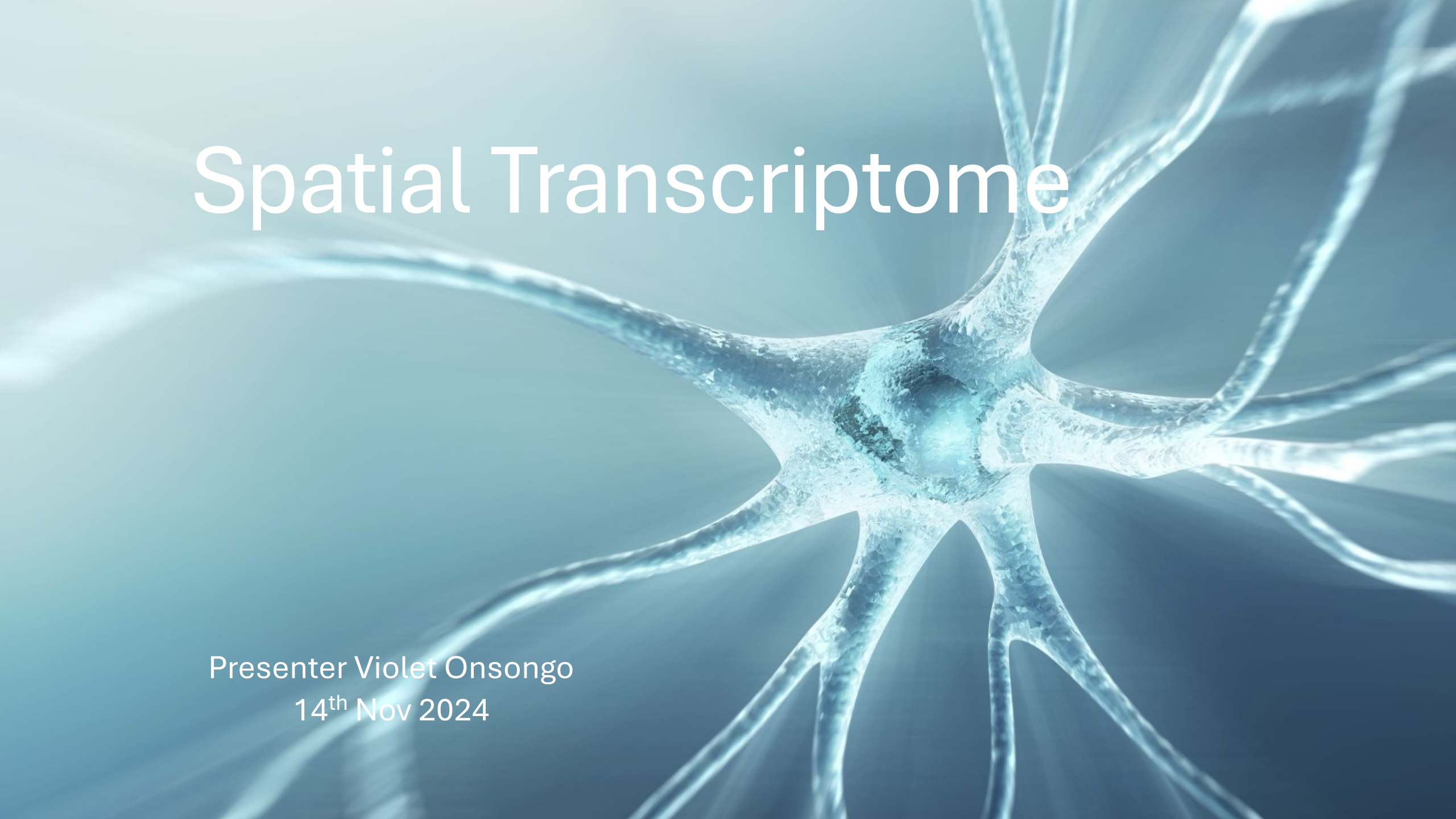
Spatial Transcriptome



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Introduction

- Spatially resolved transcriptomics was invented by Lundeberg, Frisen and Stahl at KHL Royal Institute of Technology in Sweden in 2016

