Mapping Cellular Coordinates through Advances in Spatial Transcriptomics Technology

Teves, Joji Marie; Won, Kyoung Jae

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Complex cell-to-cell communication underlies the basic processes essential for homeostasis in the given tissue architecture. Obtaining quantitative gene-expression of cells in their native context has significantly advanced through single-cell RNA sequencing technologies along with mechanical and enzymatic tissue manipulation. This approach, however, is largely reliant on the physical dissociation of individual cells from the tissue, thus, resulting in a library with unaccounted positional information. To overcome this, positional information can be obtained by integrating imaging and positional barcoding. Collectively, spatial transcriptomics strategies provide tissue architecture-dependent as well as position-dependent cellular functions. This review discusses the current technologies for spatial transcriptomics ranging from the methods combining mechanical dissociation and single-cell RNA sequencing to computational spatial re-mapping.

Keywords: cellular communication, single-cell RNA, spatial transcriptomics, tissue architecture

INTRODUCTION

Cell-to-cell communication is essential to maintain proper tissue homeostasis. Disruption of homeostatic cellular communication underlies many pathologic cellular transformations including cancer (Oktay et al., 2015). Studying the complexity of healthy tissue architecture and abnormal transformations both at the cellular and transcriptional level is important in improving the understanding of key pathways that can be targeted for therapeutic strategies. Recently, single cell RNA-sequencing (scRNAseq) technologies have revolutionized our understanding of gene expression by quantifying the transcriptome of individual cells. Moreover, the development of computational approaches to quantify large transcriptomic data alongside scRNAseq technology has provided transcriptomic information for previously uncharacterized cell types and has made it possible to study their dynamics at a population level (Grun et al., 2015; Patel et al., 2014; Pollen et al., 2014; Proserpio et al., 2016; Shalek et al., 2014; Trapnell et al., 2014).

However, scRNAseq technologies have the intrinsic limitation of losing positional information during tissue dissociation into single cells. Positional information is a critical aspect when studying tissue architecture to account for how physically interacting cells and signal exchanges maintain or alter homeostasis. Indeed, several strategies have been employed to provide positional information relative to transcriptomic data. In particular, tissue microdissection followed by RNA sequencing provides approximated positional information based on microdissected fragments (Combs and Eisen, 2013). Moreover, recent advances in a set of techniques collectively called spatial transcriptomics allow positional infor-
## Table 1. Summary of selected technologies for spatial profiling of cells

| Approach | Technology | Input material | Experimental method | Quantification method | Representative detection sensitivity | Detection range | Reference |
|----------|------------|----------------|---------------------|-----------------------|--------------------------------------|-----------------|-----------|
| LCM-seq  | LCM-seq    | Primary mouse brain and spinal cord tissue; Post-mortem human brain tissue | Laser capture microdissection | NGS data analysis (DESeq2 + GO analysis) | ~1,743 to 14,893 genes per 0.1 RPKM | SG to HT | (Combs and Eisen, 2013) |
| FISH-based | smFISH    | A549 and CHO cell line; Primary rat hippocampus neurons; Yeast, *Caenorhabditis elegans*, *Drosophila melanogaster* | Fluorescence imaging + photo-bleaching on fixed cells | Probe-based computational identification of mRNA targets | ~3 mRNA species per cell | SG | (Halpern et al., 2017) |
| MERFISH  |            | IMR90 cell line; U2OS cell line | Multiplexed fluorescence imaging of target probes on fixed cells (+ clearing) | Probe-based encoding + GO analysis | ~100 to 1,000 RNA species per cell | SG to MT | (Lee et al., 2015) |
| seqFISH+ |            | NIH3T3 cell line; Primary mouse brain tissue | Sequential fluorescence of pseudocolor probes | Probe-based encoding + scRNA-seq-based spatial localization mapping | ~10,000 genes per cell | SG to HT | (Kumar et al., 2018) |
| In situ sequencing (ISS) barcode-based | FISSEQ | HeLa, 293A, COS1, U2OS, iPSC, primary fibroblasts and bipolar neurons cell lines; iPSc-derived 3D organoids; Primary mouse embryo and brain tissue; Drosophila embryos | Reverse transcript probes + sequence-by-ligation | Probe-based calling + 3D image deconvolution | ~200 to 400 mRNA per cell; scalable 5X | MT | (McFaline-Figueroa et al., 2019) |
| STARmap  |            | Primary mouse cortical neuron cells; Primary mouse brain tissue | Hydrogel-based isolation of target probes + SEDAL sequencing | Probe-based calling + 2D/3D cell segmentation + differential gene expression analysis | ~160 to 1,020 genes simultaneously; scalable to ~30,000 cells | MT to HT | (Moffitt and Zhuang, 2016) |
| Spatial and single-cell sequencing-based | Spatial reconstruction from single-cell transcriptomics (Seurat) | *Danio rerio* embryo tissue | Tissue dissociation + strand-specific, scRNA-seq modified from SMART protocol | NGS analysis + spatial location inference | Spatial reconstruction from HT 851 single-cell reference | | (Patel et al., 2014) |
| Spatial transcriptomics | Primary mouse olfactory bulbs and brain tissue; Breast cancer biopsy tissue | Spatial oligoT barcode array + cDNA synthesis + RNA-seq | Transcriptome analysis | On surface: 9.6 M unique transcripts per 400 M reads; In solution: 18 M unique transcripts per 290 reads | | HT | (Moncada et al., 2018) |
Spatial Transcriptomics
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One of the earliest methods to study spatial transcriptomics was through the use of tissue microdissection followed by RNA sequencing. In Drosophila embryos, this approach has allowed to uncover spatial-specific gene expression patterns using coordinate-bound cyrosectioning (Fig. 1A) (Combs and Eisen, 2013). Similarly, using 50 to 100 cryosectioned thin slices from zebrafish embryos, the Tomo-Seq method has provided three-dimensional (3D) spatial expression patterns with the aid of computational reconstruction of the zebrafish tissue architecture (Junker et al., 2014). The microdissected slices were further used to the reconstruction of murine brain, providing a 3D image of gene expression (Okamura-Oho et al., 2012).

