

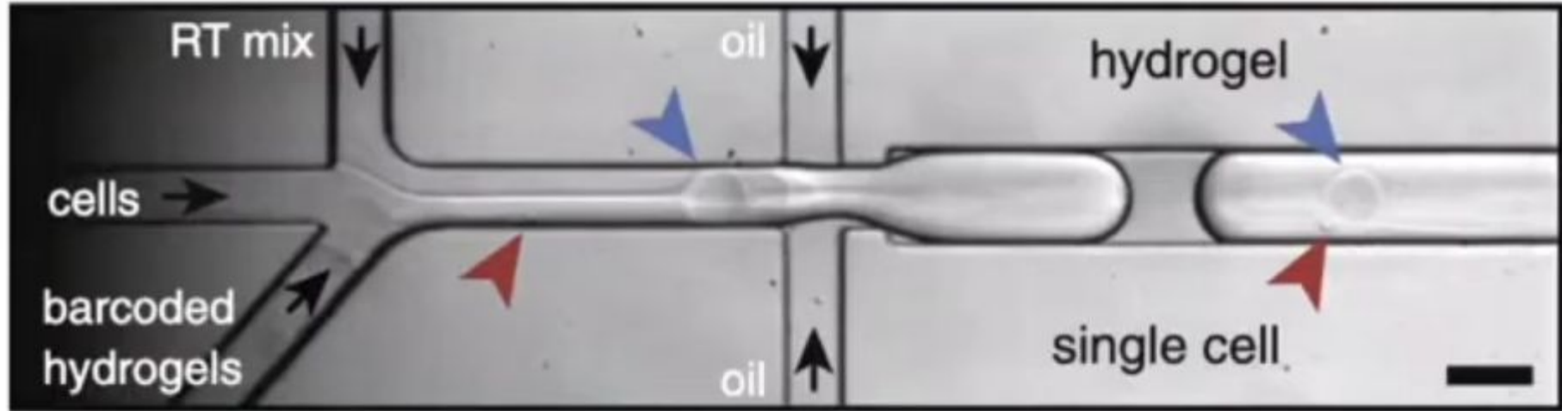
Wrap-up

-

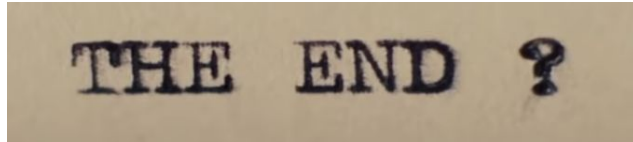
Single Cell RNAseq Analysis *... and beyond !*

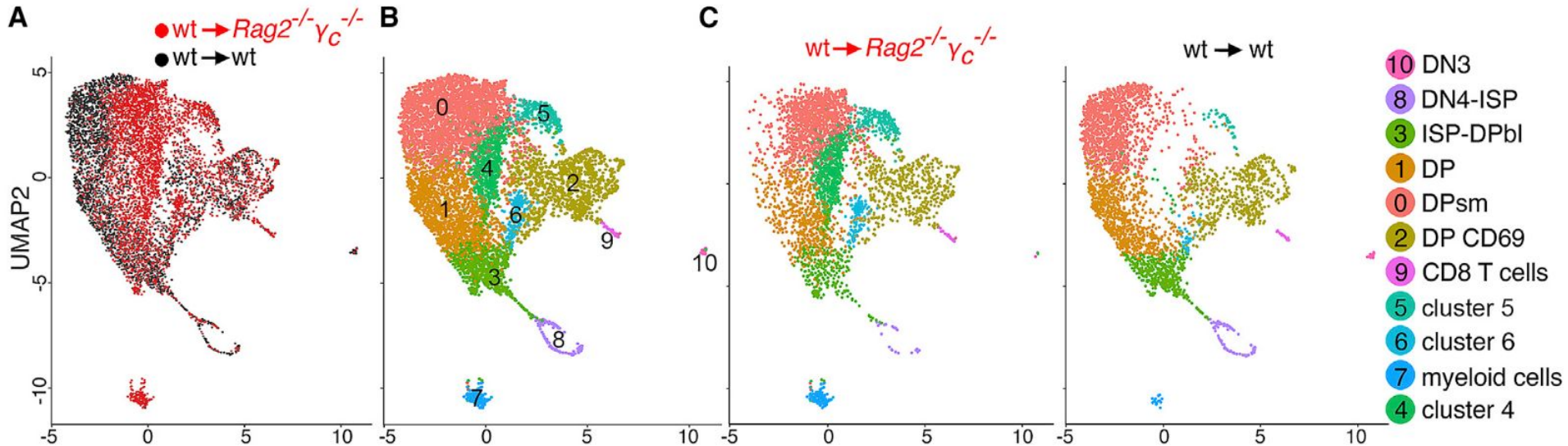


Off-topic : isolated cell size and capillary size



Training course dataset:



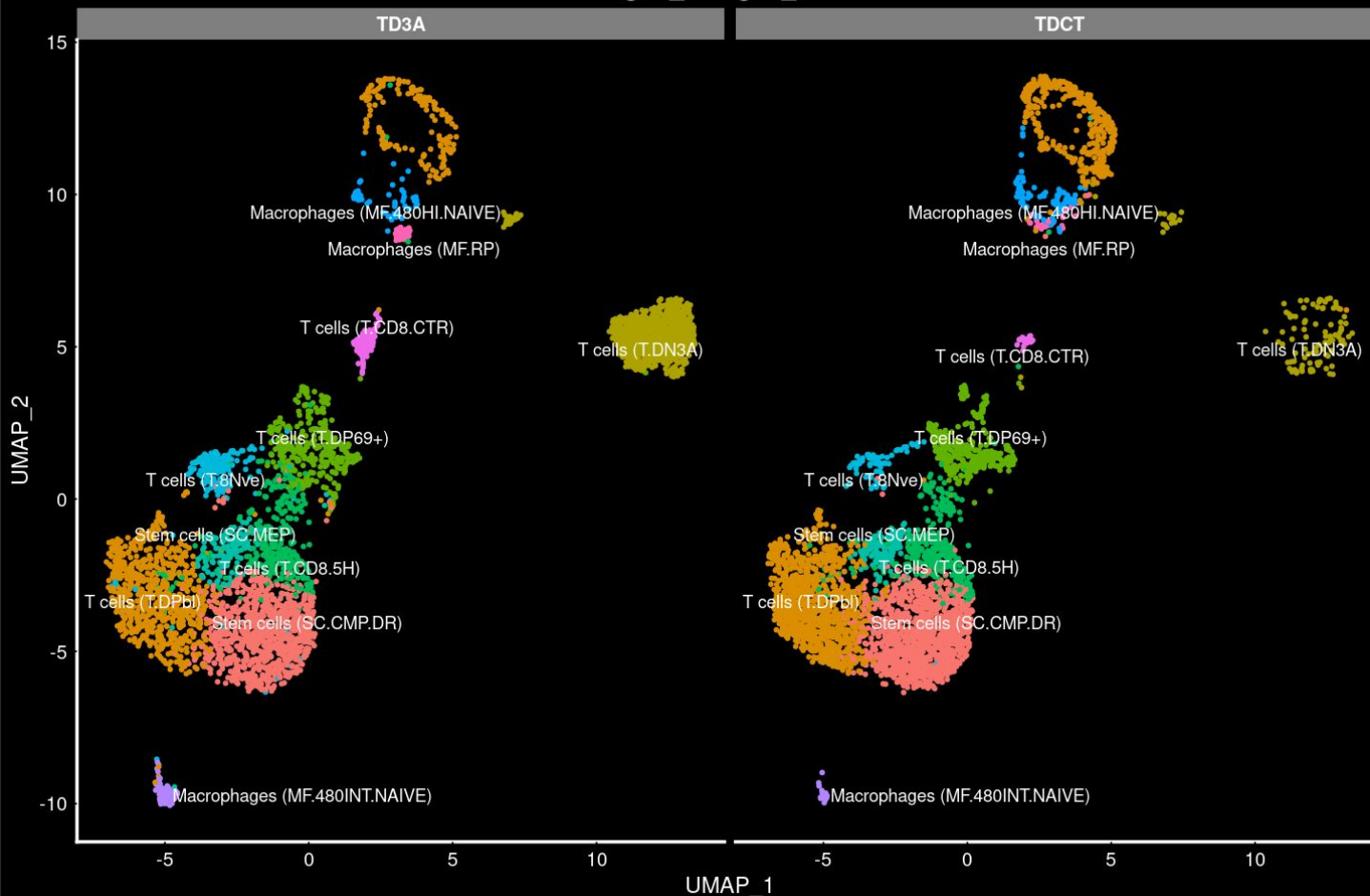


**Integration
 Seurat
 (older)**

Paiva et al, 2021, Cell Reports

<https://doi.org/10.1016/j.celrep.2021.108967>

singler_immgen_clustS

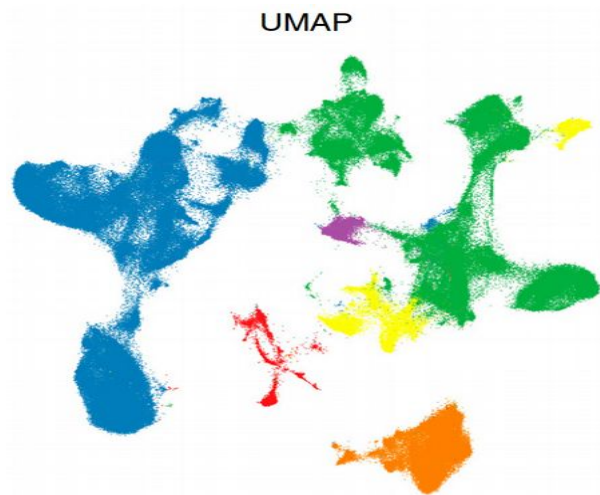


- Stem cells (SC.CMP.DR)
- T cells (T.DPbl)
- T cells (T.DN3A)
- T cells (T.DP69+)
- T cells (T.CD8.5H)
- Stem cells (SC.MEP)
- T cells (T.8Nve)
- Macrophages (MF.480HI.NAIVE)
- Macrophages (MF.480INT.NAIVE)
- T cells (T.CD8.CTR)
- Macrophages (MF.RP)

