

Single-cell and Spatial Isoform Transcriptomics

Kévin Lebrigand

Computational Biology and Omics Data Analysis

 <https://cobioda.github.io>

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FRANCE
GÉNOMIQUE

UNIVERSITÉ
CÔTE D'AZUR



ipmc



CoBiODA
Computational Biology
Omics Data Analysis
CÔTE D'AZUR

Institute of Molecular and Cellular Pharmacology

Sophia-Antipolis

ijpmc



20 research teams composed of > 220 members

- Ion channels(pain, perception, epilepsy)
- Molecular signaling(molecular trafficking,lipidomics)
- Neurodegenerative disorders(Alzheimer, Parkinson)
- Neuropsychiatric disorders(nervous breakdown, mental retardation)
- Functional genomics and bioinformatics

15 Engineers running 5 technological platforms

- MICA, Imaging and Flow Cytometry
- CAPABIO, Proteomics and Metabolomic
- ANIPRO, animal care and behavior facility
- CoBiODA, BioinformaticsHub
- UniCA GenomiX, Functional Genomics platform



FRANCE GENOMIQUE

Core member of the “France Génomique” network (2008, 60M€) > 500 academics

illumina

10X GENOMICS[®]

NANOPOR

vizgen

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2015



2016



2018



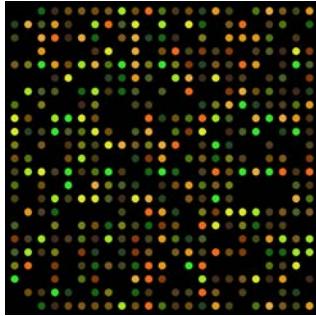
2022



2024

20 years of transcriptomics

Driven by microfluidics technological developments

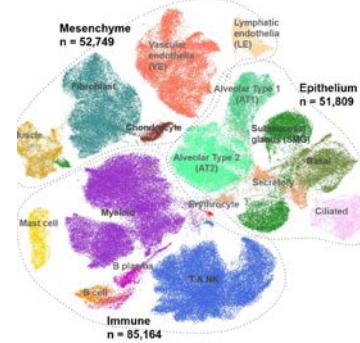
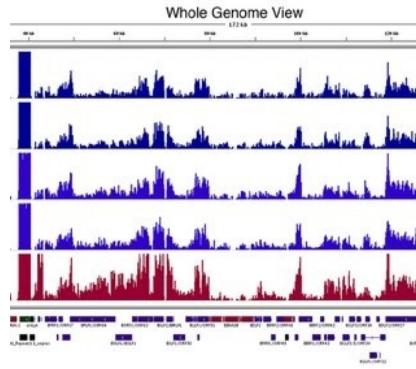


Early 2000's: DNA microarray

- 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

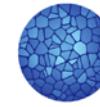
- 300-1000 gene targets
- Imaging analysis
- MultiplexingFiSH(single molecule)
- Sensitivity (3080%)
- Sub-cellular resolution



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

Human Cell Atlas

CZI initiative (2016)

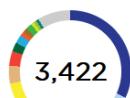


HUMAN
CELL
ATLAS

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

Members



Countries

101

Institutes

1,787

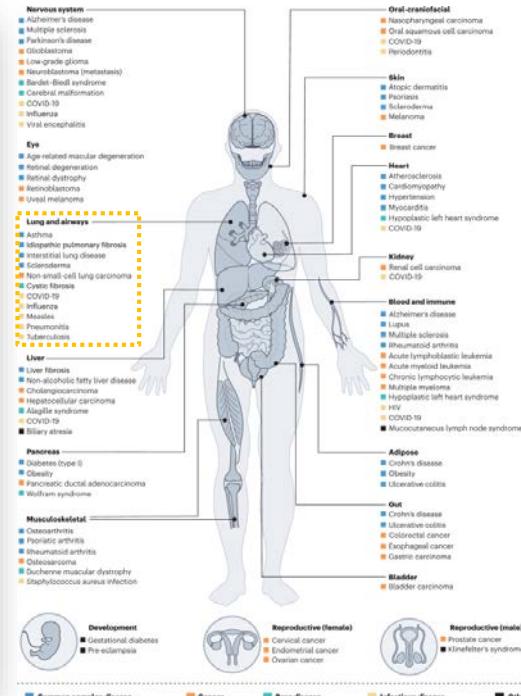
Networks

18

Publications

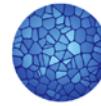
199

Global distribution of HCA members



Human Cell Atlas

Our contribution



HUMAN
CELL
ATLAS

2019

TECHNIQUES AND RESOURCES | 23 OCTOBER 2019

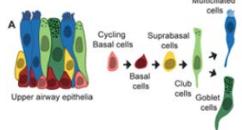
Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures

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 Barbuy², Laure-Emmanuelle Zaragozi¹

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

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+ Author Affiliations

21 125 215

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

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



80K

2020



10X
GENOMICS[®]

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

36k Accesses | 83 Citations | 67 Altmetric | Metrics



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, Gökçen Eraslan¹, Lisa Sikkema¹, Avinash Waghray¹, Graham Heimberg¹, Yoshihiko Kobayashi¹, Eeshit Dhaval Vaishnav¹, Ayswarya Subramanian¹, Christopher Smillie¹, Karthik A. Jagadeesh¹, Elizabeth Thu Duong¹, Evgenij Finkin¹, Elena Torlai 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

nature

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nature > perspectives > article

Perspective | Published: 08 September 2021

A roadmap for the Human Developmental Cell Atlas

Muzaffar Haniffa¹, Dearne 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

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2022

2022

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

Malte D. Luecken^{1,26}, Laure-Emmanuelle Zaragozi^{1,26}, Elo Madisson^{3,4,26}, Lisa Sikkema^{1,26}, 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 Powell¹⁶, Ugis Sarkans¹⁷, 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

nature medicine

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nature > nature medicine > resources > article

Resource | Open access | Published: 08 June 2023

An integrated cell atlas of the lung in health and disease

Lisa Sikkema¹, Ciro Ramirez-Svästegui¹, Daniel C. Strobl¹, Tessa E. Gillett¹, Luke Zappia¹, Elo Madisson¹, Nikolay S. Markov¹, Laure-Emmanuelle Zaragozi¹, Yuge Ji¹, Meshal Ansari¹, Marie-Jeanne Arguel¹, Leonie Apperloo¹, Martin Banchez¹, Christophe Récamier¹, Marijn Berg¹, Evgeny Chichelnitskiy¹, Mei-Ji Chung¹, Antoine Collin¹, Aurore C. A. Gay¹, Janine Gote-Schniering¹, Baharak Hooshpar Kashani¹, Kemal Incelik¹, Manu Jain¹, Theodore S. Kapellou¹, Lung Biological Network Consortium

... Fabian J. Theis¹

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

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2,4M



10X
GENOMICS[®]

2023

The spatial landscape of gene expression isoforms in tissue sections

Kevin Lebrigand¹, Joseph Bergensträhle¹, Kim Thrane¹, Annelie Mollbrink¹, Konstantinos Meletis¹, Pascal Barbuy¹, Rainer Waldmann¹, Joakim Lundeberg¹
Author Notes

Nucleic Acids Research, Volume 51, Issue 8, 8 May 2023, Page e47, <https://doi.org/10.1093/nar/gkac010>

01

Single -Cell isoform Transcriptomics

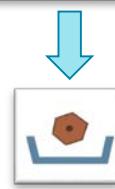
Single -cell transcriptomics

Context

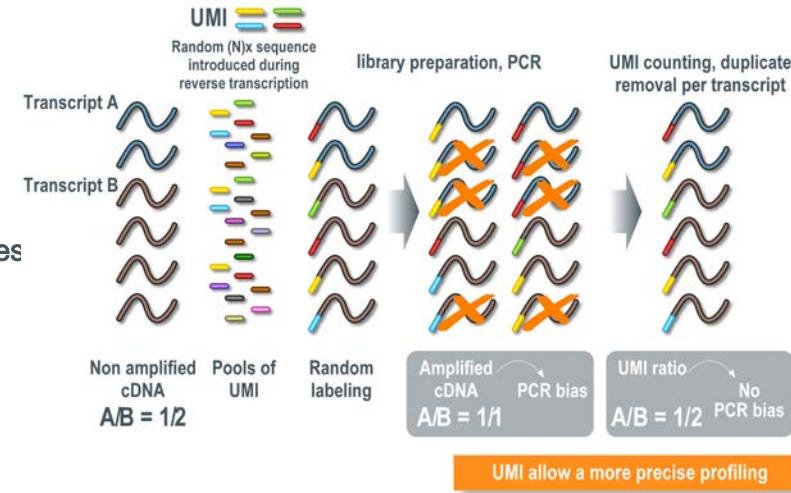
Bulk mRNA libraries
100ng total RNA
(10,000 cells)



Single cell mRNA libraries
10 pg total RNA
(<<1pg mRNA)

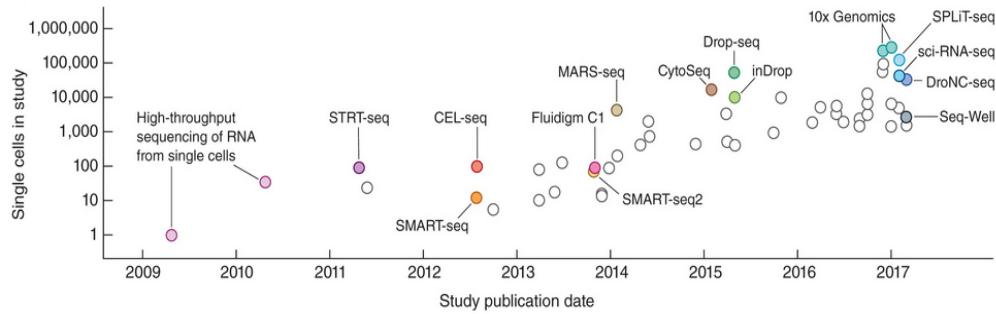
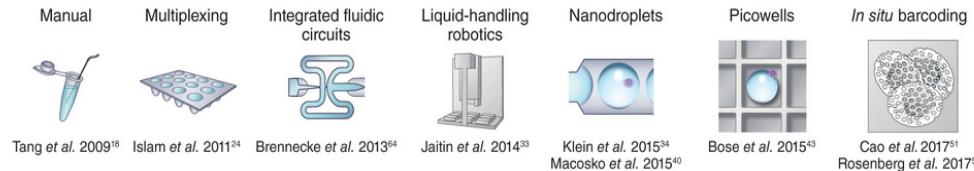


- Highly efficient library preparation techniques
- Elimination of PCR amplification bias and artefacts
- Use of Unique Molecular Identifiers (UMI) to monitor the **number of molecules**
 - Kivioja, T. et al. Counting absolute numbers of molecules using unique molecular identifiers. Nat Meth 9, 72-74 (2012)
 - Improved accuracy of molecule counting



Single -cell transcriptomics

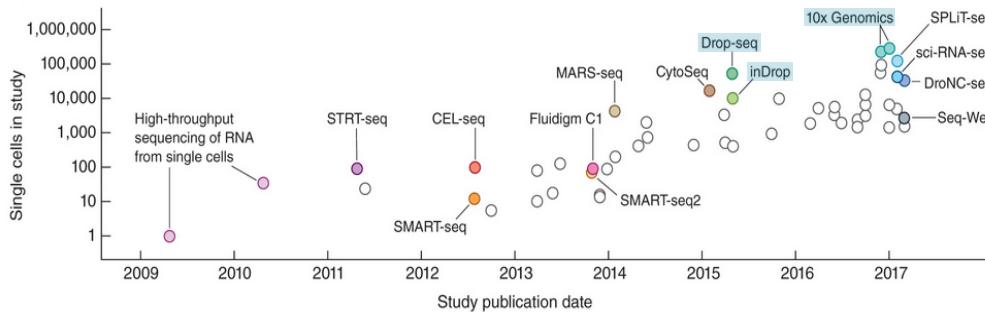
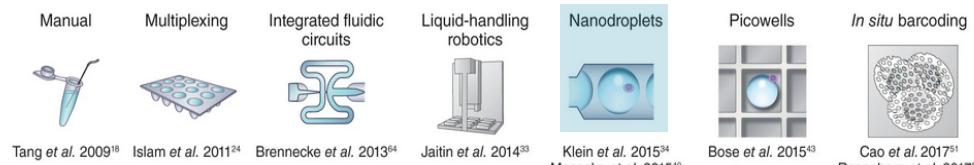
Evolution of isolation techniques and throughput



