

# SPATIAL TRANSCRIPTOMICS INFOGRAPHIC

IN FOCUS

Spatial transcriptomics tools make it possible to discover the relationship between gene expression and its natural tissue architecture at high resolution. This infographic highlights the key considerations for implementing spatial transcriptomics tools into your workflows and introduces the existing methodologies.



## Current methodologies

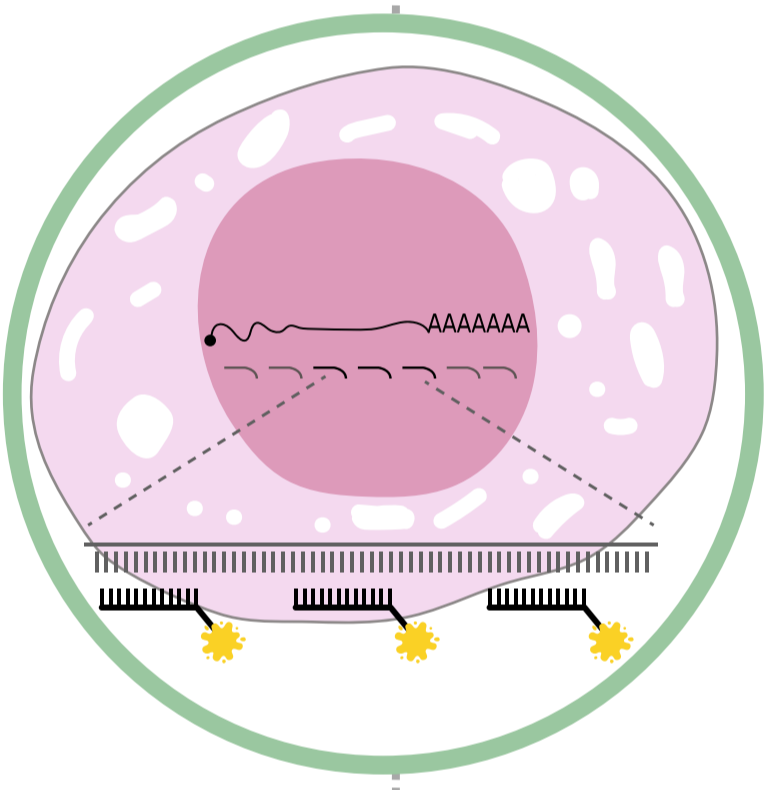
### smFISH

#### Advantages

#### Limitations

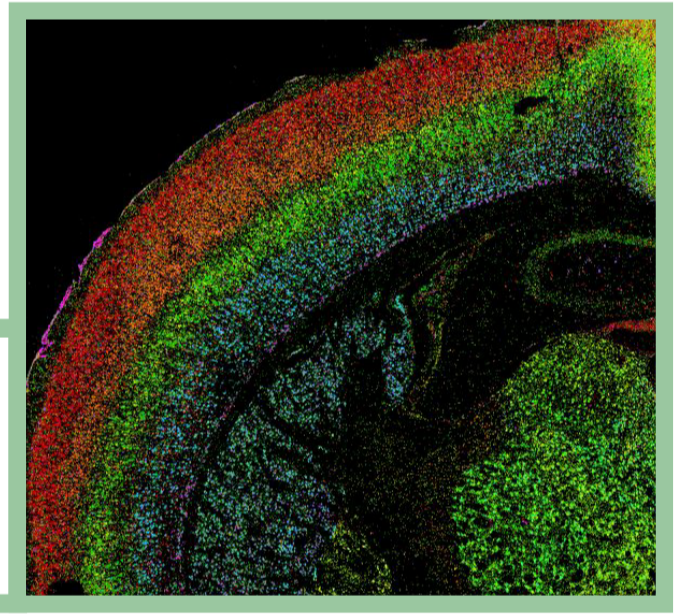
- > Flexible sample types: fresh-frozen, formalin-fixed paraffin-embedded (FFPE) and cell culture
- > Highest cellular and subcellular resolution for cell-type-specific transcript analysis
- > Multiplexing capability
- > High specificity and sensitivity
- > Robust signal for quantitative transcript measurement

- Pre-defined target required
- Integrity of tissue is disrupted



Dye-labeled oligo probes bind to a target stretch of complementary mRNA, providing the readout for smFISH

Murine cortex captured with Resolve BioSciences' Molecular Cartography™, the only smFISH platform capable of **maintaining tissue integrity**



