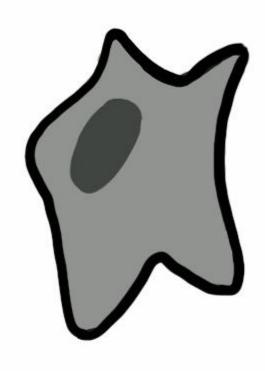


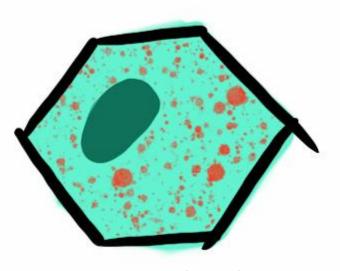


## **Generating Connectome**

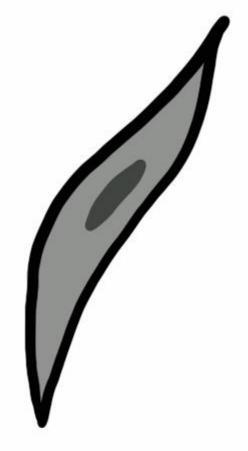


6th European Zebrafish PI Meeting Single cell Genomics approaches 31 Mar 2022





Sumeet Pal Singh IRIBHM, Brussels, Belgium



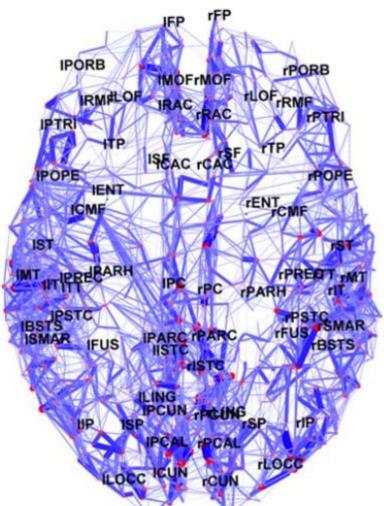
https://github.com/sumeetpalsingh/Presentations

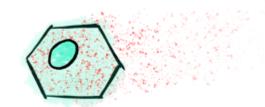
Github: sumeetpalsingh Repository: Presentations

### **Connectome**

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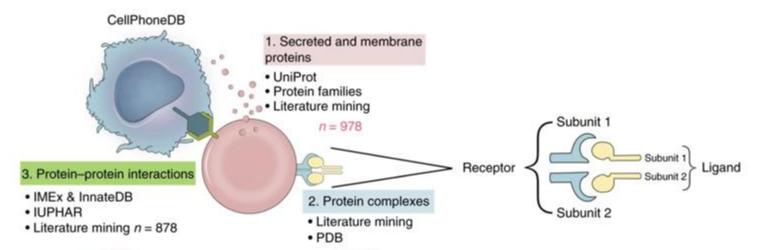
- Link between cells → communicating with each other
- 1. Neighboring each other
- 2. Signaling molecules







### In silico connectome using ligand-receptor pairs

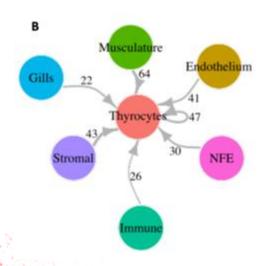


n = 112

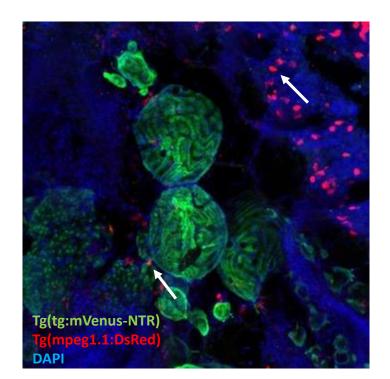


Efremova et al. Nature Protocols 15: 1484-1506 (2020). Doi: 10.1038/s41596-020-0292-x

