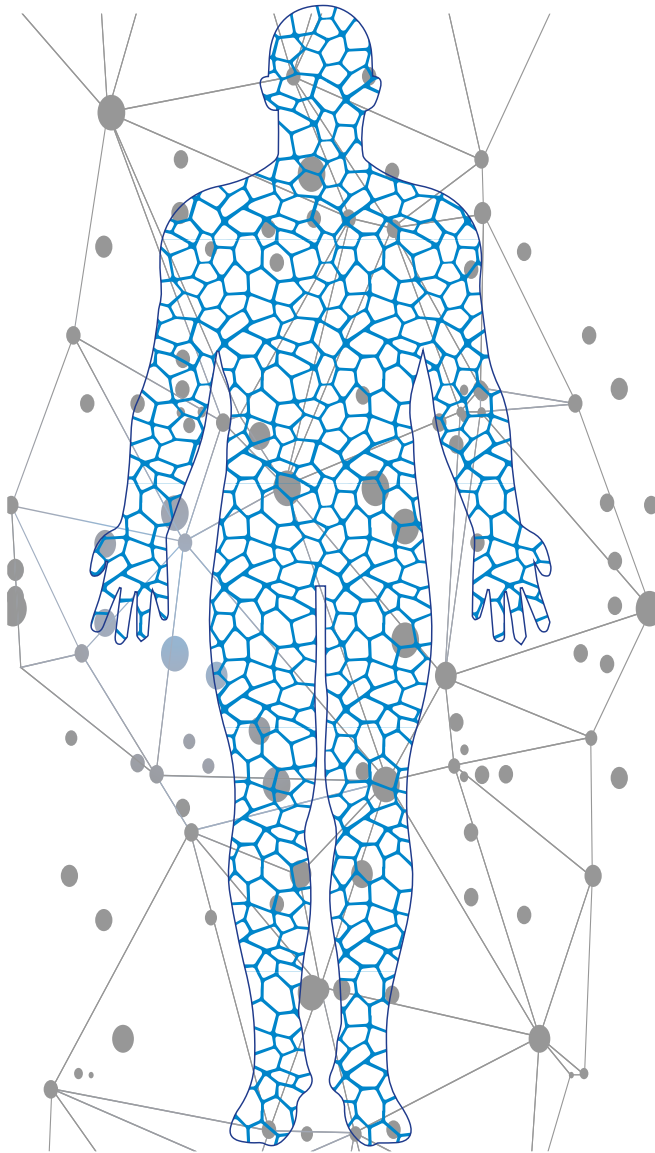re

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With spatial biology tools, scientists welcome a new era of cell atlases

Mapmaking has been a fundamental activity for centuries, but it wasn't until the late 1970s that humans created the first high-resolution map of an organism — the famous cell atlas of *Caenorhabditis elegans*. The revolutionary work from John Sulston and Robert Horvitz involved painstakingly tracking every cell in the developing nematode.[1] Each cell division was meticulously charted, revealing for the

first time not only each cell's trajectory but also its full lineage from larval stage to maturity. When the duo published their cell atlas in 1977, it accounted for nearly 1,000 cells in *C. elegans*.

This work was impressive not just for its value as a scientific breakthrough, but also for the tremendous effort it required. The first cell atlas was created by direct observation: Sulston and Horvitz spent days peering through a microscope, vigilantly watching for signs of cell activity. Each cell's offspring was noted and tracked by hand with simple drawings on paper.

Most organisms lack the biological simplicity and transparency required for this kind of observation. And, understandably, most scientists lack the willingness to spend days hunched over a microscope to repeat Sulston and Horvitz's work. For decades, the map of *C. elegans* stood as the only cell atlas available to the research community. It facilitated remarkable insights into developmental and cellular biology.

But now, new developments in microscopy, genomics, and spatial biology have finally made it possible to build a cell atlas for virtually any organism. This golden age of cell-by-cell mapmaking has ushered in critical new scientific resources. Cell atlases of key tissues or entire organisms are now available for zebrafish, mouse, and non-human primates, among others. Most recently, the Human Cell Atlas consortium published a series of papers reporting single-cell transcriptome atlases of two dozen organs and tissues in the human body.

