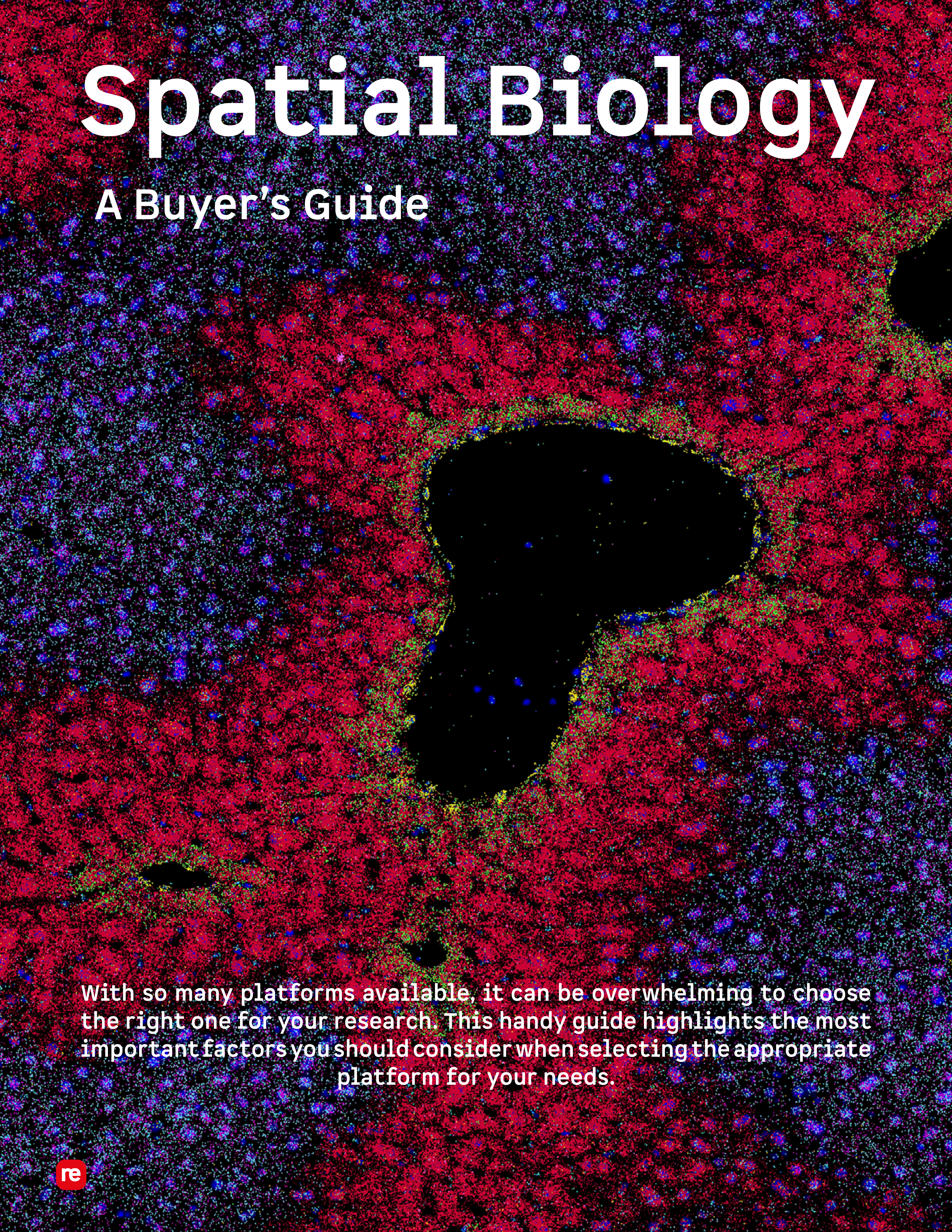
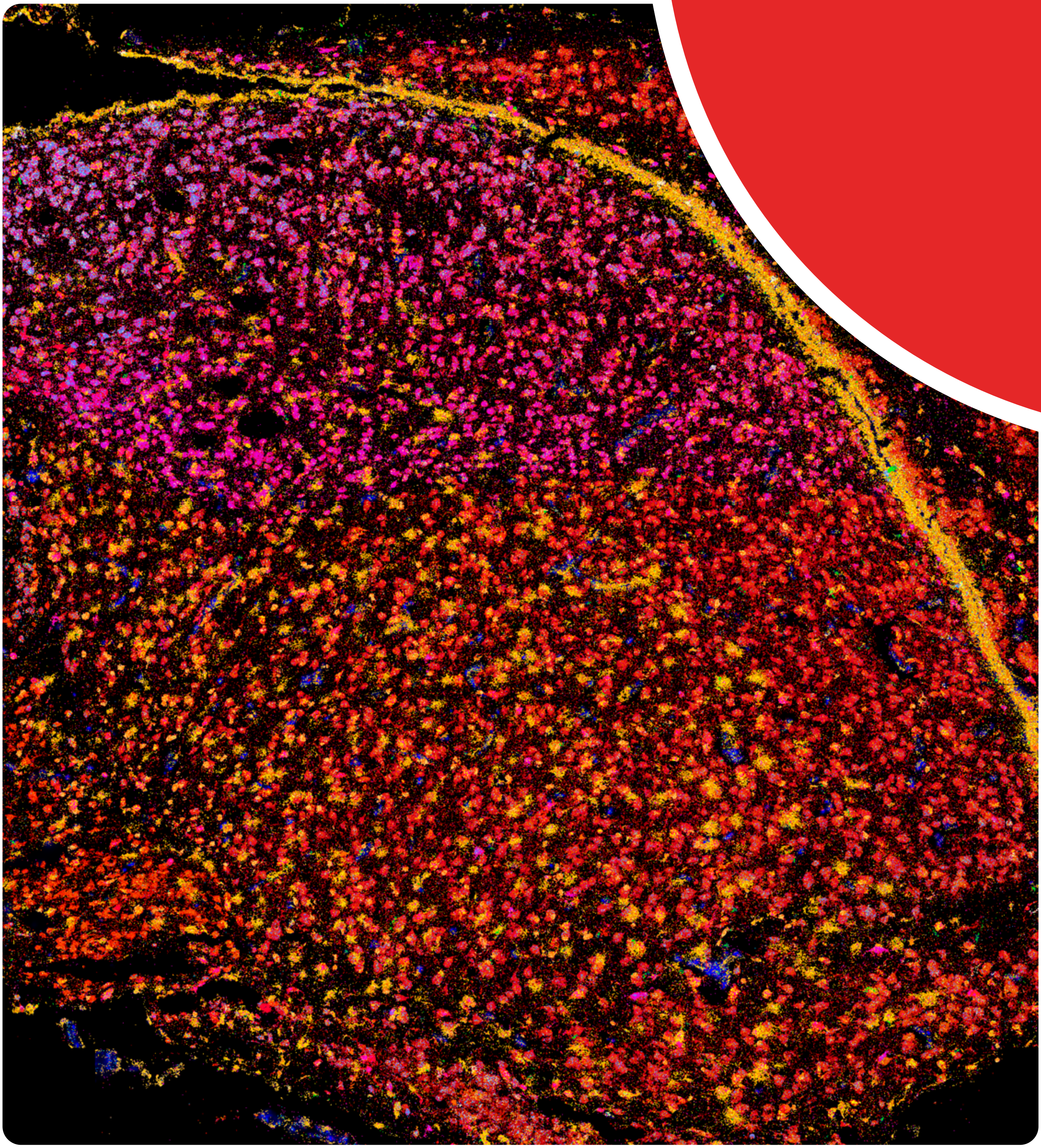


