

Single-cell spatial transcriptomics

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Computational Biology and Omics Data Analysis

 <https://cobioda.github.io>

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ipmc



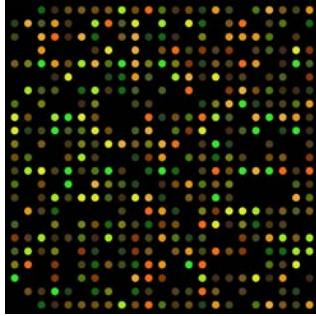
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01

Introduction

20 years of transcriptomics

Driven by microfluidics technological developments

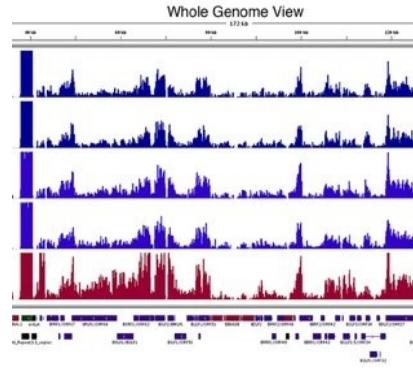


Early 2000's: DNA microarray

- Large-scale transcriptome
- Oligonucleotide probe tilling
- Fluorochromesignal analysis
- Bulk resolution



Cost : 4k€
20 samples
25k genes
0.5M matrix

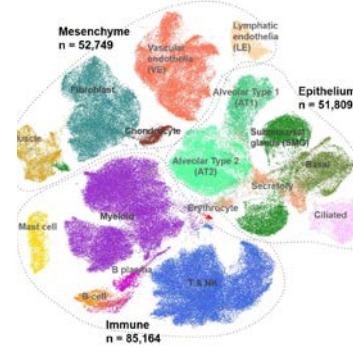


Late 2000's : RNA sequencing

- Whole transcriptome
- Next Generation Sequencing
- Full-transcript coverage
- Bulk resolution



Cost : 4k€
20 samples
50k genes
1M matrix



Mid 2010's: Single -cell

- Whole transcriptome
- Microfluidics + NGS
- 3p-end gene signal (UMI)
- Sensitivity (6%)
- Single-cell / state resolution



Cost : 4k€
5k cells
50k genes
250M matrix



2020's : Spatial

- 500-1000 gene targets
- Imaginganalysis
- MultiplexingFiSH(single molecule)
- Sensitivity (3080%)
- Sub-cellular resolution

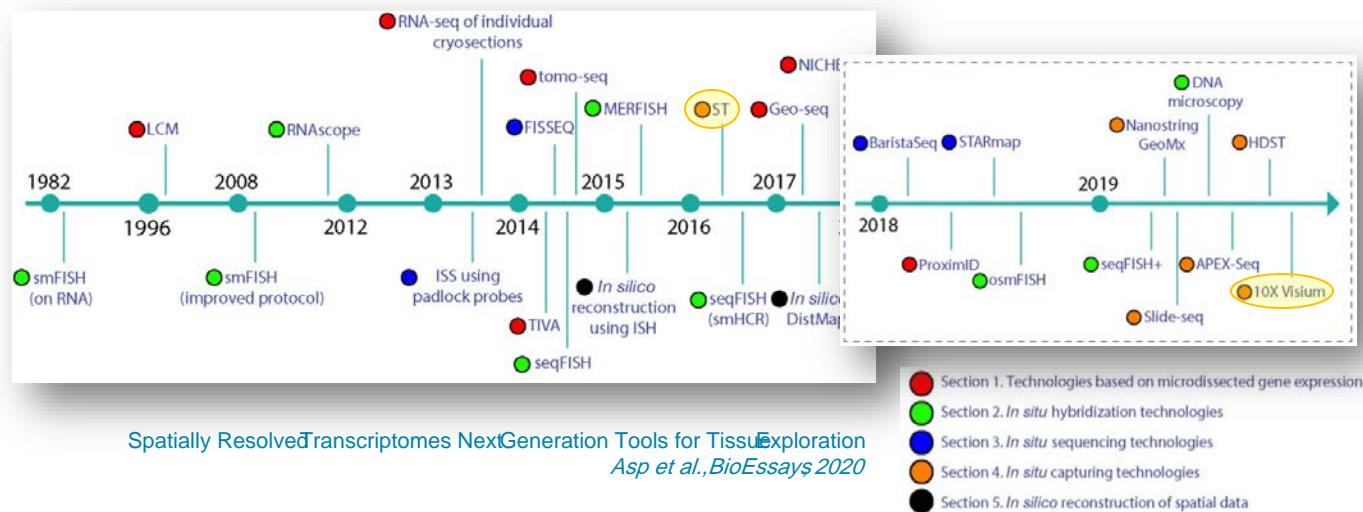


Cost : 4k€
250k cells
1k genes
250M matrix + Spatial dimension

Spatial Transcriptomics approaches

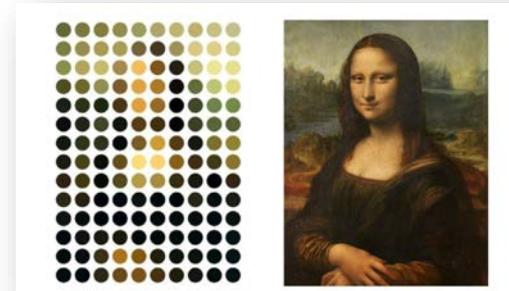
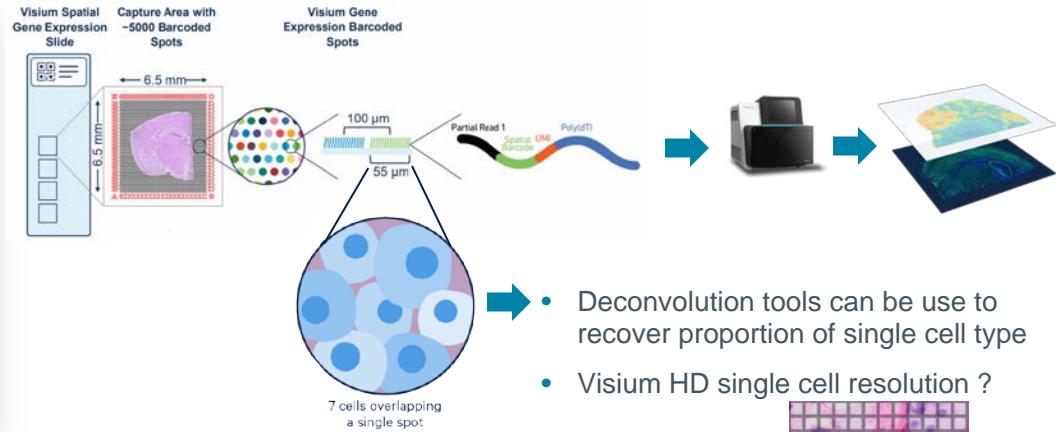
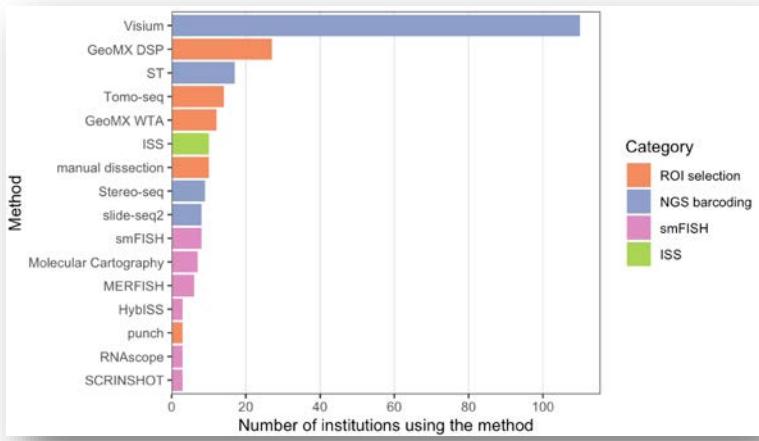
Historical timeline

- Spatial transcriptomics aims at directly visualize gene expression in their original environment,
- It tackles the main limitation of single cell experiment missing the spatial organization,
- A lot of developments in the last years thanks to recent advances in different fields,



Spatial transcriptomics (2017 -2022)

Visium is widely adopted by academics

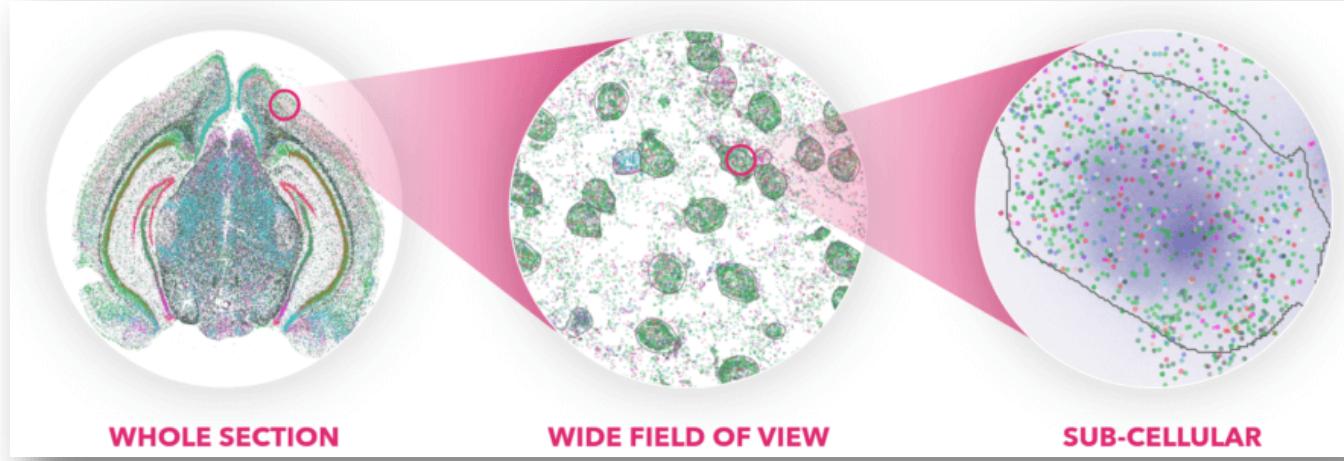


But is not the ideal readout for spatial biology
(Akoyacredit rough caricature)

Spatial imaging -based transcriptomics (2022)

No more sequencing for direct singlecell resolution

- Lower gene panel targets (from whole transcriptome to ~1,000 genes)
- Higher sensitivity (from ~6% to 30-80%)
- Larger imaging area (42 to 236 mm²)
- Higher resolution (from 55 µm to subcellular)



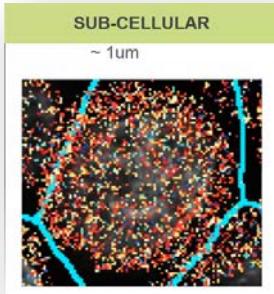
Spatial imaging -based transcriptomics (2022)

