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Spatial Transcriptomics Technologies and Analysis Tools

An overview

Harvard Medical School, 31/01/2022

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MEDICAL SCHOOL

Introduction

Spatial organization of cells in tissues are well known to be particularly relevant to understand their functions, which in turn are influenced by transcripts expression and epigenomics factors.

To unveil this relation, in the last years, multiple technologies are emerging to allow to extract this information.

We refer to these technologies as Spatially Resolved Technologies.

We focus on Spatial Transcriptomics, named method of the year 2021 in Nature Methods - Marx Vivien.
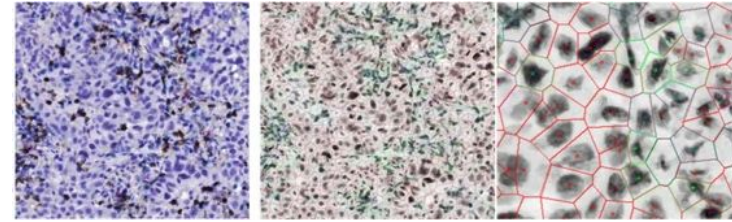
Cells in lung tumor

Spatial Transcriptomics Description

- **Spatial Transcriptomics technologies detect**
  - Spatial coordinates
  - Transcripts quantifications
  - Number of transcripts varies on the used technology
- **We distinguish two main families**
  - <u>Spot based</u> family
    - SpatialTranscriptomics (ST) now 10x-Visium
    - Slide-seq, Slide-seq v2
    - High Definition Spatial Transcriptomics (HDST)
    - others
  - <u>Molecule based</u> family
    - seqFISH, seqFISH+
    - MERFISH
    - osmFISH
   - others
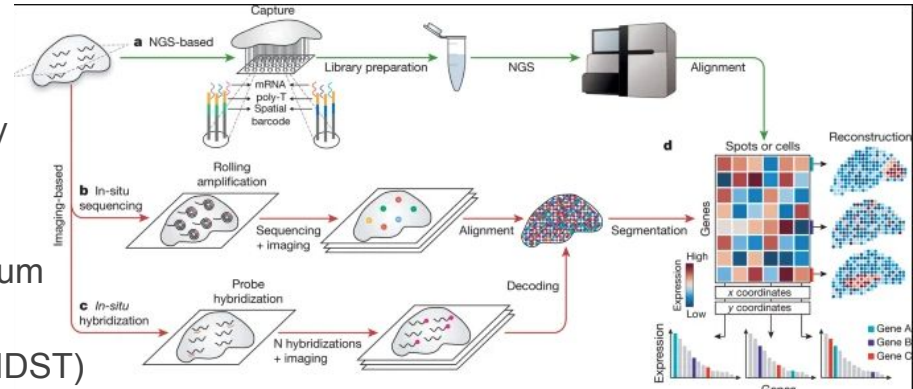
Rao A. et al, Nature, 2021

Spot Based Family

- A tissue slice is placed on a chip/slide

- Typically barcodes are associated to spots that can be holes, beads or regions.

- Typically uses scRNAseq for transcripts quantification (in loco or additionally)

- Spatial coordinates are associated to spots
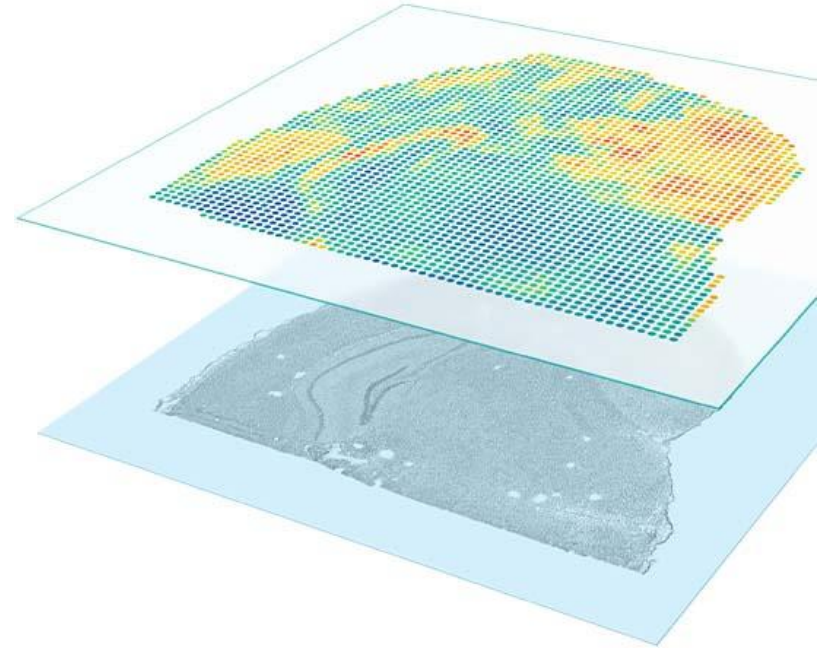
10x-Genomics Visium

