

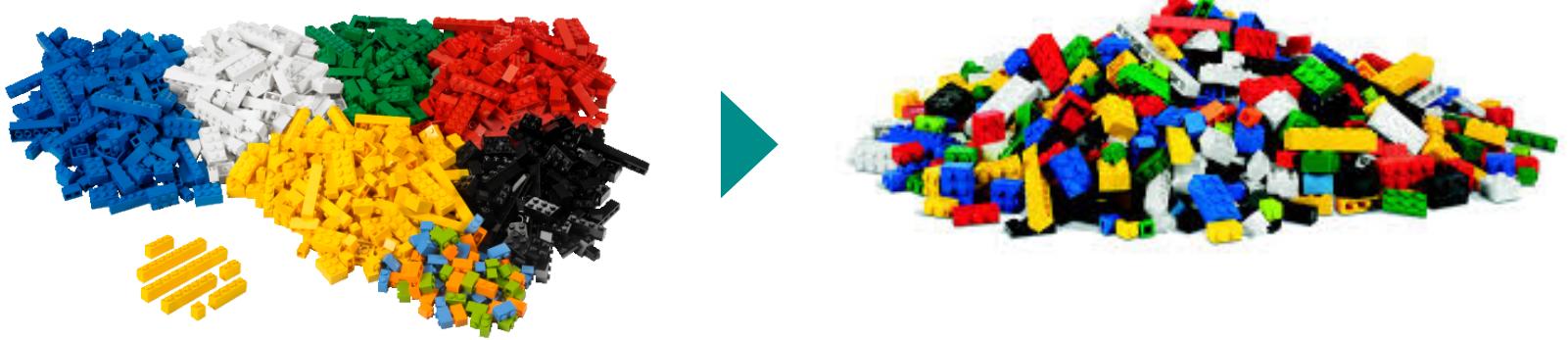
Spatial transcriptomics technologies

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Hubrecht Institute, Utrecht, The Netherlands

Single-cell analysis course – October 2019

During single-cell sequencing, spatial information is lost



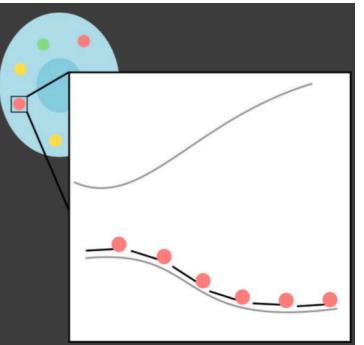
We can try to reconstruct spatial information from single-cell data
(for example using novoSpaRc (Nitzan *et al.*, *BioRxiv*, 2018))

However, such reconstructions are often not accurate
→ Better to use spatial technologies

Spatial transcriptomics technologies currently available

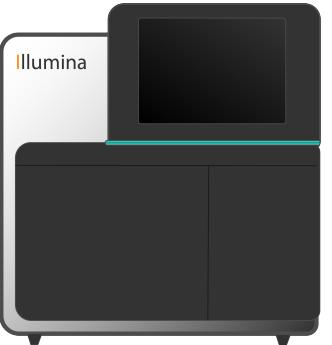
Microscopy based:

- *In situ* hybridization
- Single-molecule *in situ* hybridization
- *In situ* hybridization chain reaction
 - Cyclic fish (osmFISH)
 - Barcoded fish (SeqFISH; MerFISH)
- *In situ* sequencing (FISSEQ; STARMAP)



Sequencing based:

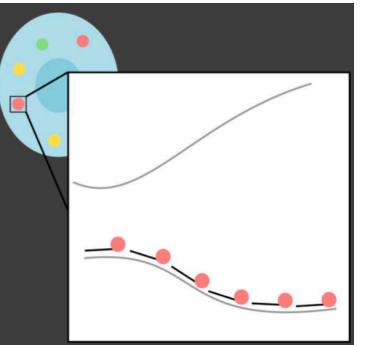
- Geo-seq
- Slide-based spatial transcriptomics
 - Slide-seq
 - Tomo-seq