No more sequencing for direct single-cell resolution



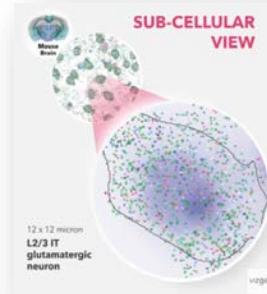
Nanostring CosMx

- Limited availability
- 960 targets (panel 20k, AGBT24)
- Sensitivity : << 30-80% (+)
- Imaging area: 16 mm² (2 days)
- Resolution: 200 nm



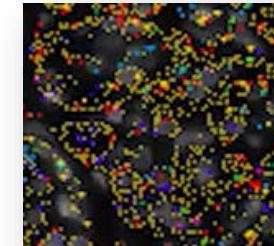
Vizgen Merscope

- Available (oct.22)
- 1.000 targets
- Sensitivity: 30-80% (+++)
- Imaging area: 100 mm² (2 days)
- Resolution: 100 nm



10xGenomics Xenium

- Available (jan.24)
- 400 targets (panel 6k)
- Sensitivity : 530% (++)
- Imaging area: 236 mm² (4 days)
- Resolution: 200 nm

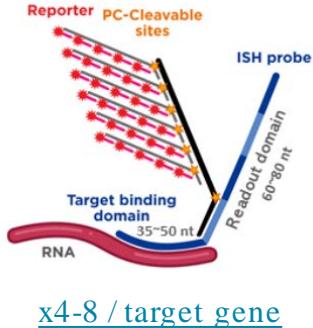


Spatial imaging -based transcriptomics (2022)

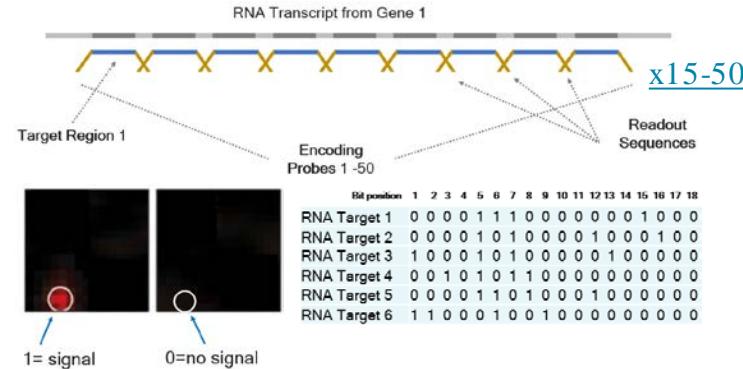
No more sequencing for direct single-cell resolution



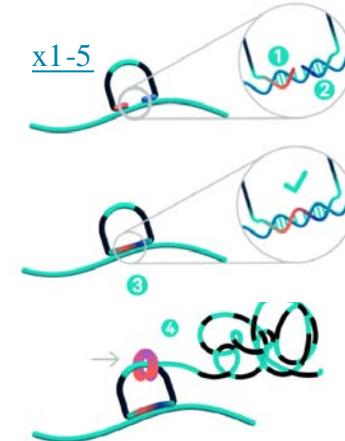
Nanostring CosMX
ISH-based



Vizgen Merscope
Multiplex ErrorRobust FISH



10xGenomics Xenium
Cartana ISS, padlock probes /RCA

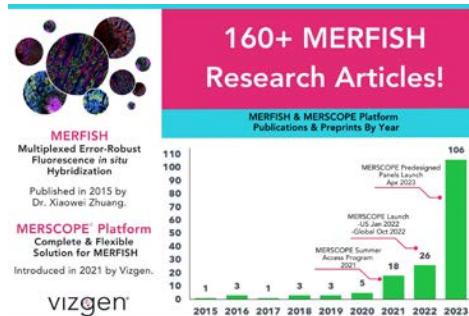


Spatial imaging -based technologies comparison

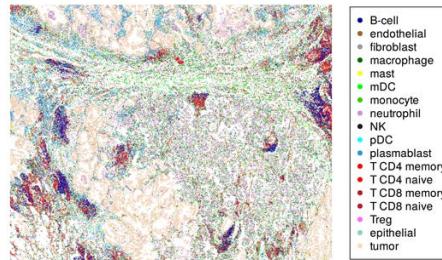
Compare available datasets

Vizgen Merscope

- XiaoweiZhuang'slab merfishpublications
 - Chen et al., Science (2015)
 - Moffitt et al., PNAS (2016), Science (2018)
 - EmanuelG et al., Nature Methods (2017)
 - Xia C. et al., PNAS (2019, Scientific Reports (2019)
 - Zhang M. et al., Nature (2021)
- Internal data release program
 - Human Immuno-oncology (**breast, colon, lung, liver, skin, prostate, uterine and ovarian**) 500 genes, >4 billion transcripts, 9 million cells
 - Mouse Liver Map(347genes)
 - Mouse brain Receptor Map (483 genes)

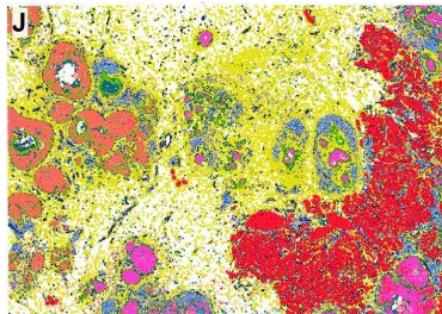


Nanostring CosMx



- Release date: 11/2021
- FFPE Human NSCLC (**Lung**)
- 960 gene targets
- 8 sections for 800k cells
- Imaging area: 8 x 16 mm²
- 259,604,214 transcripts
- Mean transcripts/cell: 265

10xGenomics Xenium



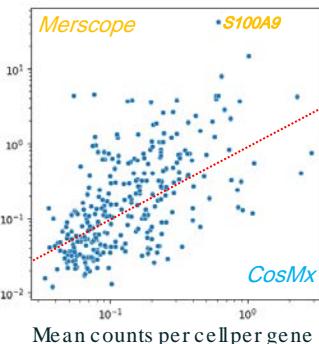
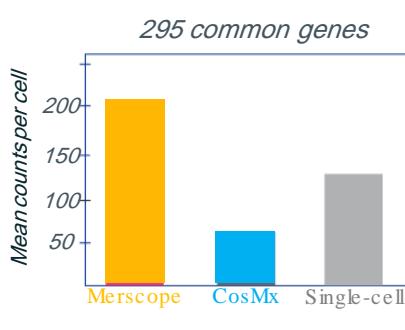
- Release date: 10/2022
- FFPE Human **Breast** cancer
- 313 gene targets
- 167,885 cells,
- 36,944,521 transcripts
- Imaging area: 40 mm²
- Mean transcripts/cell: 193

Spatial imaging technologies comparison

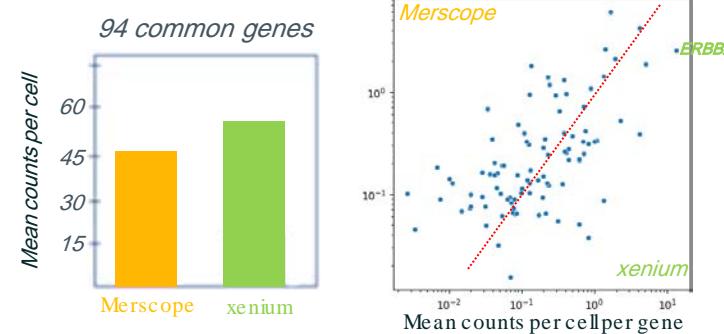
Compare available datasets: Lung and Breast cancer samples



FFPE Human Lung Cancer	Merscope	CosMx
Total cells	353 k (x4)	92 k
Detected transcripts	107 M (x4)	26 M
Gene targets	500	960
Total RPKM	9,204	61,680 (x6)
Mean transcripts/cell	302	284



FFPE Human BreastCancer	Merscope	Xenium
Total cells	713 k (x4)	168 k
Detected transcripts	353 M (x10)	32 M
Gene targets	500	313
Total RPKM	9,909	7,912
Mean transcripts/cell	495	193



Spatial imaging technologies comparison

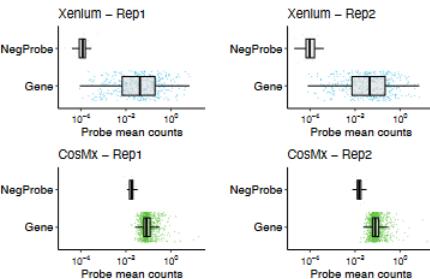
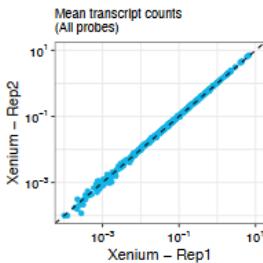
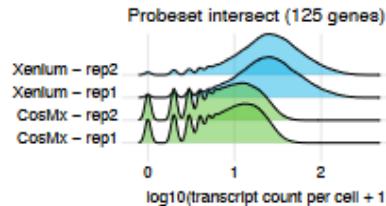
Recent biorxiv comparative studies

A Comparative Analysis of Imaging-Based Spatial Transcriptomics Platforms

David P. Cook¹, Kirk B. Jensen^{2,3,4}, Kellie Wise^{2,3}, Michael J. Roach^{2,3}, Felipe Segato Dezem^{6,7}, Natalie K. Ryan^{3,5}, Michel Zamojski⁹, Ioannis S. Vlachos^{10,11,12}, Simon R. V. Knott^{13,14}, Lisa M. Butler^{3,5}, Jeffrey L. Wrana^{1,15}, Nicholas E. Banovich¹⁶, Jasmine T. Plummer^{6,7,8*}, Luciano G. Martelotto^{2,3*}

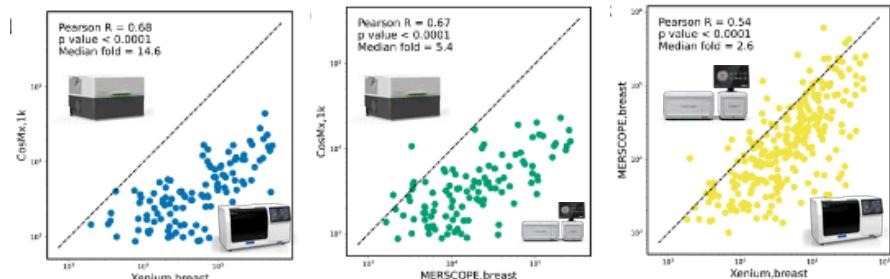


	Xenium Rep 1	Xenium Rep 2	CosMx Rep 1	CosMx Rep 2
Gene target #	377	377	1000	1000
Total cell count	99,852	102,508	98,139	98,767
Median gene count per cell	33	34	75	71
Median transcript count per cell	88	92	113	99
Median transcript count / gene target count	0.23	0.24	0.11	0.10
Median transcript count (intersecting targets only)	23	24	8	7



Systematic benchmarking of imaging spatial transcriptomics platforms in FFPE tissues

Huan Wang^{1,*}, Ruixu Huang^{2,*}, Jack Nelson^{1,*}, Ce Gao³, Miles Tran³, Anna Yeaton⁴, Kristen Felt⁵, Kathleen L. Pfaff⁶, Teri Bowman⁷, Scott J. Rodig^{6,7}, Kevin Wei^{3,7}, Brittany A. Goods^{2,**}, Samouil L. Farhi^{1,**}



MERSCOPE @ UCAGenomiX (Nice-Sophia -Antipolis)

October 2022



↪ William Amoyal Retweeted
Pascal Barbry @pbarbry · Oct 12

Happy to announce the installation of our first Merscope at @UCAGenomix. Many thanks to @vizgen_inc people for amazing work and interactions.