n = 1,396 n = 466 involving protein complexes



Gillotay et al., EMBO Reports: e50612 (2020). Doi:10.15252/embr.202050612



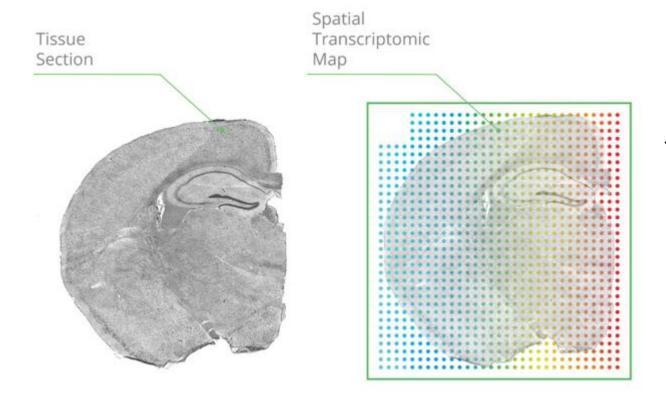
# Lack of information about position



### **Spatial transcriptomics**





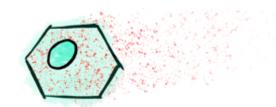


https://spatialtranscriptomics.com/technology/

- Location
- Transcriptome information

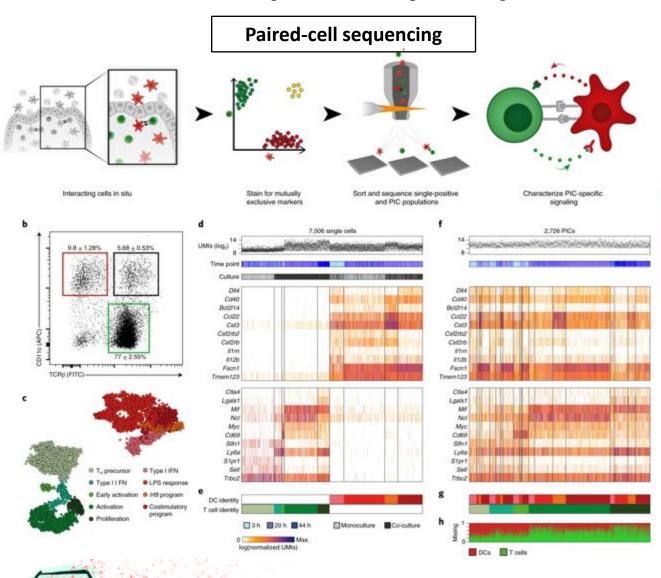
### Disadvantages

- Expense
- Spot size → 5 um there is very little transcriptome (~300 genes)

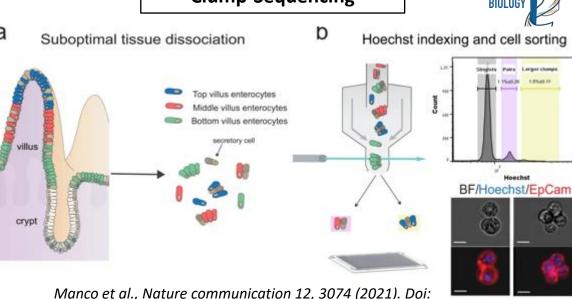




### Paired-cell Seq / Clump-Seq







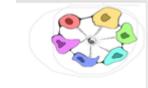
Manco et al., Nature communication 12, 3074 (2021). Doi: 10.1038/s41467-021-23245-2