Spatial transcriptomics technologies currently available

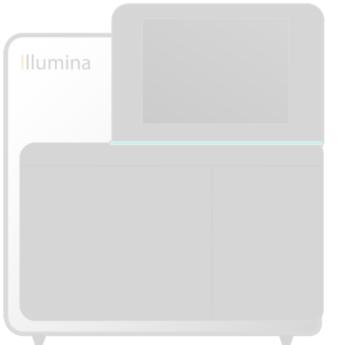
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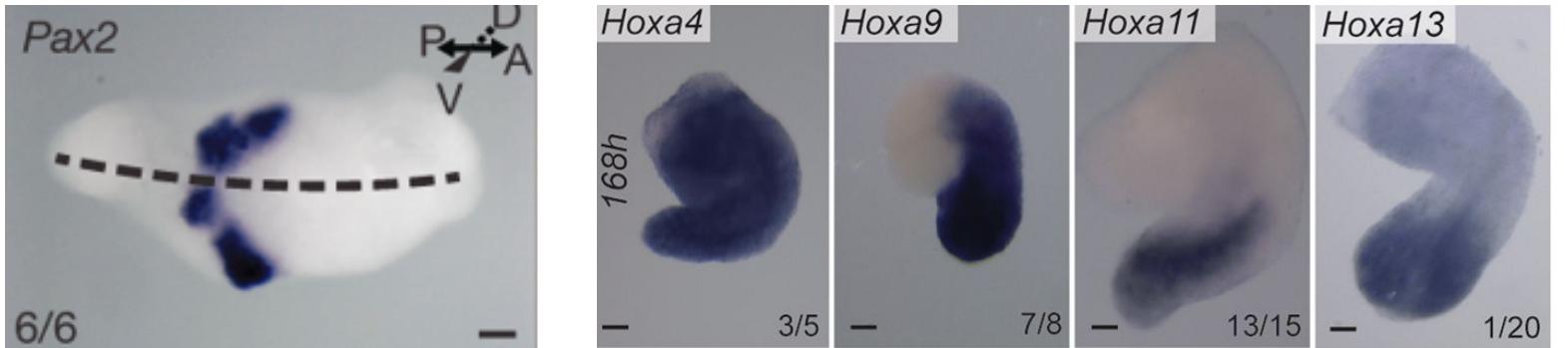
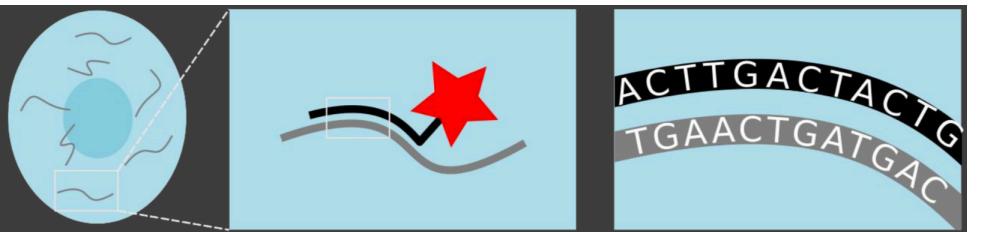
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In situ hybridization (ISH)

Spatial information, but no single-molecule resolution

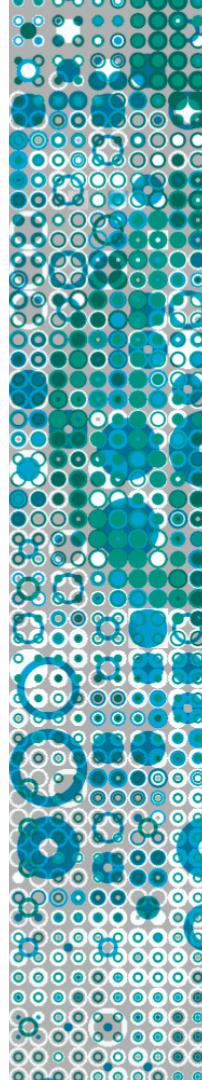
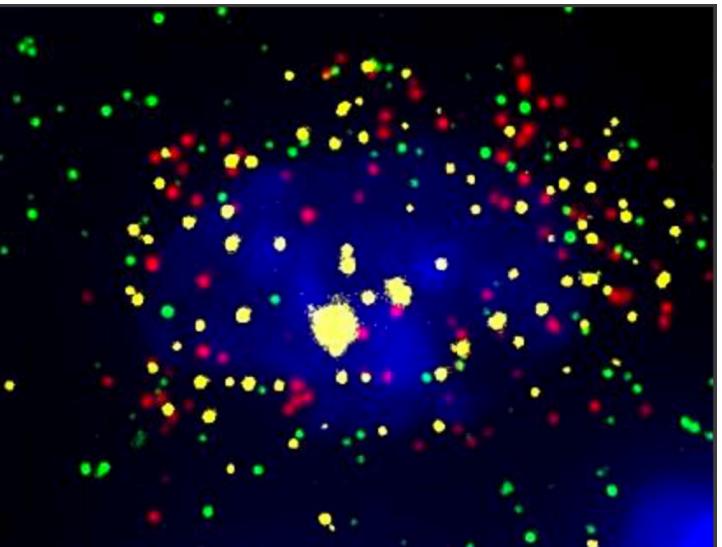
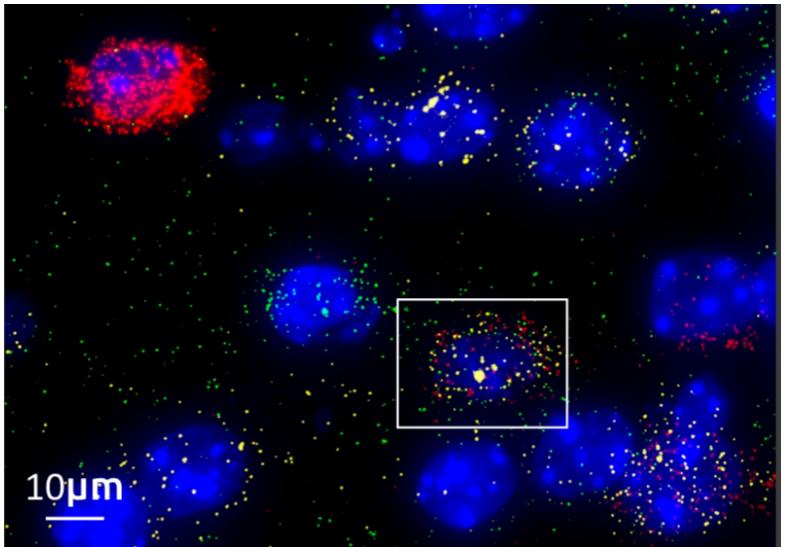
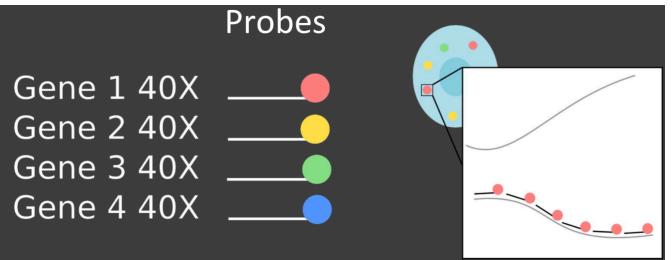


