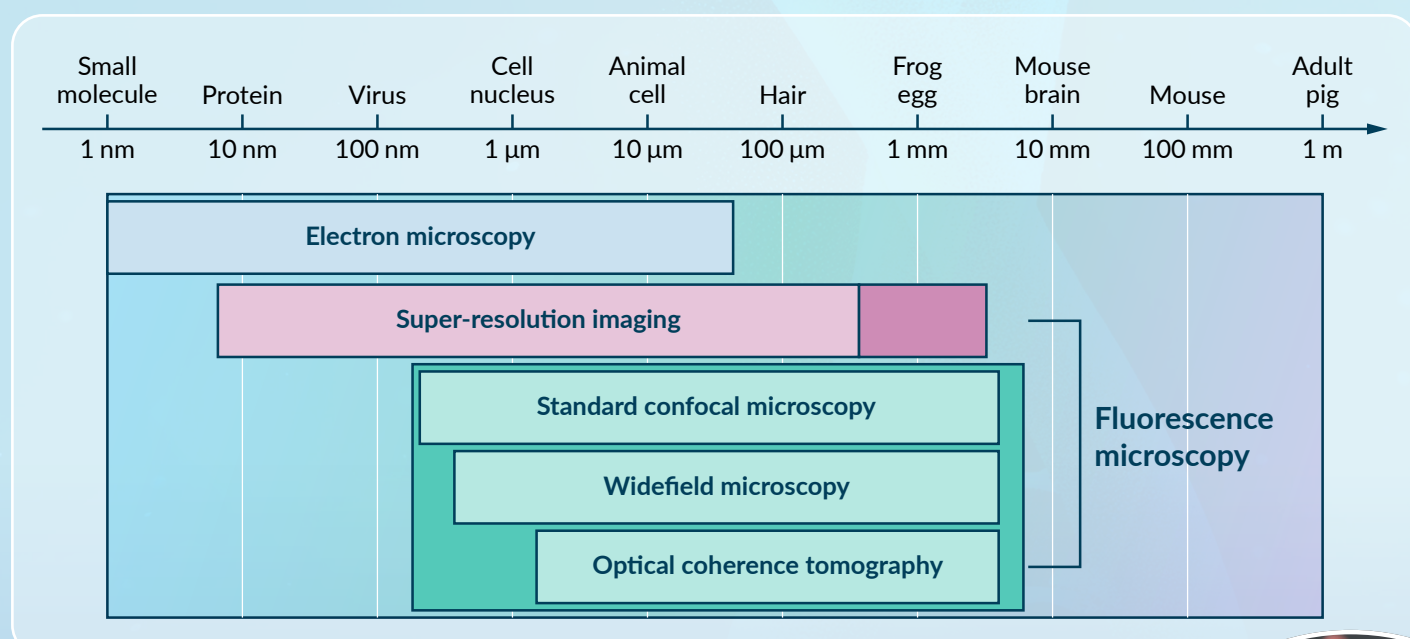


# Navigating Spatial Biology: From Techniques to Data Optimization

Spatial biology, whether its genomics, proteomics or transcriptomics, offers a geographical context to observed morphology, function, cellular interactions and signaling. Location of the cell within a tissue or sample, and location of molecular structures within a cell is known to affect cell function and has implications in disease pathology and progression. Hence, understanding where and how spatial biology techniques can be used is very important.

## Imaging Modalities and Resolutions

There are several different techniques offering multi-target strategies to image and study cell biology at varying length scales. The choice of technique used often depends on the biology being studied and the sample being used. Each technique is also associated with some tradeoffs in terms of resolution, length scales, cost, and throughput, which need to be considered.



## Applications of Spatial Techniques

### Spatial techniques can be used to study:

- Geographic distribution of cell types and markers
- Cellular changes *in vitro* and *in vivo* over time
- Changes with respect to age, genotype, location, pathology
- Changes in disease phenotype and progression in spatially distinct regions
- Cell-cell organization, interactions and signaling pathways
- Sub-cellular and molecular level localization and interactions
- Cellular response to drug, stimuli
- Histological heterogeneity and molecular subtypes
- Single-cell heterogeneity
- Localization and movement of multiple cellular targets
- DNA and RNA sequencing data in a spatial context
- Biomarkers for early detection of disease

## Sample Preparation and Experimental Design Tips for Spatial Biology

- Choose a spatial technique that works best for the biological question to be answered
- Decide on whether the study needs unbiased high-level discovery or a specific biological query
- Ensure that the technology supports the sample type (fresh frozen, biopsy, FFPE, microarrays)
- Choose regions of interest (ROIs) for study within the sample to save on time and costs
- Choose good morphology markers to identify each region
- Pick a technique that can increase detection of samples with low gene/mRNA expression
- Maximize the capacity of the platform for analyzing the sample
- Integrate microfluidic channels for multiplex imaging to study multiple targets
- Improve probe accessibility (using flow, background blockers)
- Use signal enhancers during sample preparation
- Follow recommended best practices and workflows given by the vendor
- Stay abreast of new techniques and improvements

## Optimization Tips to Improve Data Quality for Spatial Biology

- Ensure that data is high quality using visualization tools (count artifacts, signal to noise ratio)
- Use positive and negative controls
- Perform quality control to remove probes of low quality and genes that were below the limit of quantitation (LOQ)
- Analyze sample to sample correlation and batch effects between runs to identify outliers
- Use proper tools for effective integration with single-cell sequencing (scSeq) data
- Use machine learning to analyze data
- Replicate findings from literature that were obtained using other complementary technologies

## Resources for Spatial Biology Experiments and Data Analysis

### Public Datasets including:

- Digital spatial profiling—GEO DataSets—NCBI
- Datasets —10x Genomics
- CZ CELLxGENE Discover—Cellular Visualization Tool
- Single Cell Portal (broadinstitute.org)

### Spatial Data Analysis Tools in R for GeoMX DSP

- Analyzing Nanostring's GeoMx transcriptomics data using standR, limma and vissE
- GeoMXAnalysisWorkflow
- Analyzing GeoMx-NGS RNA Expression Data with GeomxTools
- GeoScript Hub | NanoString

### Spatial Data Analysis Tools in R for Visium

- Analysis, visualization, and integration of spatial datasets with Seurat
- Secondary Analysis in R -Software—Spatial Gene Expression

Alternative normalization tools (Q3, RUV4, DESeq2, CPM, Quantile) to reduce noise interference

CellChat to detect ligand-receptor and cell-cell communication between different regions

SpatialHeatmap data visualization tool for cell, tissue, organ-specific spatial images

TrendCatcher open-source R-package to systematically analyze and visualize time course data

Singscore reference database for well-defined single-sample gene expression profile scores

This infographic is excerpted from presentations given by Dr. Matthew Rames, Oregon Health and Science University; Dr. Shuai Wang, University of South Florida; and Dr. Osama Shiraz Shah, University of Pittsburgh in Biocompare's Bench Tip webinar on **Spatial Biology: Understanding the Nuances for Cancer and CNS Applications**, streamed on October 26, 2023.