Single-Cell and Spatial technologies and experimental approaches

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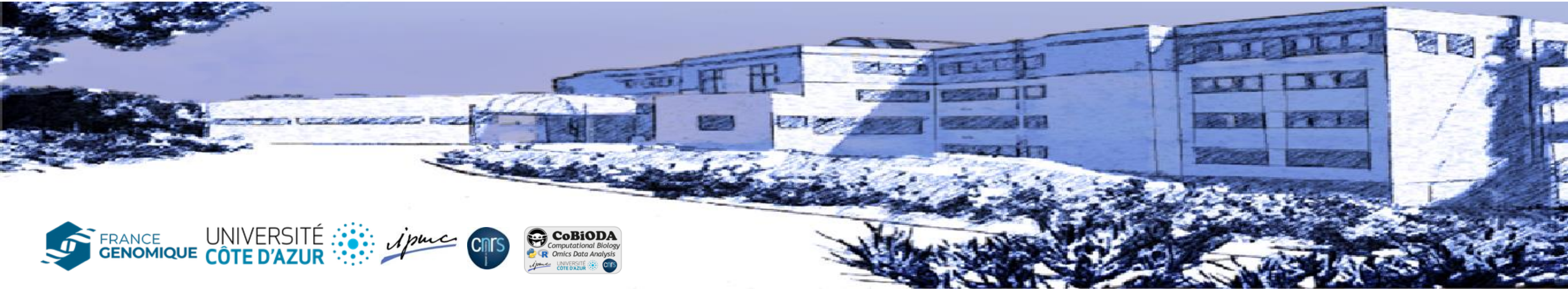


Overview

01 - Single-Cell experimental approaches

02 - Spatial in-situ capture experimental approaches

03 - Spatial imaging-based experimental approaches

Institute of Molecular and Cellular Pharmacology

Sophia-Antipolis

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 Metabolomics
- ANIPRO, animal care and behavior facility
- CoBiODA, Bioinformatics Hub
- UniCA GenomiX, Functional Genomics platform

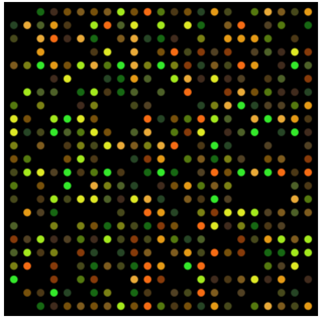
FRANCE GENOMIQUE

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

2015 2016 2018 2022 2024

20 years of transcriptomics

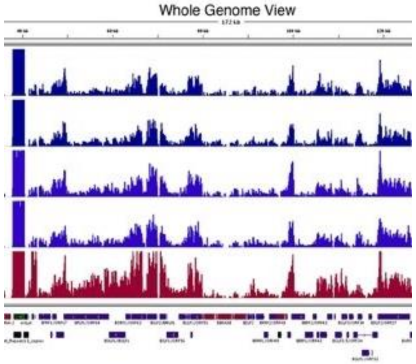
Driven by microfluidics technological developments



Early 2000’s: DNA microarray

- Large-scale transcriptome
- Oligonucleotide probe tilling
- Fluorochrome signal analysis
- Bulk resolution

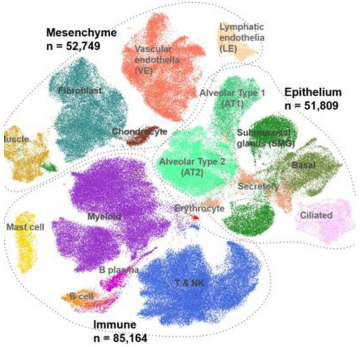
Cost : 4k€
20 samples
25k genes
0,5M matrix



Late 2000’s: RNA sequencing

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

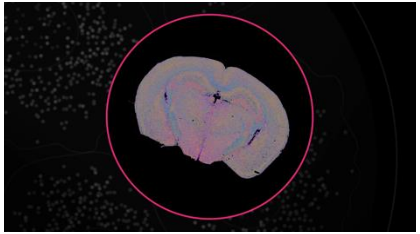
Cost : 4k€
20 samples
50k genes
1M matrix



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
- Multiplexing FiSH (single molecule)
- Sensitivity (30-80%)
- Sub-cellular resolution

