

Single-cell and Spatial Transcriptomics

From sequencing to imaging

Kévin Lebrigand

Computational Biology and Omics Data Analysis

 <https://cobioda.github.io>

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CÔTE D'AZUR



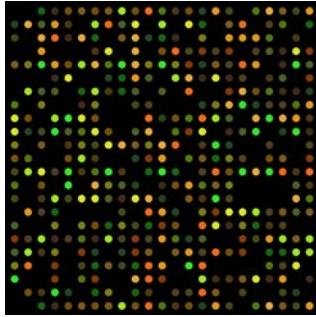
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CoBiODA
Computational Biology
Omics Data Analysis
CÔTE D'AZUR

20 years of transcriptomics

Driven by microfluidics technological developments

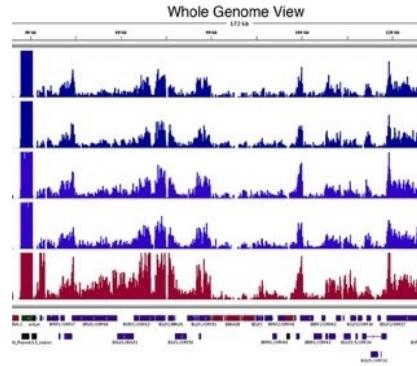


Early 2000's: DNA microarray

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



Cost : 4k€
20 samples
25k genes
0,5M matrix

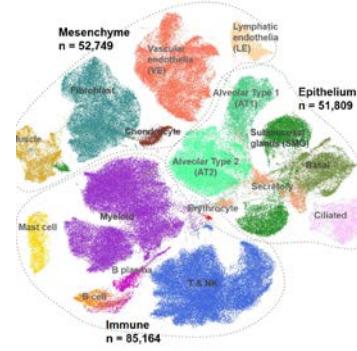


Late 2000's : RNA sequencing

- 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

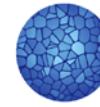
- 300-1000 gene targets
- Imaging analysis
- MultiplexingFiSH(single molecule)
- Sensitivity (3080%)
- Sub-cellular resolution



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

Human Cell Atlas

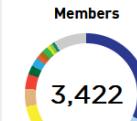
CZI initiative (2016)



HUMAN
CELL
ATLAS

Mission to create comprehensive reference maps of all human cells, the fundamental units of life, as a basis for both understanding human health and diagnosing, monitoring, and treating disease.

HCA Metrics Dashboard



Countries

101

Institutes

1,787

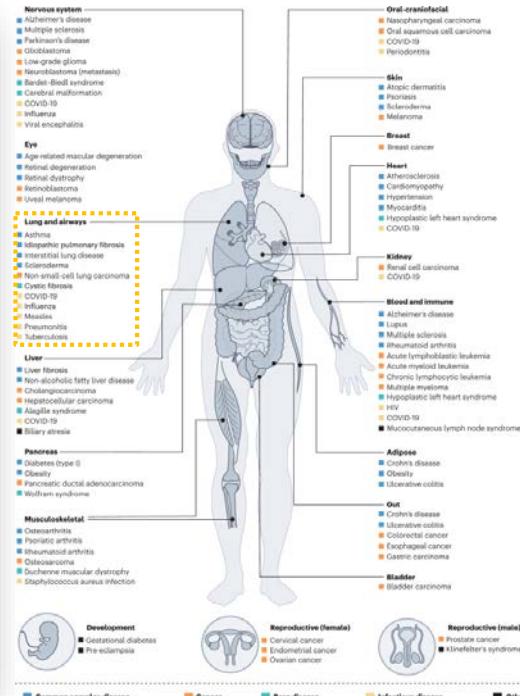
Networks

18

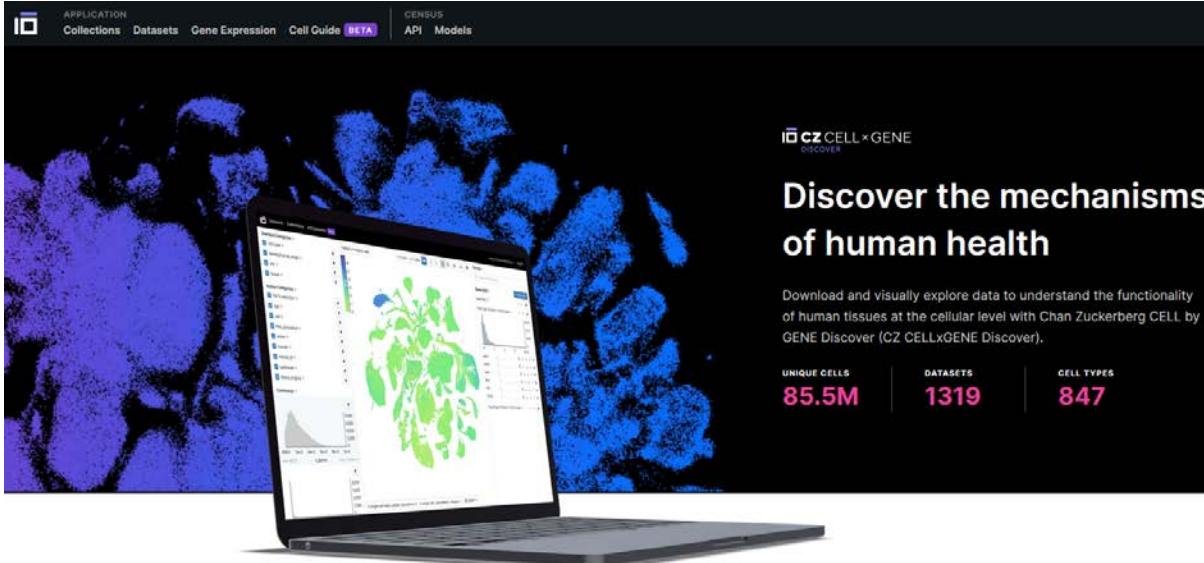
Publications

199

Global distribution of HCA members



■ Common complex disease ■ Cancer ■ Rare disease ■ Infectious disease ■ Other



The homepage features a large image of a laptop displaying the Chan Zuckerberg CELL Discover interface. The interface shows a complex tree-like network of cell types and datasets. Below the laptop, a section titled "Census" provides details about the service, including its availability as a free-to-use API and data service.

Census

Census provides access to any custom slice of standardized cell data available on CZ CELLxGENE Discover in R and Python.

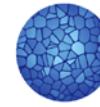
[See quick start tutorial >](#)

Free-to-use service (API + Data) that allows for querying its single-cell data corpus directly into Python or R.

Discover the mechanisms of human health

Download and visually explore data to understand the functionality of human tissues at the cellular level with Chan Zuckerberg CELL by CZ CELLxGENE Discover.

UNIQUE CELLS: 85.5M | DATASETS: 1319 | CELL TYPES: 847



New Results

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Universal Cell Embeddings: A Foundation Model for Cell Biology

Yanay Rosen, Yusuf Roohani, Ayush Agarwal, Leon Samotorčan, Tabula Sapiens Consortium, Stephen R. Quake, Jure Leskovec

doi: <https://doi.org/10.1101/2023.11.28.568918>

This article is a preprint and has not been certified by peer review [what does this mean?].



Article | Published: 26 February 2024

scGPT: toward building a foundation model for single-cell multi-omics using generative AI

Haojian Cui, Chloe Wang, Hassan Maan, Kuan Pang, Fengning Luo, Nan Duan & Bo Wang

[Nature Methods \(2024\)](#) | [Cite this article](#)

40k Accesses | 1 Citations | 126 Altmetric | [Metrics](#)

Nicheformer: a foundation model for single-cell and spatial omics

Anna C. Schaar, Alejandro Tejada-Lapuerta, Giovanni Palla, Robert Gutgesell, Lennard Halle, Maria Minaeva, Larsen Vornholz, Leander Dony, Francesca Drummer, Mojtaba Bahrami, Fabian J. Theis

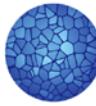
doi: <https://doi.org/10.1101/2024.04.15.589472>

This article is a preprint and has not been certified by peer review [what does this mean?].



Human Cell Atlas

Pascal Barbuy's lab contribution



HUMAN
CELL
ATLAS

2019

TECHNIQUES AND RESOURCES | 23 OCTOBER 2019

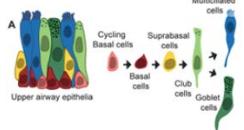
Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures

In collection: Human development

Sandra Ruiz Garcia¹, Marie Deprez¹, Kevin Lebrigand¹, Amélie Cavad¹, Agnès Paquet¹, Marie-Jeanne Arguel¹, Virginie Magnone¹, Marin Truchi¹, Ignacio Caballero¹, Sylvie Leroy¹, Charles-Hugo Marquette¹, Brice Marcat¹, Pascal Barbuy^{1,2}, Laure-Emmanuelle Zaragozi^{1,2}

+ Author and article information

Development (2019) 146 (20): dev177428.



2019

Home > American Journal of Respiratory and Critical Care Medicine > List of Issues > Volume 202, Issue 12

A Single-Cell Atlas of the Human Healthy Airways

Marie Deprez^{1*}, Laure-Emmanuelle Zaragozi^{1,2}, Marin Truchi¹, Christophe Becavin¹, Sandra Ruiz Garcia¹, Marie-Jeanne Arguel¹, Magali Plaisant¹, Virginie Magnone¹, Kevin Lebrigand¹, Sophie Abelanet¹, Frédéric Brau¹, Agnès Paquet¹, Dana Pé'er², Charles-Hugo Marquette¹, Sylvie Leroy^{1,2†}, and Pascal Barbuy^{1,2}

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21 125 215

https://doi.org/10.1164/rccm.201911-2199OC PubMed: 32726565

Received: November 15, 2019 Accepted: July 28, 2020



80K

2020

High throughput error corrected Nanopore single cell transcriptome sequencing

Kevin Lebrigand¹, Virginie Magnone¹, Pascal Barbuy¹ & Rainer Waldmann²

Nature Communications 11, Article number: 4025 (2020) | Cite this article



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2023

The spatial landscape of gene expression isoforms in tissue sections

Kevin Lebrigand, Joseph Bergensträhle, Kim Thrane, Annelie Mollbrink, Konstantinos Meletis, Pascal Barbuy, Rainer Waldmann, Joakim Lundeberg | Author Notes

Nucleic Acids Research, Volume 51, Issue 8, 8 May 2023, Page e47, <https://doi.org/10.1093/nar/gkac331>

2021

Analysis | Published: 02 March 2021

Single-cell meta-analysis of SARS-CoV-2 entry genes across tissues and demographics

Christoph Muus¹, Malte D. Luecken², Gökmen Eraslan¹, Lisa Sikkema¹, Avinash Waghray¹, Graham Heimb erg¹, Yoshihiko Kobayashi¹, Eeshit Dhaval Vaishnav¹, Ayswarya Subramanian¹, Christopher Smilie¹, Karthik A. Jagadeesh¹, Elizabeth Thu Duong¹, Evgenij Fiskin¹, Elena Torral Trigilia¹, Meshal Ansari¹, Peiven Cai¹, Brian Lin¹, Justin Buchanan¹, Sijia Chen¹, Jian Shu¹, Adam L. Haber¹, Hattie Chung¹, Daniel T. Montero¹, Taylor Adams¹, The NHBL LungMap Consortium & The Human Cell Atlas Lung Biological Network

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Nature Medicine 27, 546–559 (2021) | Cite this article

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2021

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Perspective | Published: 08 September 2021

A roadmap for the Human Developmental Cell Atlas

Muzaffar Hamza¹, Deanne Taylor¹, Sten Linnarsson¹, Bruce J. Aronow¹, Gary D. Bader¹, Roger A. Barker¹, Pablo G. Camara¹, J. Gray Camp¹, Alain Chédotal¹, Andrew Copp¹, Heather C. Etchevers¹, Paolo Giacobini¹, Berthold Göttgens¹, Guojun Guo¹, Anja Hupialis¹, Kylie R. James¹, Emily Kirby¹, Arnold Kriegstein¹, Joakim Lundeberg¹, John C. Marioni¹, Kerstin B. Meyer¹, Kathy K. Niakan¹, Mats Nilsson¹, Bayanee Olabi¹, Human Cell Atlas Developmental Biological Network

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Nature 597, 196–205 (2021) | Cite this article

65k Accesses | 87 Citations | 324 Altmetric | Metrics

2022

The discovAIR project: a roadmap towards the Human Lung Cell Atlas

Malte D. Luecken^{1,2*}, Laure-Emmanuelle Zaragozi^{1,2,3}, Elo Madisoona^{3,4,26}, Lisa Sikkema^{1,3,26}, Alexandra B. Firsova^{5,26}, Elena De Domenico^{6,26}, Louis Kümmeler^{1,26}, Adem Saglam^{5,26}, Marijn Berg^{7,8,26}, Aurore C. A. Gay^{7,8,26}, Janine Schniering^{9,26}, Christoph H. Mayr^{7,26}, Xésus M. Abalo^{10,26}, Ludvig Larsson^{10,26}, Alexandros Sountoulidis^{5,26}, Sarah A. Teichmann^{3,11}, Karen van Eunen^{12,13}, Gerard H. Kopelman^{8,12}, Kouroush Saeb-Parsy¹⁴, Sylvie Leroy¹⁵, Pippa Powell¹⁵, Ugis Sarkans¹⁶, Wim Timens^{17,8}, Joakim Lundeberg¹⁷, Maarten van den Berg^{8,18}, Mats Nilsson¹⁹, Peter Horváth¹⁹, Jessica Denning¹⁴, Irene Papathodorou²⁰, Joachim L. Schultz^{6,20,21}, Herbert B. Schiller²⁰, Pascal Barbuy²⁰, Ilya Petukhov²², Alexander V. Misharin²³, Ian M. Adcock²⁴, Michael von Papen²⁵, Fabian J. Theis¹, Christos Samakovlis², Kerstin B. Meyer³ and Martijn C. Nawijn^{1,7,8}

