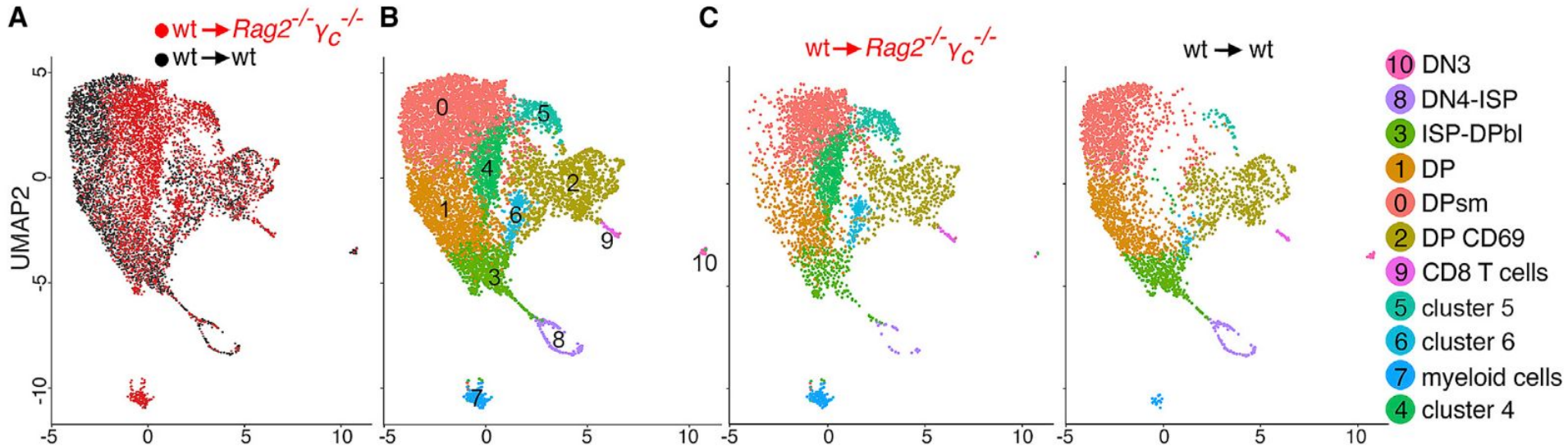


Single Cell RNAseq Analysis *... beyond !*

Training course dataset:



THE END ?

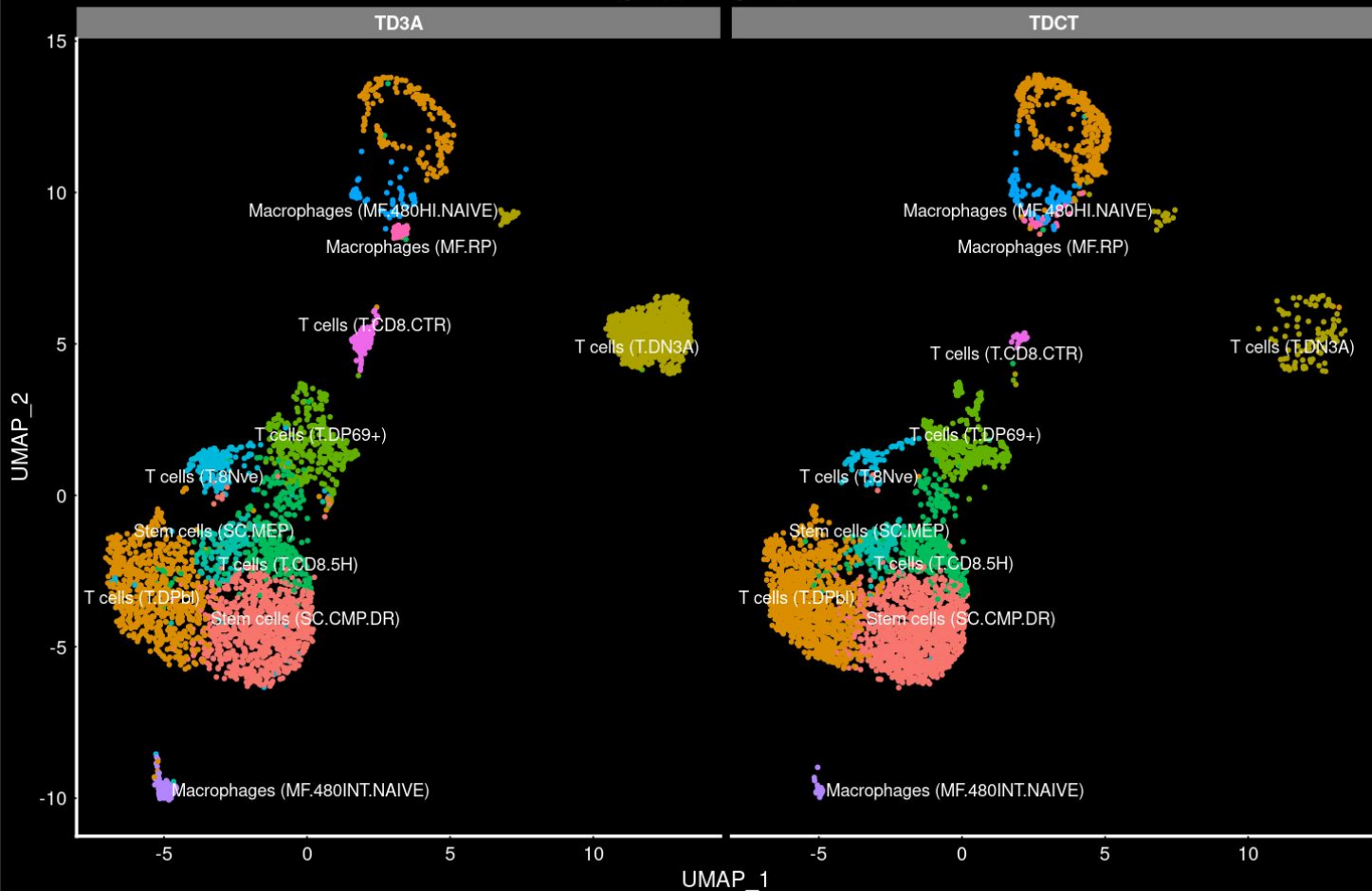


Paiva et al, 2021, Cell Reports

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

**Integration
 Seurat
 (older)**

singler_immgen_clustS

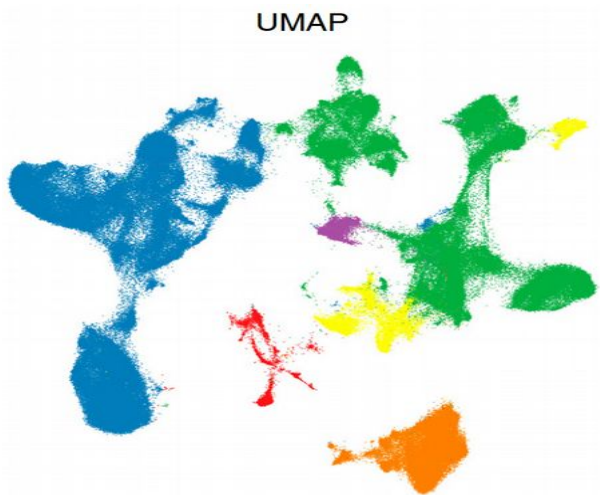


- Stem cells (SC.CMP.DR)
- T cells (T.DPbl)
- T cells (T.DN3A)
- T cells (T.DP69+)
- T cells (T.CD8.5H)
- Stem cells (SC.MEP)
- T cells (T.8Nve)
- Macrophages (MF.480HI.NAIVE)
- Macrophages (MF.480INT.NAIVE)
- T cells (T.CD8.CTR)
- Macrophages (MF.RP)