- Aim: To provide gene expression data for a large number of cells

- Making it possible to analyze tens of thousands of genes localized in a small section of tissue

Key Study Questions Addressed by Spatial Transcriptomics in Biology and Medicine

- Tissue Heterogeneity: What are the gene expression differences across distinct regions within the same tissue?
- Effects of Treatment: How do therapies impact spatial gene expression within affected tissues?
- Biomarker Discovery: Where are potential biomarkers for diseases localized within tissue?
- Developmental Biology: How does gene expression vary spatially and temporally during development?

How does spatial transcriptomics work?

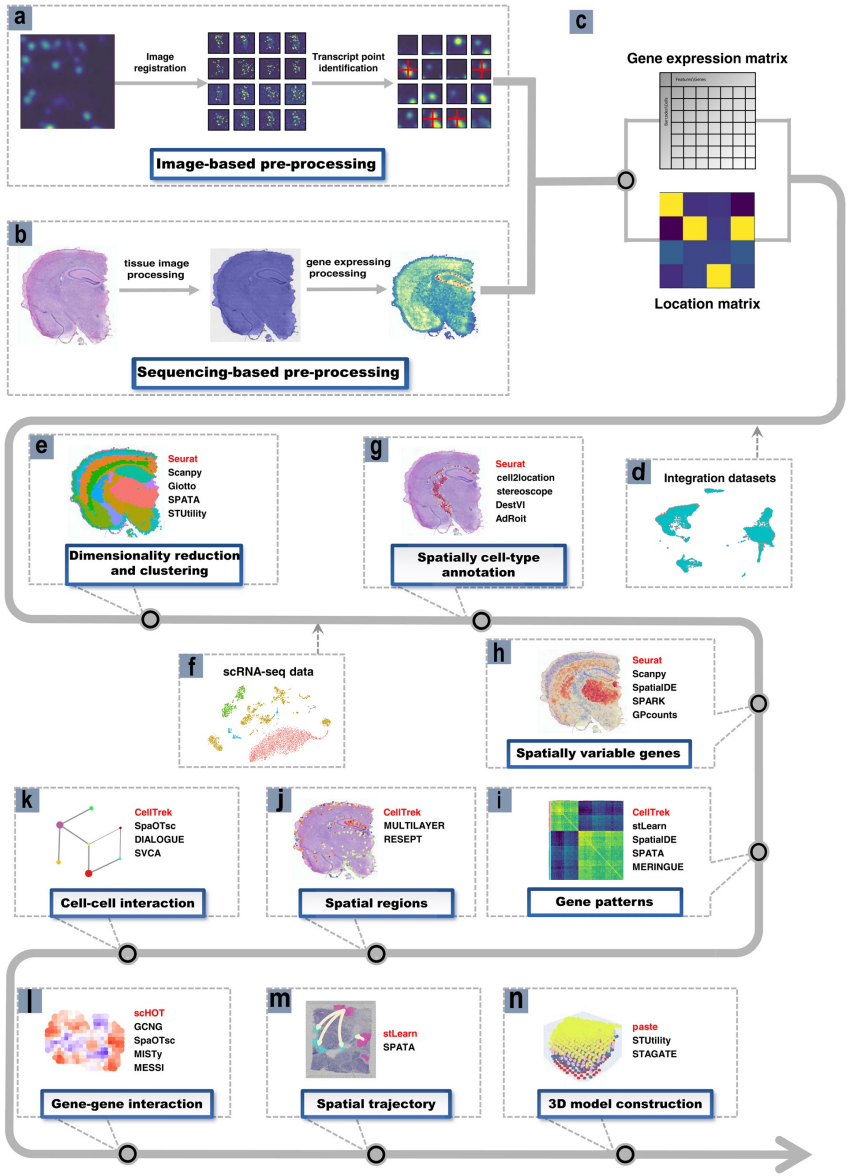
- A typical spatial transcriptomics workflow starts by isolating and staining a tissue section of interest
- The section is then placed into contact with a slide holding RNA-binding capture probes
- The bound RNA is barcoded and used to synthesize complementary DNA, which is then generated into libraries and sequenced
- Subsequently, the data is visualized, making it possible to infer where the genes of interest are expressed and in which parts of the tissue section

