DANISH SINGLE-CELL NETWORK
NEWSLETTER

JUNE - 2023

THIS MONTH'S HIGHLIGHTS:

Hallmarks of transcriptional intratumour heterogeneity across a thousand tumours

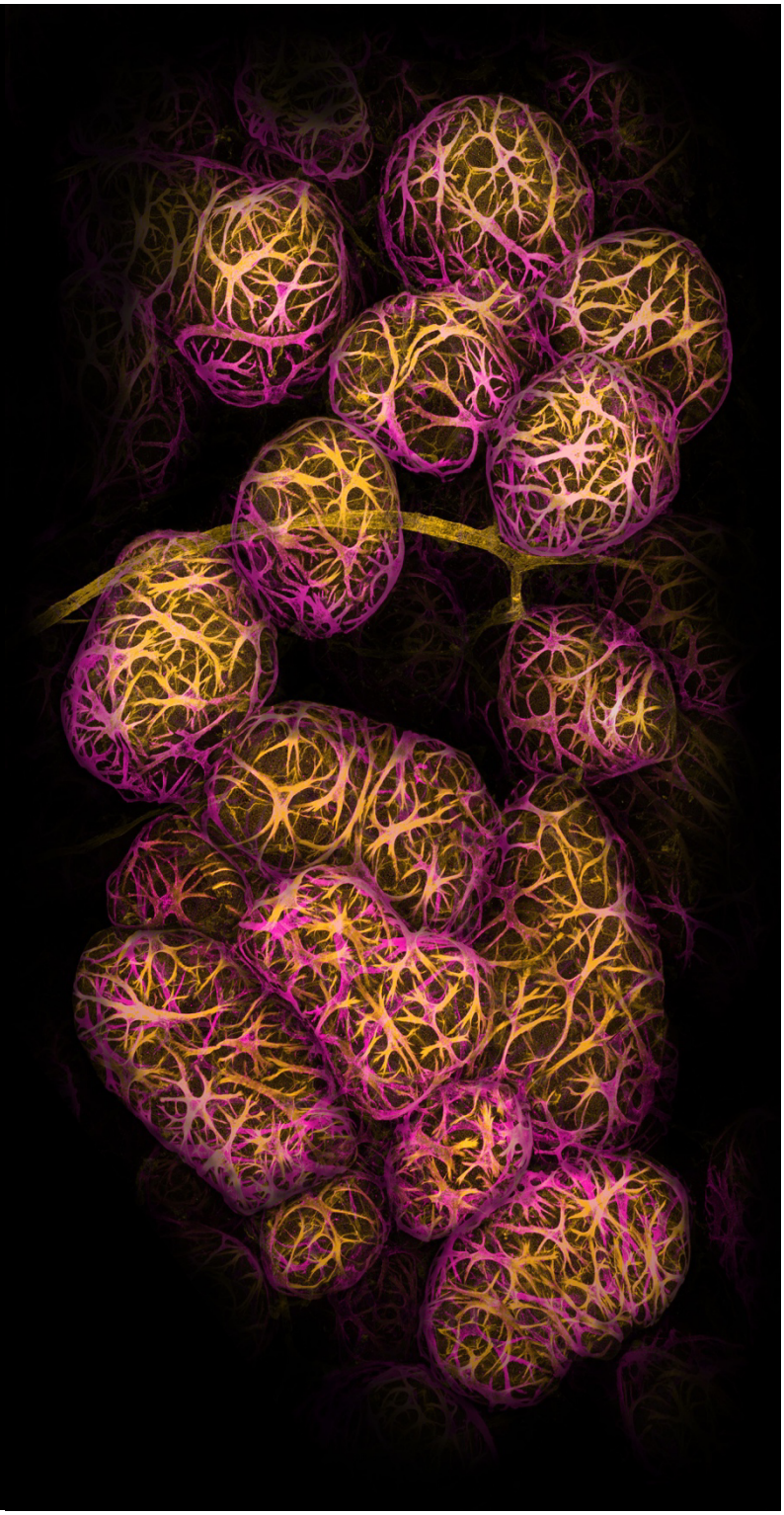
Gavish, A., Tyler, M., Greenwald, A.C. et al.

Spatial proteomics in neurons at single-protein resolution

Niu, M., Cao, W., Wang, Y. et al.