**Integration
Harmony
(standalone)**

Other types of scRNAseq analysis

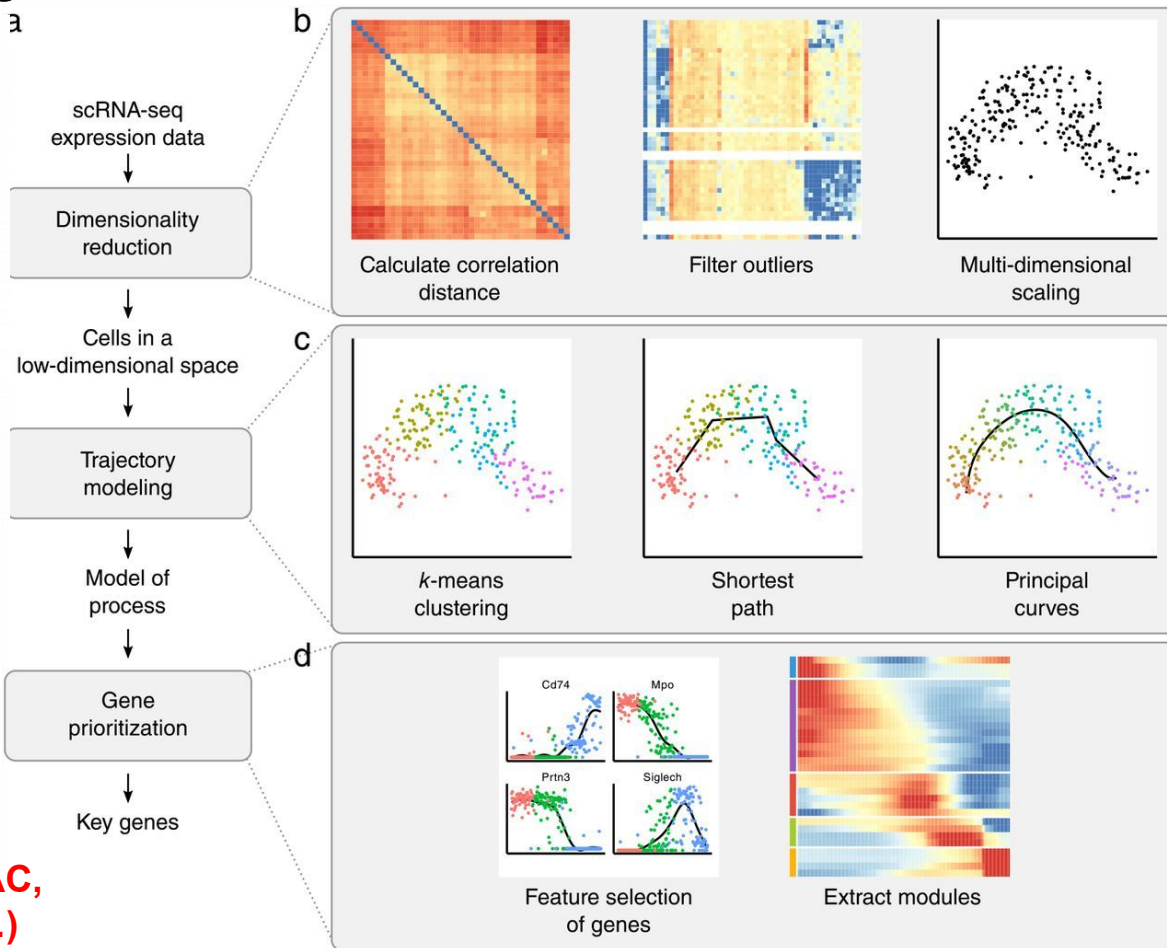
Cell trajectory : methods



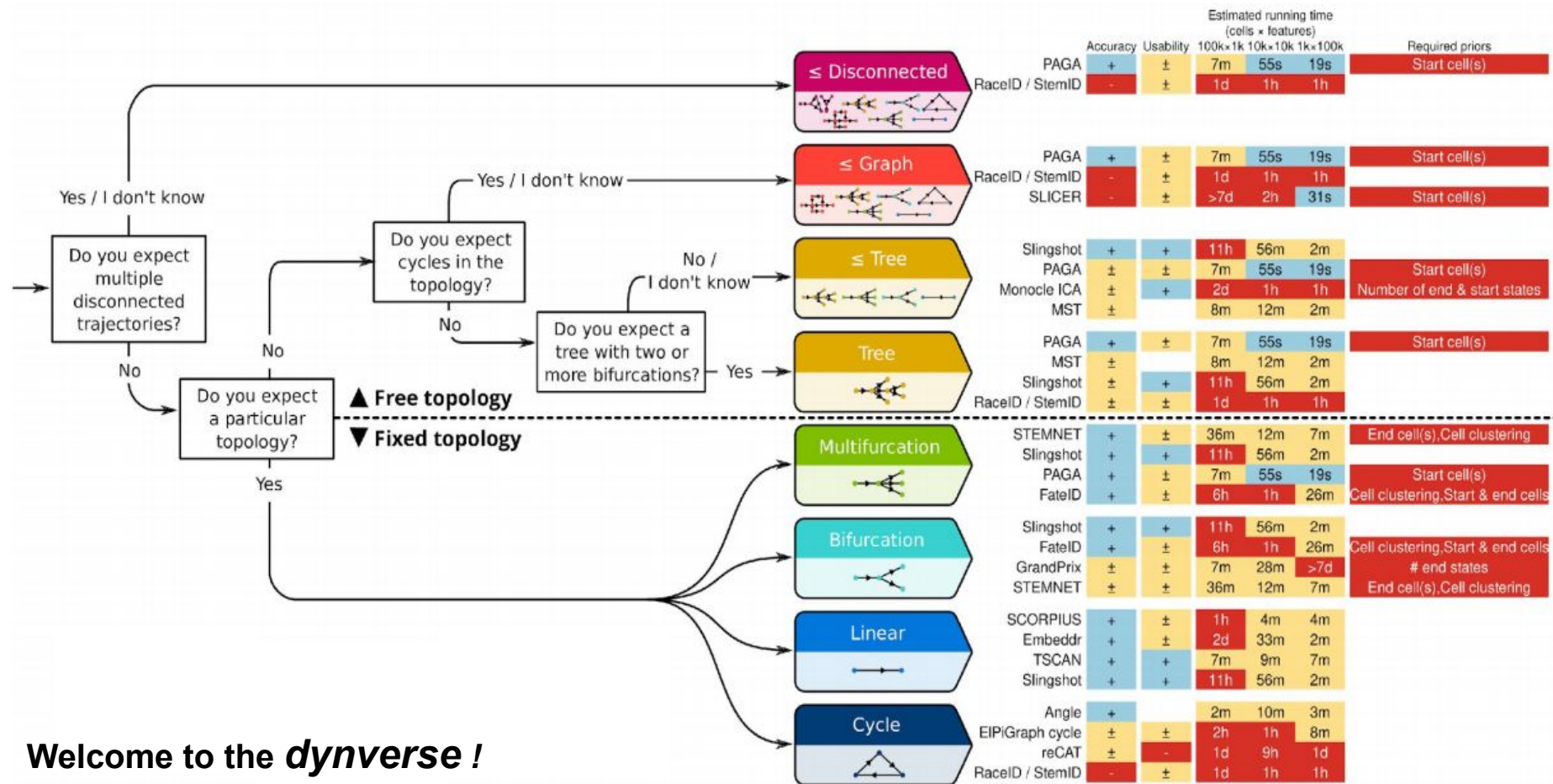
Most adopted tools :

- Monocle 3
- PAGA
- STREAM
- Scorpis
- Slingshot, TinGa, ...

