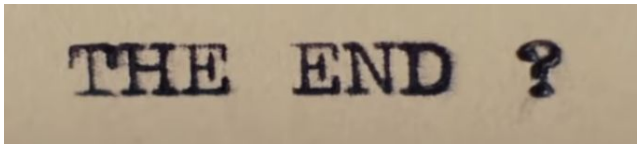
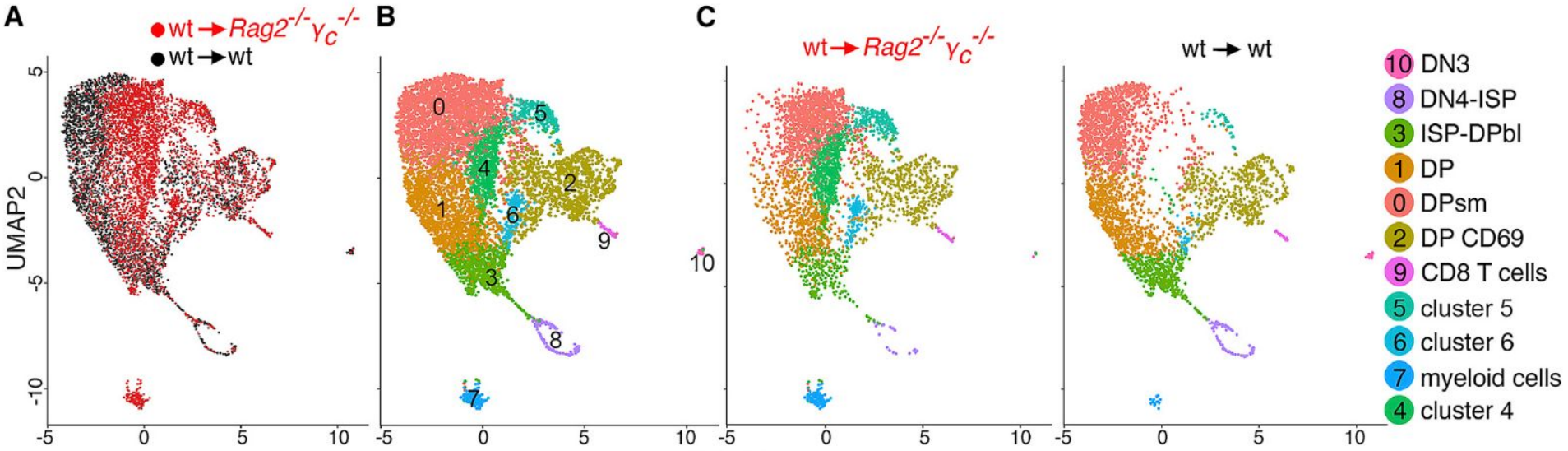
Single Cell RNAseq Analysis

… beyond !

École de bioinformatique IFB-INSERM 2024

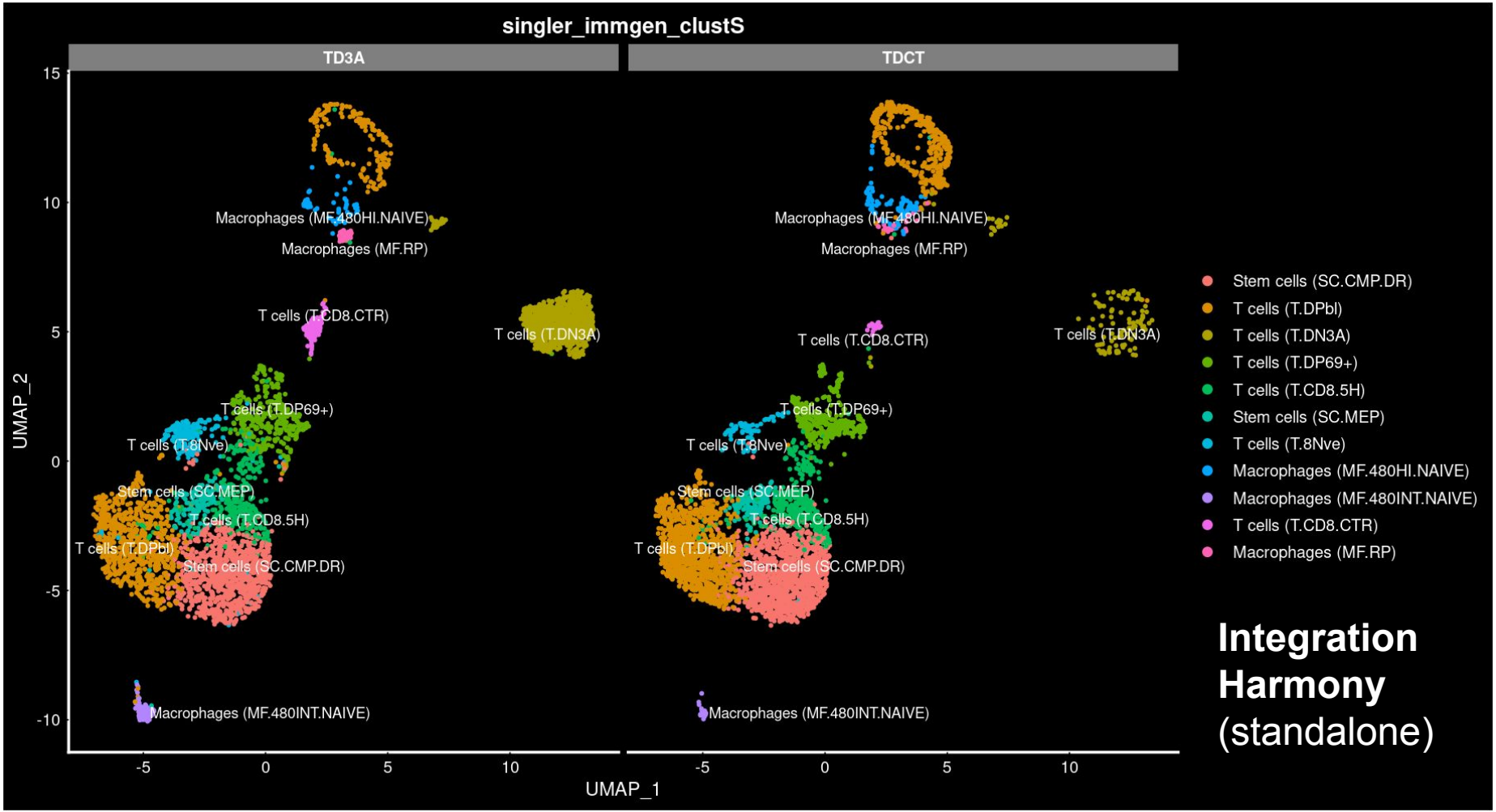
Training course dataset:





Integration
Seurat
(older)

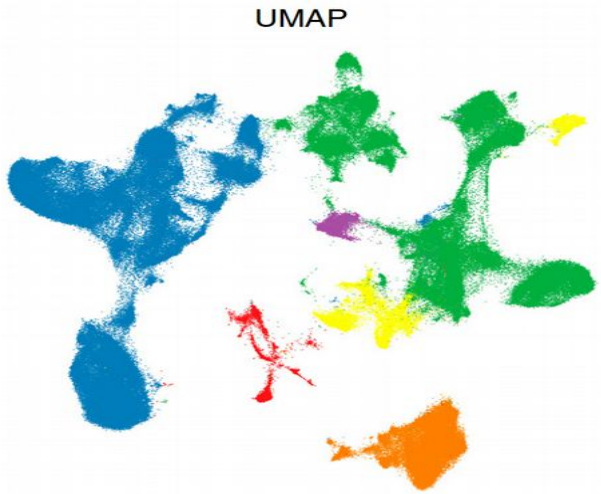
Paiva et al, 2021, Cell Reports
https://doi.org/10.1016/j.celrep.2021.108967