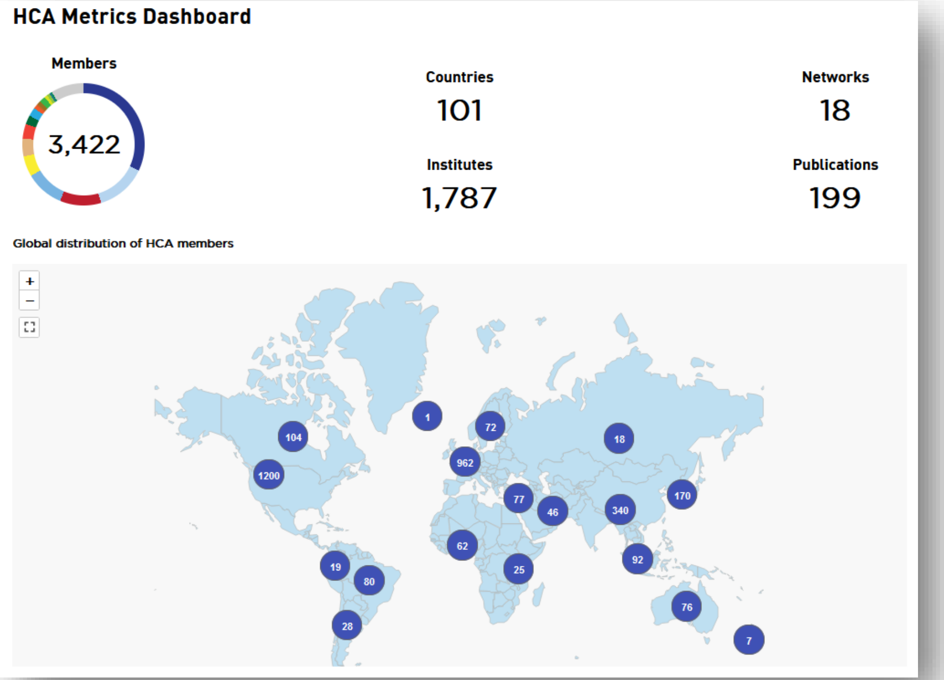
Cost : 4k€
250k cells
1k genes
250M matrix
+ Spatial dimension