500K



2023

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Resource | Open access | Published: 08 June 2023

An integrated cell atlas of the lung in health and disease

Lisa Sikkema, Ciro Ramirez-Suárez, Daniel C. Strobl, Tessa E. Gillett, Luke Zappia, Elo Madisoona, Nikolay M. Markov, Laure-Emmanuelle Zaragozi, Yuge Ji, Meshal Ansari, Marie-Jeanne Arguel, Leonie Apperloo, Martin Banchez, Christophe Récamier, Marijn Berg, Evgenij Chelnitskiy, Mei-Ji Chung, Antoine Collin, Aurore C. A. Gay, Janine Gote-Schniering, Baharak Hooshpar Kashani, Kemal Incelik, Manu Jain, Theodore S. Kapellou, Lung Biological Network Consortium ... Fabian J. Theis

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Nature Medicine 29, 1563–1577 (2023) | Cite this article

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2,4M



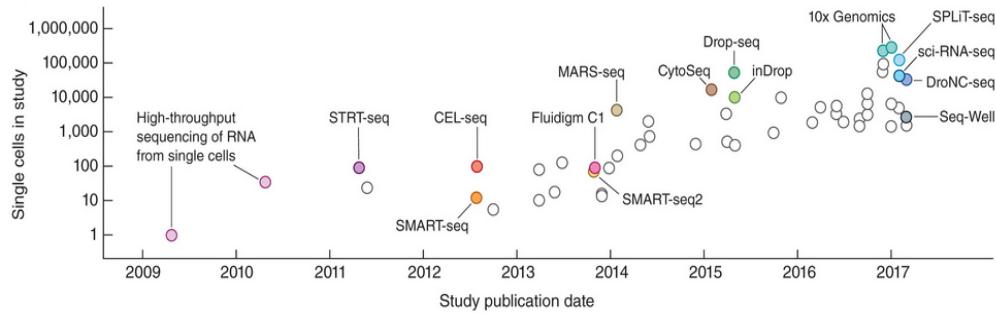
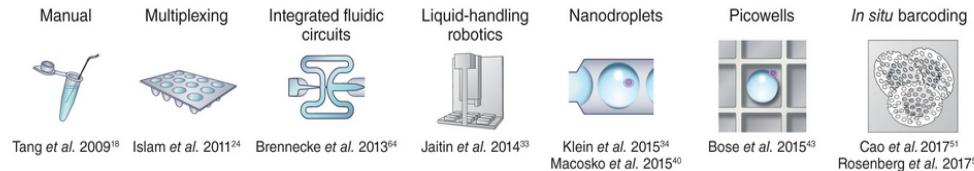
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01

Single -Cell isoform Transcriptomics

Single -cell transcriptomics

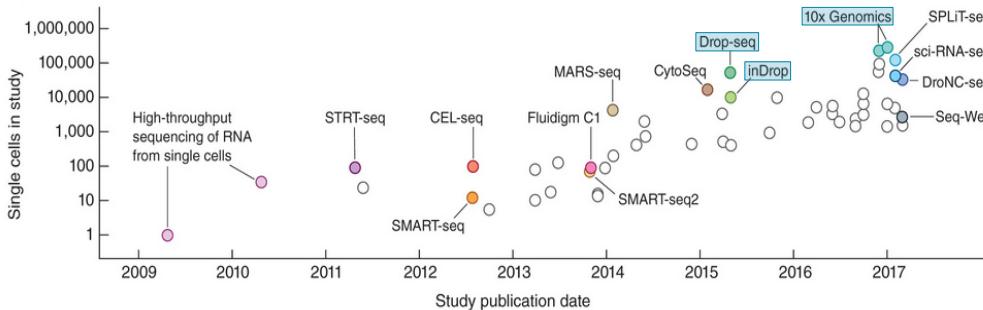
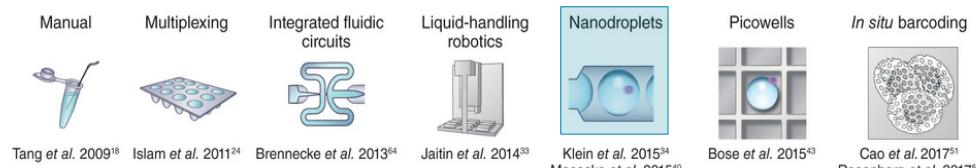
Evolution of isolation techniques and throughput



Exponential scaling of single-cell RNA-seq in the past decade
Svensson *et al.*, *Nature Protocols*, 2018

Single -cell transcriptomics

Droplet-based approaches

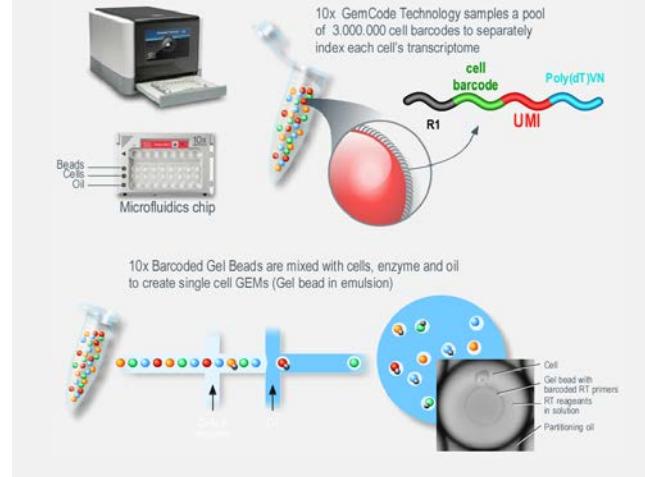


Exponential scaling of single-cell RNA-seq in the past decade
Svensson et al., *Nature Protocols*, 2018

InDrop, Klein et al, 2015
Drop-seq, Macosko et al, 2015
10x Genomics, Zheng et al, 2016

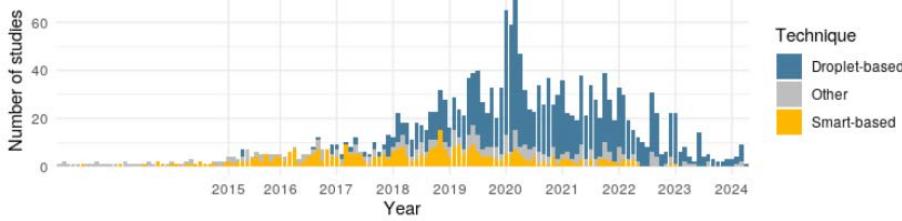
10x Genomics Chromium single cell controller (2016)

- Easy-to-set-up and robust workflow
- High scalability (1,3M cells dataset)



Single -cell transcriptomics

Single cell approaches in publications



- Huge amount of singlecell studies in the past 5 years,
- Droplet-based approaches = 61% (Chromium: 47%)

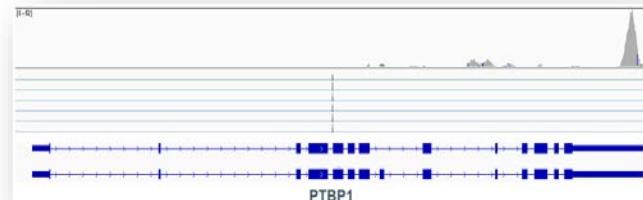
A curated database reveals trends in single cell transcriptomics
Svensson et al., Database , 2020

<https://doi.org/10.1093/database/baa073>

Technique	Count
Chromium	725
Smart-seq2	177
SMARTer (C1)	124
Drop-seq	74
SMARTer	28
InDrops	23
CITE-seq	18
CEL-seq2	17
STRT-seq	17
MARS-seq	16
Tang	15
CEL-seq	13
STRT-seq (C1)	13
Seq-Well	13
SORT-seq	12
BD Rhapsody	11
BioMark	8
GemCode	7
ICELL8	7
Perturb-seq	7
Patch-seq	6
sc-RT-mPCR	6
MERFISH	5

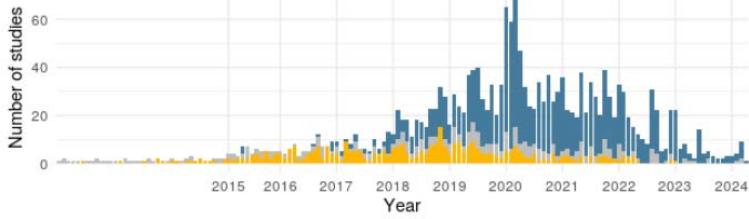
Droplet-based approaches

- Digital Gene Expression (UMI)
- High cell number throughput
- Limited capture efficiency (<10%)
- 3p or 5p signal (SAGE-like)



Single -cell transcriptomics

Single cell approaches in publications



<https://doi.org/10.1093/database/baa073>

A curated database reveals trends in single cell transcriptomics
Svensson et al., Database, 2020

- Huge amount of singlecell studies in the past 5 years,
- Droplet-based approaches = 61% (Chromium: 47%)
- Smart-based approach = 21%, <5% in the last 2 years

Technique	Count
Chromium	725
Smart-seq2	177
SMARTer (C1)	124
Drop-seq	74
SMARTer	28
InDrops	23
CITE-seq	18
CEL-seq2	17
STRT-seq	17
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SORT-seq	12
BD Rhapsody	11
BioMark	8
GemCode	7
ICELL8	7
Perturb-seq	7
Patch-seq	6
sc-RT-mPCR	6
MERFISH	5

Smart-based approach

- Lower cell number (384plate handling)
- Higher capture efficiency (~30%)
- No UMI before v3 (may 2020)
- Full-length coverage using shortreads

Article | Open Access | Published: 30 May 2022

Scalable single-cell RNA sequencing from full transcripts with Smart-seq3xpress

Michael Hagemann-Jensen, Christoph Ziegner & Rickard Sandberg

Brief Communication | Open Access | Published: 30 May 2022

Fast and highly sensitive full-length single-cell RNA sequencing using FLASH-seq

Vincent Haubus, Dinka Pavlenic, Walter Carbone, Sven Schreiner, Pierre Balmer, Mathieu Quirodz, Magdalena Reiner, Guglielmo Roma, Camerun S. Cowan & Simone Picardi

UMIs detected in HEK293 cells

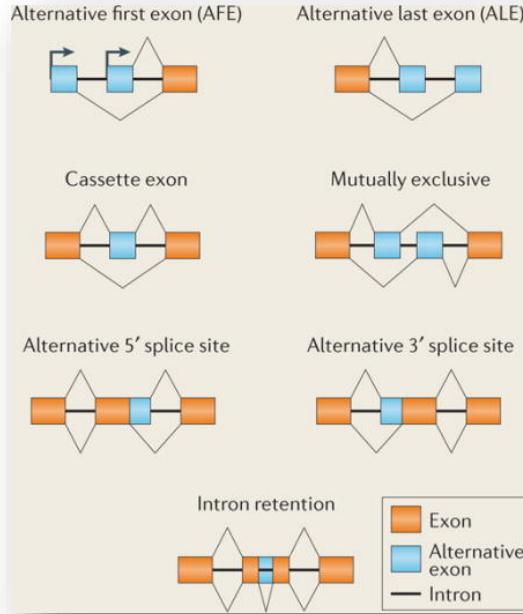
Droplets 10x: 30k (50k reads)
Plate-based : 60k (200k reads)
Smart-seq3: 150k (750k reads)



Mantis Microdispenser

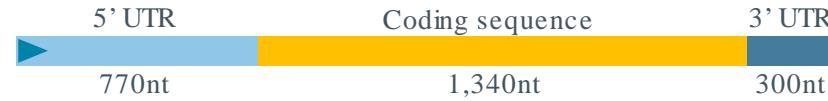
Transcriptomics

Complex outcomes of alternative splicing

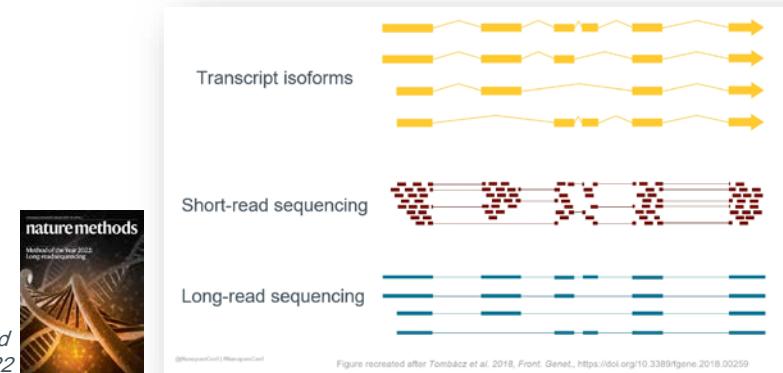


Scotti and Swanson, Nat Rev Genet., 2016

- 90% of the genes are subjected to alternative splicing,
- Gencode v42 : 252,416 distinct isoforms for 62,696 genes,
- On average, a human gene contains 8.8 exons, mean size of 145 nt,
- Average encodes mRNA 2,410 nt long :