Early cell atlases
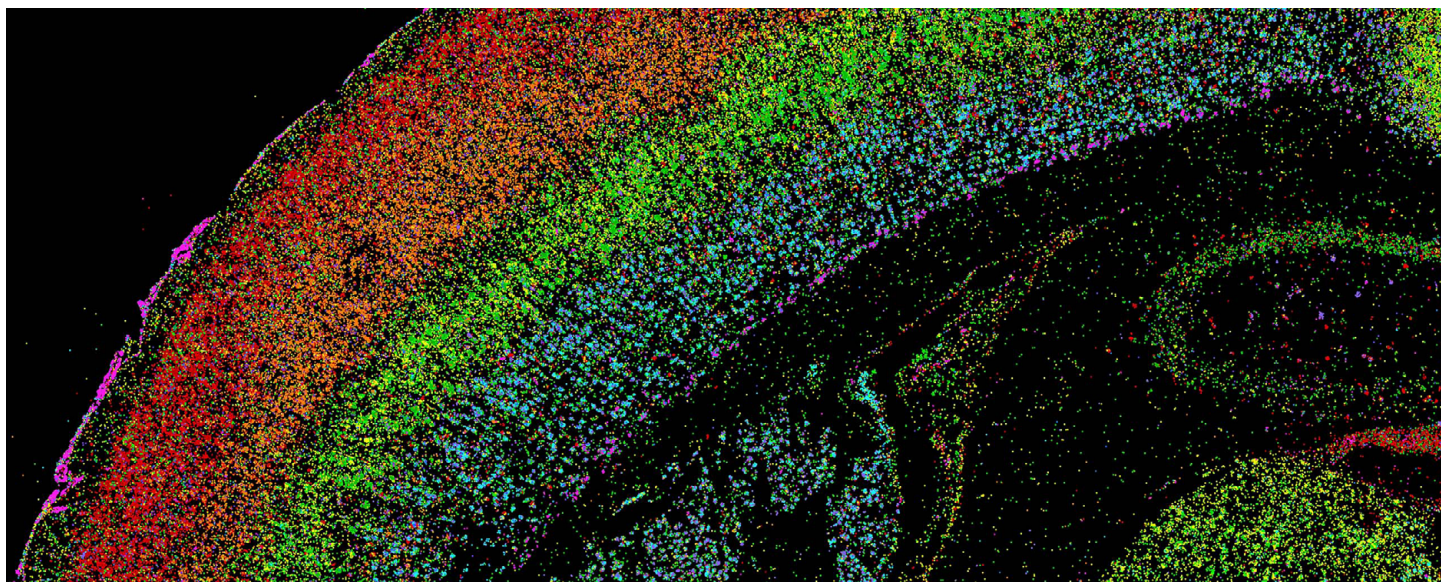
This Molecular Cartography™ image spatially resolves the layered structures of the mouse cortex as identified using layer-specific gene markers

When Sulston and Horvitz teamed up for the cell-mapping efforts that would later earn them the Nobel Prize, they based their work on a technique developed by Sulston for observing cell lineages as they emerged. *C. elegans*, the scientists learned, could be positioned on a microscope slide on top of a thin sheet of agar. Despite the unusual conditions, the worm would proceed through its normal development cycle, which takes less than four days from fertilization to maturity.

Sulston and Horvitz watched worms grow through four larval stages to maturity, tracking somatic cells as they divided and migrated with electron microscopy and differential interference contrast microscopy.

It took decades, but tools began to improve and scientists once again took up the art of creating cell atlases. In the early 2000s, researchers at the newly formed Allen Institute for Brain Science began developing tissue-specific cell maps to support the neuroscience community.[2] They started with the adult mouse brain, finely slicing tissue sections to create a 3D gene expression map with cellular resolution. Since then, the team has released cell atlases for developing mouse brain and mouse spinal cord. They have also created cell-map resources for human brain.

In addition, innovation with light-sheet microscopy allowed scientists to directly observe more organisms at high resolution. This technology supported new cell maps for zebrafish in 2008, followed by *Drosophila* and mouse.[3-6] Other improvements, such as lattice light-sheet imaging and imaging combined with tissue clearing, have given scientists better resolution and speed for their investigations.