Great spatial transcriptomics work to come

@fr_genomics @discovAIR_HCA @3IAcotedazur @IPMC_sophia @CNRS @Univ_CotedAzur @CanceropolePACA



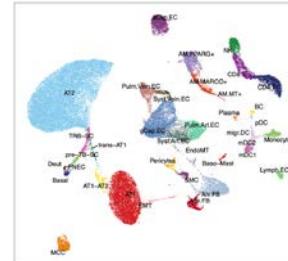
- Human Lung Cell Atlas (CZI) [discovAIR](#)
Discovering the Cellular Landscape of the Airways and Lung Tissue



- 12 control / 2 IPF / 10 COPD patients
- 415,764 cells (117 samples)
- 48 cell types



- Epileptic encephalopathy Dravet Synd. (Mouse brain, MMantegazza, Nice)
- Human embryo olfactory epithelium exploration (Pac , Jacobini, Lille)
- Pulmonary Arterial Hypertension (Christophe Guignabert, Paris Saclay)



- 7 control / 7 PAH patients
- 69,949 cells
- 39 cell types

02

Data acquisition and statistical analysis

Gene targets panel design

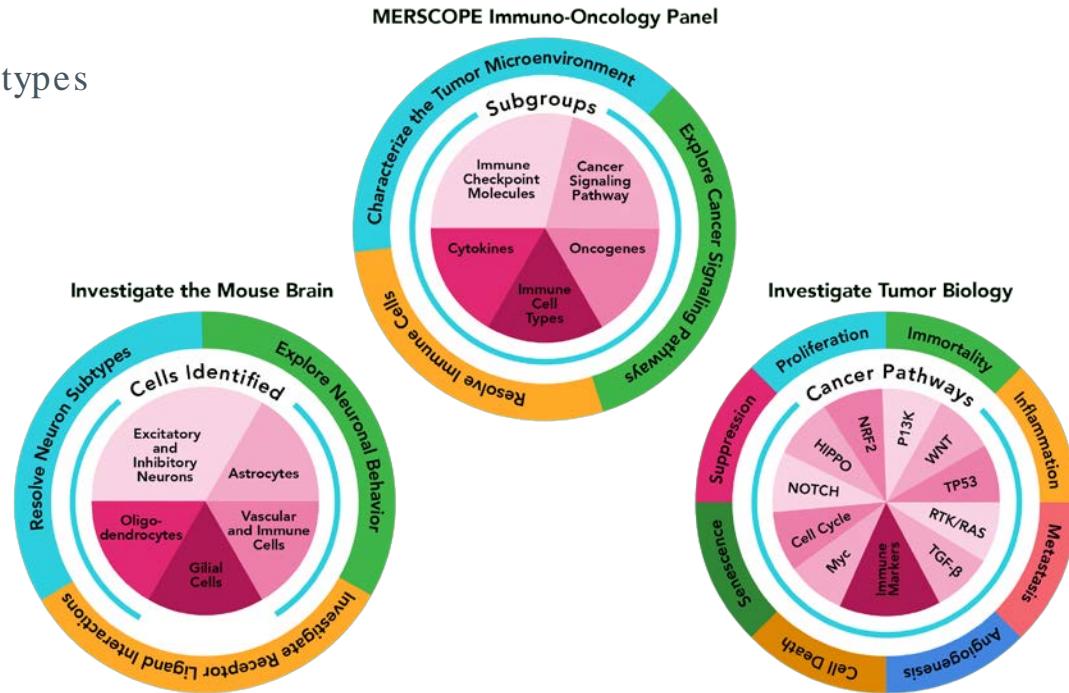
Depending on the biological question !

Depending of your specific scientific focus

- Identify all major cell types, resolve cell subtypes
- Explore functional information
- Investigate interactions between cell types
- Ligand-receptors analysis
- Explore canonical signaling pathways
- Profile immune checkpoint molecules
- ...

Satisfy technological system limitations

- Number of targets available
- Range of gene targets expression
- Total gene targets expression
- Have a nice budget to spend (~15 k€)
- ...



<https://portal.vizgen.com/>

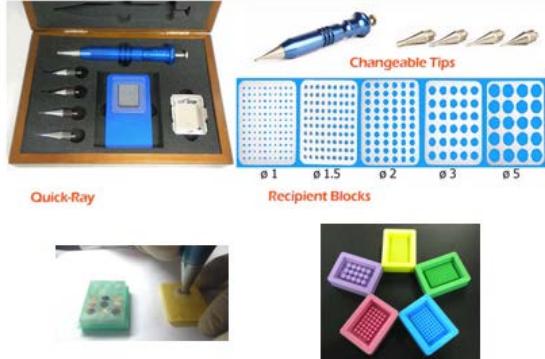
<https://cloud.10xgenomics.com/xeniumpaneldesigner>

Experimental design

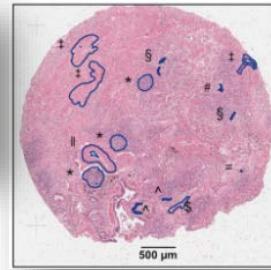
Take advantage of the large imaging area

Image-based spatial transcriptomics identifies molecular niche dysregulation associated with distal lung remodeling in pulmonary fibrosis

Annika Vannan^{1,a}, Ruqian Lyu^{2,3,a}, Arianna L. Williams¹, Nicholas M. Negretti⁴, Evan D. Mee¹, Joseph Hirsh⁴, Samuel Hirsh⁴, David S. Nichols⁵, Carla L. Calvi², Chase J. Taylor¹, Vasiliy V. Polosukhin⁶, Ana PM Serezani⁶, A. Scott McCall⁶, Jason J. Gokey⁶, Heejung Shim³, Lorraine B. Ware^{5,7}, Matthew J. Bacchetta⁸, Ciara M. Shaver⁵, Timothy S. Blackwell^{9,10}, Rajat Walia¹¹, Jennifer MS Sucre¹⁹, Jonathan A. Kropski^{5,9,10,b}, Davis J McCarthy^{2,3,b}, Nicholas E. Banovich^{1,b,*}

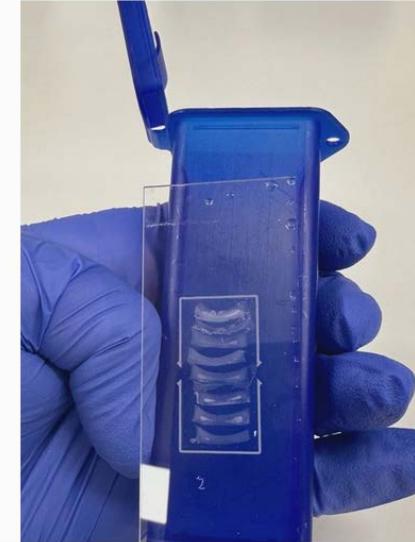


<https://www.ihcworld.com/products/Quick-Ray-Mold.htm>



Raymond Yip @rkhyp · Mar 4

Oh boy.. how lucky we are to have a histologist that can do this kind of magic ✨ #xenium

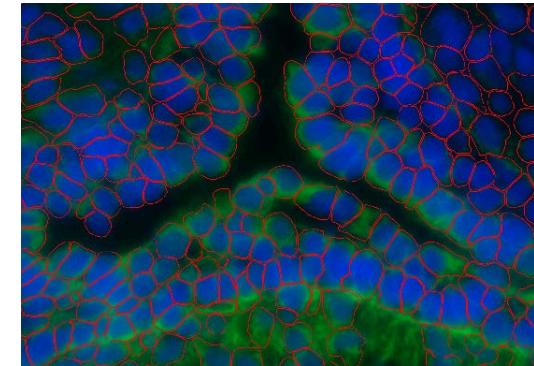
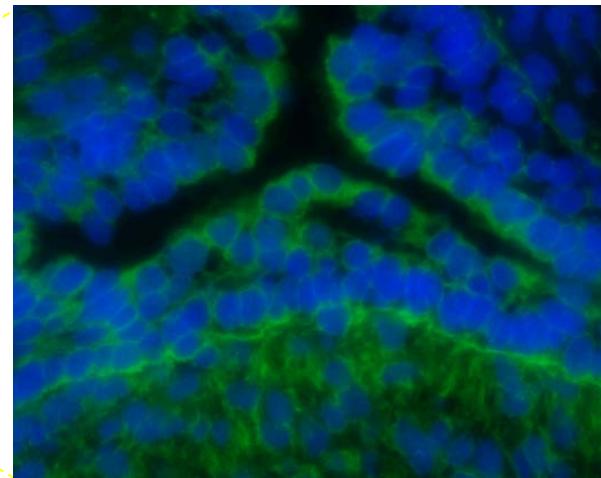
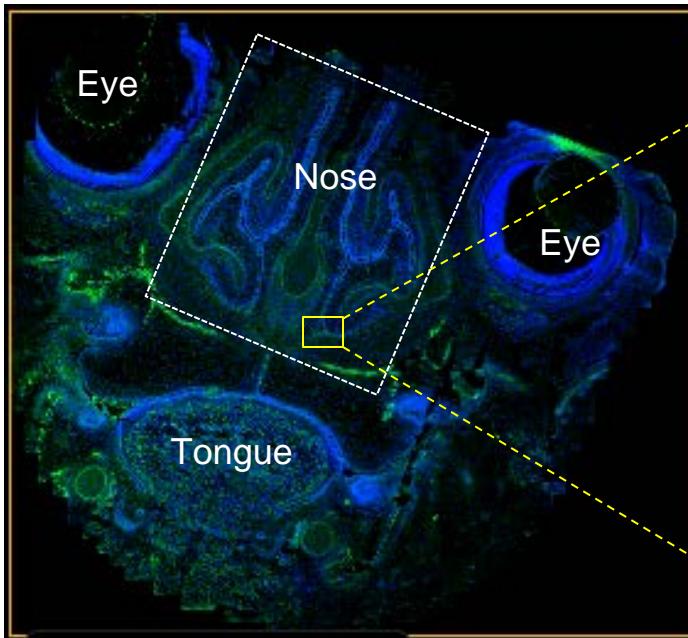


Each run is around 5 k€

multiplexing helps removing batch effect and increase replicates for a robust statistical analysis

MERSCOPE data acquisition (7 z -stack)