#### Advantages

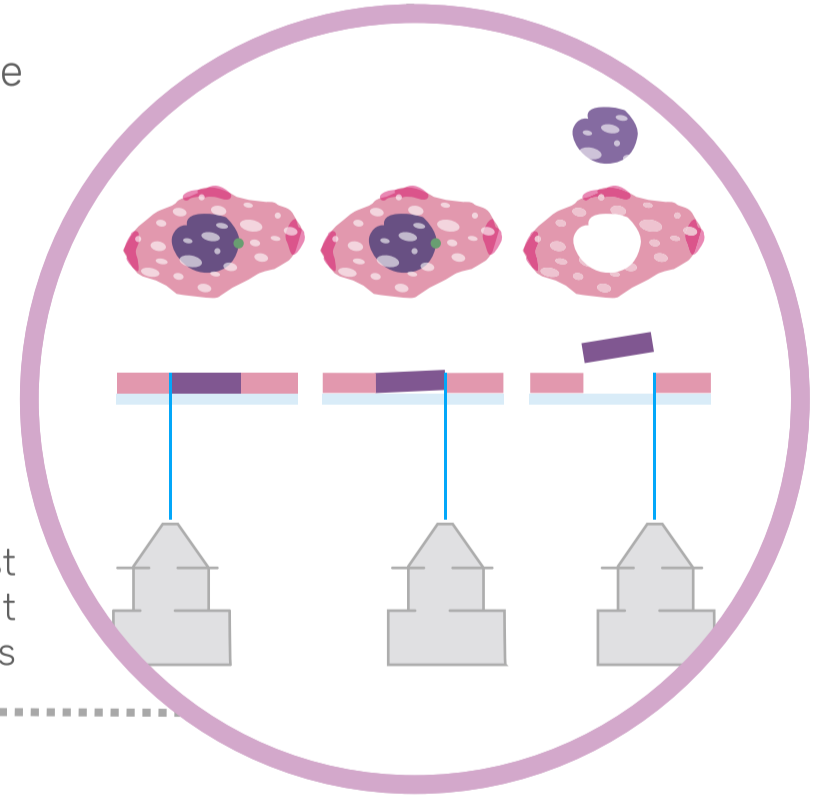
#### Limitations

- > Flexible sample types: fresh-frozen tissues, FFPE tissues and cell culture
- > Single-cell resolution for cell-type-specific transcript analysis
- > Complete extraction of cells from known tissue location for downstream analysis

- Low throughput
- Small area of tissue on slide
- No subcellular resolution

Laser excising a region of interest from a tissue for subsequent gene expression analyses

### Microdissection



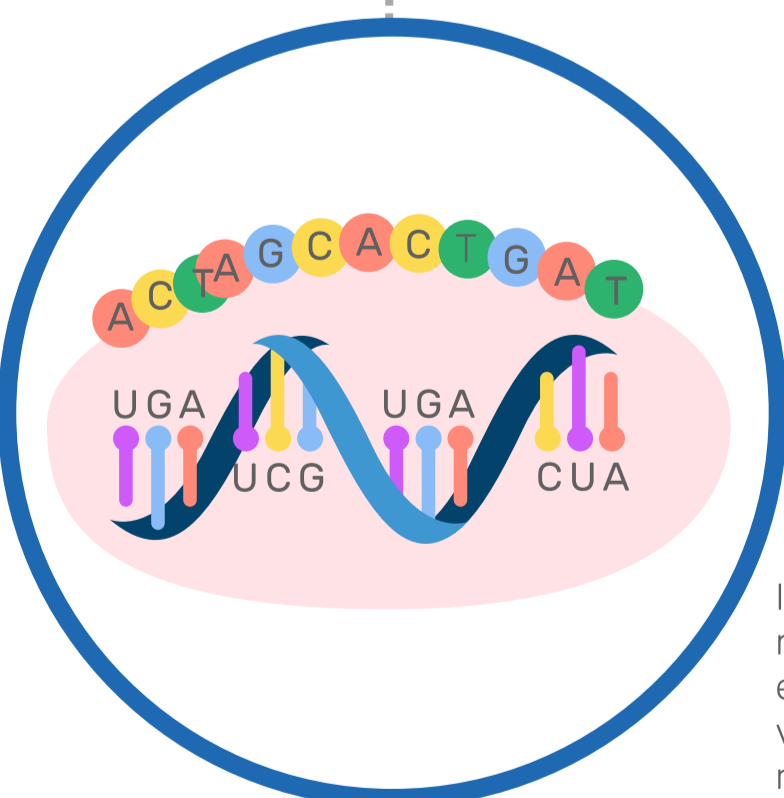
### In situ sequencing

#### Advantages

#### Limitations

- > Flexible sample types: fresh-frozen tissues, FFPE tissues and cell culture
- > Single-cell and subcellular resolution for cell-type-specific transcript analysis
- > Multiplexing capability
- > Easily applied to a large tissue section

- Low sensitivity
- Highly abundant genes involved in translation and splicing are depleted
- Integrity of sample is lost



In situ sequencing performs RNA sequencing of the transcripts within the tissue. mRNA is reverse transcribed to cDNA. Padlock probes hybridize to the cDNA and enable rolling-circle amplification. These products can then be visualized using fluorescent oligonucleotides. This method enables subcellular resolution insights into the transcripts present in the cells of the tissue.

#### Advantages

#### Limitations

- > Flexible sample types: fresh-frozen tissues, FFPE tissues and cell culture
- > Single-cell resolution for cell-type-specific transcript analysis
- > Highly scalable
- > Untargeted approach

- Low sensitivity
- No subcellular resolution
- Integrity of sample is lost

A tissue section is placed on an array containing spatial barcodes. After permeabilization, RNA molecules are captured at the barcoded location and sequenced externally. The location-specific barcodes are then used to reassemble the spatial gene-expression profiles in the sample.

### In situ capturing