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 3,422

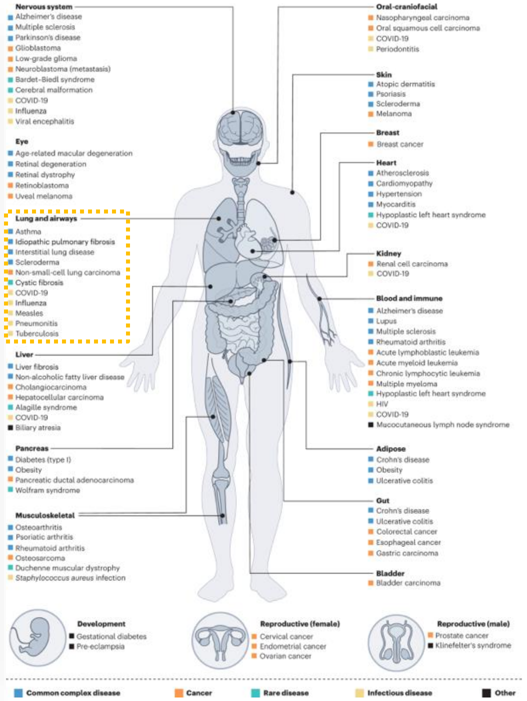
Countries 101

Networks 18

Institutes 1,787

Publications 199

Global distribution of HCA members

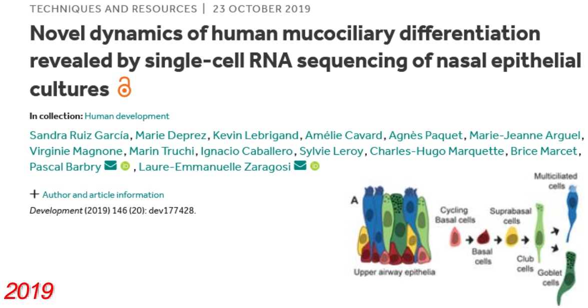


Human Cell Atlas

Contributions

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

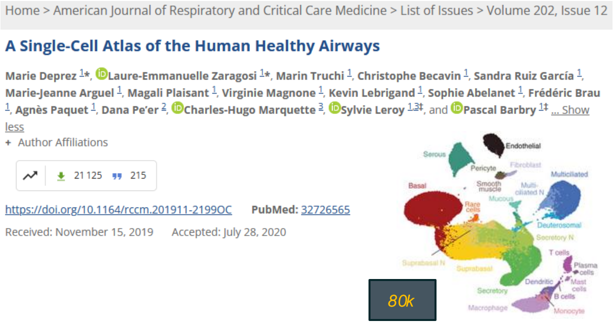
In collection: Human development

Sandra Ruiz García, Marie Deprez, Kevin Lebrigand, Amélie Cavard, Agnès Paquet, Marie-Jeanne Arguel, Virginie Magnone, Marin Truchi, Ignacio Caballero, Sylvie Leroy, Charles-Hugo Marquette, Brice Marcet, Pascal Barbry, Laure-Emmanuelle Zaragosi

\+ 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 Zaragosi [1]*, Marin Truchi [1], Christophe Becavin [1], Sandra Ruiz García [1], Marie-Jeanne Arguel [1], Magali Plaisant [1], Virginie Magnone [1], Kevin Lebrigand [1], Sophie Abelanet [1], Frédéric Brau [1], Agnès Paquet [1], Dana Pe'er [2], Charles-Hugo Marquette [3], Sylvie Leroy [1,3]‡, and Pascal Barbry [1]‡ ... Show less

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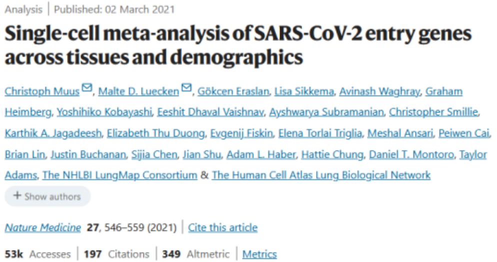
21 125 215

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

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

80k

2021



Analysis | Published: 02 March 2021

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

Christoph Muus, Malte D. Luecken, Gökcen Eraslan, Lisa Sikkema, Avinash Waghray, Graham Heimberg, Yoshihiko Kobayashi, Eeshit Dhaval Vaishnav, Ayshwarya Subramanian, Christopher Smillie, Karthik A. Jagadeesh, Elizabeth Thu Duong, Evgenij Fiskin, Elena Torlai Triglia, Meshal Ansari, Peiwen Cai, Brian Lin, Justin Buchanan, Sijia Chen, Jian Shu, Adam L. Haber, Hattie Chung, Daniel T. Montoro, Taylor Adams, The NHLBI 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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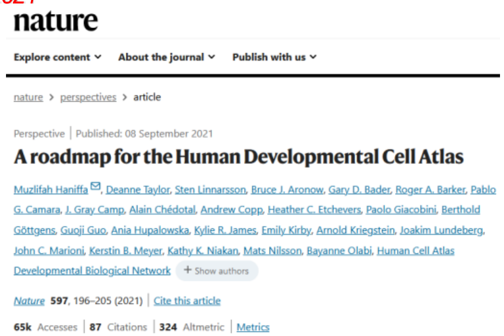
2021



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Perspective | Published: 08 September 2021

A roadmap for the Human Developmental Cell Atlas

Muzlifah Haniffa, Deanne Taylor, Sten Linnarsson, Bruce J. Aronow, Gary D. Bader, Roger A. Barker, Pablo G. Camara, J. Gray Camp, Alain Chédotal, Andrew Copp, Heather C. Etchevers, Paolo Giacobini, Berthold Göttgens, Guoji Guo, Ania Hupalowska, Kylie R. James, Emily Kirby, Arnold Kriegstein, Joakim Lundeberg, John C. Marioni, Kerstin B. Meyer, Kathy K. Niakan, Mats Nilsson, Bayanne Olabi, Human Cell Atlas Developmental Biological Network

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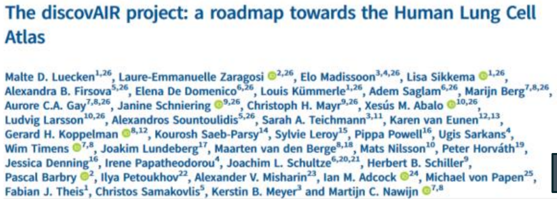
Nature **597**, 196–205 (2021) | Cite this article