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

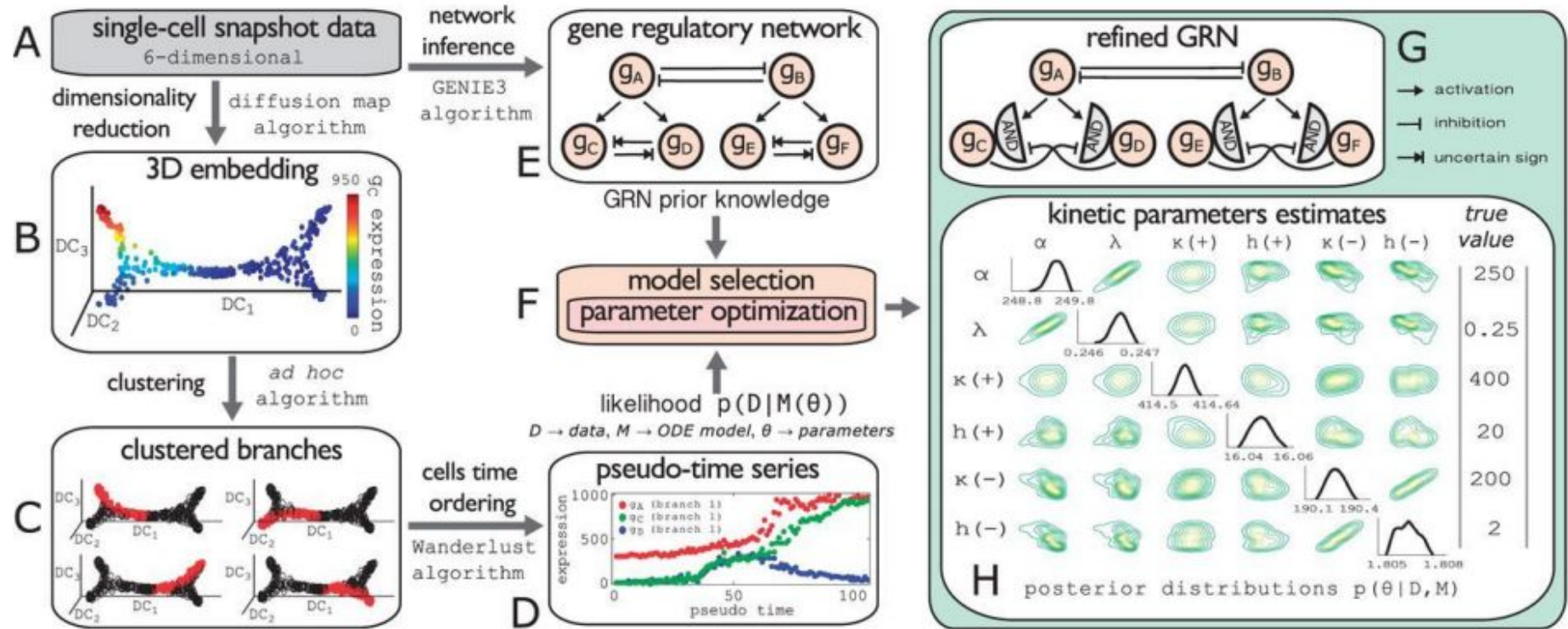


Cell trajectory : Contexts



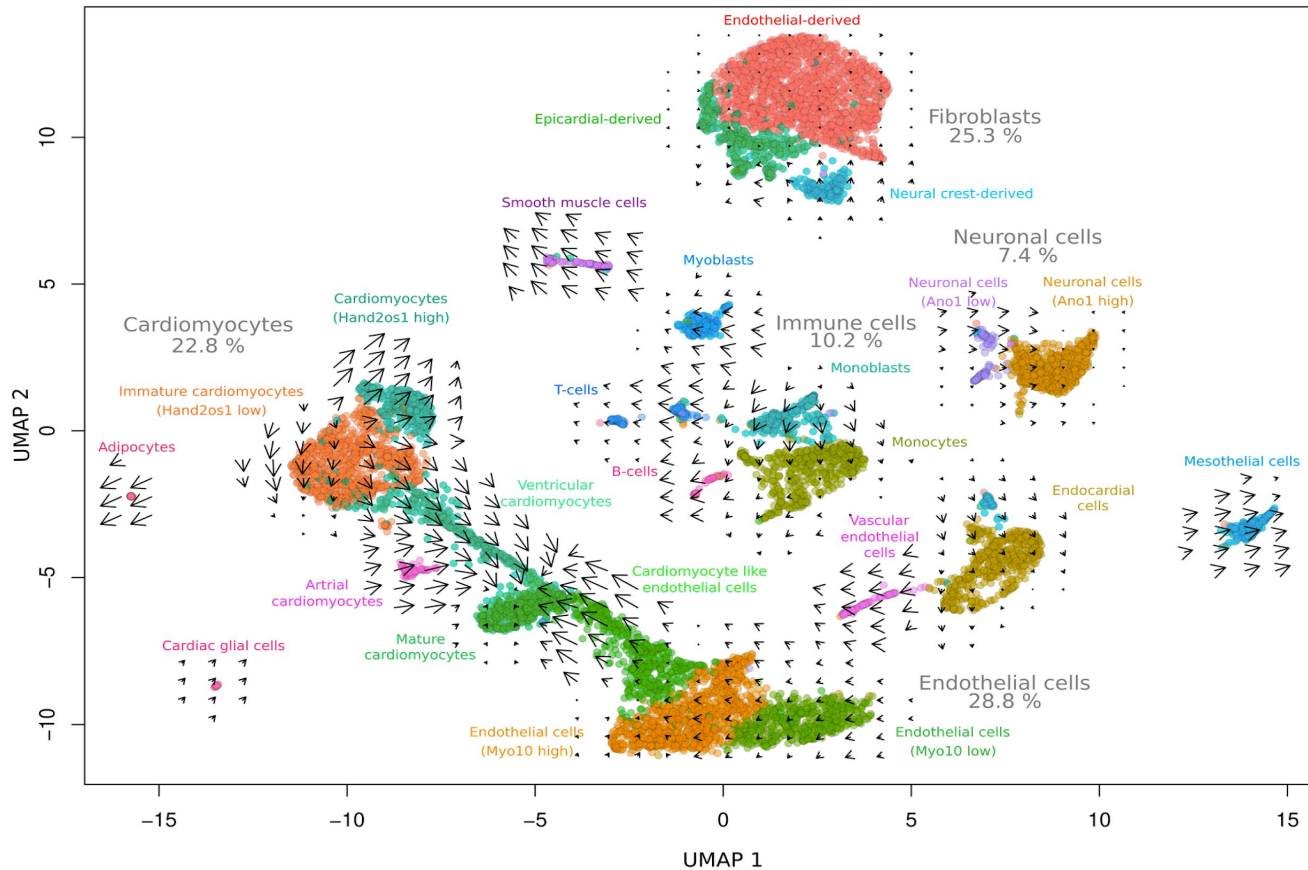
Network inference

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



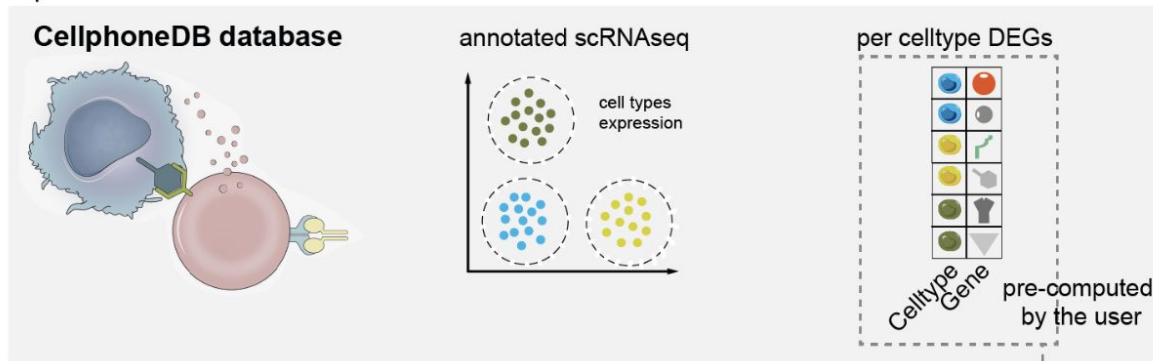
RNA Velocity

Example on an entire mouse heart profile



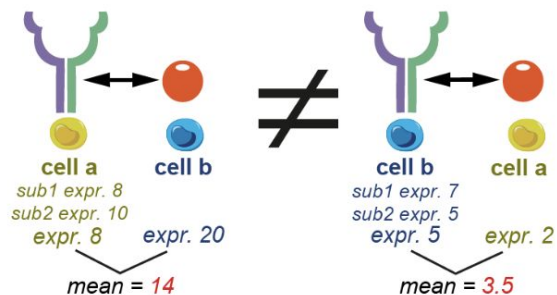
Cell-cell interactions

Input

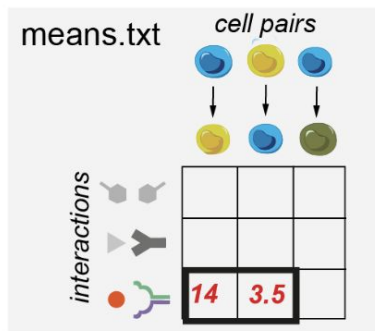


Tools : CellPhoneDB,
CellChatDB,
CellTalkDB ...

How are means computed?



NOTE that interactions are not symmetric



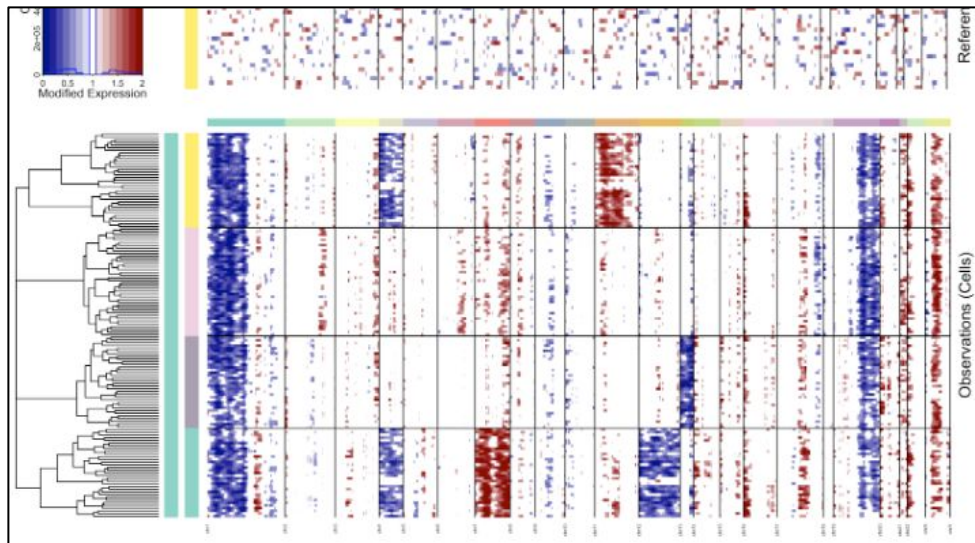
Benchmark :

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

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

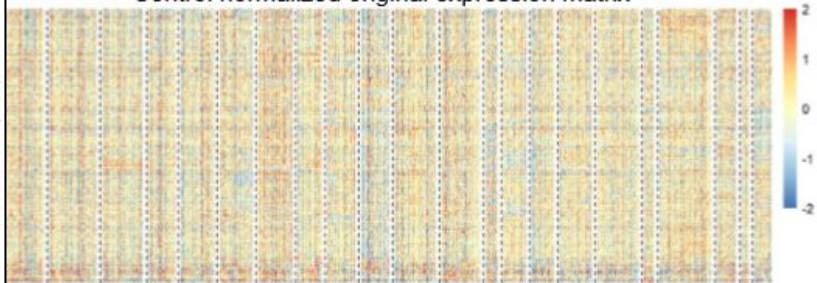
Copy number estimation from scRNASeq

InferCNV (Broad Institute)

