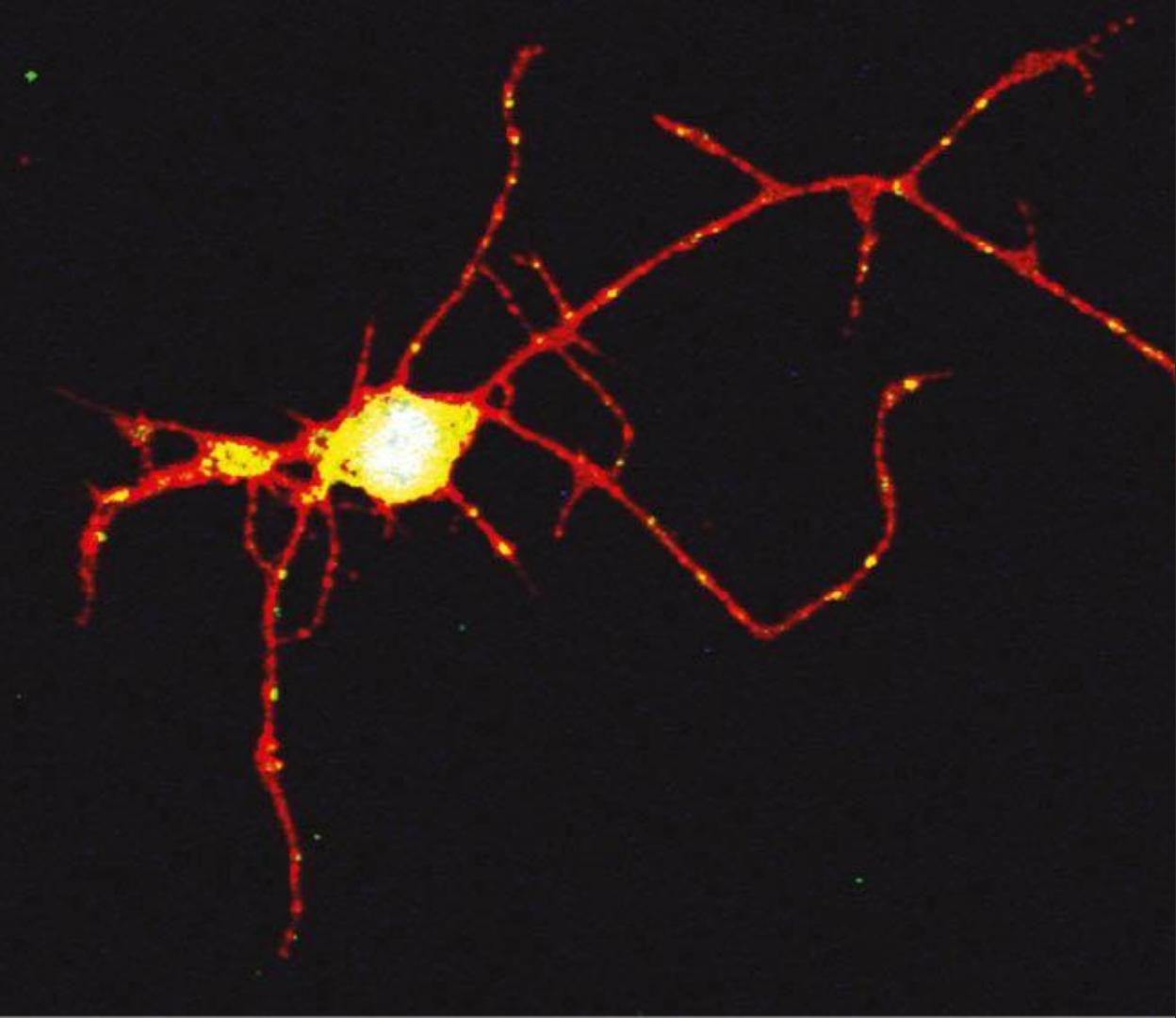


GENOMICS IN 3D

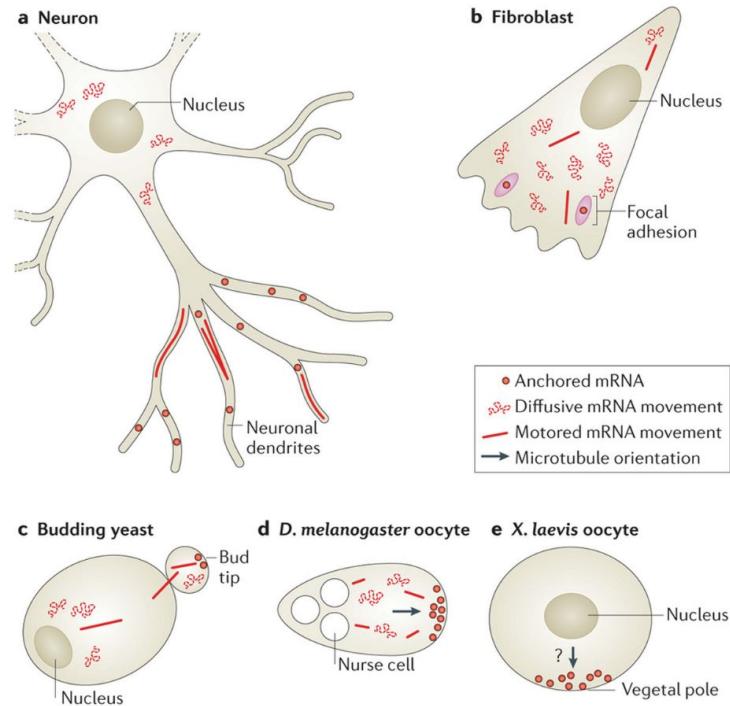
NOORSHER AHMED
CLARENCE MAH

FEB. 7, 2022



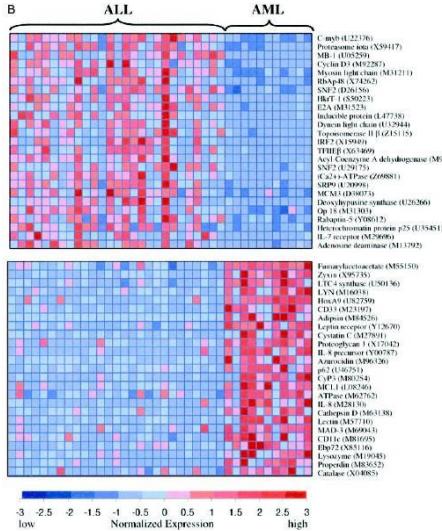
WHY IS RNA LOCALIZATION IMPORTANT?

- Locally targeted protein expression at scale (mini translation factories)
- Subcellular localization using 'zipcode' information without changing structure and function of protein
- Asymmetric cellular functionality

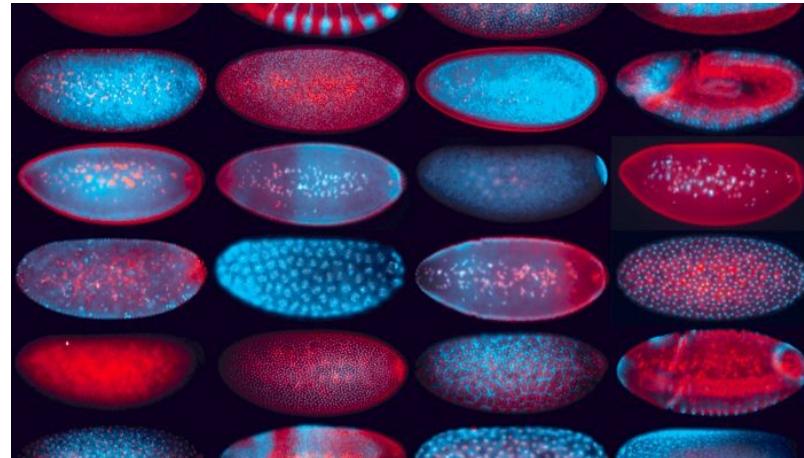


CANONICAL GENOMICS VS. SPATIAL GENOMICS

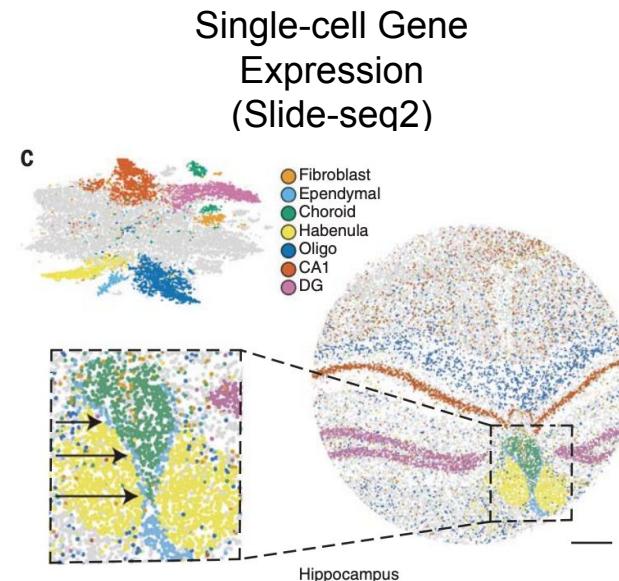
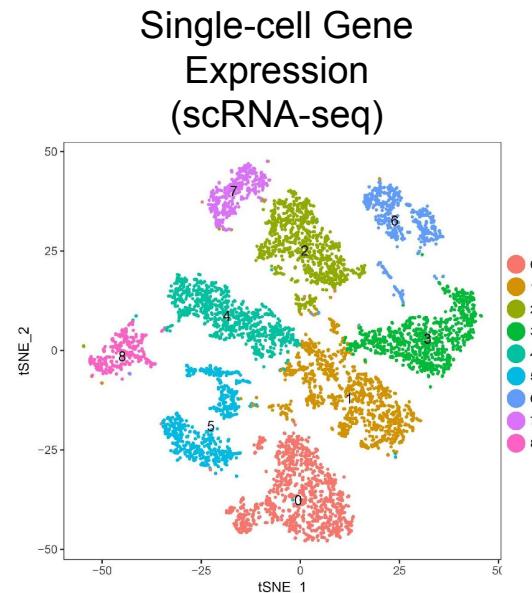
Differential Gene Expression (RNA-seq)



Differential mRNA Localization
(Fluorescent *in situ* hybridization)