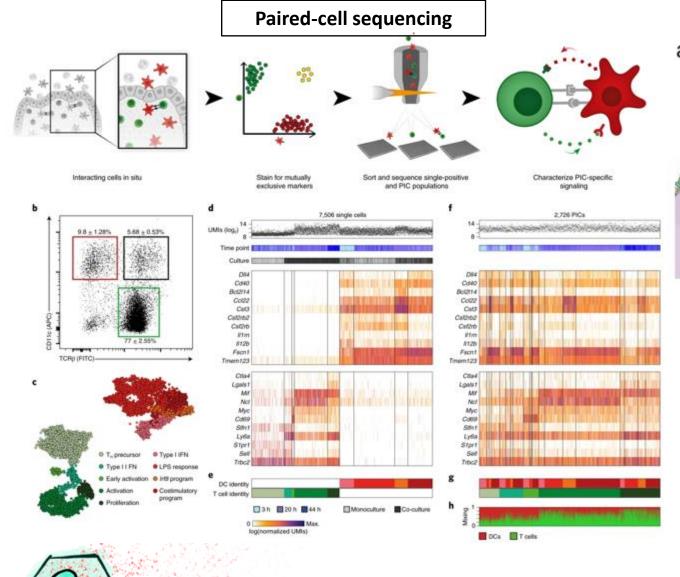


REGENERATIVE

Giladi et al., Nature Biotechnology 38: 629-637 (2020). Doi: 10.1038/s41587-020-0442-2

### Paired-cell Seq / Clump-Seq





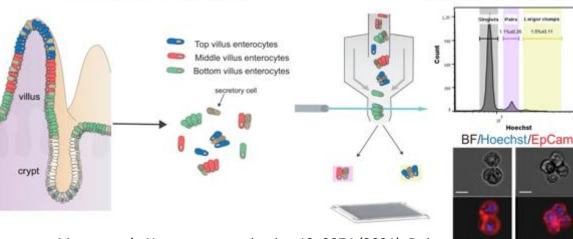
Giladi et al., Nature Biotechnology 38: 629-637 (2020). Doi:

10.1038/s41587-020-0442-2

#### **Clump-Sequencing**



Hoechst indexing and cell sorting



Manco et al., Nature communication 12, 3074 (2021). Doi: 10.1038/s41467-021-23245-2

### **Advantages**

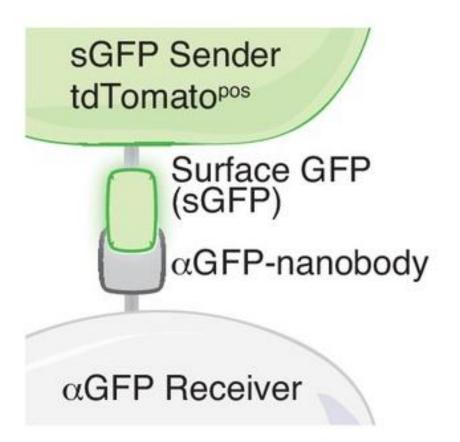
- Identification of two interacting cells.
- scRNA-Seq of two interacting cells.

### **Disadvantages**

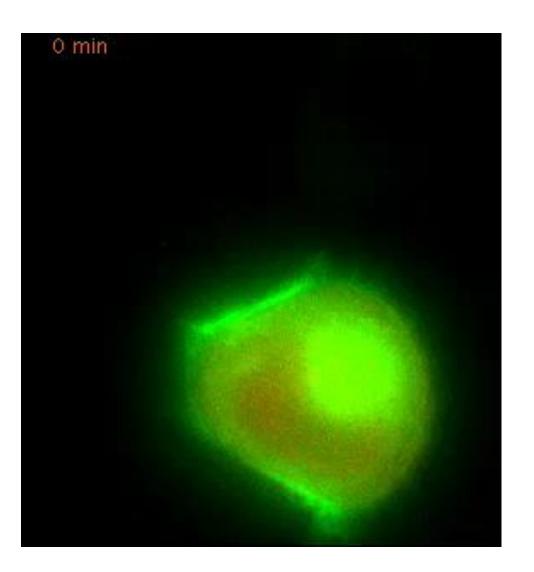
- Not possible to perform bulk RNA-Seq
- Clumps could appear because of the dissociation process.
- No possibility to perform confocal images.