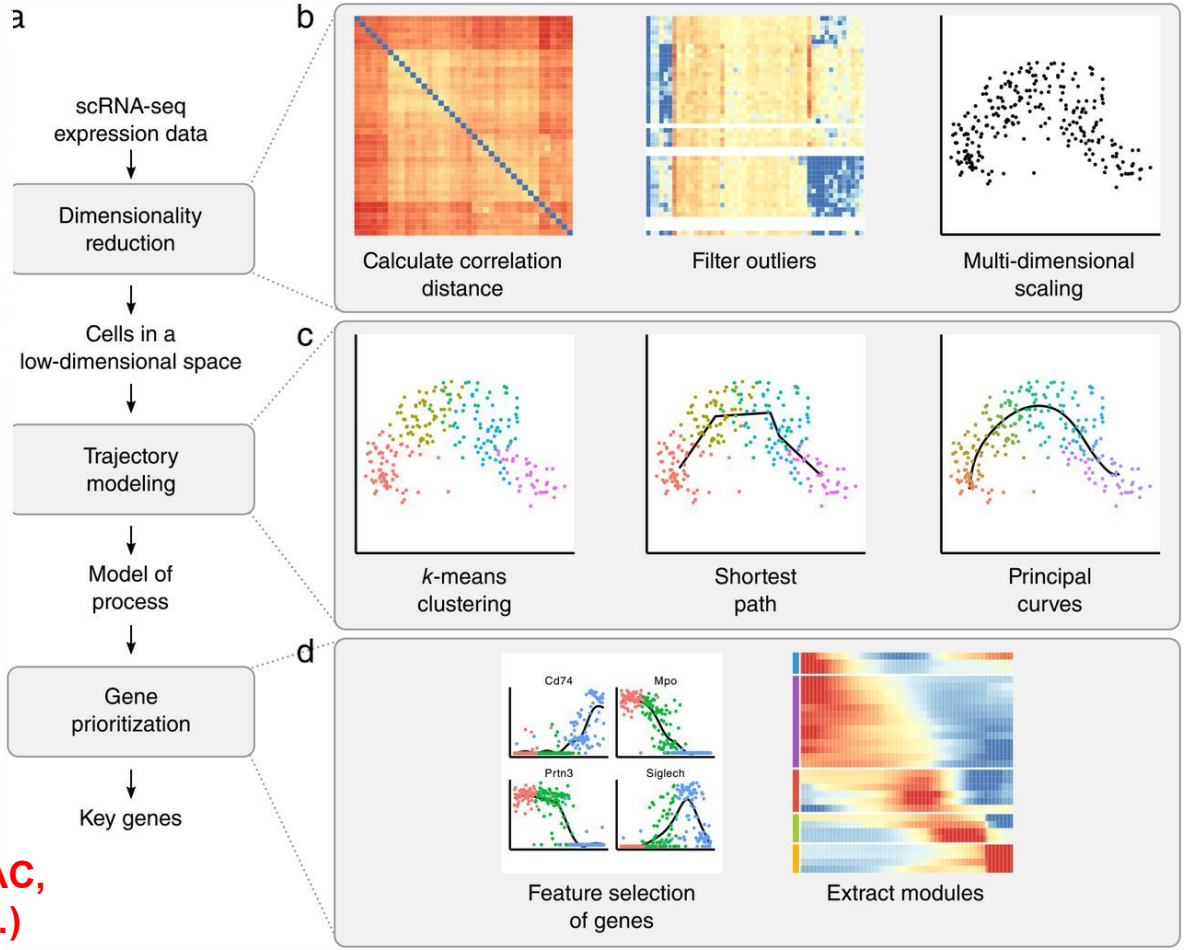


Integration
Harmony
(standalone)

Other types of scRNAseq analysis

Cell trajectory : methods





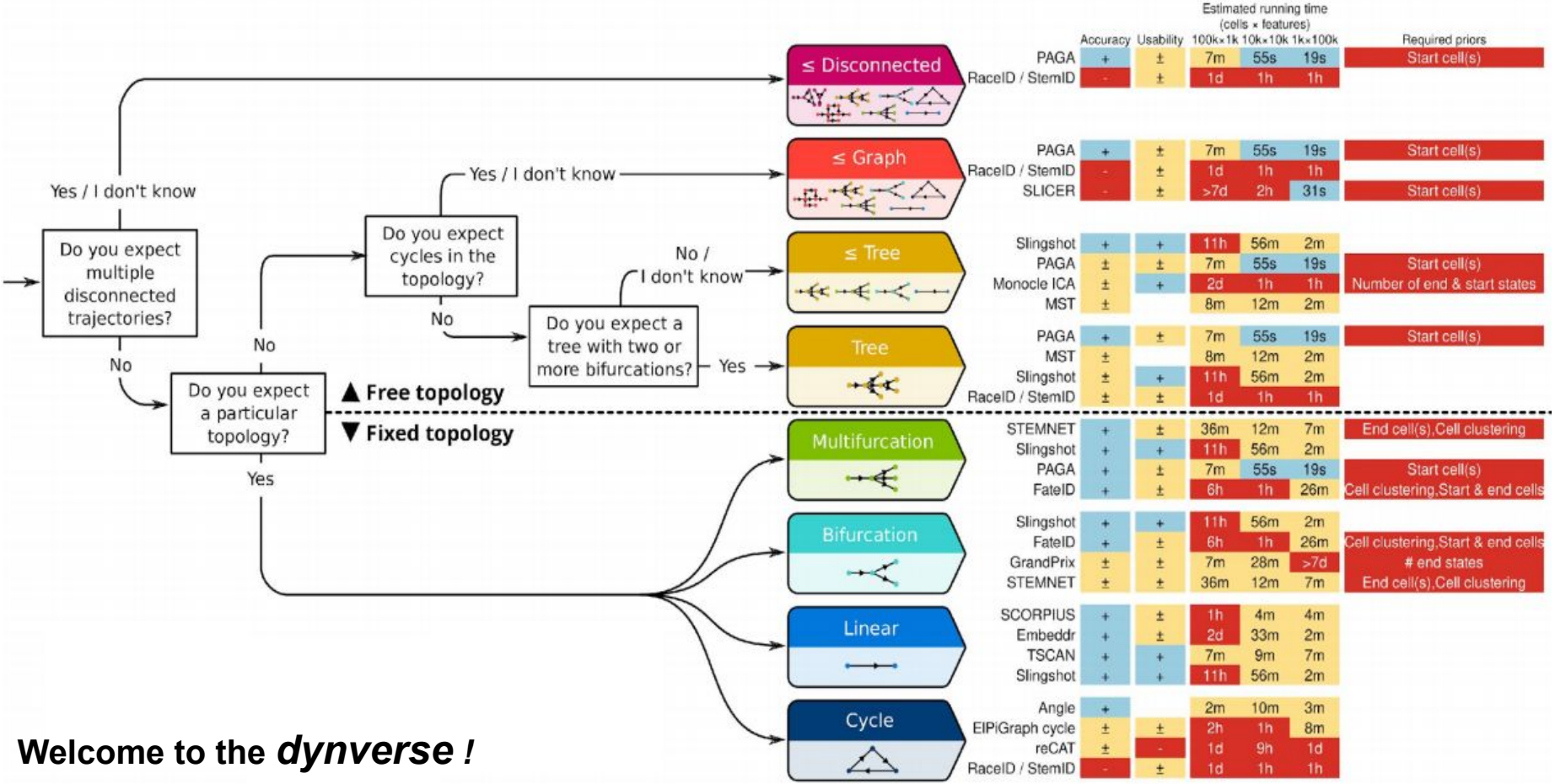
Most adopted tools :

- Monocle 3
- PAGA
- STREAM
- Scorpius
- Slingshot, TinGa, …

Not limited to scRNAseq ! (ATAC, CITE, multiomics, imagery-based …)

SCORPIUS, bioRxiv (2016)