Alternative splicing and disease
Tazi et al, 2008



Single -cell long -read transcriptomics

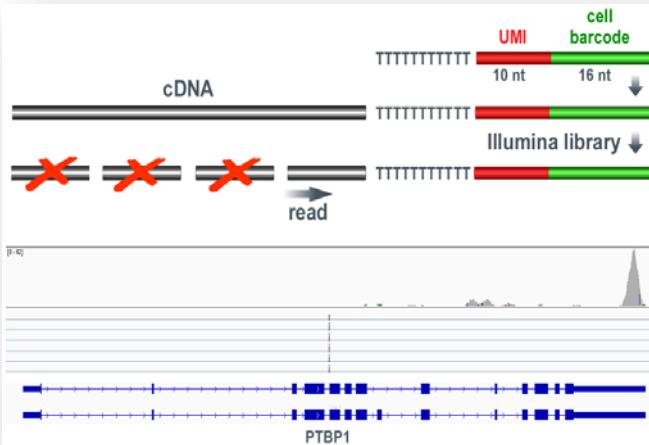
Droplets-based approach short reads vs long reads



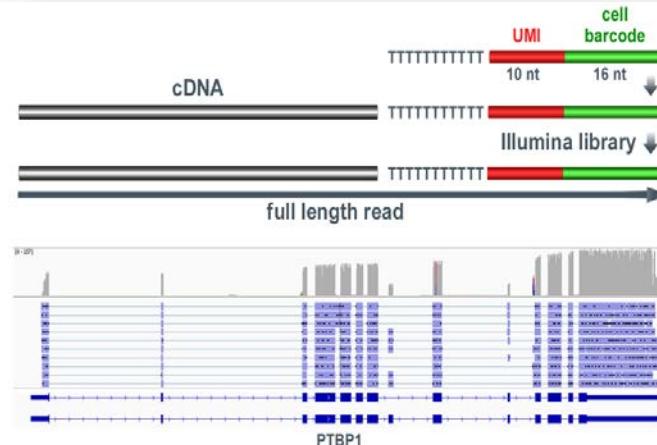
Gene-level
matrix



Standard short-read sequencing



Long-read full-length sequencing



Isoform-level
matrix



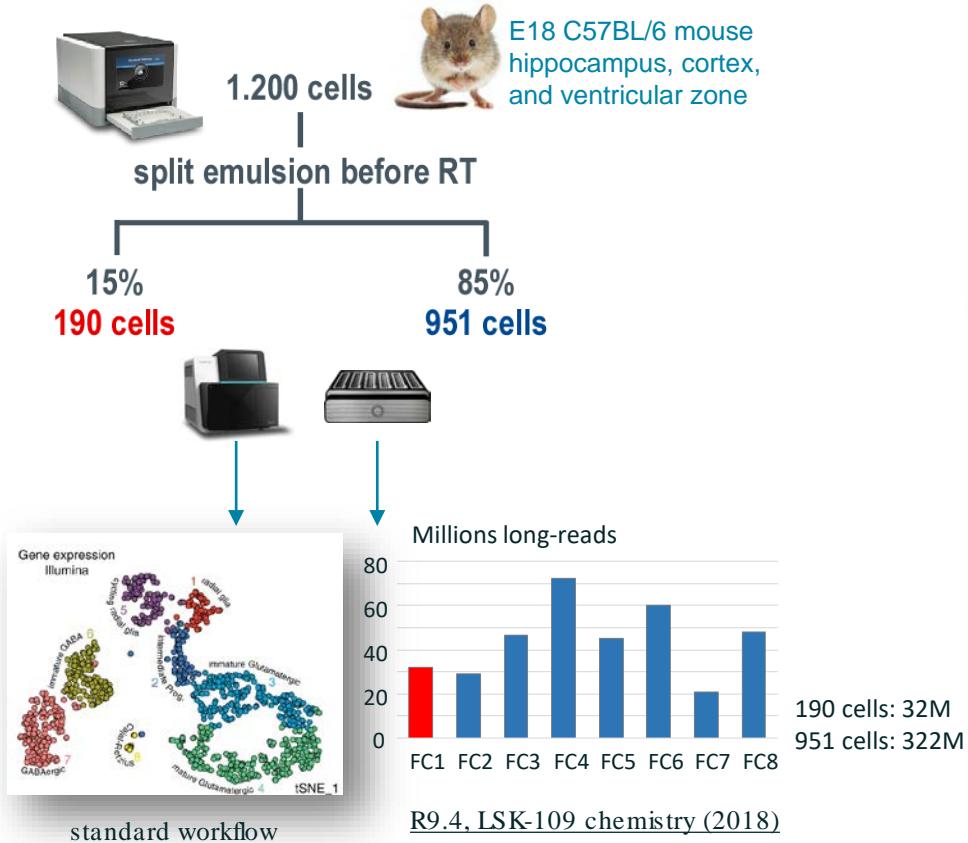
Information on alternative splicing, fusion transcripts, SNV, editing, imprinting, allelic imbalance

Is lost

Remain accessible

Single -cell long -read transcriptomics

SiCeLoRe bioinformatics for Single Cell Long Read



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Article | [Open Access](#) | Published: 12 August 2020

High throughput error corrected Nanopore single cell transcriptome sequencing

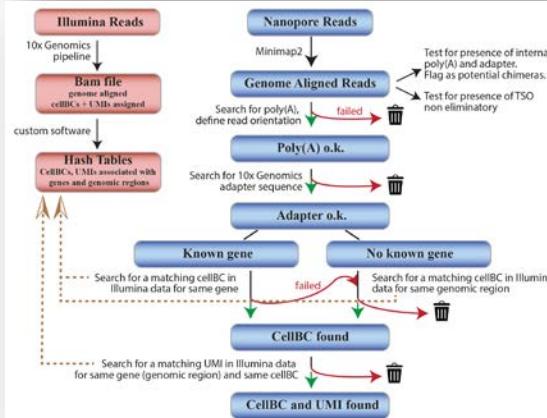
Kevin Lebrigand , Virginie Magnone , Pascal Barbuy & Rainer Waldmann

Nature Communications 11, Article number: 4025 (2020) | [Cite this article](#)

20k Accesses | 38 Citations | 58 Altmetric | [Metrics](#)



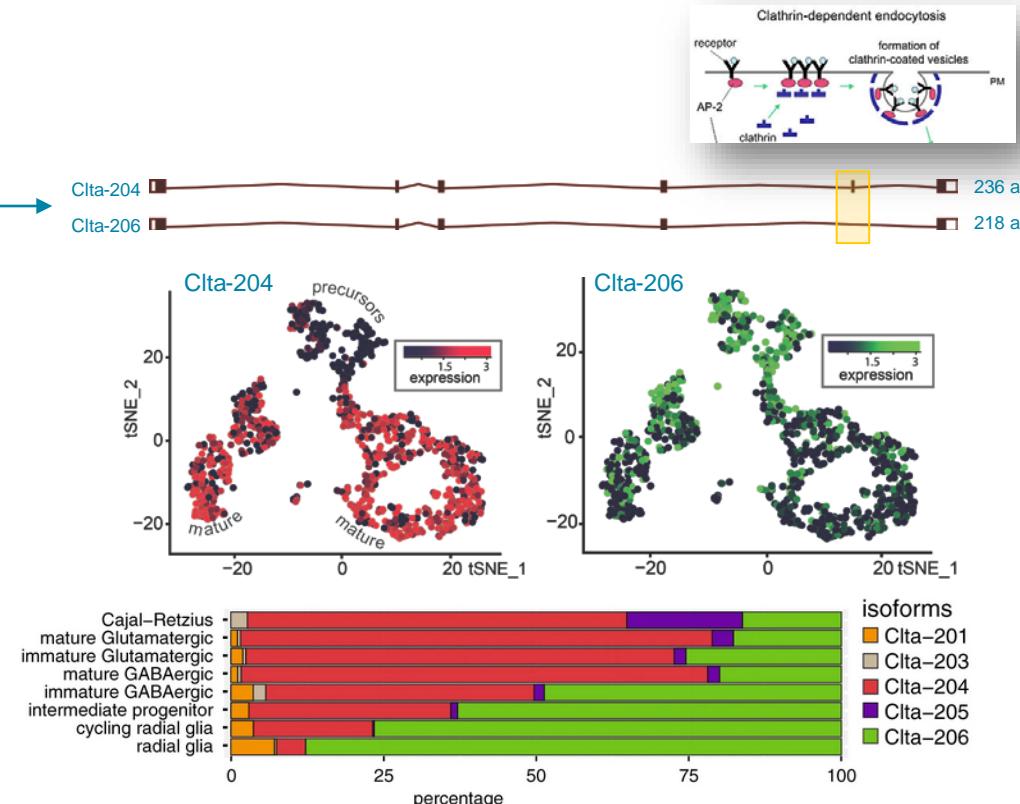
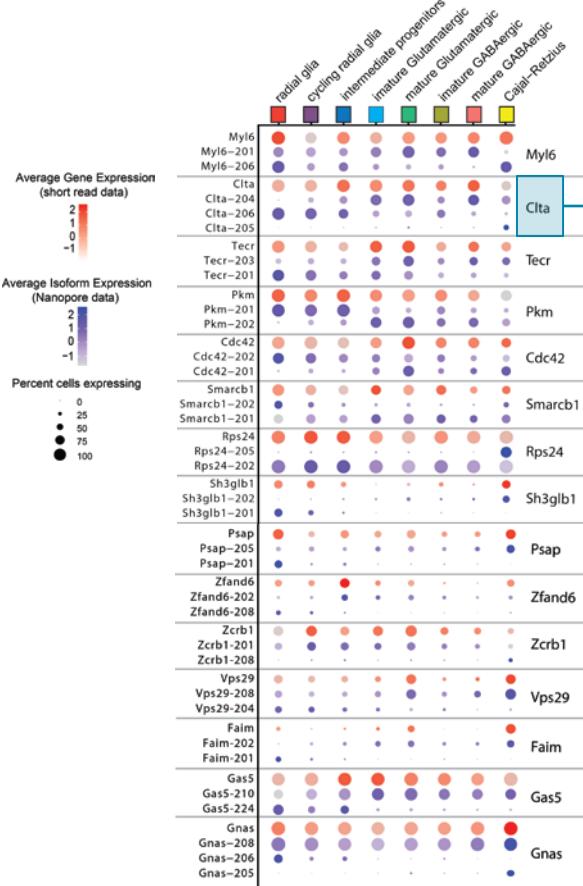
Rainer Waldmann



<https://github.com/ucagenomix/sicelore>

Single -cell long -read transcriptomics reveals diversity

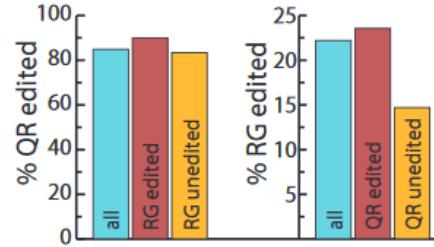
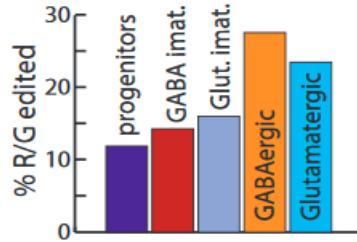
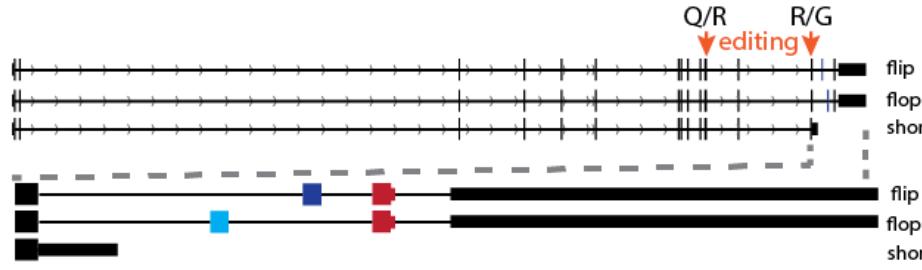
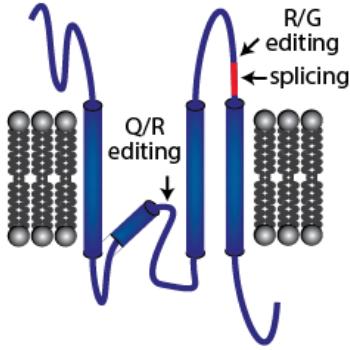
76 isoform-switching genes along neuronal maturation