- A designed chip with ~5000 spots, each of ~55μm in diameter

- Each hole corresponds (approximately) to ~one cell

- Each hole performs ~whole transcriptome cDNA amplification

- Microscopy imaging of the tissue is taken during amplification

- Coordinates are associated to the spots

- Depending on the experiment, some spots could be uncovered
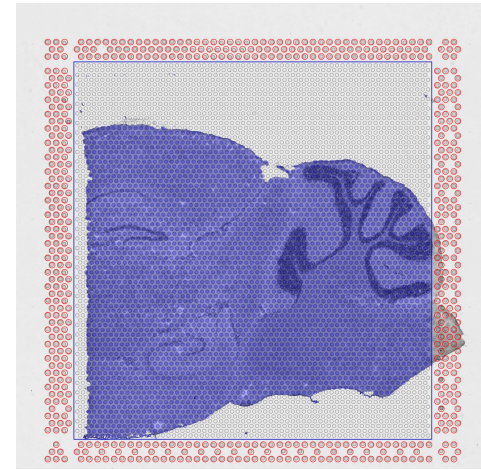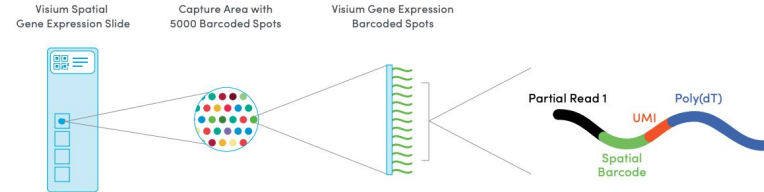

10x Genomics Visium Website

Slide-seq

- A designed coverslip is randomly covered with beads

- Each barcoded bead corresponds (approximately) to one cell

- Resolution is lower than 10x-Visium in terms of transcripts detection

- Could need parallel scRNAseq

- Coordinates are associated to the beads

- Microscopy imaging is taken from a different slice of the same tissue
  - could be a problem for tissues like tumors

- V2 improves the transcripts detection of ~10% than V1
  - ~50% of scRNAseq
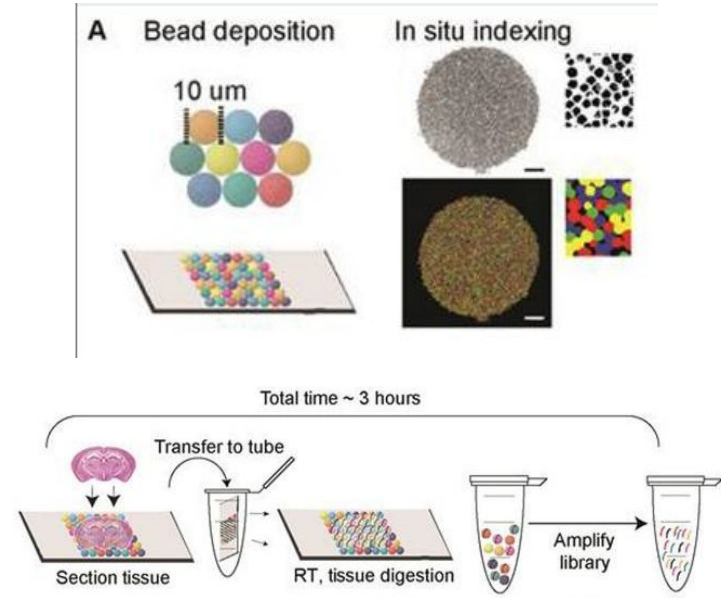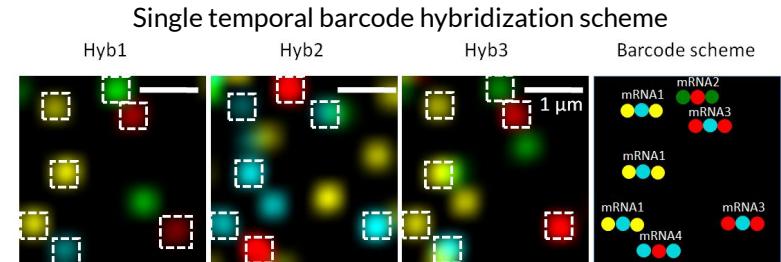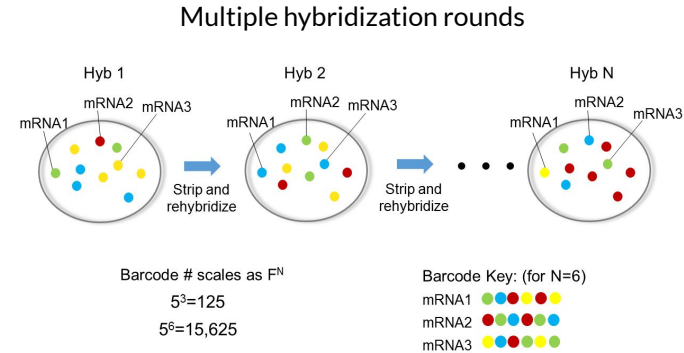