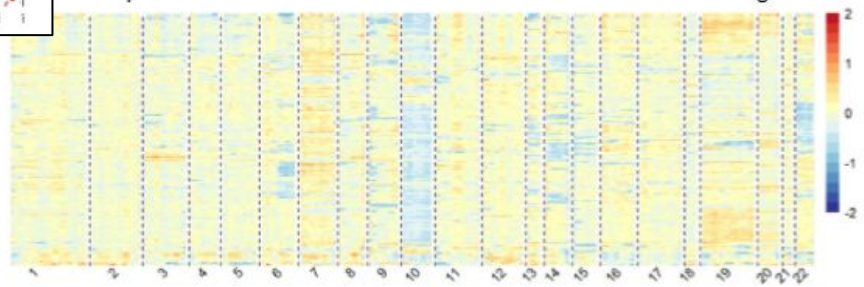


CaSpER (Armanaci et al, BioRxiv 2019)

Control normalized original expression matrix



Expression Matrix after Recursive Multiscale Median Filtering



WARNING :

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

D

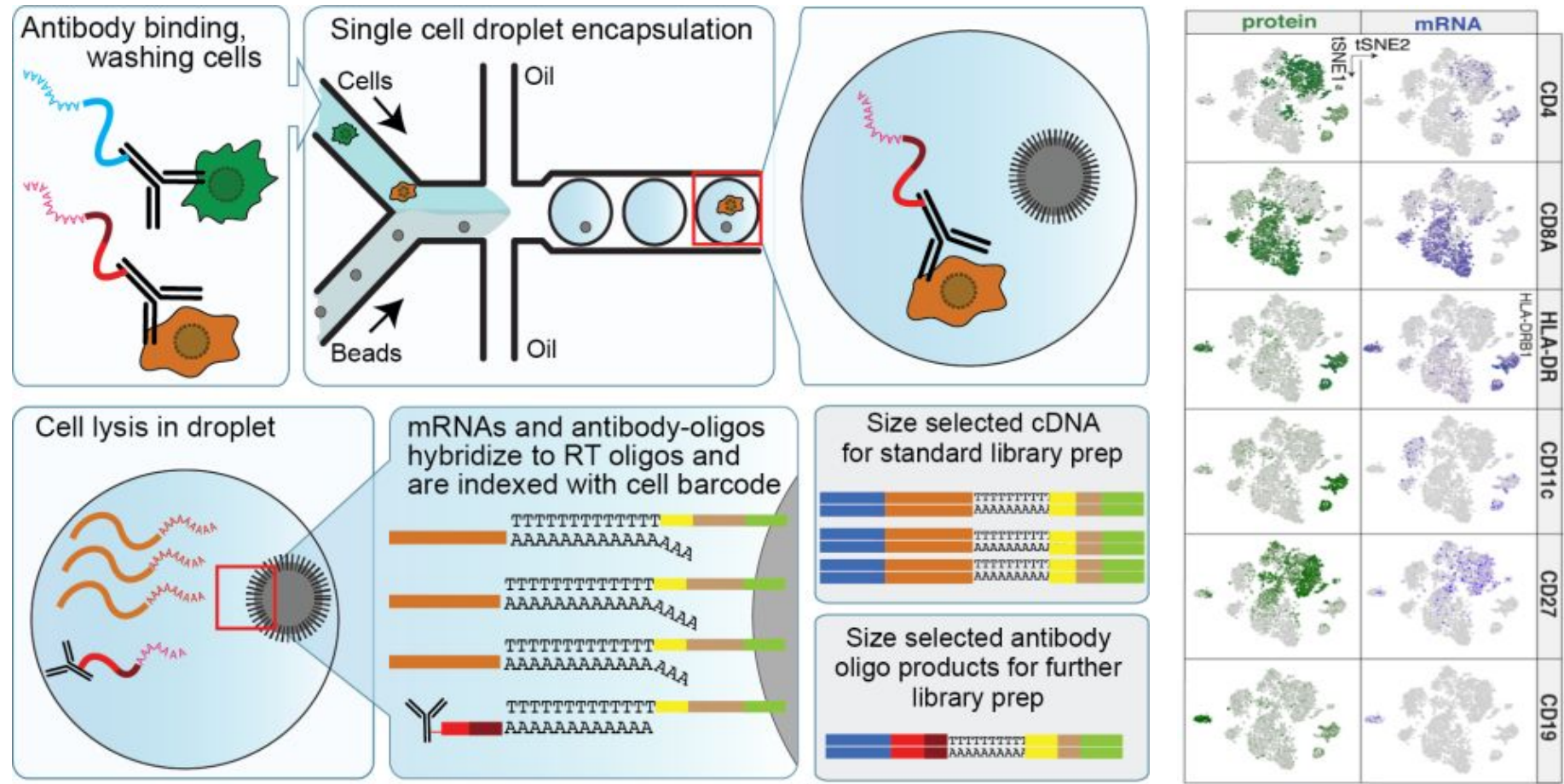
Before BAF shift correction

After BAF shift correction

Technologies

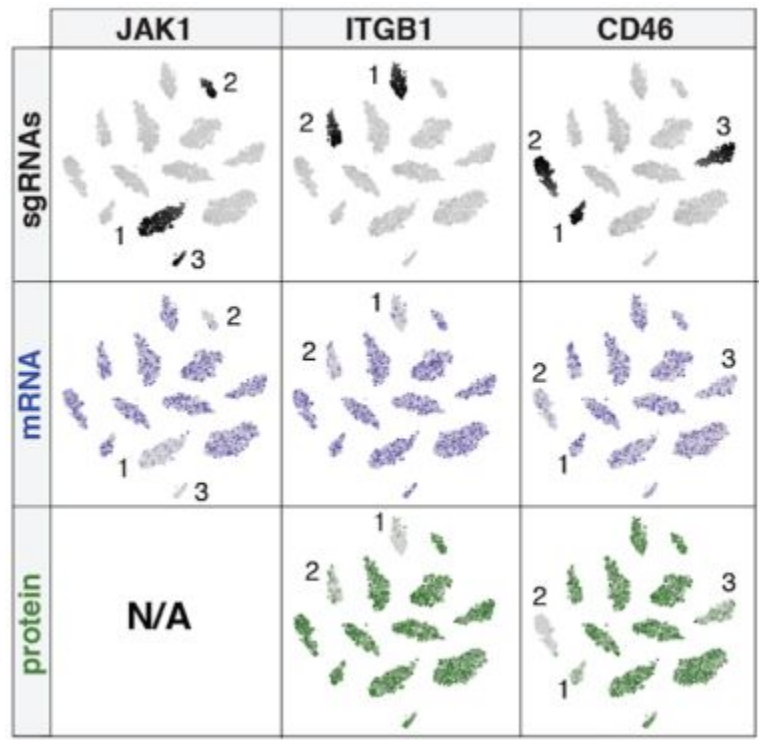
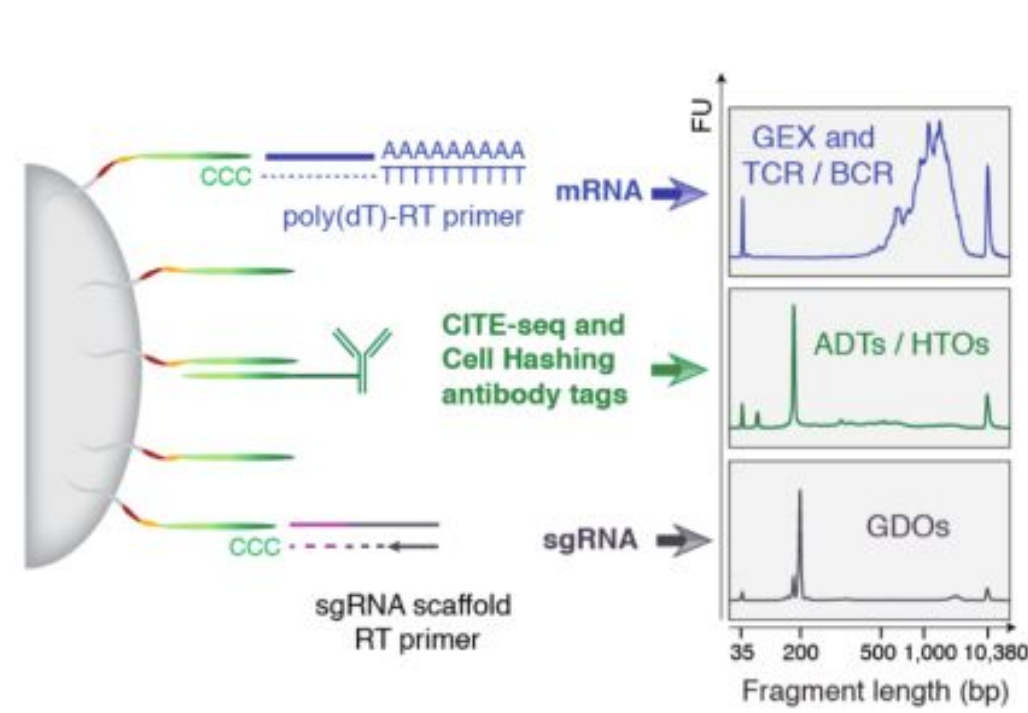
CITE-Seq (+ECCITE-Seq)