Single -cell long -read sequencing reveals sequence heterogeneity

RNA A-to-I editing of the AMPA receptor Gria2

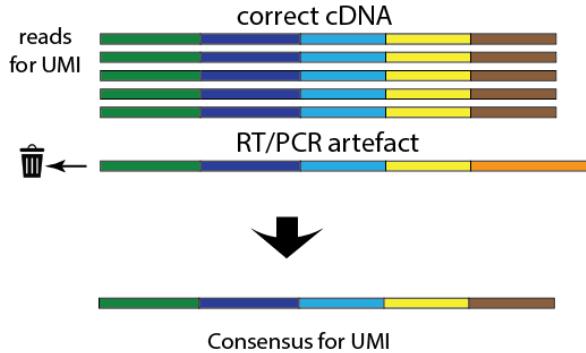
Q/R site regulates AMPA receptor Ca^{2+} -permeability
R/G site is involved in desensitization and recovery of the receptor



Single -cell long -read transcriptomics reveals sequence heterogeneity

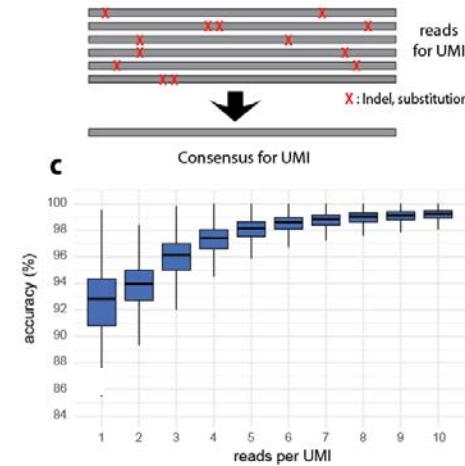
Consensus sequence computation per UMI

UMIs enable elimination of PCR artifacts

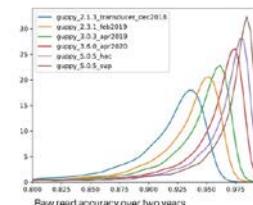


Crucial for accurate novel isoform discovery

UMIs enable correction of sequencing errors



Crucial for high accuracy SNV call



Nanopore PromethION sequencing

2018: 30M reads/FC, 92% raw read accuracy

2023: 120M reads/FC, 99% raw read accuracy



Sicelore is now short-read free:

<https://github.com/ucagénomix/sicelore2.1>

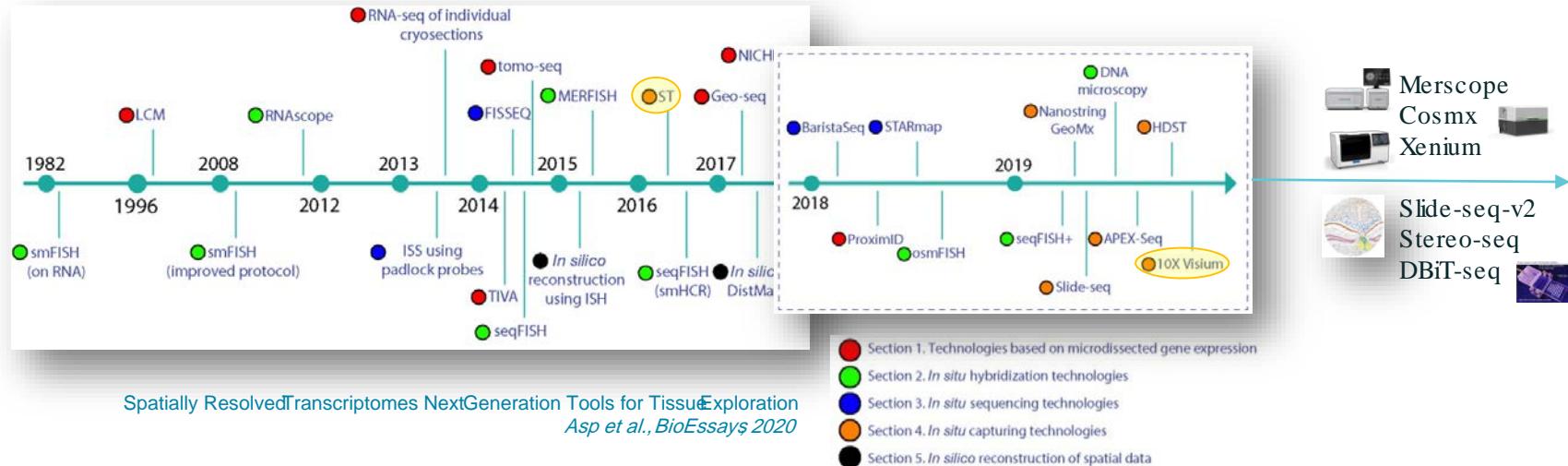
02

Spatial isoform Transcriptomics

Spatial Transcriptomics approaches

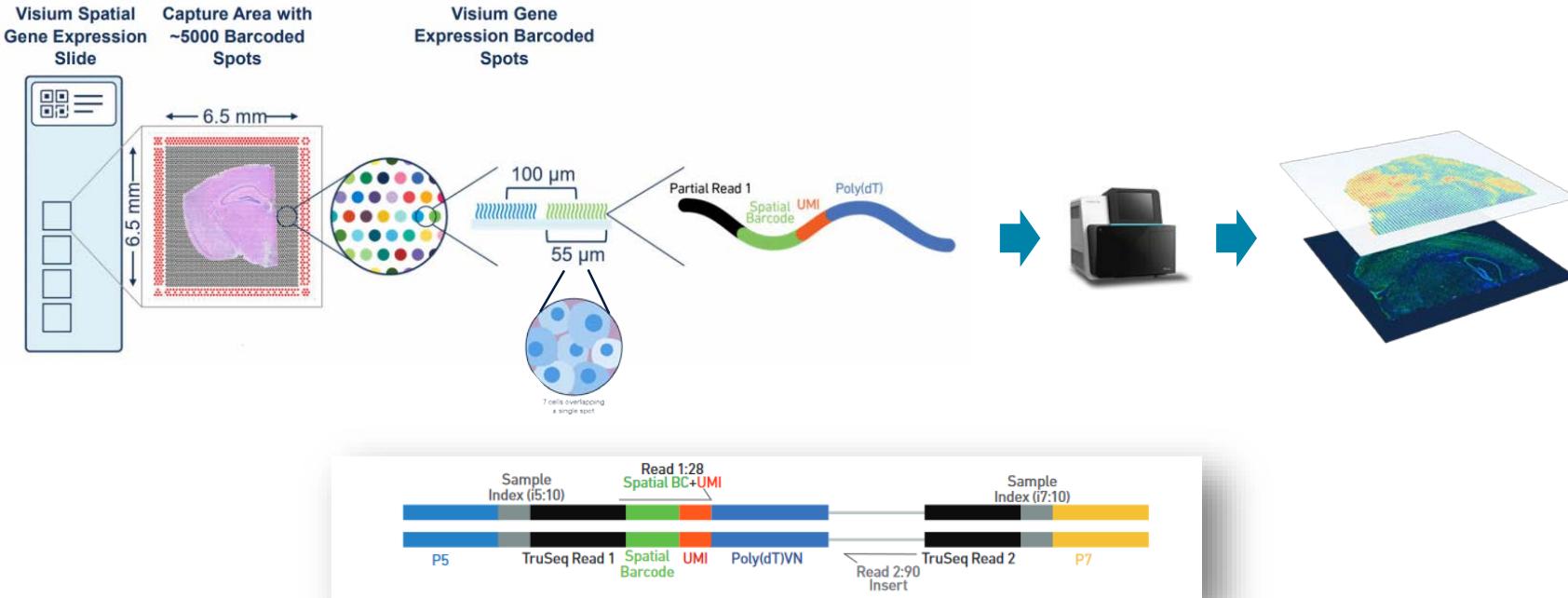
Historical timeline

- Spatial transcriptomics aims at directly visualize gene expression in their original environment,
- 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,



In-situ capture spatial transcriptomics

Ståhl et al. (2016); 10x Genomics Visium(2019)



→ Spatial barcode / UMI assignment strategy identical to single cell transcriptomics

Spatial isoform Transcriptomics (SiT)

Nucleic Acids Research, 2023

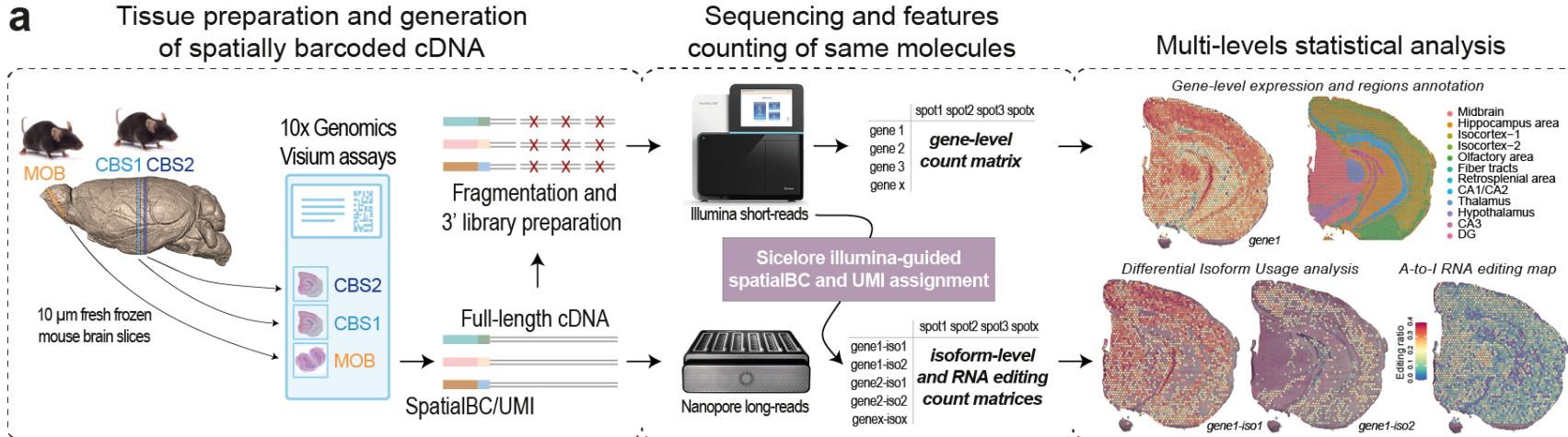
The spatial landscape of gene expression isoforms in tissue sections

Kevin Lebrigand, Joseph Bergenstråle, Kim Thrane, Annelie Mollbrink, Konstantinos Meletis, Pascal Barbuy , Rainer Waldmann, Joakim Lundeberg Author Notes

Nucleic Acids Research, Volume 51, Issue 8, 8 May 2023, Page e47, <https://doi.org/10.1093/nar/gkad169>

Published: 17 March 2023 Article history ▾

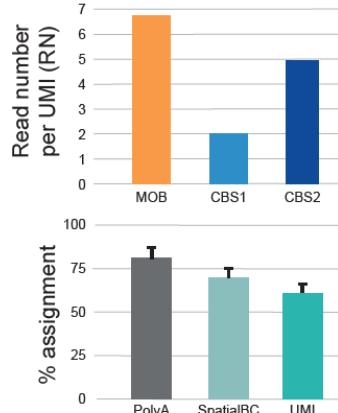
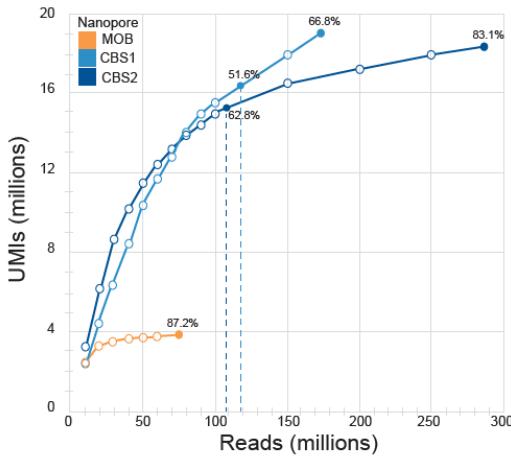
a



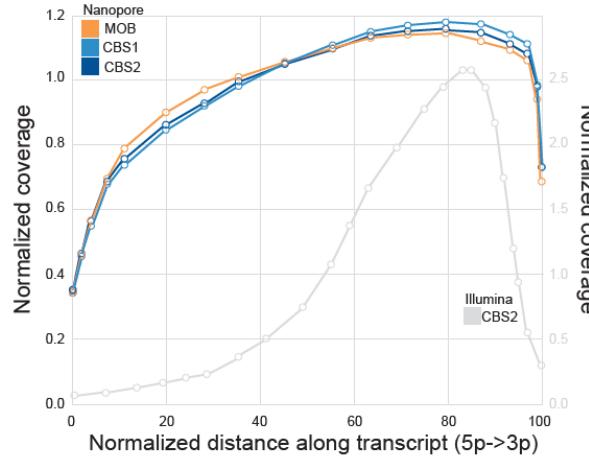
Nanopore promethION long -read sequencing