Staining for cell segmentation



Article | Published: 14 December 2020

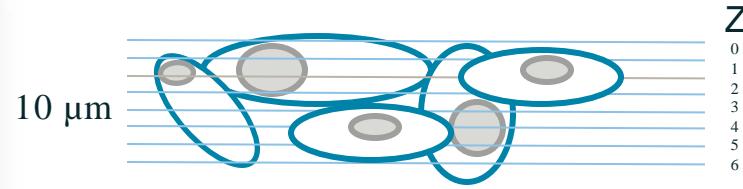
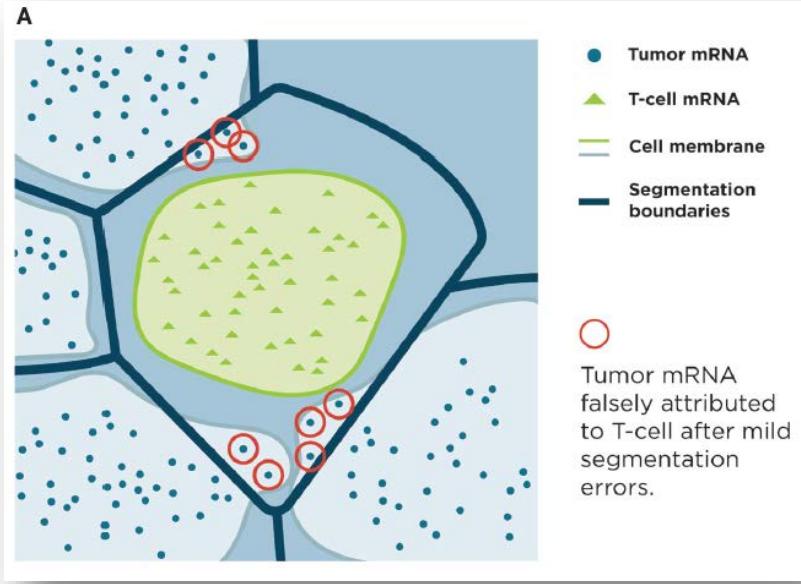
Cellpose: a generalist algorithm for cellular segmentation

Carsen Stringer, Tim Wang, Michalis Michaelos & Marius Pachitariu

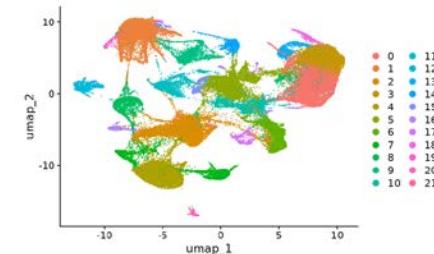
Nature Methods 18, 100–106 (2021) | [Cite this article](#)

Cell segmentation is crucial

Cell x gene matrix purity and good subsequent biology



3D segmentation required, actually not used, 2D segmentation per Z then harmonizing and summing the detected transcripts for all Z into the harmonized segmentation mask (nuclei of full cell)



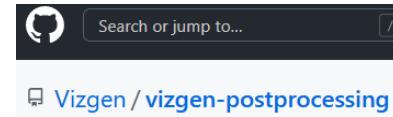
Vizgen Postprocessing tool

Run on Merscope standard analysis output folder

The Vizgen Post-processing Tool (VPT) enables users to reprocess and refine the single-cell results of MERSCOPE experiments. VPT is a command line tool that emphasizes scalable, reproducible analysis, and can be run on a workstation, a cluster, or be deployed in a cloud computing environment.

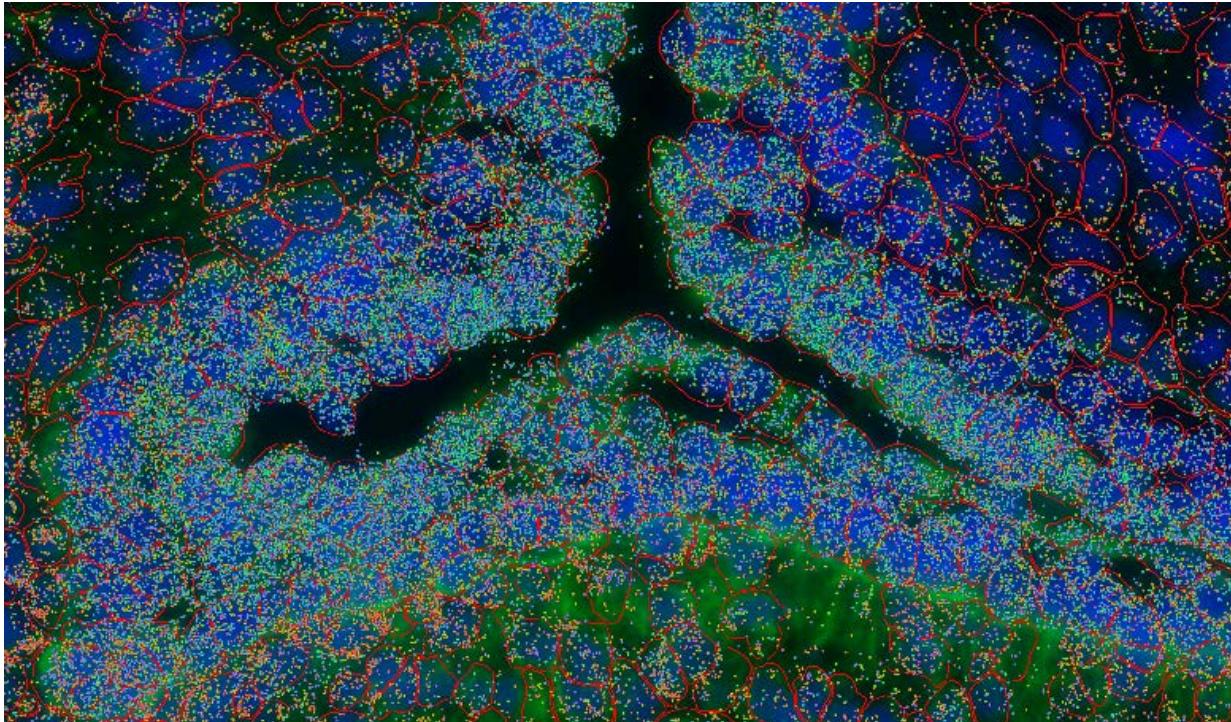
Features

- Perform cell segmentation
 - Reproduce standard Vizgen segmentation options
 - Perform reproducible custom segmentation
- Import cell segmentation from other tools
 - Supports geojson and hdf5 formats
- Regenerate single cell data with new segmentation
 - Cell by gene matrix
 - Cell spatial metadata
 - Image intensity in each cell
 - Update MERSCOPE Vizualizer file (vzg)
- Image format conversion
 - Convert large tiff files to single or multi-channel Pyramidal OME-TIFF files
- Nextflow compatible, example pipeline provided



MERSCOPE raw data

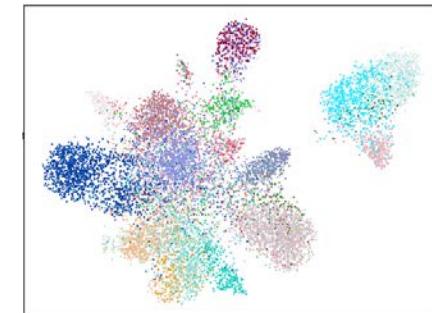
Cell x genematrix



Gene-level matrix

	100k's cells																				
Cltα	5	4	6	7	1	4	3	9	5	5	.	1	4	3	6	4	.	7	5	2	4
Myl6	5	2	5	1	2	4	13	2	5	2	4	4	1	4	8	4	2	3	3	1	1
Pkm	3	2	1	.	.	8	3	9	4	10	5	1	.	4	12	5	1	3	9	1	7
Tecr	3	1	5	2	4	6	5	9	6	3	2	2	1	4	7	1	1	1	3	2	8
Meis2	8	29	3	.	24	6	6	21	25	16	6	1	.	2	29	.	6	18	.	10	
...																					

1.000 Genes



MERSCOPE raw data

Standard Merscope output files

```
000-giacobini/000-data/202304281610_20230428-HuDeCa-Giacobini-PGW9-2-3A_VMSC06001/region_0:
```

```
total 13G
```

```
-rw-r--r-- 1 lebrigand solid 11G 3 mai 2023 202304281610_20230428-HuDeCa-Giacobini-PGW9-2-3A_VMSC06001_region_0.vzg
-rw-r--r-- 1 lebrigand solid 447M 3 mai 2023 cell_boundaries.parquet
-rw-r--r-- 1 lebrigand solid 83M 3 mai 2023 cell_by_gene.csv
-rw-r--r-- 1 lebrigand solid 46M 3 mai 2023 cell_metadata.csv
-rw-r--r-- 1 lebrigand solid 2,1G 3 mai 2023 detected_transcripts.csv
drwxr-xr-x 2 lebrigand solid 4,0K 3 mai 2023 images →
-rw-r--r-- 1 lebrigand solid 855K 3 mai 2023 summary.png
```

```
8,9K 3 mai 2023 manifest.json
227 3 mai 2023 micron_to_mosaic_pixel_transform.csv
6,6G 3 mai 2023 mosaic_Cellbound2_z2.tif
6,6G 3 mai 2023 mosaic_DAPI_z2.tif
```

0,5-2 Tb

```
1 sdata.shapes['P11_region_0_polygons']
✓ 0.0s
```

	geometry
361385220000910002	POLYGON ((4702.379 302.065, 4704.864 304.790, ...))
361385220000910005	POLYGON ((4709.067 304.517, 4709.887 305.673, ...))
361385220000910006	POLYGON ((4767.177 301.994, 4767.475 303.202, ...))
361385220000910007	POLYGON ((4723.735 306.300, 4723.939 307.090, ...))
361385220000910008	POLYGON ((4764.231 307.210, 4764.805 312.376, ...))

```
1 sdata.table.layers['counts']
✓ 0.0s
```

	0.0s
	array([[0, 1, 1, ..., 1, 0, 0], [0, 1, 0, ..., 2, 0, 0], [0, 3, 4, ..., 0, 0, 0], ..., [0, 0, 0, ..., 0, 0, 0], [0, 0, 0, ..., 0, 0, 0], [0, 2, 6, ..., 4, 0, 0]]])

```
1 sdata.table.obs
✓ 0.1s
```

	fov	volume	center.x	center.y	min.x	min.y	max.x	max.y	anisotropy
361385220000910002	NaN	841.761444	4707.689677	298.299088	4702.379316	292.031852	4713.603506	305.207677	1.990932
361385220000910005	NaN	762.740701	4713.532399	299.961526	4709.066587	293.764119	4718.738288	306.280330	1.812676
361385220000910006	NaN	1243.613805	4774.139602	300.963756	4767.176971	293.701627	4780.340526	307.566412	1.069031
361385220000910007	NaN	391.378917	4727.286292	305.141647	4723.734863	301.881750	4730.984004	308.291885	1.194130
361385220000910008	NaN	680.804739	4768.106763	308.764922	4764.065981	303.609728	4772.968368	313.933532	1.204513

```
1 sdata.points['P11_region_0_transcripts'].compute()
✓ 15.7s
```

	x	y	gene	Unnamed: 0	global.z	transcript.id	fov	barcode.id	cell.id
0	76.288345	6615.9116	CFTR	138	0.0	ENST000000003084	0	0	-1
1	-0.706320	6656.7720	CFTR	480	0.0	ENST000000003084	0	0	-1
2	56.071490	6741.6104	CFTR	1284	0.0	ENST000000003084	0	0	3613852200420100110
3	-6.401468	6764.1587	CFTR	1479	0.0	ENST000000003084	0	0	-1
4	12.010611	6604.1650	CFTR	1808	1.0	ENST000000003084	0	0	3613852200390100641