Comparative analysis of cell–cell communication at single-cell resolution

Wilk, A.J., Shalek, A.K., Holmes, S. et al.



COVER IMAGE
Author:
Caleb Dawson
The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia

Title: Breast tissue showing contractile myoepithelial cells wrapped around milk-producing alveoli (2022)

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Hallmarks of transcriptional intratumour heterogeneity across a thousand tumours

Gavish, A., Tyler, M., Greenwald, A.C. et al. *Nature* (2023), https://doi.org/10.1038/s41586-023-06130-4

Cancer is a complex disease, and understanding the diverse cellular states within tumours is crucial for developing effective treatments. This article presents a comprehensive analysis of intratumour heterogeneity (ITH) in cancer through the integration of data from 77 different studies, covering 1,163 tumour samples across 24 tumour types. The authors curated, annotated, and integrated the single-cell RNA sequencing data to reveal patterns of transcriptional ITH. They identified 41 consensus meta-programs, consisting of genes that are coordinately upregulated in subpopulations of cells within many tumours. These meta-programs represent diverse cellular processes, including both generic and lineage-specific patterns, which are mapped into 11 hallmarks of transcriptional ITH. The study finds that most meta-programs of carcinoma cells resemble those identified in non-malignant epithelial cells, suggesting that a significant proportion of malignant ITH programs exist even before oncogenesis, reflecting the biology of the cells' origin. The analysis is extended to non-malignant cell types, allowing for the mapping of cell-cell interactions within the tumour microenvironment. The authors argue that dividing global cell states into intertumour and intratumour heterogeneity helps to make sense of the high degree of cellular heterogeneity observed in cancer. Intertumour differences reflect accumulated genetic and epigenetic aberrations acquired during the oncogenic process, while intratumour differences primarily reflect recent events shaping the state of cells. The study highlights predictable subpopulations of cells in certain tumours, suggesting the potential for new therapeutic strategies targeting these specific cellular states. The authors also acknowledge potential limitations, such as the inability to capture rare patterns, limitations in detecting small-scale expression programs, and the focus on mRNA rather than proteins or metabolites.

