



Perspective

Single-cell and spatially resolved omics: Advances and limitations

Recent advances in experimental and computational single-cell and spatially resolved omics have opened new avenues for research in biology and medicine. These technologies allow for the study of individual cells in unprecedented detail, providing insights into the heterogeneity within tissues and organs, and how different cells interact with each other. Humans and other eukaryotes are composed of billions of cells, each with vastly heterogeneous cell types and functional cell states determined by intrinsic and extrinsic factors. Robust technologies for mono-omics measurements of individual cells have already revolutionized the discovery and understanding of various cell types, while multi-omics methodologies at single-cell and spatial resolution are necessary to develop fundamental understanding of the molecular hierarchy from genome to phenome in individual cells [1]. Recent developments in single-cell and spatially resolved omics technologies enable investigation of intermolecular dynamics and the impact of genetic variation on cell function and tissue function. The field is rapidly advancing both technologically and computationally, enabling broad applications to understand cell biology.

Recently, the throughput of single-cell RNA sequencing (scRNA-seq) technology has rapidly increased, allowing for the analysis of hundreds of thousands of cells with a significant decrease in cost. Different technologies, such as microfluidics and microwells have enabled fast and accurate scRNA-seq. Thanks to the scRNA-seq technology, research has been able to establish the Human Cell Atlas since 2017 to map the characteristics of every cell type in the human body. The key steps involved in scRNA-seq include single-cell isolation and capture, cell lysis, reverse transcription, cDNA amplification, and library preparation. Advancements in microfluidics technology have led to more efficient and reliable methods for isolating single cells, including droplet and solid microfluidic techniques. The droplet microfluidic method separates individual cells using water-in-oil emulsions. The widely used 10X Genomics platform applies this technique in their kit. Alternatively, the solid microfluidic method uses physical partitions through diffusion and is often chosen for very limited clinical samples due to its lower throughput. Examples of solid microfluidic platforms include Fluidigm C1, which can capture a few hundred to around a thousand cells, and the higher throughput BD Rhapsody Single-Cell Analysis System, which uses a microwell technology to partition individual cells via diffusion. Recently, the scope of samples for scRNA-seq has been dramatically broadened. The 10X Chromium Gene Expression Flex platform, which is a probe-based method, can be applied to formalin-fixed paraffin-embedded (FFPE) tissues. M20 Genomics developed random primer based scRNA-seq platform that can be applied to FFPE tissues and bacteria [2].

Metabolomics, the study of small molecules in cells, is now shifting towards the single-cell level. The single cell metabolomics

was even named as the seven technologies to watch in 2023 by *Nature*. However, there are significant challenges due to the vast number of molecules with diverse chemical properties in the metabolome, and most metabolic analyses cover only a few metabolites, which could miss crucial information [3]. Despite the challenges, currently, the single-cell metabolomics can be achieved with mass Spectrometry (MS). We have techniques like matrix-assisted laser desorption/ionization (MALDI), secondary ion mass spectrometry (SIMS), and laser ablation electrospray ionization (LAESI) to release and ionize metabolites in cells from solid surface, while we also have electrospray ionization (ESI) to alternatively sample and ionize metabolites in cells from liquid phase (Fig. 1). These techniques also enable capturing the spatial coordinates from which the metabolites originated in the sample.

Single-cell proteomic technologies are in an earlier stage than other omics technologies. One limitation is that proteins, unlike DNA or RNA, cannot directly be amplified. Mass spectrometry-based single-cell proteomics has had the most success thus far, such as nanoPOTS and single cell proteomics by mass spectrometry (SCOPE-MS) (Fig. 1). Advanced automated sample preparation methods coupled with label-free or multiplexed data collection on highly sensitive mass spectrometry instruments now allow researchers to detect as many as 3,000 proteins per cell [4].