**Integration
Harmony
(standalone)**

Other types of scRNAseq analysis

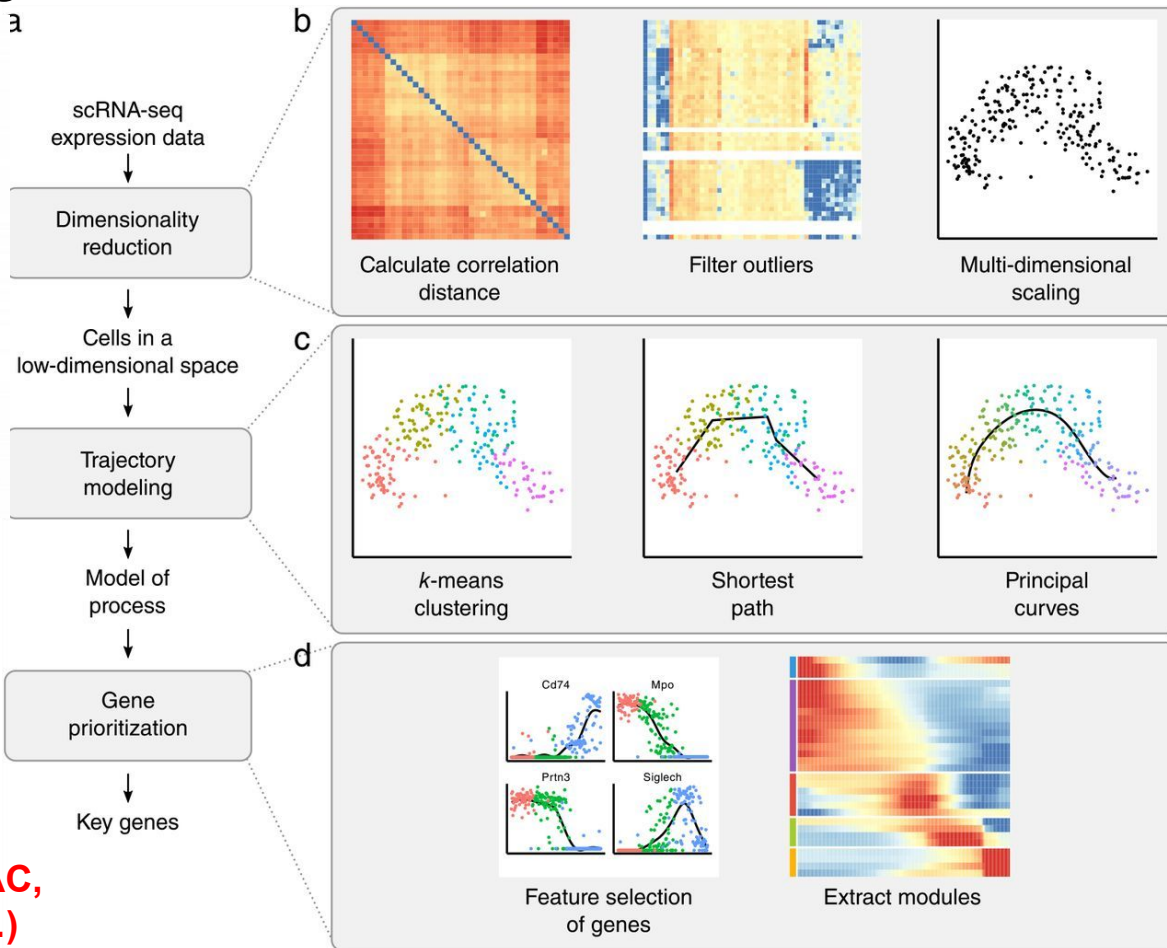
Cell trajectory : methods



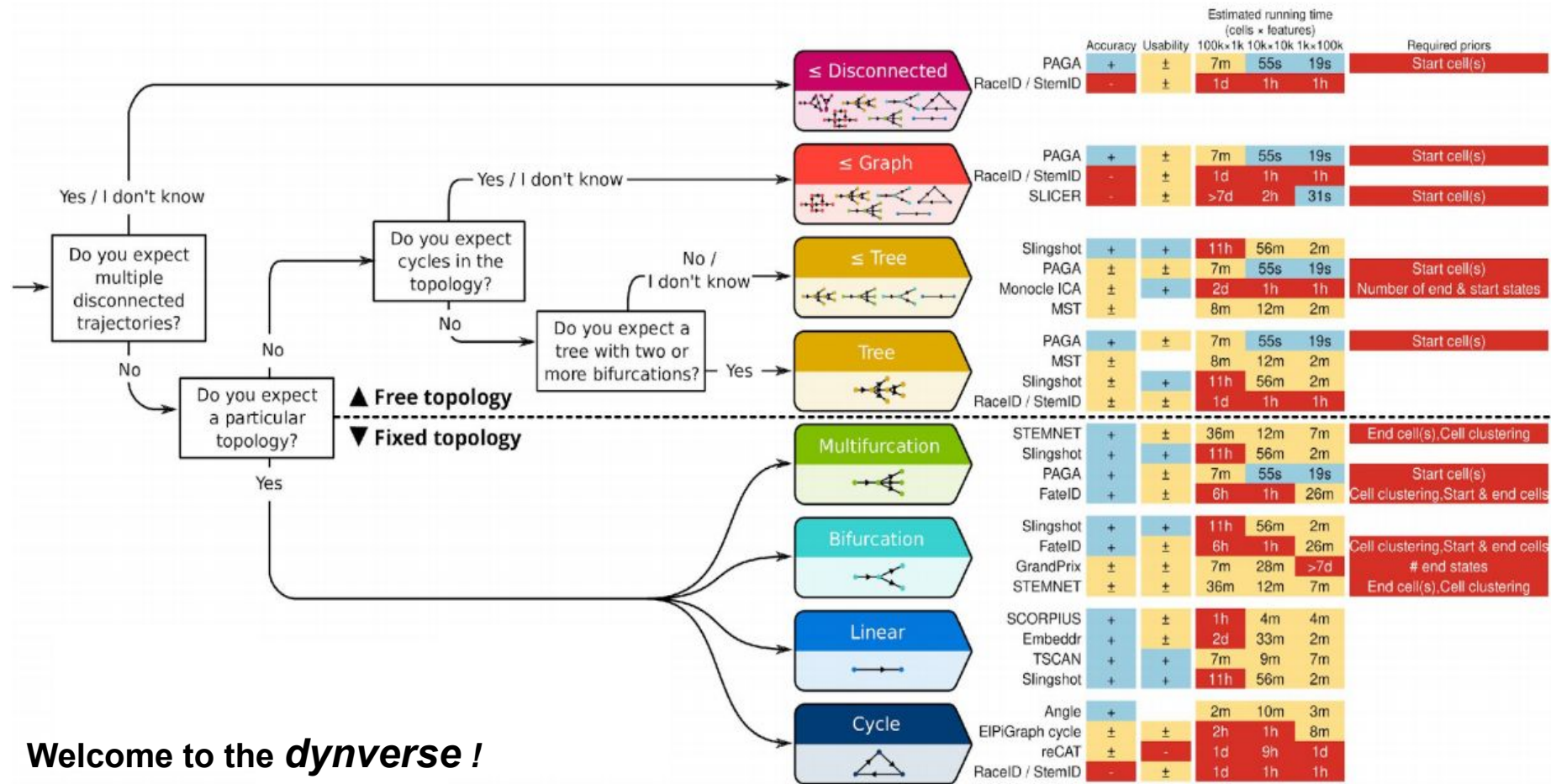
Most adopted tools :

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

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



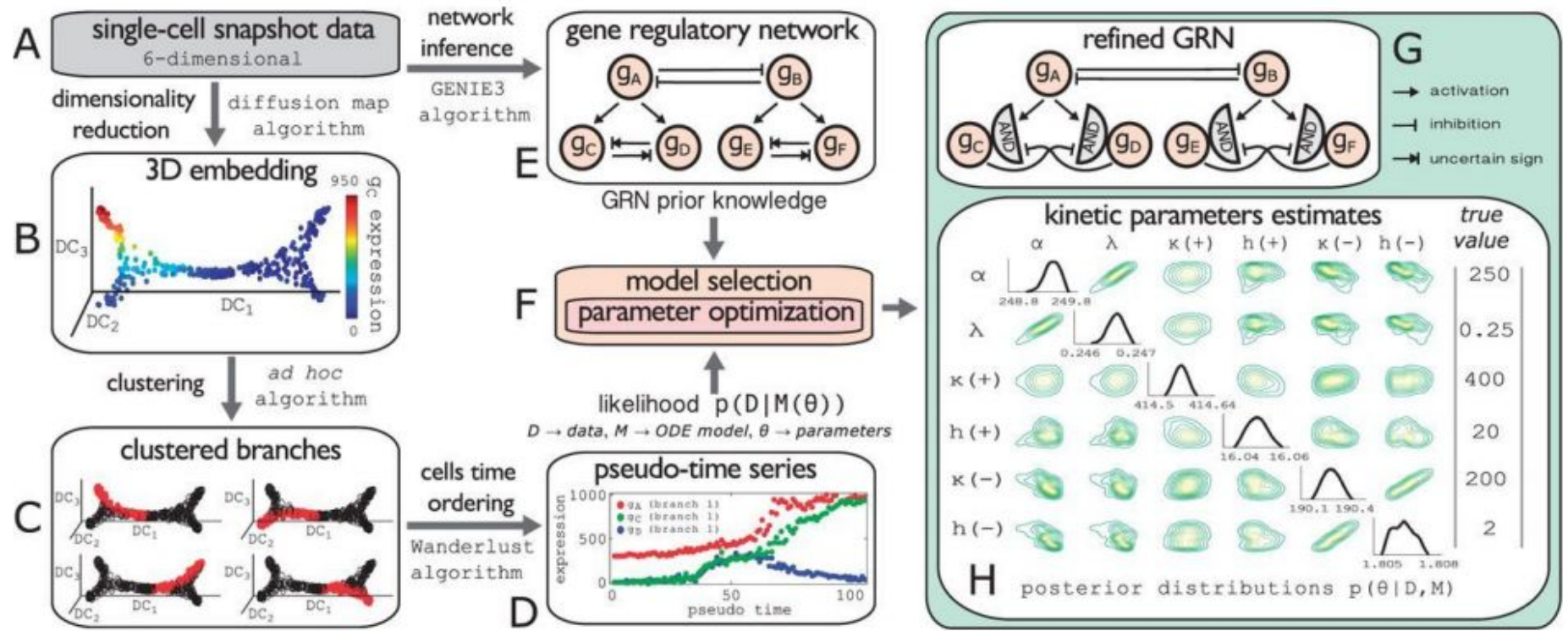
Cell trajectory : Contexts



Welcome to the *dynverse* !