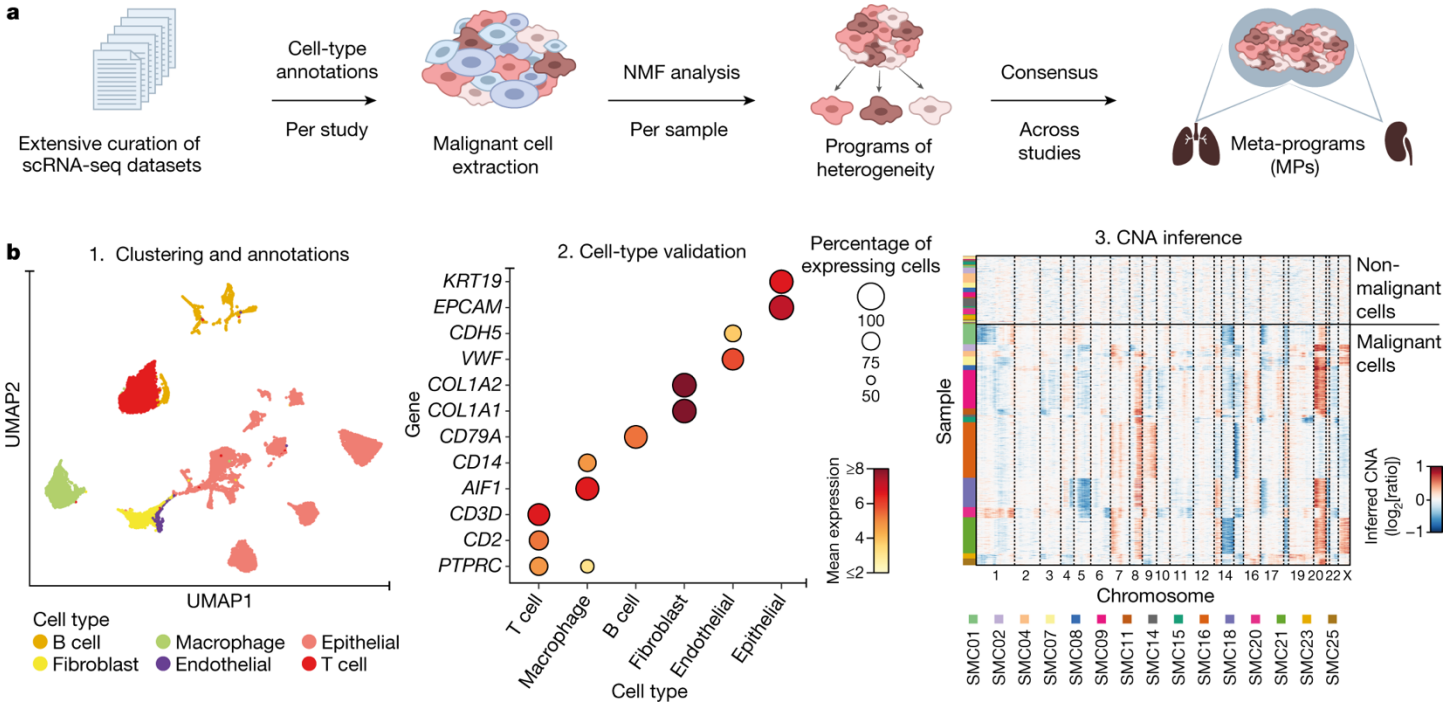

Figure 1. Defining MPs from a curated and annotated collection of scRNA-seq datasets.

Spatial proteomics in neurons at single-protein resolution

Unterauer, E.M., Shetab Boushehri, S., Jevdokimenko, K. *et al.* *bioRxiv* (2023),
https://doi.org/10.1101/2023.05.17.541210

The researchers developed Secondary label-based Unlimited Multiplexed PAINT (SUM-PAINT), a method that revolutionizes super-resolution imaging. SUM-PAINT decouples the DNA barcoding of target molecules from the imaging process using a primary barcode and secondary label. This approach enables virtually unlimited multiplexing while maintaining the throughput improvements achieved through optimized sequence design. By transiently binding dye-labeled "imager" strands to complementary "docking" strands on target molecules, SUM-PAINT allows for conceptually unlimited multiplexing and high-resolution imaging. With SUM-PAINT, the researchers achieved unparalleled spatial resolution and throughput for a virtually unlimited number of protein targets. They developed an analysis workflow that incorporated advanced machine learning and omics-inspired approaches to map the multiparameter features of highly multiplexed, high-resolution datasets. This analysis revealed the nano-arrangements of individual proteins at the single-protein level, enabling the most extensive multiprotein dataset of neurons to date. The dataset comprised up to 30 distinct protein targets, with an average localization precision of 6.6 nm.