Provides isoform-level spatial transcriptomics

Sequencing saturation curves per samples



Transcripts fulllength coverage

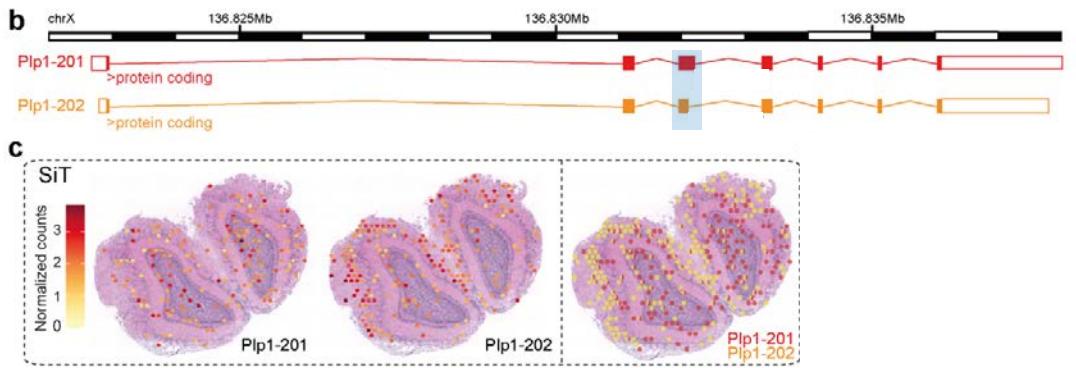


Reads	MOB			CBS1			CBS2							Total	%age	of Total passed reads	
Date	18 feb. 20	20 mar. 20		18 feb. 20	20 mar. 20	24 feb. 21	12 may 20	13 may 20	19 may 20	25 may 20	26 may 20	27 may 20	09 feb. 21				
Flow cells	PAE06474	PAE59649		PAE01745	PAE59645	PAG52067	PAE59606	PAE59231	PAE32756	PAE32753	PAE31188	PAE21339	PAD99555	PAG56368	13		
Total reads (fastq_pass)	27628000	47272000		24980000	31736000	117280000	22897702	30405384	27492770	18534938	31506774	19108718	25596387	110916000	535354673		
PolyA and Adapter found reads	21318117	47970311		17980183	27286678	80516212	18536047	25199992	22871198	16088962	26777546	15983663	21682530	85837208	428048647	79,96	of Total passed reads
SpatialIBC found reads	14506264	29316718		12554655	19051597	54323311	14613934	19867830	14666481	11403706	19099469	11266930	14090779	60154119	294915793	68,90	of PolyA found reads
UMIs found reads	10445006	19328468		7323748	10517081	27584331	8616415	11714126	9347072	7557944	12657620	7448718	9031708	34225619	175797856	59,61	of SpatialIBC found reads

CBS1: One flow cell, 117 M reads → 51.6% sequencing saturation
 CBS2: One flow cell, 111 M reads → 62.2% sequencing saturation
 → 1 or 2 PromethION flow cells per slice

SiT reveals specific splicing pattern across MOB regions

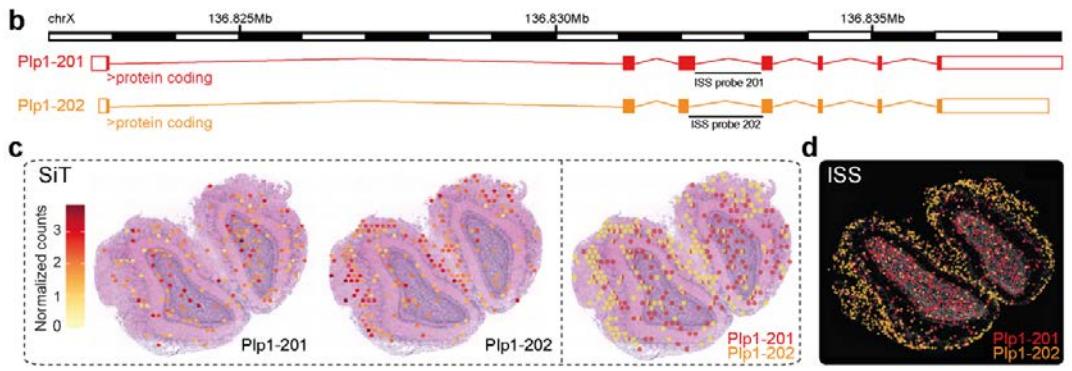
Plp1 Differential TranscriptUsage (DTU)



Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination

SiT reveals specific splicing pattern across MOB regions

Plp1 Differential TranscriptUsage (DTU)



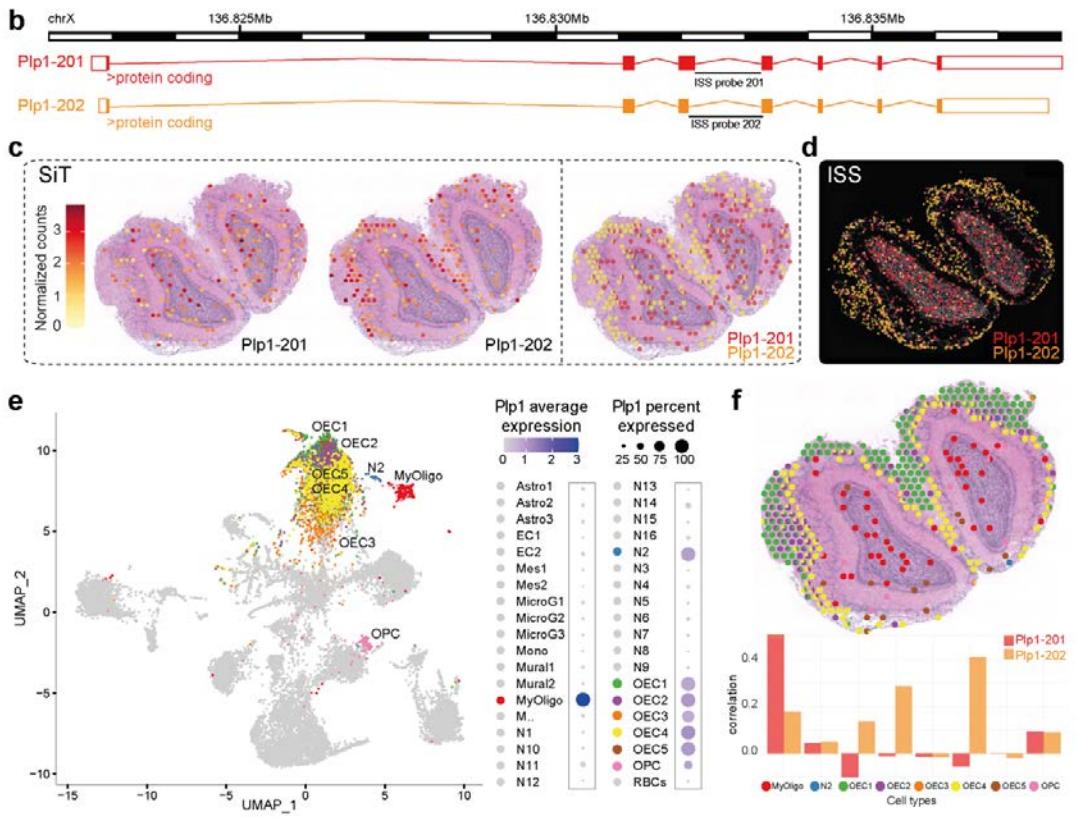
Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination



In Situ Sequencing Data

SiT reveals specific splicing pattern across MOB regions

Cell type deconvolution using single cell external dataset (Tépeet al., 2018)



Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination



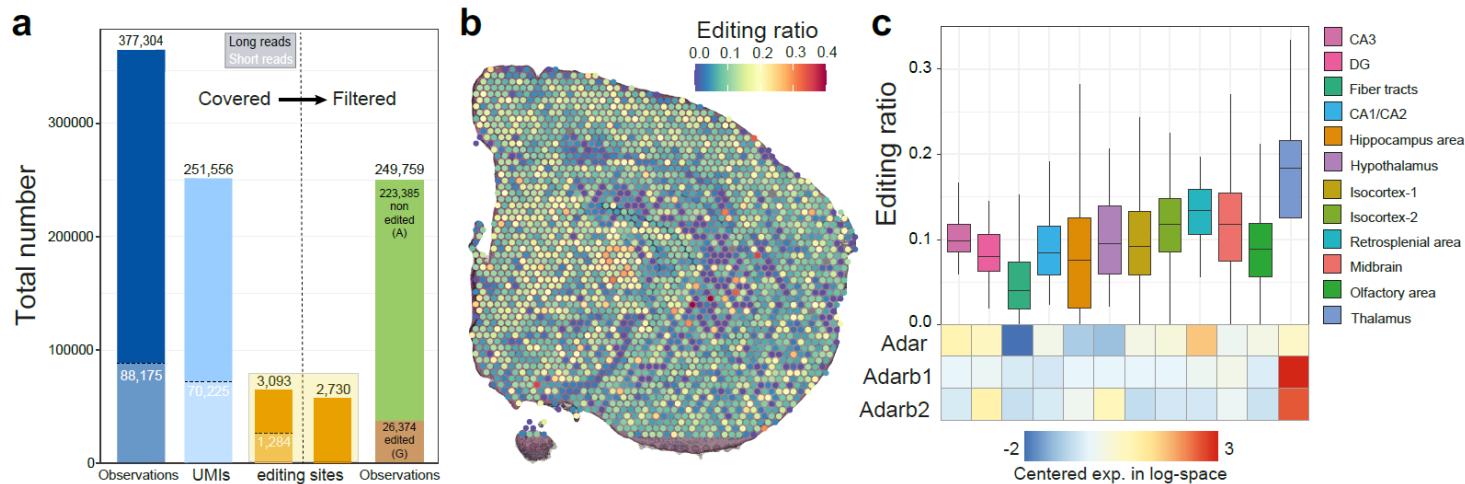
In Situ Sequencing Data

Spatial spot deconvolution of prominent *Plp1* expresser cell types. Correlation Deconvolution score / *Plp1* isoforms expression correlation shows that *Plp1* is predominantly expressed as *Plp1-202* by olfactory ensheathing cells (OEC) in the ONL and as *Plp1-201* isoform by myelinating oligodendrocytes (MyOligo) in the GCL.

SiT reveals full -length sequence heterogeneity (CBS)

Global A-to-I RNA editing spatial map

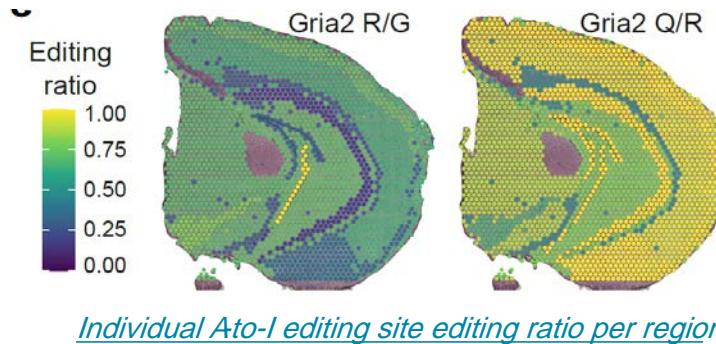
- Exploration of 5,817 A-to-I RNA editing sites described in the literature (Ramaswami et al., 2013 (RADAR) Licht et al., 2019)
- Long read high confidence call thresholding looking at agreement between long and short read base calls for 88,175 shared UMIs
 - number of reads per UMI ≥ 3
 - consensus Phred score QV ≥ 6



SiT reveals full -length sequence heterogeneity (CBS)

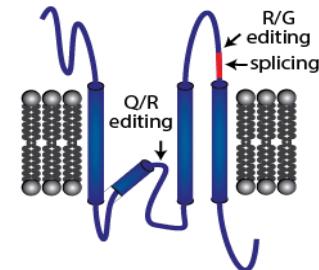
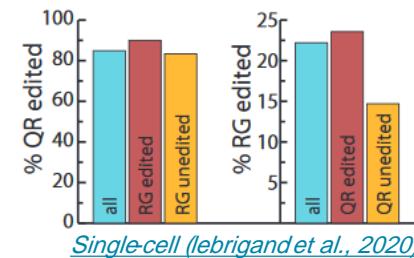
Global A-to-I RNA editing spatial map