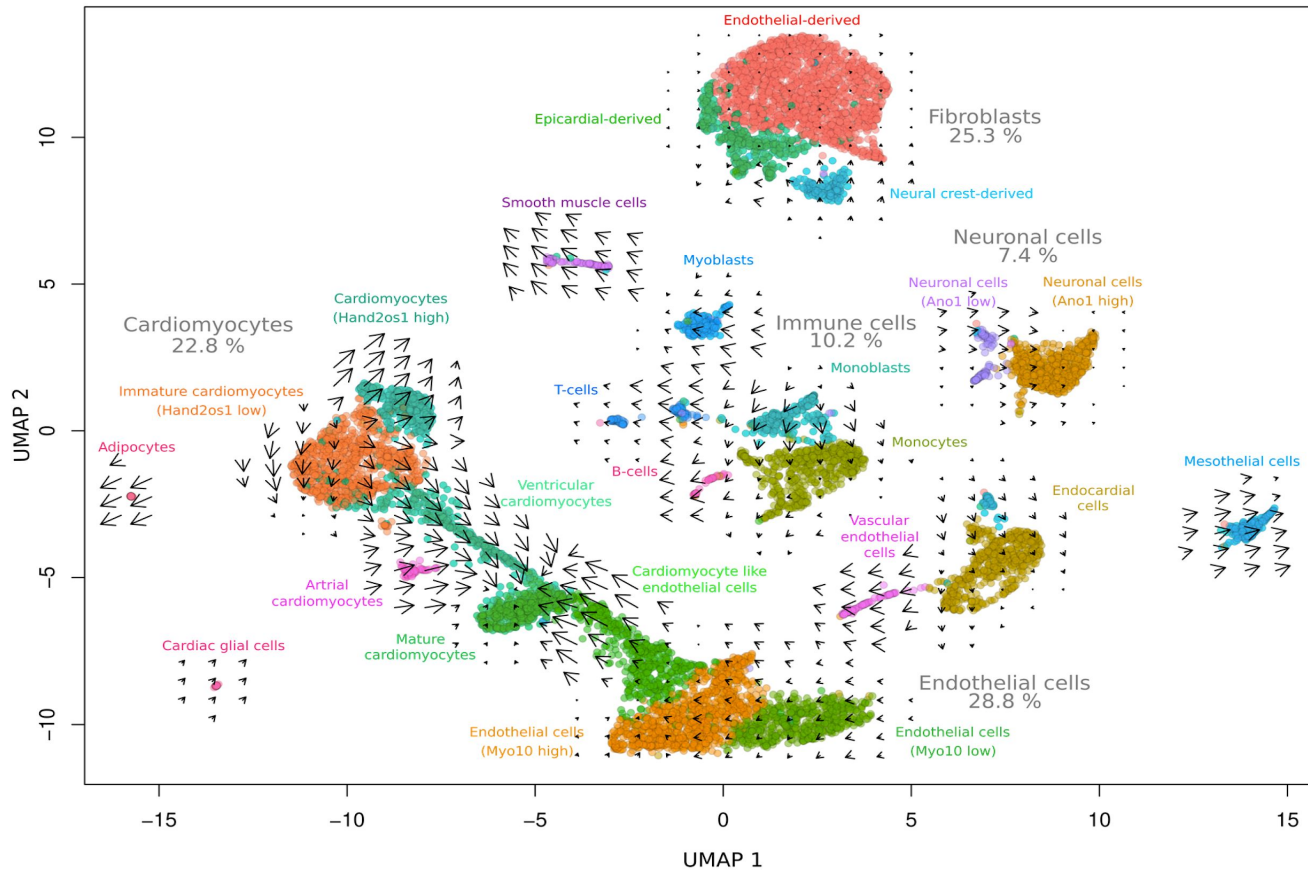
Network inference

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



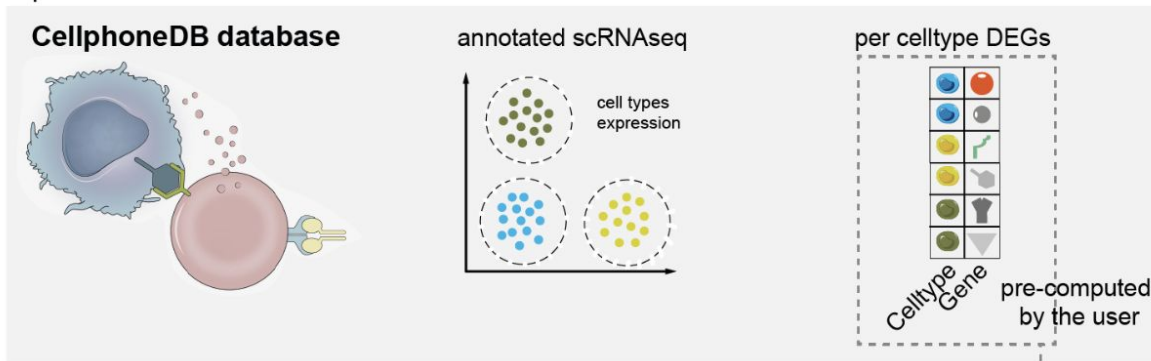
RNA Velocity

Example on an entire mouse heart profile



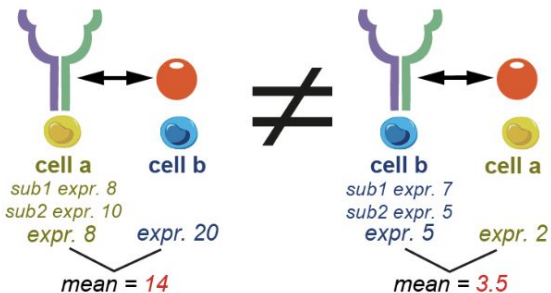
Cell-cell interactions

Input

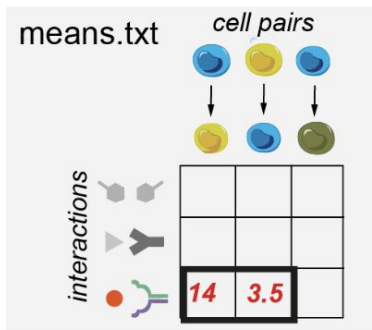


Tools : CellPhoneDB,
CellChatDB,
CellTalkDB ...

How are means computed?



NOTE that interactions are not symmetric



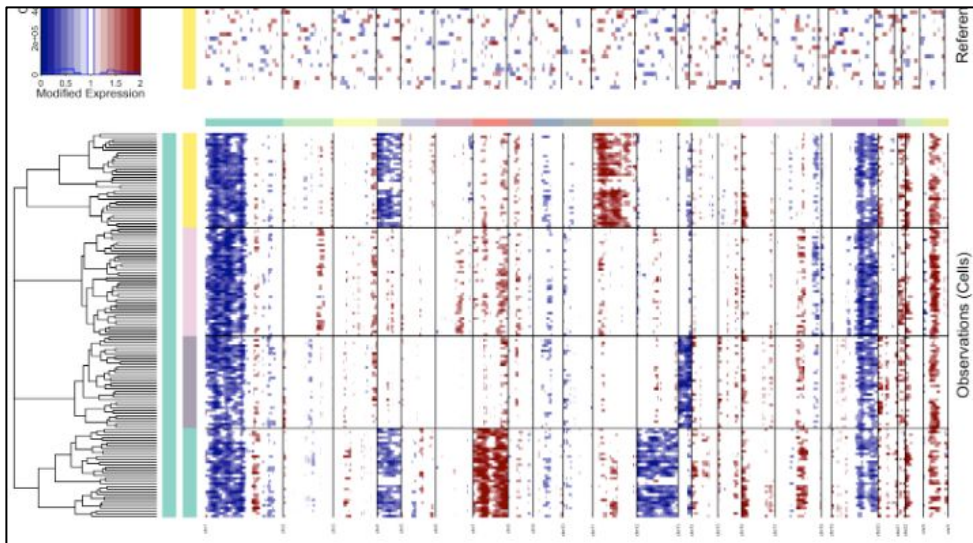
Benchmark :

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

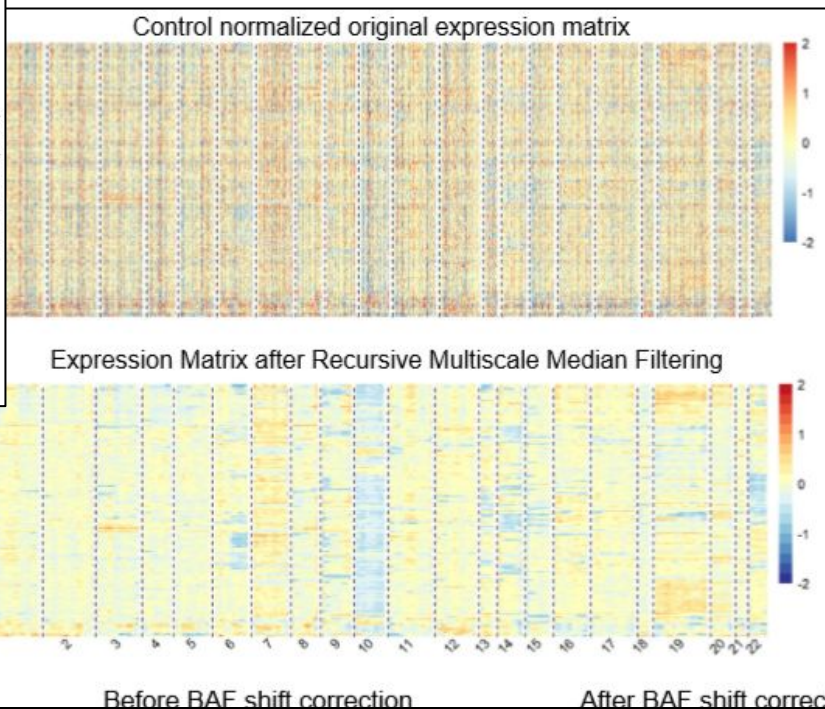
Figures from : <https://cellphonedb.readthedocs.io/>

Copy number estimation from scRNAseq

InferCNV (Broad Institute)



CaSpER (Armanci et al, BioRxiv 2019)



WARNING :