Spatial Biology

A Buyer's Guide

With so many platforms available, it can be overwhelming to choose the right one for your research. This handy guide highlights the most important factors you should consider when selecting the appropriate platform for your needs.



Molecular Cartography™ was used on *Pogona vitticeps* to compare brain architecture of amniotes, at the cell-type level, and gain insights into brain evolution (In collaboration with Prof. Gilles Laurent, Max Planck Institute for Brain Research, Frankfurt).

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Why spatial biology?

In recent years, spatial biology tools have delivered a critical new dimension of information to help scientists better characterize biology, understand disease, and predict the progression of cancers and infectious diseases. This powerful data is writing an entirely new chapter in biology.

To understand how spatial data makes a difference, consider this analogy, which represents each individual cell as a person for the sake of simplicity: An infectious disease is detected at a restaurant, and high-throughput 'omics tools are deployed to assess the problem. Sequencing technologies report that some but not all of the people in the restaurant are infected. Single-cell analysis tools indicate that four of the 20 people are infected, but cannot pinpoint which four, as there is no location identifier. High-resolution spatial biology identifies the four infected people, precisely where they are located, and consequently which of the remaining 16 people have the highest risk of exposure.

Spatial biology fills a key gap in most genomic or proteomic analyses: context. An analysis based on RNA sequencing can report which genes are expressed in a sample, but cannot provide information about where they are located and how they interact. As a result, there is a limit to what can be inferred. To understand the *why* in biology, it is important to understand the *where*, *what*, and *when* of its molecular components. Spatial biology aims to provide that information.

For many scientists, the question is not whether to incorporate spatial biology into their research, but how to choose from a dizzying array of instruments and approaches. This buyer's guide aims to help. We'll walk through the most important factors to consider — from your target of interest to sample integrity and more — so you can select the platform that's best suited for your needs, both now and in the future.

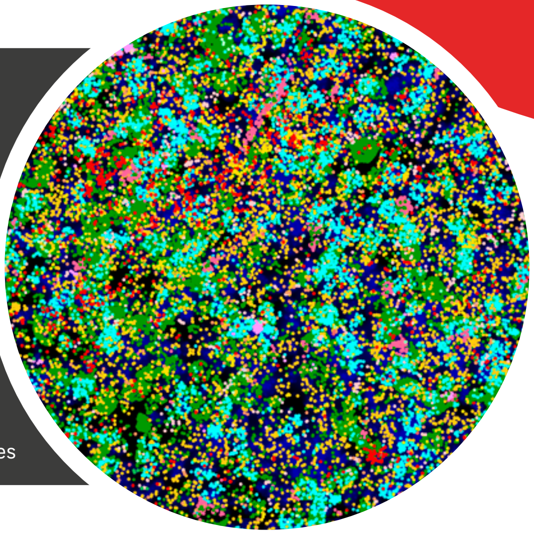
Key applications for spatial biology tools

Before we get to the specific factors to consider in a platform, let's take a quick look at the fields of research where spatial biology is already having an impact.

Cancer

Scientists in the field of immuno-oncology are always looking for better ways to predict which patients would benefit from new therapies. Spatial transcriptomics and spatial proteomics investigations have both been used to visualize the tumor microenvironment, which has provided new insights into the interaction between tumor and immune cells in addition to an improved ability to identify responders and non-responders.

