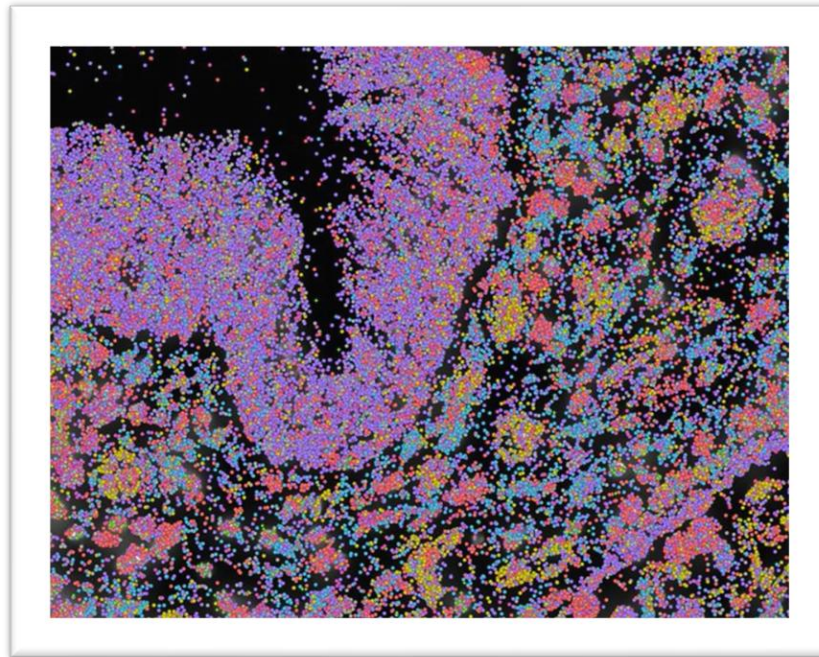


Introduction to Spatial Transcriptomics



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Single-Cell Transcriptomics (scRNA-seq)

- hundreds of types of cells that comprise the human body possess essentially the same DNA
- Cellular diversity and cell-specific function, is best assessed not at the DNA level, but at the protein level
- However, there are no commercially available methods for quantifying the thousands of proteins within individual cells of our bodies
- Heterogeneity and dynamic change in eukaryotic cells is studied using scRNA-seq.

Limitations of scRNA-seq

- Most commercially available protocols require cells to be recovered intact and viable from tissue
- which are then assessed and labelled individually using microfluidic, droplet-based, or limiting dilution methods
- This has precluded many cell types from study and largely destroys the spatial context that could otherwise inform analyses of cell identity and function

What is Spatial Transcriptomics?

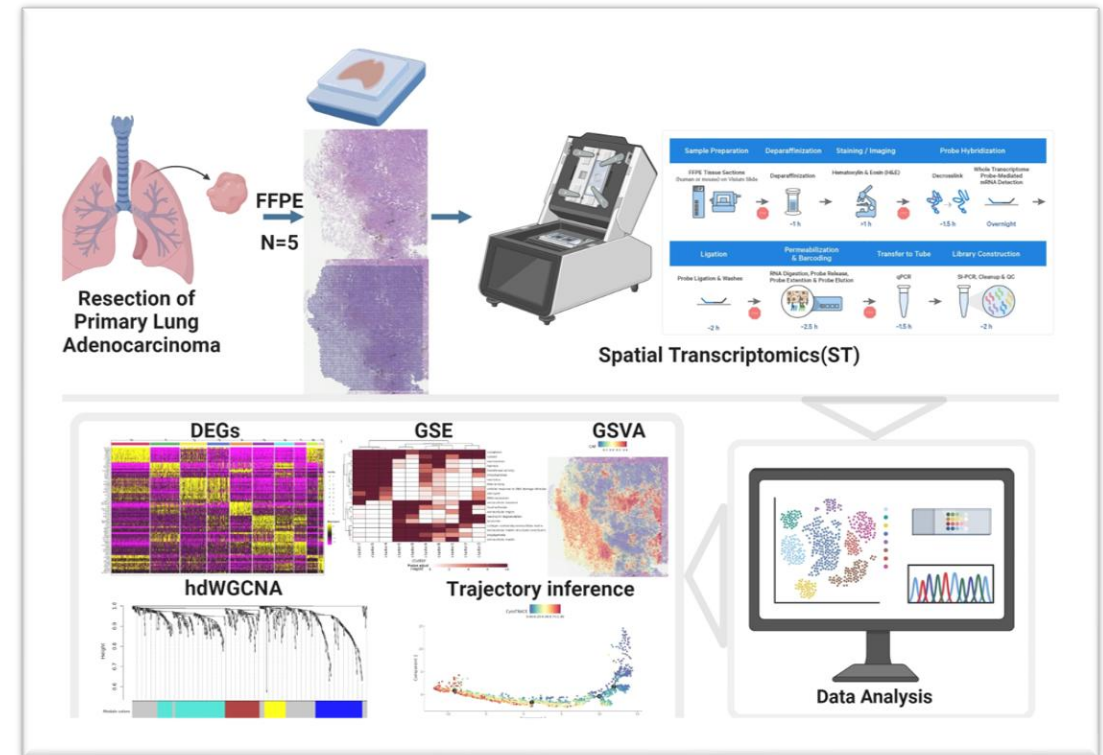
- Commercially available platforms now facilitate spatially resolved, HD assessment of gene transcription known as ‘spatial transcriptomics’
- These either record the locations of hybridized mRNA in tissue, image the positions of cells themselves prior to assessment, or employ spatial arrays of mRNA probes of pre-determined location.
- The aim is to count the number of transcripts of a gene at distinct spatial locations in a tissue
- Leading to improved understanding of human tissue in research, diagnostics, and therapeutic settings
- Unlike traditional scRNA-seq, spatial transcriptomics reveals where genes are expressed within the tissue microenvironment