Cell trajectory : Contexts

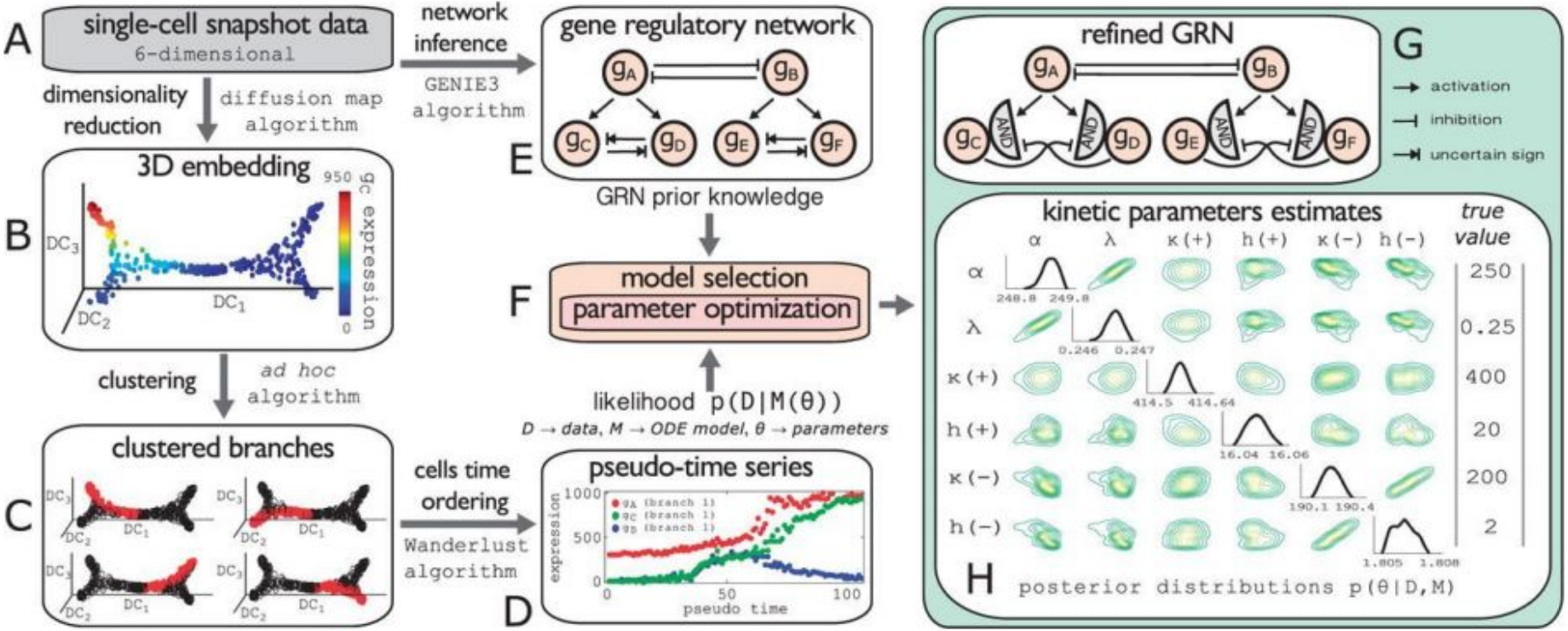


Saoutens et al, Nature Biotech, 2019

Welcome to the *dynverse !*

Network inference

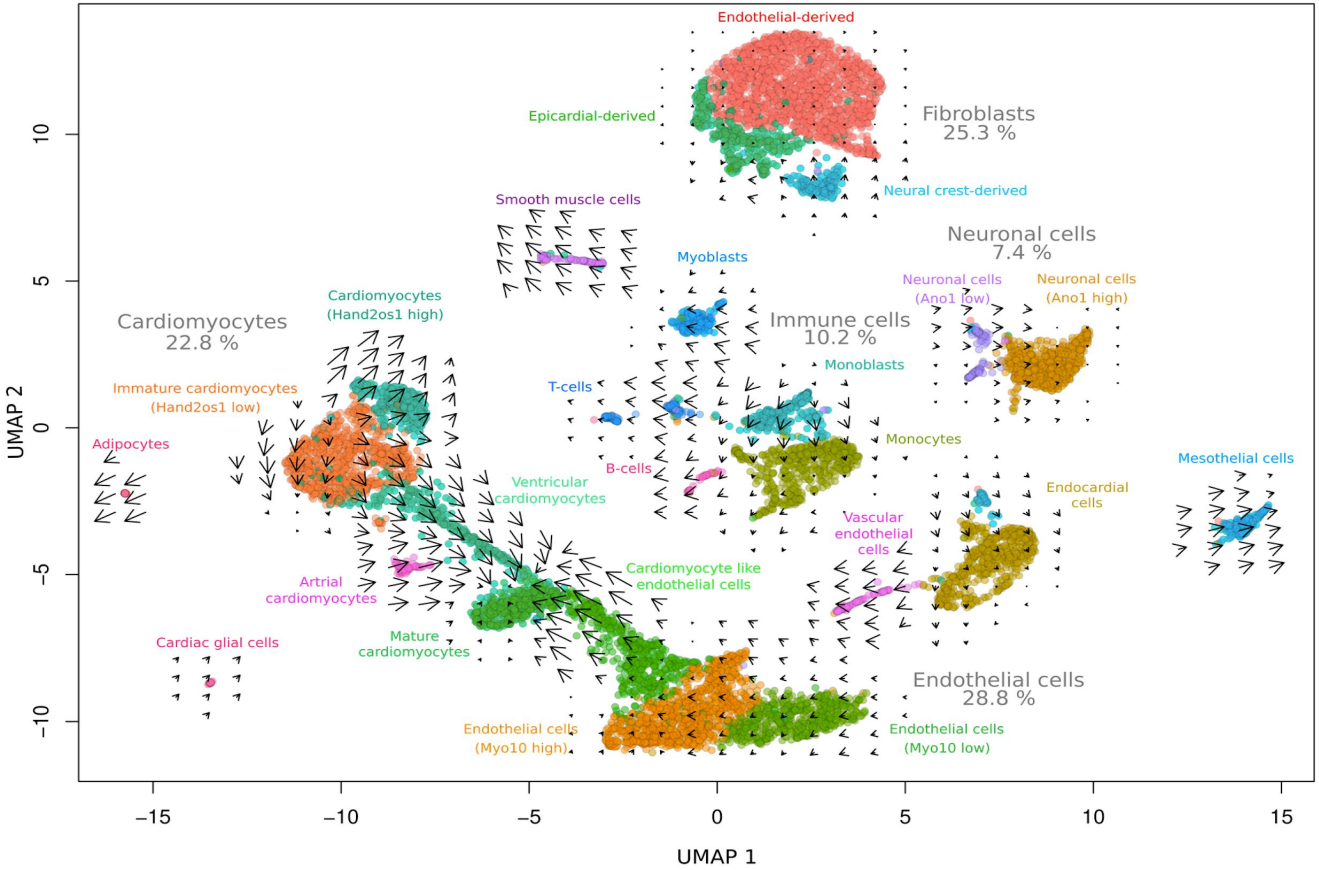
Using cell ordering from trajectory analysis + co-occurring / correlated genes



Ocone et al. Bioinformatics (2015)

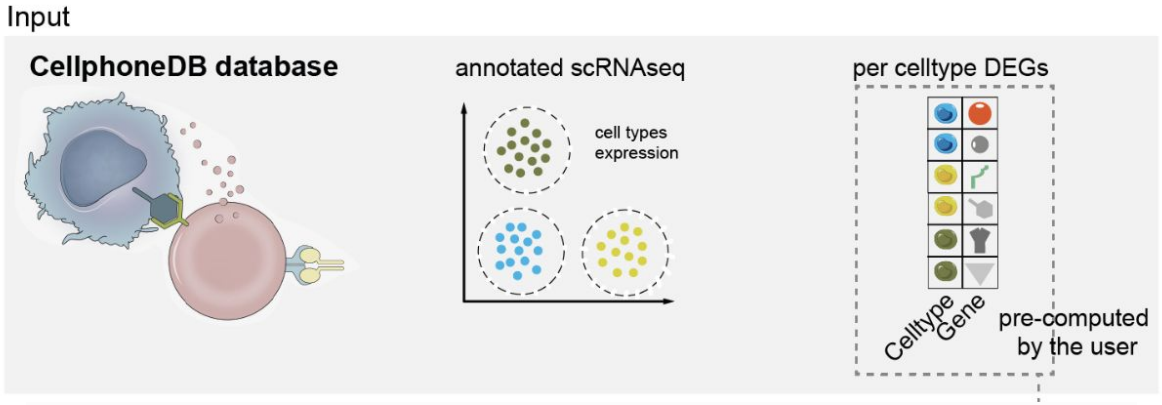
RNA Velocity

Example on an entire mouse heart profile

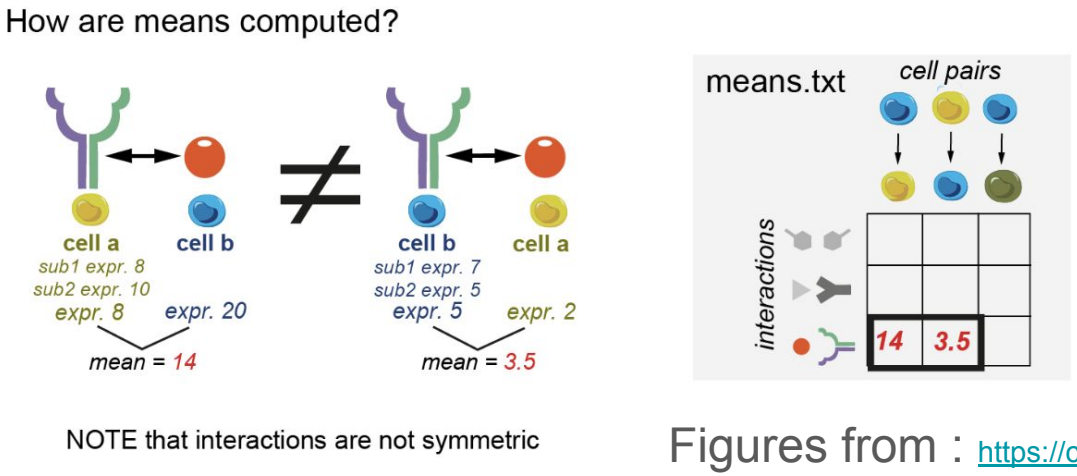


https://doi.org/10.3390/cells9020318

Cell-cell interactions



Tools : CellPhoneDB, CellChatDB, CellTalkDB …

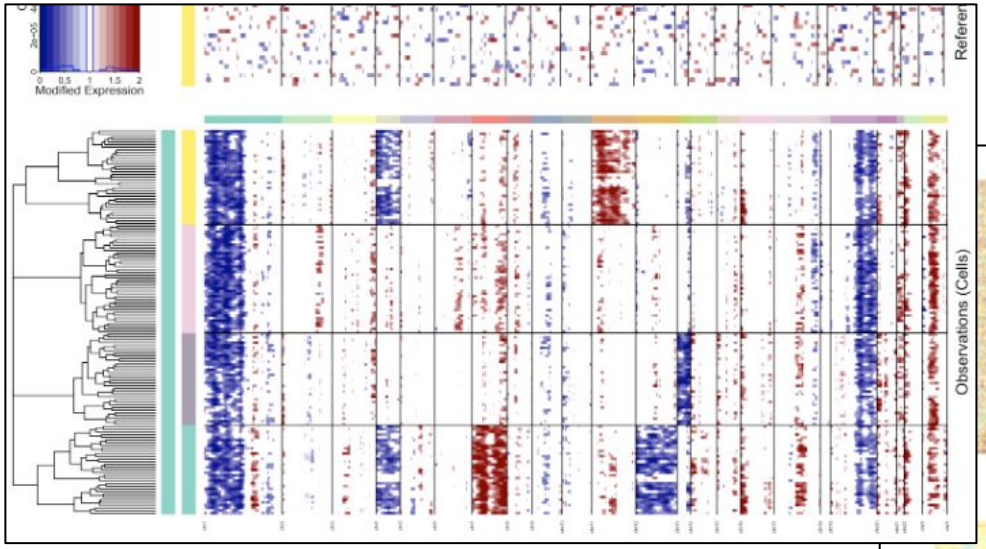


Benchmark : https://doi.org/10.1038/s41467-022-30755-0

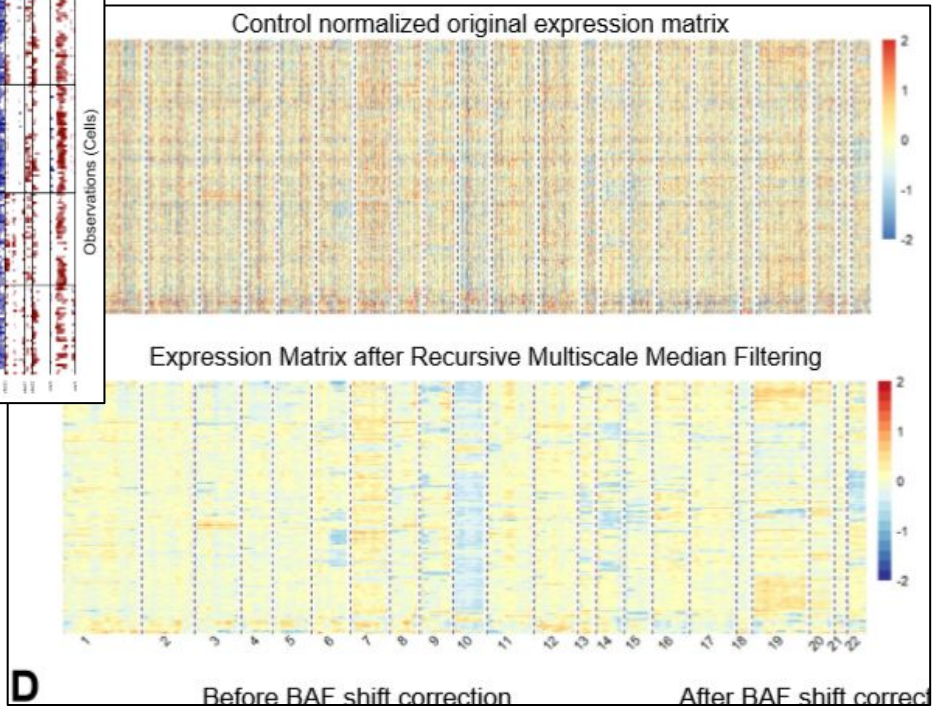
Figures from : https://cellphonedb.readthedocs.io/