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

Single-cell transcriptomics

Droplet-based approaches

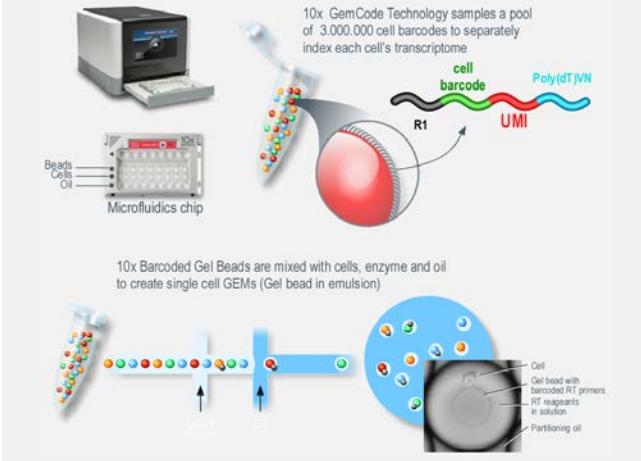


Exponential scaling of single-cell RNA-seq in the past decade
Svensson et al., *Nature Protocols*, 2018

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

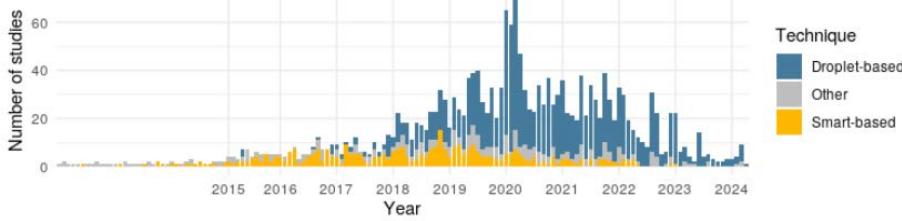
10x Genomics Chromium single cell controller (2016)

- Easy-to-set-up and robust workflow
- Generalize UMI usage
- Shows 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

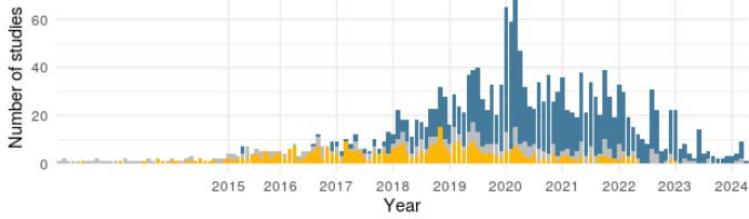
Droplet-based approaches

- 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/baaa073>

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
MARS-seq	16
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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

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 Quirodoz, Magdalena Reiner, Guglielmo Roma, Camerun S. Cowan & Simone Picardi



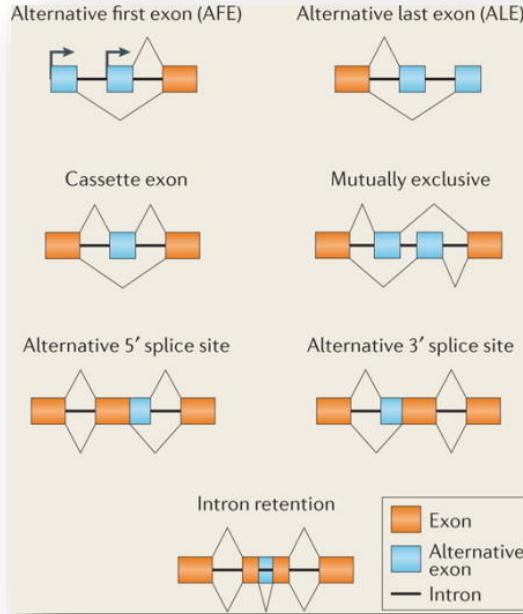
Mantis Microdispenser

UMIs detected in HEK293 cells

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

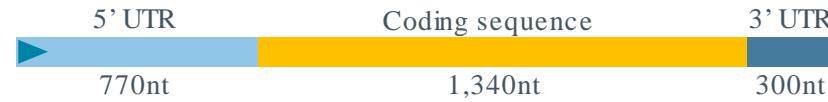
Transcriptomics

Complex outcomes of alternative splicing

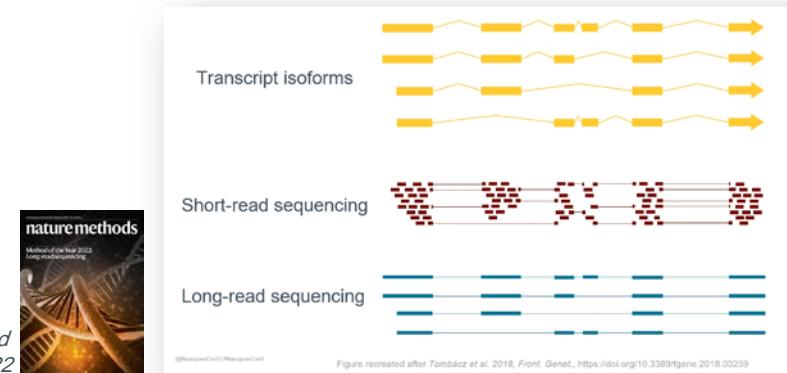


Scotti and Swanson, Nat Rev Genet., 2016

- 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



→ Inference required

→ Direct full exonic layout
One read is one molecule

Single -cell long -read transcriptomics

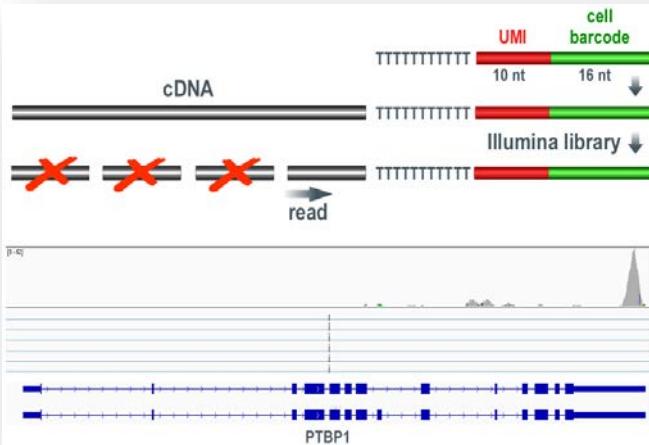
Droplets-based approach short reads vs long reads



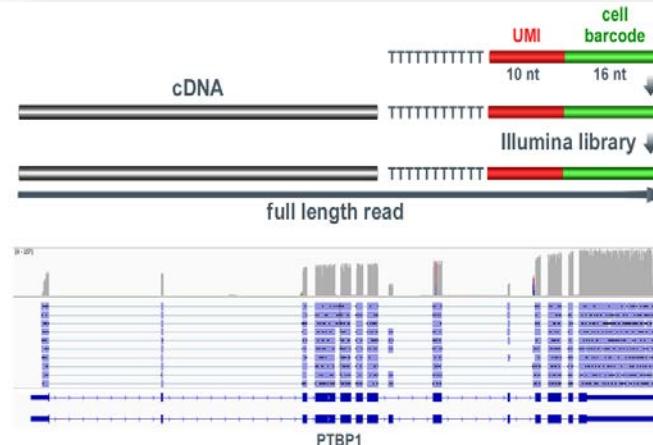
Gene-level
matrix



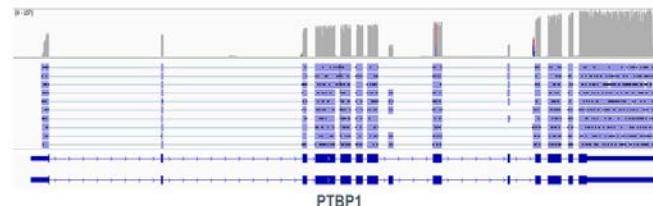
Standard short-read sequencing



Long-read full-length sequencing



Isoform-level
matrix



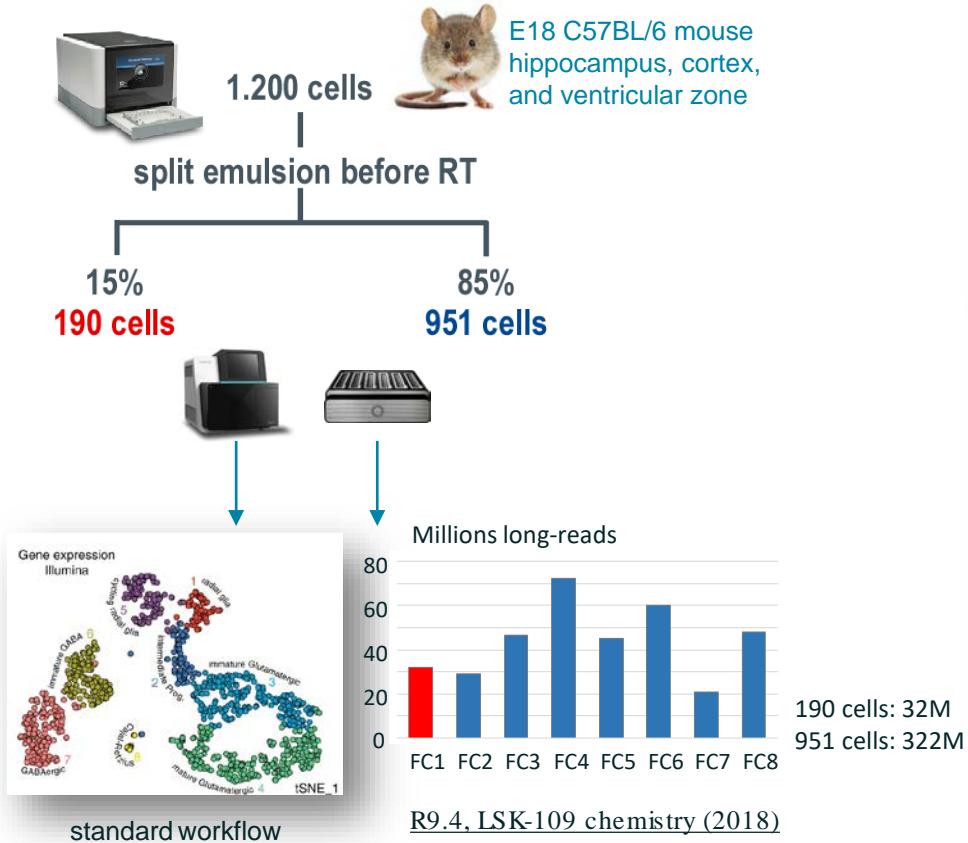
Information on alternative splicing, fusion transcripts, SNV, editing, imprinting, allelic imbalance

Is lost

Remain accessible

Single -cell long -read transcriptomics

SiCeLoRe bioinformatics for Single Cell Long Read



nature communications

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Article | Open Access | Published: 12 August 2020