Advancements such as laser capture microdissection (LCM) enabled a precise capture of targeted cells, or even single cells, while retaining intact tissue structure (Fig. 1B) (Datta et al., 2015). Subsequently, combining LCM and RNA sequencing was used to resolve spatially bound transcriptomic profiles of rare cell population (Nichterwitz et al., 2016). Comparably, geographical position sequencing (Geo-seq) is technique combining LCM with scRNAseq (Chen et al., 2017; Xue et al., 2019). Moreover, LCM has been used in various applications to provide position-based transcriptional information. For instance, LCM followed by RNAseq in mouse intestinal epithelium revealed the transcriptome of spatially zoned areas along the villus axis, which leads to spatial reconstruction of the tissue from scRNAseq data (Moor et al., 2018). LCM enables accurate separation of a small number of cells while preserving the tissue morphology. However, LCM procedures are labor-intensive and expensive to perform (Chung and...
FLUORESCENCE IN SITU HYBRIDIZATION (FISH) APPROACHES FOR SPATIAL TRANSCRIPTOMICS

Multiplexed image-based transcriptomics is an emerging technology for spatial detection of RNAs. Particularly, in situ hybridization followed by microscopic analysis of intact tissues has the potential to provide direct information regarding the spatial organization of the cell’s transcriptome (Levsky and Singer, 2003). This approach utilizes fluorescent-labelled nucleotide probes to detect specific mRNAs localized at different spatial coordinates in a cell or tissue. Single molecule FISH (smFISH) is a pioneering method that uses many short oligonucleotide probes to target mRNAs (Raj et al., 2008). smFISH provides the cell-to-cell variation in transcript abundance and even the subcellular localization of a given RNA (Raj et al., 2010). However, the number of RNA species that can be simultaneously measured by smFISH is limited. To increase the number of detected RNA species, combinatorial fluorescence has been developed to visualize 10 or more genes simultaneously (Jakt et al., 2013). smFISH can obtain spatial information of targeted mRNA with high sensitivity and low false positives or false negatives. However, smFISH is limited by technical factors regarding probe design and binding such as relatively low signal intensity and tendencies towards nonspecific probe binding. This limitation was improved through the development of click-amplifying FISH (clampFISH), which utilizes bio-orthogonal click-chemistry to fix a padlock-style probe/target interaction that subsequently enhances the specificity and fluorescence of signal amplification (Rouhanifard et al., 2018).