F. L., & Mobley, 2021

Step-by-step: Spatial transcriptomics workflow

1. All cells are permeabilized
2. A dense carpet of specialised probes on the surface of a glass slide capture and bind to the mRNA. Each capture probe contains a spatial barcode, unique to that spot on the slide.
3. Fluorescent labelling of the mRNA creates a footprint that mirrors the morphology of the tissue.
4. The tissue that is attached to the slide acts as a template for a reverse transcription reaction, generating a complementary DNA library.
5. The complementary DNA, with the spatial barcodes incorporated, is cleaved from the surface of the slide and collected for standard sequencing.
6. The uniquely barcoded capture probes then link the resulting RNA sequences back to their previous spots on the slide – a process called ‘de-multiplexing’.
7. The RNA data is aligned to a high-resolution microscope image of the tissue section so that it can be mapped back to its point of origin.
8. The expression of mRNA can then be visualised with a spatial dimension.

Workflow of data analysis



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Spatial transcriptomic technologies

- The choice of spatial transcriptomic technology requires a trade-off between gene throughput, sequence information, sensitivity, resolution, area size, and feasibility

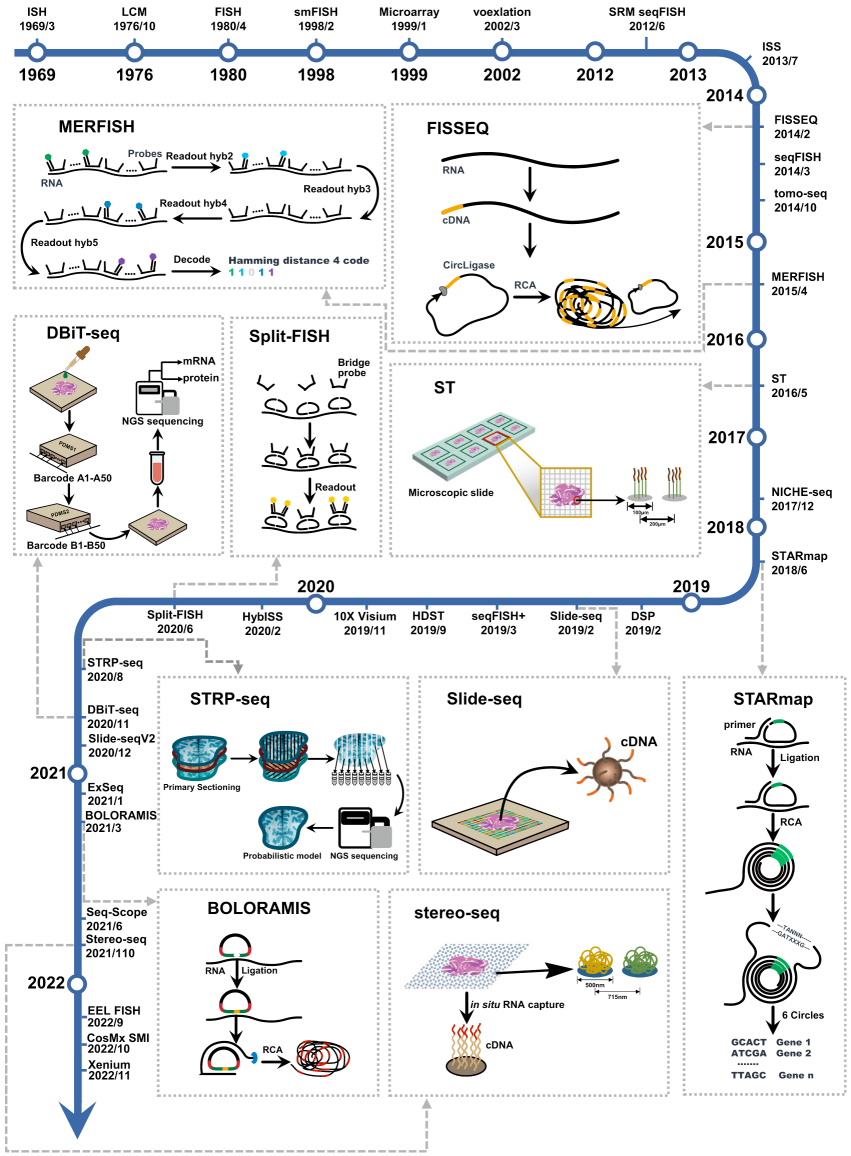
Classification of the spatial transcriptomic technologies