MERSCOPE data analysis

Data analysis workflows

Seurat 5.0.1
Install Get started Vignettes Extensions FAQ News Reference Archive

SEURAT
squidpy stable

GENERAL
Installation API Classes Release Notes References

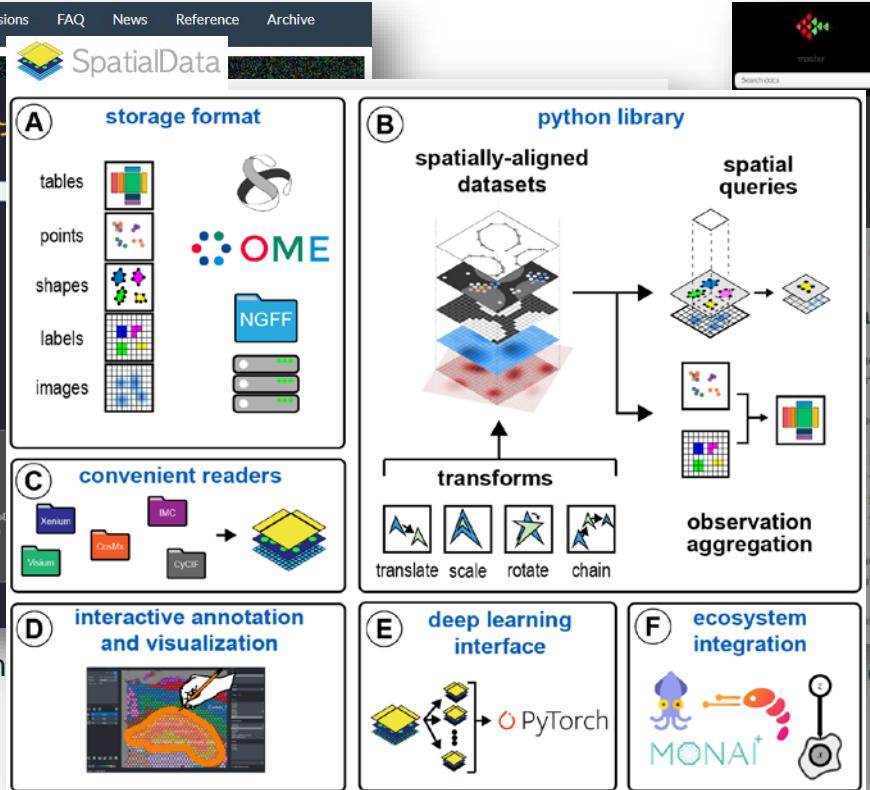
GALLERY
Tutorials Examples

We are excited to release Seurat v5! To ins new features and functionality:

Satija'slab, NYGC

Simplify infrastructure with MongoDB Atlas, the leading developer data platform. Ad by EthicalAds

Theis'slab, h



Scverse ecosystem, Oliver Stegle & Fabian J. Theis

+ Giotto Edit on GitHub

Spatial transcriptomic and proteomic technologies have provided new opportunities to investigate cells in their native microenvironment. Here we present Giotto, a comprehensive and open-source toolkit for spatial data analysis and visualization. The analysis module provides end-to-end analysis by implementing a wide range of algorithms for characterizing tissue composition, spatial expression patterns, and cellular interactions. Furthermore, single-cell RNAseq data can be integrated for spatial cell-type enrichment analysis. The visualization module allows users to interactively visualize analysis outputs and imaging features. To demonstrate its general applicability, we apply Giotto to a wide range of datasets encompassing diverse technologies and platforms.

Example Functionalities

monkeybread

monkeybread is a Python package that facilitates the analysis of single-cell resolution spatial transcriptomics data such as those generated by the MERSCOPE or Xenium platforms.

monkeybread provides tools that enable:

- Identification of cellular niches (i.e., regions with distinct compositions of cell types)
- Visualization of density of cell types across the tissue
- Statistical tests for testing for colocalization between cell types
- Statistical tests and visualization for ligand-receptor co-expression between neighboring cells

monkeybread operates on datasets stored as AnnData objects and thus, can be integrated into analyses that use packages from the scverse such as scanpy or squidpy.

monkeybread was developed at Immunitas Therapeutics.

CS

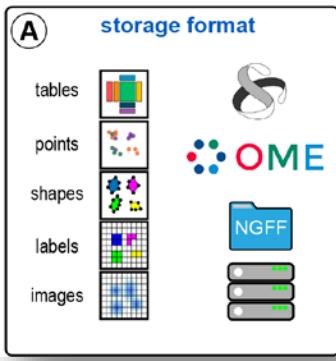
Product Solutions Open Source Pricing

cobioda / scisipy Public

<https://github.com/cobioda/scisipy/>

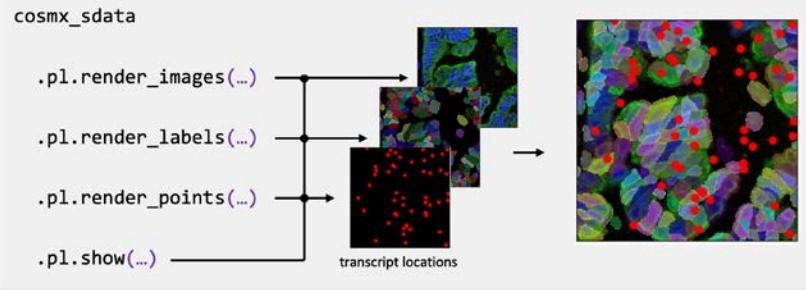
spatialdata data architecture

Scverse ecosystem

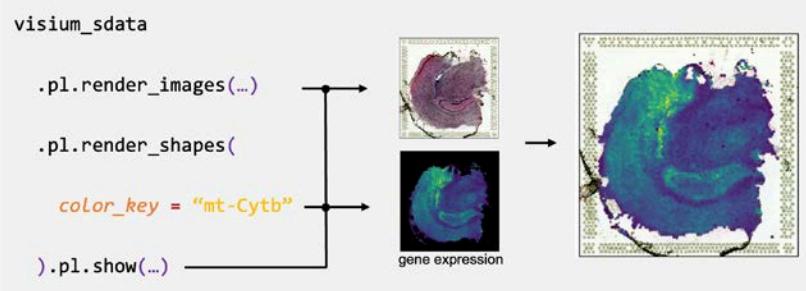


```
1 sdata
✓ 2.1s
SpatialData object with:
├── Images
│   └── 'P11_region_0_z2': MultiscaleSpatialImage
├── Points
│   ├── 'P11_region_0_transcripts': DataFrame
│   └── 'celltypes': DataFrame with shape: (22, 1)
├── Shapes
│   ├── 'P11_region_0_polygons': GeoDataFrame
│   ├── 'anatomical': GeoDataFrame shape: (22, 1)
│   └── 'arteries': GeoDataFrame shape: (22, 1)
└── Table
    AnnData object with n_obs × n_vars = 22 × 22
    ├── obs: 'fov', 'volume', 'center_x', 'center_y'
    ├── var: 'mean', 'std'
    ├── uns: 'leiden', 'leiden_colors', 'neighbors'
    ├── obsp: 'connectivities', 'distances': AnnData
    ├── layers: 'counts', 'scaled'
    └── microns, with elements:
        P11_region_0_z2 (Images), P11_region_0_z2 (Shapes)
```