Another FISH technology development includes a cyclic approach to single-molecule fluorescence in situ hybridization methodology (osmFISH) wherein multiple smFISH rounds are repeatedly applied to increase the number of detected RNA species (Fig. 1C) (Codeluppi et al., 2018). In osmFISH, the number of targets becomes the number of fluorochrome channels multiplied by the number of hybridization cycles thus, significantly increasing the number of transcripts quantified in each round. In a recent study, osmFISH profiled 33 genes over 13 imaging rounds in mouse somatosensory cortex (Codeluppi et al., 2018). Similarly, sequential FISH (seqFISH) utilizes sequential labelling of mRNAs using a set of FISH probes designed with a single type of fluorophore, generating a barcode of color labels (Fig. 1C) (Lubeck et al., 2014). For this, mRNA hybridization is performed at each round using the same FISH probes but labeled with a different dye. Therefore, seqFISH can scale the number of targets exponentially for each round. SeqFISH has been applied to detect thousands of transcripts per cells in mouse hippocampus using multiple colors (Shah et al., 2016). However, seqFISH is hampered by the optical density of each targeted transcript, which limits the number of detectable RNAs despite the multiplexing capacity. In an improved version, SeqFISH+ made it possible to probe 10,000 genes by solving the optical density problem using ‘pseudocolours’ to dilute cellular RNAs and enabling spatial reconstructions at high resolution (Eng et al., 2019).

Alternatively, the multiplexed error-robust FISH (MERFISH) technology, instead of barcoding with different color combinations, determines the presence or absence of fluorescence using a two-stop hybridization protocol (Fig. 1C) (Moffitt and Zhuang, 2016). Each RNA specie is encoded with a N-bit binary word from N rounds of hybridization that shows the presence or absence of a color. MERFISH, as well as other multiplexed smFISH-based RNA profiling methods, requires non-overlapping signals from individual RNAs. Improvements in microscopic detection combined with MERFISH has been used to overcome technical drawbacks such as signal detection and background correction (Wang et al., 2018a). Spatial information resolution was increased when MERFISH was combined with high-content imaging of sub-cellular structures to accurately determine the compartmentalization of RNAs (Xia et al., 2019).

HYBRID COMBINATIONS OF IN SITU AND SEQUENCING-BASED TRANSCRIPTOMIC PROFILING

To maximize the features of in situ profiling, while also obtaining multi-omic spatially resolved information from the same biological samples, combined adaptations of in situ profiling and biochemical modifications of sequencing library preparations have been developed. One alternative to single cell extraction from tissues or multiplexed FISH is sequencing of transcripts directly on tissue sections using nucleotide-fluorescent sequencing or enzymatic amplification (Ke et al., 2013; Lee et al., 2014). In particular, the fluorescent in-situ sequencing (FISSEQ) technology utilizes in situ conversion of RNA into cross-linked cDNA amplicons followed by manual sequencing by fluorescent tag detection on a confocal microscope. This results in the detection of thousands of spatially resolved nucleotide sequences of genes from high-content-images (Lee et al., 2015). Additionally, RNA sequencing of intact tissue using STARmap (spatially-resolved transcript amplicon readout mapping) provides 3D sequence information by labeling cellular RNA with pairs of DNA probes followed by enzymatic amplification. The resulting amplification produces a DNA nanoball that eliminates background signal caused by mislabeling of single probes (Wang et al., 2018b). CARTANA is a company that employed in situ sequencing for customizable gene panels (Hernandez et al., 2019). CARTANA can profile more than 100 genes in a high throughput pipeline (10 cm² [1 million cells] per week per microscope).

Barcode based approaches for spatial transcriptomics

Recent strategies using barcodes on native tissue on a slide, namely Spatial transcriptomics and Slide-seq, have been developed for high-resolution spatial resolution (Rodrigues et al., 2019; Stahl et al., 2016). This approach dissecta histological section with a grid, where each spot is labelled with barcoded oligonucleotide primers to capture adjacent tissue mRNA. The resulting transcripts are then reverse transcribed to cDNA and positional information is contained within their respective barcodes (Fig. 1D). The Visium Spatial Gene Expression Solution (10x genomics) is based on the barcode-based approach (https://www.10xgenomics.com/products/spatial-gene-expression/). The current resolution of
Fig. 1. Diverse approaches to associate spatial information with transcriptomics. (A) Cryosection provides positional information. (B) LCM provides fine resolution (even to single cell) positional information. (C) Image-based single cell level spatial transcriptomic approaches. osmFISH labels RNA with a number of colors each time for different genes, seqFish uses a combination of colors to mark RNAs. MERFISH labels presence or absence of fluorescence. (D) Spatial transcriptomics uses barcodes to spatially distinguish each spot. (E) RNAseq for interacting cells provides relative spatial information. (F) Spatial reconstruction uses transcriptomic information to reconstruct original spatial information.
commercial Spatial Transcriptomics is limited to 100 µm, capturing an average of 3 to 30 cells per regular grid. High-definition spatial transcriptomics (HDST) uses 2-µm beads to enhance the resolution (Vickovic et al., 2019) while the Slide-seq technology uses 10-µm beads containing individual position barcode (Rodrigues et al., 2019).

