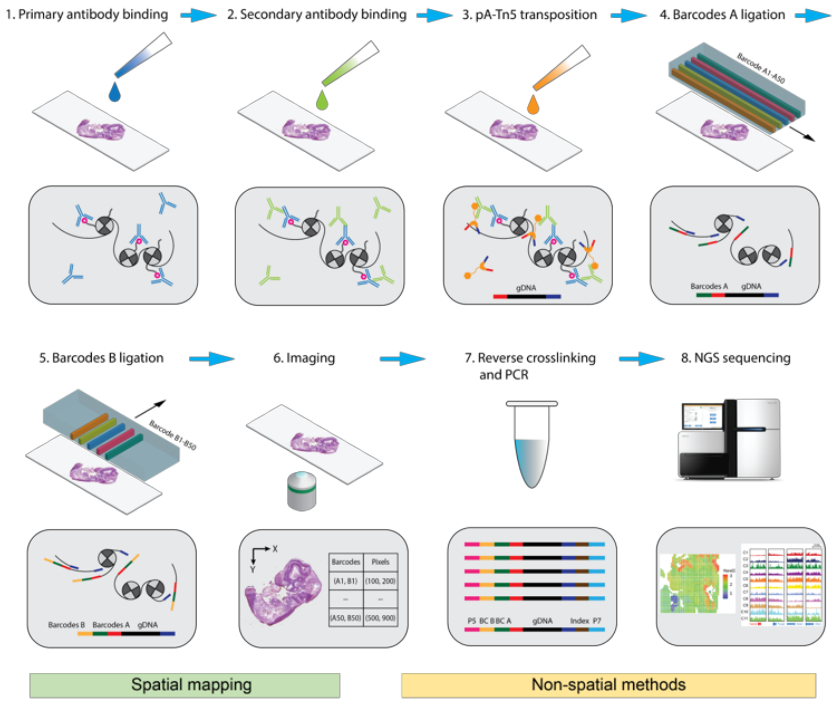
Single Cell Omics

Newsletter April 2021

Paper 1.

Spatial Epigenome Sequencing at Tissue Scale and Cellular Level

Deng et al., *bioRxiv*, 2021.

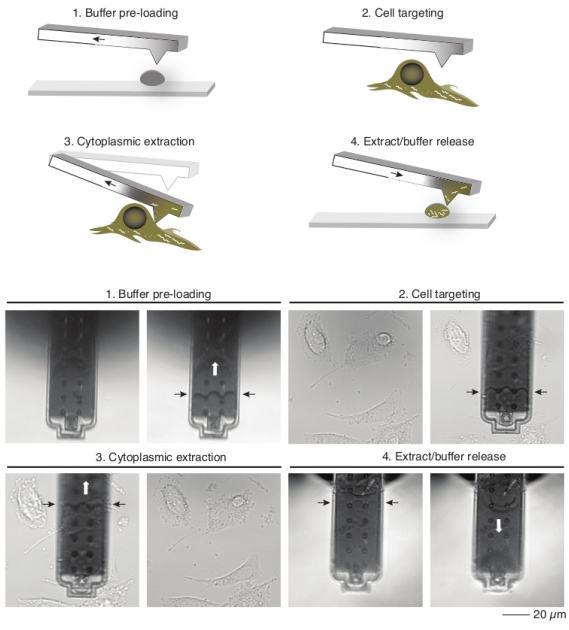


Rong Fan lab released extension to their previous microfluidics-based spatial transcriptomics (DBiT-seq) protocol this time focusing on spatial epigenomics. hsrChST-seq is the first tool to allow access to epigenetic modifications in a spatial tissue context. Spatial epigenome profiling was achieved by combining in-tissue CUT&Tag of the three histone modifications (H3K27me3, H3K4me3, H3K27ac) and microfluidic deterministic barcoding. Spatial chromatin states in mouse embryos or olfactory bulbs revealed tissue type specific epigenetic regulations, in concordance with ENCODE reference data, confirming robustness of the protocol. However, the smallest pixel size used in the protocol was 20 um. Thus, to be truly single cell technique investigators used DAPI staining to select pixels containing only a single nucleus. Thus, implementation of 5-10 um pixel sized chips with bigger surface areas (quite limited as of now) would be logical next step for a wider adoption of this technology.