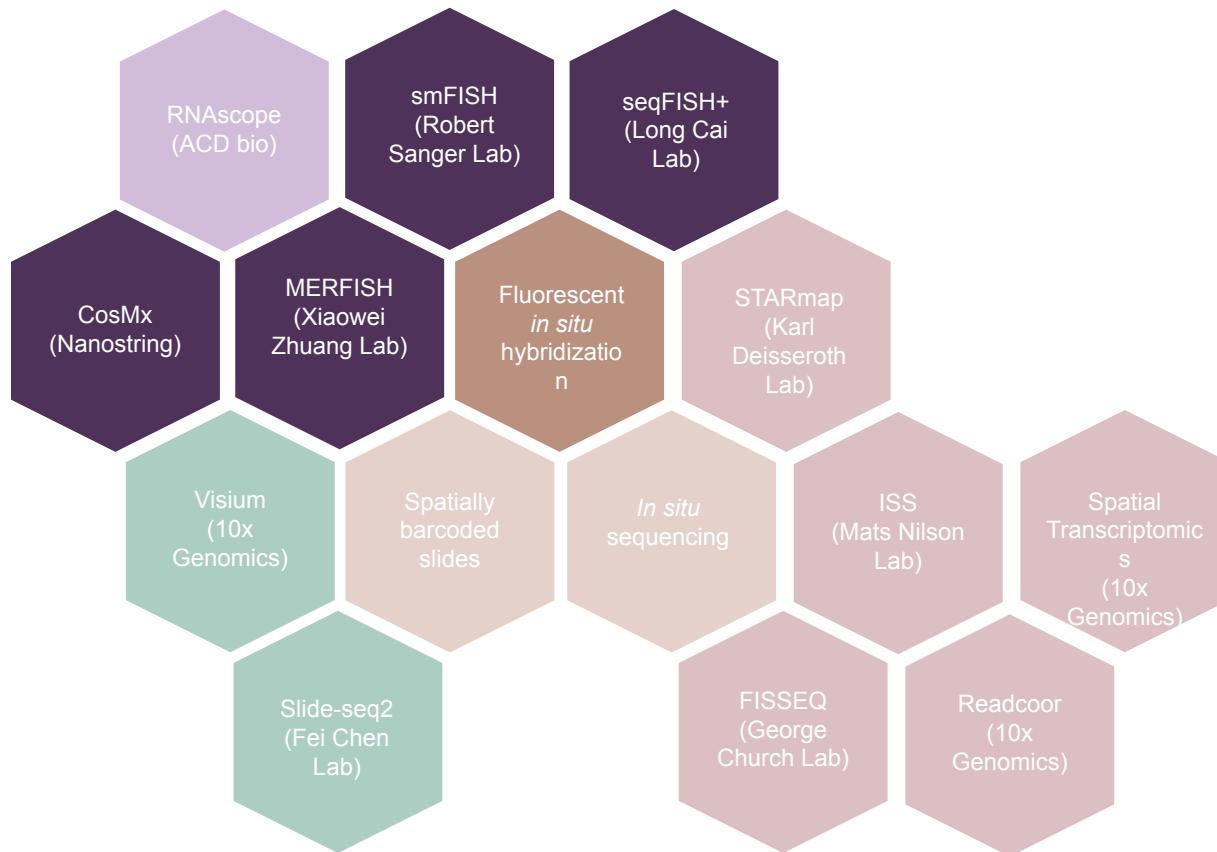
CANONICAL GENOMICS VS. SPATIAL GENOMICS



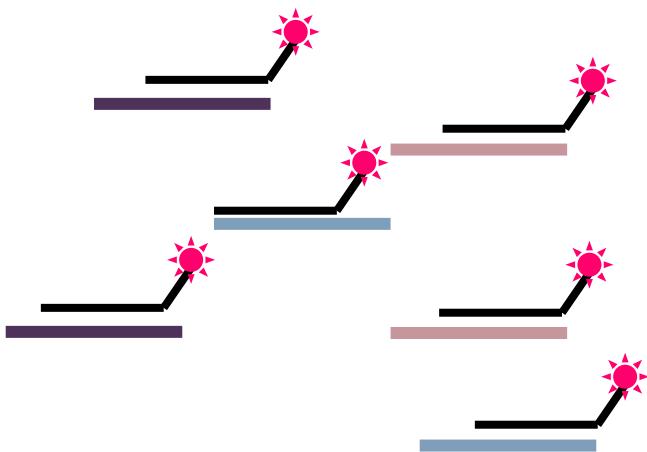
HOW TO STUDY RNA LOCALIZATION?



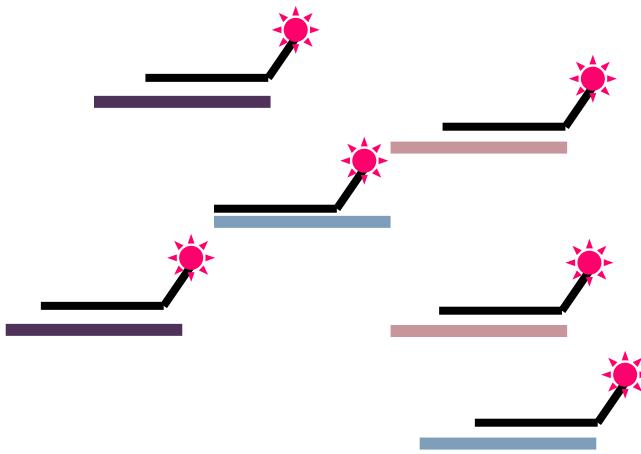
HOW TO STUDY RNA LOCALIZATION?



SPACE-TX WITH FISH-BASED APPROACHES

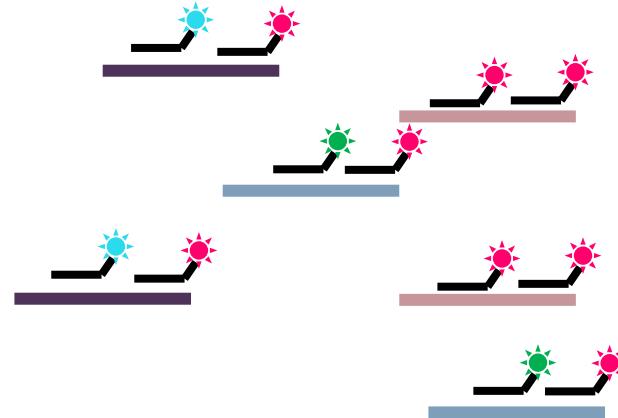
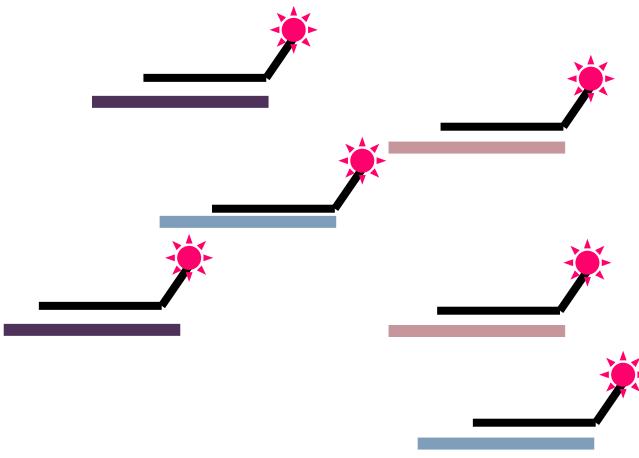


SPACE-TX WITH FISH-BASED APPROACHES



- Only needs a minimum of one color
- Scales poorly when increasing number of target genes (even if you multiplex with several colors)

SPACE-TX WITH FISH-BASED APPROACHES



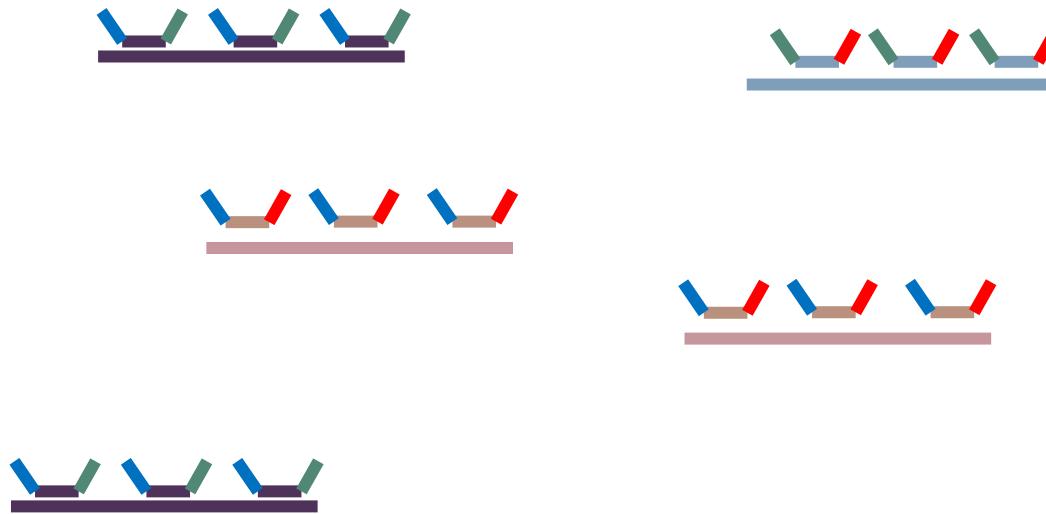
- Only needs a minimum of one color
- Scales poorly when increasing number of target genes (even if you multiplex with several colors)
- Multiplex with many color combinations (**seqFISH+**) or ON/OFF patterns (**MERFISH**)
- Can scale to ~10k genes
- Does require targeted probe design