**Sequencing direct cell contact**

Although FISH and barcode-based approaches provide cellular positioning within a tissue, approaches to use cell contact information (thus provide relative positional information) have also been developed. This approach utilizes transcriptomic information obtained from physically-interacting cells. ProximID is an approach where cells are gently dissociated to retain cells that are in physical contact, and then subsequently processed for RNAseq to obtain transcriptomic information of doublets or triplets of interacting cells (Fig. 1E) (Boisset et al., 2018). In parallel, RNAs from single cells are also sequenced (scRNAseq), which provide the reference information of the doublets and triplets. Without microdissection, ProximID identified that Tac1+ enteroendocrine cell-Lgr5+ stem cells interact in the intestinal crypt (Boisset et al., 2018), thus providing insights to previously uncharacterized interactions that may play a role in stem cell driven regeneration. ProximID collects the interacting cells that survived mild dissociation therefor this method can potentially be biased towards collecting strongly interacting cells. Similarly, paired cell RNAseq (pcRNAseq) sequenced RNA from cellular doublets composed of liver endothelial cells (LEC)s and hepatocytes generated from extensive flow cytometric sorting (Halpern et al., 2018). pcRNAseq and parallel scRNAseq allowed the identification of zone-dependent LEC gene expression relative to the hepatocyte gradient (Halpern et al., 2018). These approaches utilize the innate interactions between cells in the tissue. Similarly, PIC-seq sequences RNAs from physically interacting cells and identified the composition of the interacting cells and investigated genes that are differentially expressed when interacting with other cell types (Giladi et al., 2020).

**Computational reconstruction of spatial information from scRNAseq data**

Tissue architecture reconstruction is an algorithm-based strategy used for reverse identification of a cell’s spatial origin based on gene expression data and a positional reference (Fig. 1F). Tomo-Seq used slices of cryosectioned zebrafish embryo to reconstruct 3D expression patterns (Junker et al., 2014). Similarly, scRNAseq information was used to reconstruct zoned areas along the villus axis of the intestinal epithelium with respect to the location information provided by LCM (Moor et al., 2018). Computational algorithms have been developed to predict the positional origin of cells using a model trained using the in situ hybridization (ISH)-based gene expression data as reference (Achim et al., 2015; Satija et al., 2015). Lobule zonation markers identified using smFISH in the liver has been used to discover zonation-specific genes (Halpern et al., 2017). Additionally, Distmap enabled the reconstruction of Drosophila embryo using scRNAseq and gene expression of 84 marker genes and performed virtual in situ hybridization (Karaiskos et al., 2017). Similar to pseudo-time analysis for scRNAseq data, pseudo-spatial trajectory analysis using scRNAseq from the dissected colon samples identified location-dependent gene expression that controls epithelial-to-mesenchymal transition (EMT) (McFalone-Figueroa et al., 2019). Recently, architecture reconstruction has been achieved without a spatial reference in novoSpaRc under that assumption that gene expression between nearby cells is generally more similar than gene expression between cells separated by a larger distance (Nitzan et al., 2019). Computational strategies in spatial transcriptomics complement the developments in biochemical and mechanical techniques.

**Cellular coordinates as a critical component for studying cell-cell interactions**

Cellular location is important to understand how cells interact with each other within a tissue. Current technologies in spatial transcriptomics such the ones mentioned in this review have provided vital information on the cellular identity, transcriptional changes, critical gene markers for cell communication and even previously uncharacterized cellular interactions relative to its position in the tissue. Spatial reconstruction allowed the detection of a comprehensive set of liver genes differentially expressed across the lobule (Halpern et al., 2017). The LECs, the interacting partner with hepatocytes, also exhibited differential gene expression across the lobule (Halpern et al., 2018). Moreover, proximity-based reconstruction of hematopoietic stem/progenitor cells (HSPC) microanatomy in bone marrow followed by scRNAseq post-transplantation allowed the retrieval of osteolinesage cells (OLCs) that were proximal or distal to HPSCs (Silberstein et al., 2016). Through proximity-based profiling, the study identified differentially expressed genes in OLCs affected by nearby HSPC and niche factors regulating HSPC quiescence in vivo (Silberstein et al., 2016). Likewise, proximity-based “clumplet” cell sequencing technique ProximID identified the physical interaction of hormone-producing Tac-enteroendocrine cells with Lgr5 stem cell in the intestinal crypt (Boisset et al., 2018). These results suggest a previously uncharacterized hormone-related secretory pathway involved in homeostatic maintenance of intestinal crypts. Furthermore, spatial transcriptomics could also spatially resolve interactions between foreign cells as demonstrated through scDual-Seq by quantifying host-pathogen interactions (Avital et al., 2017). Indeed, accounting for a cell’s positional information allows an extra component to help resolve the complex interplay of key regulatory pathways and cellular interactions involved in homeostatic tissue maintenance. Advancements in spatial transcriptomics technologies ranging from mechanical cell capture, in situ biochemistry, enhanced microscopy and/or combinations thereof, as well as sequencing innovations along with advanced computational algorithms will further shed light on complex tissue architecture in the context of tissue homeostasis and pathologies.