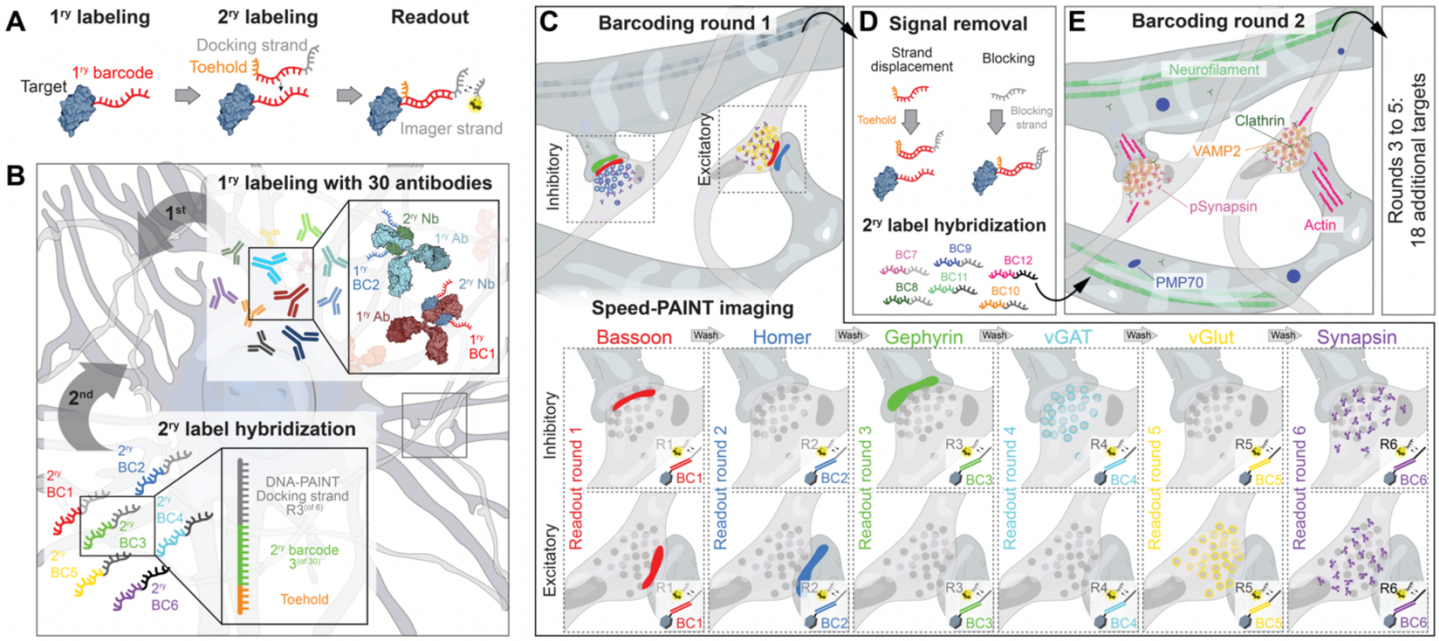

Figure 2. Secondary-label-based DNA-PAINT enables highly multiplexed imaging of single proteins

Applying SUM-PAINT to neurons, the researchers uncovered remarkable details of neuronal architecture, including spectrin rings, clathrin pits, and synapses, with exceptional spatial resolution. Notably, they discovered a novel mixed synaptic subtype that combined elements of both excitatory and inhibitory synapses. This subtype exhibited a precise molecular alignment on a single-protein level, challenging traditional understanding of neurotransmission. The researchers hypothesized that these synapses may be chemically inactive or transitioning to a fully excitatory state through undiscovered mechanisms. Additionally, the researchers characterized the details of excitatory, inhibitory, and mixed synapse types using various features such as interprotein cluster distances, protein distribution shape and volume, and correlation matrices. They found that certain proteins displayed varying correlations with different synaptic subtypes, indicating their potential differential expression and role in human pathologies.

Comparative analysis of cell–cell communication at single-cell resolution

Wilk, A.J., Shalek, A.K., Holmes, S. *et al. Nature Biotechnology* (2023), https://doi.org/10.1038/s41587-023-01782-z

This article describes a framework called Scriabin that enables comparative analysis of cell-cell communication (CCC) at the level of individual cells in single-cell RNA sequencing (scRNA-seq) data. Existing CCC methodologies often aggregate expression values at the cell type or cluster level, potentially obscuring valuable information. Scriabin leverages the single-cell resolution of the data to maintain the full structure of CCC heterogeneity and specificity. The framework allows the identification of rare communication pathways and the analysis of early dynamic communication events. It addresses the challenge of data inflation in CCC analysis, where pairwise calculations between cells become computationally prohibitive. Scriabin implements two workflows that avoid subsampling and aggregation, preserving single-cell resolution data. By doing so, it increases the likelihood of detecting biologically meaningful differences in CCC pathways.