65k Accesses | **87** Citations | **324** Altmetric | Metrics

2022

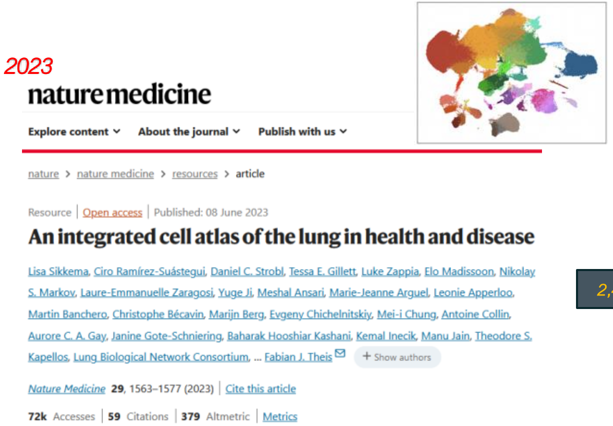


The discovAIR project: a roadmap towards the Human Lung Cell Atlas

Malte D. Luecken[1,26], Laure-Emmanuelle Zaragosi[2,26], Elo Madissoon[3,4,26], Lisa Sikkema[1,26], Alexandra B. Firsova[5,26], Elena De Domenico[6,26], Louis Kümmerle[1,26], Adem Saglam[6,26], Marijn Berg[7,8,26], Aurore C.A. Gay[7,8,26], Janine Schniering[9,26], Christoph H. Mayr[9,26], Xesús M. Abalo[10,26], Ludvig Larsson[10,26], Alexandros Sountoulidis[5,26], Sarah A. Teichmann[3,11], Karen van Eunen[12,13], Gerard H. Koppelman[8,12], Kourosh Saeb-Parsy[14], Sylvie Leroy[15], Pippa Powell[16], Ugis Sarkans[4], Wim Timens[7,8], Joakim Lundeberg[17], Maarten van den Berge[8,18], Mats Nilsson[10], Peter Horváth[19], Jessica Denning[16], Irene Papatheodorou[4], Joachim L. Schultze[6,20,21], Herbert B. Schiller[9], Pascal Barbry[2], Ilya Petoukhov[22], Alexander V. Misharin[23], Ian M. Adcock[24], Michael von Papen[25], Fabian J. Theis[1], Christos Samakovlis[5], Kerstin B. Meyer[3] and Martijn C. Nawijn[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 Ramírez-Suástegui, Daniel C. Strobl, Tessa E. Gillett, Luke Zappia, Elo Madissoon, Nikolay S. Markov, Laure-Emmanuelle Zaragosi, Yuge Ji, Meshal Ansari, Marie-Jeanne Arguel, Leonie Apperloo, Martin Banchero, Christophe Bécavin, Marijn Berg, Evgeny Chichelnitskiy, Mei-i Chung, Antoine Collin, Aurore C. A. Gay, Janine Gote-Schniering, Baharak Hooshiar Kashani, Kemal Inecik, Manu Jain, Theodore S. Kapellos, Lung Biological Network Consortium, ... Fabian J. Theis

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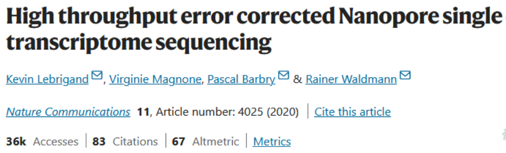
Nature Medicine **29**, 1563–1577 (2023) | Cite this article

72k Accesses | **59** Citations | **379** Altmetric | Metrics

2,4M

2020

10x GENOMICS



High throughput error corrected Nanopore single cell transcriptome sequencing

Kevin Lebrigand, Virginie Magnone, Pascal Barbry & Rainer Waldmann

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

36k Accesses | **83** Citations | **67** Altmetric | Metrics

Oxford NANOPORE Technologies

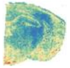
2023

The spatial landscape of gene expression isoforms in tissue sections

Kevin Lebrigand, Joseph Bergenstråhle, Kim Thrane, Annelie Mollbrink, Konstantinos Meletis, Pascal Barbry, Rainer Waldmann, Joakim Lundeberg