### **Cell contact labeling system**

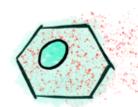


Tang et al, eLife 2020;9:e61080 DOI: 10.7554/eLife.61080



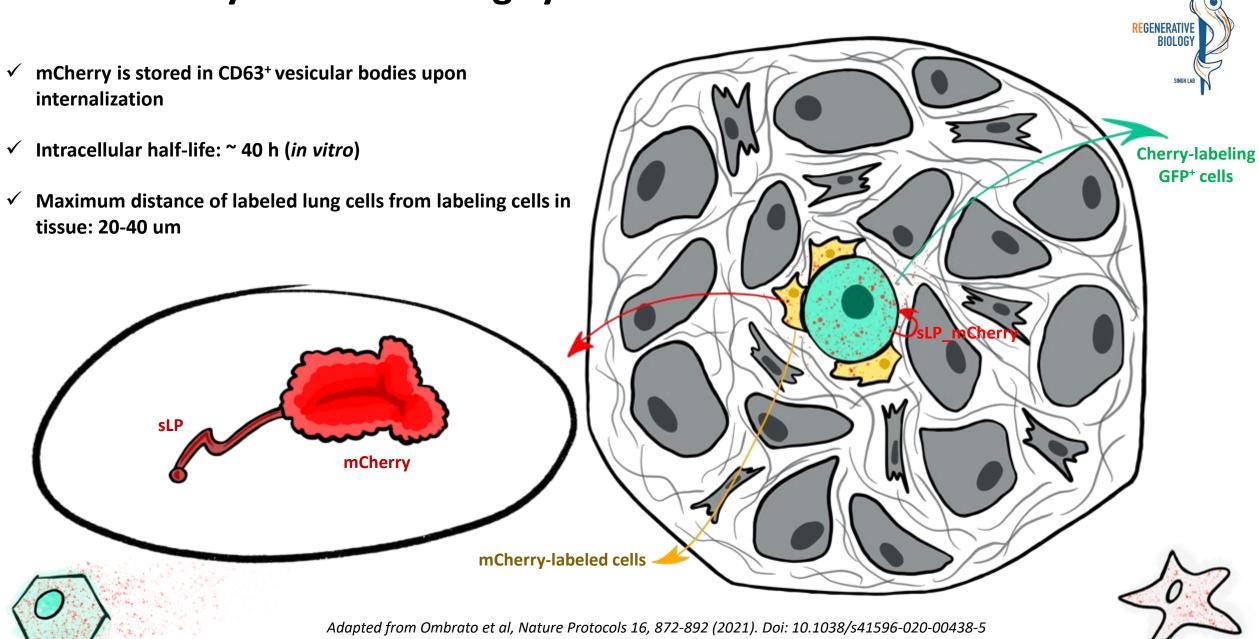






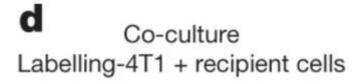
# The mCherry niche-labelling system REGENERATIVE BIOLOGY sLP-mCherry mCherry NLS **Cherry-labeling GFP**<sup>+</sup> cells **mCherry** mCherry-labeled cells Adapted from Ombrato et al, Nature Protocols 16, 872-892 (2021). Doi: 10.1038/s41596-020-00438-5