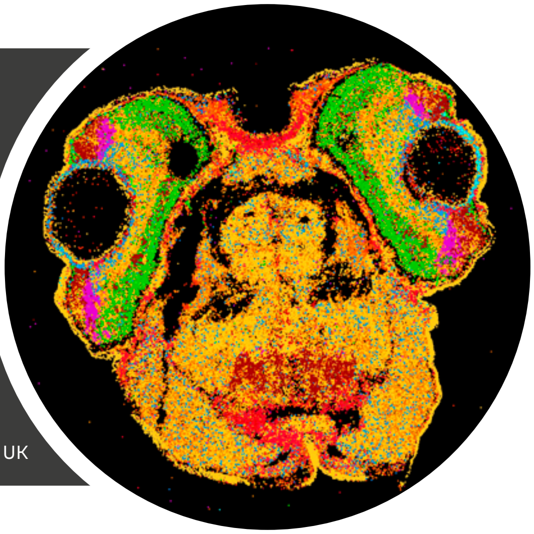
Image by Resolve Biosciences



Developmental biology

In developmental biology studies, scientists have used spatial biology data to watch how specific environments change over time. For example, in unpublished work, researchers studied the spatial expression of 48 genes in a zebrafish to observe retinal development, and then monitored the same genes throughout its entire lifespan to identify genes that become dysregulated in the aging process.

Image courtesy of Dr. Ryan MacDonald, University College London, UK



Neuroscience

Scientists have deployed spatial biology tools for neuroscience research to generate comprehensive catalogs of molecularly characterized cell types in mouse and human brains. In theory, identifying cell signatures could make it possible to develop better therapeutics and diagnostics for neurodegenerative diseases.

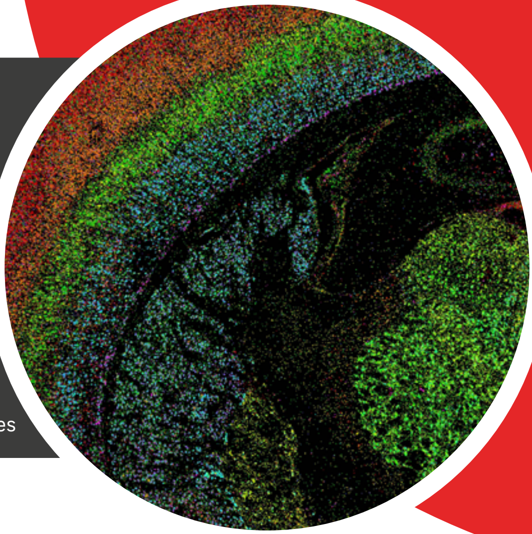


Image by Resolve Biosciences

Toxicology

Spatial biology tools are giving researchers new insights into drug toxicity, revealing molecular mechanisms related to cell sensitivity, drug tolerance, drug resistance, and adverse reactions. For example, teams have applied spatial biology methods to longitudinal studies of PD-1 inhibitors to characterize the development of toxicities over time.

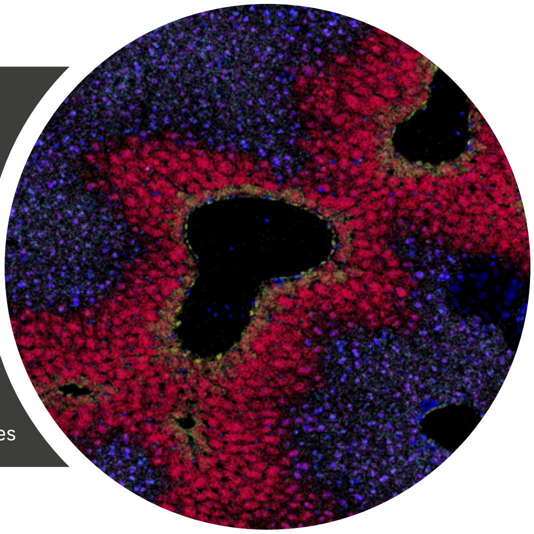


Image by Resolve Biosciences

Infectious disease

In the COVID-19 pandemic, scientists implemented spatial biology to understand the molecular pathology behind SARS-CoV-2 infection and the subsequent inflammatory response on the mRNA level that leads to severe disease. These analyses gave researchers a better view of subcellular gene regulation and how the infection affects neighboring cells over time.