High throughput error corrected Nanopore single cell transcriptome sequencing

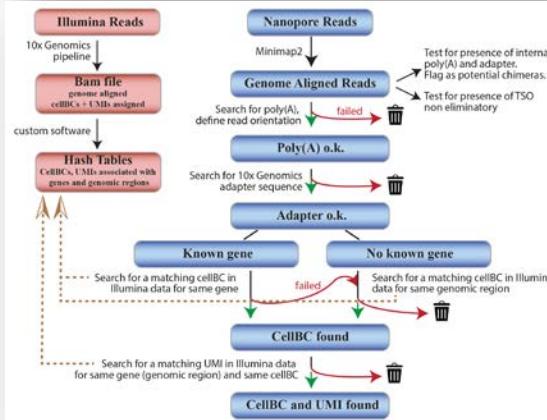
Kevin Lebrigand , Virginie Magnone , Pascal Barbuy & Rainer Waldmann

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

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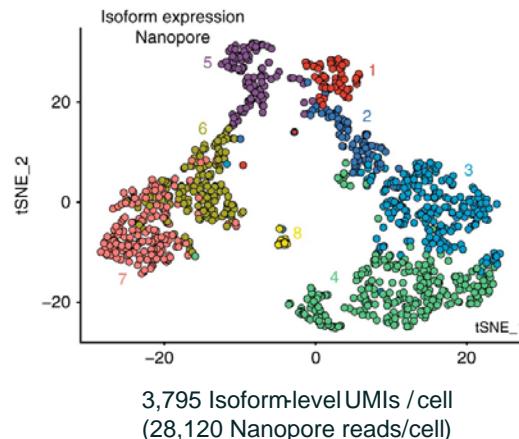
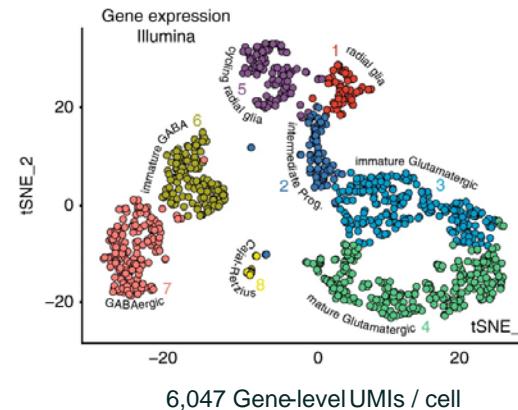
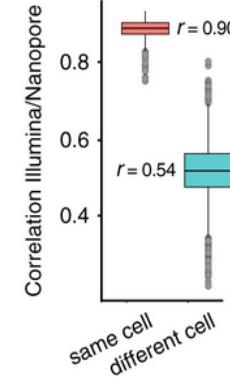
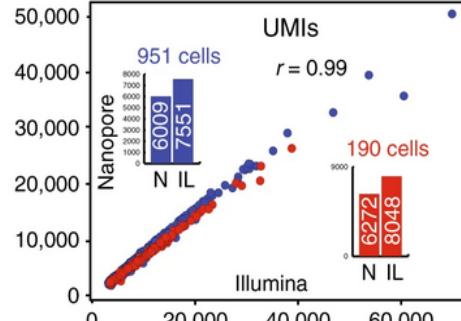
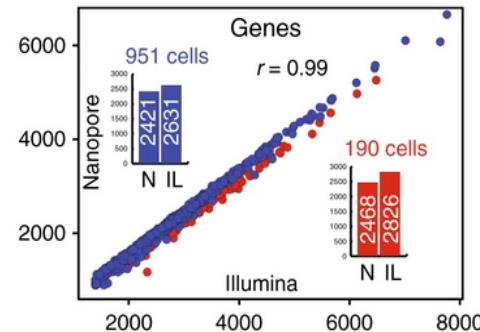
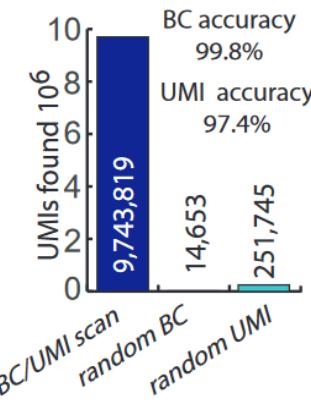
Rainer Waldmann



<https://github.com/ucagenomix/sicelore-2.1>

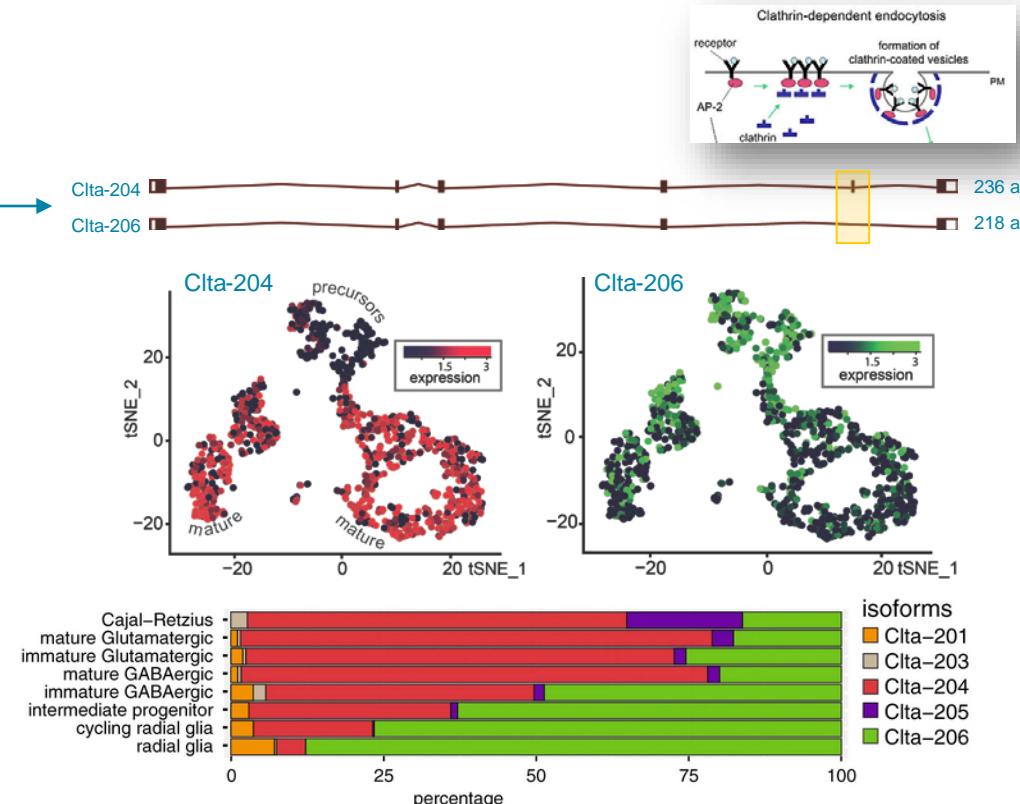
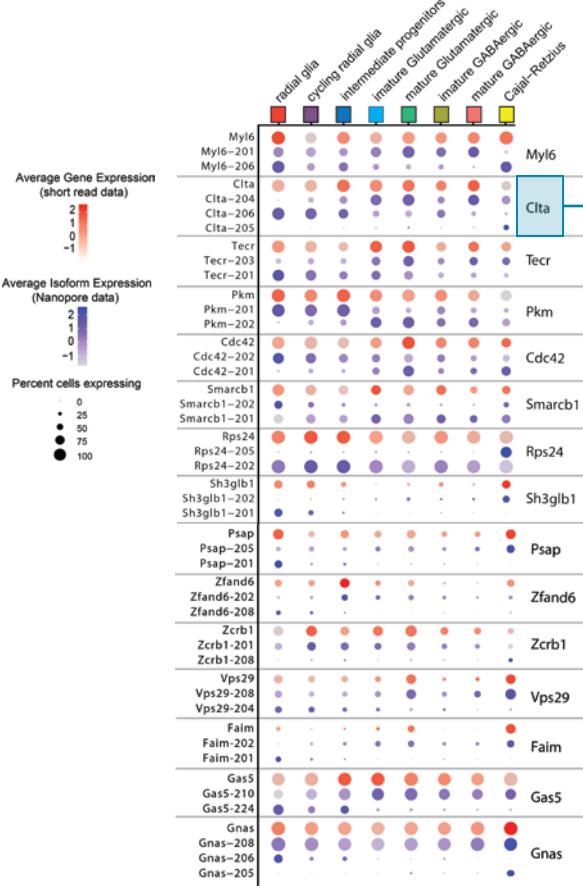
Single -cell long -read transcriptomics

Shows high accuracy, high correlation with short-read and high reproducibility



Single -cell long -read transcriptomics reveals diversity

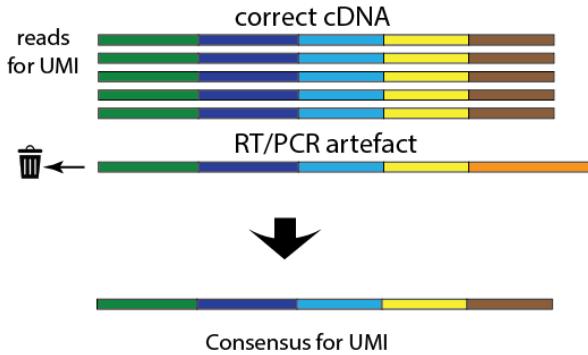
76 isoform-switching genes along neuronal maturation



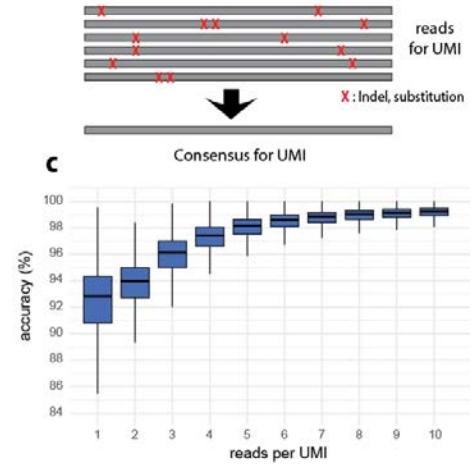
Single -cell long -read transcriptomics reveals sequence heterogeneity

Consensus sequence computation per UMI

UMIs enable elimination of PCR artifacts



UMIs enable correction of sequencing errors



Crucial for accurate novel isoform discovery

Identification of 4.388 novel mouse isoforms

- Backed by > 5 UMIs in more than 2 cells
- All SJs confirmed by SR dataset
- 5' end < 50 nt. from CAGE-seq
- 3'end < 50 nt. from polyA site

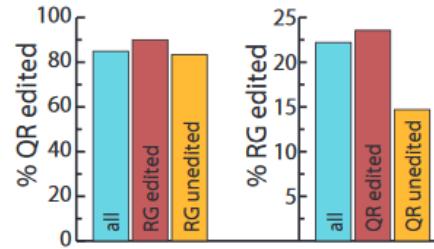
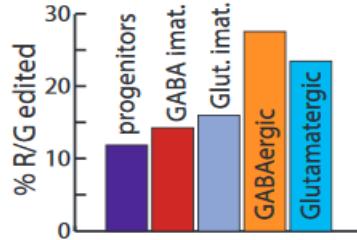
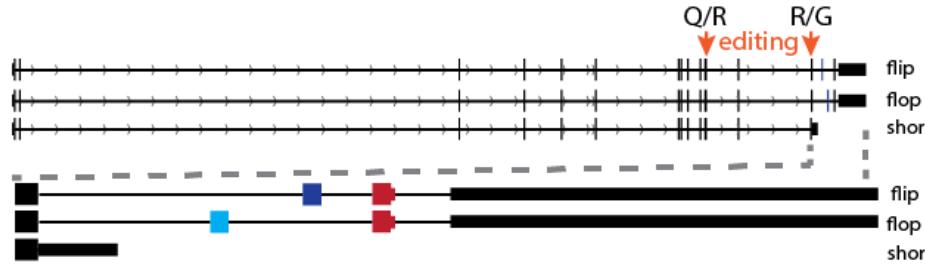
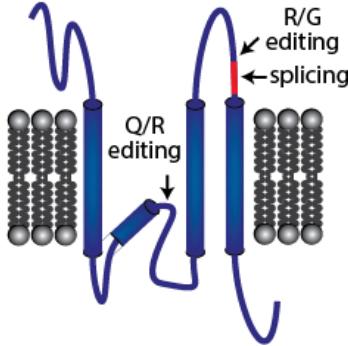
Crucial for high accuracy SNVcall

Single -cell long -read transcriptomics 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



RG site less edited in non-edited QR molecules
→ Editing process synchronization ?