Spatial Transcriptomics Platforms

Platform Type	Examples	Strengths	Limitations
Sequencing-based	Visium, Slide-seq	Whole transcriptome coverage	Lower spatial resolution
Imaging-based	MERFISH, Xenium, CosMx	High spatial resolution	Limited gene panels
In situ hybridization	seqFISH	Single-cell detail	Computationally intensive

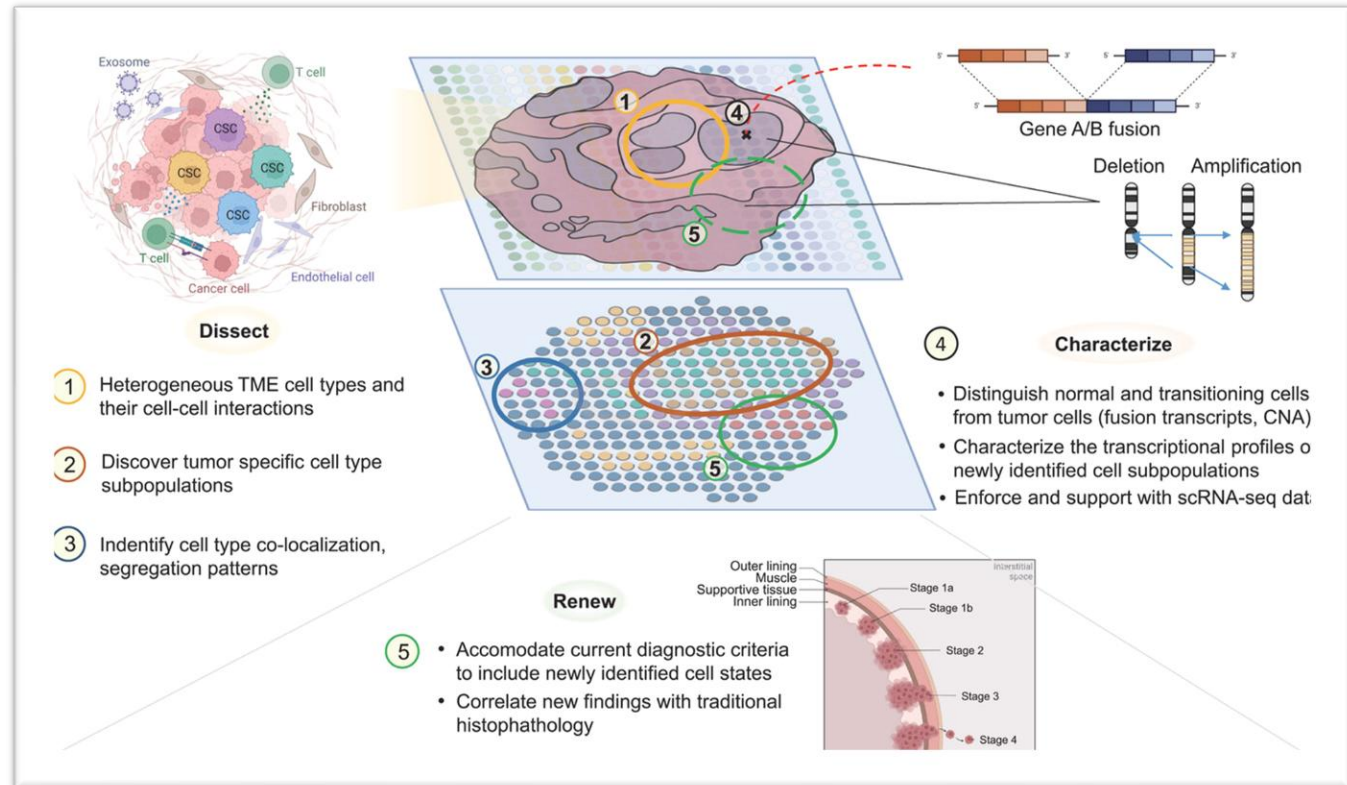
Why Spatial Context Matters

- The position of any given cell, relative to its neighbours and non-cellular structures, can provide helpful information for defining cellular phenotype, cell state, and ultimately cell and tissue function.
- Location can determine the signals to which cells are exposed



Spatial Transcriptomics in Biomedical Research

- Tissue differentiation and developmental patterning
- Spatial mapping of tumour histology and protein gradients
- Disease microenvironment analysis
- Identification of immunosuppressive tumour niches
- Discovery of novel cell types in tissues
- Cell-cell interaction and signalling pathway mapping



Spatial Transcriptomics Technologies

- Different techniques have different technical parameters
- Tissue size can vary from a small ($<1\text{ mm}^2$) section to whole organ sections
- The number of genes counted can vary from tens to thousands or even the whole genome
- A spatial location may range from a whole tissue domain to a single cell or even finer
- Currently, there are two main ways to profile transcriptomes while preserving spatial information:
 1. Imaging mRNAs *in situ* via microscopy (Imaging based technologies)
 2. *in situ* sequencing (ISS) of amplified mRNAs (Sequencing based)

Imaging-based technologies

- When imaging mRNAs in situ, you also need a way to distinguish different mRNA species – there are two options available
 1. *in situ* hybridization
 - hybridization of mRNAs to fluorescently labelled, gene-specific probes
 - Eg: *seqFISH*, *seqFISH+*, and *MERFISH*
 2. *in situ* sequencing
 - Amplified transcripts are directly sequenced inside a tissue block by sequencing by ligation technology
 - Eg: *FISSEQ*, *ExSeq*, and *STARmap*

Sequencing based technologies