Rodriques et al, 2019, Science

Molecule Based Family

- Direct visualization of transcripts in their original environment

- Use Fluorescence In Situ Hybridization (FISH) for targeting genes

- A priori knowledge of target transcripts

- Number of transcripts varies on the used technology
  - close to whole trancriptome capacity

- Cellular or subcellular resolution

- Coordinates are associated to the transcripts
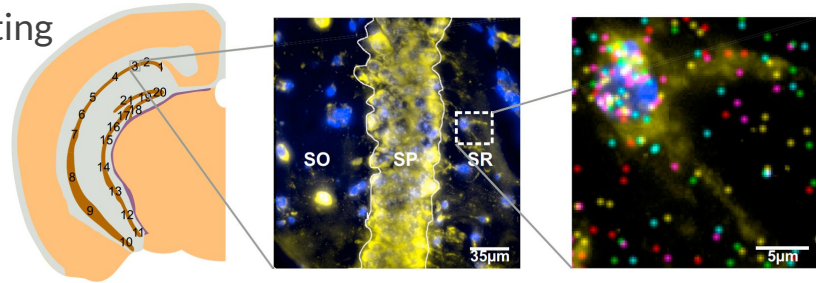
Sequential Hybridization – seqFISH

- Uses temporal barcodes of transcripts multiple hybridization rounds in combination with image microscopy and FISH approach
  - seqFISH+ not requires super resolution equipment
    - microscope could be standard confocal

- Localization of the transcripts at each barcoded time

- For each Barcode we have multiple hybridization rounds to co-localize the transcripts

- Transcripts abundance is detected as the number of colocalizing spots per each transcript (no cDNA or PCR amplification needed).

- Spatial coordinates are associated to each molecule

- seqFISH+ is able to detect thousands of transcripts (~whole transcriptome)

Multiple hybridization rounds

Barcode # scales as F^N

$5^3 = 125$

$5^6 = 15{,}625$

Barcode Key: (for N=6)

mRNA1
mRNA2
mRNA3

Single temporal barcode hybridization scheme

Hyb1 Hyb2 Hyb3 Barcode scheme

1 μm

Lubeck E. et al., Nature Methods, 2014 - Eng C-HL et al, Nature, 2019

Multiplexed Error Robust FISH - MERFISH

- Similar principle behind seqFISH
  - multiple hybridization rounds for targeted transcripts

- A computational error correction for imperfect hybridizations

- Less time consuming than seqFISH (first version)

- Used in combination with other technologies by the Brain Initiative Cell Census Network (BICCN)
  - building an atlas for the Mouse Brain
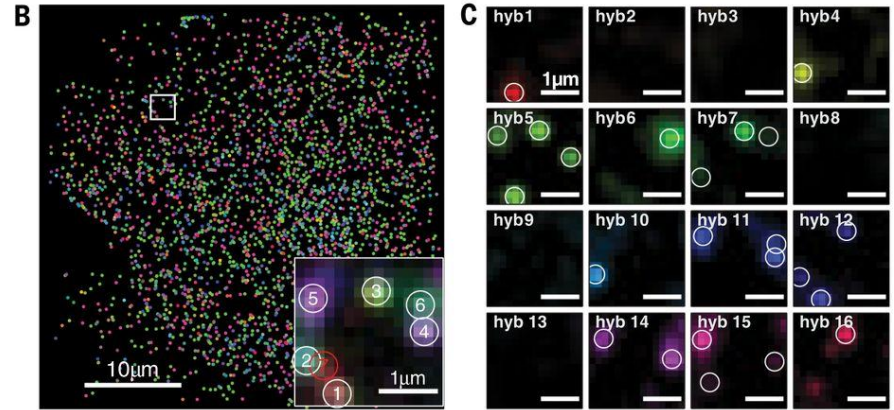