Author Notes

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



10x GENOMICS

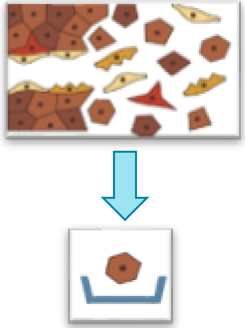
01

Single-Cell transcriptomics experimental approaches

Single-cell transcriptomics

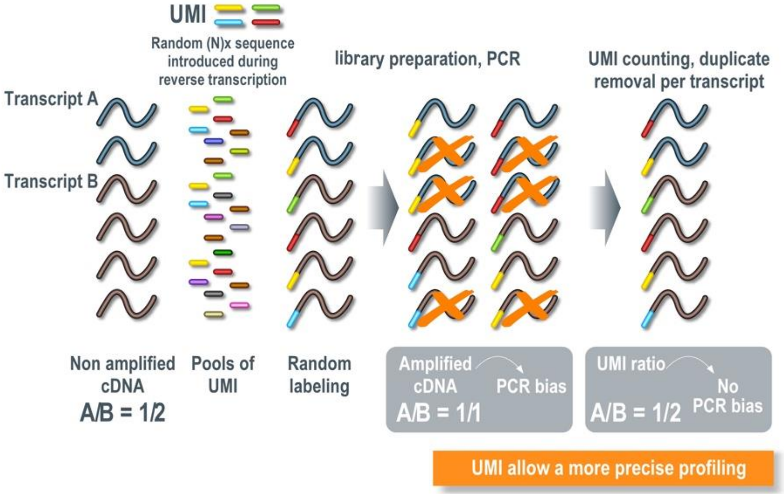
Context

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



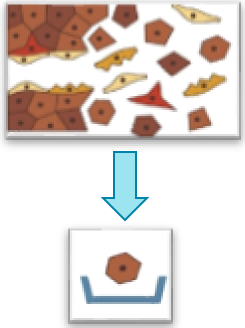
Single cell mRNA libraries
10 pg total RNA
(<<1pg mRNA)

- Elimination of PCR amplification bias and artefacts
- Highly efficient library preparation techniques
- 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

Context

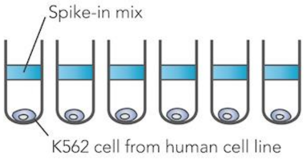


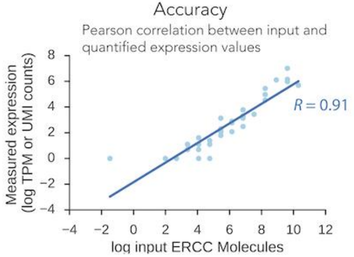
Bulk mRNA libraries
100ng total RNA
(10,000 cells)

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

- Elimination of PCR amplification bias and artefacts
- Highly efficient library preparation techniques
- Spike-in ERCC molecules allow yield and **capture efficiency** evaluation

ERCC (External RNA Controls Consortium)
  — set of 92 RNA sequences,
  — of varying length and GC content,
  — mixed at known concentrations,
  — 22 abundance levels that are spaced one fold change apart from each other





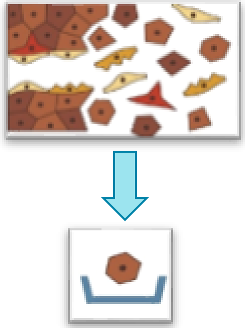
Accuracy

Pearson correlation between input and quantified expression values

Power Analysis of Single Cell RNA-Sequencing Experiments, Svensson et al., 2018

Single-cell transcriptomics

How much RNA does a typical mammalian cell contain?

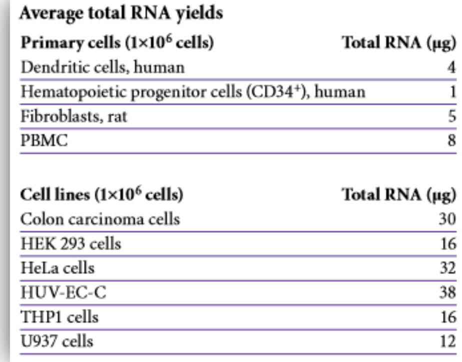


Bulk mRNA libraries
100ng total RNA
(10,000 cells)

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

- Cell RNA content depend on its cell type and developmental stage
- Majority of RNA molecules are tRNAs and rRNAs, mRNA accounts for only 1-5%
- Approximately 360,000 mRNA molecules are present in a single mammalian cell
- ~ 12,000 different transcripts with a typical length of around 2 kb,
- Some comprise 3% of the mRNA pool whereas others account for less than 0.1%. These rare or low-abundance mRNAs may have a copy number of only 5-15 molecules per cell.

https://www.qiagen.com/fr/resources/faq?id=06a192c2-e72d-42e8-9b40-3171e1eb4cb8&lang=en