1. In situ hybridization (ISH
2. In situ sequencing (ISS
3. Next-generation sequencing (NGS
4. The spatial information reconstruction technologies

Table 1
Technical highlights of four technical routes in spatial transcriptomics.

In situ hybridization (ISH)-based technologies				
Method	Application	Detection efficiency	Features	Refs
seqFISH	Fresh-frozen	84 %	Targeted, expensive experiments	[9,42]
MERFISH	Fresh-frozen	80 %	Targeted, high robustness of probe design method, expensive experiments	[9,17]
seqFISH+	Fresh-frozen	49 %	Targeted, expensive experiments	[9,18]
DSP	Fresh-frozen or FFPE	NA	Targeted, commercially available	[19]
Split-FISH	Fresh-frozen	NA	Targeted, no tissue clearance required , expensive experiments	[9,20]
EEL FISH	Fresh-frozen	13.2 %	Targeted, transferring RNA using electrophoresis, low-cost experiments	[21]
SMI	Fresh-frozen or FFPE	One or two copies per cell	Targeted, high signal to noise ratio detection, commercially available	[22]
In situ sequencing (ISS)-based technologies				
Method	Application	Detection efficiency	Features	Refs
ISS	Fresh-frozen or FFPE	< 1 %	Targeted, low throughput, commercially available	[42,103]
FISSEQ	Fresh-frozen or FFPE	< 0.005 %	Unbiased, whole transcriptome, low capture efficiency , commercially available	[42,103,187]
STARmap	Fresh-frozen	higher than single-cell RNA sequencing	Targeted, reverse transcription-free	[25]
HybISS	Fresh-frozen or FFPE	higher than ISS	Targeted, higher throughput than ISS	[26]
BOLORAMIS	Cell lines	10–30 %	Targeted, reverse transcription-free	[29]
Next generation sequencing (NGS)-based technologies				
Method	Application	Resolution	Features	Refs
ST/Visium	Fresh-frozen/Fresh-frozen or FFPE	100/55 μm	Unbiased, whole transcriptome, low capture efficiency, commercially available	[31,188,189]
slide-seq	Fresh-frozen	10 μm	Unbiased, whole transcriptome, low capture efficiency	[33]
HDST	Fresh-frozen	2 μm	Unbiased, whole transcriptome, low capture efficiency	[34]
DBiT-seq	Fresh-frozen or FFPE	10 μm	Unbiased, whole transcriptome, low capture efficiency	[35]
slide-seqV2	Fresh-frozen	10 μm	Unbiased, whole transcriptome, higher capture efficiency than slide-seq	[36]
Seq-Scope	Fresh-frozen	0.5–0.8 μm	Unbiased, whole transcriptome, low capture efficiency	[37]
stereo-seq	Fresh-frozen	500/715 nm	Unbiased, whole transcriptome, low capture efficiency, largest detection area	[38]
Spatial information reconstruction technologies				
Method	Application	Algorithm	Features	Refs
tomo-seq	Fresh-frozen	Iterative Proportional Fitting (IPF)	Unbiased, imaging-free, whole transcriptome, low capture efficiency	[40]
STRP-seq	Fresh-frozen	Tomographer	Unbiased, imaging-free, whole transcriptome, low capture efficiency	[41]