1 0 1

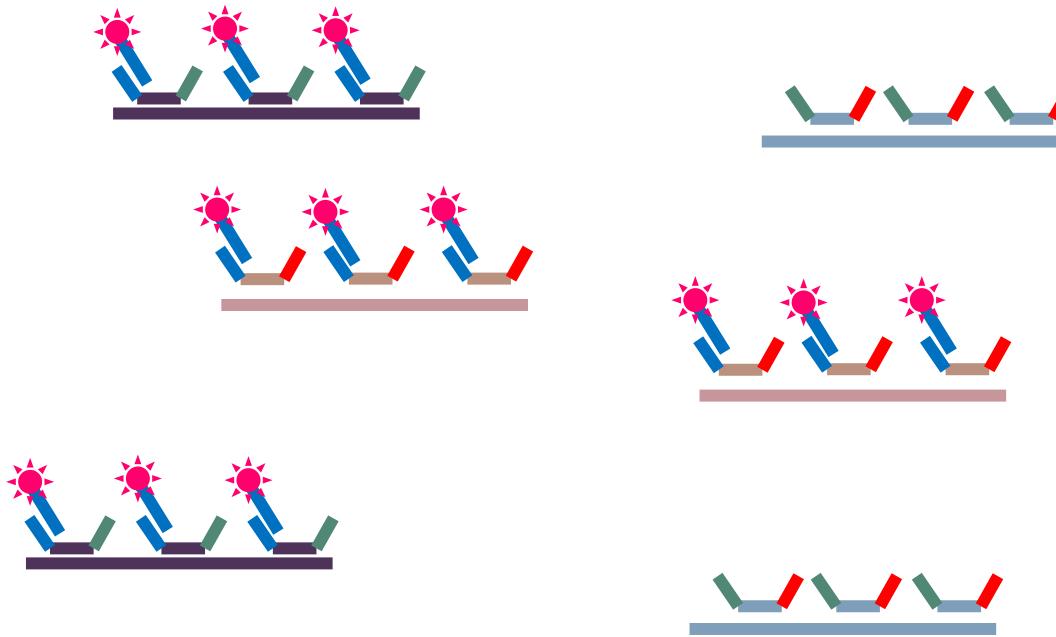
1 1 0

0 1 1



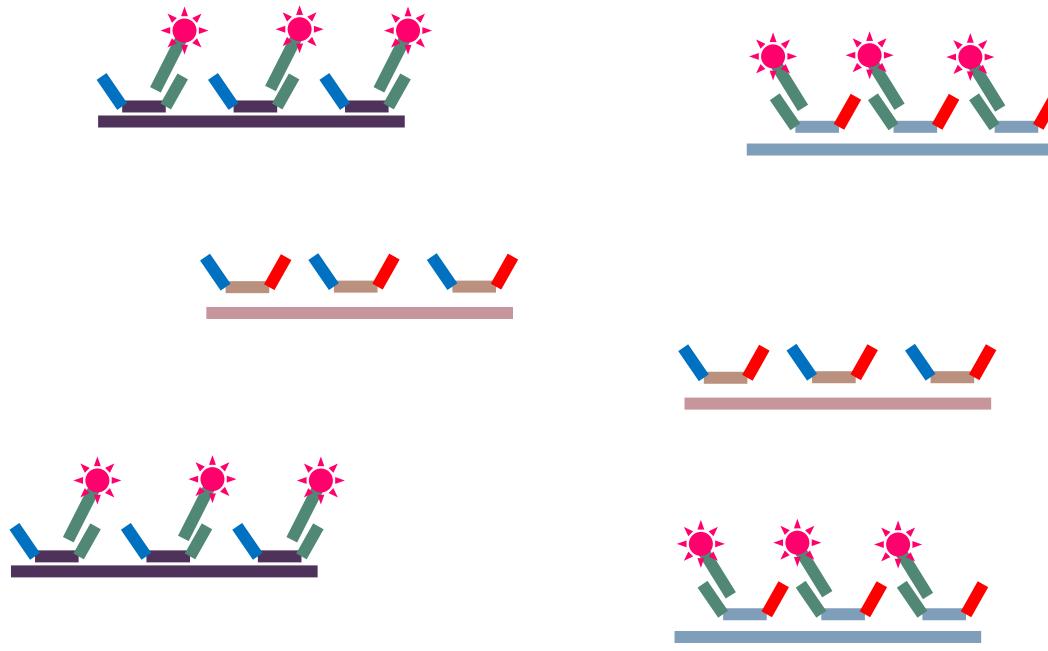


- 1 0 1
- 1 1 0
- 0 1 1



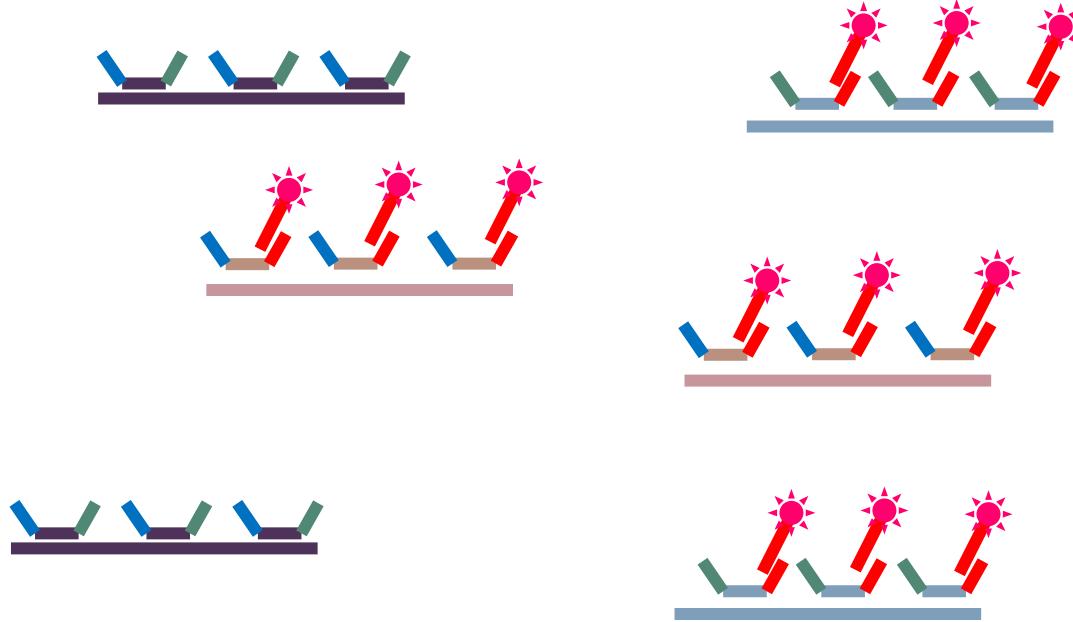


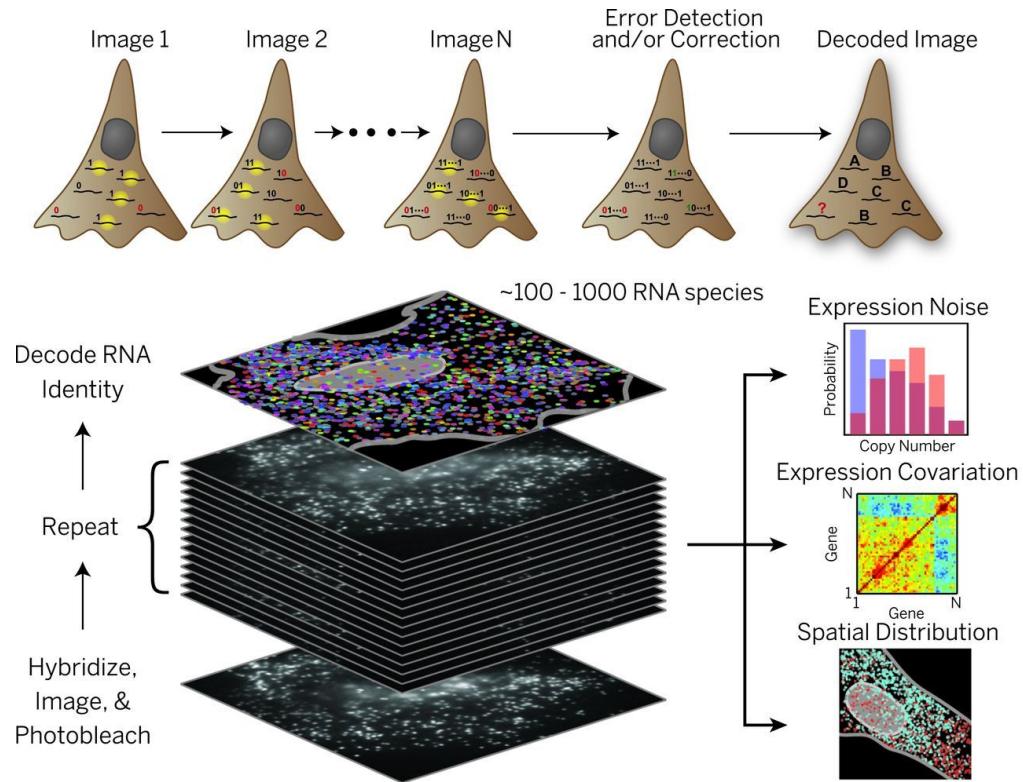
1 0 1
1 1 0
0 1 1



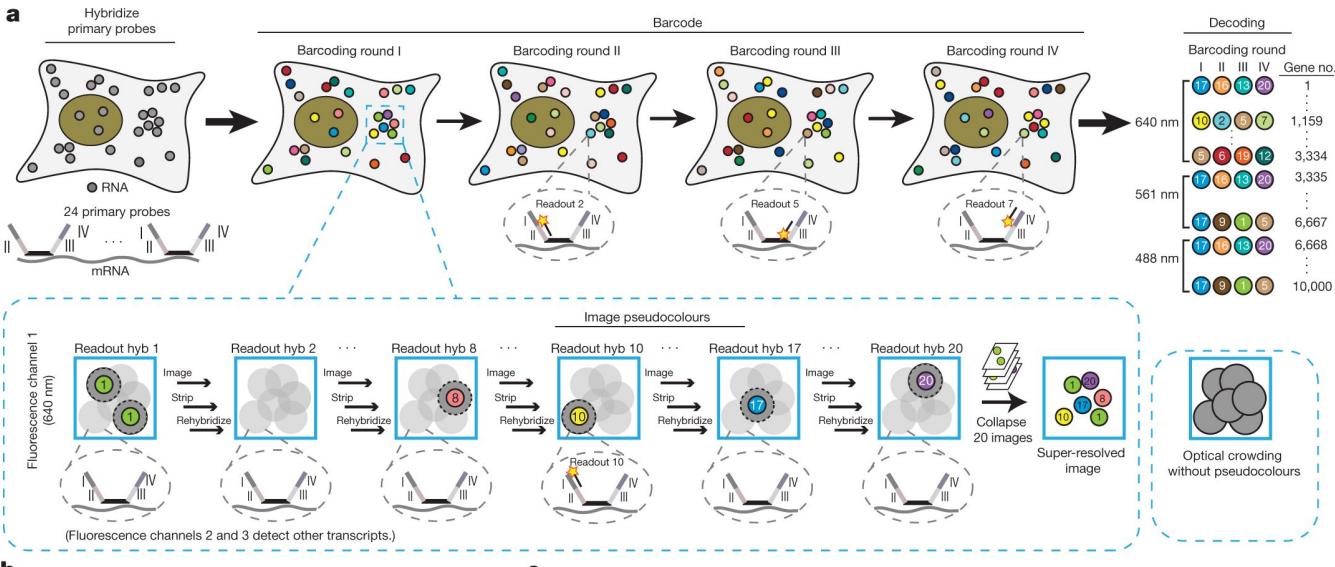


1 0 1
1 1 0
0 1 1



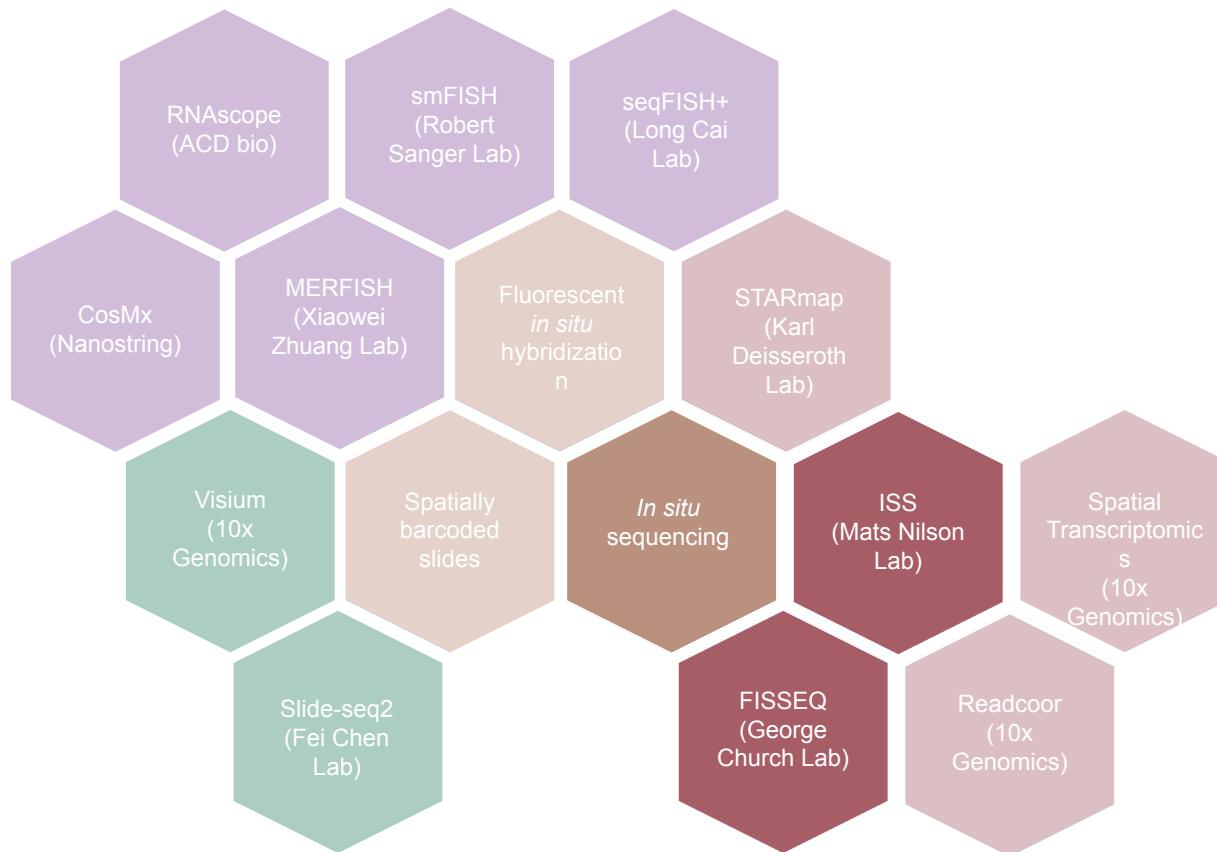


The logo consists of a dark gray circle with a thick white double-line border. Inside the circle, the word "SEQFISH" is written in a bold, white, sans-serif font, centered horizontally and vertically.