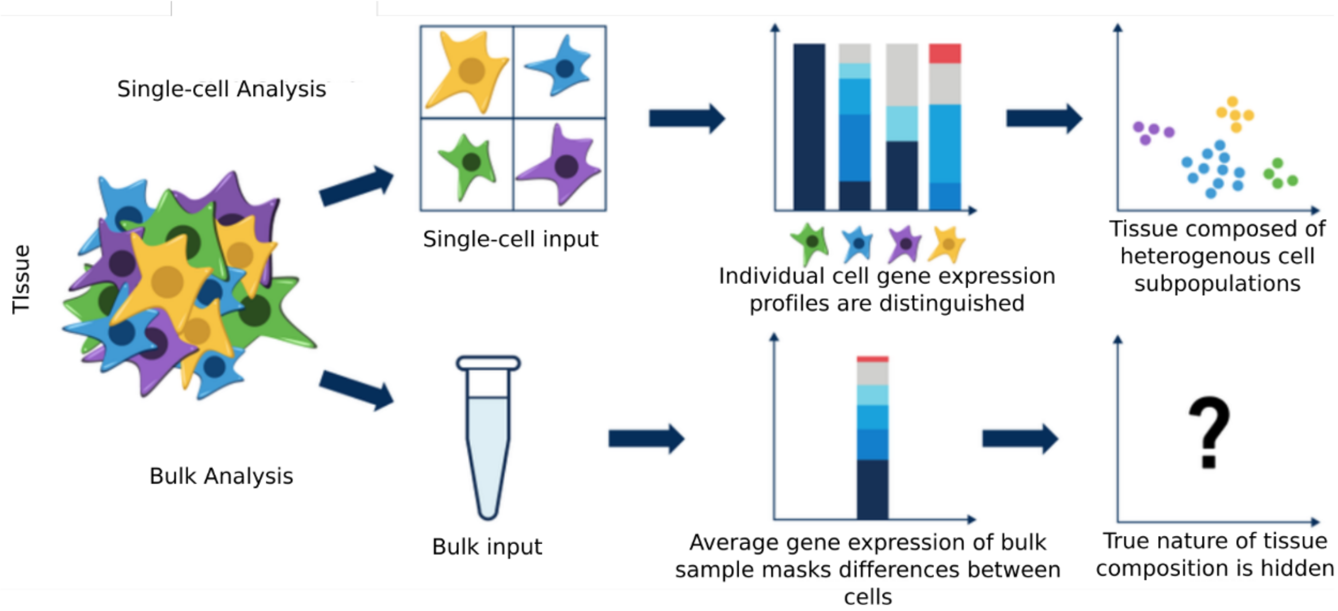
Average total RNA yields

Primary cells (1×10^6 cells)	**Total RNA (µg)**
Dendritic cells, human	4
Hematopoietic progenitor cells (CD34⁺), human	1
Fibroblasts, rat	5
PBMC	8

Cell lines (1×10^6 cells)	**Total RNA (µg)**
Colon carcinoma cells	30
HEK 293 cells	16
HeLa cells	32
HUV-EC-C	38
THP1 cells	16
U937 cells	12

Single-cell transcriptomics

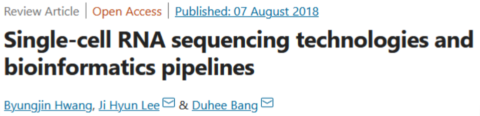
Why single-cell profiling ?