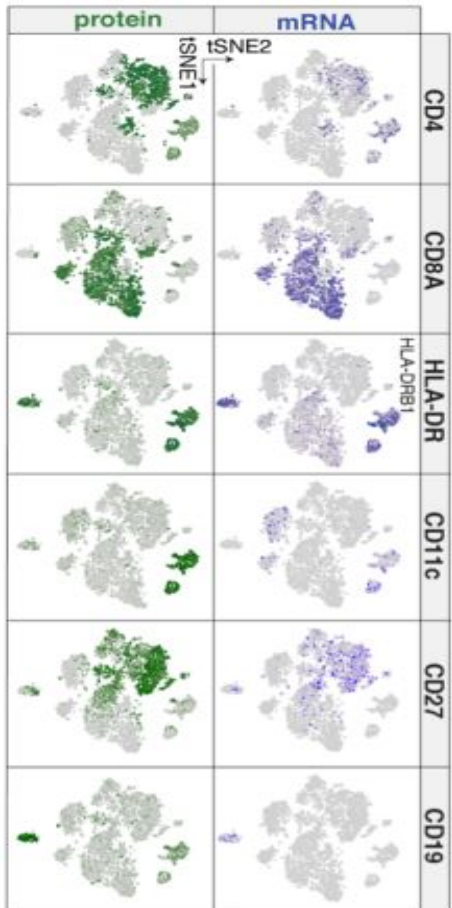
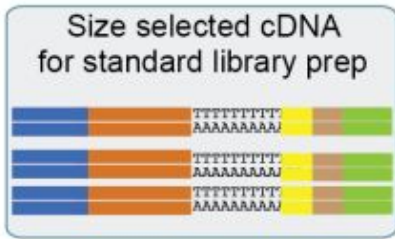
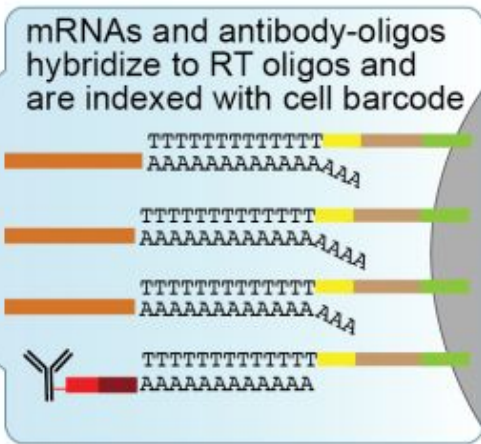
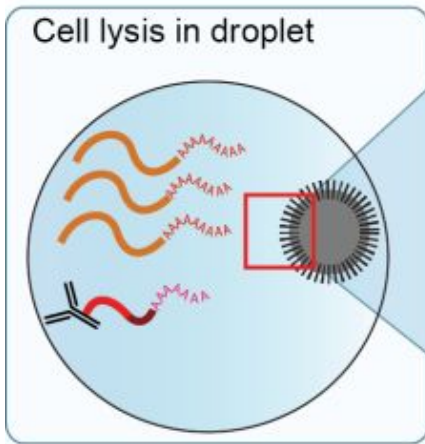
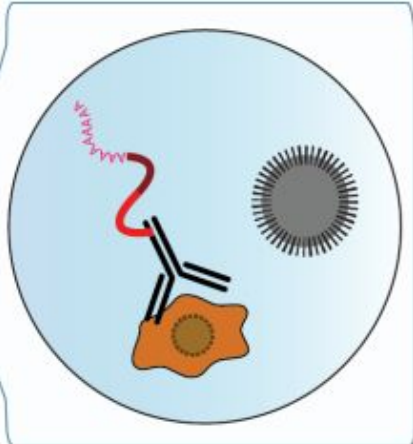
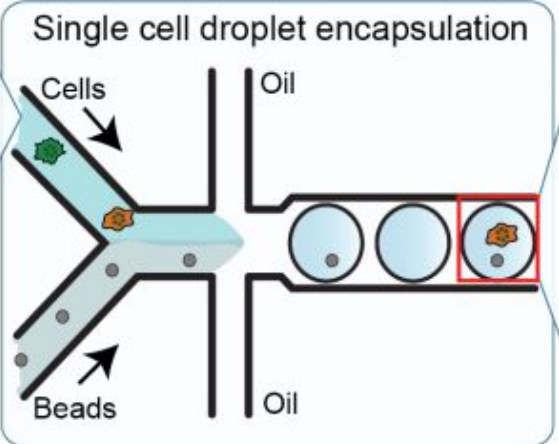
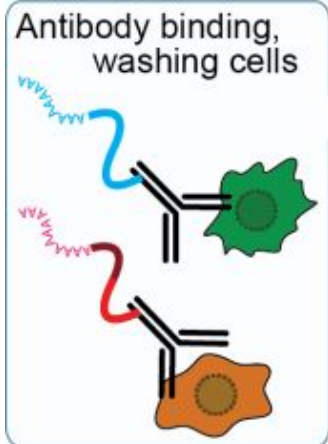
- Coarse grain (> 10 Mb)
- Requires $> 75,000$ reads / cell

D

Technologies

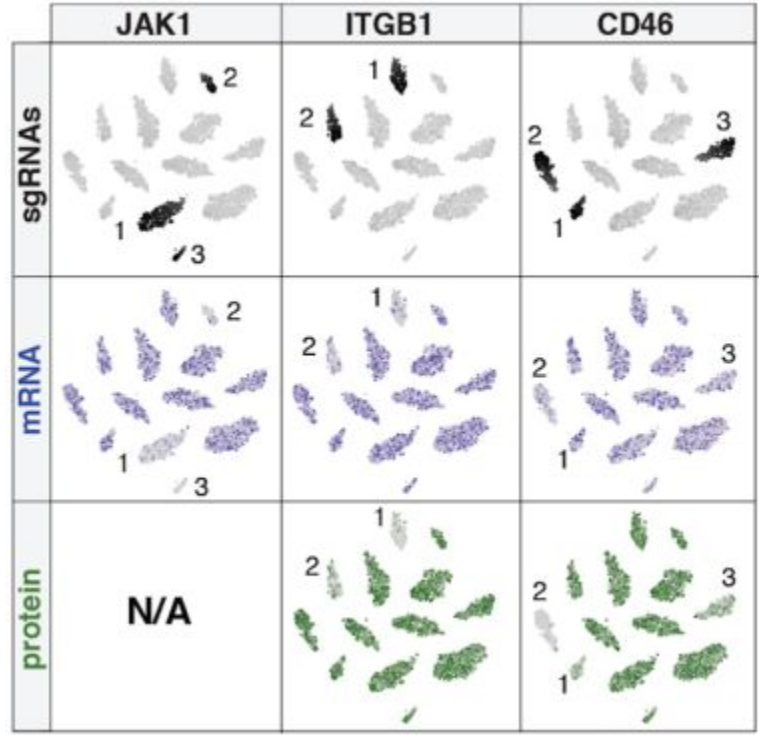
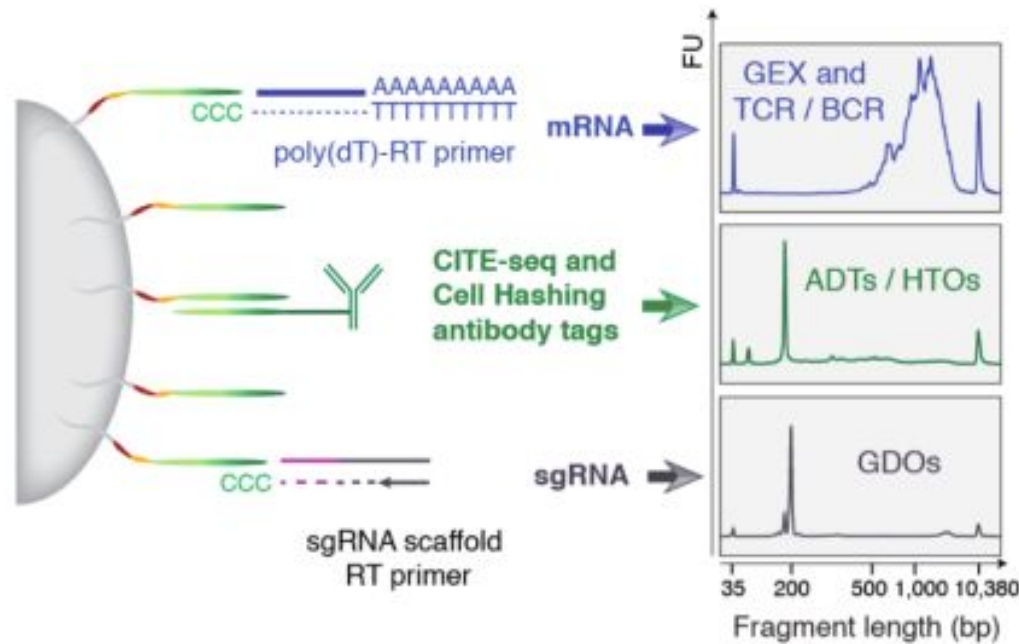
CITE-Seq (+ECCITE-Seq)