Copy number estimation from scRNAseq

InferCNV (Broad Institute)



CaSpER (Armanci et al, BioRxiv 2019)



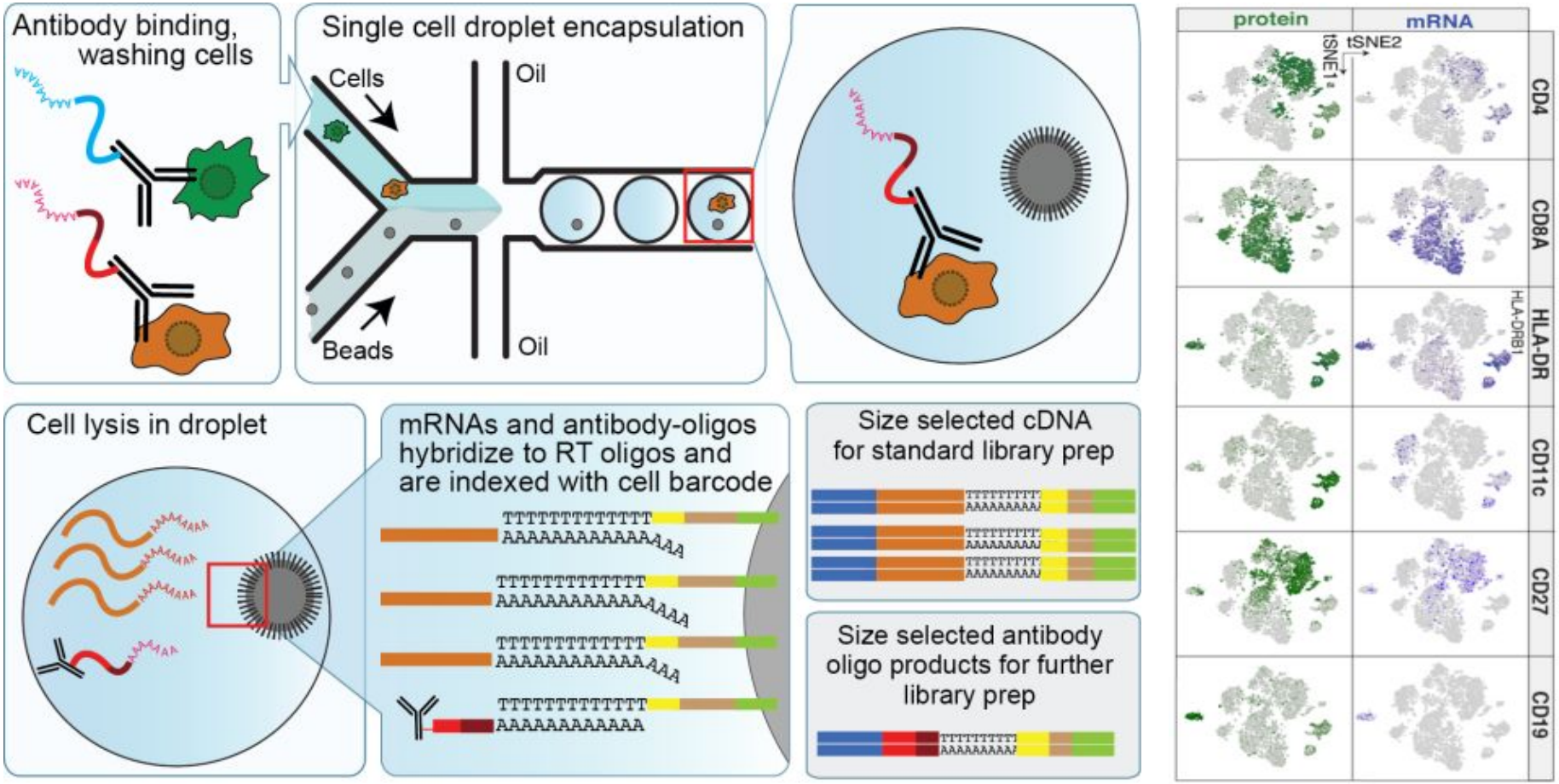
WARNING :

- Coarse grain (> 10 Mb)
- Requires > 75,000 reads / cell

Technologies

CITE-Seq (+ECCITE-Seq)

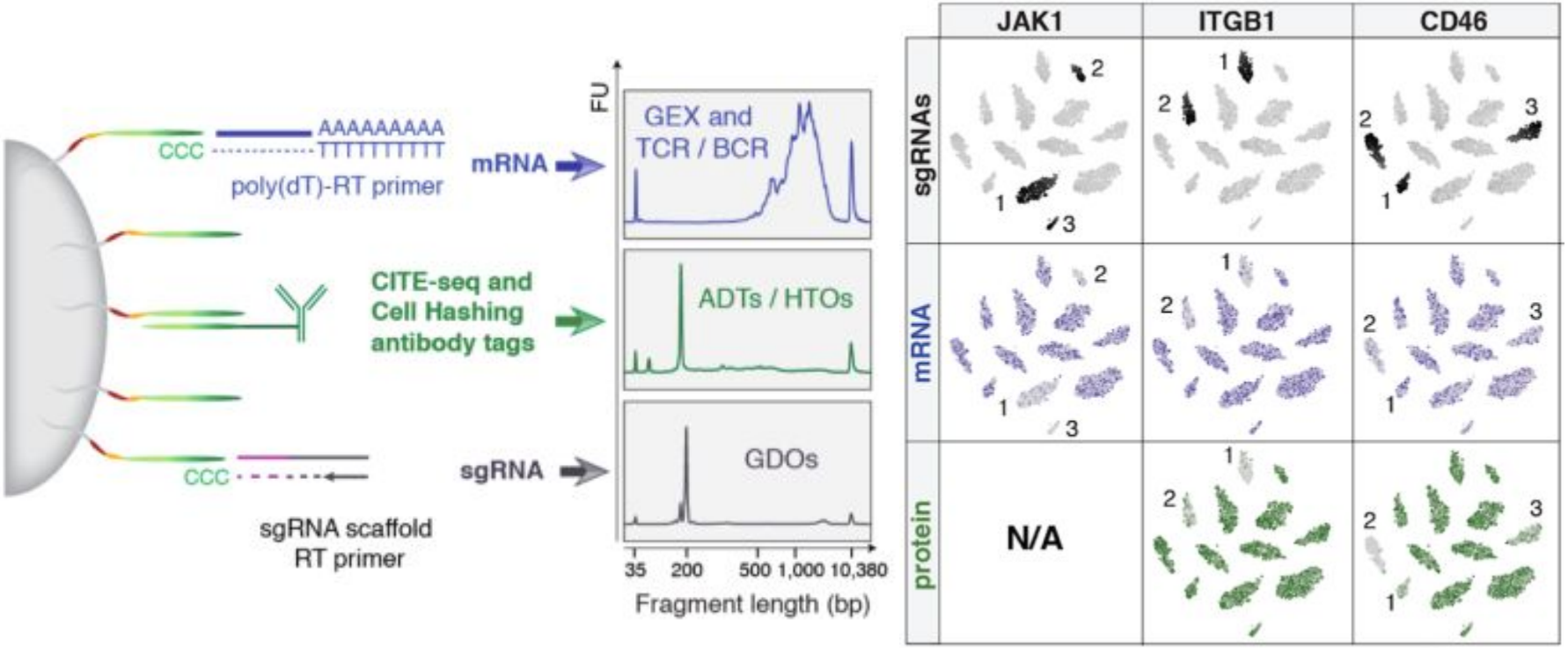
Cellular **I**ndexing of **T**ranscriptomes and **E**pitopes by **Seq**uencing