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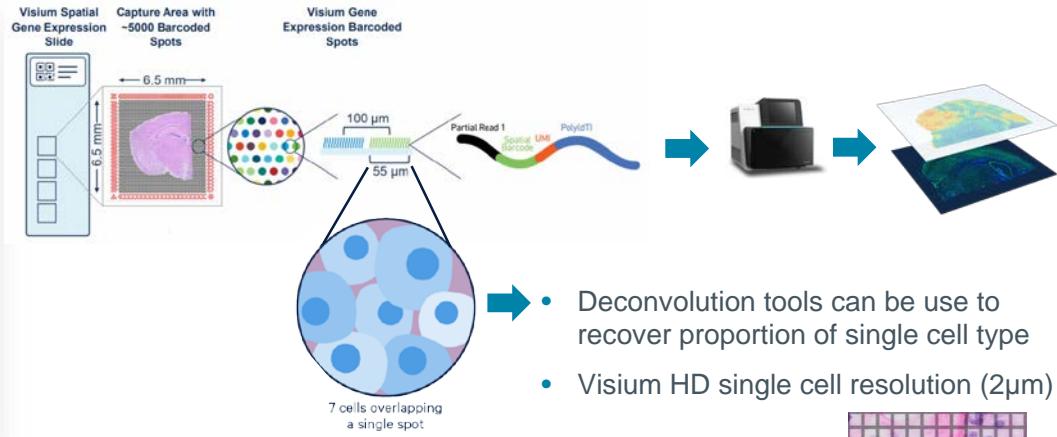
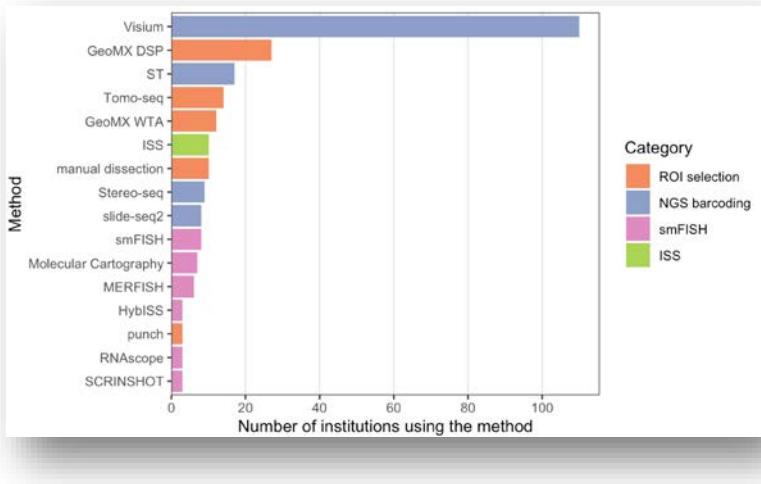
Gria2

- R/G site is involved in desensitization and recovery of the receptor
- Q/R site regulates AMPA receptor Ca^{2+} -permeability

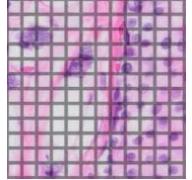
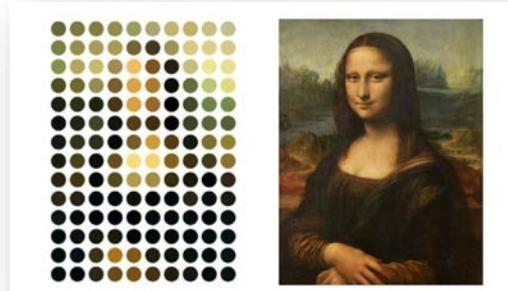


Spatial transcriptomics (2017 -2022)

Visium is widely adopted by academics



- Deconvolution tools can be used to recover proportion of single cell type
- Visium HD single cell resolution (2μm)



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

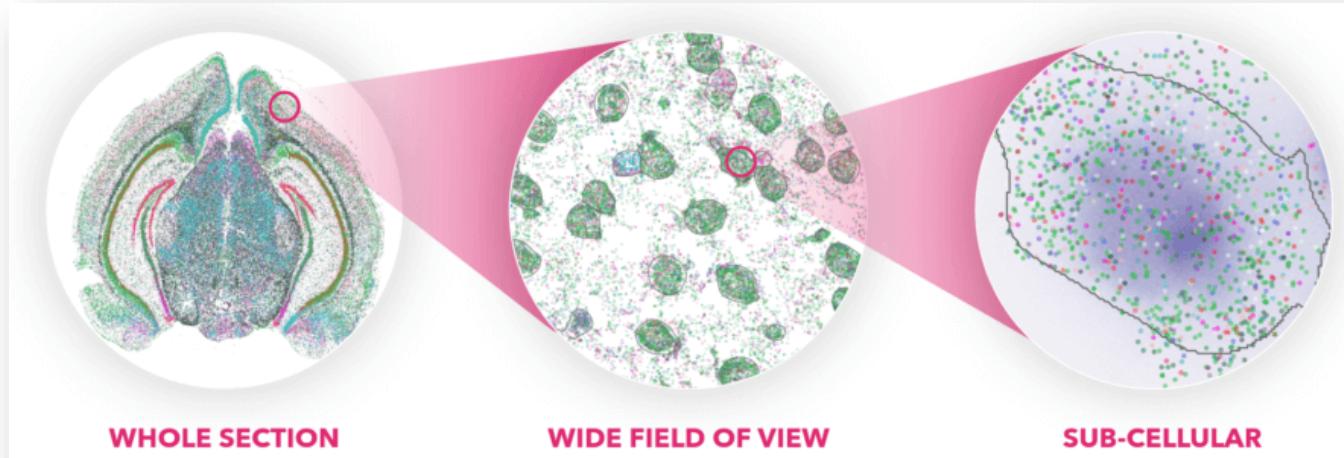
03

Spatial imaging -based Transcriptomics

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)



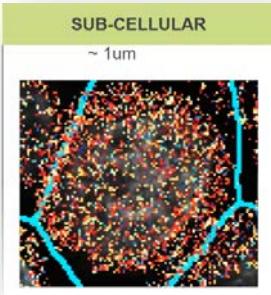
Spatial imaging -based transcriptomics (2022)

No more sequencing for direct single-cell resolution



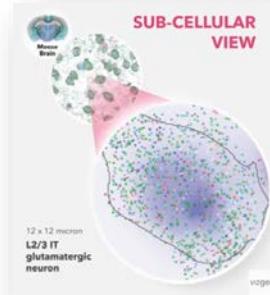
Nanostring CosMx

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



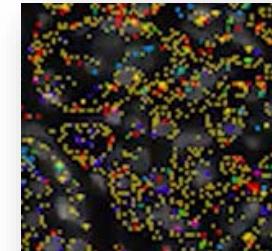
Vizgen Merscope

- 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 : 5-30% (++)
- Imaging area: **236 mm²** (4 days)
- Resolution 200 nm

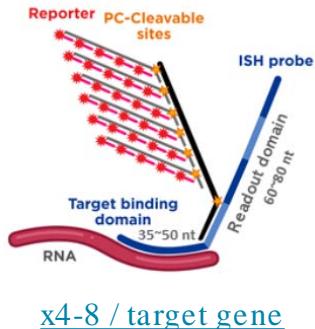


Spatial imaging -based transcriptomics (2022)

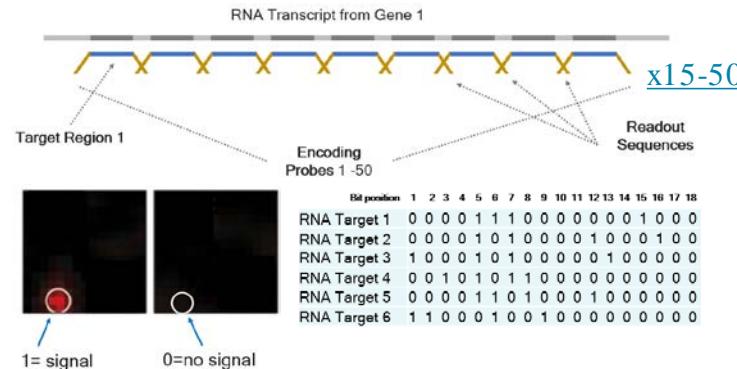
No more sequencing for direct single-cell resolution



Nanostring CosMX
ISH-based



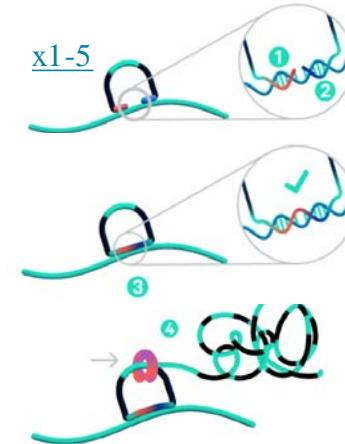
Vizgen Merscope
Multiplex ErrorRobust FISH



Cyclic *in situ*Hybridization Chemistry



10xGenomics Xenium
Cartana ISS, padlock probes / RCA

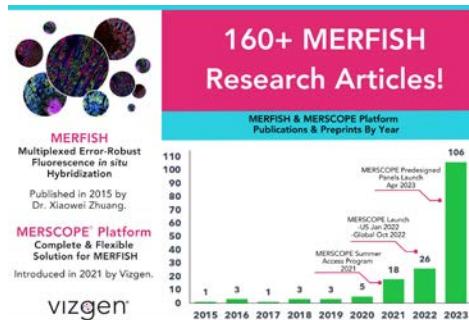


Spatial imaging -based technologies comparison

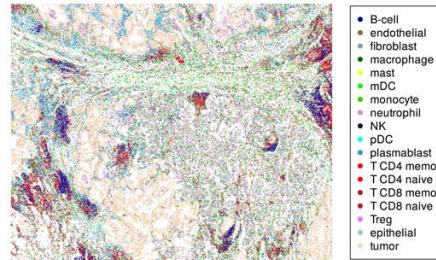
Compare available datasets

Vizgen Merscope

- Xiaowei Zhuang's lab mersfish publications
 - Chen et al., Science (2015)
 - Moffitt et al., PNAS (2016), Science (2018)
 - Emanuel G 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)

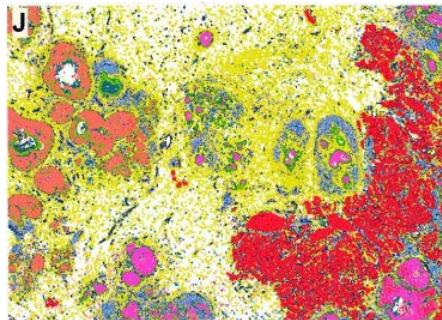


Nanostring CosMx



- 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 -based technologies comparison

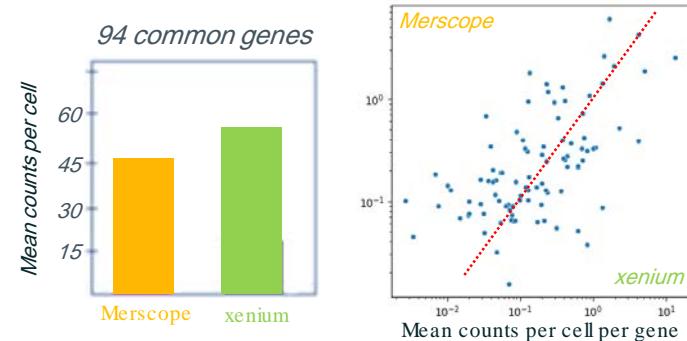
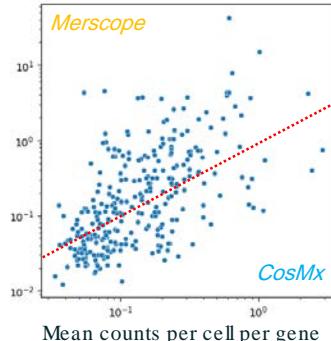
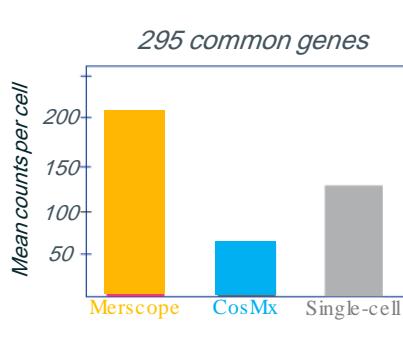
Compare available datasets: Lung and Breast cancer samples



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 Breast Cancer	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 -based technologies comparison

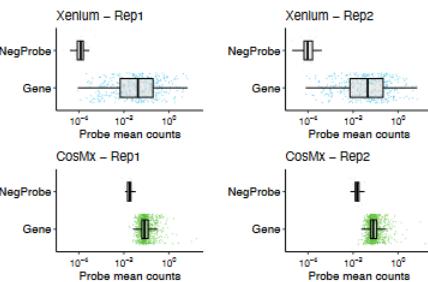
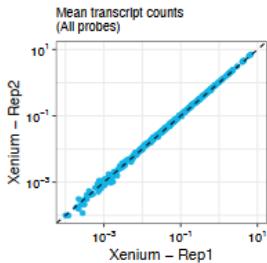
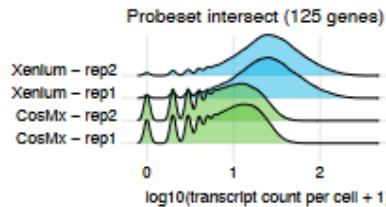
Recent biorxiv comparative studies

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*}

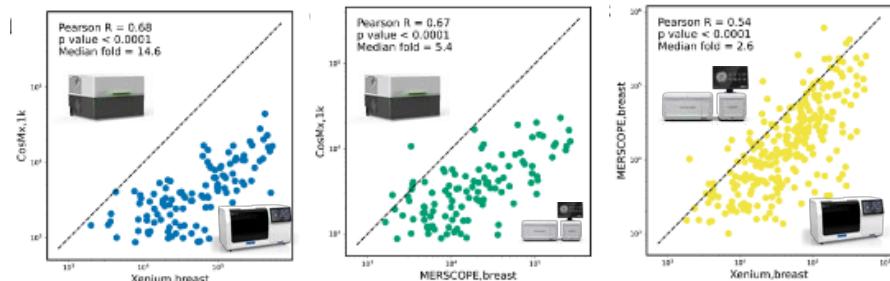


	Xenium Rep 1	Xenium Rep 2	CosMx Rep 1	CosMx Rep 2
Gene target #	377	377	1000	1000
Total cell count	99,852	102,508	98,139	98,767
Median gene count per cell	33	34	75	71
Median transcript count per cell	88	92	113	99
Median transcript count / gene target count	0.23	0.24	0.11	0.10
Median transcript count (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,**}