(ISH stainings on gastruloids; Beccari *et al.*, *Nature*, 2018)

Single-molecule *in situ* hybridization (smFISH)

Single-molecule resolution; however, whole-mount imaging not possible

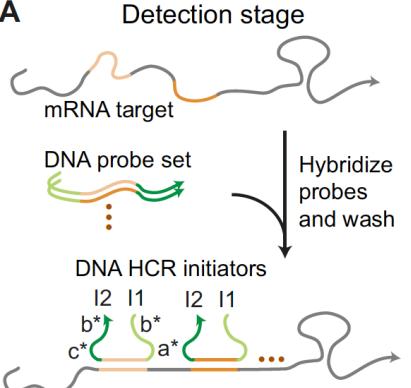
Raj et al., *Nature Methods*, 2008



HCR: *in situ* hybridization chain reaction

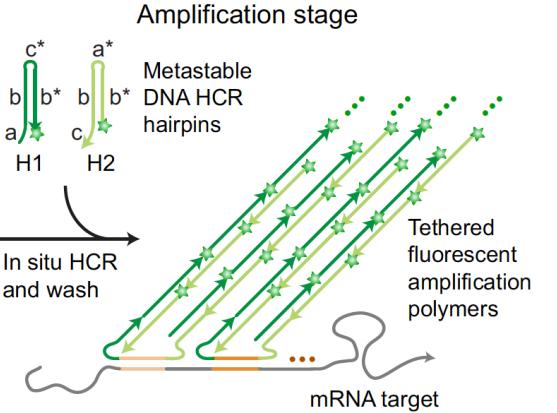
Simple and fast; whole-mount imaging possible

A Detection stage

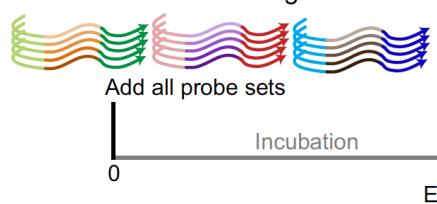


Hybridize probes and wash

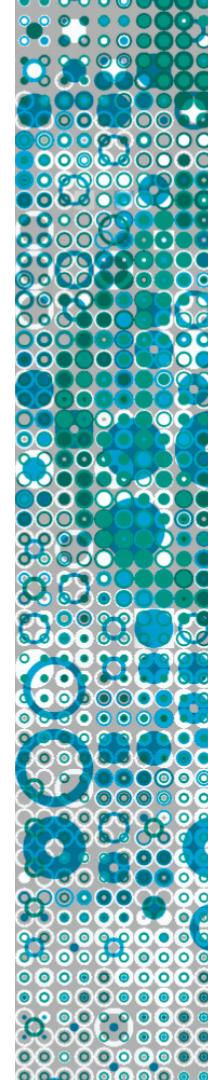
Amplification stage



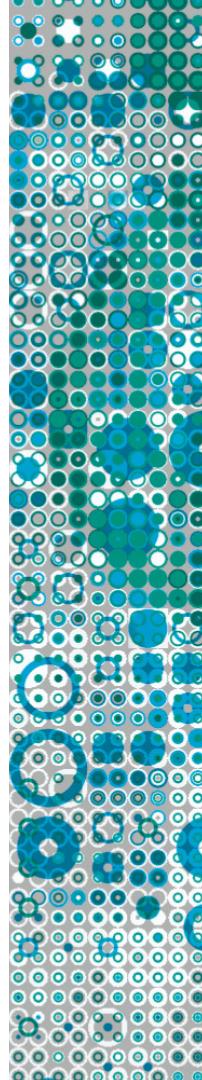
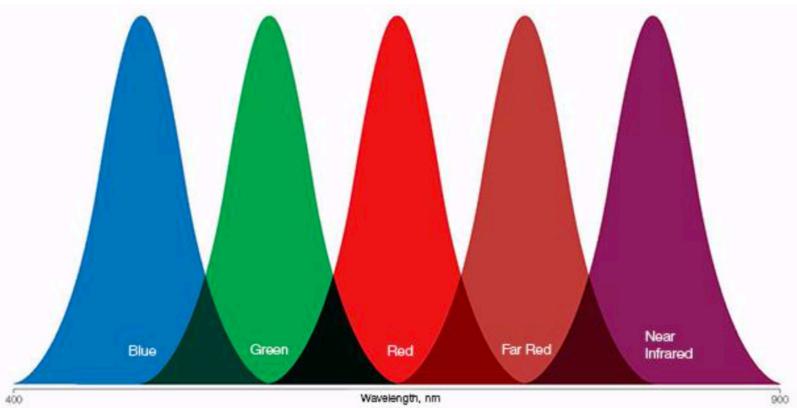
B Detection stage