Cellular Indexing of Transcriptomes and Epitopes by Sequencing

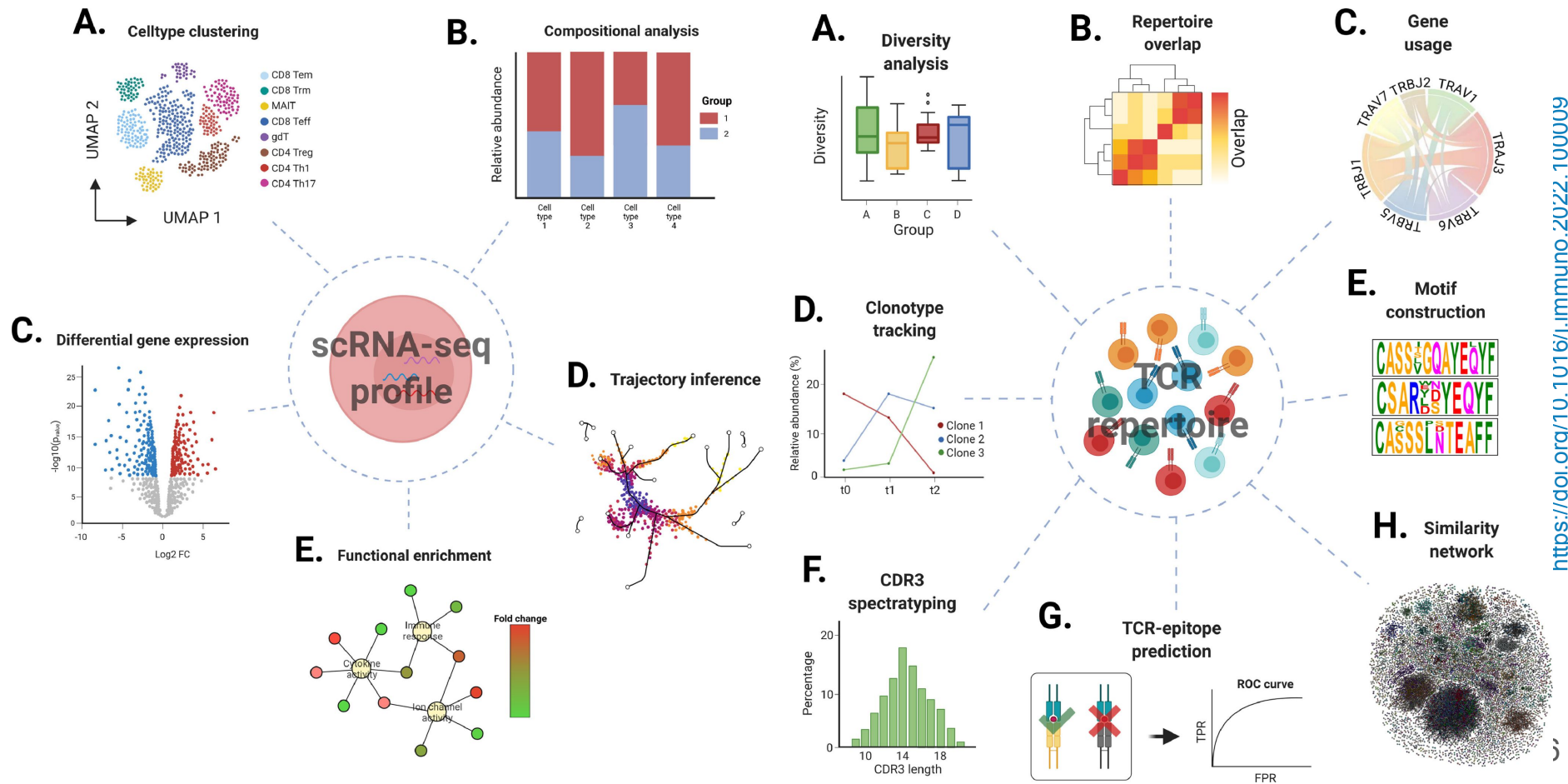


ECCITE-Seq

Extended CRISPR-compatible Cellular Indexing of Transcriptomes and Epitopes by Sequencing (5')



scRNAseq + TCR repertoire



Why so much hype ?