Cellular Indexing of Transcriptomes and Epitopes by Sequencing

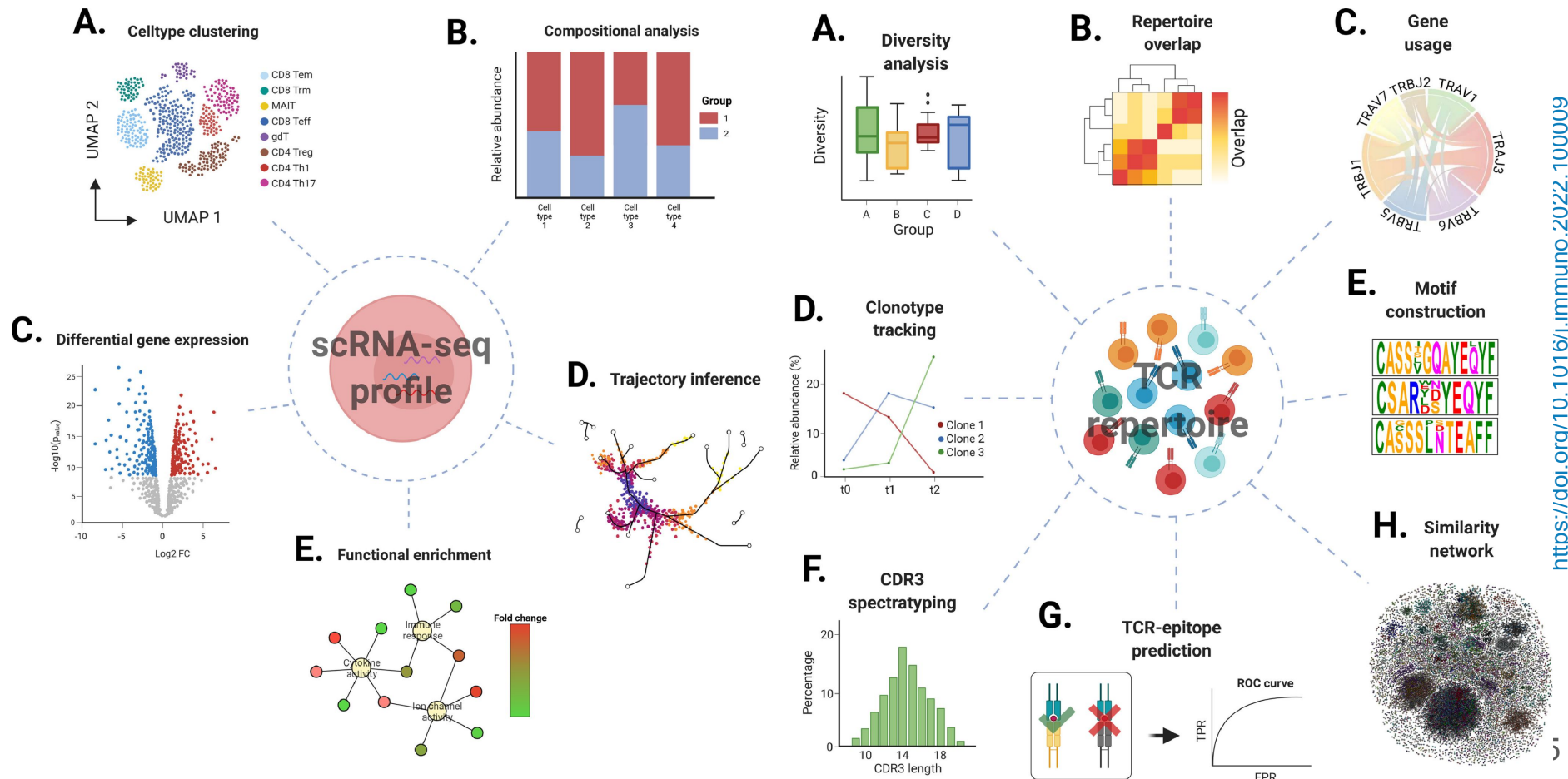


ECCITE-Seq

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



scRNAseq + TCR repertoire



Why so much hype ?

Bulk



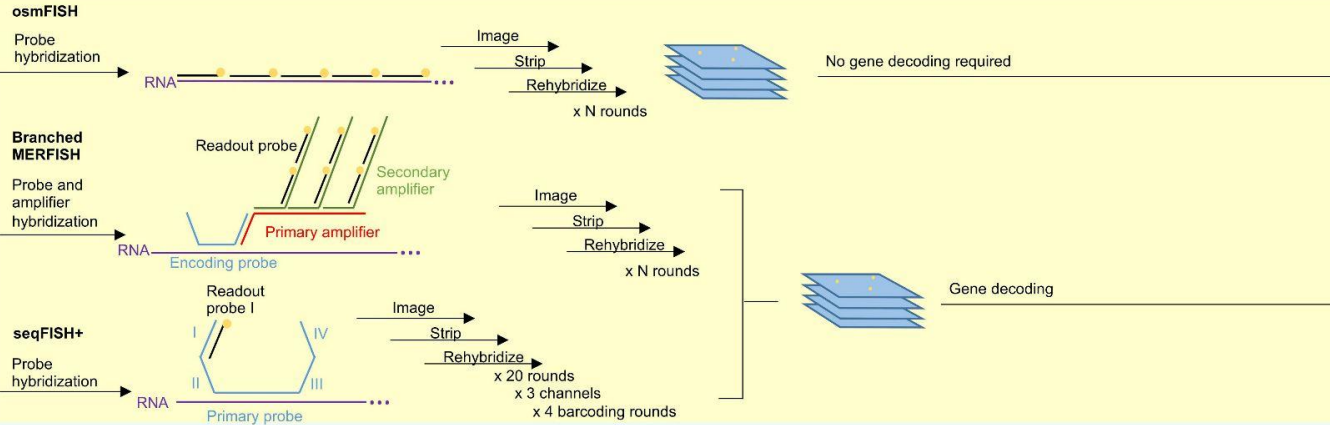
Single cell



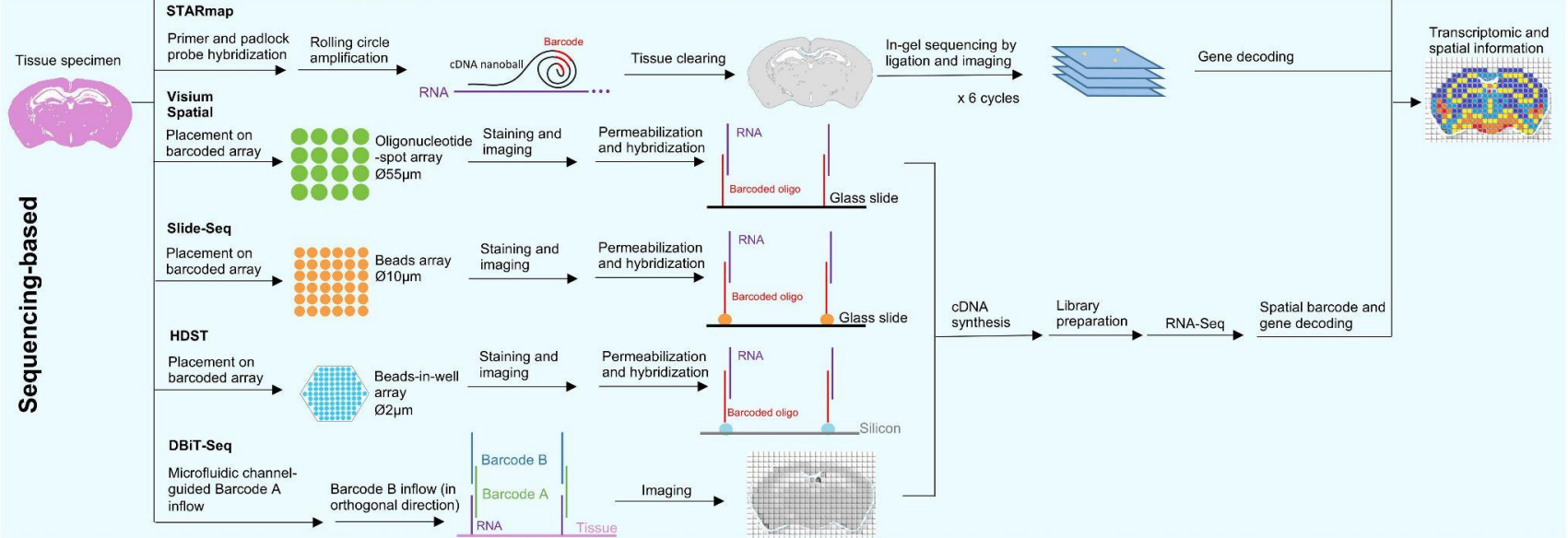
Spatial single cell



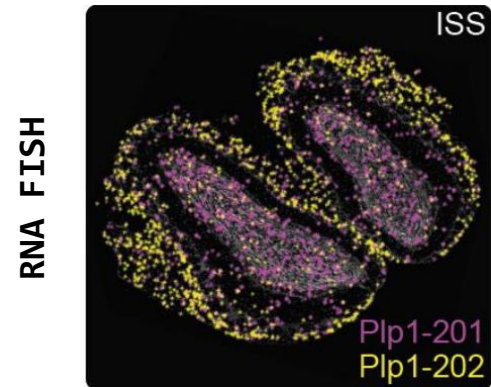
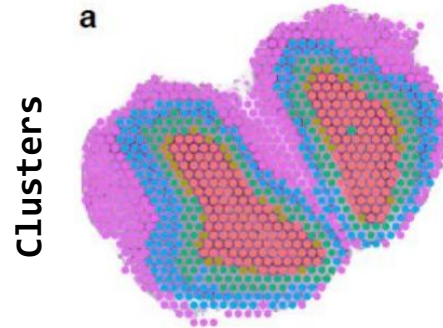
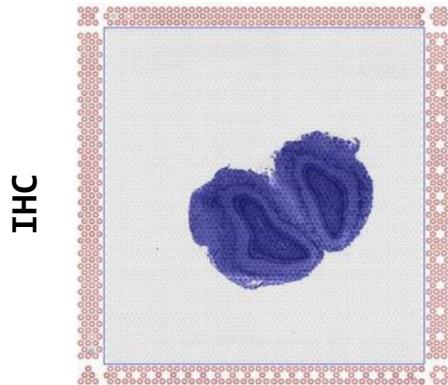
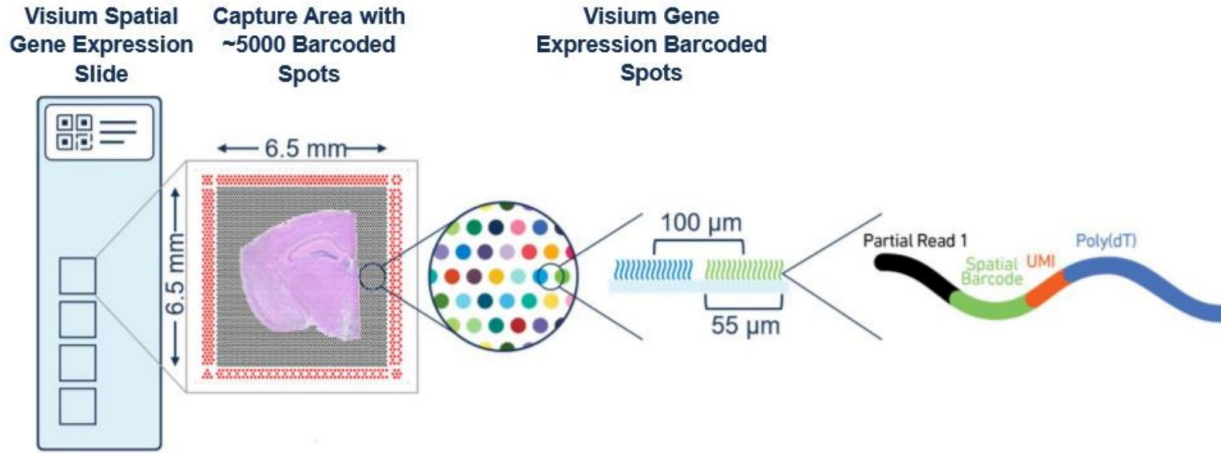
FISH-based