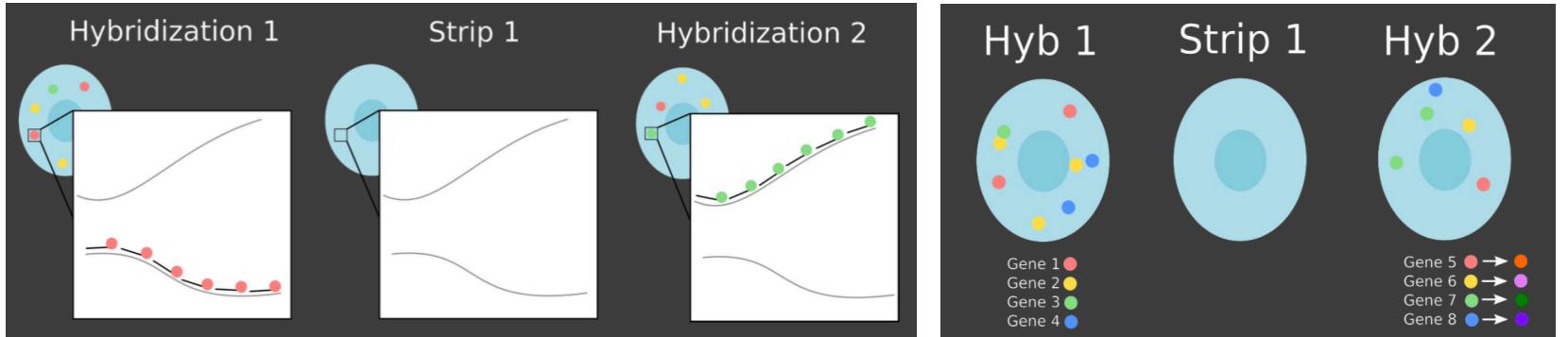
(Choi et al., Development, 2018)



Most microscopy approaches are limited by the amount of distinguishable fluorophores

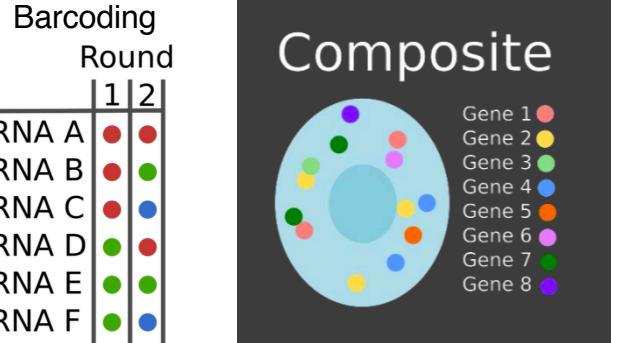


Cyclic and barcoded FISH methods provide spatial information about larger numbers of genes