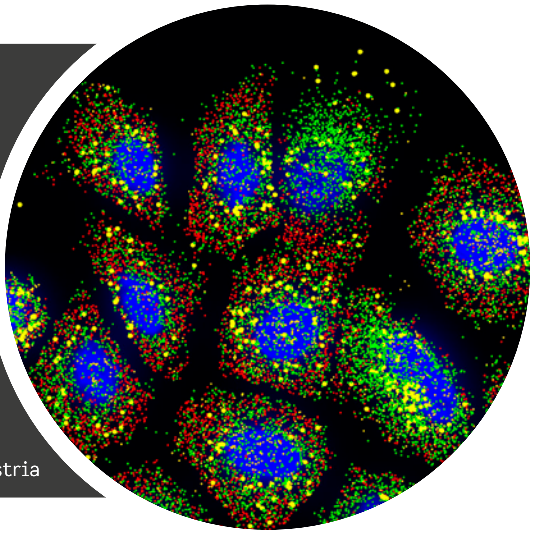
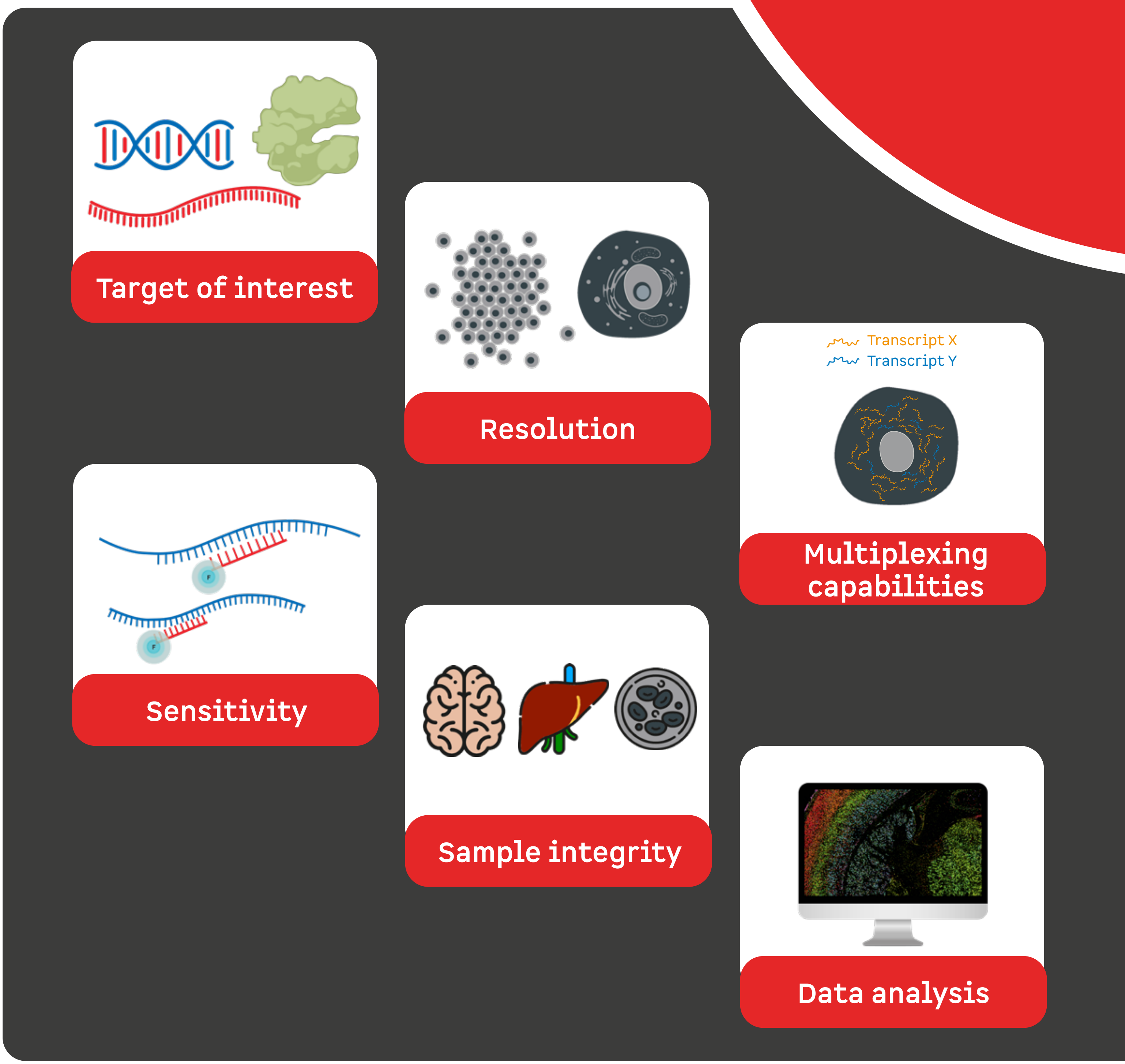


Image courtsey of Prof. Kurt Zatloukal, Medical University Graz, Austria

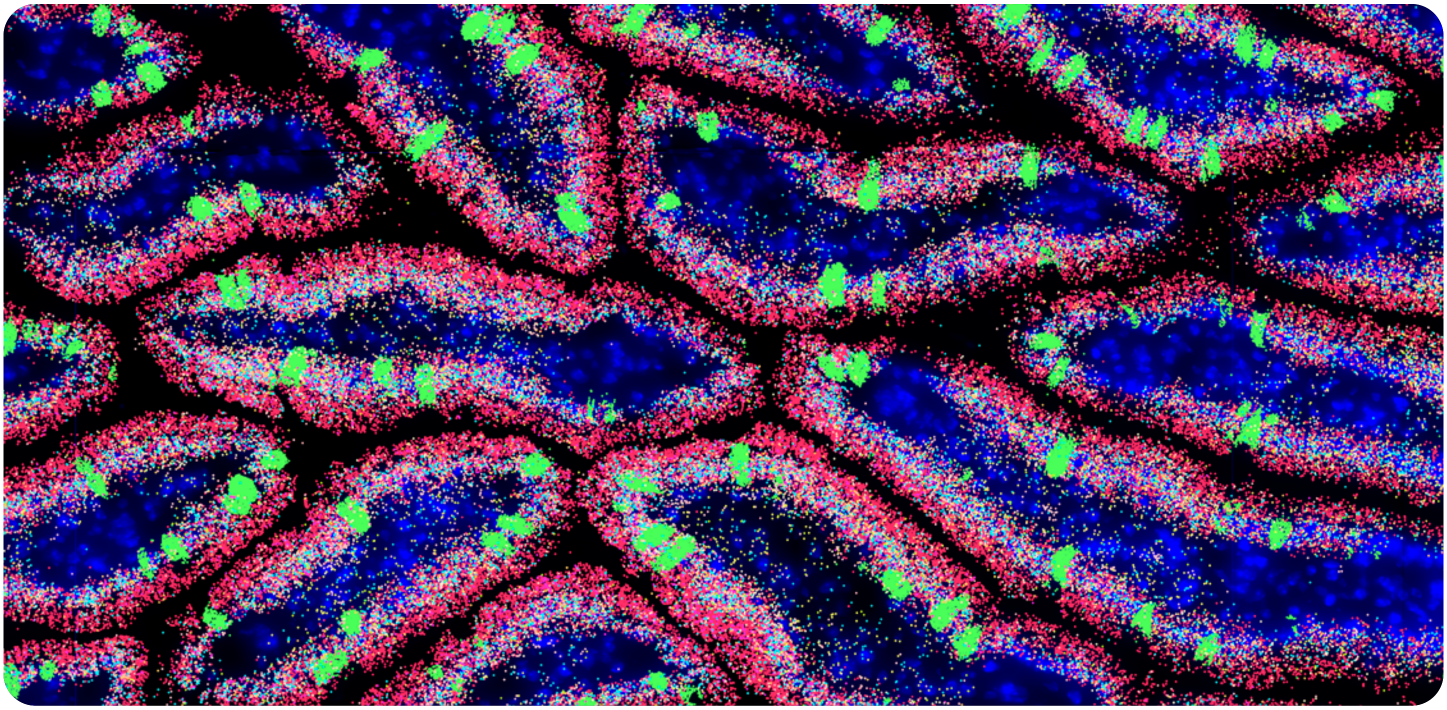
Factors to consider when incorporating spatial biology tools