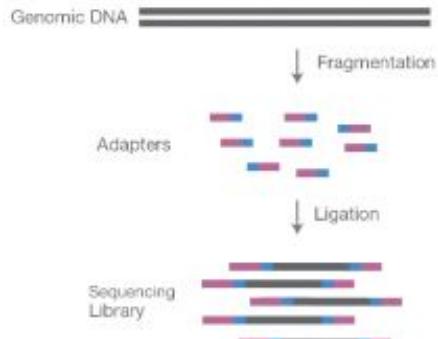
Very similar method, but using “pseudocolors” instead of binary “bits”

HOW TO STUDY RNA LOCALIZATION?



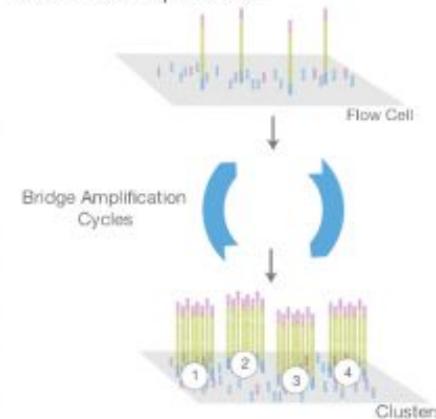
WHAT IS SEQUENCING?

A. Library Preparation



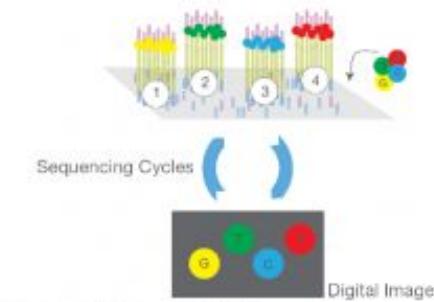
NGS library is prepared by fragmenting a gDNA sample and ligating specialized adapters to both fragment ends.

B. Cluster Amplification



Library is loaded into a flow cell and the fragments are hybridized to the flow cell surface. Each bound fragment is amplified into a clonal cluster through bridge amplification.

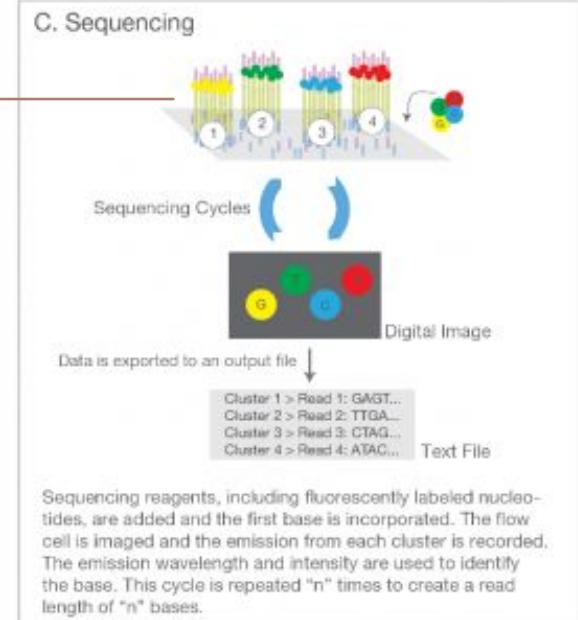
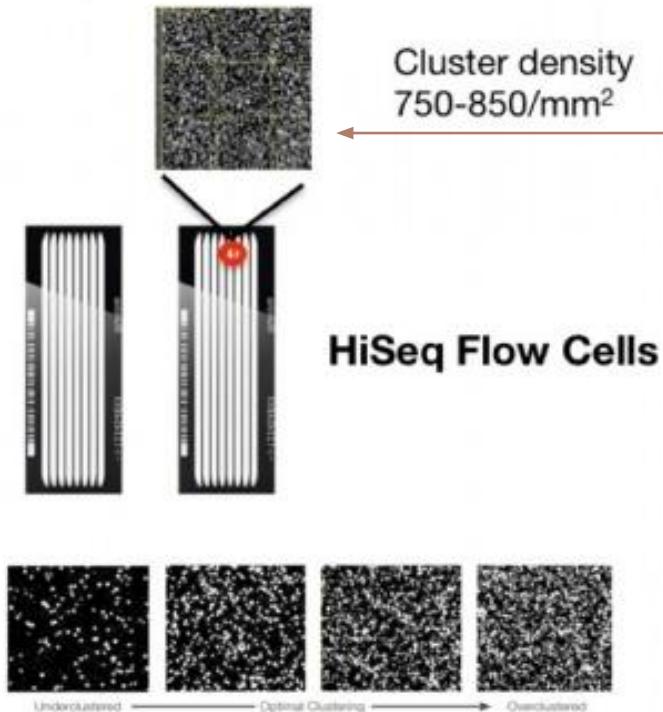
C. Sequencing



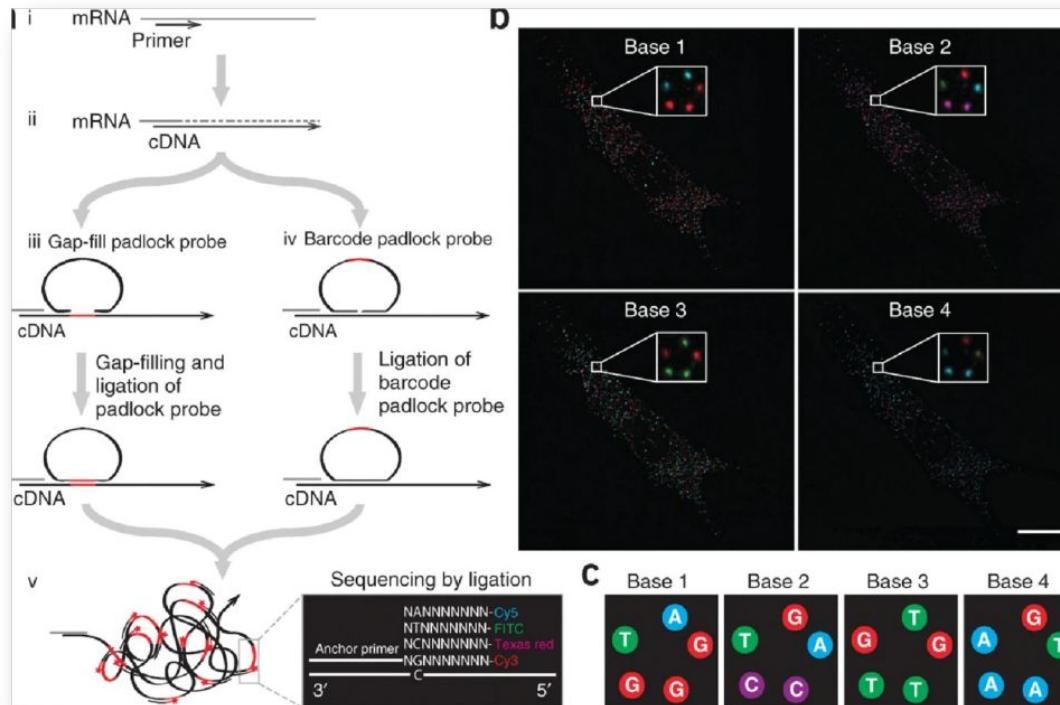
Sequencing reagents, including fluorescently labeled nucleotides, are added and the first base is incorporated. The flow cell is imaged and the emission from each cluster is recorded. The emission wavelength and intensity are used to identify the base. This cycle is repeated "n" times to create a read length of "n" bases.

WHAT IS SEQUENCING?

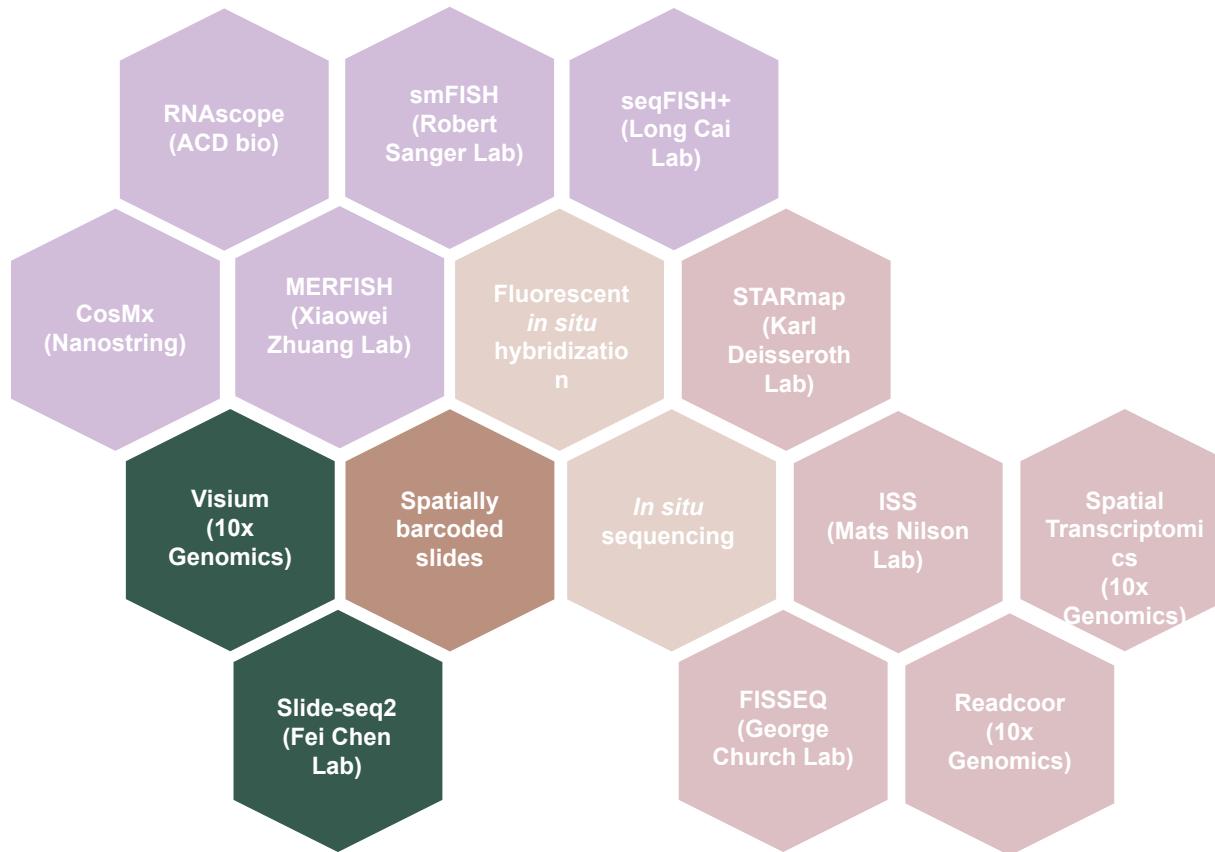
Can we do this
inside cells??