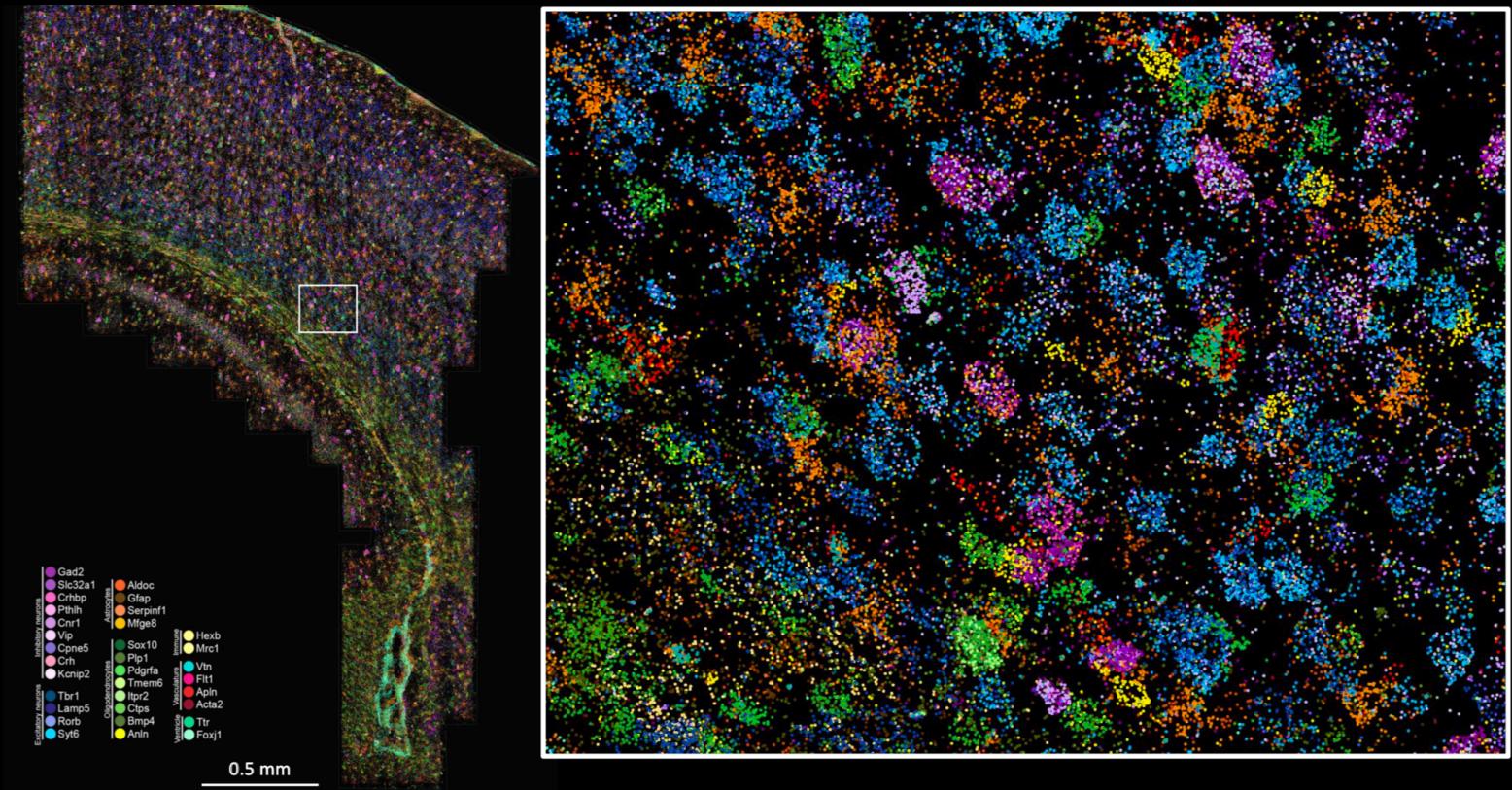


Cyclic FISH:
osmFISH (Codeluppi *et al.*, *Nature Methods*, 2018)

Barcoded FISH (reprobing; more genes):
SeqFISH(+) (Lubeck *et al.*, *Nature Methods*, 2014;
Eng *et al.*, *Nature*, 2019)
MerFISH (Chen *et al.*, *Science*, 2015)



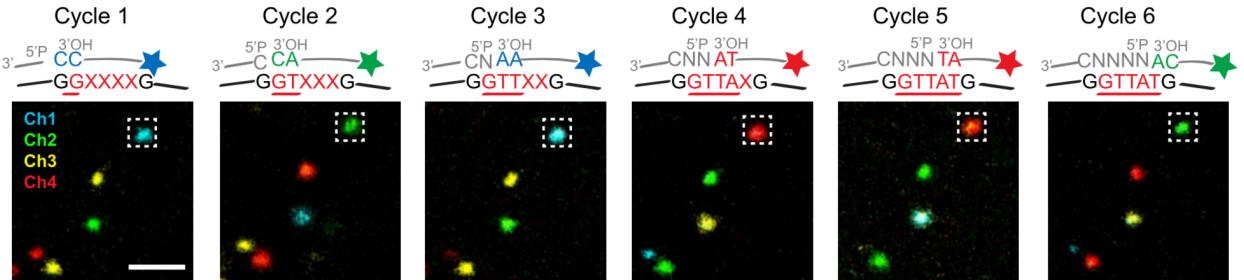
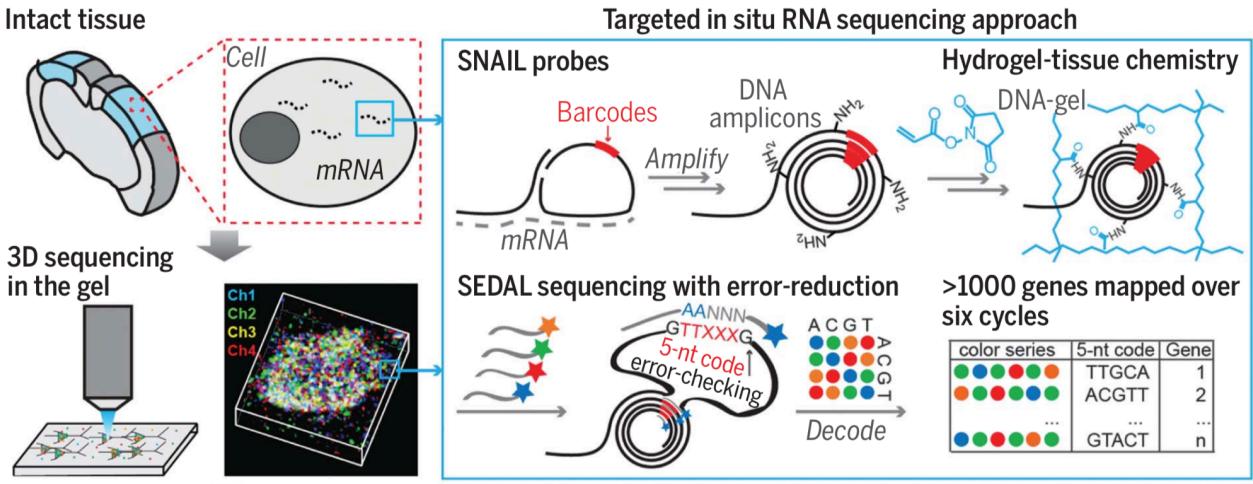
osmFISH example: sequential FISH imaging of mouse sensory cortex



(Codeluppi *et al.*, *Nature Methods*, 2018)

In situ sequencing:

STARMAP (spatially-resolved transcript amplicon readout mapping)