Wikipedia / citeseq.com

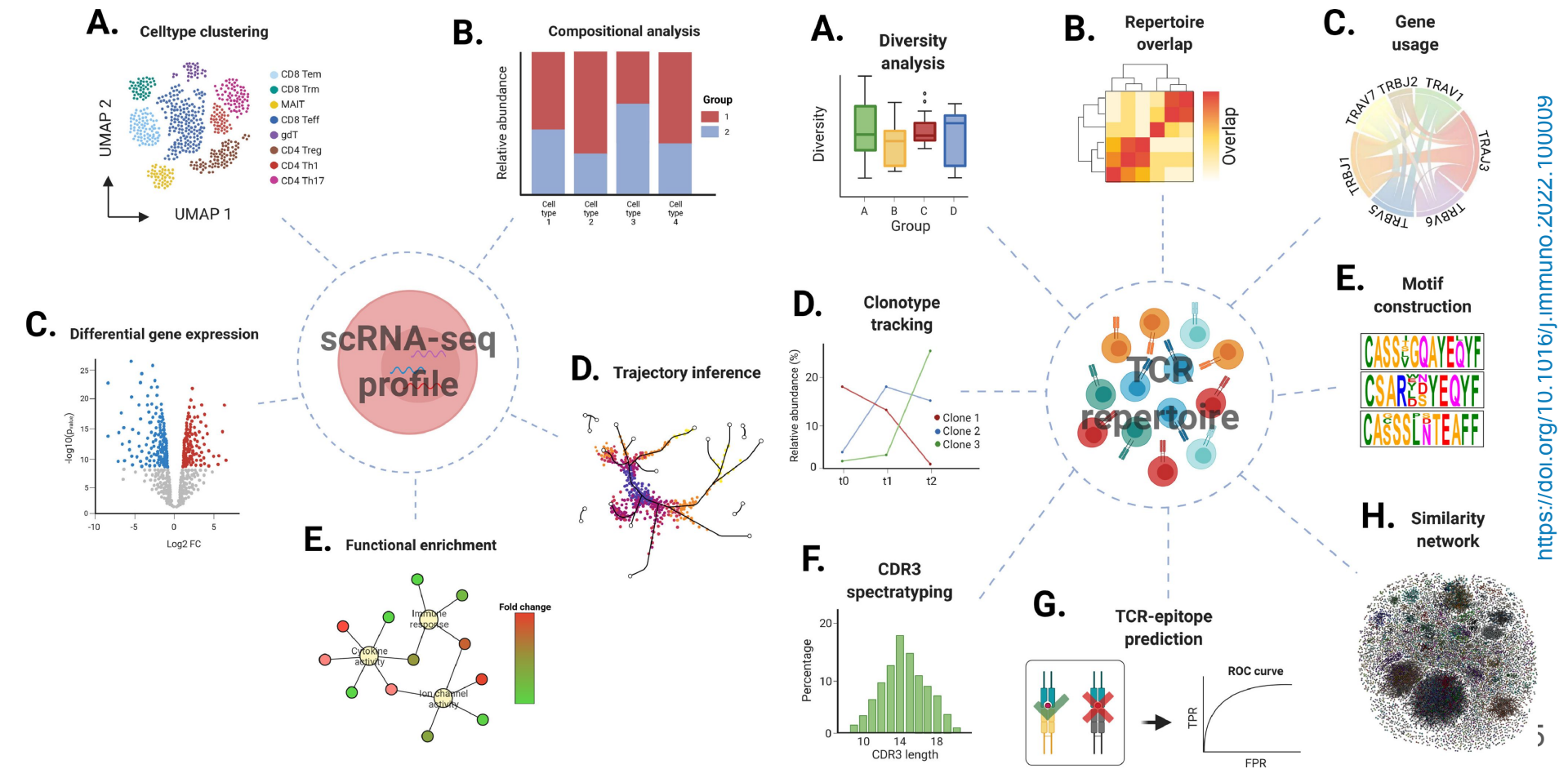
ECCITE-Seq

Extended **C**RISPR-compatible **C**ellular **I**ndexing of **T**ranscriptomes and **E**pitopes by **Seq**uencing (5’)



Wikipedia / citeseq.com

scRNAseq + TCR repertoire



https://doi.org/10.1016/j.immuno.2022.100009

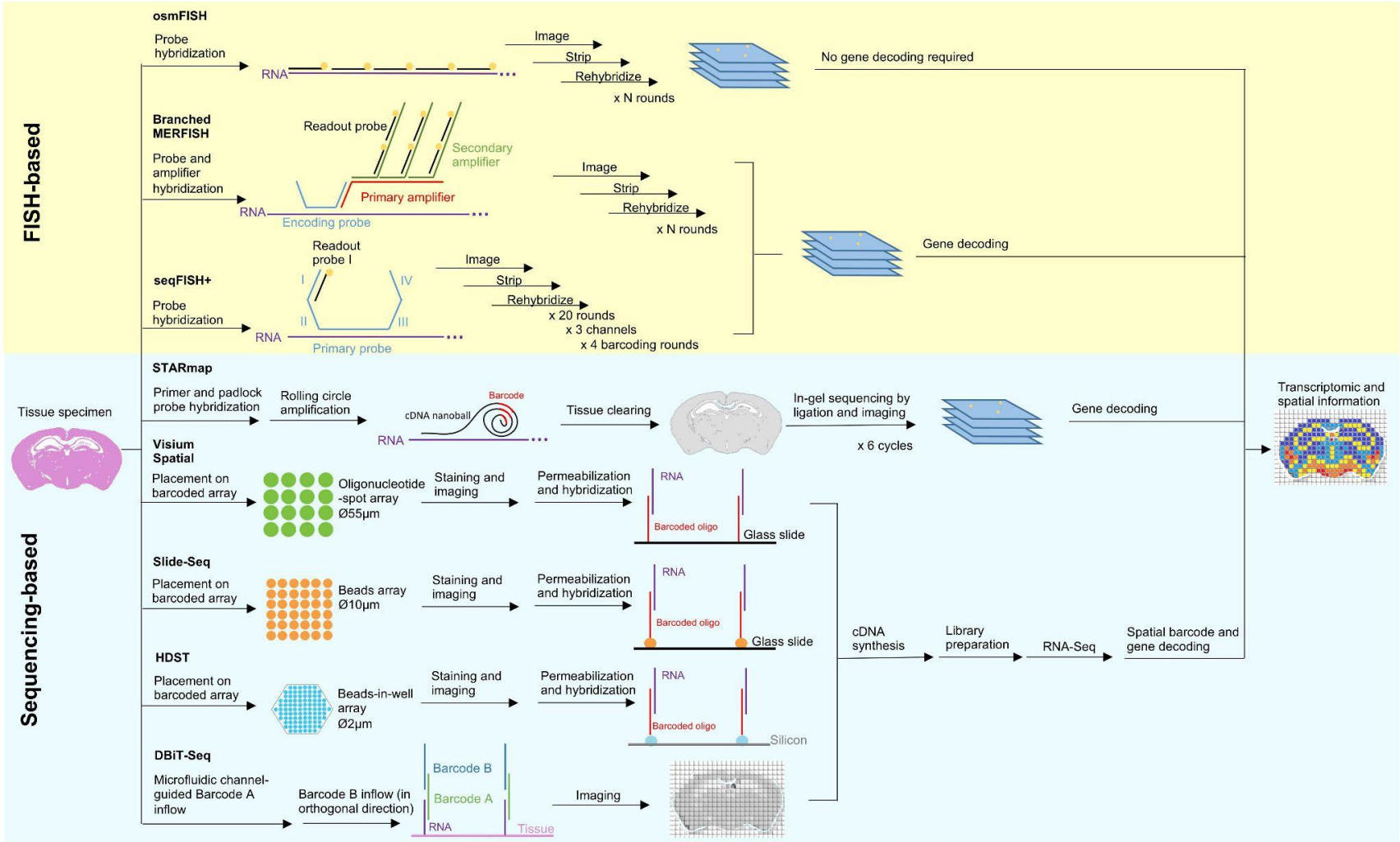
Why so much hype ?

Bulk

Single cell

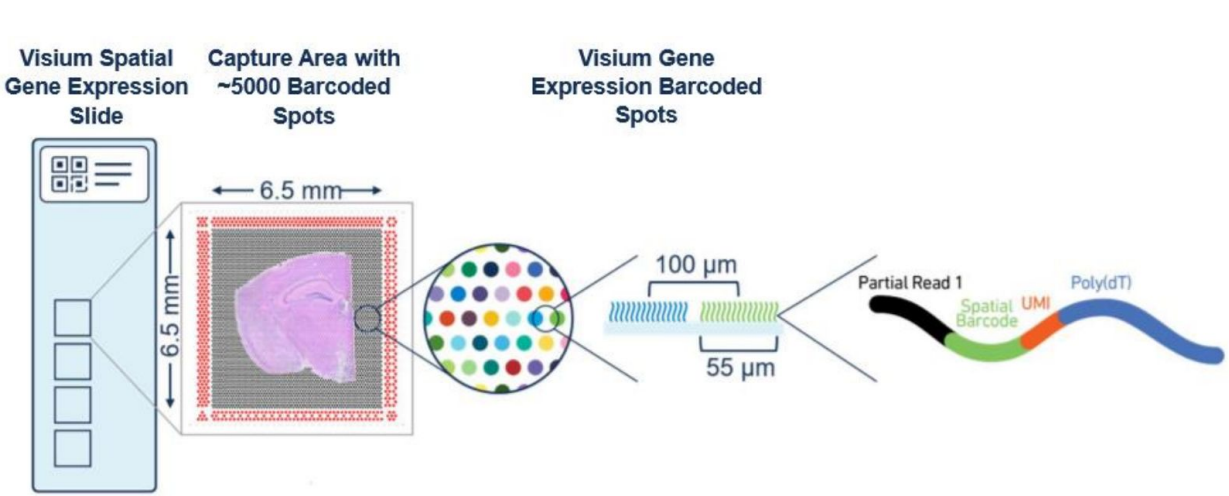
Spatial single cell

Based upon Shalek and Regev, 2016

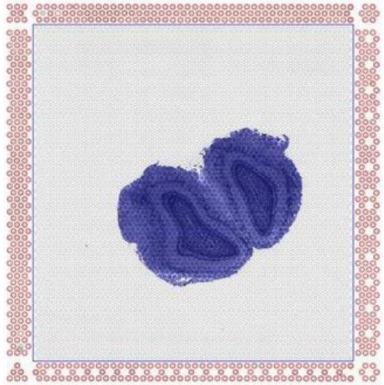


https://doi.org/10.3389%2Ffgene.2020.00662

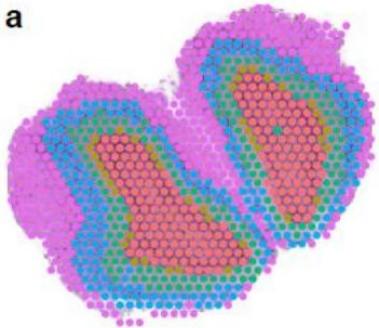
Seq-based spatial scRNAseq : 10x Genomics Visium