Rapidly visualise different modalities

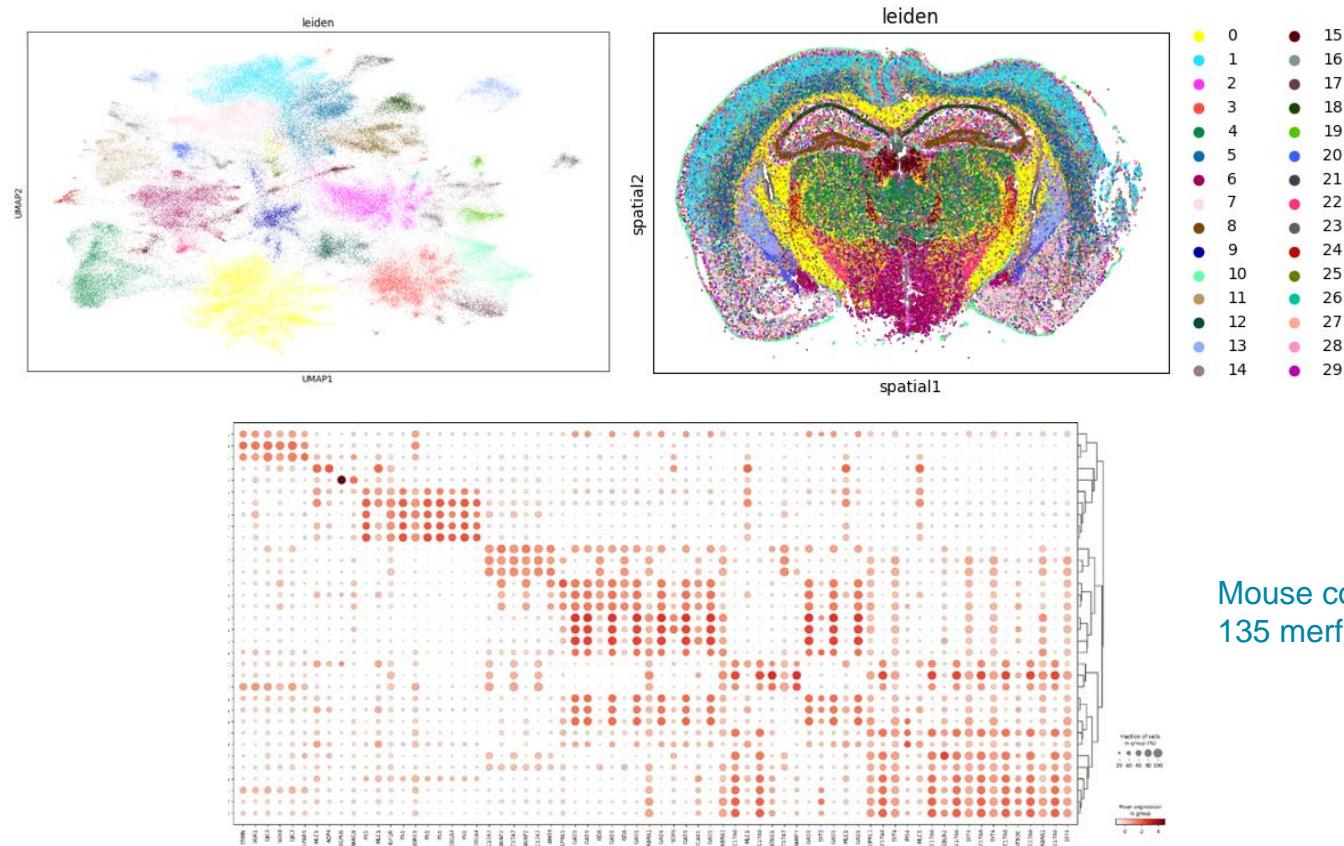


Enrich visualisation with metadata



Cell type labeling

Directly from your target gene panel statistical analysis



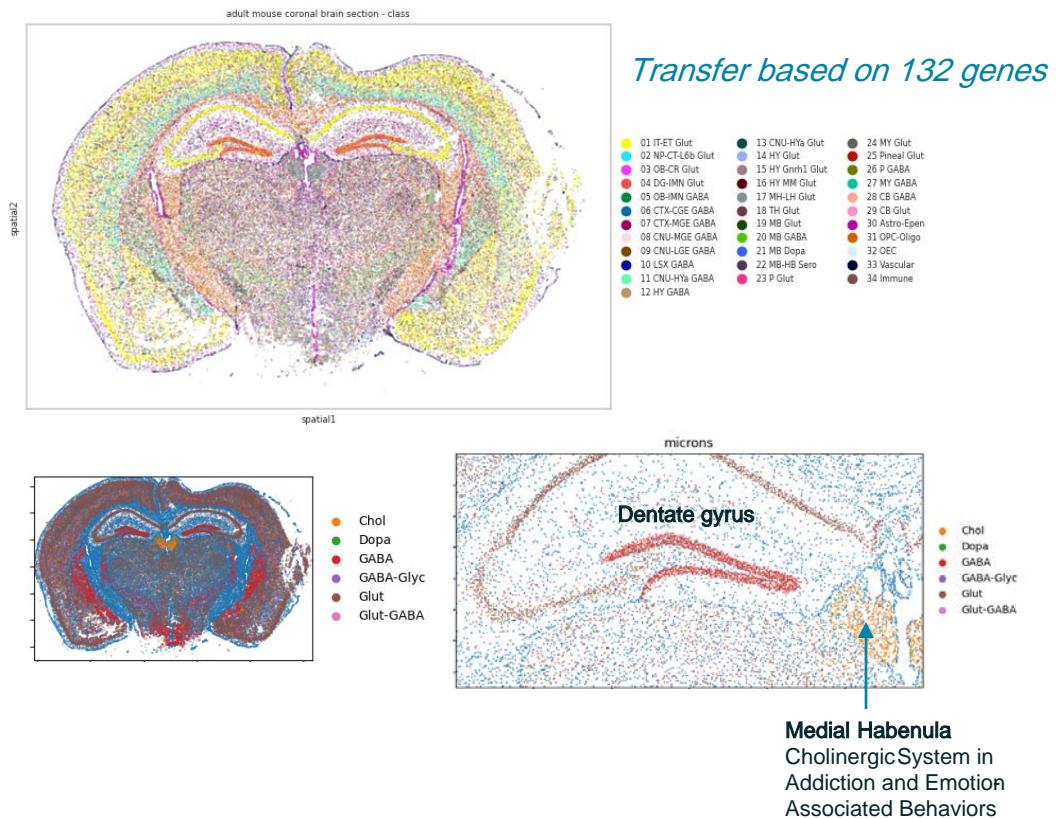
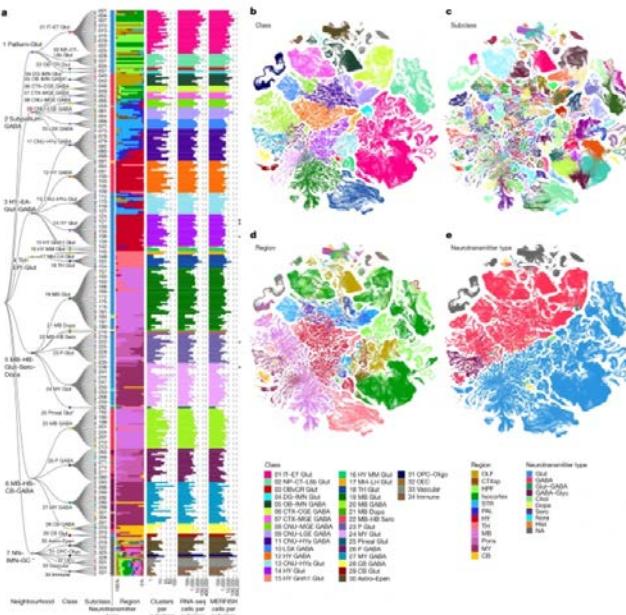
Cell type labeling using annotation transfer

Based on a reference singlecell dataset using SCANVI

Article | Open access | Published: 13 December 2023

A high-resolution transcriptomic and spatial atlas of cell types in the whole mouse brain

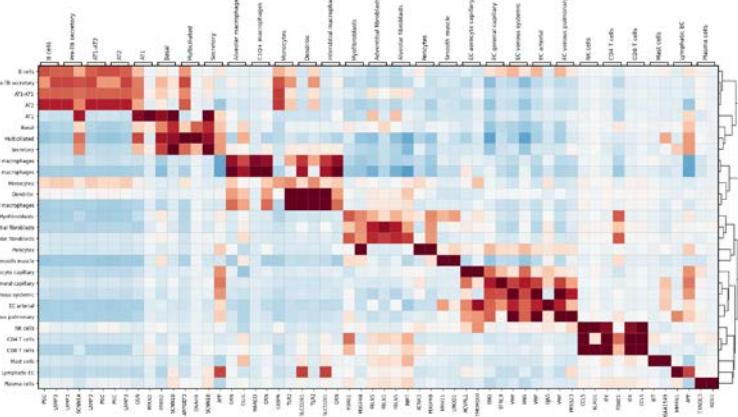
Zizhen Yao , Cindy T. J. van Velthoven, Michael Kunst, Meng Zhang, Delissa McMillen, Changkyu Lee, Won Jung, Jeff Goldy, Aliya Abdelhak, Matthew Aitken, Katherine Baker, Pamela Baker, Eliza Barkan, Darren Bertagnoli, Ashwin Bhandiwad, Cameron Bielstein, Prajal Bishwakarma, Jazmin Campos, Daniel Carey, Tamara Casper, Anish Bhaswanth Chakka, Rushil Chakrabarty, Sakshi Chavan, Min Chen, ... Hongkui Zeng



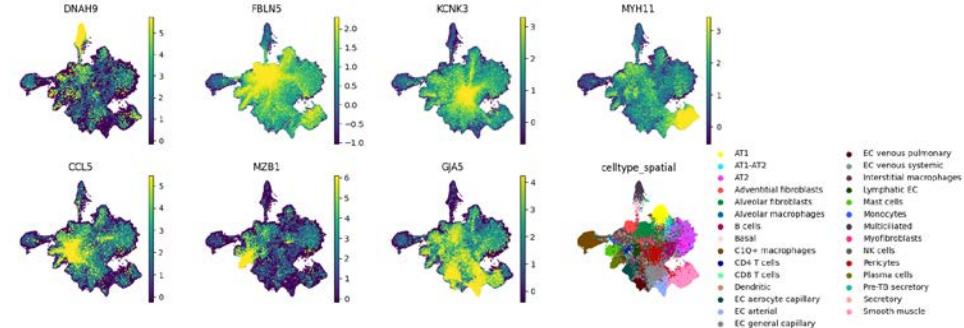
Standard single -cell data analysis

Scanpy package

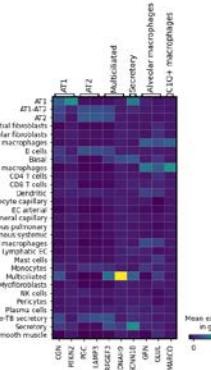
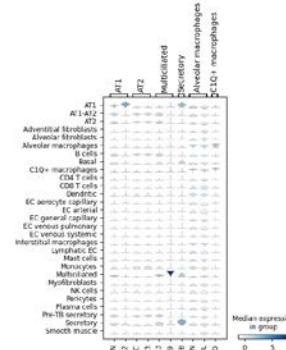
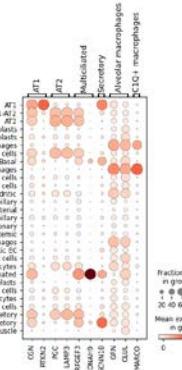
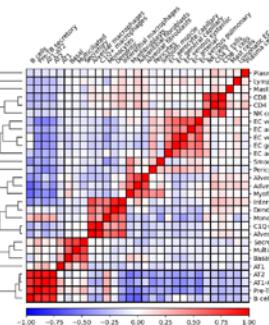
Gene markers



Gene markers

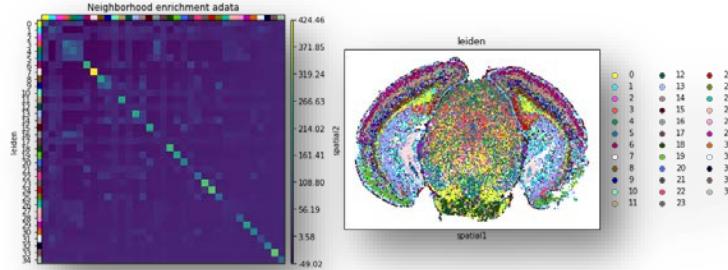


Cell type correlation



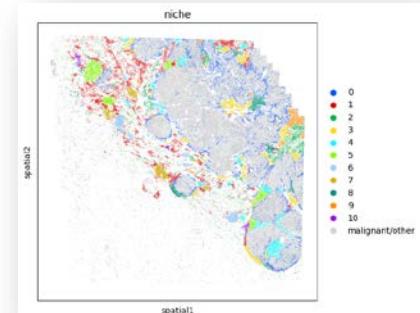
Standard single -cell spatial data analysis

New vast area for computationalbiologists (just like single-cell 5 years ago !)



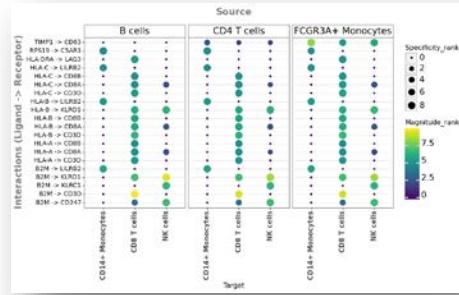
Neighbors enrichment analysis

Test if cells belonging to 2 clusters are close to each other more often than expected (**co-occurrence probability**)



Cellular niches analysis

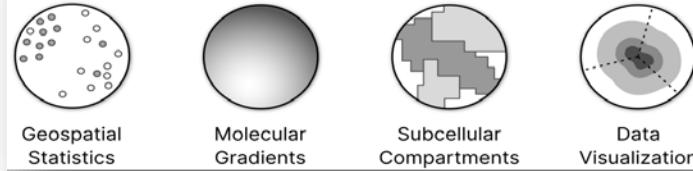
for each cell, we count the number of neighbors that are of each cell type thus forming a “neighborhood profile” vector of length C, where C is the number of cell types. We then cluster all neighborhood profiles and call each cluster a “niche”.



Ligand-Receptor analysis

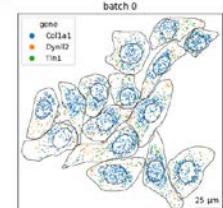
- Need to be in gene panel !!
- CellPhoneDB[Efremova et al., 2020]
- Omnipath[Türeli et al., 2016].