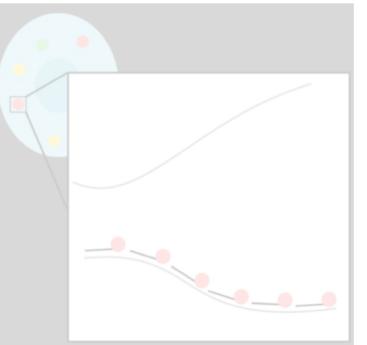
(Wang *et al.*, *Science*, 2018)

Alternative method without barcodes: FISSEQ
(Lee *et al.*, *Science*, 2014)

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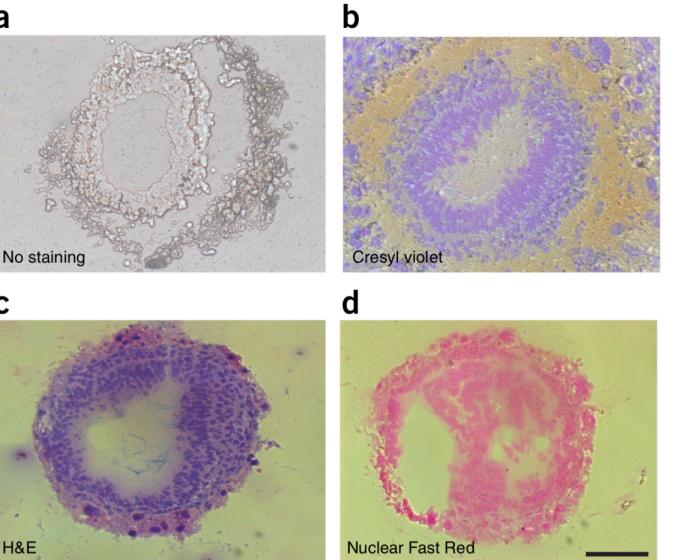
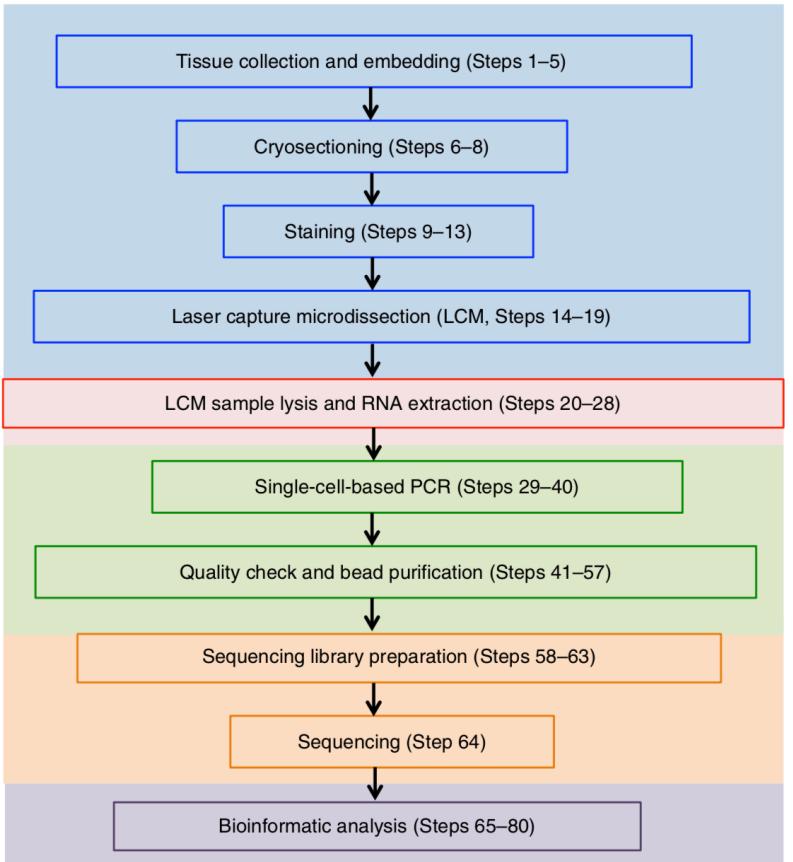