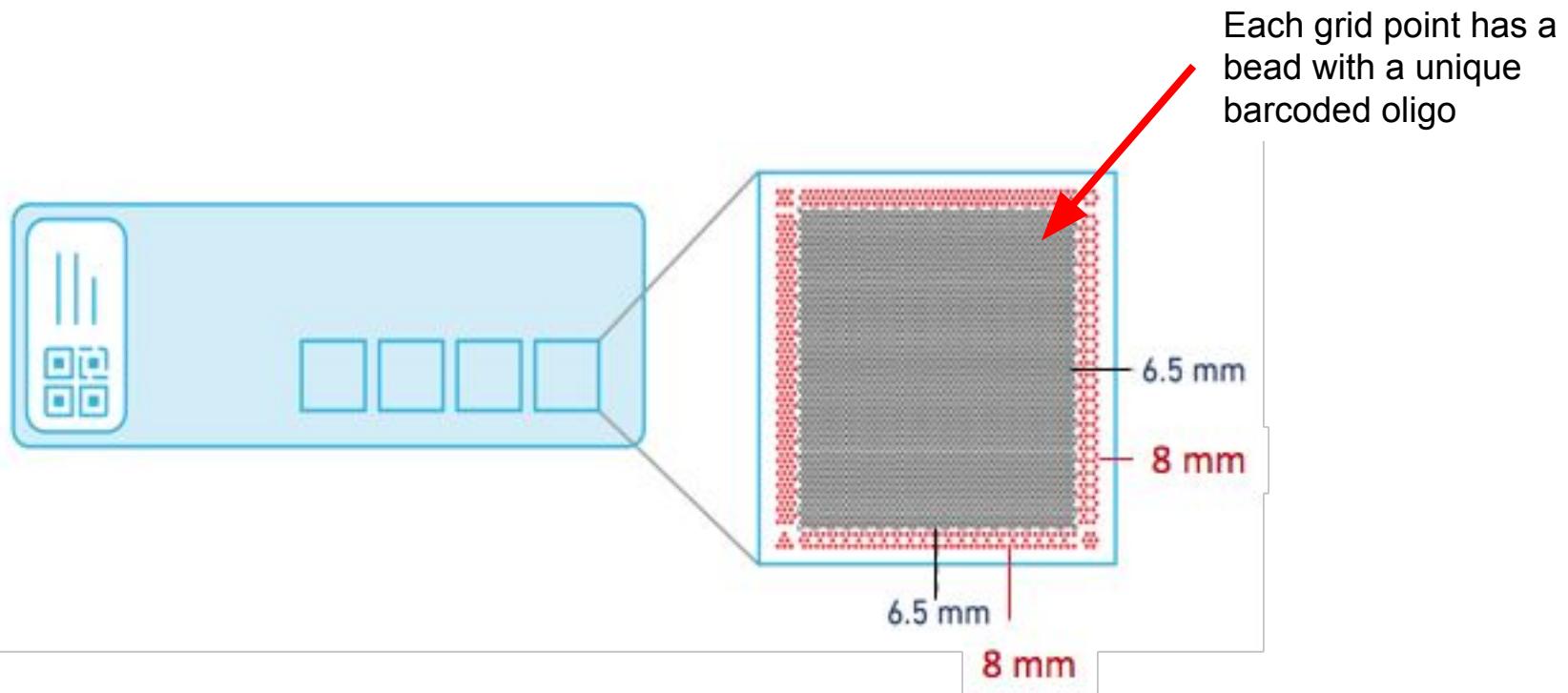
In Situ Sequencing



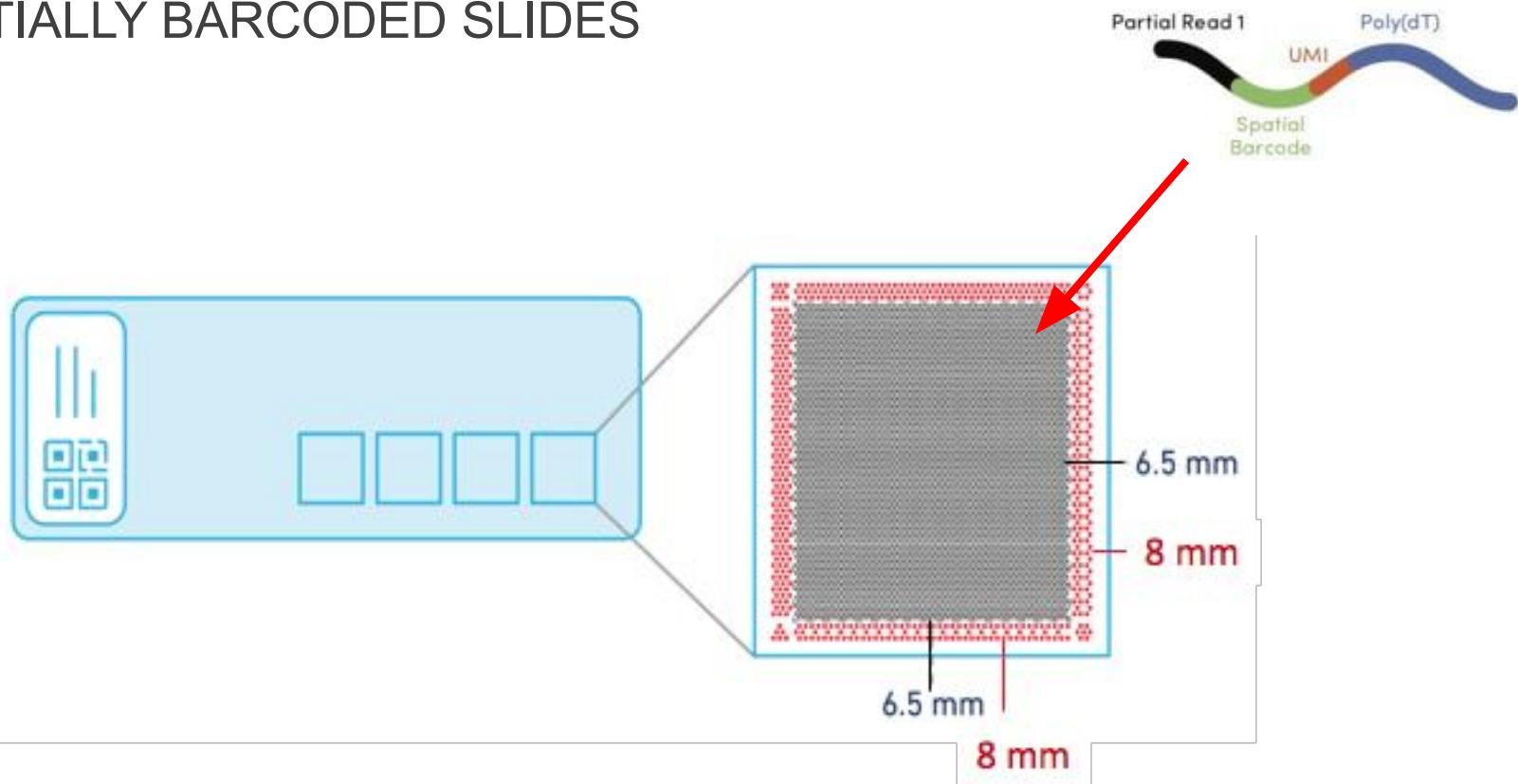
HOW TO STUDY RNA LOCALIZATION?