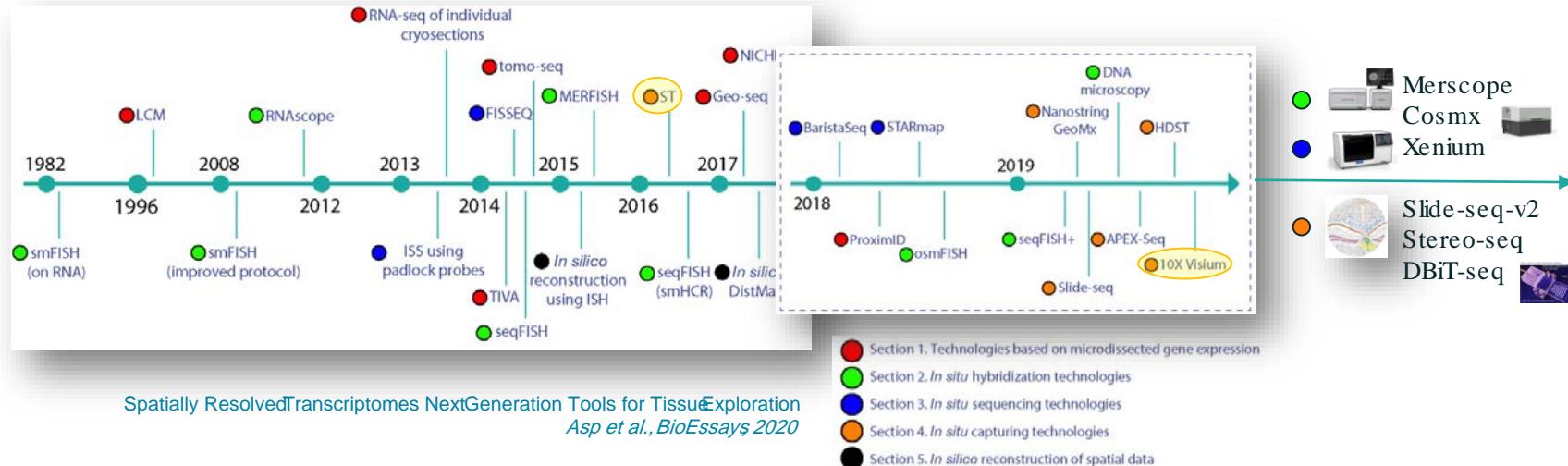
02

Spatial isoform Transcriptomics

Spatial Transcriptomics approaches

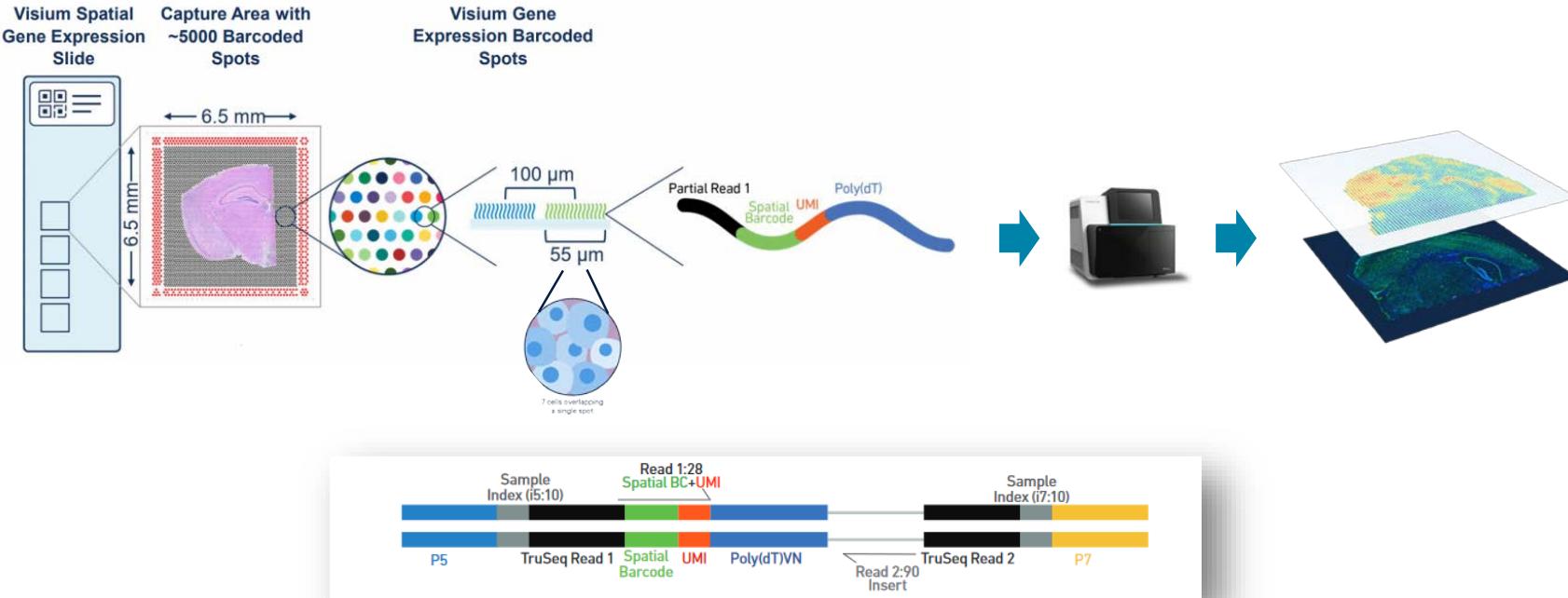
Historical timeline

- 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 as the droplet-based single-cell approach

Spatial isoform Transcriptomics (SiT)

Nucleic Acids Research, 2023

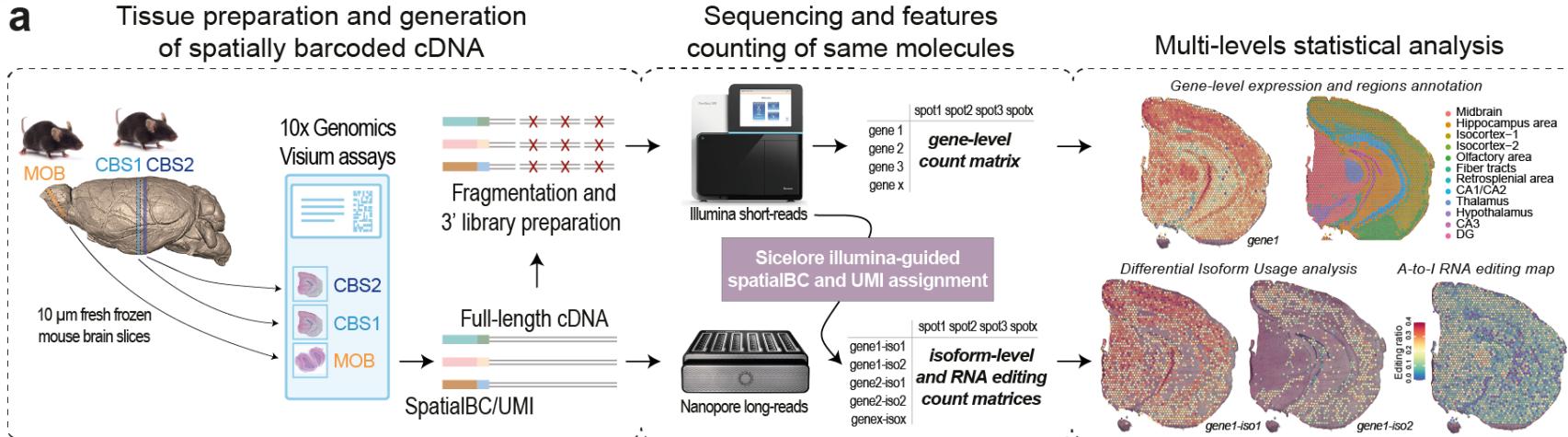
The spatial landscape of gene expression isoforms in tissue sections

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 ▾

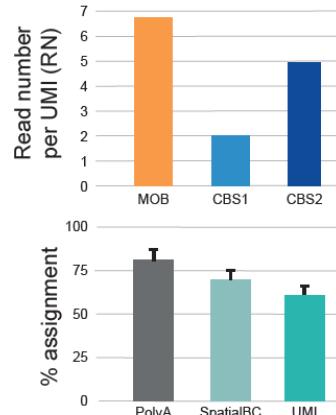
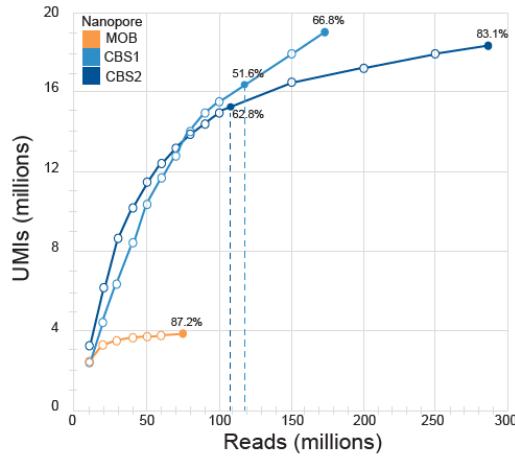
a



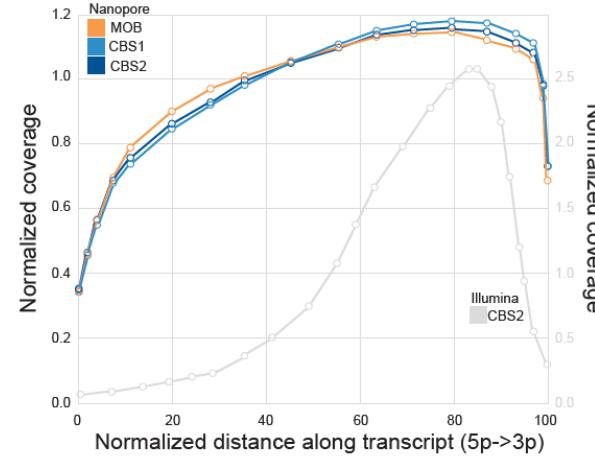
Nanopore promethION long -read sequencing

Provides isoform-level spatial transcriptomics

Sequencing saturation curves per sample



Transcripts fulllength coverage



Reads	MOB	
Date	18 feb. 20	20 mar. 20
Flow cells	PAE06474	PAE59649
Total reads (fastq_pass)	27628000	47272000
PolyA and Adapter found reads	21318117	47970311
SpatialBC found reads	14506264	29316718
UMIs found reads	10445006	19328468

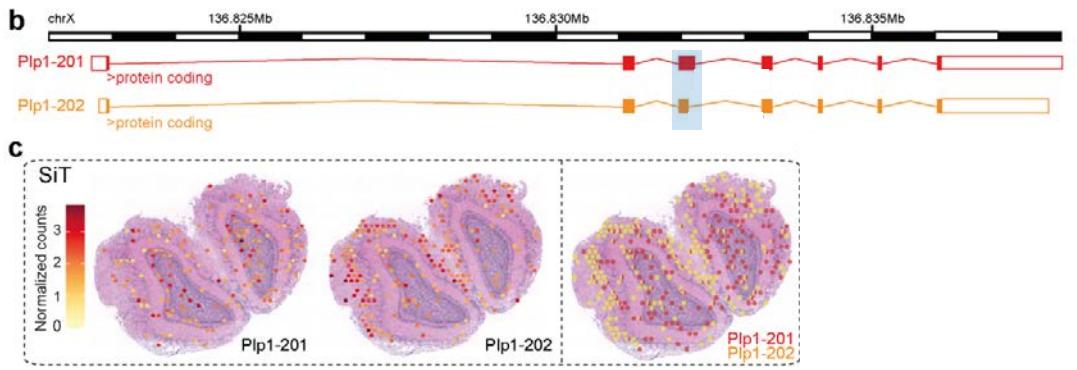
Reads	CBS1		
Date	18 feb. 20	20 mar. 20	24 feb. 21
Flow cells	PAE01745	PAE59645	PAG52067
Total reads (fastq_pass)	24980000	31736000	117280000
PolyA and Adapter found reads	17980183	27286678	80516212
SpatialBC found reads	12554655	19051597	54323311
UMIs found reads	7323748	10517081	27584331

