us to utilise a novel cohort of multi-region LR-snRNAseq paired with multi-omic bulk sequencing data from the TRACERx NSCLC cohort to perform highly detailed genotypephenotype mapping in single cells and query the evolution of non-genetic events in primary lung tumours.

Poster

**Process and validation of an RNA barcode library**

**Yuxuan Lan**, Eleonora Tassinari, Erica Hawkins, Sonia Fonseca, Edyta E. Wojtowicz, Carolina Grandellis, Wilfried Haerty

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Stem cells are essential for maintaining tissue homeostasis and repair in multicellular organisms, as they provide a renewable source for replenishing differentiated cells throughout life. To study stem cell activity, it is necessary to label individual stem cells and track the progeny they produce—known as a clone. Several genetic approaches have been developed to enable such labelling, one of which involves delivering a synthetic DNA fragment, or barcode, into the genome of a stem cell using a viral vector. To construct a medium-sized collection of barcodes, we have implemented engineering biology techniques combined with automation. Here, we analyse a library of approximately 2,000 barcodes to assess its complexity, the representation of individual barcodes, and the efficiency of barcode integration into cells. In our pipeline we subtract barcodes using regular expression, followed by merging similar sequences using the Levenshtein distance. Next, we include unique barcodes in the list of barcodes present in the library.

Poster

**An Interactive Shiny App for Biomarker Identification: Cell-Type-Specific HVGs & Functional Analysis in Single-cell RNA-Sequencing**

**Omid Mohajeri,** Independent Bioinformatics Researcher

Single-cell RNA sequencing is a powerful approach for uncovering cellular diversity and functional states in complex tissues. I present a custom-built Shiny application designed to make scRNA-seq analysis more accessible and interactive, with a focus on immune checkpoint blockade response in kidney cancer.

The app is built on over 2,000 lines of R code, integrating widely used packages for single-cell analysis, enrichment, annotation, and interactive visualisation. The app allows users to upload standard 10X Genomics output files (gene matrix, barcodes, gene names, and metadata), automatically merge the metadata, and perform preprocessing steps, including QC filtering and normalization. Next, users can access the “Inclusion” tab, which enables metadata-based subsetting. This feature dynamically displays metadata columns (e.g. ICB Response, Cell Types, etc.), allowing users to filter cells by specific values. For instance, selecting ICB_PD includes only progressive disease cells, enabling the identification of highly variable genes (HVGs) within that biological context. The selected subset of cells is then used to identify HVGs. In the HVG tab, users can define the number of HVGs to identify, download them as a CSV file, and explore them through visualisations, including scatter plots of all selected genes and top features, and a summary table for further inspection. The final step performs comprehensive functional analysis, offering both Gene Set Enrichment Analysis (GSEA) and Over-Representation Analysis (ORA) for Gene Ontology (GO) terms and KEGG pathways, with visual outputs including dot plots, Cnet plots, enrichment maps, heatmaps, upset plots, barplots, word clouds, bubble plots, and chord diagrams.

Poster

**Gaining Clinically-Relevant Insight into Early-Stage Autoimmune Liver Diseases using Single Cell Technologies**

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Primary sclerosing cholangitis (PSC) is a rare liver disease affecting 7 in 100,000 people in the UK. Is it an inflammatory condition affecting the biliary tree, where progressive scarring of the bile ducts can lead to cirrhosis and end-stage liver disease. Previous research to date has suggested PSC is an autoimmune disease, however there are no known effective treatments. Liver transplantation is the only option (accounting for 11% of UK liver transplantation) and up to 16% recur in their new liver. 70-80% of patients with PSC have inflammatory bowel disease (IBD), a clinically and genetically different disease to IBD without PSC, however the link between the liver and colon in PSC is poorly understood. Previous single-cell and spatial research in PSC and other autoimmune liver diseases, including primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH), have focused on end-stage disease, where liver explant tissue is used for experimental work. Research studies into ‘early’ stages of autoimmune liver diseases are lacking. My PhD project has focused on optimising the primary human research workflow at Norwich Research Park for collecting and processing very small clinical samples for single-cell research. I have recruited patients under the care of Norfolk and Norwich University Hospitals (NNUH) with PSC, PBC and AIH to collect disease-affected tissues from clinical biopsies: liver, colon and bile duct tissue. After optimising tissue dissociation protocols, I have used the 10x Genomics platform at Earlham Institute for single-cell RNA sequencing. We aim to gain clinically relevant insight into these diseases at an ‘early’ stage. We have focused on characterising tissue-resident immune cells, comparing immune cell infiltrate across all disease-affected tissues, aiming to contribute to finding a drug target for this devastating disease.

Poster

**Profiling Stomatal Cell-Specific Immune Responses in Wheat Against Zymoseptoria tritici through Single-Nucleus RNA Sequencing**

**Wirya Suputra**

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*Zymoseptoria tritici* is the causal agent of septoria tritici blotch (STB), a major threat to global wheat production, leading to substantial yield losses. Since the infection of *Z. tritici* depends on stomatal penetration, stomatal resistance plays a crucial role as a frontline against pathogen invasion. Gene-for-gene interactions within the *Z. tritici*–wheat pathosystem, exemplified by *Stb6* and *AvrStb6*, have demonstrated that *Stb*-mediated resistance functions specifically at the point of stomatal penetration. Previous studies have employed bulk RNA-seq on infected leaf tissues to explore stomatal resistance. However, they lack the resolution required to capture cell-specific dynamics, particularly within guard and subsidiary cells during pathogen invasion. This study aims to investigate stomatal immunity during infection by utilizing several methods. Beginning with developing a methodology for leaf infections and enriching for stomata-fungal contacts, followed by epidermal peels of infected leaves. Subsequently, we will develop the protocol for snRNA-seq that works well with wheat leaves. Ultimately, the single-nucleus sequencing data will be processed and analysed using bioinformatics pipelines to interpret differentially expressed genes and cell-type-specific transcriptional patterns related to stomatal immunity. Keywords: Single-nuclei RNA sequencing, plant resistance, *Zymoseptoria tritici*, stomatal immunity, transcriptomic.