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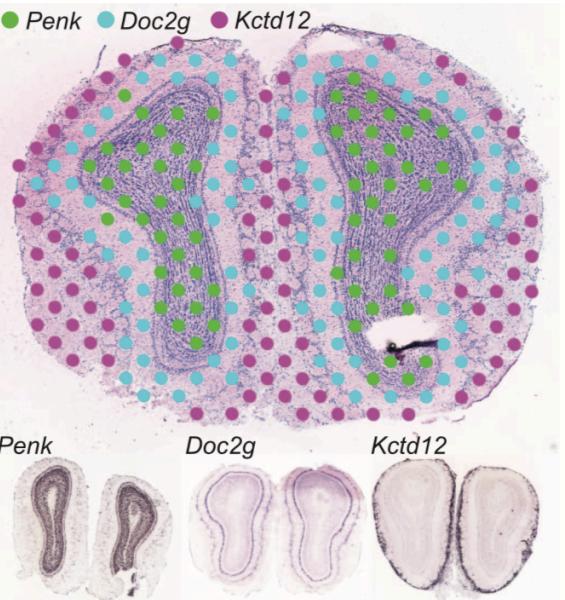
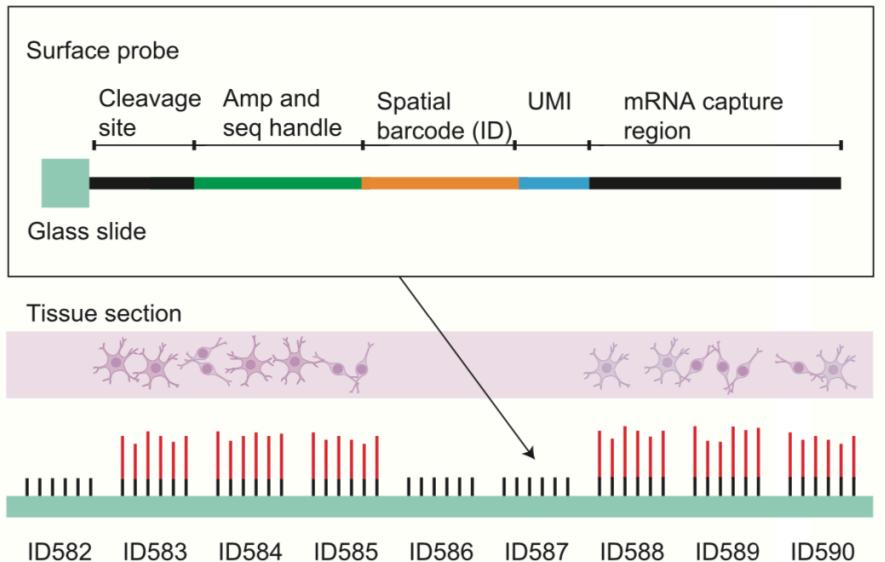
Geo-seq combines laser capture microdissection with sequencing



Labor-intensive

(Chen *et al.*, *Nature Protocols*, 2017)

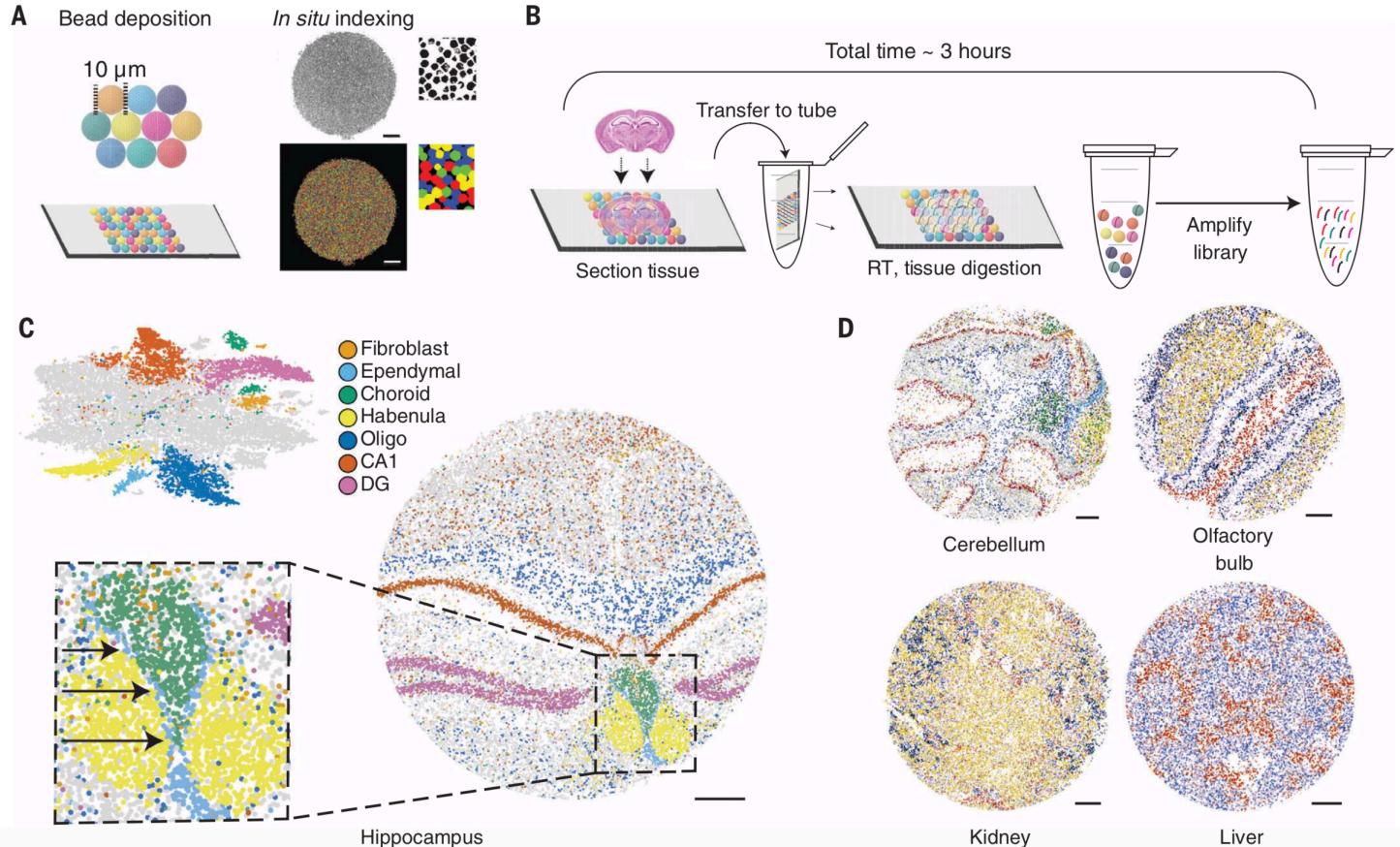
Glass-slide based spatial transcriptomics