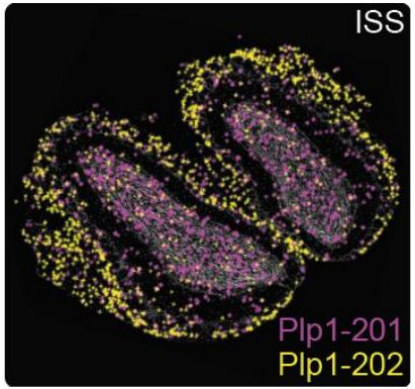
IHC



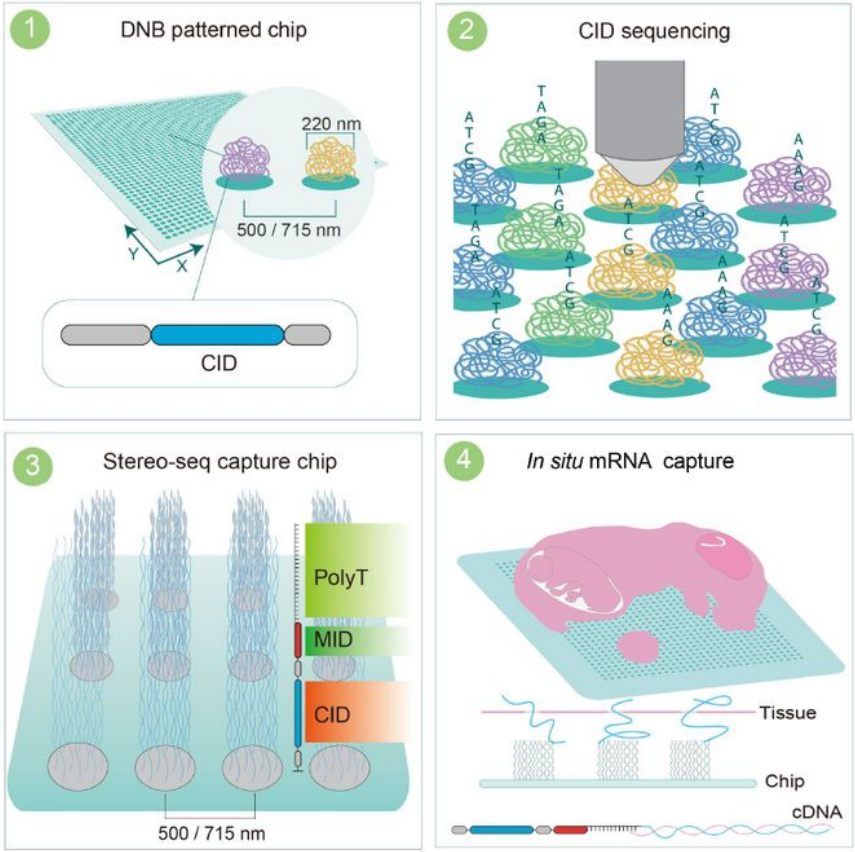
Clusters



RNA FISH

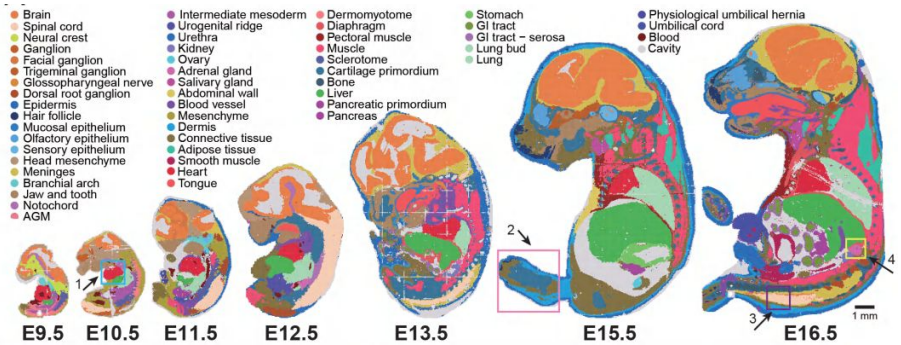


Seq-based spatial high-res long-read : StereoSeq



- **Based upon "DNB" (DNA Balls) arrays by MGI/BGI**
- **Claimed resolution of 500-715 nm**
- **Active surface of 200 mm²**
- **Performed a developmental analysis of FULL mice embryos !**