Recent advances have also enabled parallel profiling of a cell's omics. Tagmentation-based methods are commonly used to jointly profile the transcriptome and chromatin accessibility, where they recover accessible DNA as transposon-insertion-flanked regions using assay for transposase-accessible chromatin (ATAC)-seq assay. The joint profiling of transcriptome and chromatin accessibility enables the evaluation of the link between gene expression and transcription factor (TF) binding, which facilitates the assignment of TF activity to target genes. Besides, other methods, such as REAP-seq and CITE-seq, rely on DNA barcoded antibodies that recognize a limited set of cell surface or intranuclear proteins, which can be captured and amplified alongside the transcriptome (Fig. 1). These methods enable the profiling of protein abundances, stability, post-translational modifications (PTMs), and protein isoform expression, in addition to gene expression or other modalities. However, these methods are limited by the availability of specific antibodies. Moreover, the BD Rhapsody™ Single-Cell Analysis System enables the capture of multimodal information from thousands of single cells in parallel. The system covers mRNA expression levels, protein expression levels, T-cell and B-cell receptor immune repertoire, and identification of antigen-specific T and B cells.

Transcriptomic technologies, in particular, have greatly advanced single-cell sequencing technology, allowing scientists to better understand complex biological systems. However, most single-cell RNA sequencing (scRNA-seq) approaches involve