Reads	CBS2										Total	%age
Date	12 may 20	13 may 20	19 may 20	25 may 20	25 may 20	26 may 20	27 may 20	09 feb. 21				
Flow cells	PAE59606	PAE59231	PAE32756	PAE32753	PAE31188	PAE21339	PAD99555	PAG56368	13			
Total reads (fastq_pass)	22897702	30405384	27492770	18534938	31506774	19108718	25596387	110916000	535354673			
PolyA and Adapter found reads	18536047	25199992	22871198	16088962	26777546	15983663	21682530	85837208	428048647	79,96	of Total passed reads	
SpatialBC found reads	14613934	19867830	14666481	11403706	19099469	11266930	14090779	60154119	294915793	68,90	of PolyA found reads	
UMIs found reads	8616415	11714126	9347072	7557944	12657620	7448718	9031708	34225619	175797856	59,61	of SpatialBC found reads	

CBS1: Oneflow 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 Visium slice

SiT reveals specific splicing pattern across MOB regions

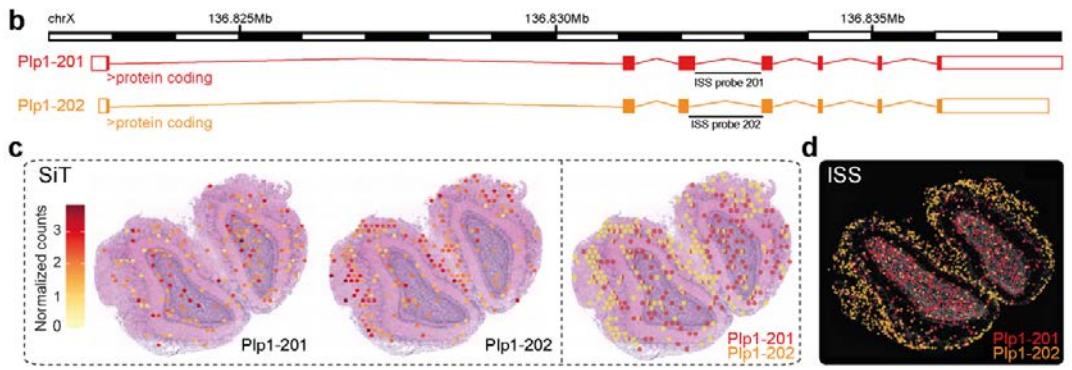
Plp1 Differential TranscriptUsage (DTU)



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)



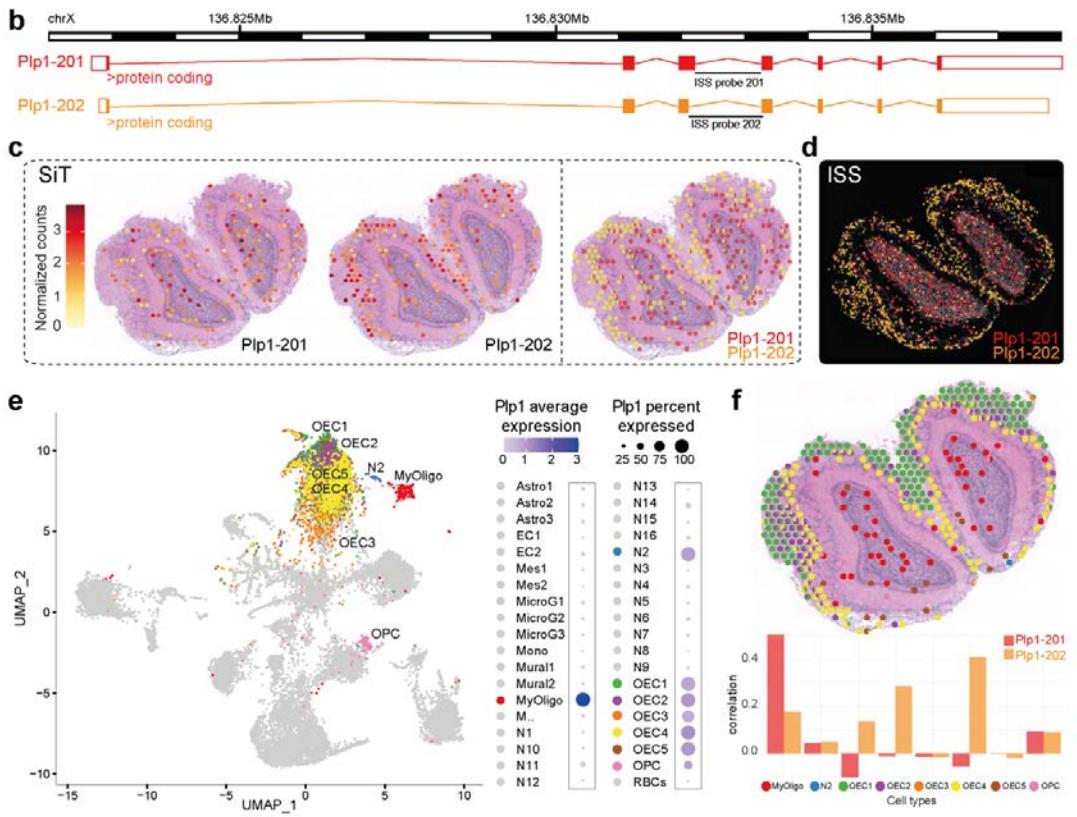
Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination



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



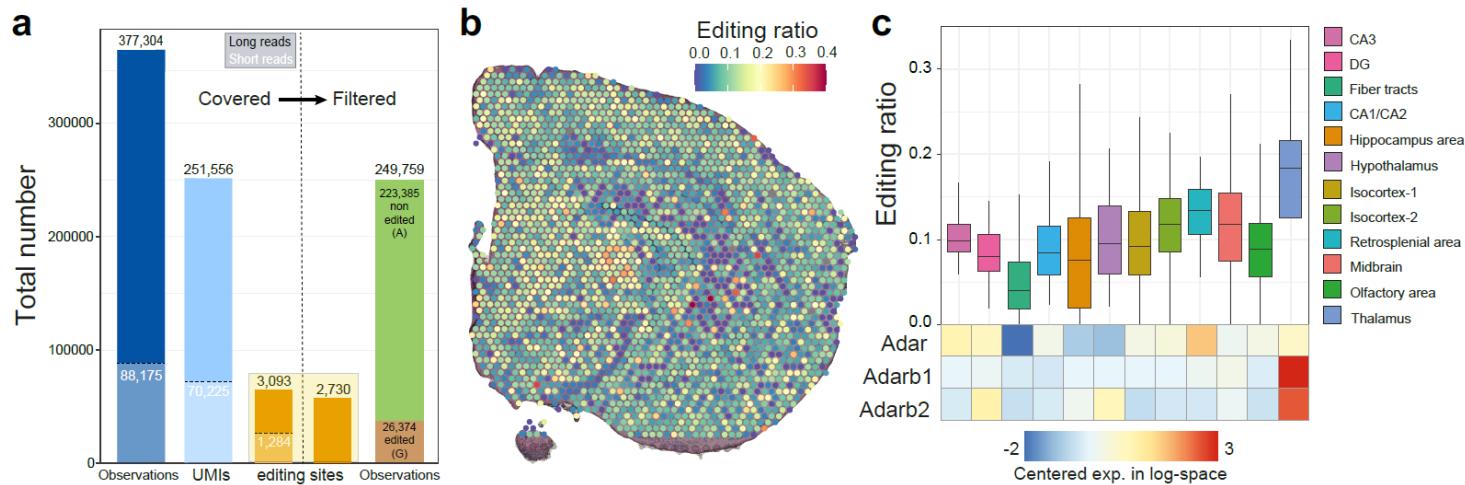
In Situ Sequencing Data

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

Global A-to-I RNA editing spatial map

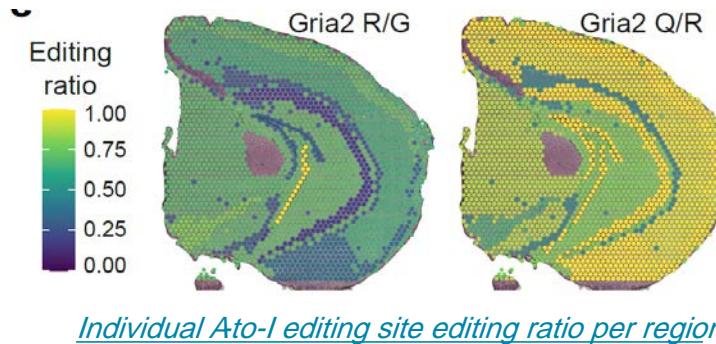
- Exploration of 5,817 Ato-I RNA editing sites described in the literature (Ramaswami et al., 2013 (RADAR); Licht et al., 2019)
- Long read high confidence call thresholding looking at agreement between long and short read base calls for 88,175 shared UMIs
 - number of reads per UMI ≥ 3
 - consensus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



SiT reveals full -length sequence heterogeneity

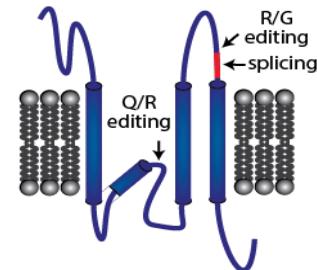
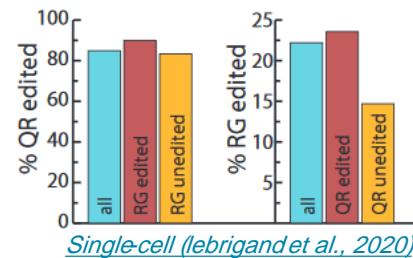
Global A-to-I RNA editing spatial map

- Exploration of 5,817 A-to-I RNA editing sites described in the literature (Ramaswami et al., 2013 (RADAR) Licht et al., 2019)
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Gria2

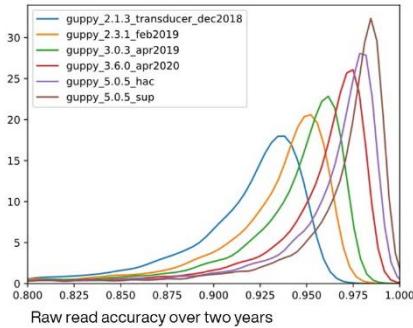
- R/G site is involved in desensitization and recovery of the receptor
- Q/R site regulates AMPA receptor Ca^{2+} -permeability



Single cell and Spatial isoform transcriptomics

Summary

- Accurate single-cell and spatial transcriptomics using Nanopore long-read sequencing is feasible
- Long reads sequencing reveal transcript diversity that is missed with standard short reads workflows
- Single Nucleotide Variation calls (SNV, editing) in single-cell and in a spatial context can be achieved
- **Sicelore-2.1** : we don't need short reads anymore