Bulk



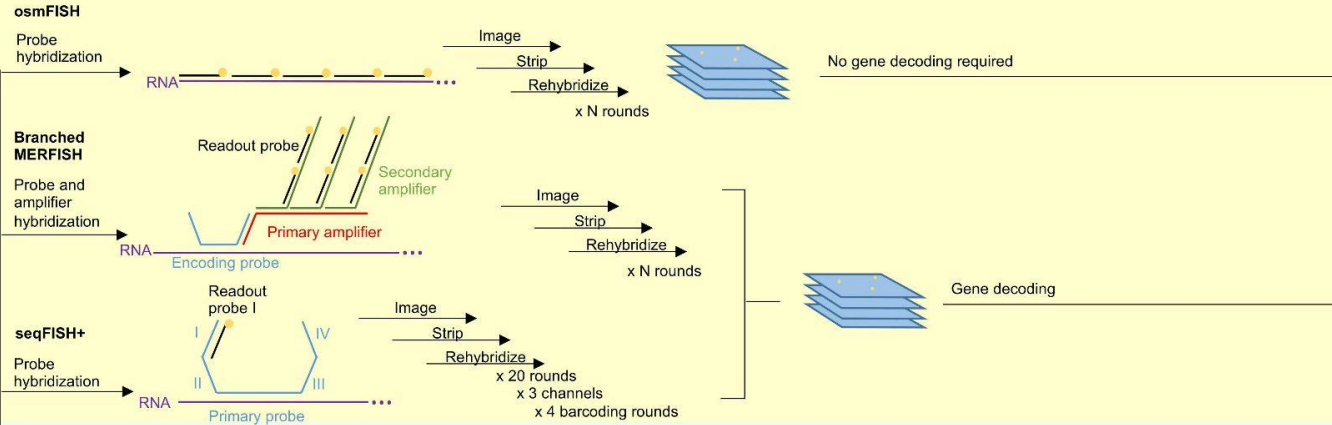
Single cell



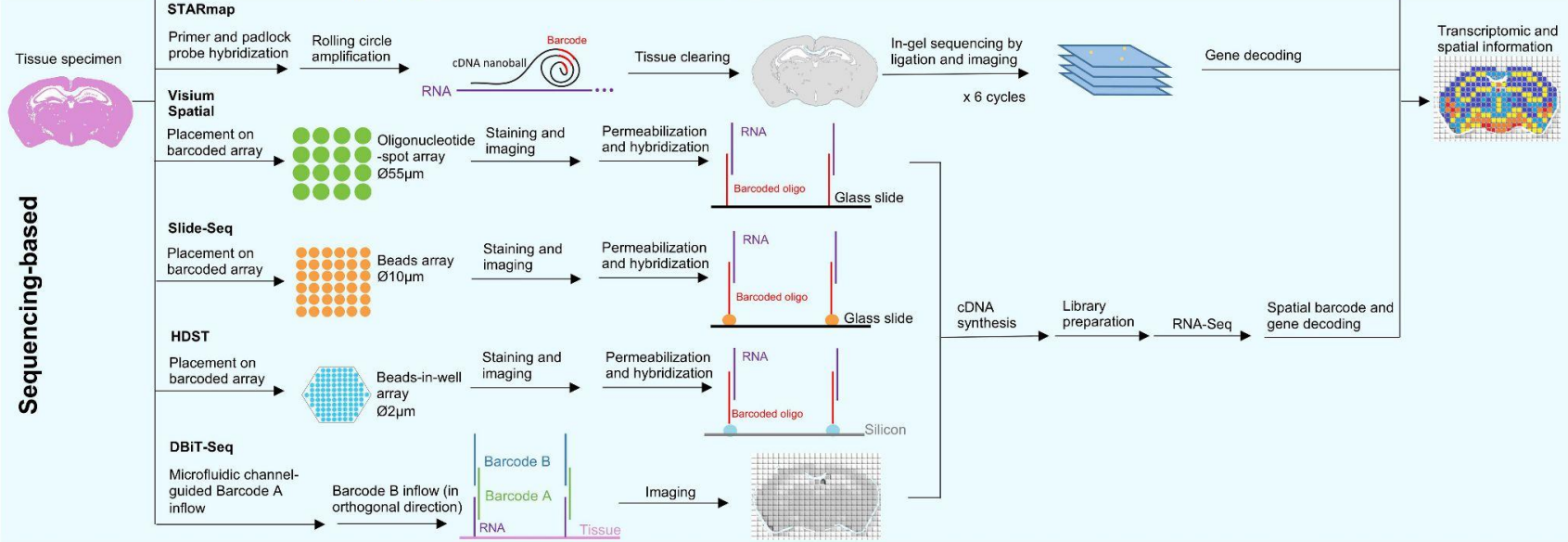
Spatial single cell



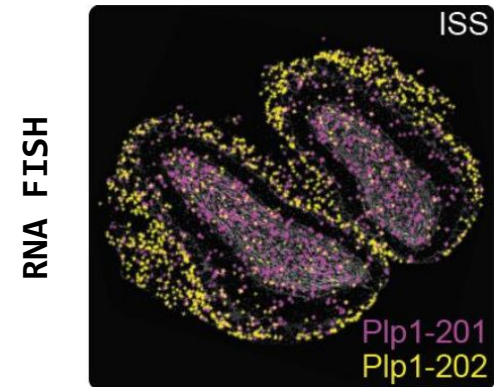
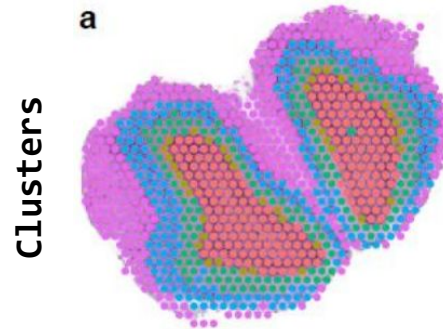
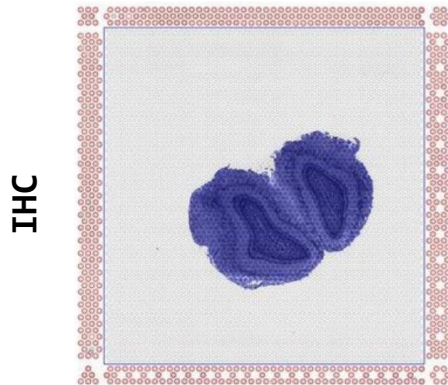
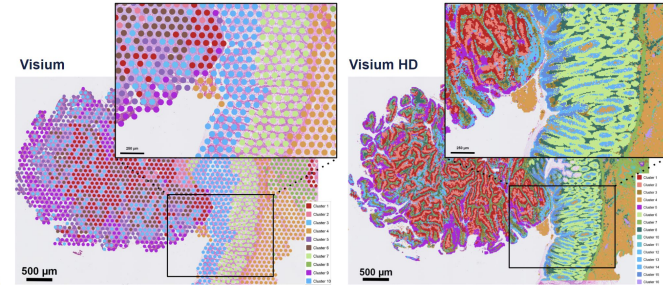
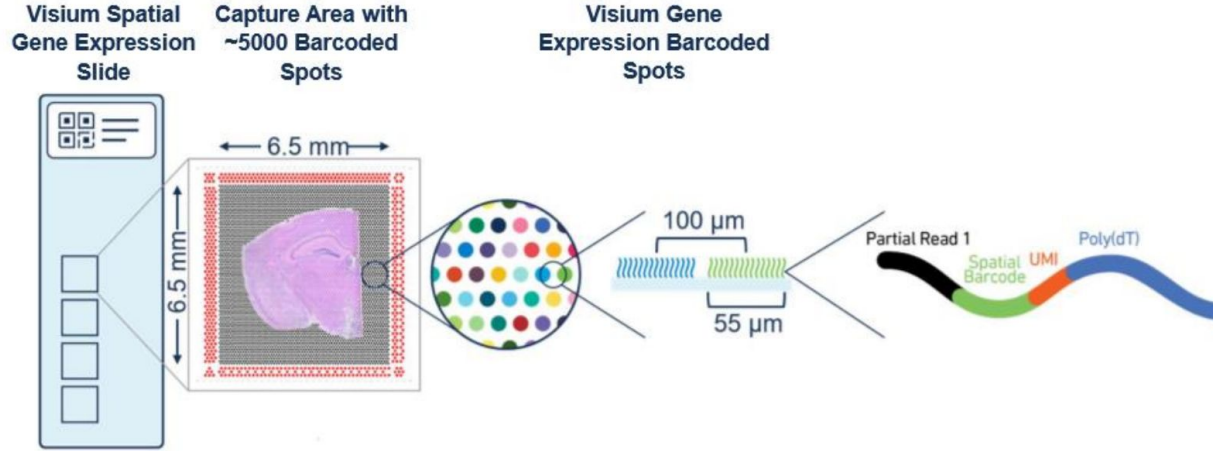
FISH-based



Sequencing-based



Seq-based spatial scRNAseq : 10x Genomics Visium (HD)



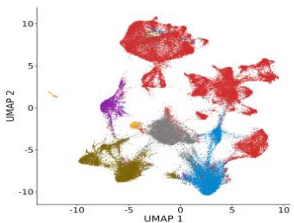
Imagery-based Spatial scRNA expression by imagery : *NanoString CosMx-SMI*



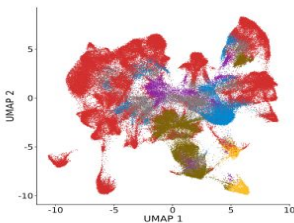
3D view : <https://www.youtube.com/watch?v=LI-gvIribDU&t=14s>

Cell Segmentation as the one point to improve

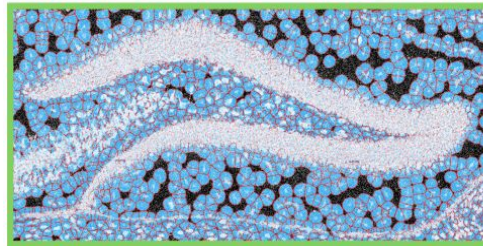
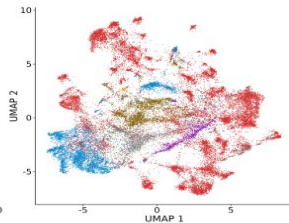
MERSCOPE



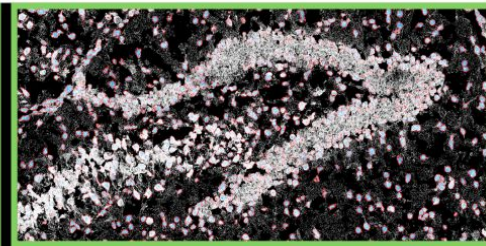
Xenium



Molecular Cartography

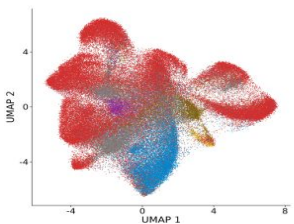


Xenium

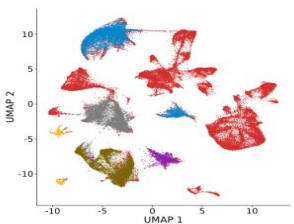


MERSCOPE

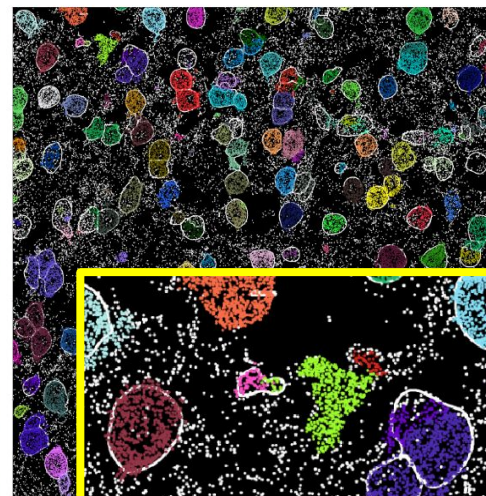
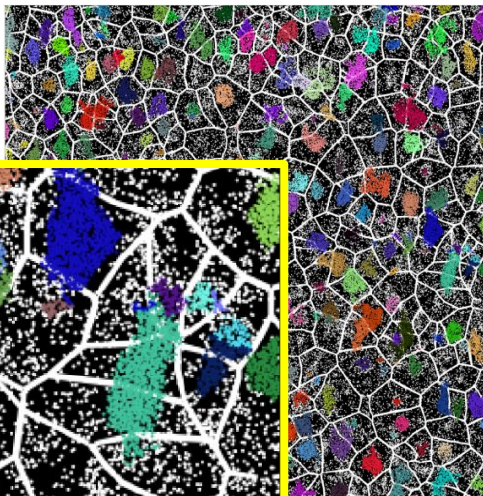
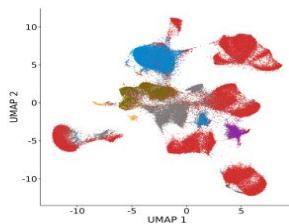
EEL FISH