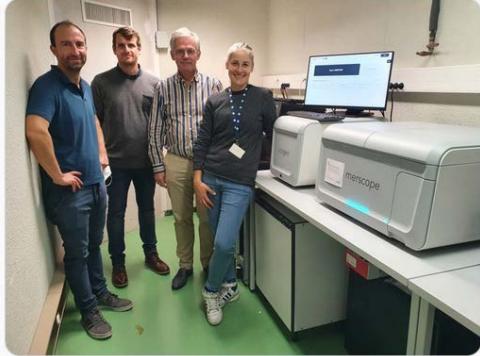
MERSCOPE @ UCAGenomiX (Nice-Sophia -Antipolis)

October 2022

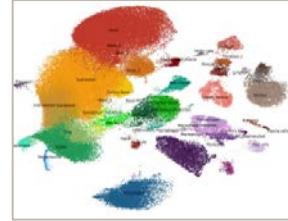


↪ William Amoyal Retweeted
Pascal Barbry @pbarbry · Oct 12

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



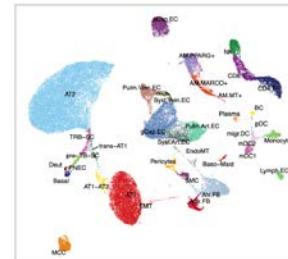
- Human Lung Cell Atlas (CZI)  
Discovering the Cellular Landscape of the Airways and Lung Tissue



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



- Human embryo olfactory epithelium exploration (Paolo Diacobini, Lille)
- Pulmonary Arterial Hypertension (Christophe Guignabert, Paris-Saclay)



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

Gene targets panel design

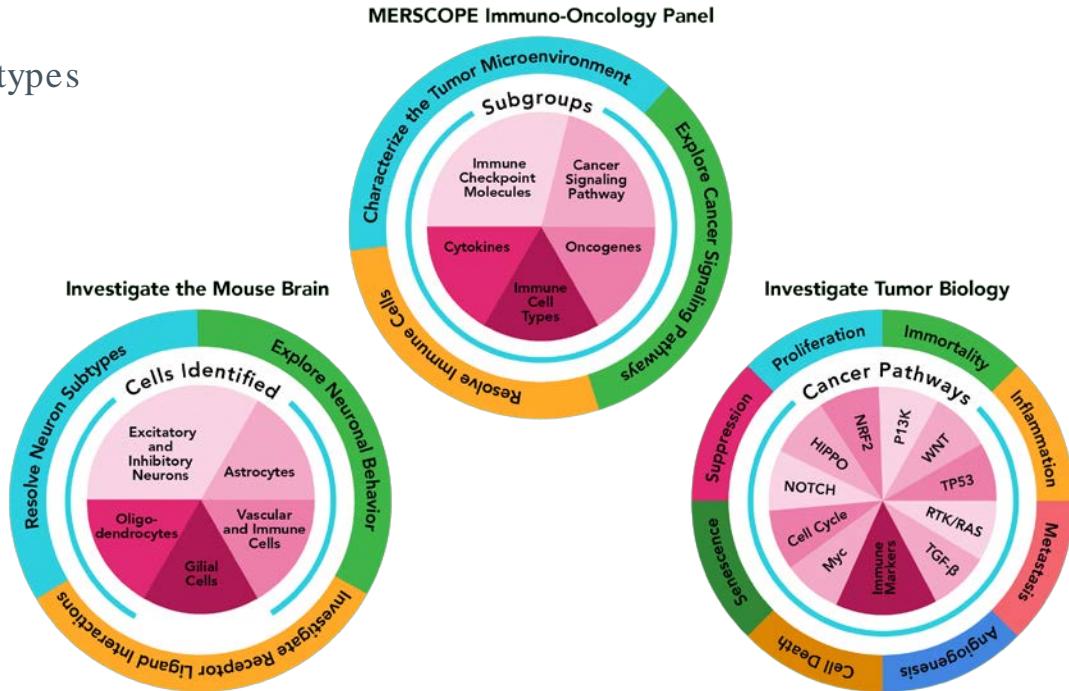
Depending on the biological question !

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/>

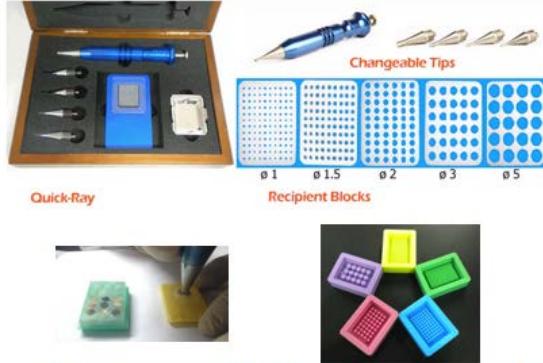
<https://cloud.10xgenomics.com/xeniumpanel-designer>

Experimental design

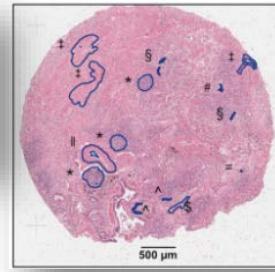
Take advantage of the large imaging area

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. Taylor⁶, 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^{5,9,10}, Rajat Walia¹¹, Jennifer MS Sucre^{4,9}, 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 @rkyip · Mar 4

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

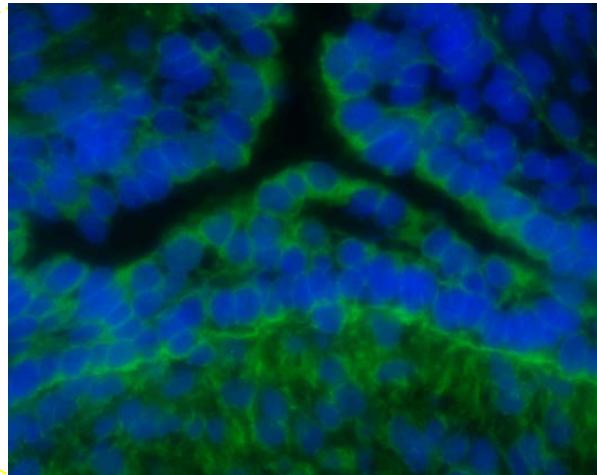
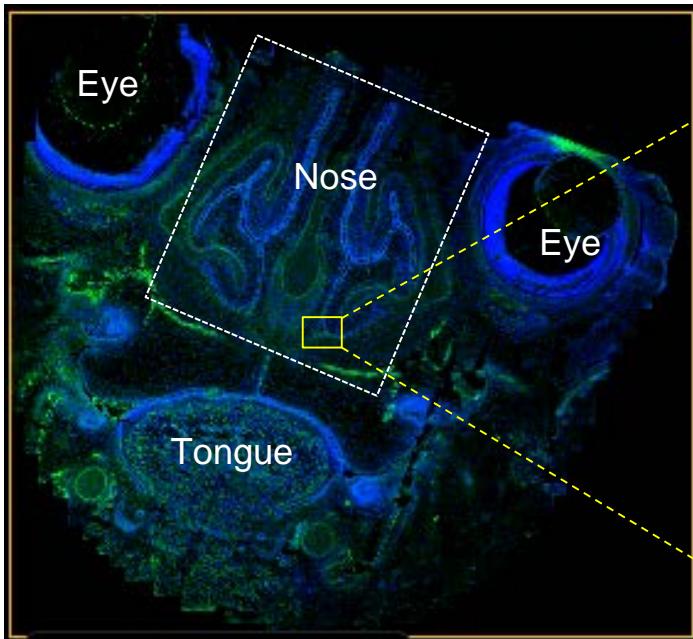


Each run is around 5 k€

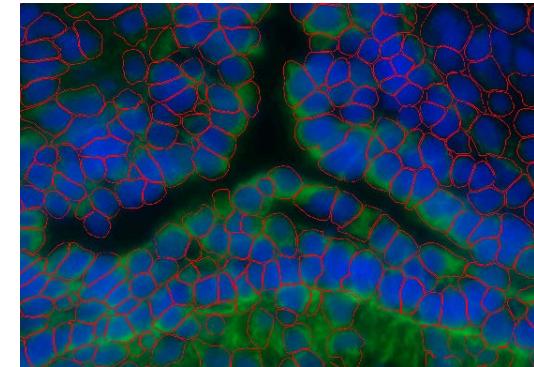
multiplexing helps removing batch effect and increase replicates for a robust statistical analysis

Data acquisition (7 z -stack)

Staining for cell segmentation



DAPIchanel
Cell boundarieschanel



Article | Published: 14 December 2020

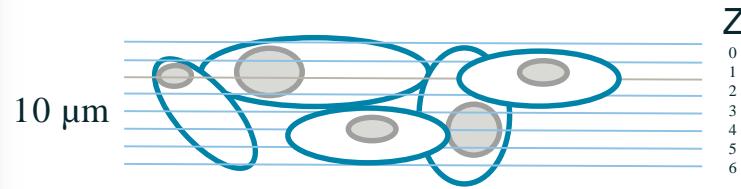
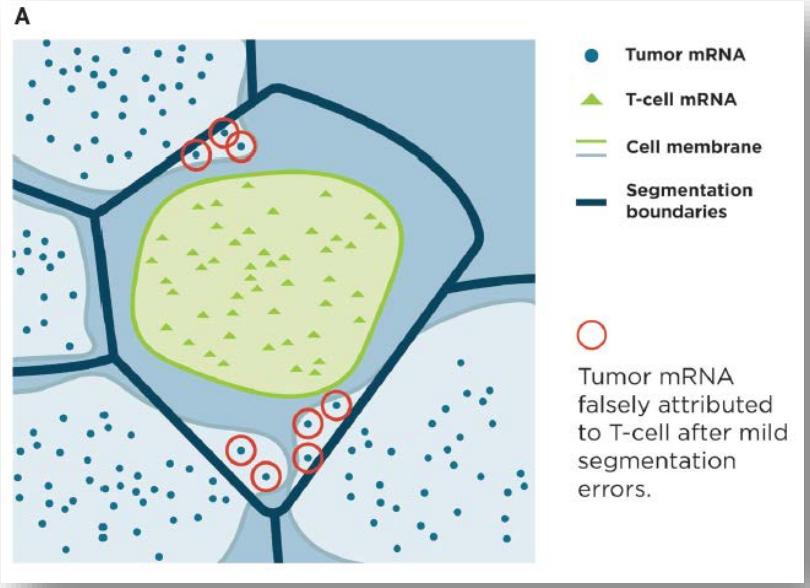
Cellpose: a generalist algorithm for cellular segmentation

Carsen Stringer, Tim Wang, Michalis Michaelos & Marius Pachitariu

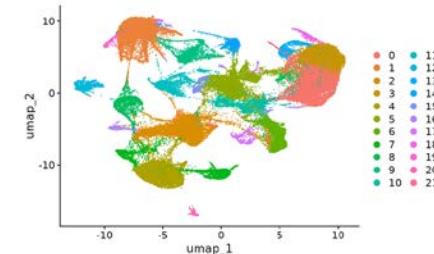
Nature Methods 18, 100–106 (2021) | [Cite this article](#)

Cell segmentation is crucial

Cell x gene matrix purity and good subsequent biology

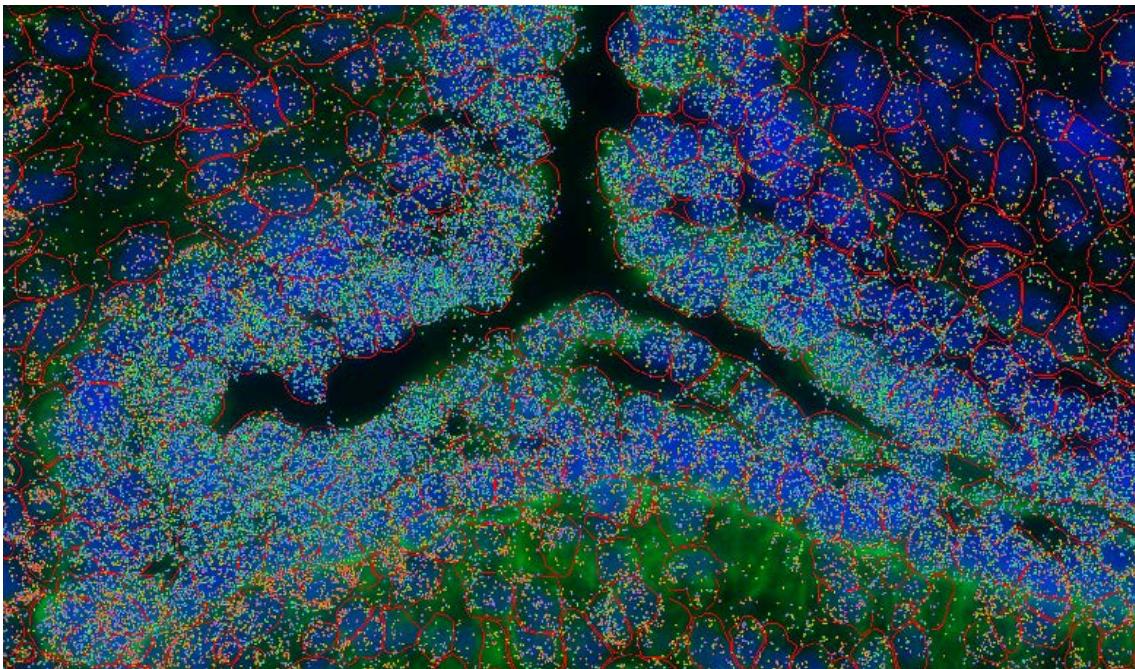


3D segmentation required, actually not used, 2D segmentation per Z then harmonizing and summing the detected transcripts for all Z into the harmonized segmentation mask (nuclei of full cell)