Machine Learning & Statistical Analysis



Sub-cellular exploration

Bento is a Python toolkit for performing subcellular analysis of spatial transcriptomics data.



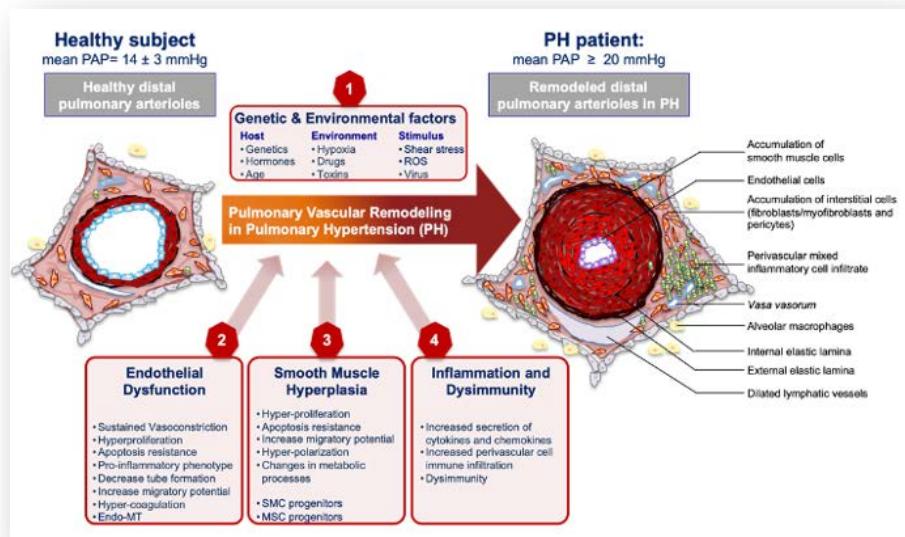
03

Ongoing projects

PAH : Pulmonary Arterial Hypertension

A rare vascular disorder

Characterized by the presence of occluded pulmonary arterioles resulting from the proliferation of pulmonary artery endothelial cells (PAECs), pulmonary artery smooth muscle cells (PASMCs) and fibroblasts, which leads to right heart hypertrophy and eventual cardiac failure

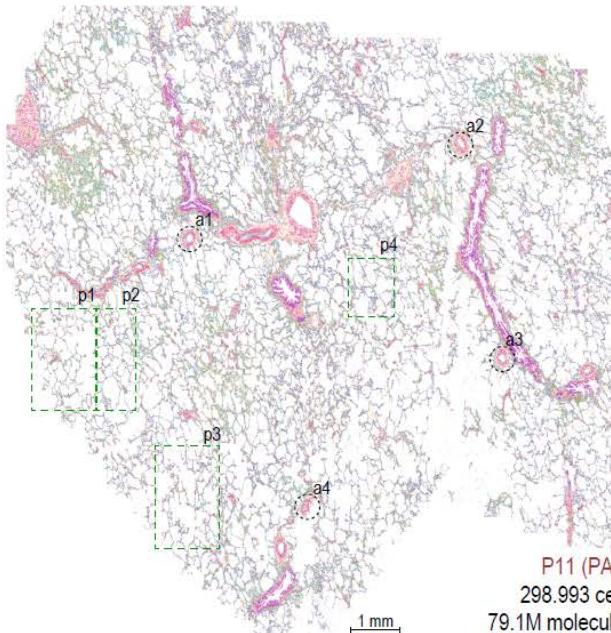
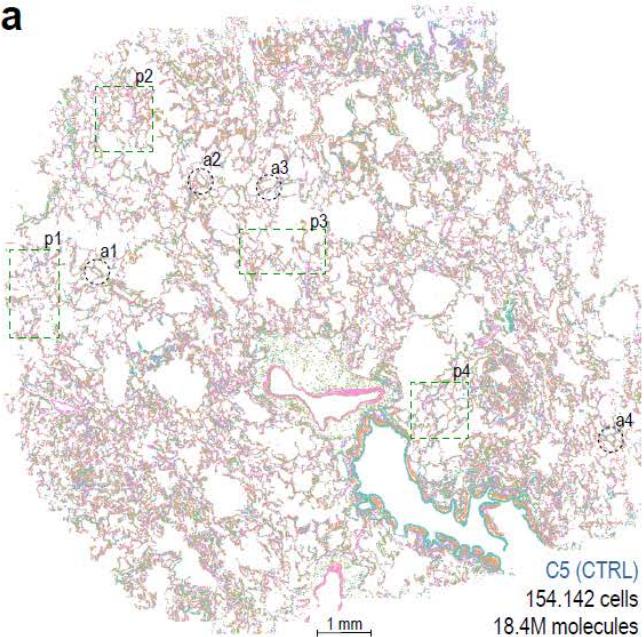


- Defined by a mean pulmonary arterial pressure >20mmHg
- More frequent in women to men (2:1 to 4:1)
- Different origins:
 - IPAH (idiopathic or sporadic cases),
 - HPAH (heritable case family history) 6-10%
monogenic autosomal-dominant - 14% ♂, 42% ♀
 - APAH (associated forms), anorexigens / liver / congenital heart / connective tissue disease

PAH : Pulmonary Arterial Hypertension

A rare vascular disorder

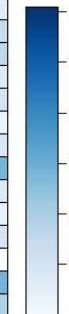
a



b

C5		P11	
6.8	3		
12	8.8	5.2	6.3
6.6	9.7		
5.3	6.4		
5.7	3.7		
5.5	4.5		
7.4	10		
7	1.5		
2.3	5.7		
1.4	5.8		

cell type prop.

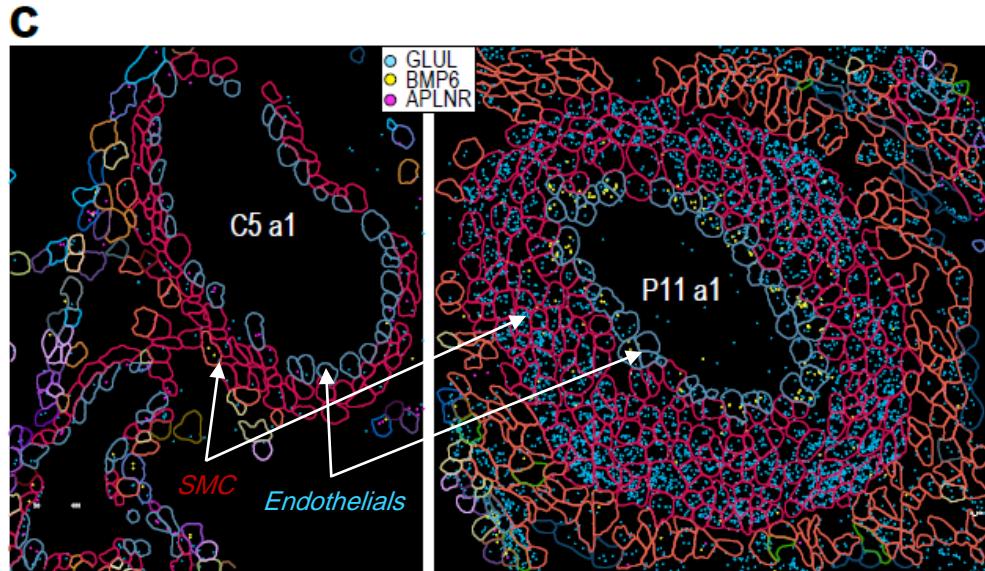


1 mm

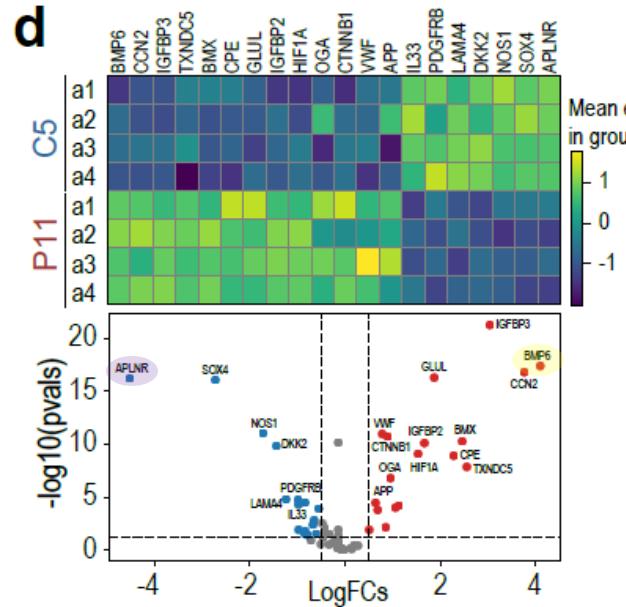
1 mm

PAH : Pulmonary Arterial Hypertension

A rare vascular disorder



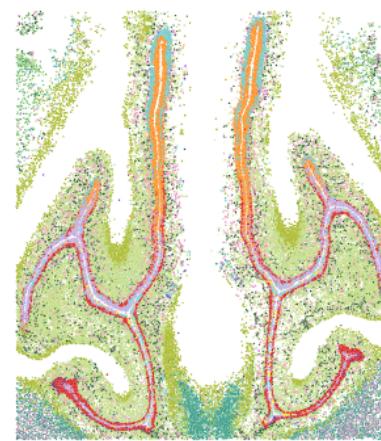
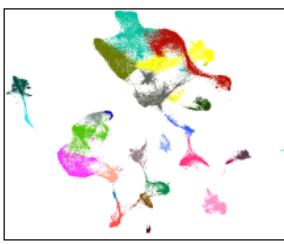
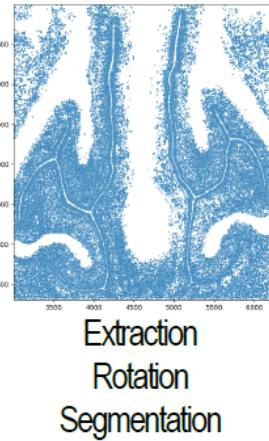
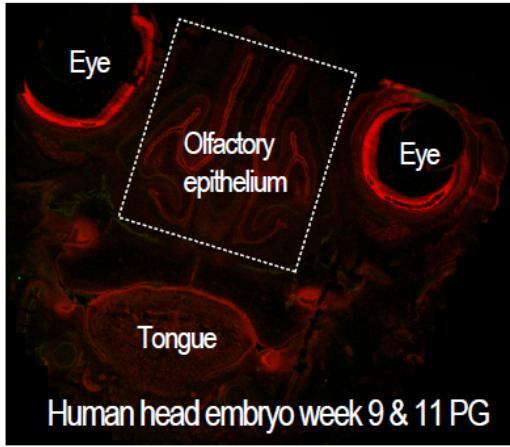
GLUL up in PAH artEC SMC
BMP6 up in PAH artEC
APLNR down in PAH artEC



Artery Endothelial Cells
pseudo-bulk analysis

HuDeCa project

human fetal nose from 7 to 12 post-conceptional weeks (PCW) at singlecell resolution