Map of spatial transcriptomics and related technological developments



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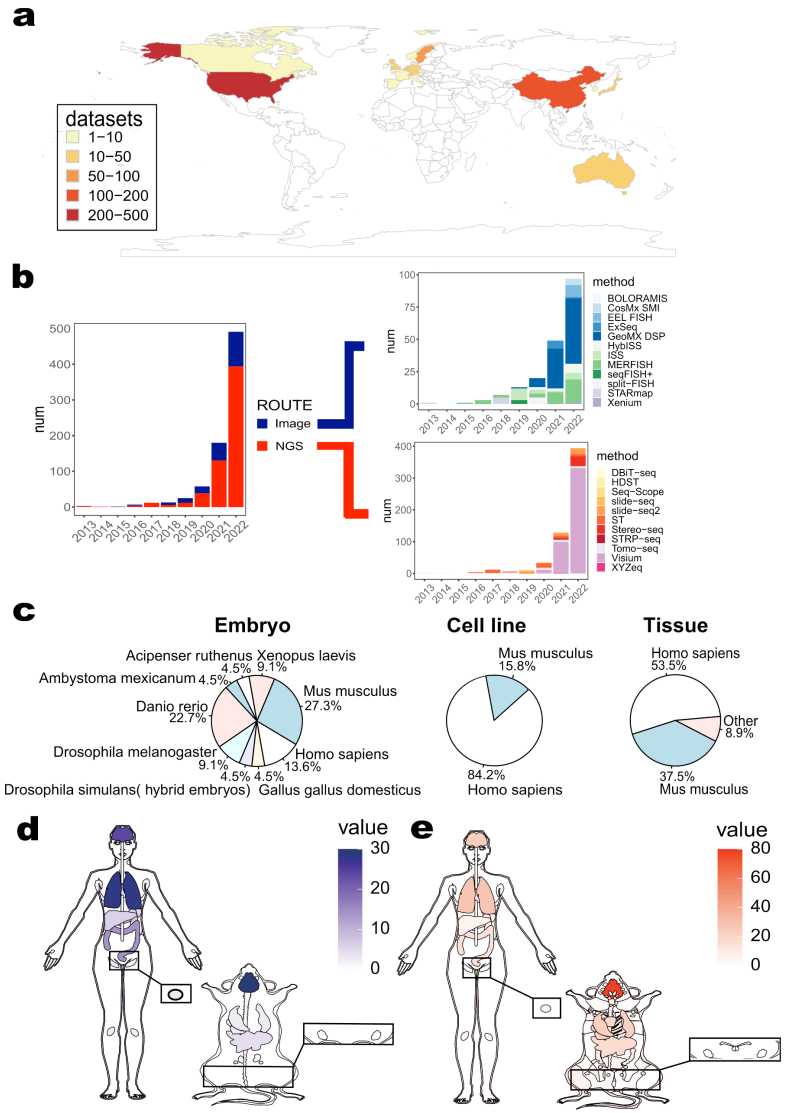
Statistics of spatial transcriptomic datasets



Differences in the distribution of papers in the database across countries

Trend graphs of the number of articles issued in both technical lines
Data are available until 31 December, 2022

Categories of experimental material used in spatial transcriptomic experiments

Percentage of human and mouse organs or tissues used in imaging-based technologies

Percentage of human and mouse organs or tissues used in sequencing-based technologies

Challenges facing spatial transcriptomics

1. **Resolution and Cell Distinction**: Limited resolution makes it hard to capture cell-specific gene expression

2. **Data Complexity**: Large, complex datasets require significant computational resources and expertise

3. **Technical Artifacts**: Sample preparation biases can affect data accuracy

4. **High Costs**: Expensive equipment and reagents limit accessibility

5. **Limited Transcript Coverage**: Low sensitivity for some genes may miss important details

6. **Data Integration**: Combining spatial transcriptomics with other data types is challenging

7. **Standardization**: Lack of standard protocols impacts reproducibility

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Class question

How Can You Use Spatial Transcriptomic Data in Your Research?