1 // Target of interest

Across the 'omic landscape, spatial biology tools evolved to detect a range of analytes for which they can provide key context. At the moment, many platforms are limited to a single modality, typically gene expression or protein expression. A few platforms allow for the detection of genes and proteins, while some single-analyte platforms are actively under development to be able to incorporate other analytes.

Scientists whose research focuses solely on genes or solely on proteins can look for a spatial biology platform designed for their specific analyte. However, even the diehard protein scientists or the most committed geneticists should consider the possibility of their work evolving over time to cover other analytes. Spatial biology platforms are serious investments, and users will want to ensure they are flexible enough to meet their needs both now and in the future.



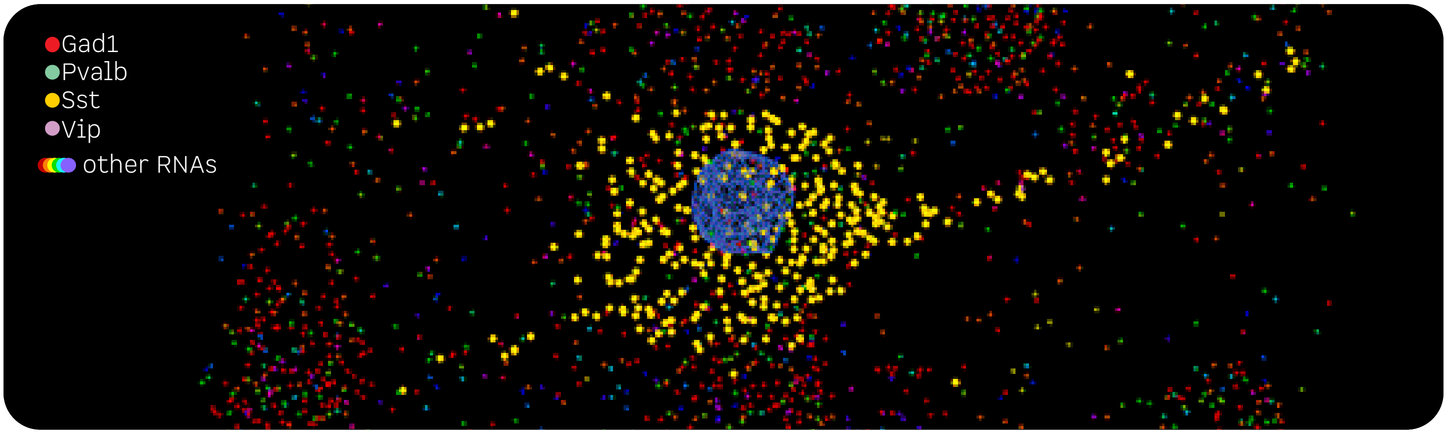
This Molecular Cartography image shows the spatial expression pattern of nine genes out of a possible 100 gene panel in the crypt region of the intestine. Ace2 (red), Ceacam 18/20 (light blue), and Muc2 (green) are showing the highest expression in this panel. The Ace2 receptor (main receptor of COVID-19) exhibits a polarized spatial localization in the epithelium towards the lumen of the intestine. Muc2 is the major mucin produced by Goblet cells of the colon, forming a protective mucus barrier.

2// Resolution

Spatial biology platforms use different approaches when creating the desired image of gene or protein expression activity *in situ*, which results in different levels of resolution. Some can offer crystal clear views all the way down to subcellular structures, while others produce pixelated images with poor resolution.

The key to resolution typically comes from how a given platform creates its view of biomarkers in a sample. Some systems incorporate microscopy or other advanced optics paired with *in situ* hybridization to directly observe fluorescent signals in a tissue sample. For these, resolution is dictated by the quality of the optical lens used and by how clearly cell boundaries can be delineated; the better the cell segmentation, the more likely that a fluorescent signal can be attributed to its exact cell of origin rather than having signals averaged across tens of cells.

Other spatial biology platforms were not designed for direct observation. Instead, they tag the genes or proteins of interest in the sample with barcodes that include location information. Samples are then analyzed with a sequencer, mass spectrometer, or other analysis tool, and the resulting data points are mapped back to their site of origin based on the barcoded location coordinates. The "image" that is generated by these platforms tends to be lower-resolution because it is a computational representation of the sample, rather than a direct view of it.