Paper 2.

Genome-wide molecular recording using Live-seq

Chen et al., *bioRxiv*, 2021.



Single-cell transcriptomics (scRNA-seq) has advanced the ability to characterize cellular heterogeneity in health and disease. However, scRNA-seq requires cell lysis, which makes impossible direct cell state transition tracing. Currently, several available tools to infer cell transitions (RNA velocity, Monocle, etc) are only based on statistical approximations. While tools such as labeling of novel RNA or state tracing with DNA barcodes require cell lysis and are end-point measurements. Thus, authors established Live-seq protocol. It is based on cytosol biopsy from a single cell using fluidic force microscopy that preserves cell viability after RNA extraction.

To analyze cytosolic biopsy authors went all the way and optimized Smart-seq2 protocol (used Maxima H Minus RT, biotin-TSO, biotin-oligo-dT) to work with as low as 1 pg of RNA input.

Based on cell division, functional responses and whole-cell transcriptome read-outs, authors estimate that Live-seq does not induce major cellular perturbations (however it seems logical different cell will have different sensitivity to such treatment). Live-seq was able to distinguish different cell lines from each other and to record cell states, such as LPS activation of macrophages. Furthermore, Live-seq was used to sequentially profile transcriptomes of the same individual macrophages before and after stimulation with LPS, thus enabling the direct mapping of a

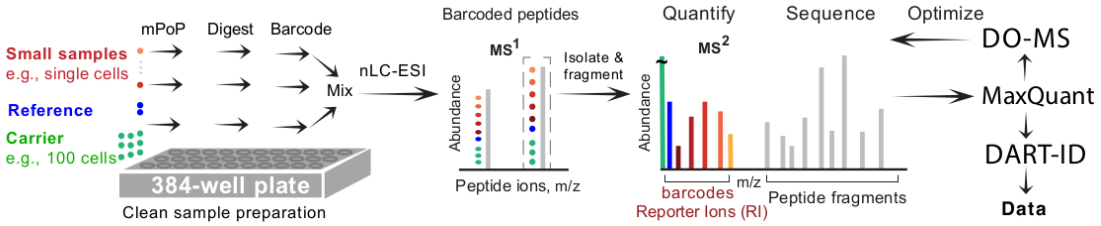
cell's trajectory.

This tool highlights very promising direction of a repeatable transcriptome interrogation of viable cells. But currently it is low throughput technique. It can be used to calibrate other high-throughput trajectory inference techniques, but will benefit from cell sampling automation, and applicability on tissue slices in addition to *in vitro* cell cultures.

Paper 3

Multiplexed single-cell proteomics using SCoPE2

Petelski et al., *BioRxiv*, 2021.



Nikolai Slavov group released new protocol for single cell proteome interrogation. Comparing to the previous ScoPE-MS protocol ScoPE2 introduces enhanced data analytics for optimizing experimental designs and data interpretation (DART-ID and Data-driven Optimization of MS (DO-MS)) and minimal ProteOmic sample Preparation (mPOP) allowing automation while reducing sample volumes. SCoPE2 has increased throughput, sensitivity and quantitative accuracy with lower cost per cell and lower barriers to adoption. It uses inexpensive reagents and is applicable to any sample that can be processed to a single-cell suspension. SCoPE2 is an automated protocol that allows analyzing 200 single cells per 24 hours using standard commercial equipment.

Due to a progress in recent years several protocols of single cell proteome interrogation appeared (including from Matthias Mann and Bo Porse groups), thus the need of in depth comparison between ease of use, sensitivity, accuracy and biological sample applicability of such tools is needed.

Next Single Cell Seminar

30th April 2021, 09:00-10:00, zoom

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