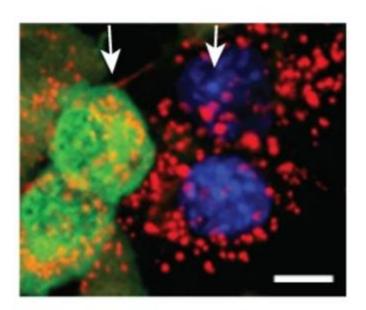
## The mCherry niche-labelling system

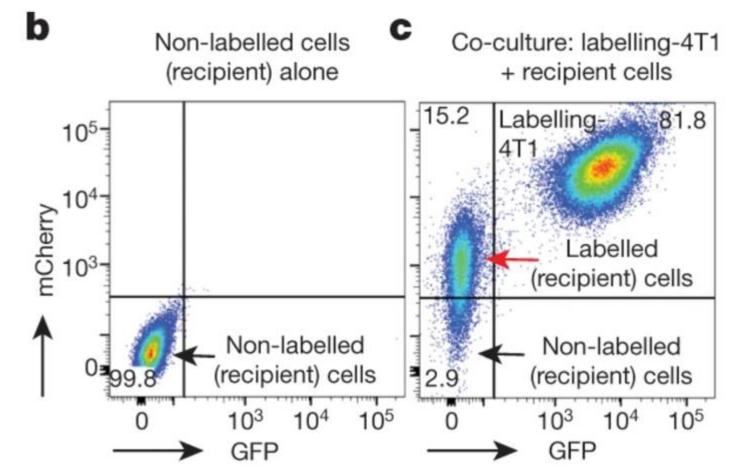


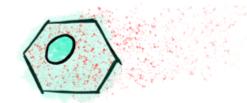
### The mCherry niche-labelling system







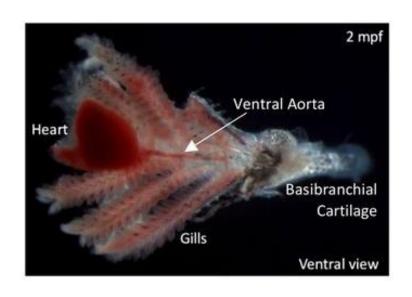


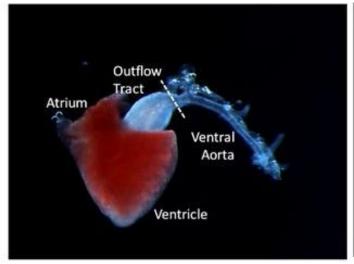


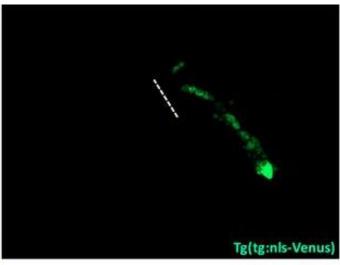


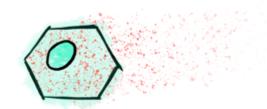
## The zebrafish thyroid gland







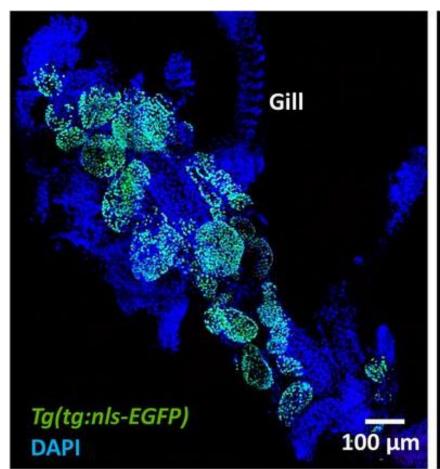


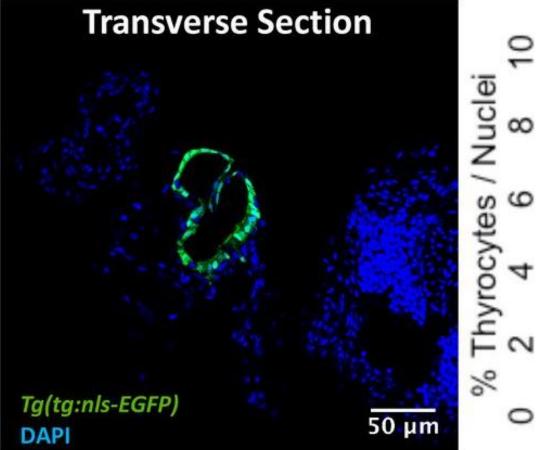


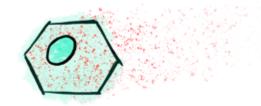


### The thyroid gland is surrounded by cell-rich gills





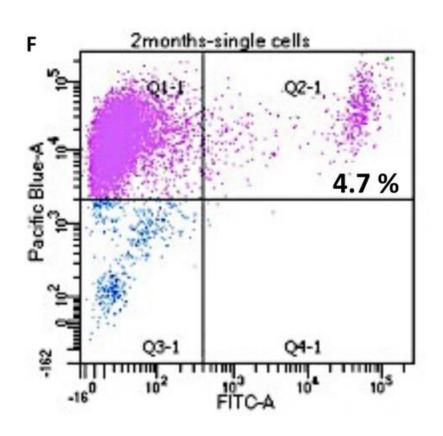


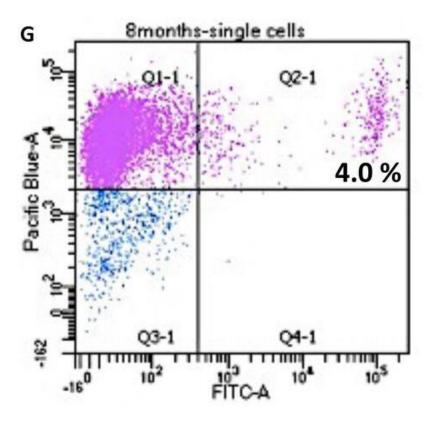


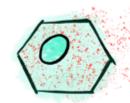