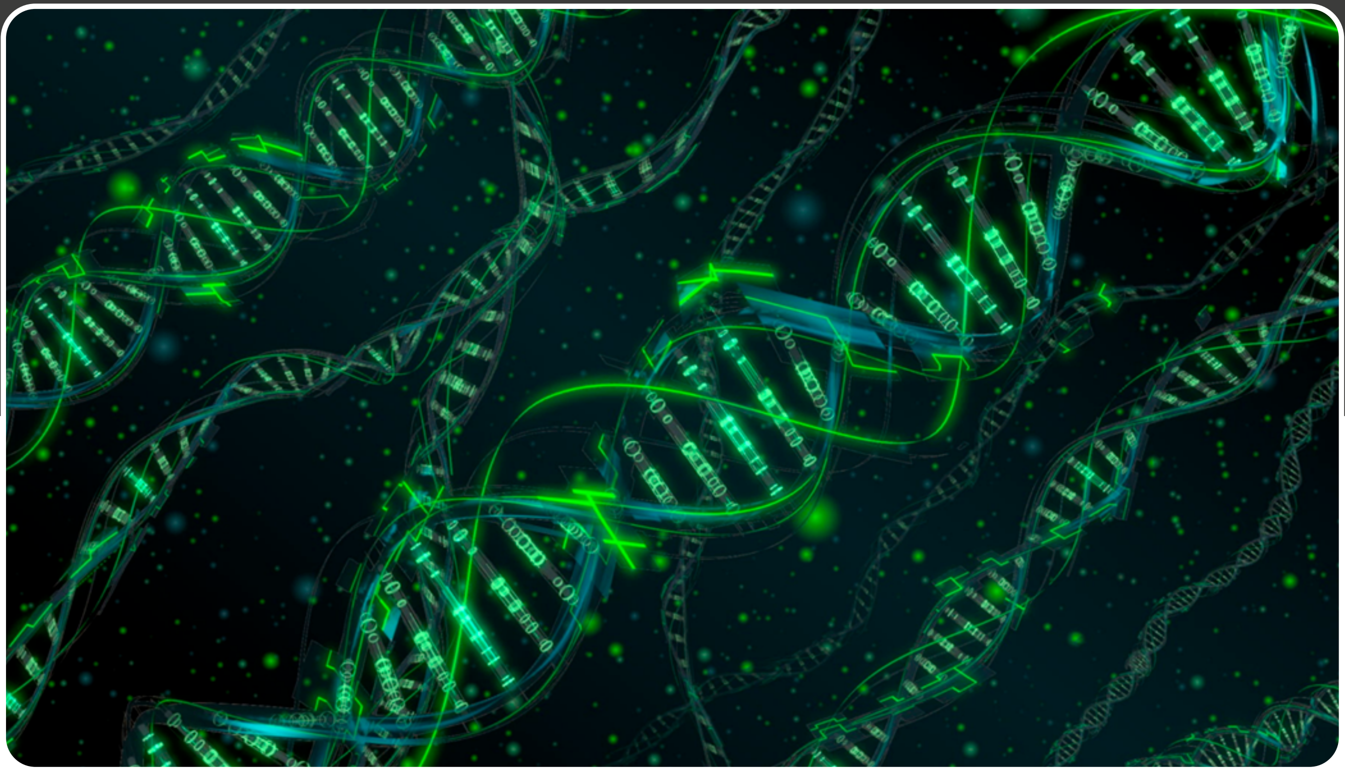
In this image, researchers used Molecular Cartography to visualize and quantify RNA localization, mapped with subcellular resolution in a mouse brain. Each of the 322 yellow dots in this image accounts for one Somatostatin (Sst) transcript, which translates to 322 Sst transcripts in this particular cell. Importantly, researchers can do this for each gene in their panel for each cell in the tissue. The exquisite resolution provides novel insights into the subcellular location where the transcript resides.

3// Multiplexing capabilities

One of the biggest differentiators among spatial biology platforms is their capacity, both for number of analytes that can be queried and for the number of samples that can be analyzed simultaneously. Grouping platforms by type of application can be a handy way of reviewing their multiplexing capabilities.

3.1// True discovery science

For cases where scientists have no prior knowledge about biomarkers or biological activity and would like to use an unbiased approach across an entire genome, transcriptome, or proteome, spatial biology platforms with the most flexible analyte analysis platform are essential. These platforms should have virtually no limit on the number of analytes that can be detected at once and often require next-generation sequencing instruments to enable the broadest biological view of a sample.



3.2// Translational science

Once scientists have a core set of genes or proteins of interest, they can streamline the analysis process significantly. Appropriate spatial biology platforms at this stage may allow for the detection of dozens of proteins or up to 100 or so genes. By targeting only the genes or proteins of interest, it is possible to generate results faster and more affordably. There are several spatial biology platforms available that meet these needs.