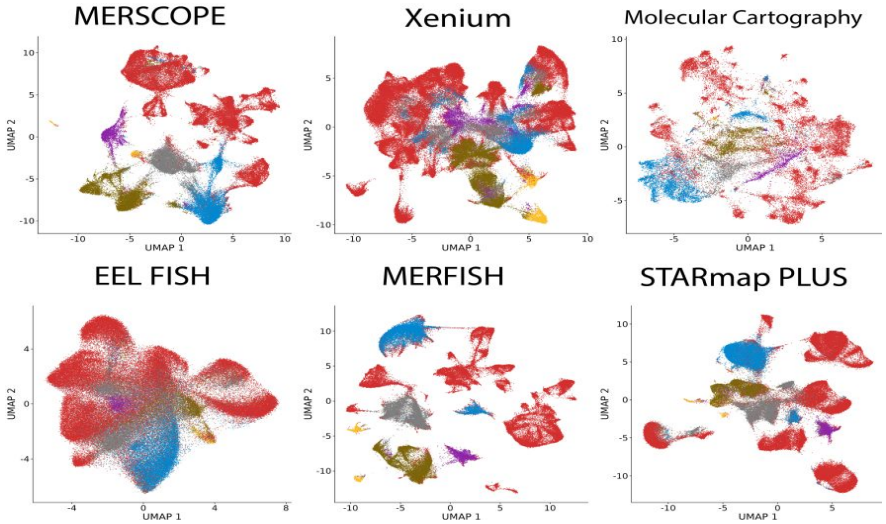
Cheng et al, BiorXiv 2021.01

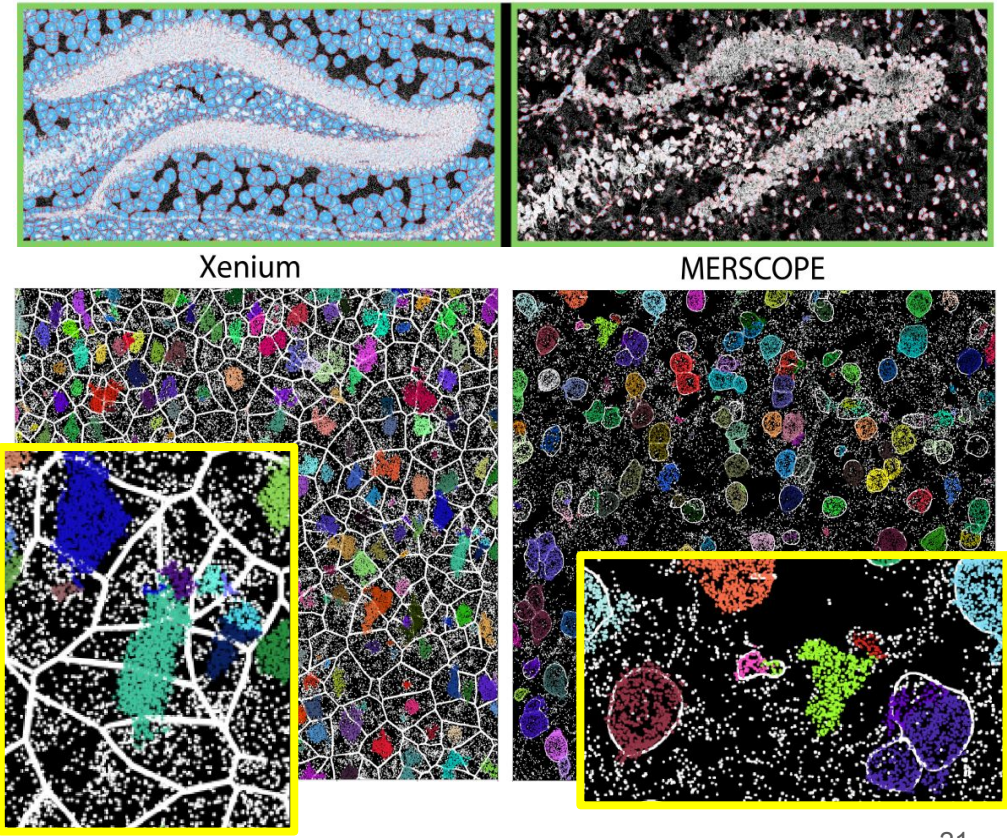


Imagery-based Spatial scRNA expression by imagery : NanoString CosMx-SMI

3D view : https://www.youtube.com/watch?v=LI-gvIribDU&t=14s

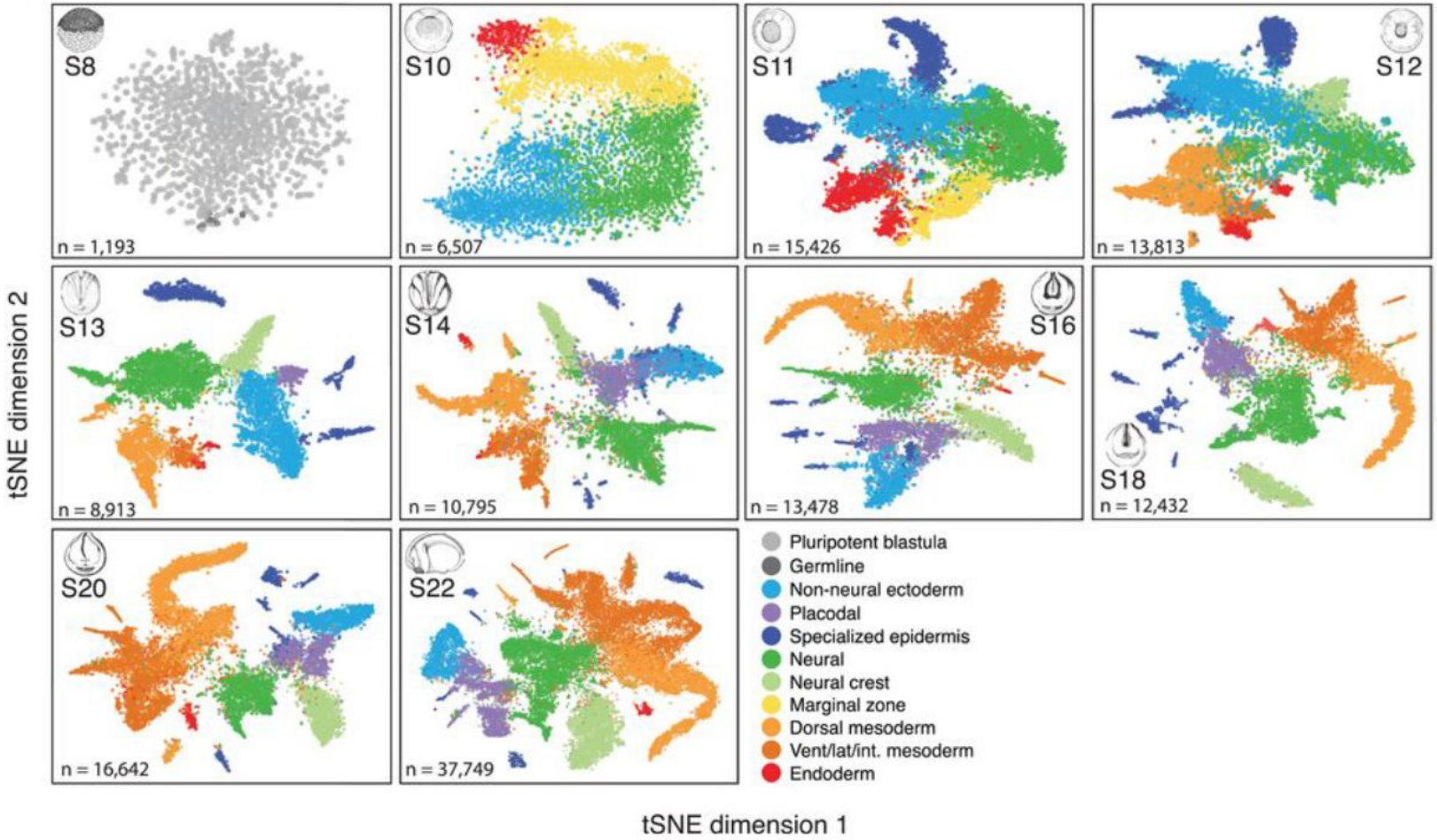
Cell Segmentation as the one point to improve