## Less than 5% of total cells belong to the thyroid follicle



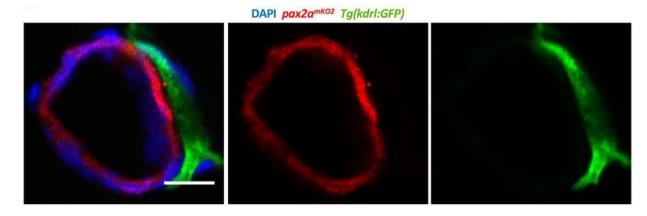


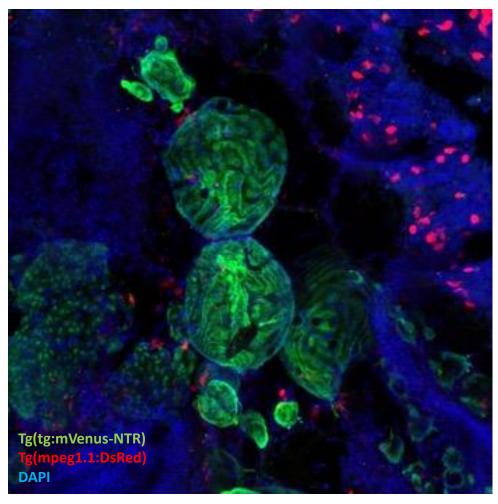


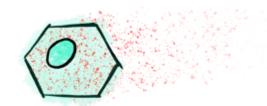




## Difficult to identify cells in proximity of the gland

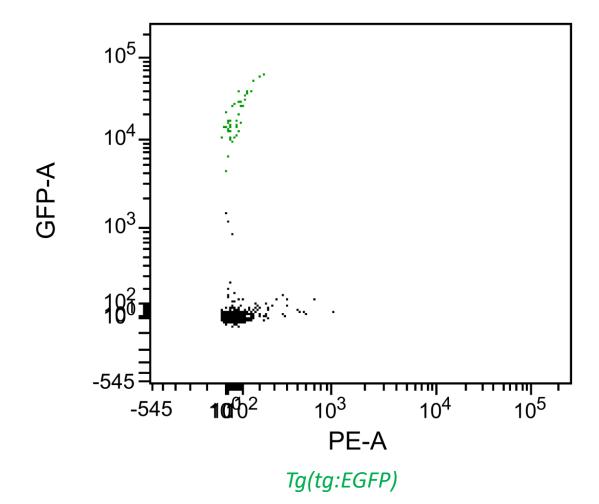


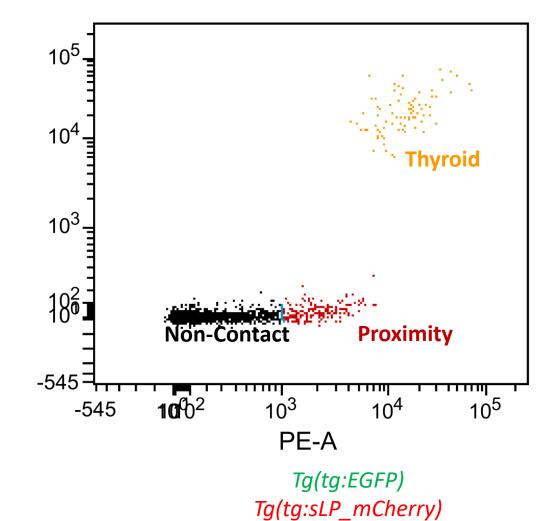


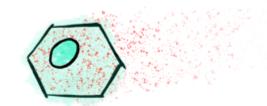




### Niche labeling of the thyroid gland

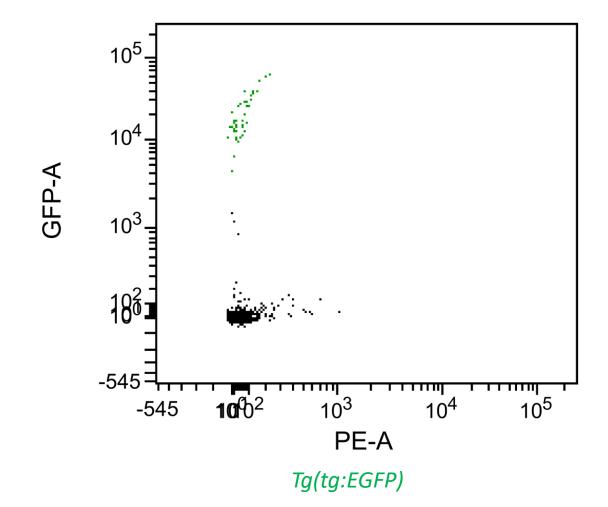


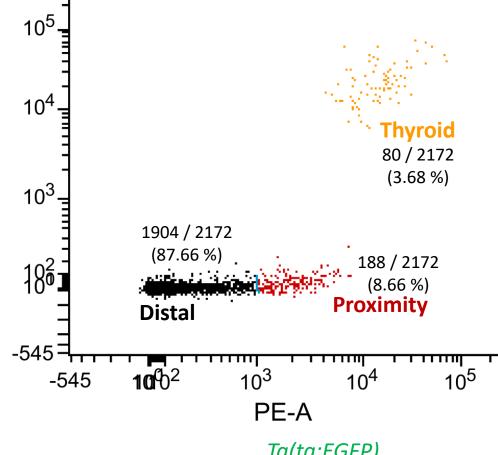




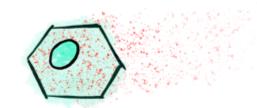
### Niche labeling of the thyroid gland







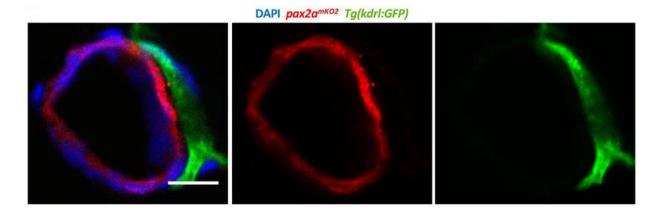
Tg(tg:EGFP)
Tg(tg:sLP\_mCherry)





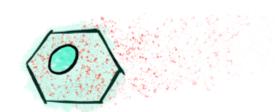