Chen KH et al, Science, 2015

The spatial technology world

Spatial Transcriptomics is an emerging technology which aims to be always more adopted in the future

We are seeing an evolving of these technologies during the years

- multiple cells for one spot (ST/10x-Visium) to multiple spots for one cell (stereo-seq) in the spot-based family

- a few molecules (seqFISH) to thousands of molecules (seqFISH+) could be detected nowadays in the molecule-based family

More in the future where both spatial information and multiple modalities will be detected from the same cell
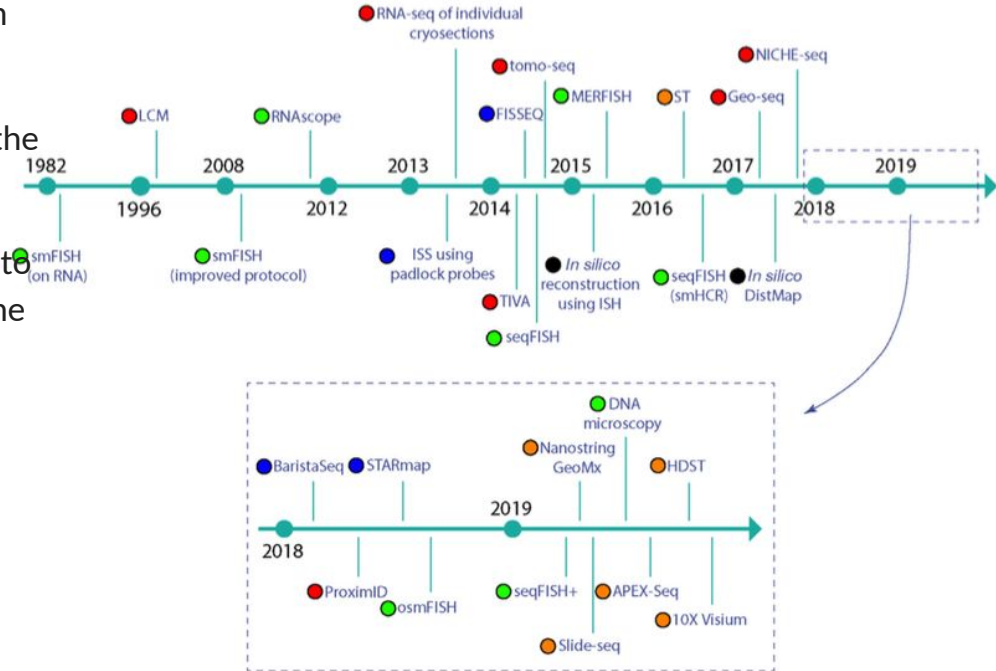
Asp M. et al, BioEssays, 2020

Current Tools for spatial analysis

- R based tools
  - *sf/sp* packages
    - tools for general spatial analysis
    - have their own S3 classes
    - people are starting to use these tools for the single cell spatial analysis
  - *Giotto*
    - specific for spatial transcriptomics analysis
    - uses Giotto class
  - *Seurat v4*
    - more general single cell analysis framework
    - vignette shows only spot based technologies analysis
    - uses seurat class
- Python based tools
  - *squidPy*
    - specific for spatial trancriptomics analysis
    - uses AnnData class
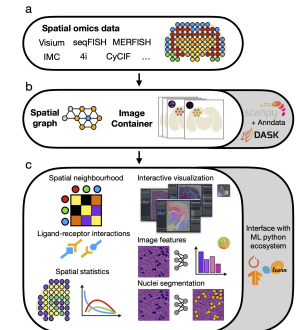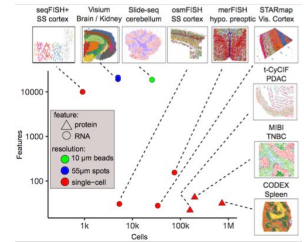
Bioconductor

- Statistical analysis & comprehension of genomic data

- 2083 R packages developed by a core team and more than 1200 researchers

- Widely used ~¾ million distinct IP downloads annually

- Well-respected ~ 50000 PubMed Central full text citations

- Resources:
  - https://bioconductor.org
  - https://support.bioconductor.org
  - community slack (sign up; participate)