MERFISH



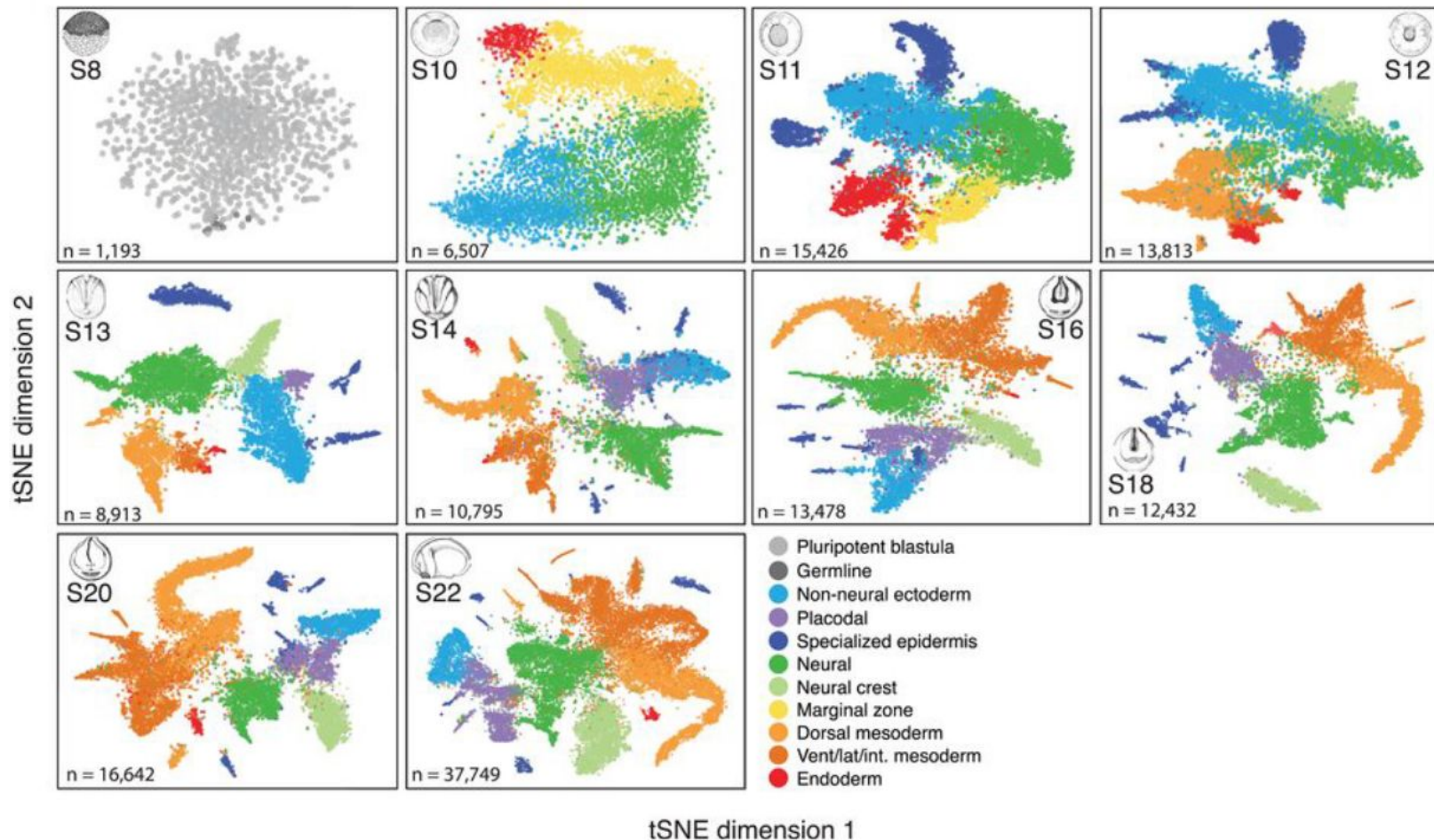
STARmap PLUS



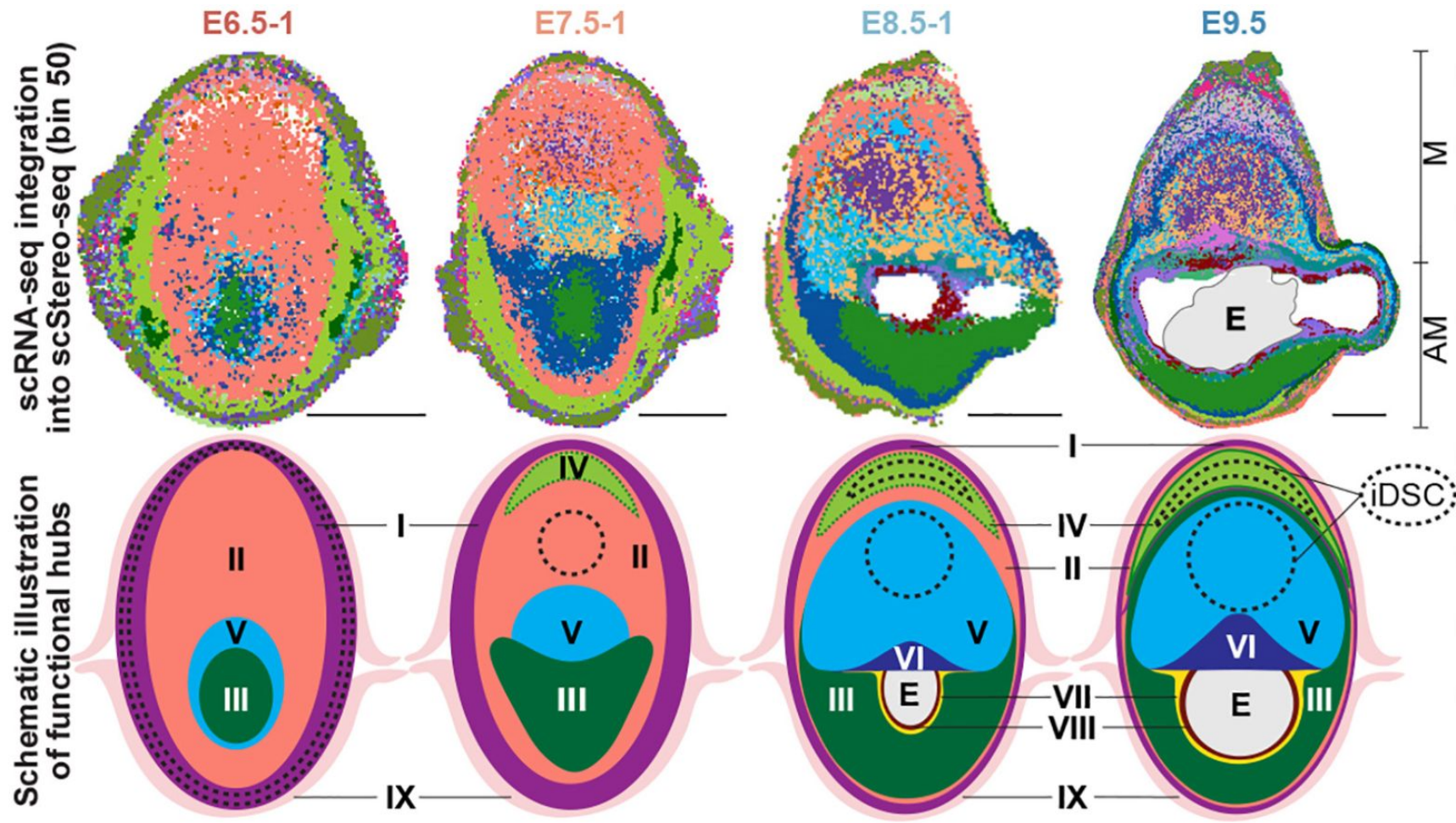
Some sweets



Xenopus embryo development



Spatial SC : Polar differentiation of mouse uterus

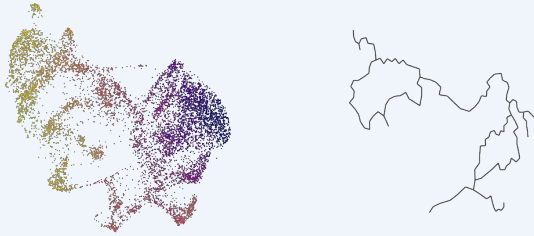




Vertebrate embryonic neural tube development



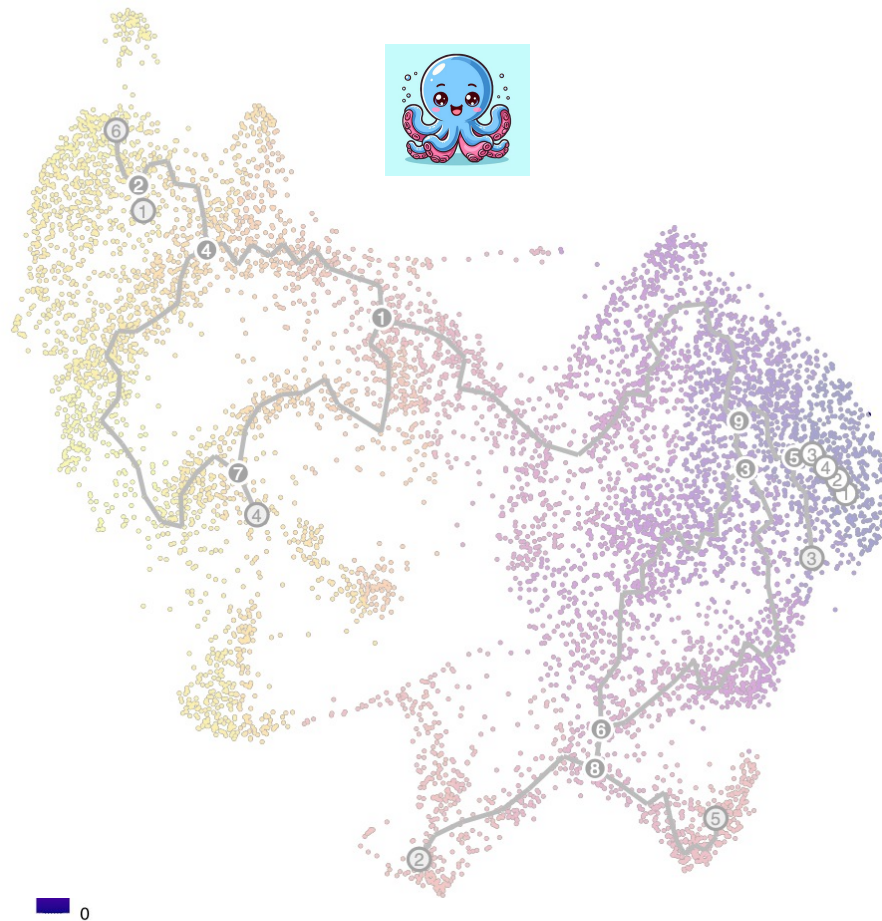
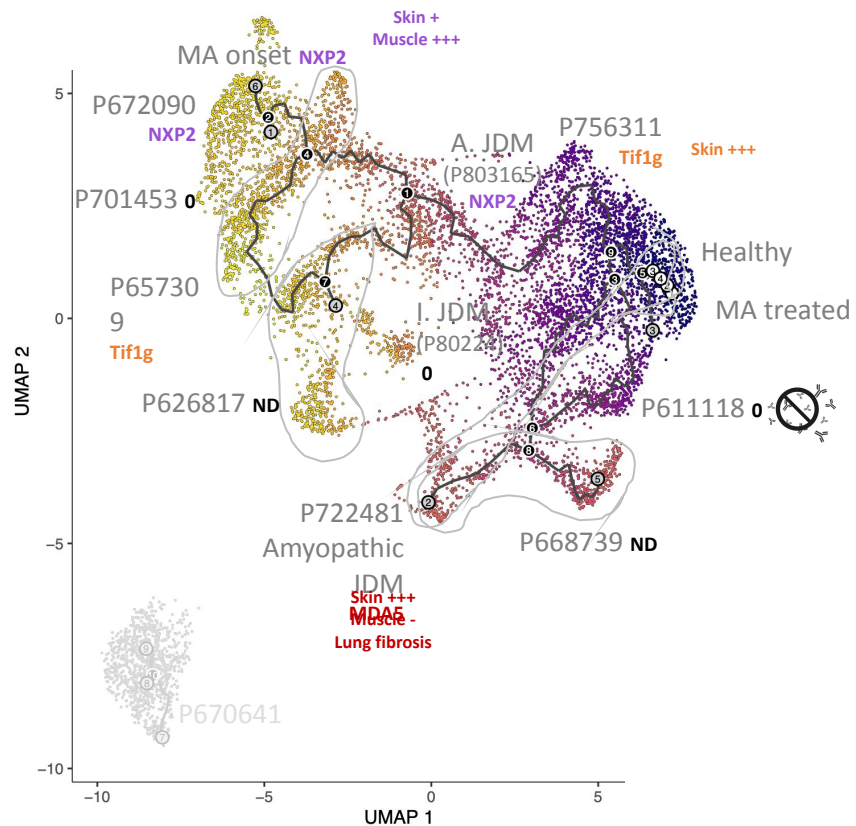
Trajectories and pseudotime with Monocle3



- **Learning** gene expression changes **trajectories**:
 - Reversed Graph Embedding
 - Lineage structure using Minimum spanning tree
- **Pseudotime**: quantification evolution along a processus/trajectory
- Pseudotime supervised or unsupervised: first cluster?