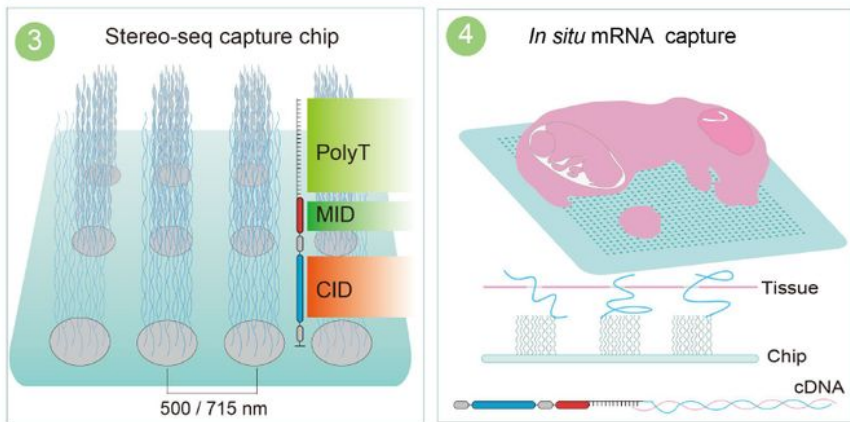
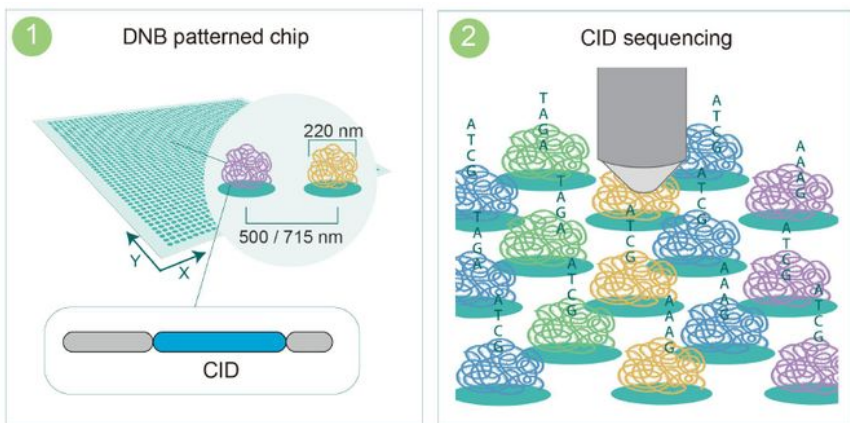
Sequencing-based



Seq-based spatial scRNAseq : 10x Genomics Visium

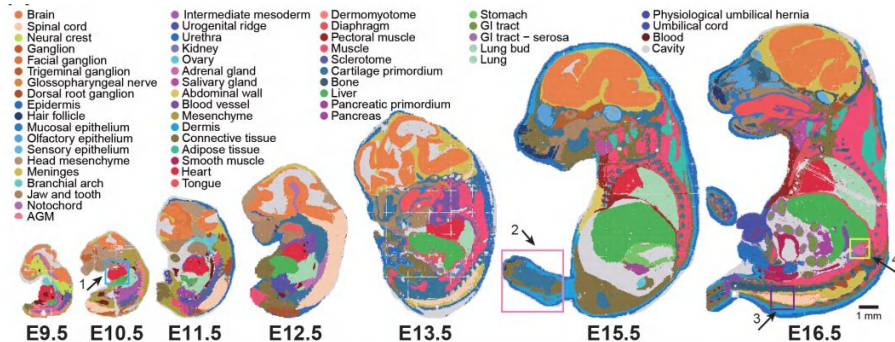


Seq-based spatial high-res long-read : StereoSeq

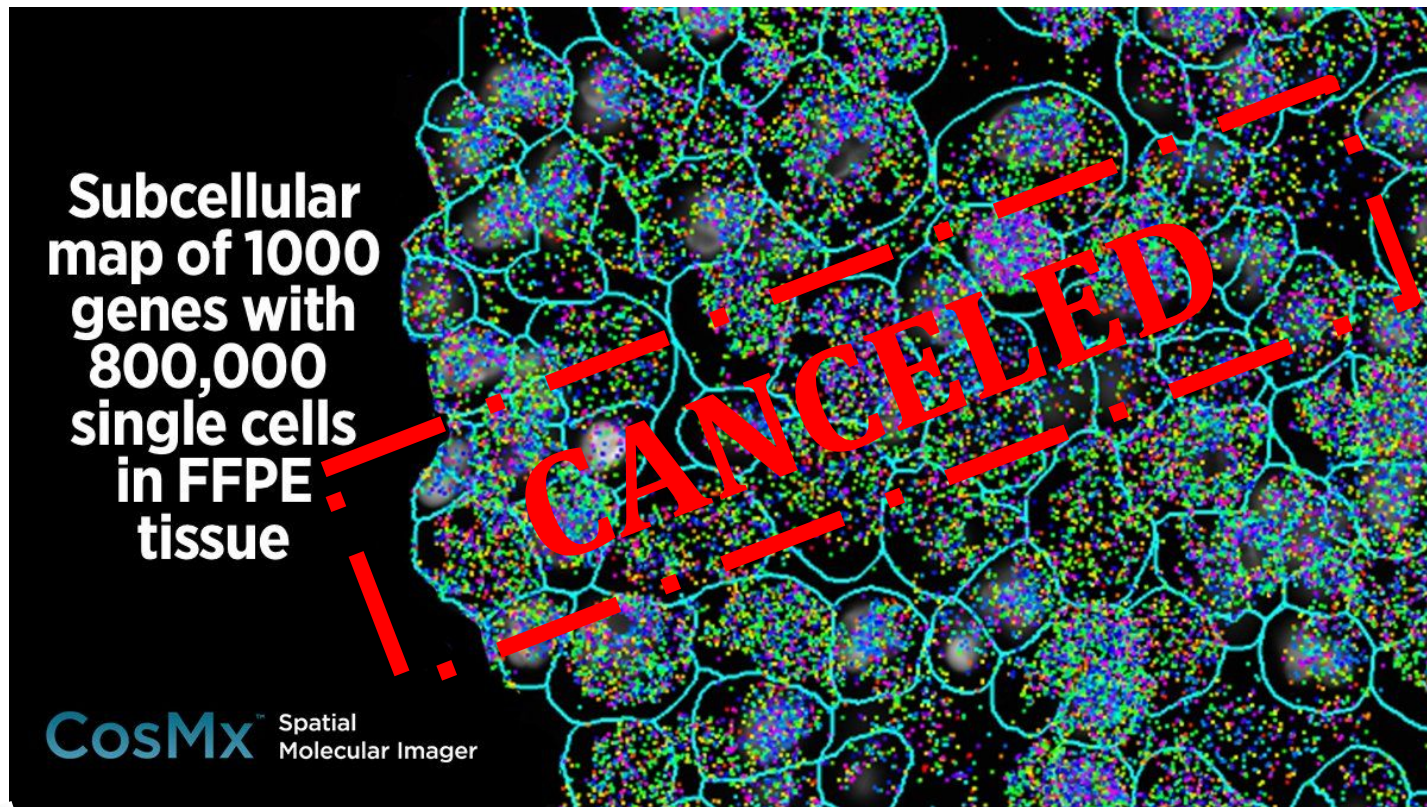


- Based upon “DNB” (DNA Balls) arrays by MGI/BGI
- Claimed resolution of 500-715 nm
- Active surface of 200 mm²
- Performed a developmental analysis of FULL mice embryos !