Nanopore PromethION sequencing

2018: **20M** reads/FC, **92%** raw read accuracy

2022: **150M** reads/FC, **98%** raw read accuracy



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

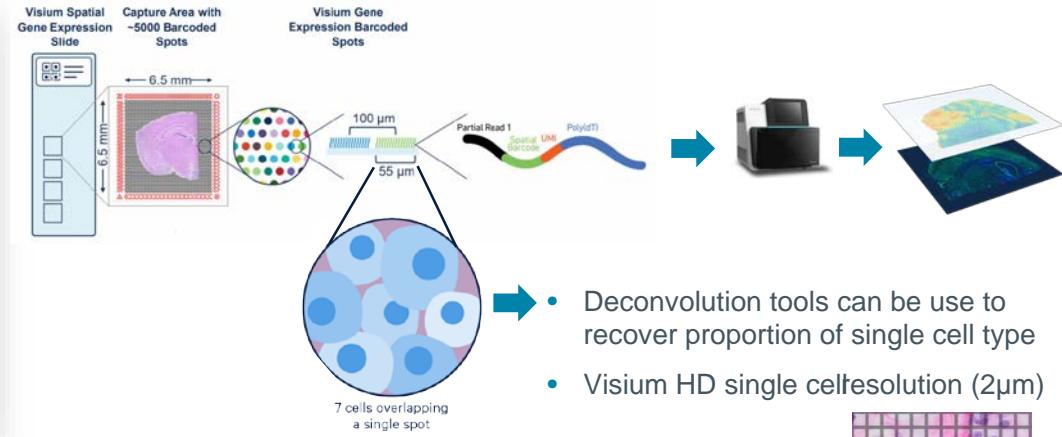
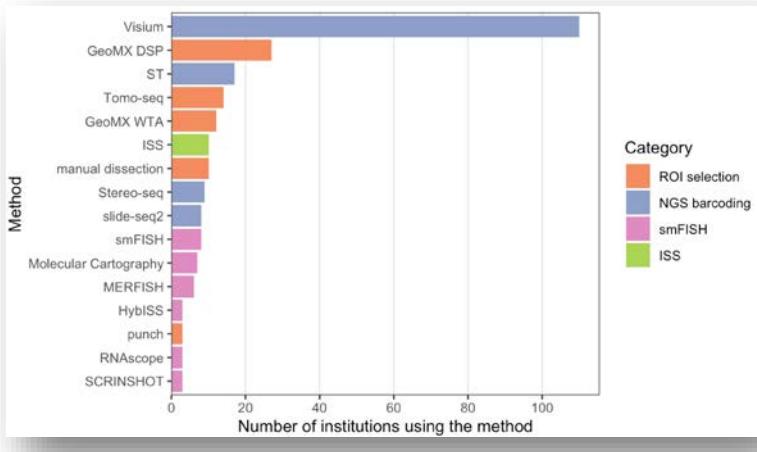
- Visium and singlecell 3' and 5' libraries
- Illumina-free profiling available

03

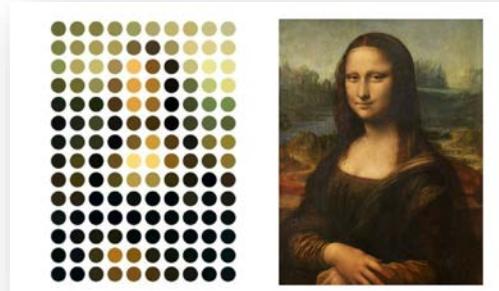
Spatial imaging -based Transcriptomics

In-situ capture Spatial Transcriptomics (2017 -2022)

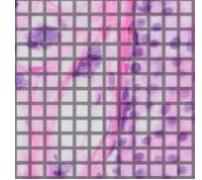
Visium is widely adopted by academics



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



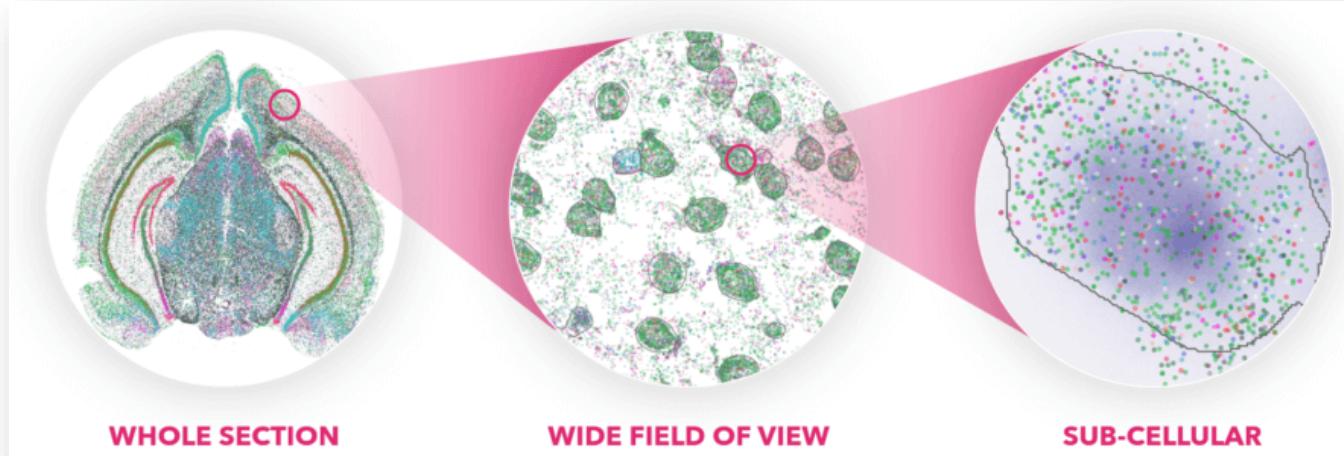
But is not the ideal readout for spatial biology
(Akoya credit rough caricature)



Imaging -based Spatial 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)



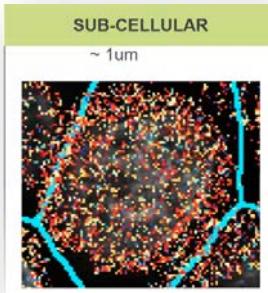
Imaging -based Spatial Transcriptomics (2022)

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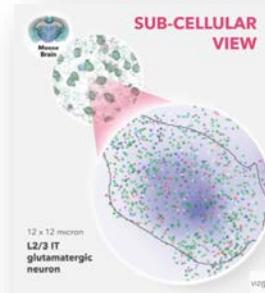
Nanostring CosMx

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



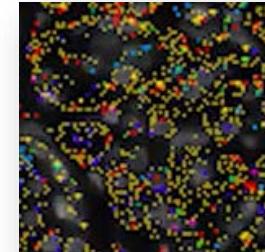
Vizgen Merscope

- 1.000 targets
- Sensitivity: 30-80% (++)
- Imaging area: 100 mm² (2 days)
- Resolution **100 nm**



10xGenomics Xenium

- 400 - 6,000 targets
- Sensitivity : 530% (++)
- Imaging area:**236 mm²** (4 days)
- Resolution 200 nm

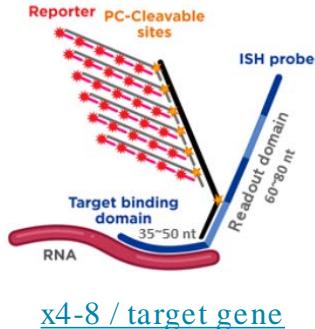


Imaging -based Spatial Transcriptomics (2022)

No more sequencing for direct single-cell resolution



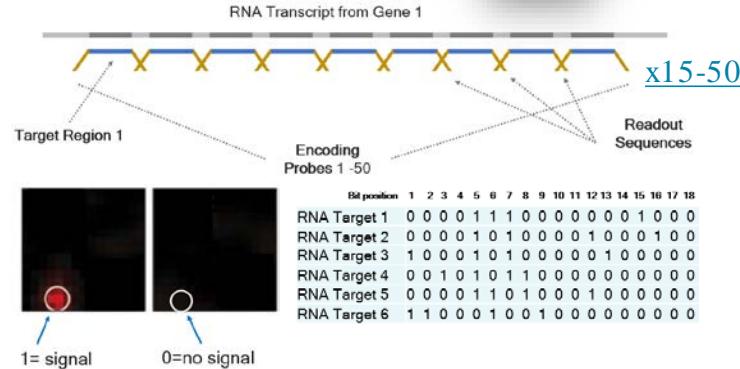
Nanostring CosMx
ISH based



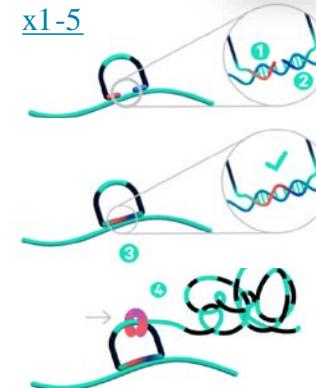
Vizgen Merscope
Multiplex Error Robust FISH
Available (oct.2022)



10xGenomics Xenium
Cartana ISS, padlock probes / RCA
Available (jan.2024)



Cyclic *in situ* Hybridization Chemistry



Imaging -based Spatial Transcriptomics platforms comparison

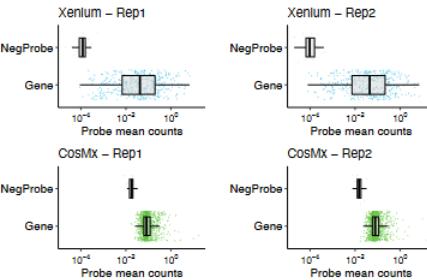
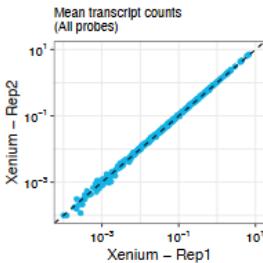
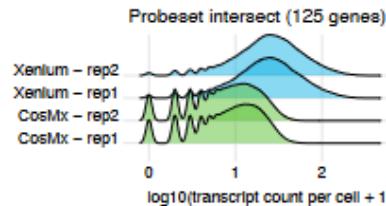
2 recent bioRxiv comparative studies

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*}

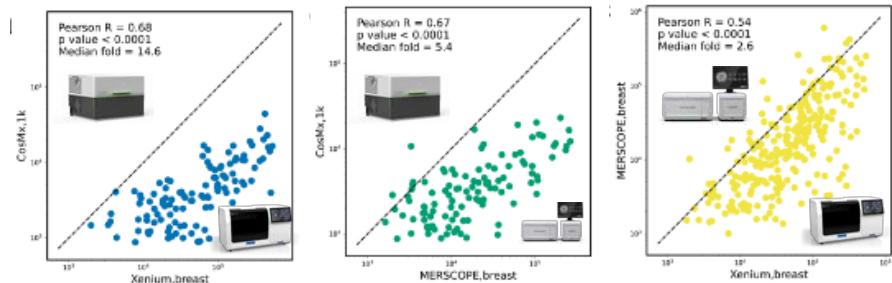


	Xenium Rep 1	Xenium Rep 2	CosMx Rep 1	CosMx Rep 2
Gene target #	377	377	1000	1000
Total cell count	99,852	102,508	98,139	98,767
Median gene count per cell	33	34	75	71
Median transcript count per cell	88	92	113	99
Median transcript count / gene target count	0.23	0.24	0.11	0.10
Median transcript count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



- CosMx is much less sensitive (high FPR)
- Merscope / Xenium for Fresh frozen slice
- Xenium optimal for FFPE slice