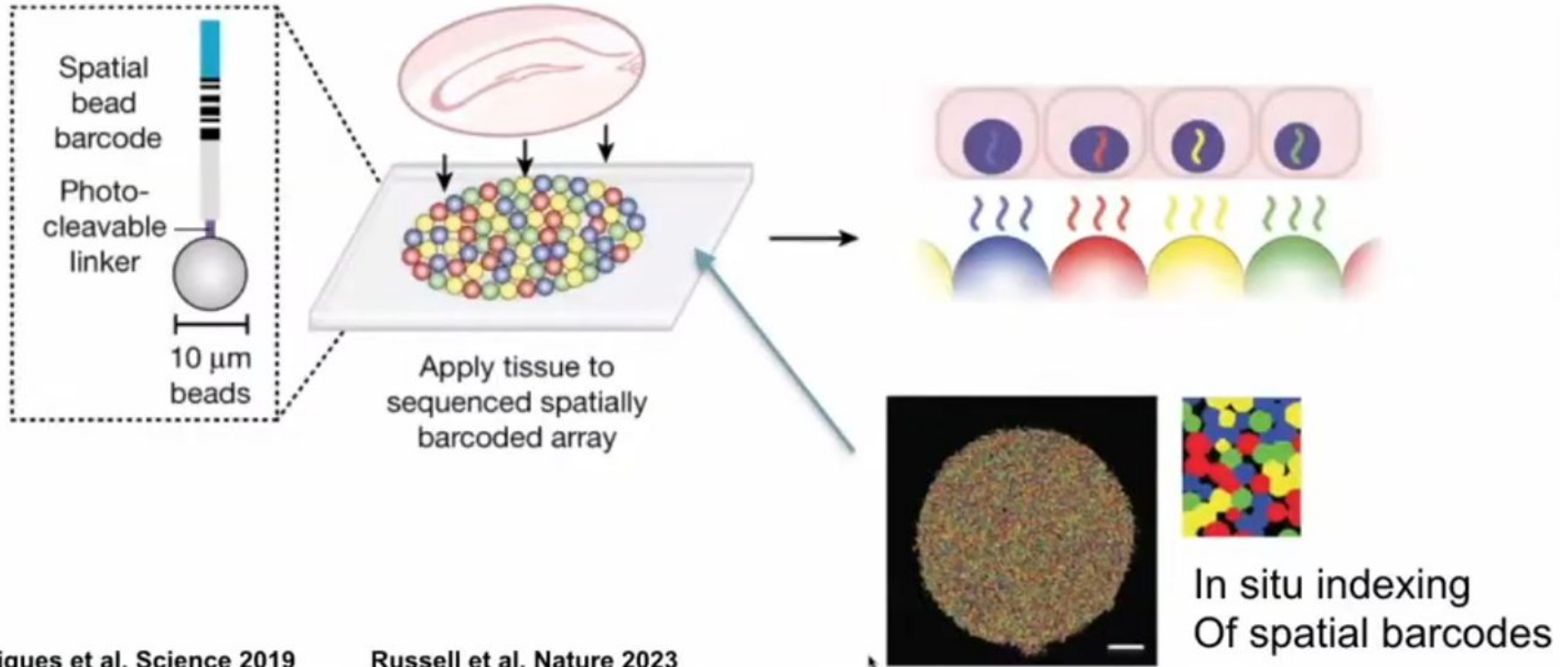


UMAP representation of 14 biopsies



Spatial single cell, without imagery nor spots !

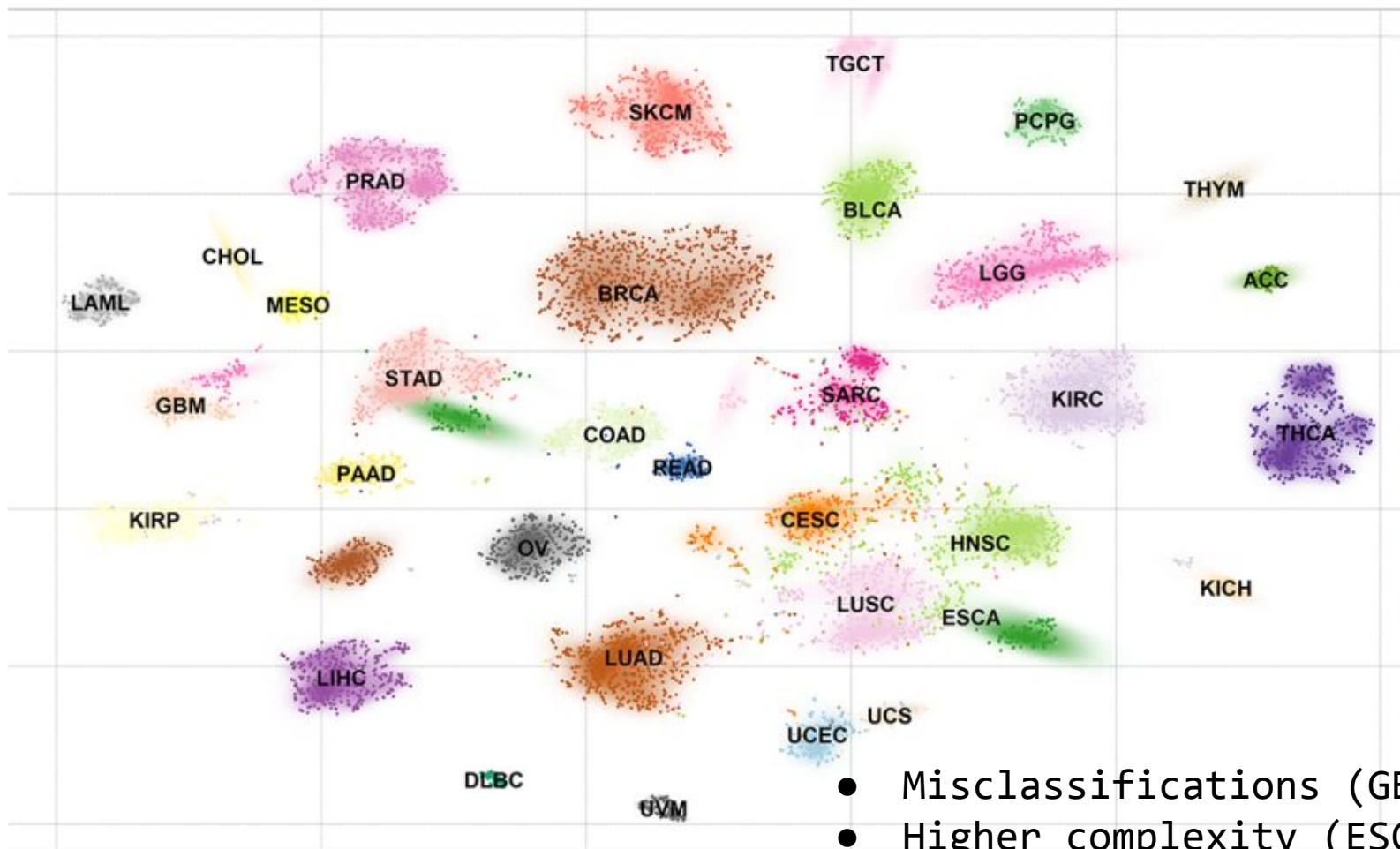
Slide-Seq, Slide-Tags



Rodrigues et al, Science 2019

Russell et al, Nature 2023

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



***Thanks and congratulations
from your devoted team***



... and you !

Lucie Khamvongsa
Morgane Thomas-Chollier
Margot Tragin
Audrey Onfroy
Lilia Younsi
William Jarassier
Bastien Job
Sébastien Mella

& our alumni



SINGLE-cellING IN THE RAInaseq (1952)*

* play on words courtesy of
Jacques van Helden



Yearly talks by Raul Satija on single cell novelties
<https://www.youtube.com/@satijalab3189>

Single cell results and the community

TYPES OF SINGLE-CELL SEQUENCING PAPER

Overclustering the data reveals unexpected levels of cellular heterogeneity



We disproved the null hypothesis that the human brain consists of a single cell type



The data made more sense when we averaged all the cells together



We're not sure why our RNA velocity arrows are pointing in the wrong direction either



We ran imputation without telling anyone, but look how nice our heatmaps look now!



In retrospect, we could probably have just used immunofluorescence



I alone can normalize your scRNA-seq data



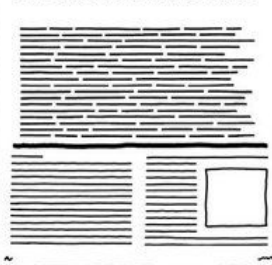
It turns out that spatial transcriptomics brings us no closer to actually curing disease



Prepare to spend your weekend searching for our count matrices



No, its not suspicious that our benchmarking study highlighted that our method is the best



Our new algorithm solves all your analysis problems, or at least it would if we made the code available



We don't have replicates but its OK since we analyzed the data with a neural network