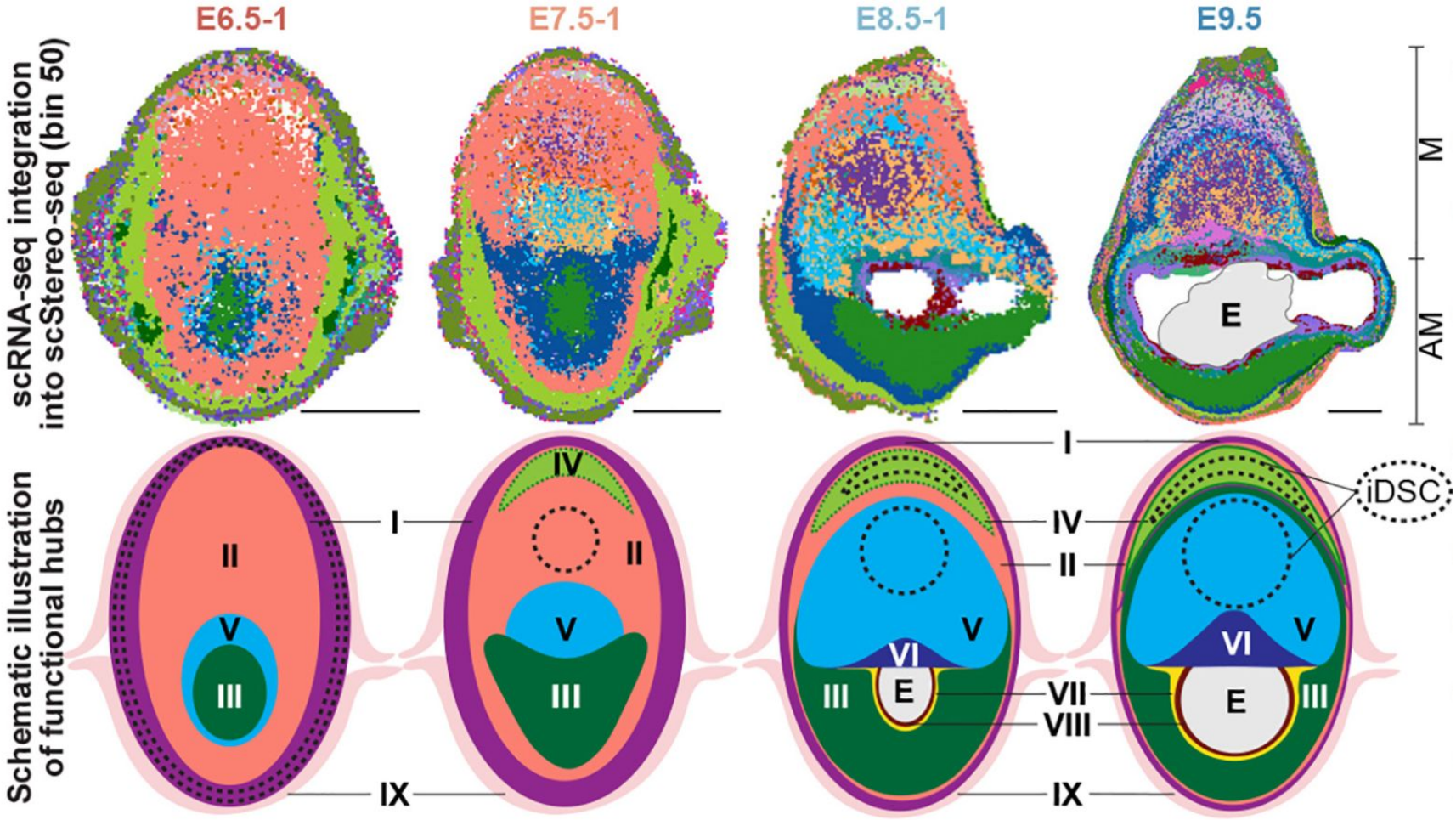


Some sweets

Xenopus embryo development

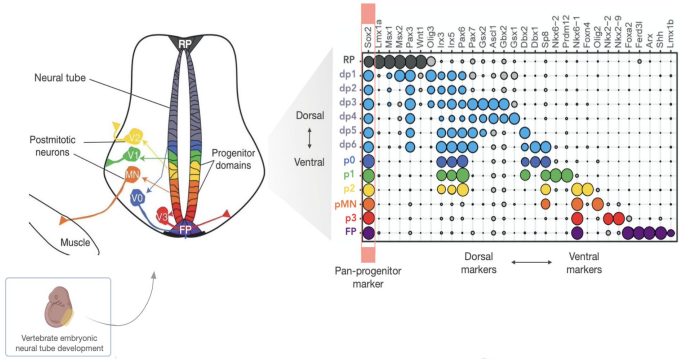


Spatial SC : Polar differentiation of mouse uterus

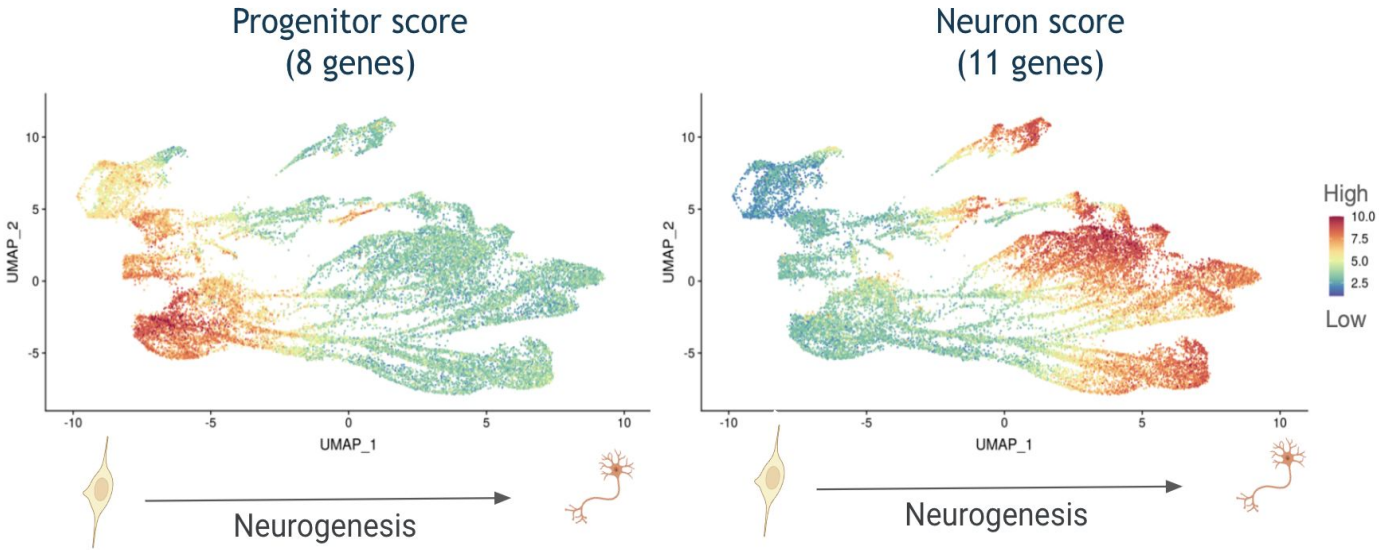


https://doi.org/10.1016/j.cell.2023.08.020

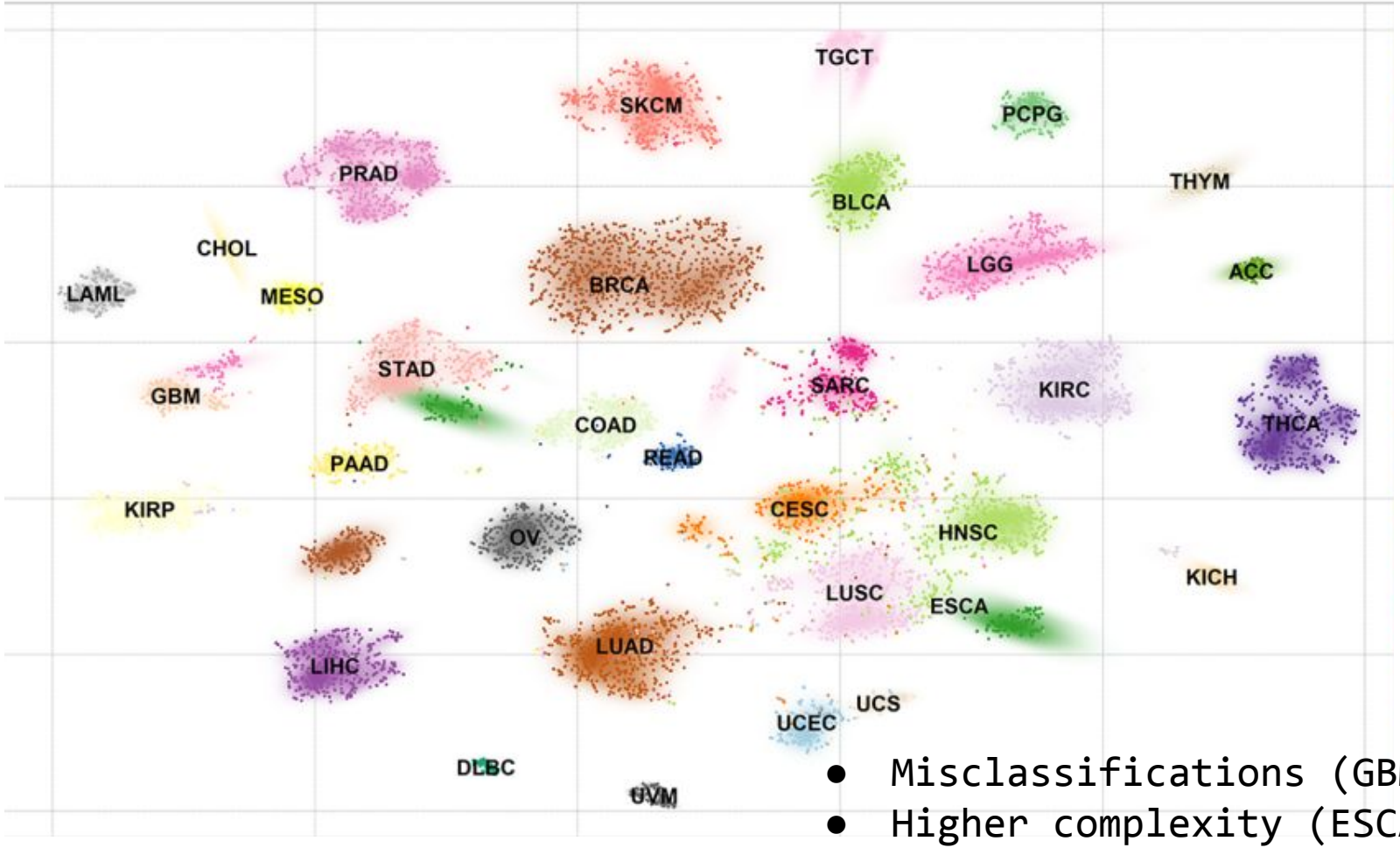
Single cell RNA-seq of mouse embryo neural progenitors



Nathalie's PhD thesis &
Delile et al, Development, 2019



t-SNE of the whole TCGA project **bulk** expression



- Misclassifications (GBM-LGG)
- Higher complexity (ESCA-STAD)

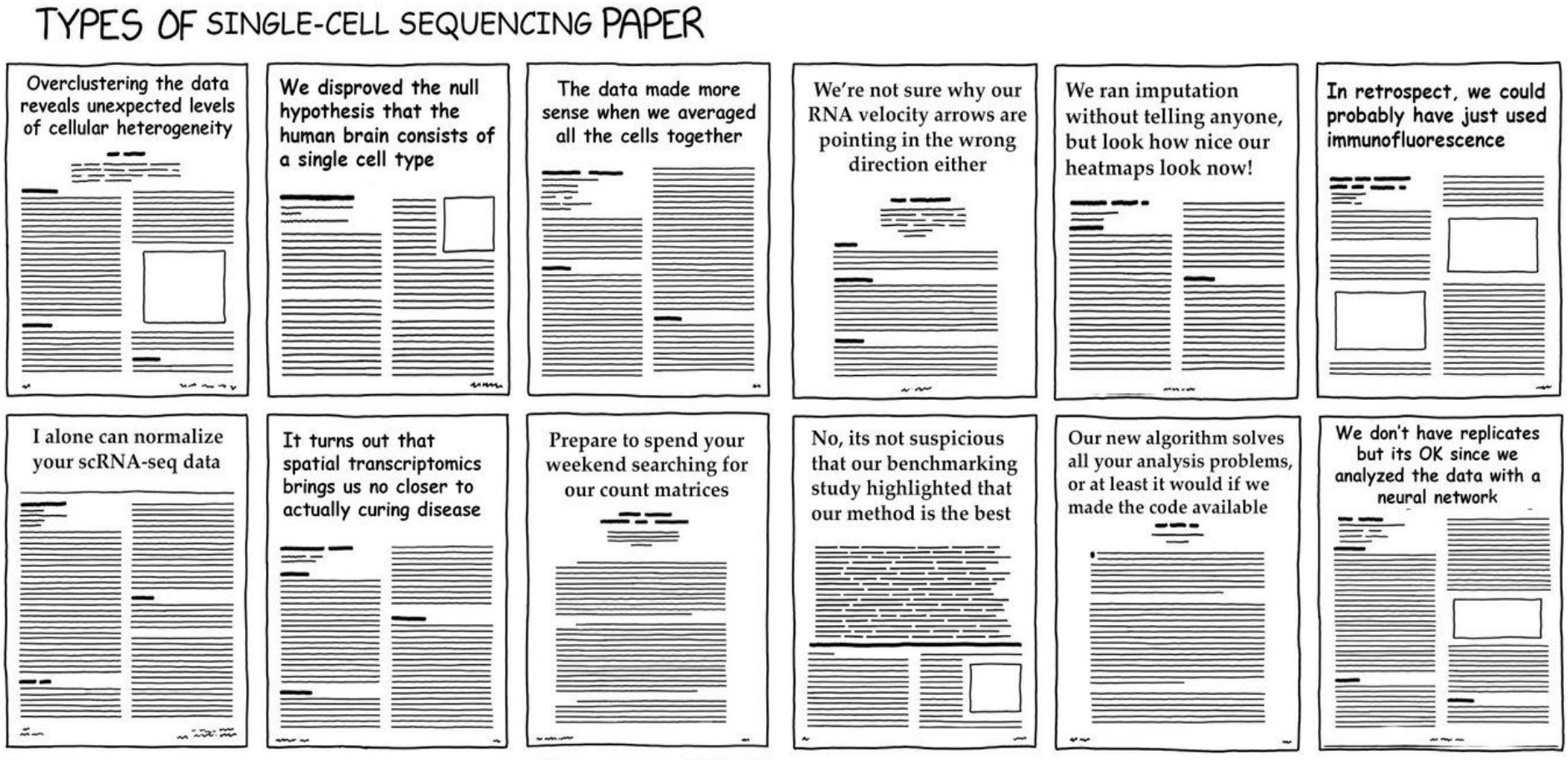
Thanks and congratulations from your devoted team

Emilie Drouineau
Nathalie Lehmann
Eulalie Liorzou
Morgane Thomas-Chollier
Audrey Onfroy
Thibault Dayris
Bastien Job

SINGle-cellING IN THE RAiNaseq (1952)*

* play on words courtesy of Jacques van Helden

AS SEEN ON YouTube

Yearly talk by Raul Satija on single cell novelties (2024)
https://www.youtube.com/watch?v=eQ7wzmzWaEk

Single cell results and the community