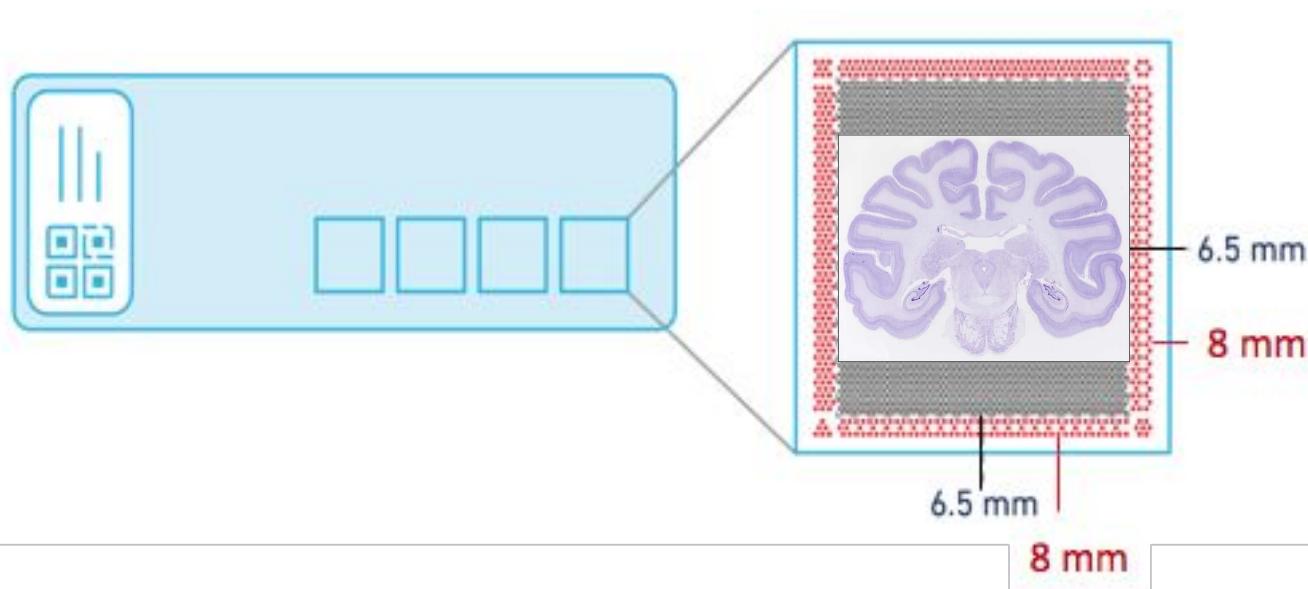
SPATIALLY BARCODED SLIDES



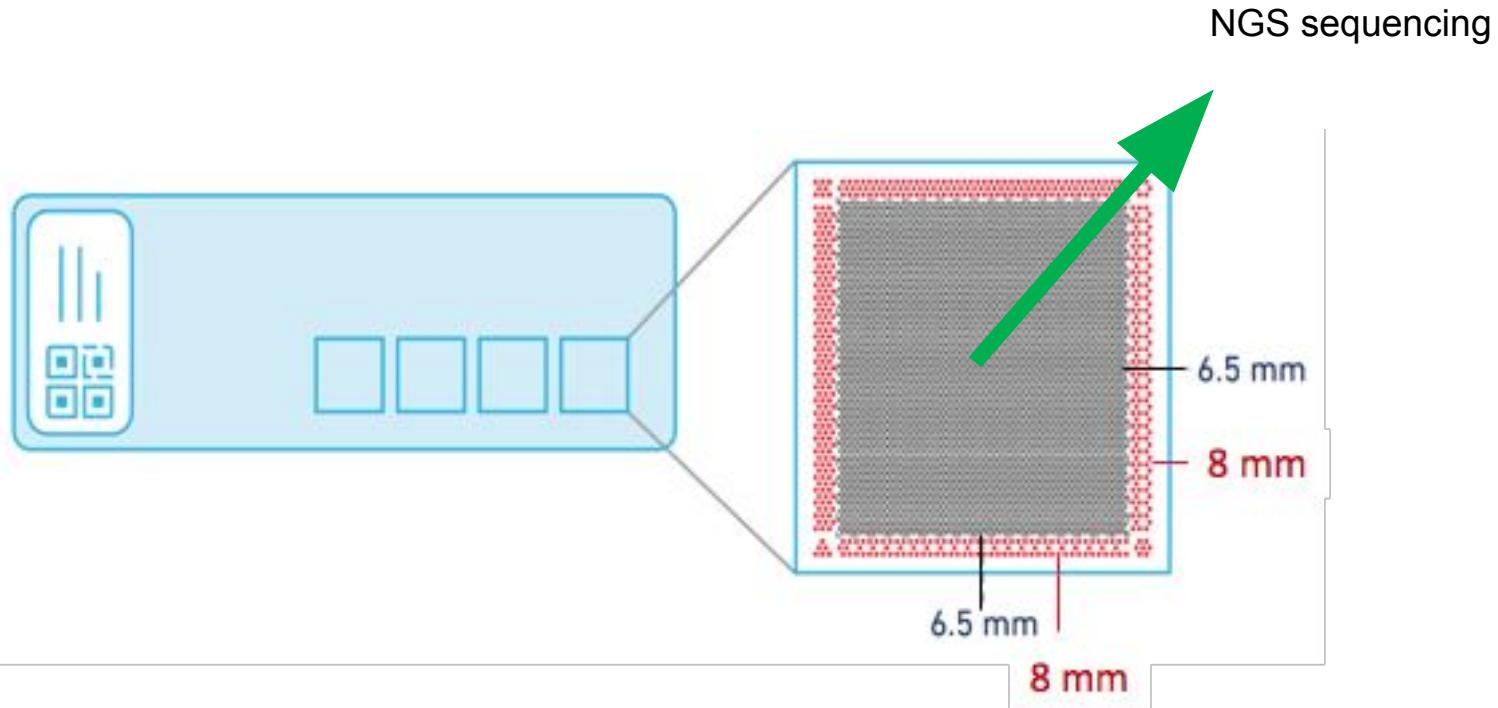
SPATIALLY BARCODED SLIDES



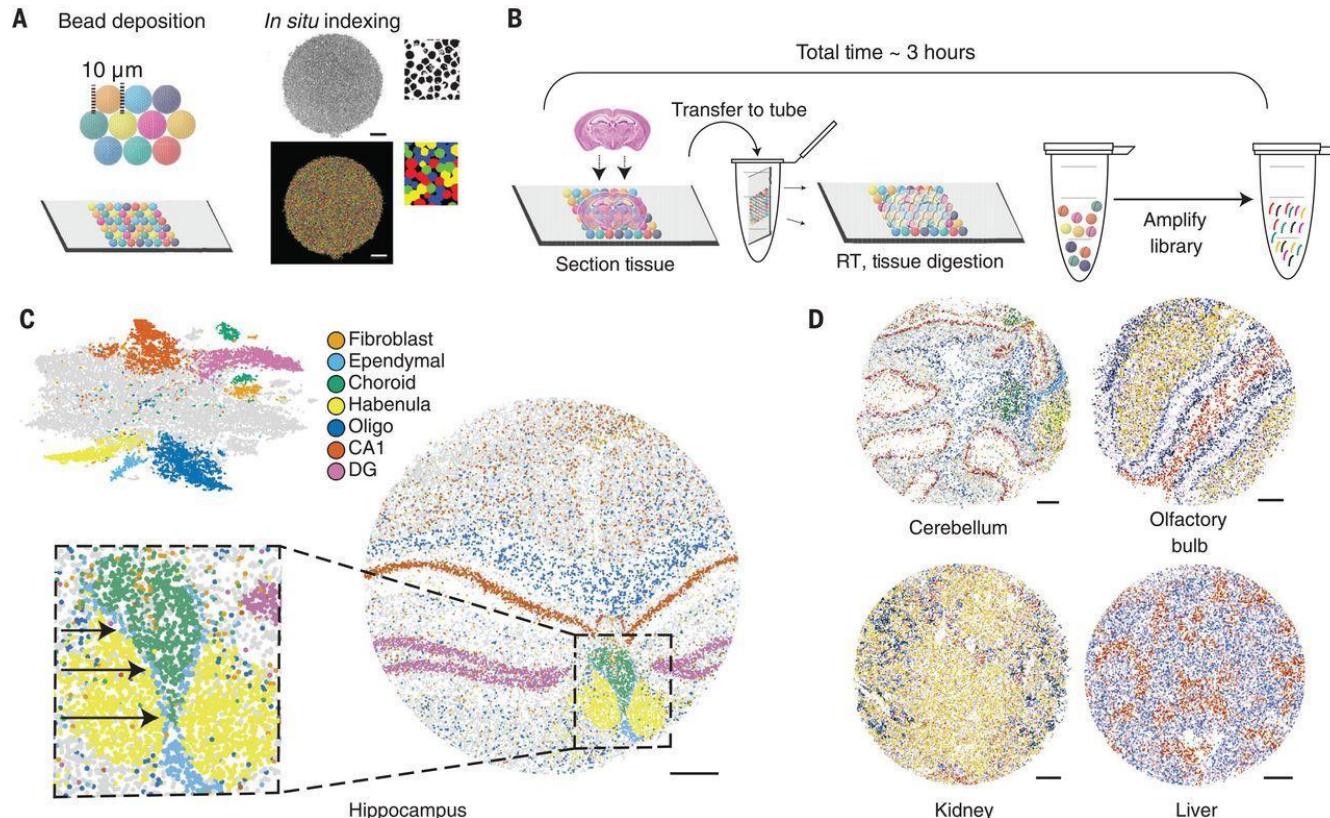
SPATIALLY BARCODED SLIDES



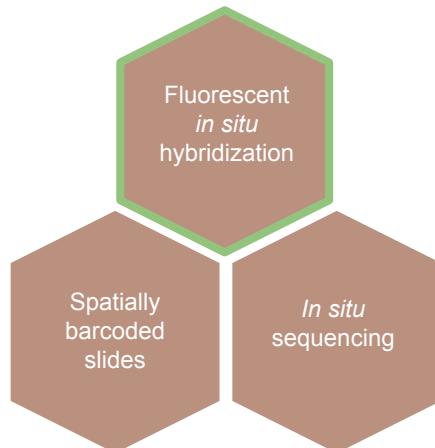
SPATIALLY BARCODED SLIDES



SPATIALLY BARCODED SLIDES

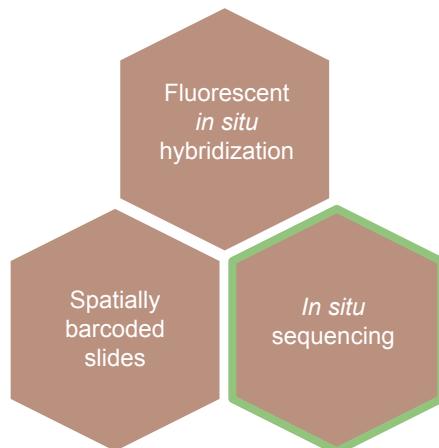


WHAT TO USE??



| Pros | Cons |
|---|--|
| <ul style="list-style-type: none">• Single-molecule resolution• Very high detection efficiency• Up to 10k genes multiplexing demonstrated | <ul style="list-style-type: none">• Very long imaging and experimental time• Very very very expensive...• Biased probe design• Lacks isoform specificity* |
| Good for hypothesis driven experiments | |
| Good for cell “fingerprinting” in tissue | |
| Bad for discovery based screens | |

WHAT TO USE??



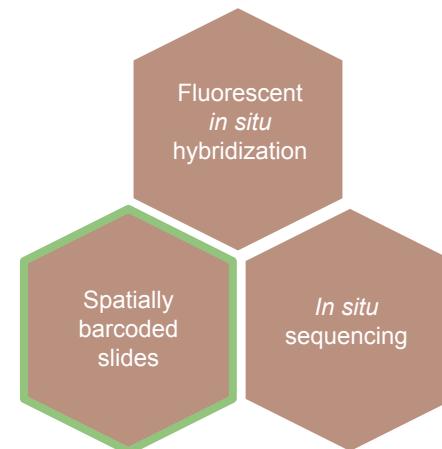
Cheaper than FISH based methods

Still has some catching up to do for quality of data

| Pros | Cons |
|---|--|
| <ul style="list-style-type: none">• Single-molecule resolution• Whole transcriptome (unbiased)*• Isoform specificity* | <ul style="list-style-type: none">• Significantly lower detection efficiency than FISH based methods• Half of detected spots/reads are rRNA (FISSEQ)• Biased probe design* (ISS) |

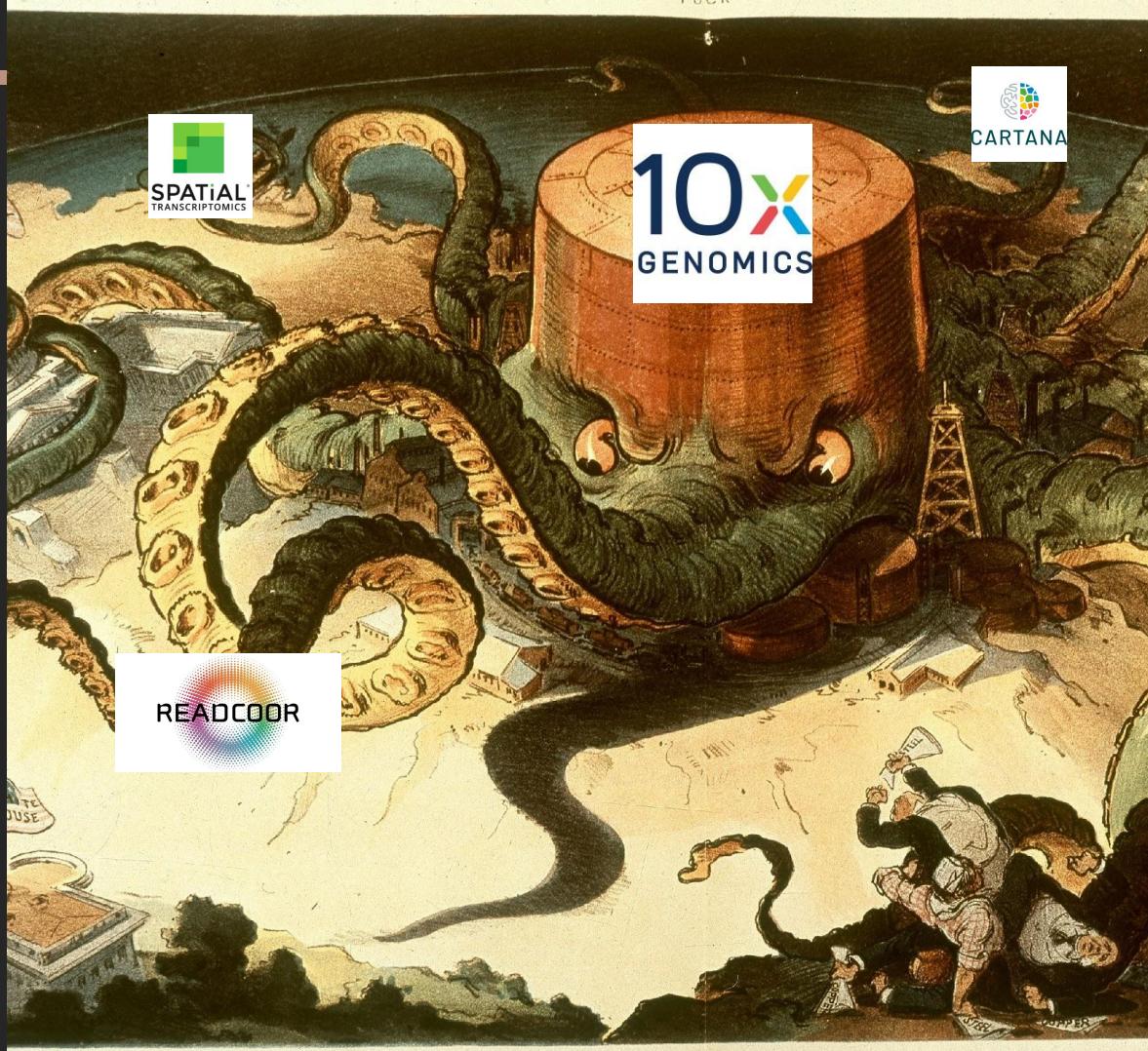
WHAT TO USE??