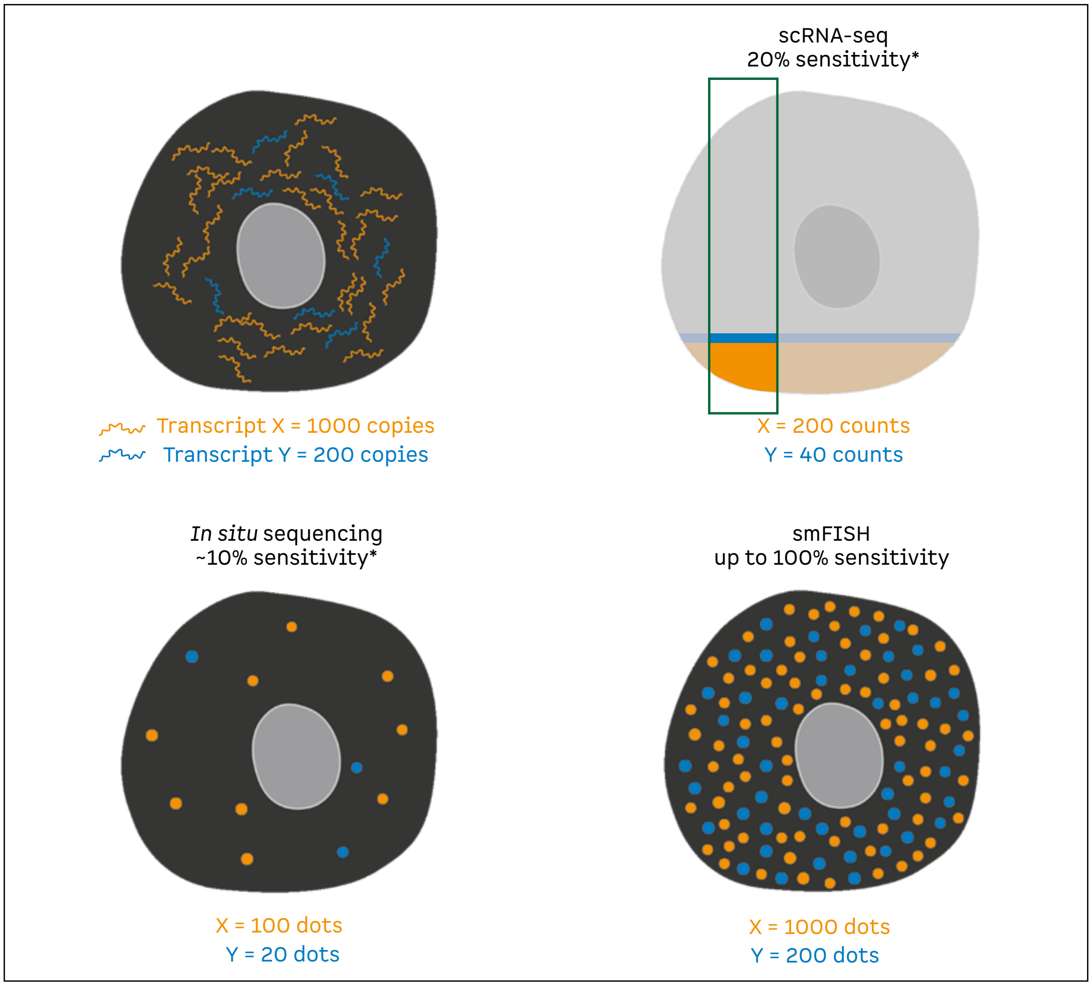
3.3// Clinical research and pathology

Further downstream, scientists tend to focus on a small number of genes or proteins. On the other hand, they may need to study more samples at this stage, which makes it important to look for a platform that can process multiple samples at once.

4// Sensitivity

For any application, sensitivity matters. But for some applications, the ability to detect every target transcript or protein in a sample — down to a single copy per cell — can make all the difference. These rare markers might be essential for detecting a disease or for accurately characterizing a specific biological mechanism.

When selecting a spatial biology platform, be sure to check out sensitivity performance. Ideally, the manufacturer will offer not just metrics but also real-world data showing how sensitive the platform is in detecting the type of analyte that matters most to your research.