Chris Harris, 2020

Single-cell isolation techniques

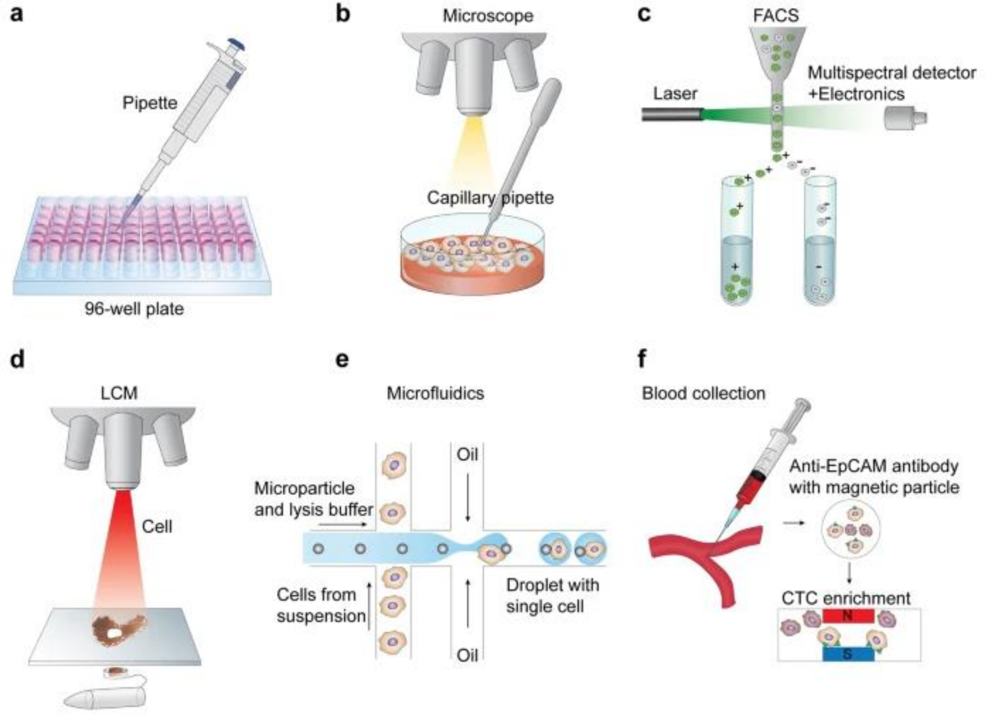
To measure sequences in individual cells, we need method that capture one cell at a time



Review Article | Open Access | Published: 07 August 2018

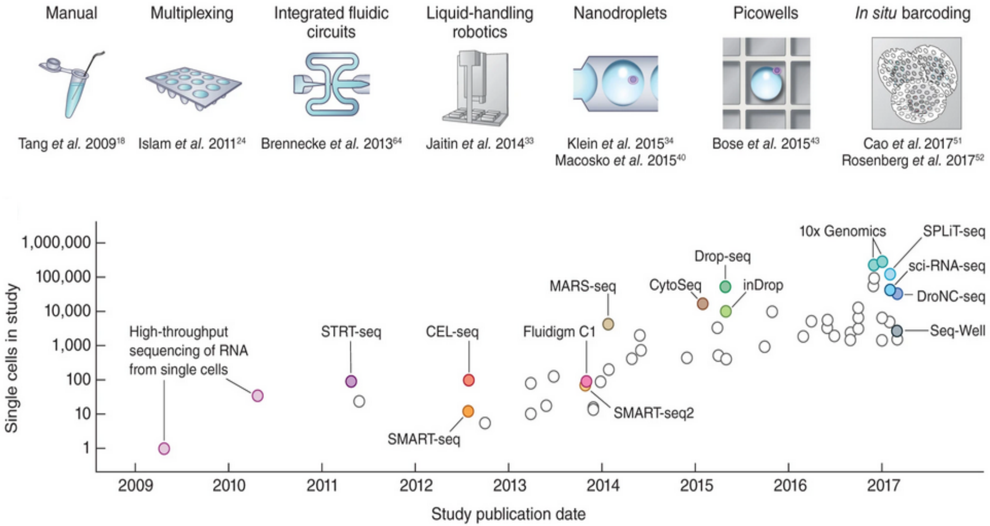
Single-cell RNA sequencing technologies and bioinformatics pipelines