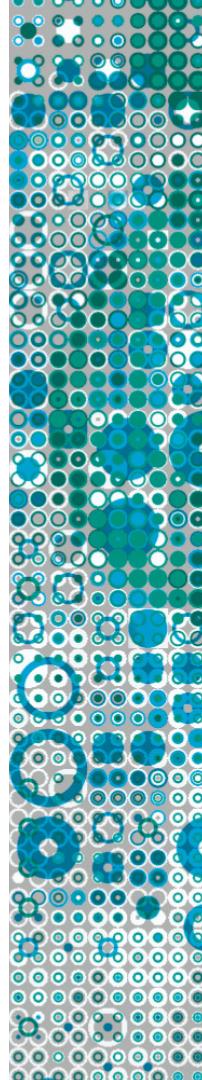
Only a small percentage (19%)
of the surface area is sampled

(Stahl *et al.*, *Science*, 2016)

Slide-seq: spatial transcriptomics using barcoded beads on coverslip

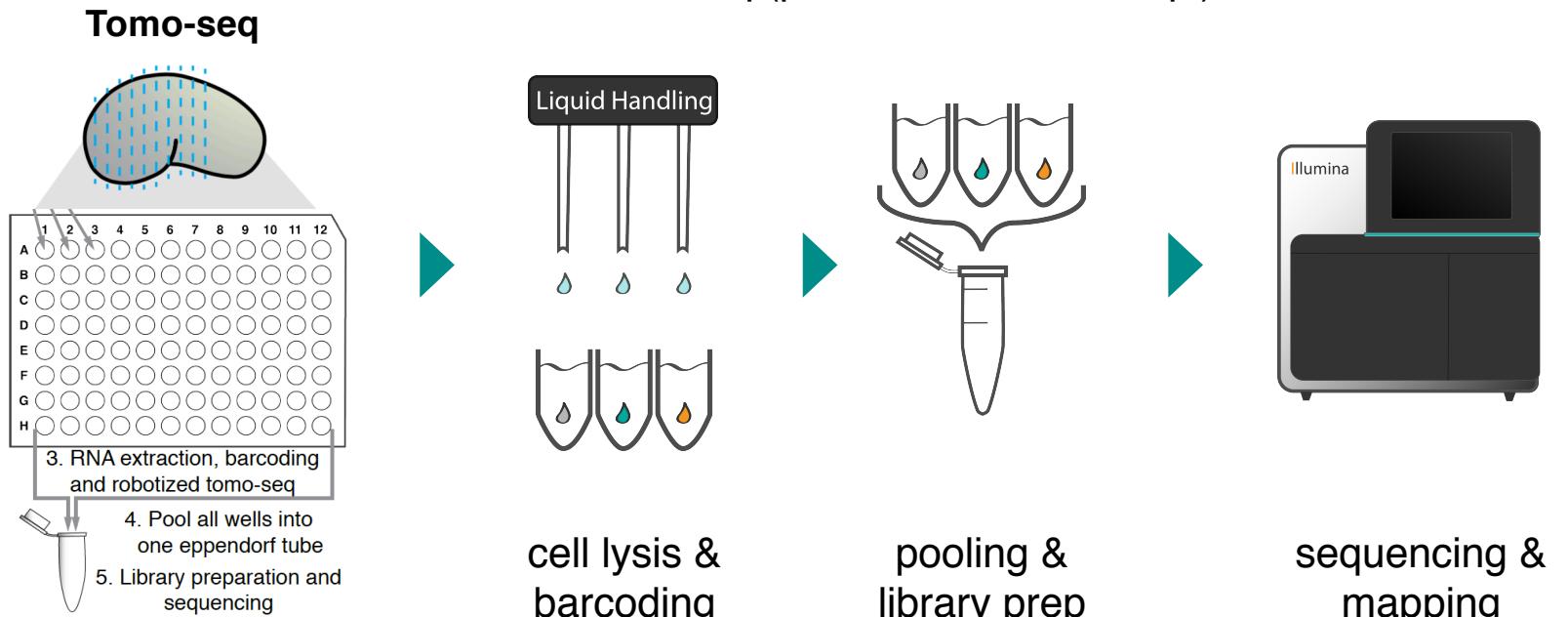


(Rodrigues *et al.*, *Science*, 2019)



Tomo-seq: genome-wide RNA tomography

Based on SORT-seq (plate-based CEL-Seq2)



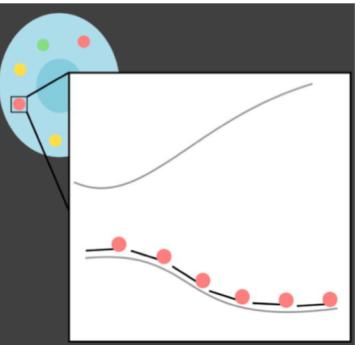
(Junker *et al.*, Cell, 2014)

Easy to implement in labs that are using plate-based scRNA-seq methods

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