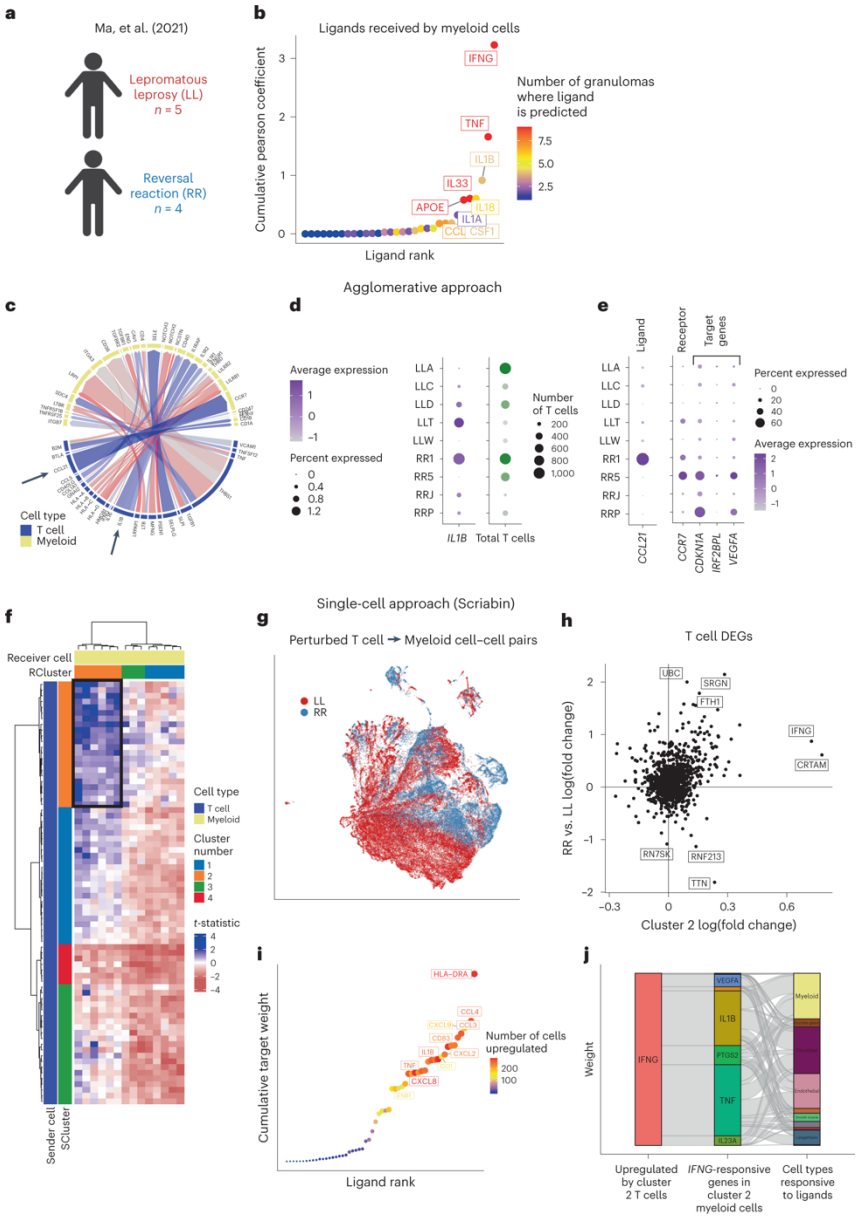

Figure 3. Scriabin reveals communicative pathways obscured by agglomerative techniques.

The study demonstrates how aggregation and subsampling can obscure subsets of T cells in SCC (Spatial Cell Composition) and in leprosy granulomas, potentially leading to inaccurate conclusions. Scriabin introduces workflows for large-scale comparative analyses and dataset alignment strategies to identify cells of interest and maximize communication perturbation detection. It also introduces a scalable workflow that focuses on common patterns of ligand-receptor pair co-expression, enabling downstream analyses with a comprehensive view of CCC structure. The framework can be applied to longitudinal datasets, allowing the analysis of CCC pathways within and between timepoints. This enables the observation of how uninfected cells initiate inflammatory pathways, which are later amplified by infected cells. Scriabin also addresses challenges related to zero values in CCC matrices and recommends the use of denoising algorithms for scRNA-seq data.

New papers from Danish researchers:

Pærregaard, S.I., Wulff, L., Schussek, S. *et al*. The small and large intestine contain related mesenchymal subsets that derive from embryonic Gli1+ precursors. Nat Commun 14, 2307 (2023). https://doi.org/10.1038/s41467-023-37952-5

Virshup, I., Bredikhin, D., Heumos, L. *et al*. The scverse project provides a computational ecosystem for single-cell omics data analysis. Nat Biotechnol 41, 604–606 (2023). https://doi.org/10.1038/s41587-023-01733-8

Job announcement

Postdoc in spatial transcriptomic technologies at Department of Veterinary and Animal Sciences

"We are looking for a highly motivated and dynamic researcher for a 21-month position, who holds a PhD and has a background in neuroscience or neurophysiology and single cell/spatial transcriptomics or bioinformatics to commence on the 1st September 2023 or as close to this date as possible."

For more details and how to apply: https://lnkd.in/eDdzSad3 **Deadline to apply, 1st July 2023.**

If you would like to announce anything single cell related, e.g. job announcement, event, your published paper, technology development etc., please contact us.

Contact: katarina.dragicevic@bric.ku.dk

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