Around this time, the advent of single-cell RNA sequencing allowed scientists to identify the full transcriptome of individual cells, yielding unprecedented insight into gene expression. This approach has been widely adopted, but unfortunately, the results do not include the essential context of each cell's location.
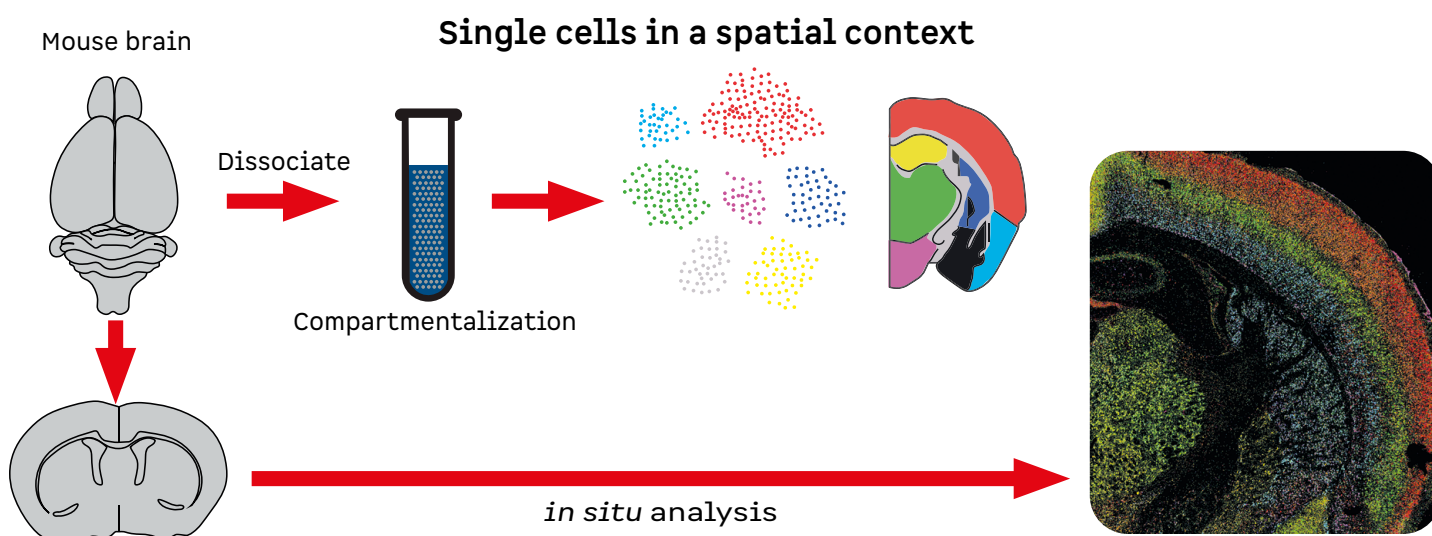
Spatial biology tools

The recent emergence of spatial biology techniques finally gave scientists what they needed all along: single-cell analysis with spatial context. Previous techniques could answer the *what* and *when* of a biological question, but spatial biology tools made it possible to answer the *where, how*, and *why*.

Because the need for spatial context has been so pressing, several different approaches emerged to meet the demand. Most allow for the interrogation of a single analyte — either expressed genes (spatial transcriptomics) or proteins (spatial proteomics). Cell atlas projects often rely on gene expression to identify cell populations or cell states.

Among spatial transcriptomic technologies, there are two common approaches. Sequencing-based methods begin by barcoding the cells in a sample with location-specific tags. The sample is then processed and analyzed in a standard sequencing workflow. The resulting data looks much like that from a typical single-cell RNA-seq pipeline, except that scientists can use the barcode tags to map each transcript back to its original location in the tissue sample. The other approach is based on fluorescent *in situ* hybridization. Typically, transcript-specific probes are added to a sample, sometimes simultaneously and other times in iterative cycles. The sample is directly imaged throughout the process. Telltale fluorescent tags attached to each probe light up to show researchers exactly where their transcripts of interest can be found throughout the sample. Technologies that generate this information at high resolution are known as smFISH, short for single-molecule fluorescent *in situ* hybridization.

One of the biggest breakthroughs enabled by spatial biology recently came from the Human Cell Atlas consortium, which generated a series of cell atlases based on the transcriptomic analysis of nearly half a million cells from 24 different tissues and organs.[7] The team was able to define 475 cell types at the molecular level with the information produced. The international consortium continues to work toward its ultimate goal of producing a full cell atlas for the entire human body.[8]

Single cells in a spatial context

Mouse brain

Dissociate

Compartmentalization

in situ analysis

Current methodologies for interrogating cellular functions and gene expression profiles, such as bulk sequencing and single-cell RNA sequencing, often involve tissue dissociation and homogenization during sample preparation, which results in the loss of spatial context. Spatial transcriptomics provides granularity regarding cell subtypes and cell states in situ, fully visualizes, and quantifies the transcript, allowing researchers to interrogate the interactions amongst cells, and their expressed genes.

The Human Cell Atlas resources join a growing number of cell atlases enabled by spatial biology tools. Some map cells across an entire organism, such as the Spatial Mouse Atlas from scientists at the European Molecular Biology Laboratory and their collaborators.[9] Others characterize a specific tissue or organ, such as the spatial lung atlas produced by researchers at the Wellcome Sanger Institute and their teammates or the cell atlas of human and mouse liver from scientists at VIB.[10,11] Collectively, these cell atlases are dramatically expanding scientists' understanding of biology from the single-cell level on up.

Technical considerations

While spatial biology tools are transforming scientists' ability to create cell atlases, some technologies offer more advantages than others. There are a number of areas that should be evaluated when considering whether a spatial biology platform is appropriate for the desired outcome.