**BEYOND CELLULAR COORDINATES: SPATIAL TRANSCRIPTOMICS ACROSS BIOLOGY**

Spatial transcriptomic methods can serve as a powerful tool...
in capturing cell-to-cell properties at a tissue-level resolution. Using spatial transcriptomics enabled the quantitative elucidation of important cellular dynamics. Besides the cell and the tissues that were used to develop the methodology in Table 1, the number of applications for spatial transcriptomics are increasing. For instance, spatial quantification of neural and mesodermal lineage trajectories from Tomo-Seq was associated with the anterior-posterior axis of gastruloids developmental (van den Brink et al., 2020). Barcode based spatial transcriptomics have been applied to detect spatial crosstalk in Alzheimer’s disease wherein novel cellular interactions were uncovered between microglia and astroglial cells in amyloid-ß plaque niches (Chen et al., 2019). Also, these approaches have been applied to study spatial localization of cell population in pancreatic ductal adenocarcinoma (Moncada et al., 2018) and HER2+ breast tumors (Salmén et al., 2018). In situ sequencing has been applied to study spatio-temporal cell fate decisions in murine neural crest (Soldatov et al., 2019), the immunological architecture of the murine tuberculosis granulomas (Carow et al., 2019), mouse brain (Tiklova et al., 2019) and human heart development (Asp et al., 2019) as well as primary colorectal cancers (Mathot et al., 2017). PIC-seq detected preferential interaction between two immune cells (T-cell and dendritic cells) which revealed interaction-specific phenomena important during pathogen stimulation (Giladi et al., 2020). Ligand-partner expression patterns were used to extract cross-interactions between breast cancer and cancer-associated fibroblast (CAF) by iCELLNet (Noël et al., 2020). A similar approach was implemented in NichNet describing the interactions between tumor and immune cell microenvironment (Brownes et al., 2020).

**DISCUSSION**

Studies on cellular identity combined with their native location has been significantly advanced by scRNAseq and spatial transcriptomic technologies. This review highlights the current technological developments that have refined spatial transcriptomics. Pioneering image-based approaches use FISH to visualize cellular localization. However, overlaps between fluorescent signals have been a major limitation in image resolution (Burgess, 2019). This was surmounted by barcoding-based improvements that lead to the development of 2-µm beads for HDST to measure single cell transcriptomic profiles (Vickovic et al., 2019). Although combining scRNA-seq and barcoding-based approaches can determine transcriptome-wide profiles, they are still currently limited by their lack of sensitivity towards RNA detection (Kolodziejczyk et al., 2015) thus providing opportunities for further technological refinements.

As cells constantly communicate with other cells within a tissue, the study of cell communication will become more popular using scRNAseq and spatial transcriptomics. Previous approaches to study cell communication relied on the co-expression of ligand-receptor pairs between cell types (Kumar et al., 2018; Skelly et al., 2018). Neighboring cells communicate via complex paracrine signaling networks (Roy and Kornberg, 2015). Studying paracrine signaling using single cell resolution spatial transcriptomics provides an opportunity to enhance our understanding of cell communication.

Subsequently, we can then begin to question spatio-temporal changes of cells during development or disease progression. The majority of current spatial transcriptomics required a section of a tissue and cannot reveal spatio-temporal changes effectively. Attempts such as 4D-seq (unpublished: https://techtransfer.universityofcalifornia.edu/NCD/30312.html) uses two-photon microscopy and DNA-labelling to capture cellular spatio-temporal variation within a tissue prior to scRNA-seq analysis. As technical resolution continually improves, it is possible to foresee studies integrating high-content 4D image data with scRNAseq in order to gain a deeper understanding of spatio-temporal cellular regulation.

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**AUTHOR CONTRIBUTIONS**

Both J.M.T. and K.J.W. wrote the manuscript.

**CONFLICT OF INTEREST**

The authors have no potential conflicts of interest to disclose.

**ORCID**

Joji Marie Teves https://orcid.org/0000-0001-9289-2357

Kyoung Jae Won https://orcid.org/0000-0002-2924-9630

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