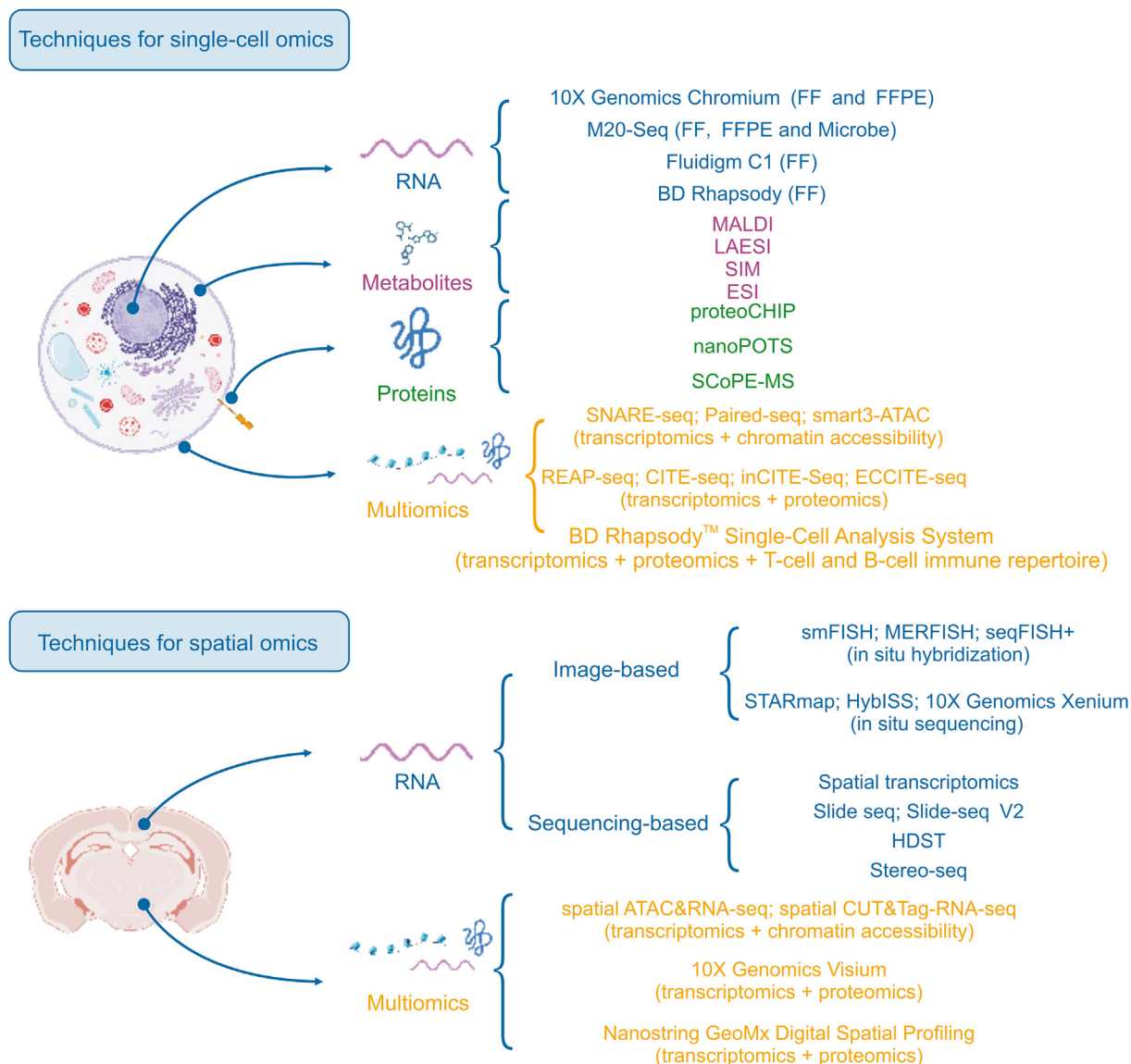


Fig. 1. Methods for single-cell and spatial omics. FF: fresh frozen; FFPE: formalin-fixed paraffin-embedded; MALDI: matrix-assisted laser desorption/ionization; LAESI: laser ablation electrospray ionization; SIMS: secondary ion mass spectrometry; ESI: electrospray ionization; SCoPE-MS: single cell proteomics by mass spectrometry; ATAC: assay for transposase-accessible chromatin; smFISH: single-molecule fluorescence in situ hybridization; MERFISH: multiplexed error-robust fluorescence in situ hybridization; seqFISH+: sequential fluorescence in situ hybridization; STARmap: spatially resolved transcript amplicon readout mapping; STARmap: spatially resolved transcript amplicon readout mapping; HDST: high-definition spatial transcriptomics; CUT&Tag: cleavage under targets and tagmentation.

isolating cells from their original position, thereby losing spatial information during transcriptome profiling. To overcome this limitation, spatially resolved transcriptomics (SRT) aims to determine gene expression profiles while preserving information about the spatial context of the tissue. SRT has opened new areas of spatially resolved omics, including genomic, transcriptomic, proteomic, and potentially other omics data with retained positional information. The application of SRT has changed the way we understand complex tissues and was named Method of the Year 2020 in *Nature Methods* [5]. By combining information from single-cell and spatially resolved data, researchers can build up a detailed picture of how cells are organized within a tissue, and how this organization changes over time.

Current SRT have two major categories: (1) image-based methods including in situ hybridization (ISH) and in situ sequencing (ISS), and (2) sequencing-based methods that capture

mRNA following with sequencing (Fig. 1). In image-based methods, ISH technique detects mRNA molecules in tissue samples using a complementary probe, but has limitations due to high autofluorescence background. Single-molecule fluorescence in situ hybridization (smFISH) methods with multi-color and multi-RNA imaging overcome this limitation by fluorescently amplifying probes complementary to the target mRNA. Multiplexed fluorescence FISH technique has been widely used for direct imaging of individual RNA molecules within intact cells and tissues that have improved over time, including multiplexed error-robust fluorescence in situ hybridization (MERFISH) and sequential fluorescence in situ hybridization (seqFISH+). Another strategy is ISS which can detect more genes than ISH in a non-targeted manner. The spatially resolved transcript amplicon readout mapping (STARmap) method detects and quantifies multiple gene sets simultaneously, while the hybridization-based in situ sequencing (HybISS) detected genes

using sequencing-by-hybridization (SBH) and autofluorescence quenching. Probabilistic cell typing by in situ sequencing (pciSeq) used scRNA-seq classification to identify cell types through multiplexed in situ RNA detection [1]. The NanoString CosMx, Vizgen MERSCOPE, and 10X Genomics Xenium platforms are now commercially available to automate imaging-based spatial transcriptome profiling of tissue sections at single-cell resolution. These platforms use smFISH-based, MERFISH-based, and ISS-based methods, respectively. They can co-profile a few to tens of proteins through imaging, making these technologies more accessible.