Gene targets panel design

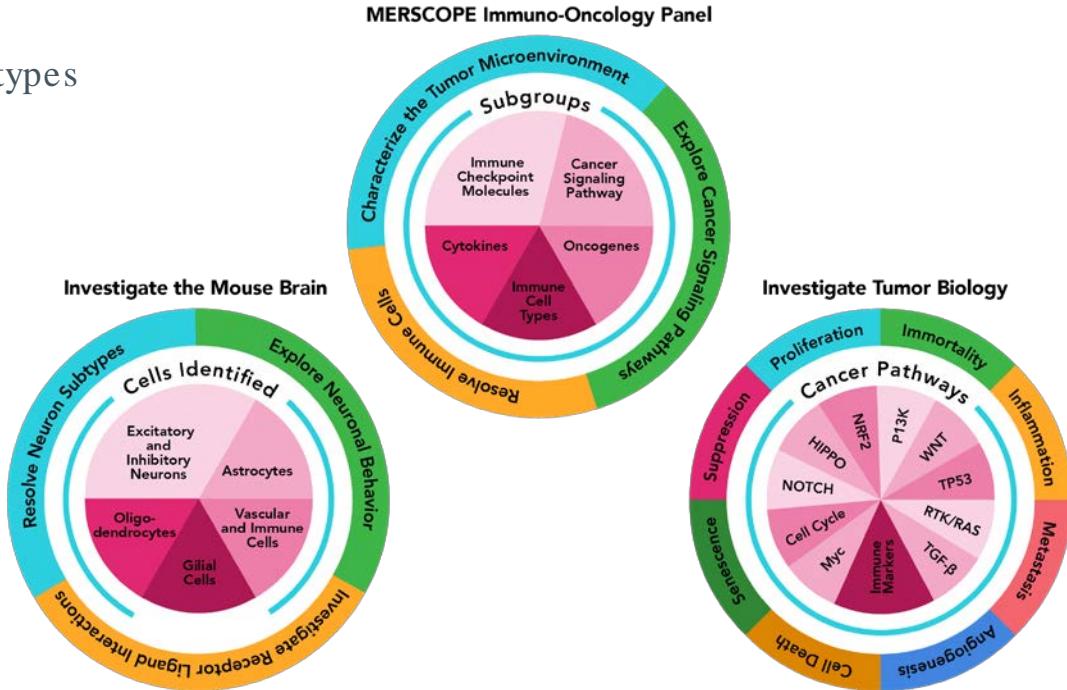
Depending on the biological question !

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
- Budget around **15 k€** for 10 reactions



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

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

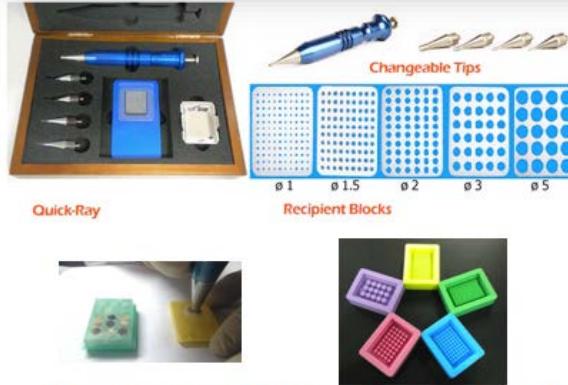
Experimental design

Take advantage of the large imaging area

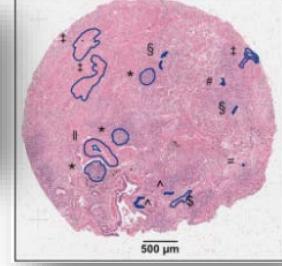


Image-based spatial transcriptomics identifies molecular niche dysregulation associated with distal lung remodeling in pulmonary fibrosis

Annika Vannan^{1,8}, Ruqian Lyu^{2,3,8}, Arianna L. Williams¹, Nicholas M. Negretti⁴, Evan D. Mee¹, Joseph Hirsh⁴, Samuel Hirsh⁴, David S. Nichols⁵, Carla L. Calvi⁶, Chase J. Taylor⁶, Vassily V. Polosukhin⁶, Ana PM Serezani³, A. Scott McCall³, Jason J. Gokey⁶, Heejung Shim³, Lorraine B. Ware^{5,7}, Matthew J. Bacchetta⁸, Clara M. Shaver⁵, Timothy S. Blackwell^{5,9,10}, Rajat Walia¹¹, Jennifer MS Sucre^{4,9}, Jonathan A. Kropski^{5,9,10,12}, Davis J McCarthy^{2,3,10}, Nicholas E. Banovich^{1,8,*}



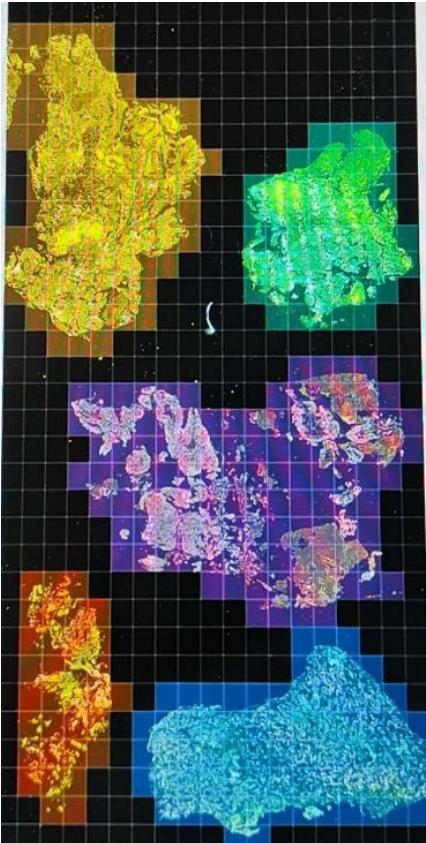
<https://www.ihcworld.com/products/Quick-Ray-Mold.htm>



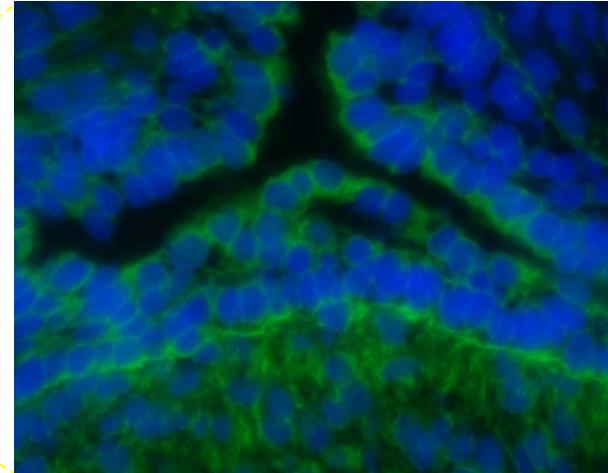
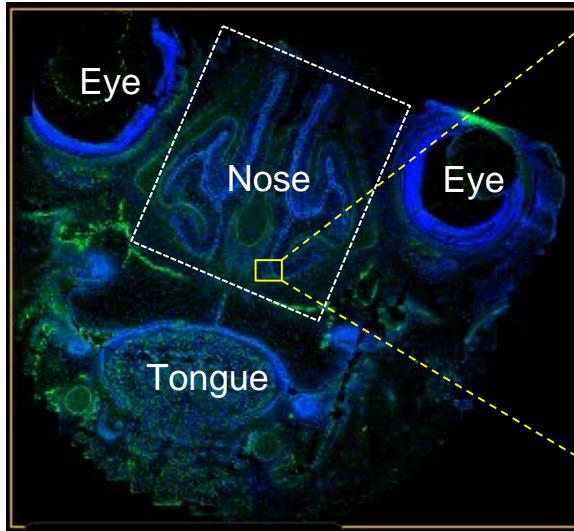
Each slide cost around 5 k€
multiplexing to remove batch effect and increase replicates for robust statistical analysis

Data acquisition

DAPI and cell boundaries staining for cell segmentation



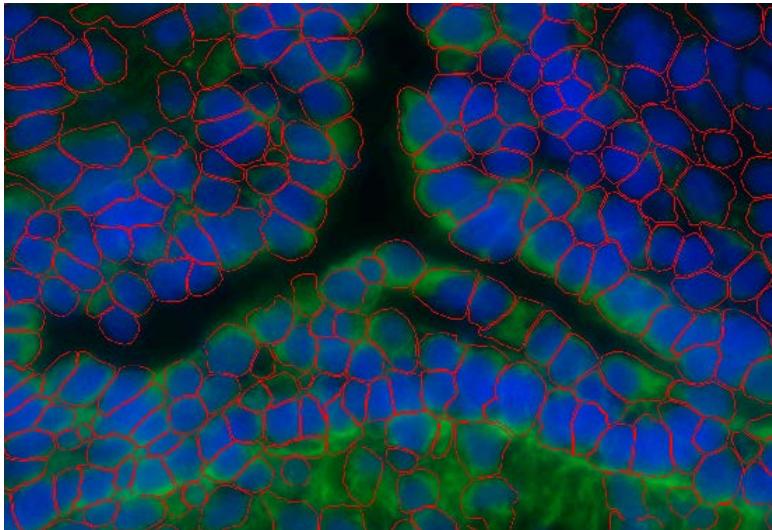
Human fetal head section (PCW9)



DAPI channel
Cell boundaries channel

Data acquisition

Cell segmentation

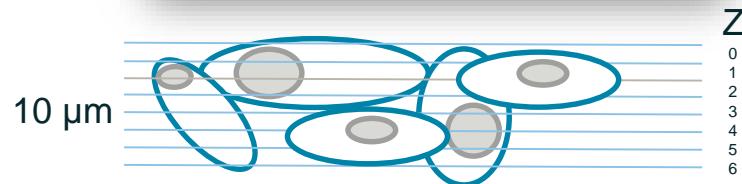
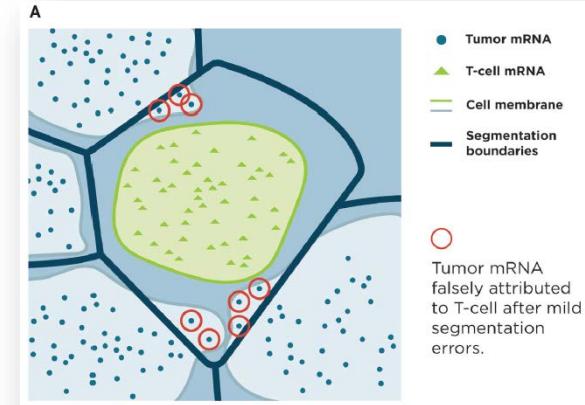


Cellpose: a generalist algorithm for cellular segmentation

Carsen Stringer, Tim Wang, Michalis Michaelos & Marius Pachitariu

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

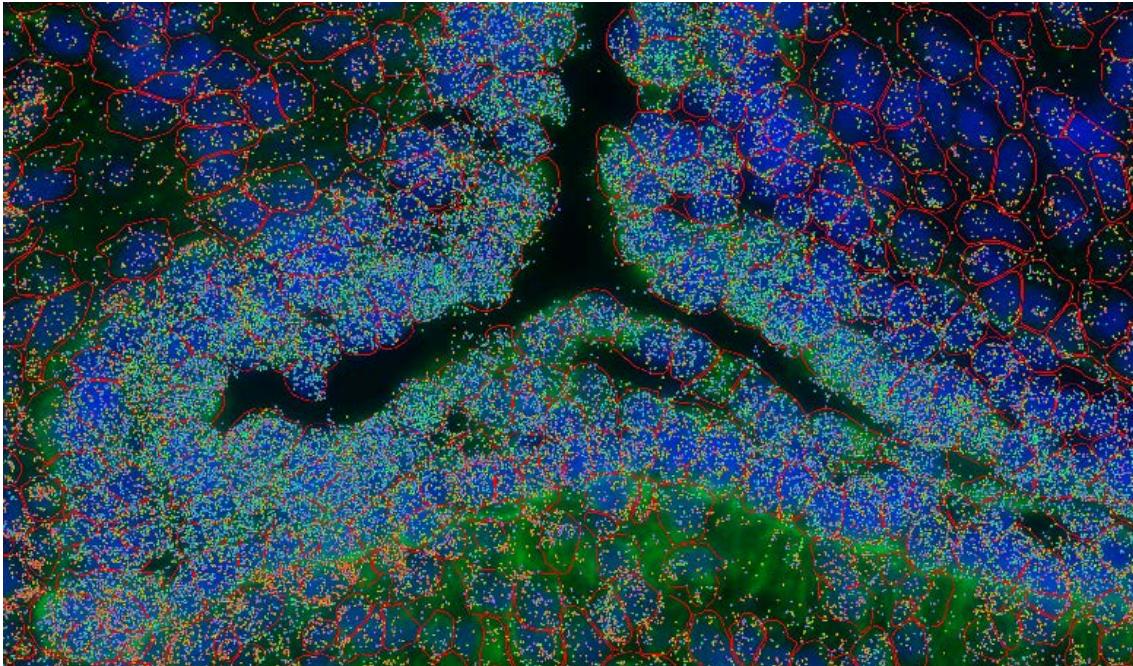
Cell segmentation is crucial to ensure cell x gene matrix purity for good subsequent biology