| Pros | Cons |
|--|--|
| <ul style="list-style-type: none">• Relatively easy and cheap• Whole transcriptome• Very scalable at tissue level• Well developed bioinformatics analytical methods | <ul style="list-style-type: none">• Does not have subcellular resolution (SlideSeq2 can do single-cell)• Detection efficiency is ~ the same as scRNA-seq...so much lower than FISH based methods |

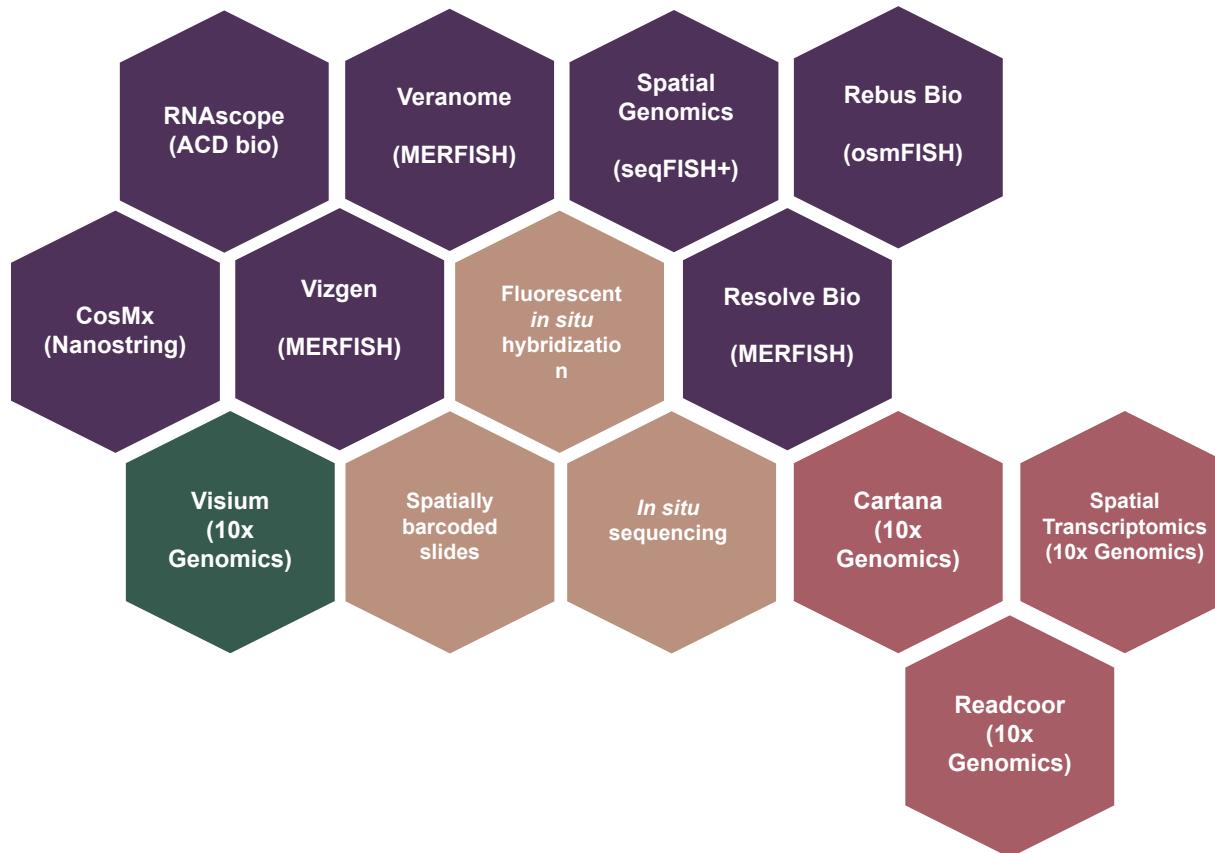


COMMERCIAL METHODS

SAVE YOUR SOUL,
PAY WITH CASH



HOW TO STUDY RNA LOCALIZATION?



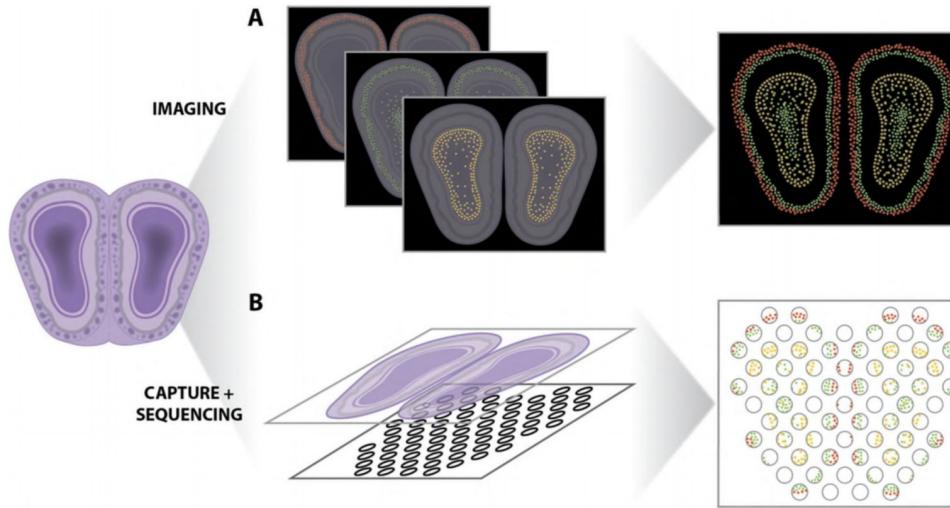
A photograph of a white ceramic cup filled with dark coffee, resting on a matching saucer. The cup is positioned in the center-left of the frame, with a thin plume of steam rising from the surface. The background is a solid, dark brown color. In the foreground, the edge of a light-colored window sill is visible. The overall composition is minimalist and focused on the coffee.

COFFEE BREAK

SPATIAL ANALYSIS

DOES IT MEAN
ANYTHING??

Technologies capture different levels of information

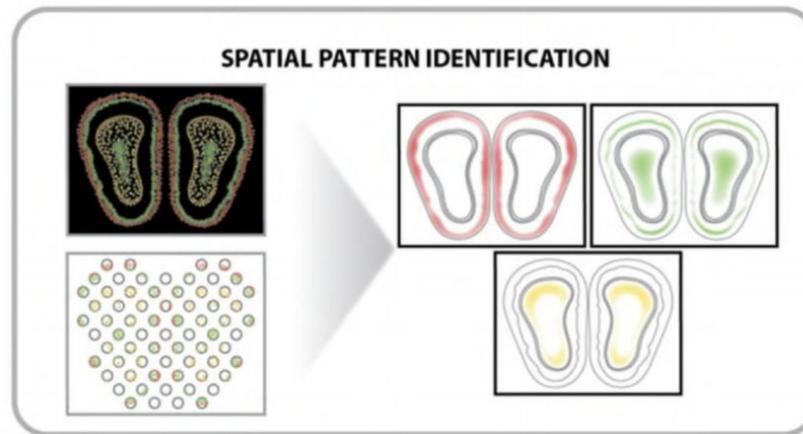


| | <u>Resolution</u> | <u>Genes</u> | <u>Detection efficiency</u> |
|--------------------------------------|-------------------|--------------|-----------------------------|
| FISH-based (imaging) | ● | | ● |
| Slides (capture + sequencing) | | ● | |

Spatial pattern identification

Why?

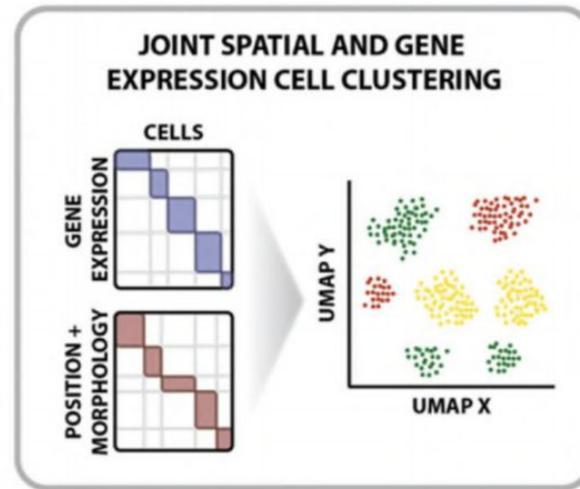
- Identify linear and periodic spatial expression
- Identify developmental and migration gradients



Integrating data types

Why?

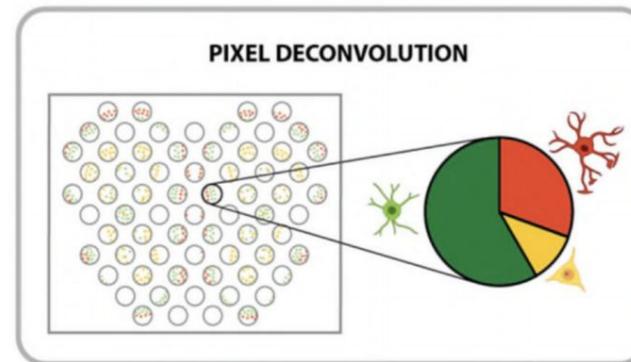
- More data types = more sources of heterogeneity
- Identify position/morphology driven cell states



Cell type deconvolution

Why?