In sequencing-based methods, the spatial transcriptomics (ST) technique obtains spatially resolved transcriptomic data by delivering slide sections to a glass slide with pre-arranged sets of bar-coded reverse transcription primers. ST's spatial resolution is limited to 100 μm center-to-center distance, but the 10X Genomics Visium platform achieves finer resolution, making it a popular choice for mapping spatial profiles of tissues in various fields [1]. The spatial transcriptomics techniques with barcoded oligonucleotide capture array have a resolution of up to 55–100 μm . To address the issue of low spatial resolution, several bead-based capturing sequencing methods have been developed, including Slide-seq, high-definition spatial transcriptomics (HDST), Slide-seqV2, Seq-Scope, and Stereo-seq (Fig. 1). These methods utilize next generation sequencing (NGS) technology and even DNA-barcoded beads to achieve higher spatial resolution comparable to individual cells or subcellular levels. Among those methods, Stereo-seq appears to be the finest. Stereo-seq relies on DNA nanoball-patterned array and in situ RNA capture, which achieves genome-wide coverage, high sensitivity, cellular resolution, and large field-of-view simultaneously [6].

New methods have been developed for simultaneous profiling of chromatin accessibility or specific histone modifications and gene expression in tissue cryosections. These methods, which include spatial ATAC&RNA-seq and spatial cleavage under targets and tagmentation (CUT&Tag)-RNA-seq, rely on combining microfluidic deterministic barcoding in tissue (DBiT) strategies (Fig. 1) [7]. However, there are limitations associated with these assays, such as near-single-cell resolution, small analyzable area, uncharacterized spaces in between adjacent pixels (depending on channel distances), and the expertise required in fabricating and handling microfluidics chips for implementation.

Current methodologies for parallel spatial profiling of both the transcriptome and proteome often involve serial characterization in a limited number of proteins and lacking single-cell resolution. The commercial array-based 10X Genomics Visium technology currently allows immunofluorescence protein detection of one or two targets on the same tissue section, but at the expense of hematoxylin and eosin (H&E) staining for spatial mapping. Alternatively, NanoString GeoMx Digital Spatial Profiling (DSP) allows quantification of RNAs and/or proteins by counting unique indexing oligonucleotides, which are covalently linked with probes or antibodies that target transcripts or proteins of interest.

Spatially resolved transcriptomic technology has the potential to discover spatial heterogeneity in disease states, characterize

spatial expression blueprints during development, and elucidate spatial architecture at the molecular level. However, currently available technologies have limitations, and the choice of method depends on study design, the biological question, and a balance between throughput and spatial resolution. In the future, improvements in throughput, cost reduction, and multimodal integration are expected. The development of new modalities such as the epitranscriptome is also anticipated. Spatial multi-omics will enable profiling of cell-intrinsic and -extrinsic molecular features, while multimodal assays will transform our understanding of organismal development, stem cell biology, and disease processes. Computational algorithms that combine spatially resolved transcriptomic data with other data modalities have significantly contributed to overcoming key challenges and gaining biological insights. The development of novel computational tools will continue to play a significant role in exploring large-scale datasets and elucidating principles of underlying biology, ultimately shedding light on differences in spatial architectures of healthy and diseased tissues.

In conclusion, single-cell and spatially resolved omics are powerful tools that are transforming our understanding of biology and medicine. These technologies offer unprecedented insights into the complexity of living organisms and have the potential to revolutionize many areas of research and clinical practice. However, there are also challenges that need to be addressed. With continued investment and innovation, we can harness the full potential of these technologies to improve human health and well-being.

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