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)

Raw data

Cell x genematrix

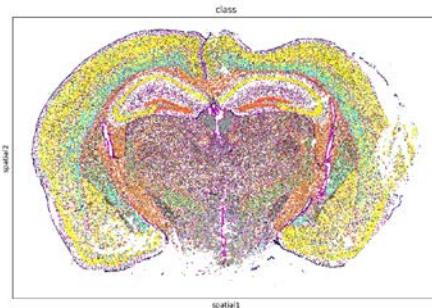


Gene-level matrix

→ 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	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



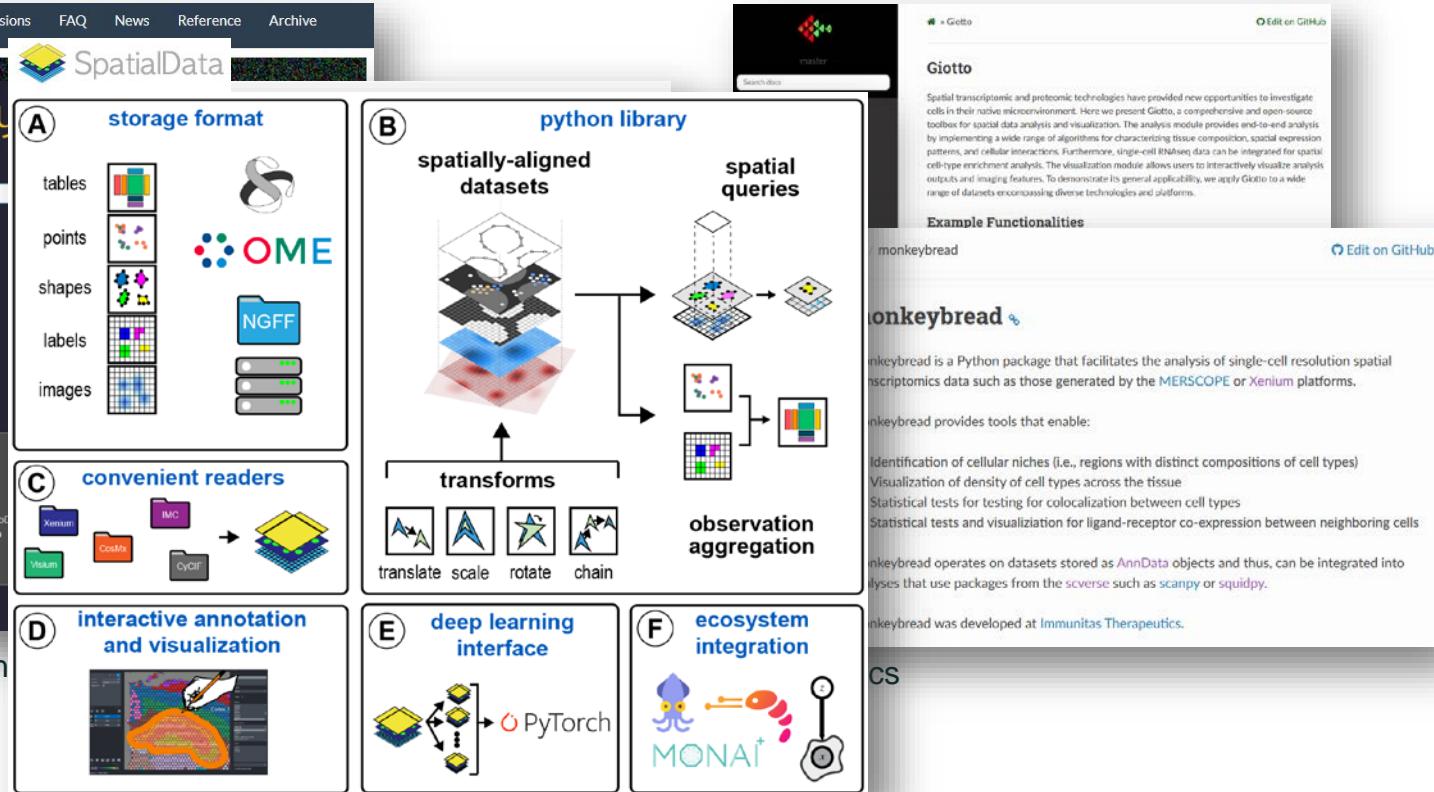
Statistical data analysis

Standardized workflows + packages development

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

Satija'slab, NYGC

Theis'slab, h

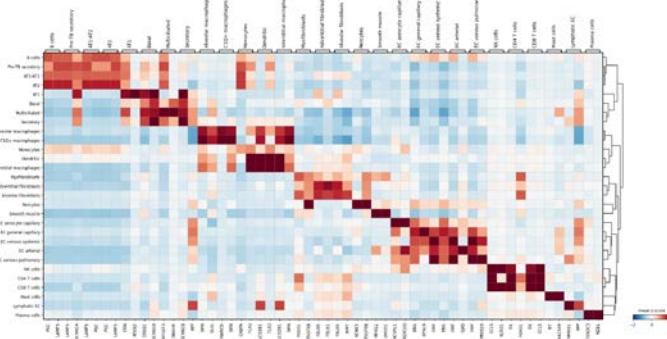


Scverse ecosystem, Oliver Stegle & FabianJ. Theis

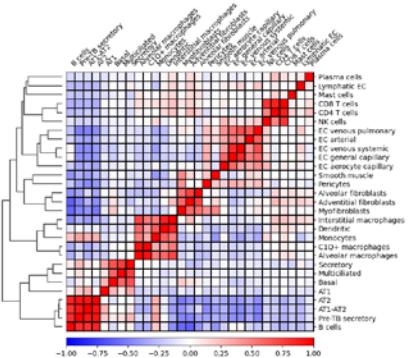
Single -cell data analysis

Scanpy and Squidpy toolkits

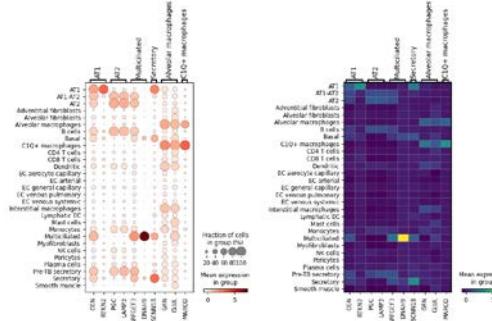
Gene marker detection, manual or automatic cell type identification



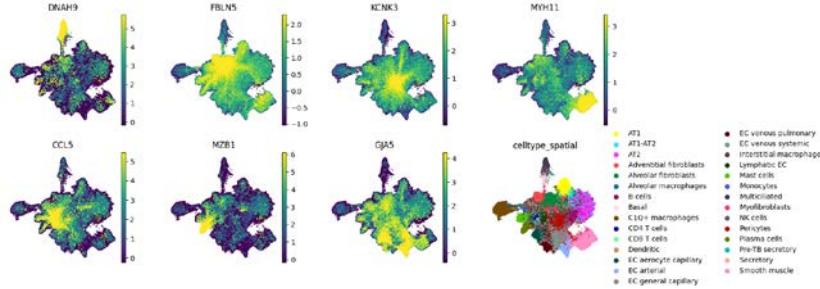
Cell type correlation



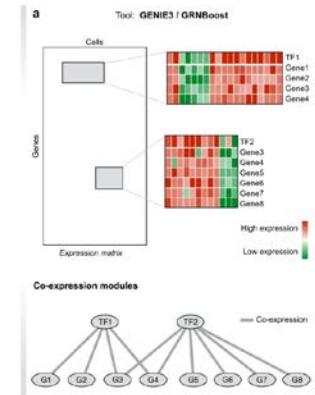
Differential expression analysis Gene set functional enrichment



Batch effect correction, sample integration, cell type labeling transfer from single-cell references dataset

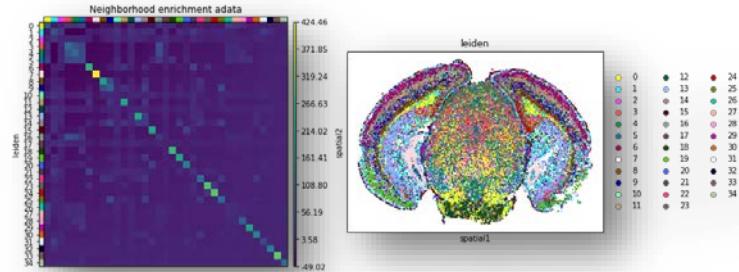


Transcription Regulatory Network



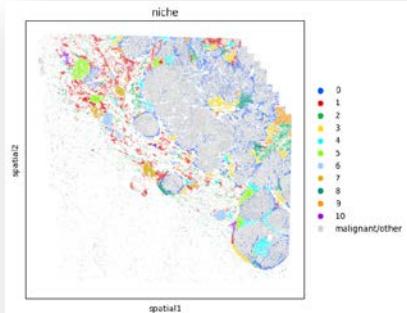
Single -cell data analysis including spatial resolution

New vast area for computational biologists (just like single-cell 5 years ago)



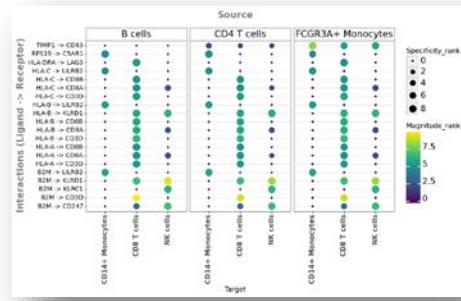
Neighbors enrichment analysis

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



Cellular niches analysis

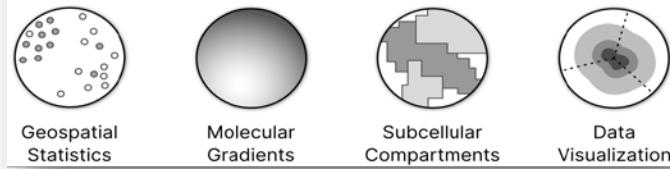
for each cell, we count the number of neighbors that are of each cell type thus forming a “neighborhood profile” vector of length C, where C is the number of cell types. We then cluster all neighborhood profiles and call each cluster a “niche”.



Cell-cell communication Ligand-Receptor analysis

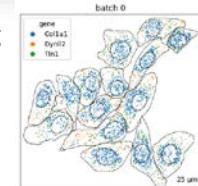
- Need to be in gene panel or inferred
- CellPhoneDB [Efremova et al, 2020]
- Omnipath [Türe et al, 2016].

Machine Learning & Statistical Analysis



Sub-cellular exploration

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



Acknowledgments

Institut de Pharmacologie Moléculaire et Cellulaire



Pascal Barbry's Lab (IPMC, CNRS, France)

- Rainer Waldmann
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- Marie-Jeanne Arguel

Joakim Lundeberg's Lab (KTH Royal Institute of Technology, Sweden)

- Joseph Bergenstråhlé
- Kim Thrane

CoBiODA IPMC bioinformatics

- Kévin Lebrigand
- Marin Truchi
- Eamon McAndrew