Cheng et al, BiorXiv 2021.01



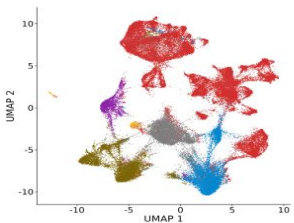
Imagery-based Spatial scRNA expression by imagery : NanoString CosMx-SMI



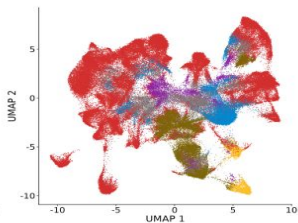
3D view : <https://www.youtube.com/watch?v=LI-gvIribDU&t=14s>

Cell Segmentation as the one point to improve

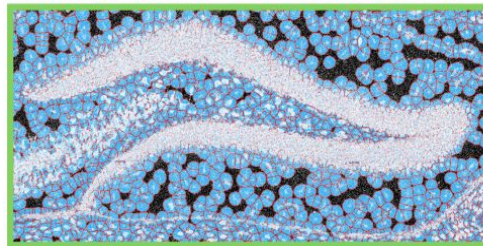
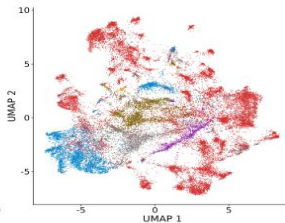
MERSCOPE



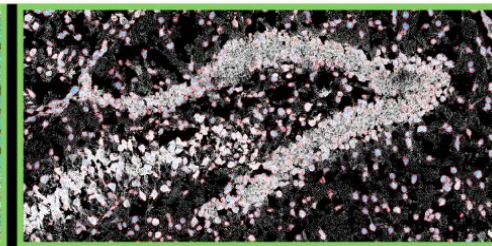
Xenium



Molecular Cartography

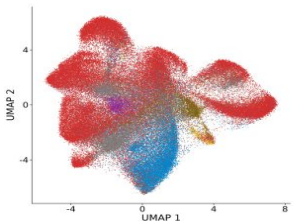


Xenium

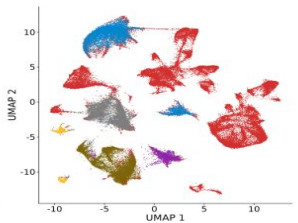


MERSCOPE

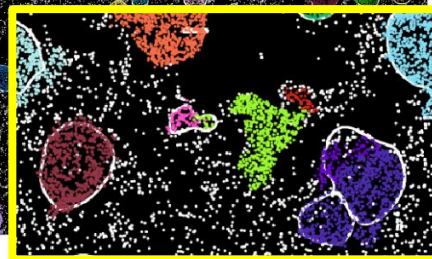
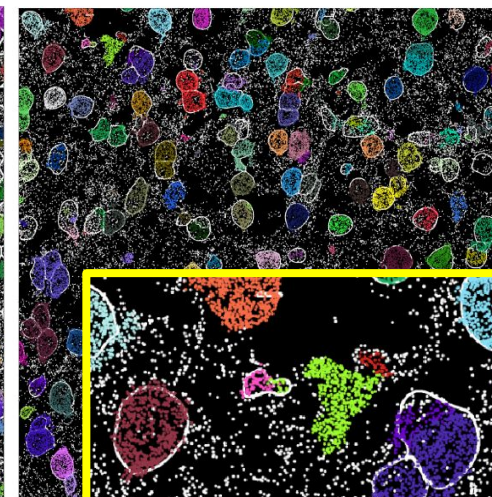
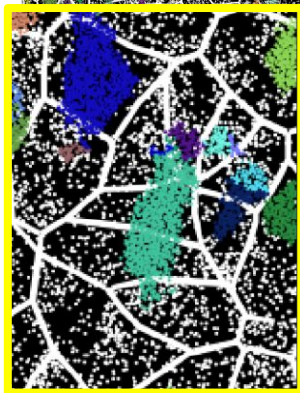
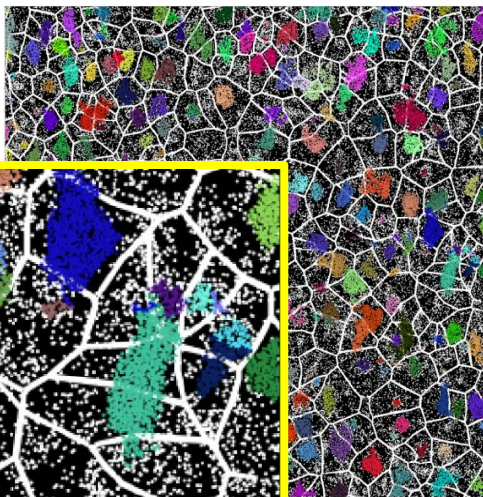
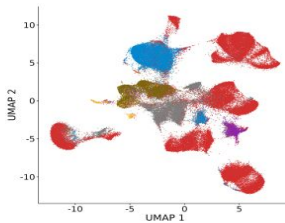
EEL FISH