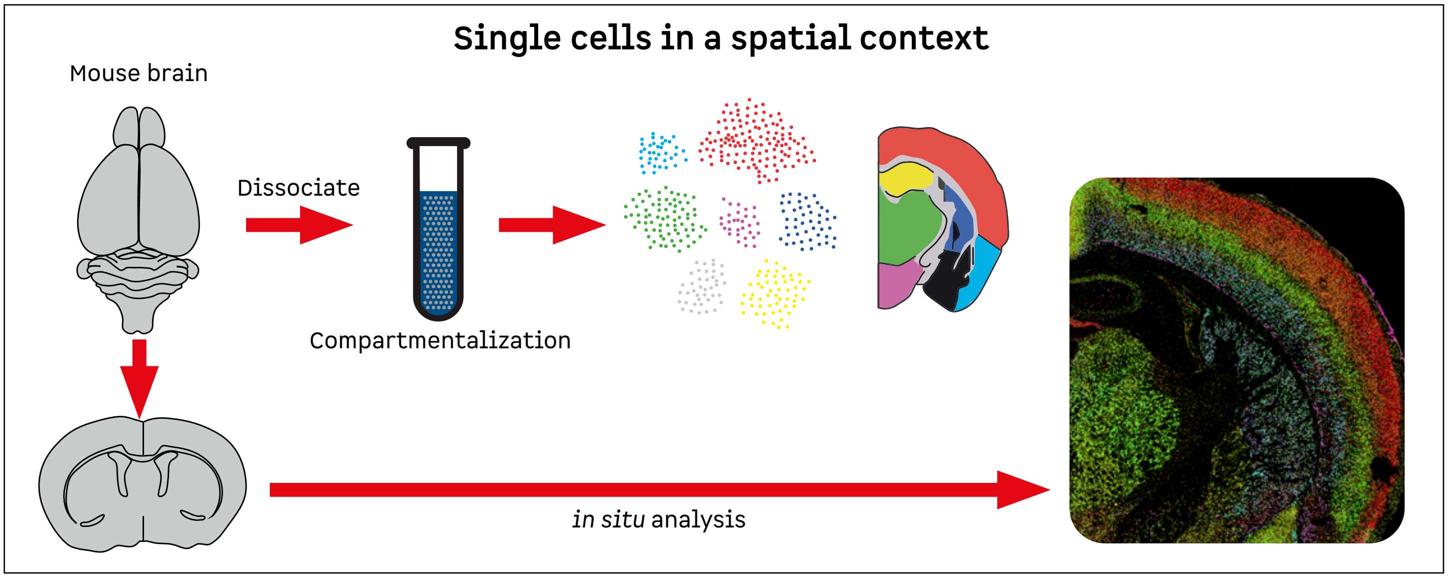


Comparison of transcript sensitivity between single-cell RNA sequencing (scRNA-seq), *in situ* sequencing, and single-molecule fluorescence *in situ* hybridization (smFISH). *Compared to smFISH, which is considered the gold standard for sensitive spatial transcriptomics.

5// Sample integrity

For many spatial biology workflows, there are significant threats to sample integrity. Used in certain applications, these may not be a problem for an experiment — but for others, they can jeopardize both current and future results. Make sure you understand how a sample will be processed for the spatial biology platform you're considering. Some workflows involve a matrix clearing step used to anchor RNAs for analysis while removing proteins, lipids, and other cellular materials to reduce background noise. While this can be effective for generating a snapshot of transcriptome activity, it prevents scientists from interrogating the sample again, such as to collect data about proteins, metabolites, and other analytes.

Even without matrix clearing, many workflows destroy the original sample as an unavoidable part of the analysis process. This is not a problem for one-time sample use, but for scientists interested in having the opportunity to go back to the sample later and re-interrogate it — perhaps to confirm anomalous findings or to check a biomarker that wasn't part of the first analysis — losing the entire sample may not be acceptable. In that case, it's important to focus on platforms that preserve the sample through the use of non-destructive processing steps.

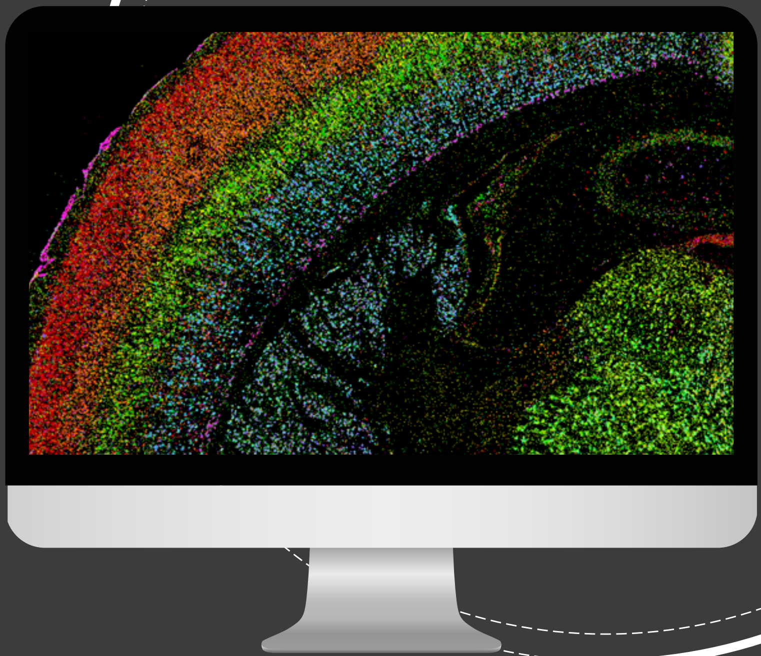


Current methodologies for interrogating cellular functions and gene expression profiles, such as bulk sequencing and single-cell RNA sequencing, often involve tissue dissociation and homogenization during sample preparation, which results in the loss of spatial context. Spatial transcriptomics provides 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.

6// Data analysis

No spatial biology workflow is complete without data analysis. As the detection technologies evolve rapidly, so too are the analytical tools needed to make sense of this new type of data. Software solutions might be needed to process images, reconstruct images from barcode data, segment cells or populations of cells, and visualize specific genes, proteins, or cell types as well as interactions between them. Some data analysis tools are made for specific platforms, while others are meant to be used more generally. A few incorporate artificial intelligence features, particularly to automate challenging tasks such as cell segmentation.

When evaluating data analysis options, be sure to identify the specific tasks you'll need so you can make sure the tool or tools you select can handle the entire analysis process. Check with the manufacturer of your spatial biology platform and other customers for recommendations about which data analysis tools perform best for that particular workflow. It may also be important to consider whether the tool can analyze other types of data — perhaps you plan to merge spatial data with single-cell data, for instance. Many tools that emerged from the single-cell RNA-seq field have been adapted to work with spatial data, and having that flexibility can be a real benefit.



Scientific publications

1. Spatial proteogenomics reveals distinct and evolutionarily conserved hepatic macrophage niches
   Guilliams *et al.*
   Cell, January 11, 2022
2. Diversity and function of motile ciliated cell types within ependymal lineages of the zebrafish brain
   D'Gama *et al.*
   Cell Reports, October 05, 2021
3. Highly resolved spatial transcriptomics for detection of rare events in cells
   Groiss *et al.*
   BioRxiv, October 12, 2021

Download white papers

1. The Rise of Spatial Biology: Why a Better Approach Is Needed
2. Molecular Cartography™ of brain organoids to unravel the spatial logic of neural tissue development

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