### Labeling specific cell-population within the niche





Are there any distinguishing transcriptomics features in vasculature between thyroid and gills

Technique	Cost: Euros / sample (Total)	Depth: genes / sample
10x	1 (4,000 Euros)	1,500
Smart-Seq (80-cells)	50 (4,000 Euros)	4,000
Bulk (100 cells)	200 (1,200 Euros)	14,000
Bulk (>500 cells)	200 (1,200 Euros)	Full Transcriptome



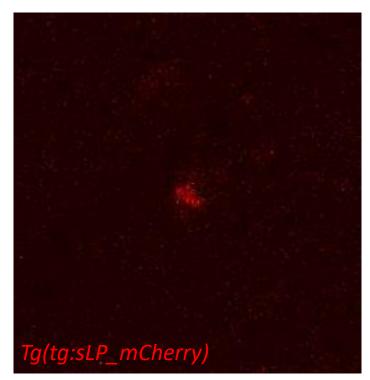


### Labeling specific cell-population within the niche



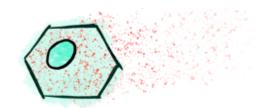






Niche labelling system can be combined with complementary labelling to sort interacting population for bulk RNA-Seq

➤ Unique advantage of the system











Singh Lab, IRIBHM, Brussels





Github: sumeetpalsingh Repository: Presentations