MERFISH



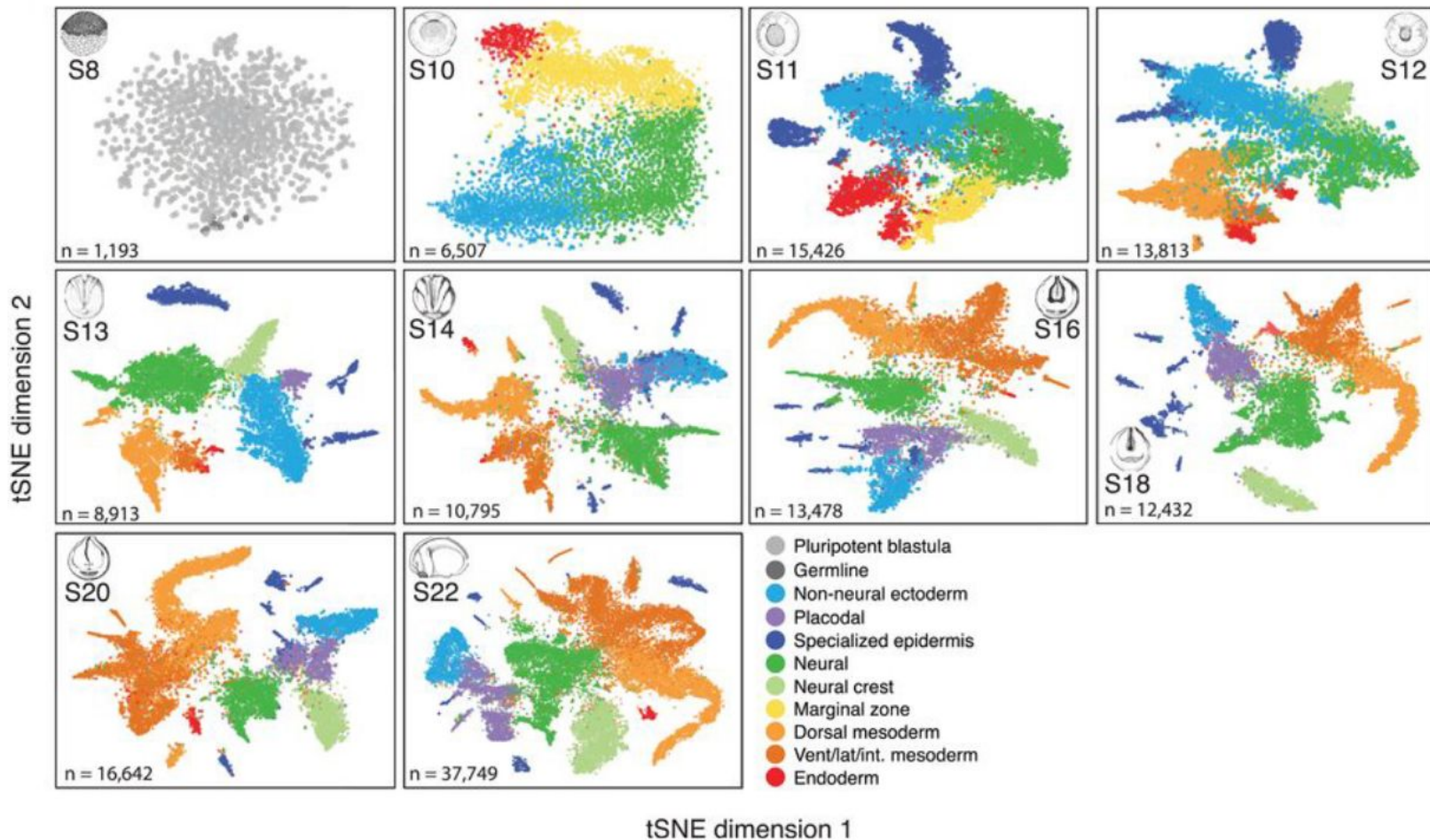
STARmap PLUS



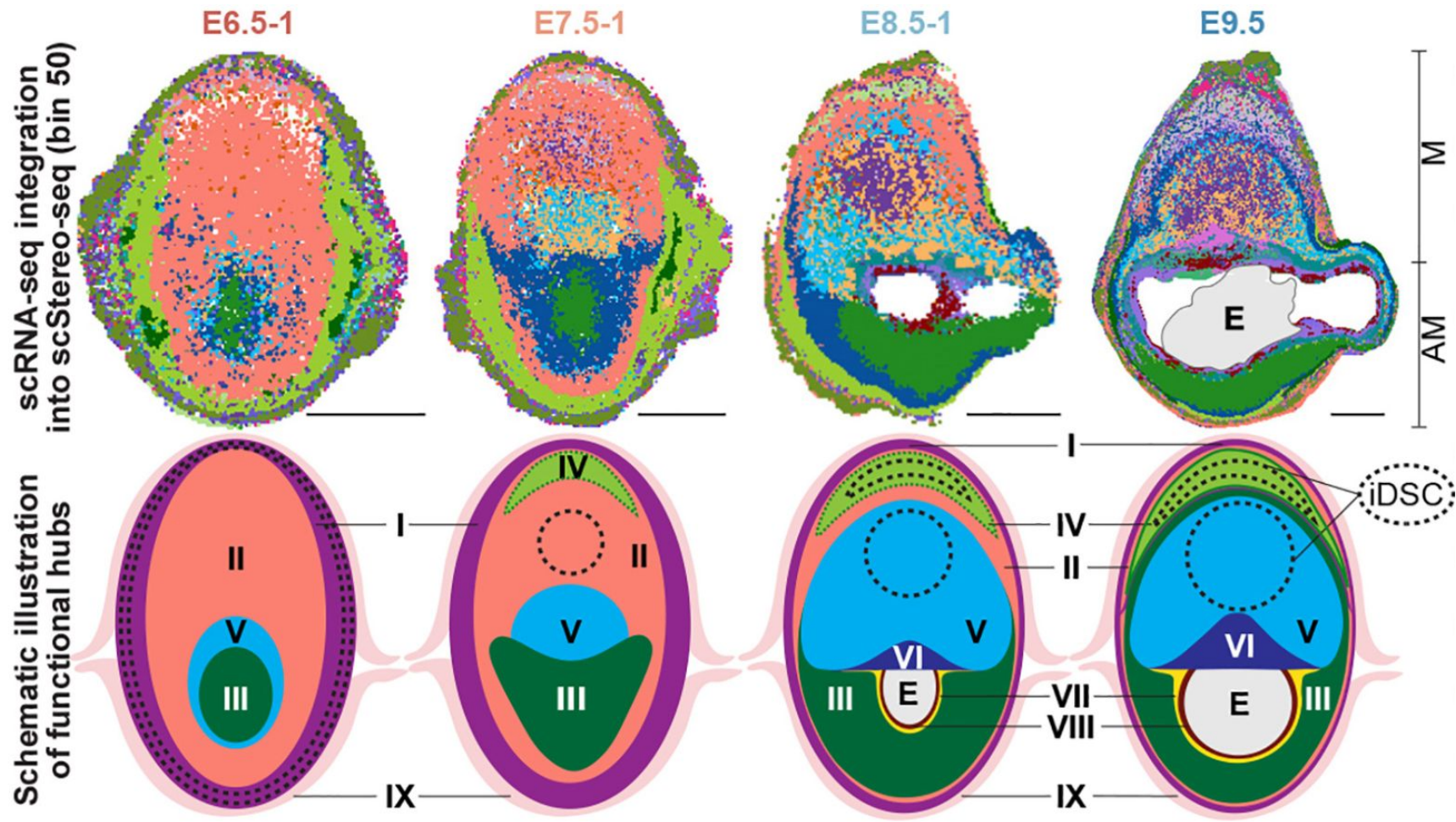
Some sweets



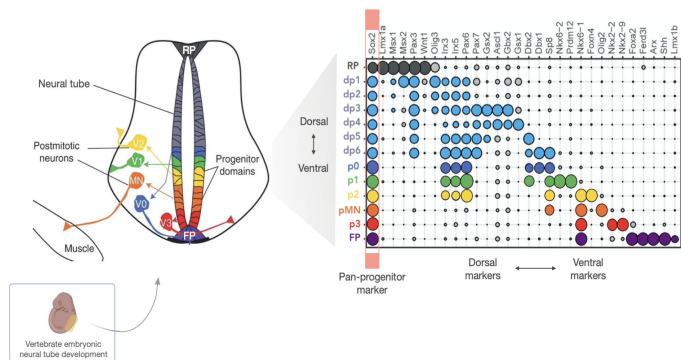
Xenopus embryo development



Spatial SC : Polar differentiation of mouse uterus

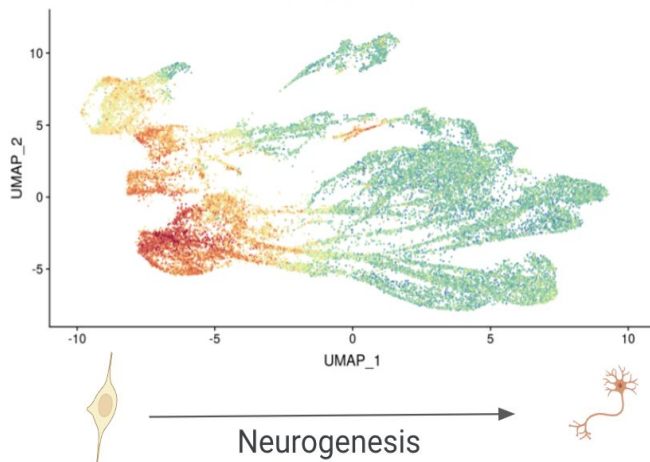


Single cell RNA-seq of mouse embryo neural progenitors

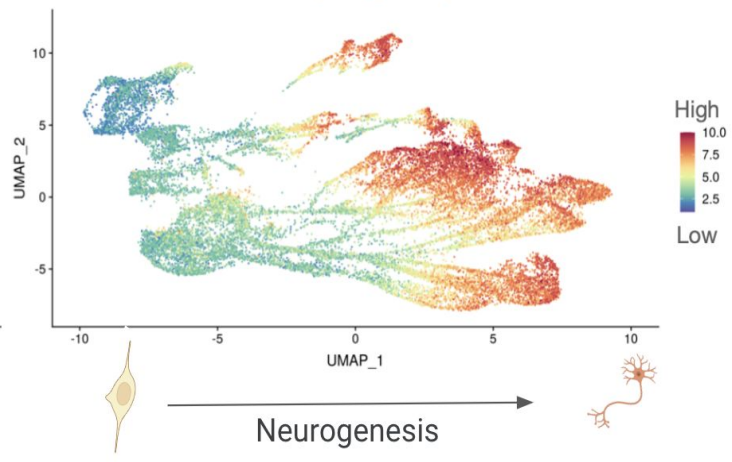


Nathalie's PhD thesis & Delile et al, Development, 2019

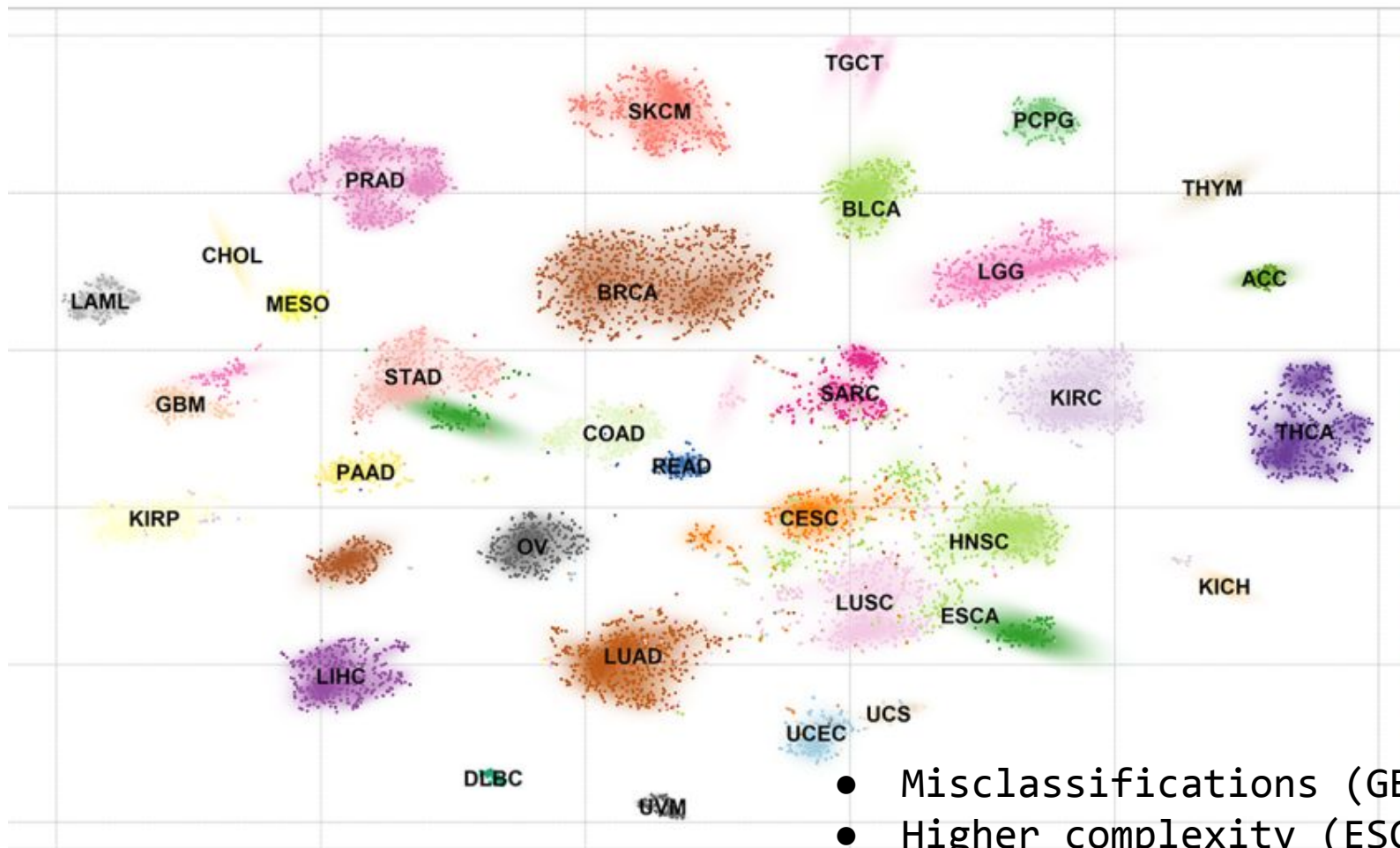
Progenitor score
(8 genes)



Neuron score
(11 genes)



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

... and you !



SINGLE-celling IN THE RAInaseq (1952)*



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

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