Detected transcripts to segmentation mask

Cell x genematrix

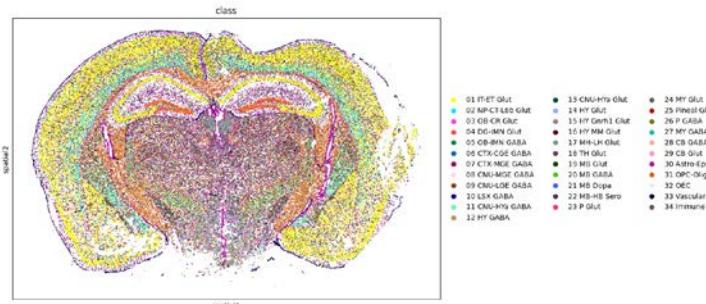


Gene-level matrix

→ 100k's cells

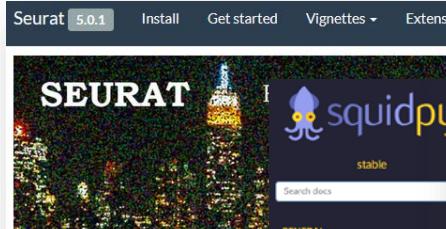
Cita	5	4	6	7	1	4	3	9	5	5	.	1	4	3	6	4	.	7	5	2	4
Myl6	5	2	5	1	2	4	13	2	5	2	4	4	1	4	8	4	2	3	3	1	1
Pkm	3	2	1	.	.8	3	9	4	10	5	1	.	4	12	5	1	3	9	1	7	
Tecr	3	1	5	2	4	6	5	9	6	3	2	2	1	4	7	1	1	1	3	2	8
Meis2	8	29	3	.	24	6	6	21	25	16	6	1	.	2	29	.	6	18	.	10	
...																					

1.000 Genes



Statistical data analysis

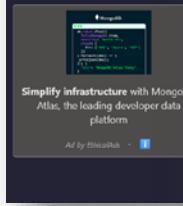
Several available suites



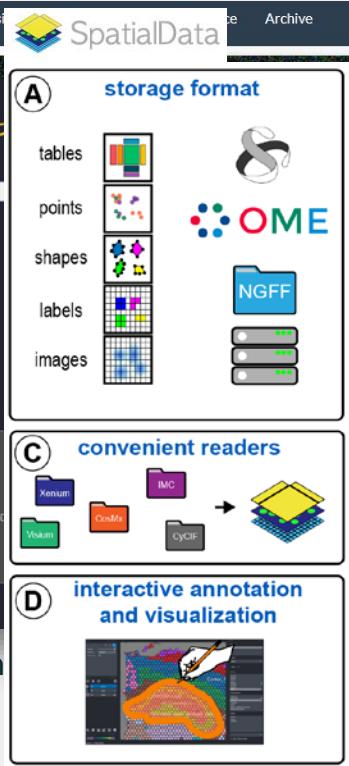
Seurat v5

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

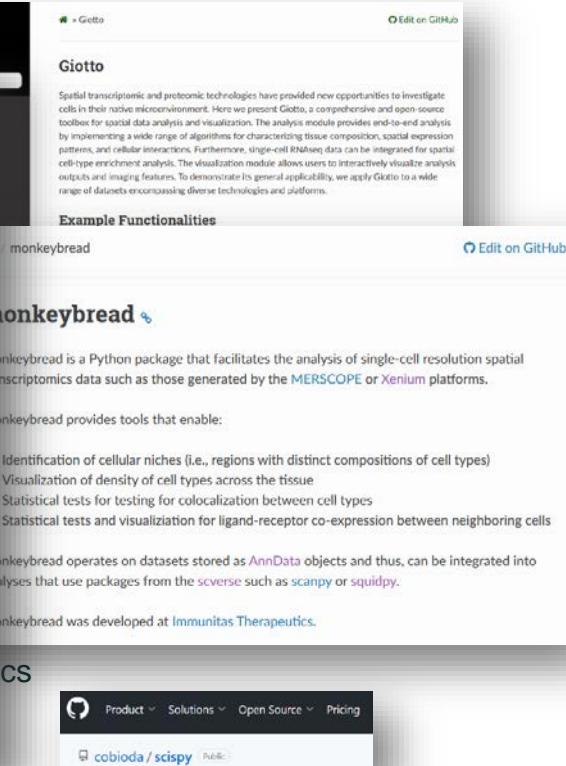
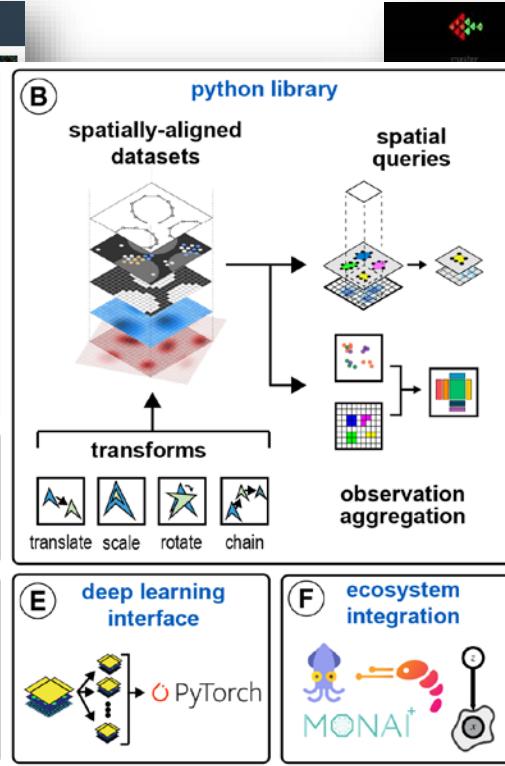
Satija'slab, NYGC



Theis'slab, h



Scverse ecosystem, Oliver Stegle & Fabian J. Theis

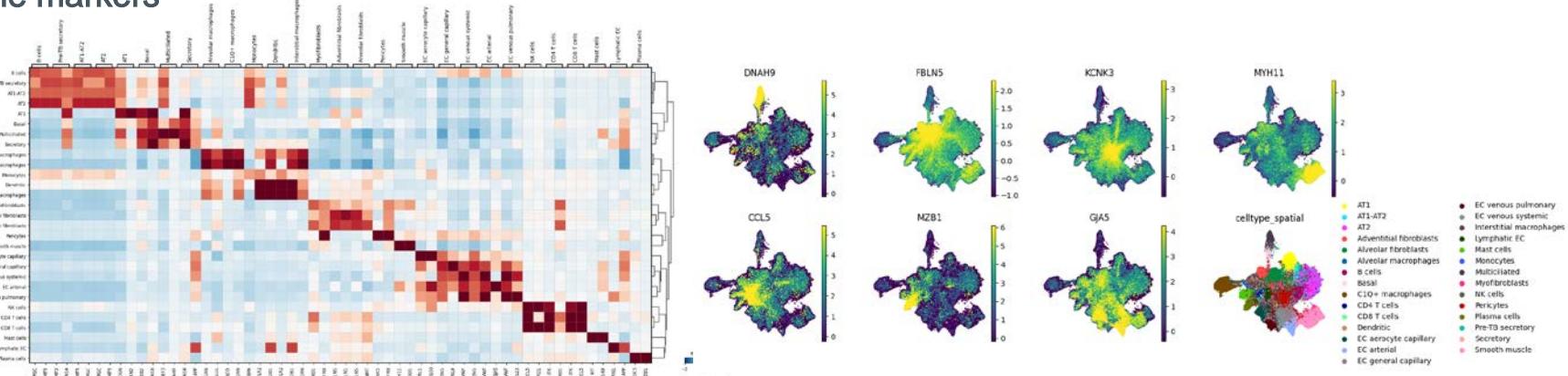


<https://github.com/cobioda/scispy/>

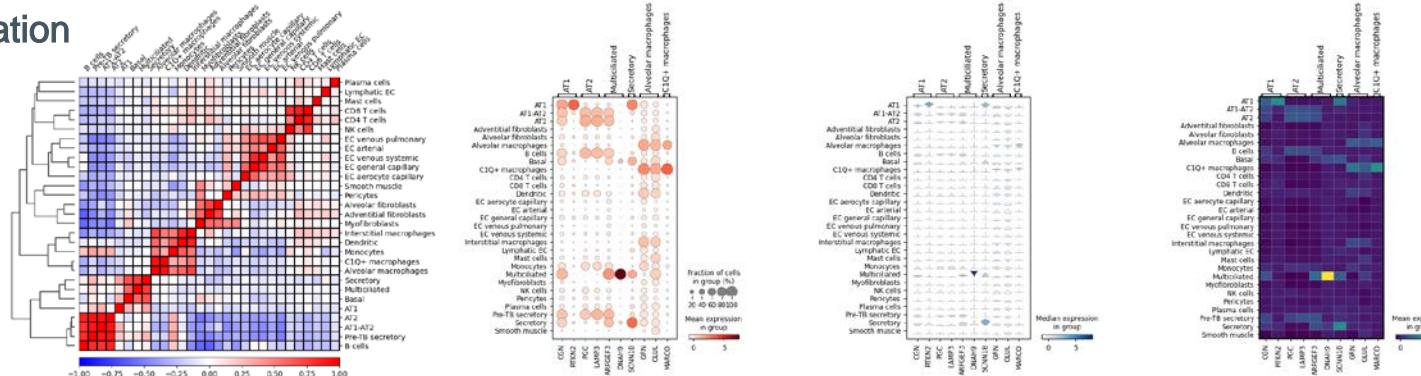
Single -cell standard data analysis

Access to 100's of packages described in the last 5 years

Gene markers

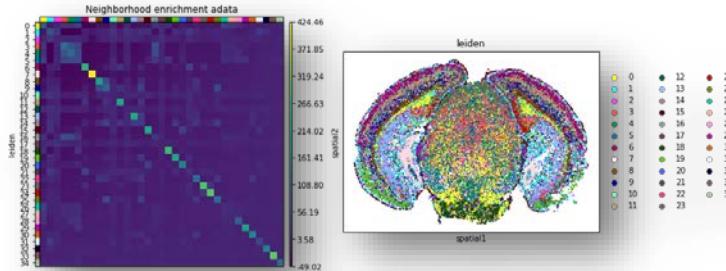


Cell type correlation



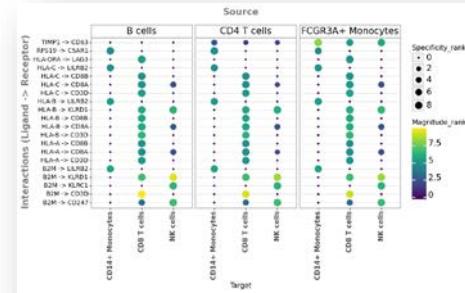
Single -cell data analysis including the spatial resolution

A new vast area for computational biologists (just like single-cell 5 years ago !)



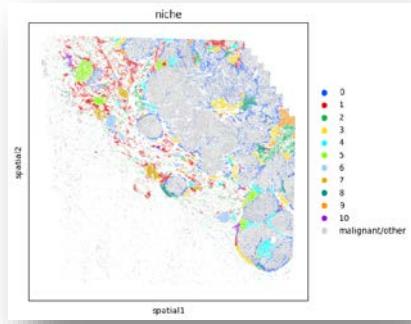
Neighbors enrichment analysis

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



Ligand -Receptor analysis

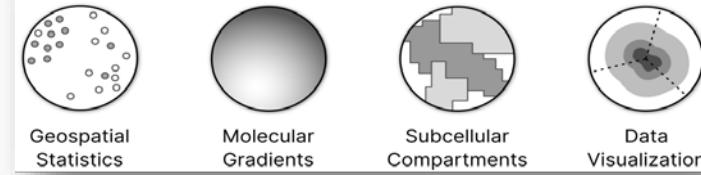
- Need to be in gene panel !!
- CellPhoneDB[Efremova et al., 2020]
- Omnipath [Türei et al., 2016].



Cellular niches analysis

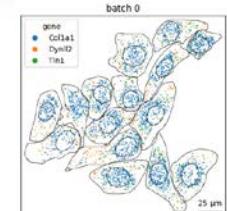
for each cell, we count the number of neighbors that are of each cell type thus forming a “neighborhood profile” vector of length C, where C is the number of cell types. We then cluster all neighborhood profiles and call each cluster a “niche”.

Machine Learning & Statistical Analysis



Sub-cellular exploration

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



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