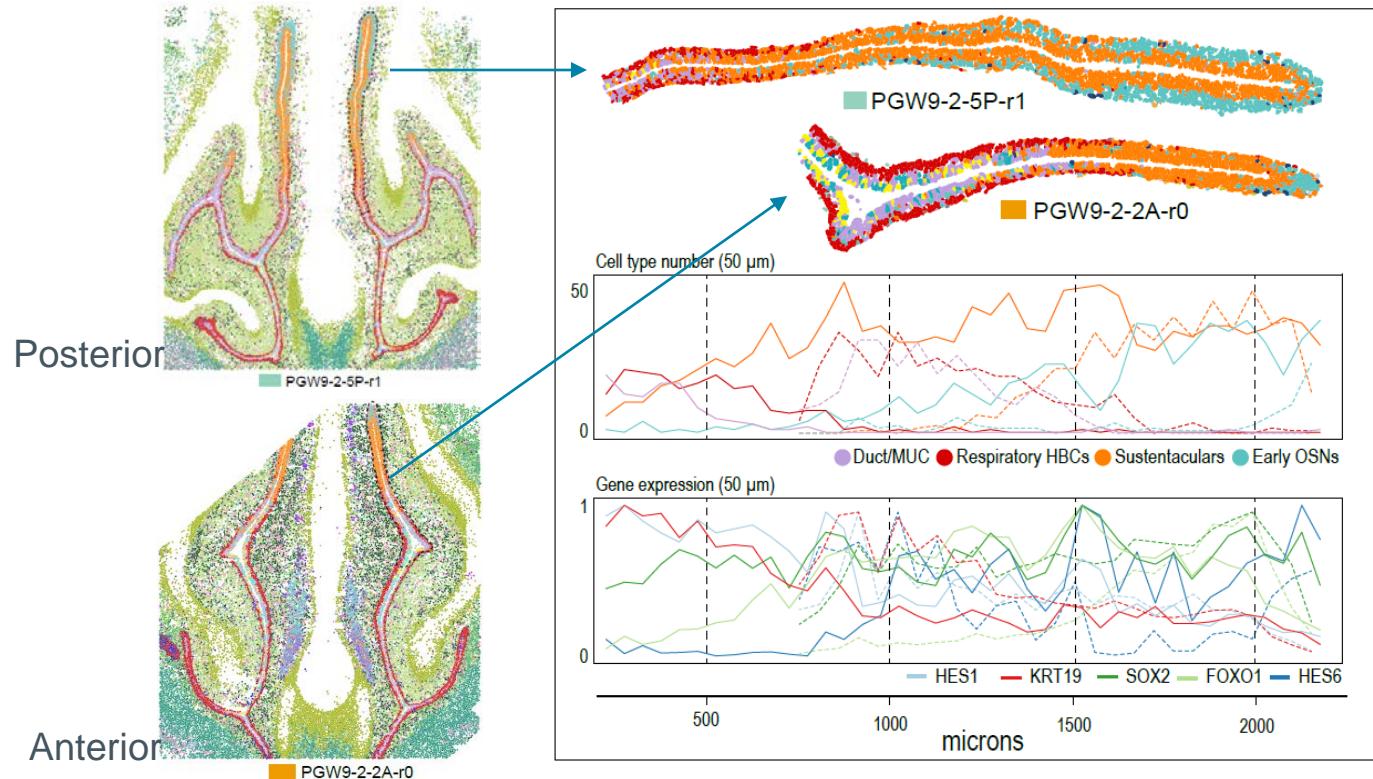


- Cell types
- Cartilage
 - Stromal
 - Lymphatic EC
 - Vascular EC
 - Pericytes
 - Respiratory HBCs
 - Olfactory HBCs
 - Duct/MUC
 - Multiciliated
 - Deuterosomal
 - Sustentaculars
 - GBCs
 - Early OSNs
 - Excitatory neurons
 - Inhibitory neurons
 - GnRH neurons

HuDeCa project

human fetal nose from 7 to 12 post-conceptional weeks (PCW) at singlecell resolution

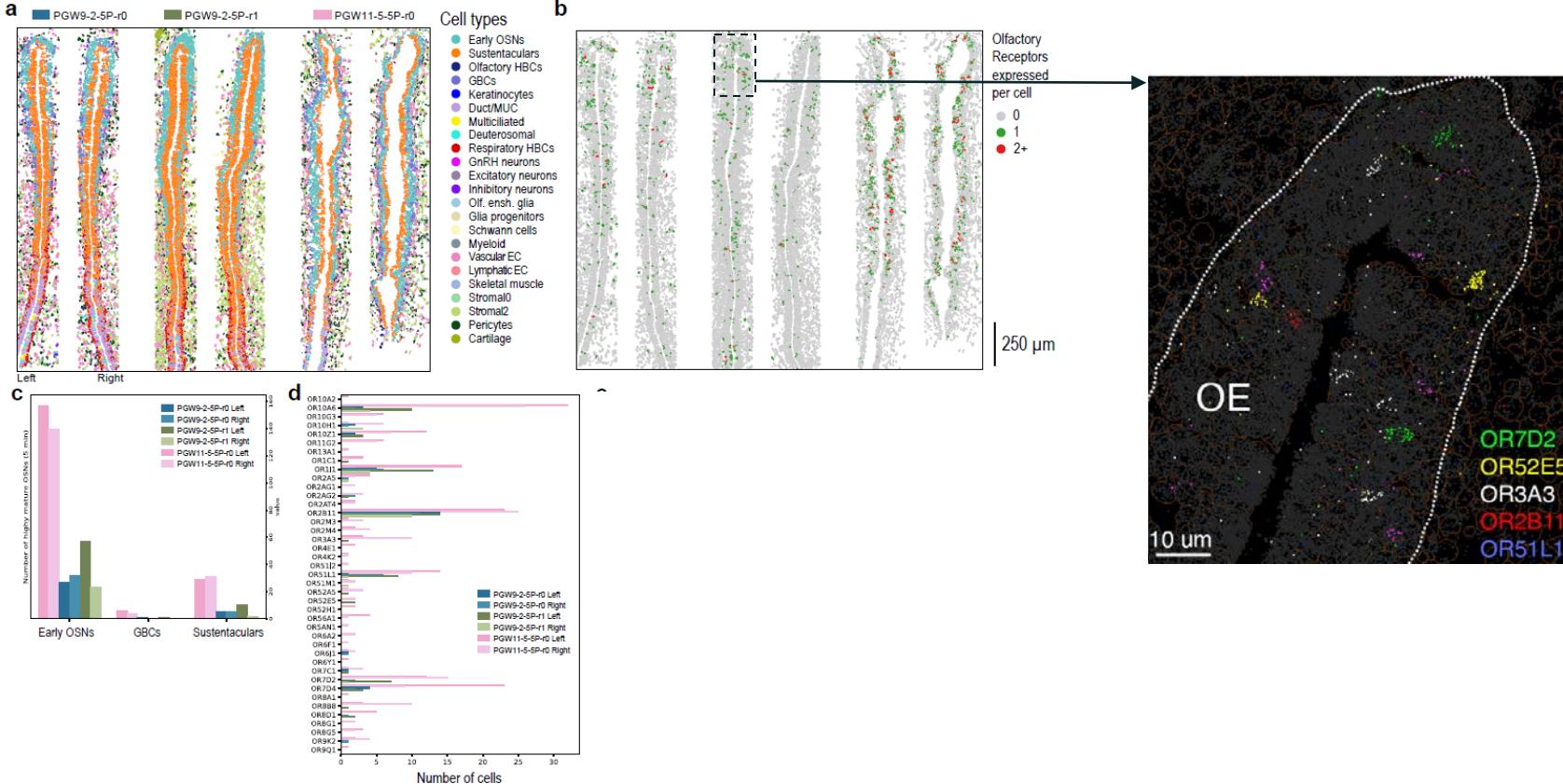
Posterior versus Anterior Olfactory epithelium comparison



HuDeCa project

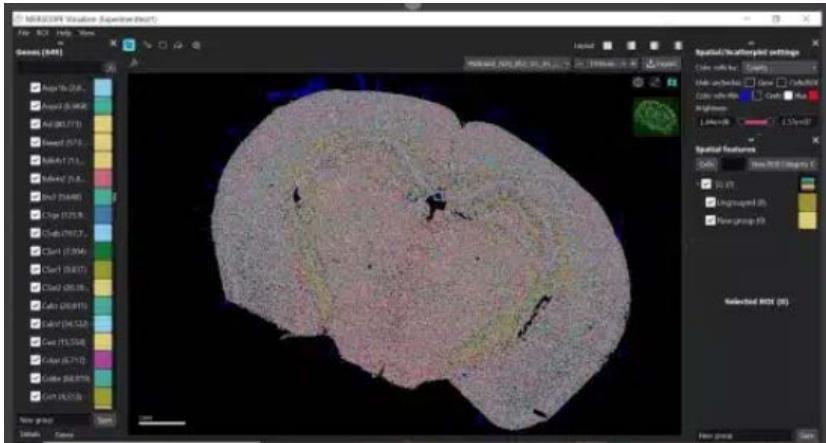
human fetal nose from 7 to 12 post-conceptional weeks (PCW) at single-cell resolution

Posterior Olfactory epithelium Week9 versus week11 comparison

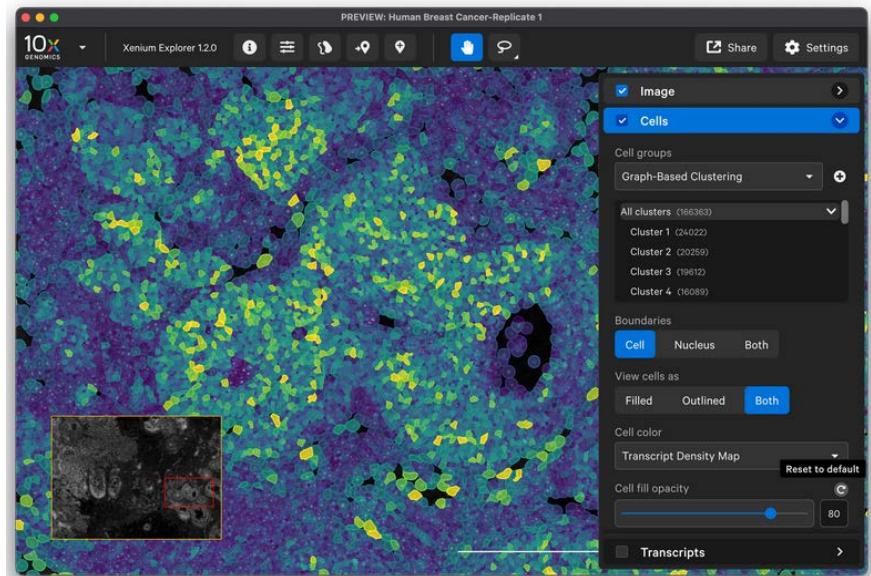


Spatial transcriptomics explorer

MERSCOPEvizualizer software



Xeniumexplorer



https://github.com/quentinblampey/spatialdata_xenium_explorer

Acknowledgments

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