- Involves the extraction mRNAs from the tissue while preserving spatial information and subsequently profile mRNA species via next-generation sequencing
- Eg: *Visium*, *Slide-seq*, and *Stereo-seq*
- Common methods of preserving spatial information are:
 1. Direct capture and recording of location, such as via microdissection and microfluidics,
 2. Ligation of mRNAs to spatially-barcoded probes in a microarray

Summary

- scRNA-seq has improved our understanding of cellular heterogeneity, but it does not preserve spatial information within tissues.
- Spatial transcriptomics allows gene expression to be studied while maintaining the original tissue structure and cellular location.
- A range of spatial transcriptomics platforms are now available, each with different strengths and limitations.
- Understanding where cells are located within tissues is important for studying cell function, communication, and disease processes.
- Spatial transcriptomics is becoming an important tool in areas such as cancer research, neuroscience, developmental biology, and precision medicine.

References

- Chen *et al.* (2020). Spatial transcriptomics and in situ sequencing to study Alzheimer's disease. *Cell*, 182(4), 976-991.
- Coy *et al.* (2022). Single cell spatial analysis reveals the topology of immunomodulatory purinergic signalling in glioblastoma. *Nature Communications*, 13(1), 4814.
- Garcia-Alonso *et al.* (2021). Mapping the temporal and spatial dynamics of the human endometrium in vivo and in vitro. *Nature genetics*, 53(12), 1698-1711.
- Kadur Lakshminarasimha Murthy *et al.* (2022). Human distal lung maps and lineage hierarchies reveal a bipotent progenitor. *Nature*, 604(7904), 111-119.
- Lin *et al.* (2023). Multiplexed 3D atlas of state transitions and immune interaction in colorectal cancer. *Cell*, 186(2), 363-381.
- McNamara *et al.* (2021). Spatial proteomic characterization of HER2-positive breast tumours through neoadjuvant therapy predicts response. *Nature cancer*, 2(4), 400-413.
- Nirmal *et al.* (2022). The spatial landscape of progression and immunoediting in primary melanoma at single-cell resolution. *Cancer discovery*, 12(6), 1518-1541.