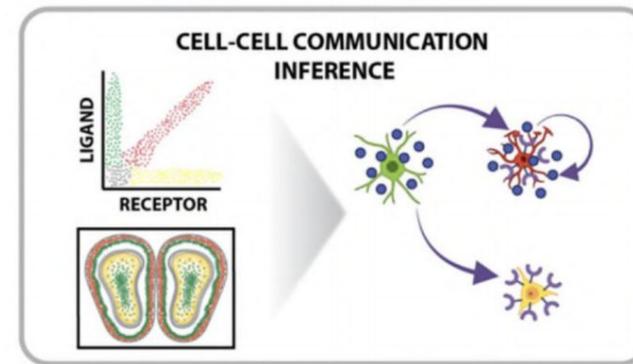
- Each spot captures the expression of multiple cells → multiple cell types
- Allows detection of cell type specific patterns



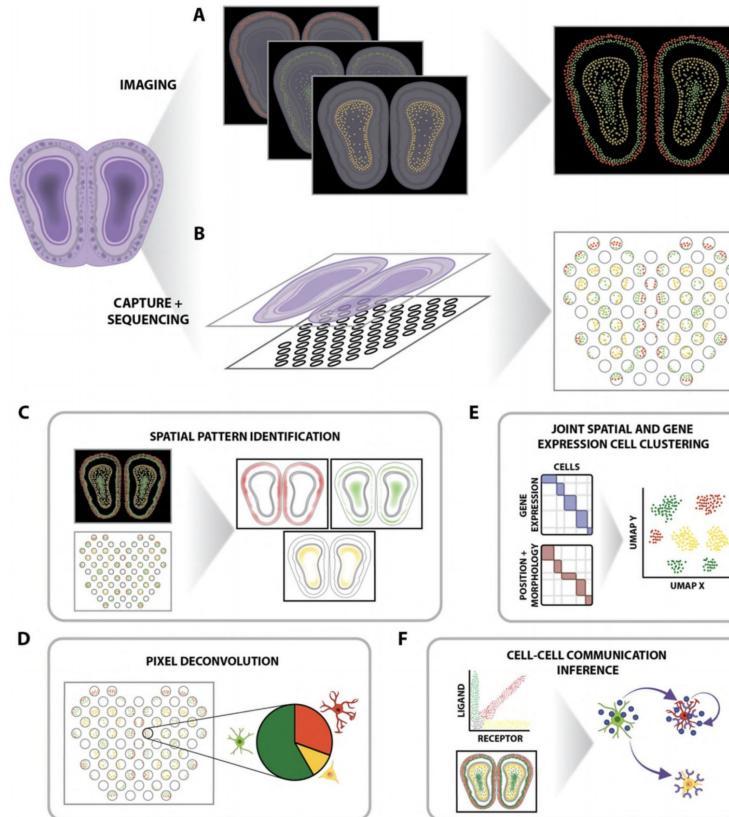
Cell-cell communication inference

Why?

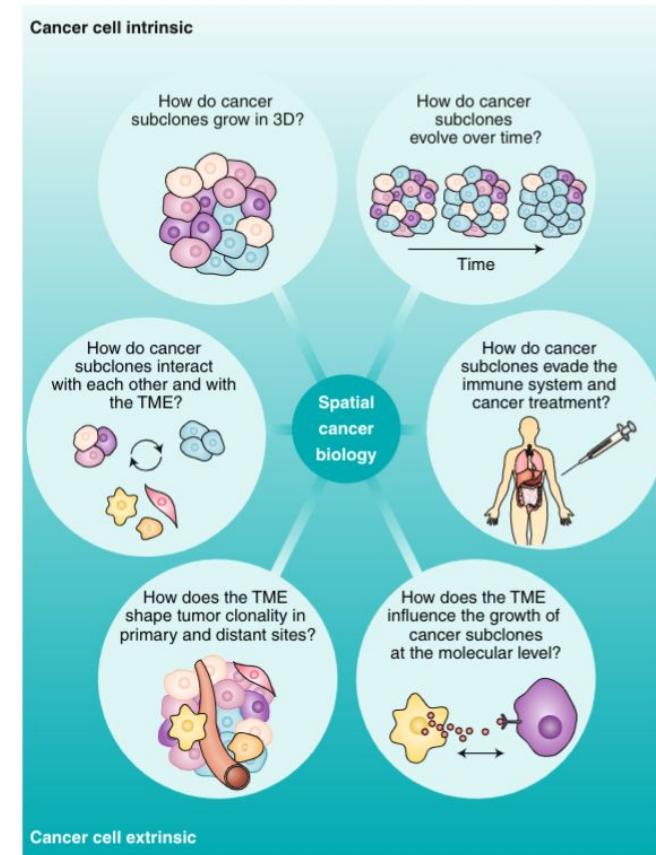
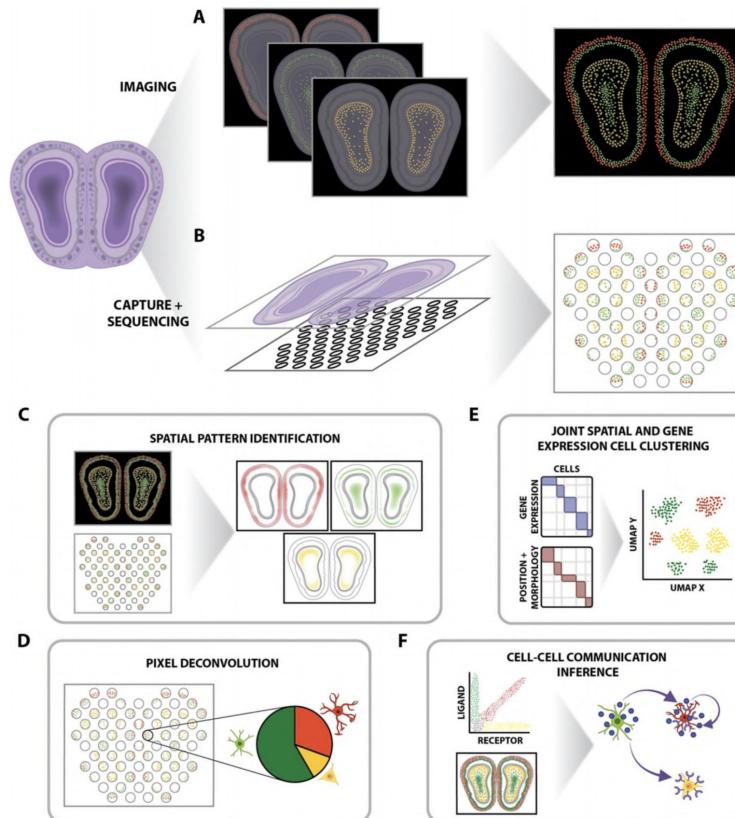
- Spatial colocalization narrows down ligand-receptor pairs that are active
- Indicates cell signaling networks



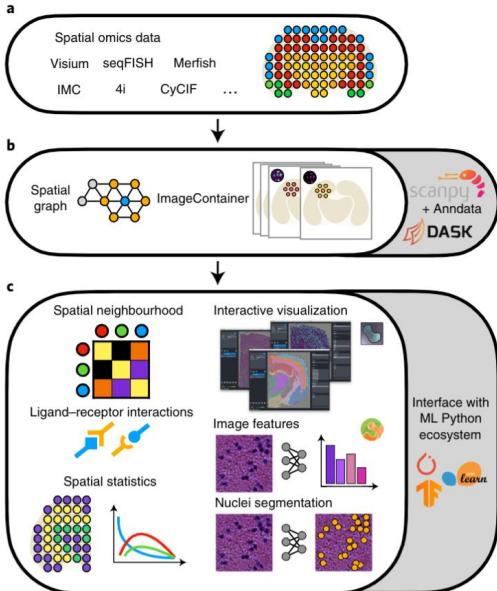
Summary of existing analyses



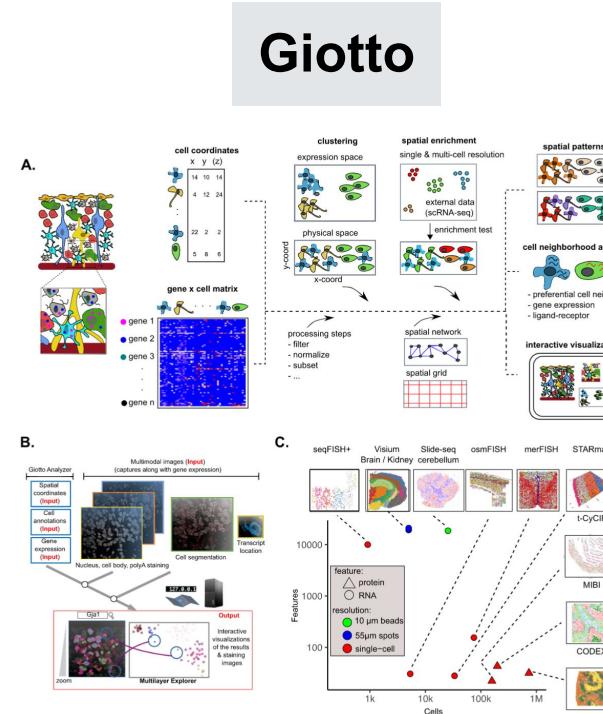
Application: Spatial exploration of cancer biology



Recommendations



Palla, G. et al. Squidpy: a scalable framework for spatial omics analysis. *Nat. Methods* (2022) doi:10.1038/s41592-021-01358-2



Dries, R. et al. Giotto: a toolbox for integrative analysis and visualization of spatial expression data. *Genome Biol.* **22**, 78 (2021)

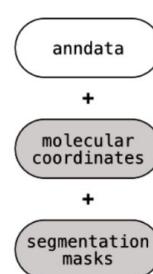


Bento: a toolkit for subcellular analysis of spatial transcriptomics data

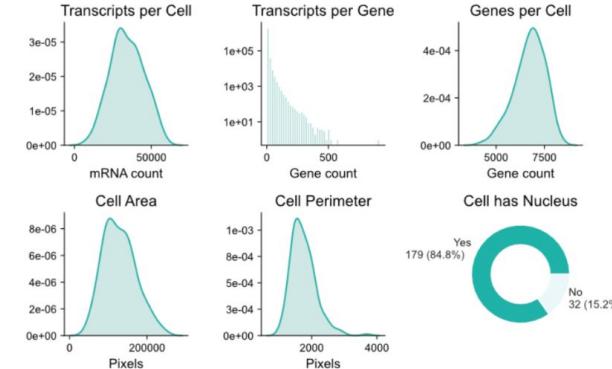
a Spatial transcriptomics data



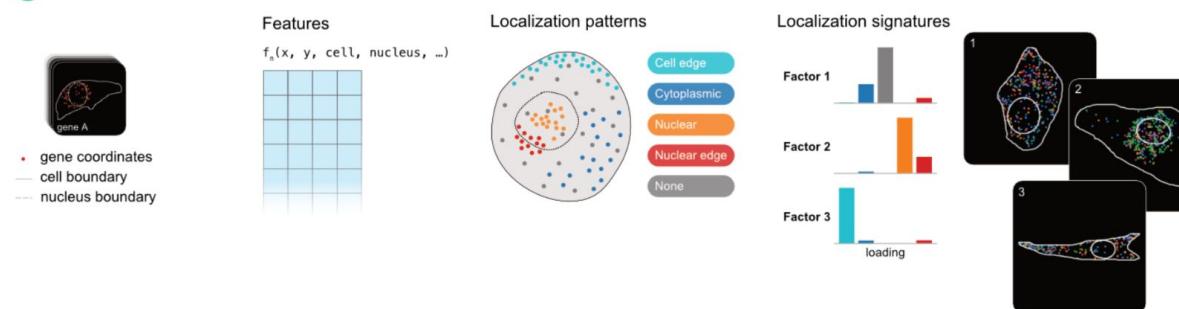
b Data ingestion



c Quality control



d Subcellular spatial analysis



Spatial bioinformatics is a young but growing field