Resolution. Even as imaging technologies have begun to offer extraordinarily high resolution in other applications, many spatial biology tools cannot truly take advantage of these innovations. Resolving down to the single-cell level can be challenging — especially when cell boundaries are difficult to identify — and subcellular resolution is essential for correctly phenotyping individual cells to develop a cell atlas. At this point, sequencing-based spatial analyses typically have lower resolution than smFISH-based data, have empty space between capture areas, and have to trade resolution against sensitivity. Systems that rely on direct observation with advanced optics deliver the highest resolution.

Multiplexing. For projects designed to lay the foundation for a cell atlas, multiplexing is important — both for the number of genes that can be tracked and for the number of samples that can be run at once. For biological utility of the atlas, scientists will need systems capable of detecting dozens of genes. To build the atlas in a reasonable period of time, being able to run several samples simultaneously is particularly helpful.

Sensitivity. In a typical cell, thousands of genes may be expressed — but most of their gene products are rare, with fewer than 50 transcripts. Spatial transcriptomics tools therefore need exquisite sensitivity to detect these rare transcripts reliably and to help differentiate lineages of cells based on gradients of gene expression. Even the rarest transcripts, present at just one copy per cell, may hold critical information about human health and disease.
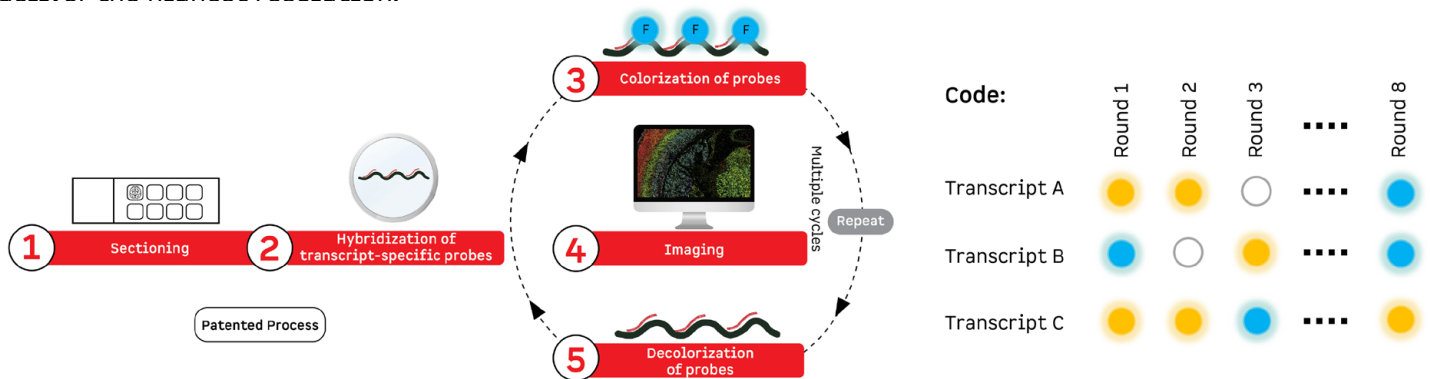
An overview of the Molecular Cartography system

Sample preservation. In classic histology workflows, a tissue section mounted on a slide can be viewed more than once if needed. Unfortunately, spatial biology methods based on next-gen sequencing and other technologies can destroy the sample in the analysis process. This prevents scientists from going back to the same sample to perform a different assay, or to confirm data that might seem anomalous. This is especially limiting for the development of a cell atlas, which could serve as a reference for an entire scientific community and must be as accurate as possible. Spatial biology platforms that preserve the original sample are important for making results more robust and reproducible.

The Molecular Cartography™ approach

A new approach to spatial biology analysis comes from the Molecular Cartography platform developed by Resolve Biosciences. The technology relies on extremely high-quality optics for imaging as well as smFISH. Its resolution is exquisite, delivering not only single-cell data but also the fine detail of subcellular activity.

Gene expression in the adult zebrafish brain at subcellular resolution. Image courtesy of the NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY.

This approach was designed for the multiplexing needs of most scientists for basic, translational, and clinical research. Resolve's platform uses transcript-specific probes to analyze the expression of as many as 100 genes at once — detecting even the rarest transcripts — without destroying the tissue section or cell culture sample. Unlike other spatial biology techniques, the Molecular Cartography platform provides exquisite sensitivity, specificity, sample throughput, and workflow convenience to elucidate the cell's complex transcriptional landscape.

This platform is currently being used by academic and pharma scientists for spatial transcriptomics, but the underlying technology is modular and flexible enough to incorporate interrogation of DNA, proteins, and metabolites. Already, it has allowed users to make new discoveries in oncology, neuroscience, and infectious disease. The technology has also been deployed by researchers who are actively building tissue-based cell atlases for human, mouse, zebrafish, and more.

To learn more about the Molecular Cartography platform, please visit
https://resolvebiosciences.com

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