Byungjin Hwang, Ji Hyun Lee ✉ & Duhee Bang ✉



Single-cell transcriptomics

Evolution of isolation techniques and throughput

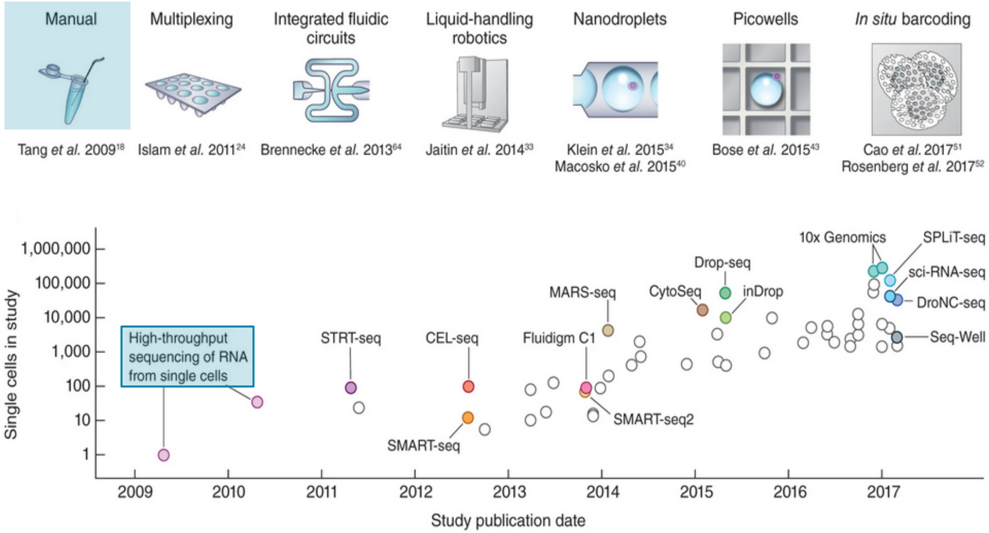


Exponential scaling of single-cell RNA-seq in the past decade
Svensson et al., *Nature Protocols*, 2018

Single-cell transcriptomics

Manual pipetting

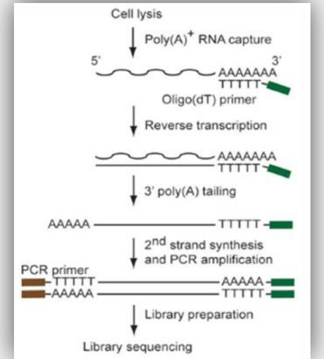
mRNA-Seq whole-transcriptome analysis of a single cell, Tang et al., 2009
RNA-Seq analysis to capture the transcriptome landscape of a single cell, Tang et al., 2010



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

Tang's initial protocol

- Total RNA isolated and fragmented,
- cDNA conversion oligodT primer + anchor
- 2nd strand synthesis polyT primer + anchor
- PCR amplification 2 anchor sequences.
- 100 million SOLiD reads per cell

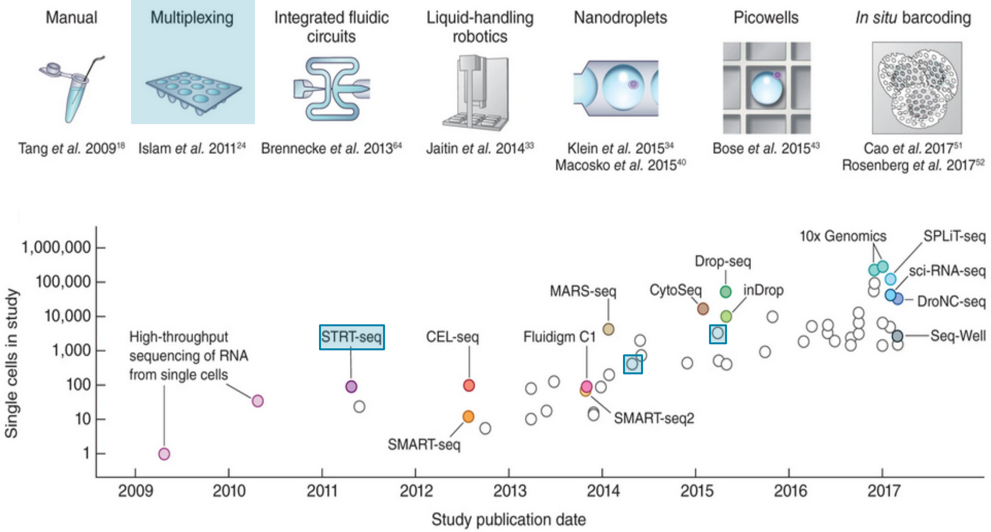


- 2009: 1 mouse blastomere
- 2010: 16 early mouse embryo

Single-cell transcriptomics

Plate-based protocols

Single cell tagged reverse transcription, Islam et al., 2011
Quantitative single-cell RNA-seq with unique molecular identifiers, Islam et al., 2014
Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq, Zeisel et al., 2015



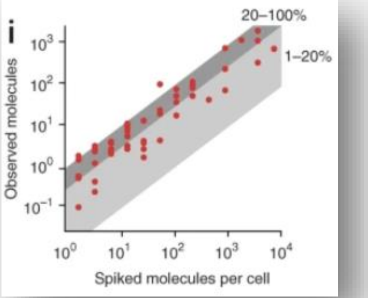
Exponential scaling of single-cell RNA-seq in the past decade
Svensson et al., *Nature Protocols*, 2018

Sten Linnarsson's Lab (Karolinska Institutet)

- Plate-based protocols,
- based on template switching (TSO),
- 5' end cDNA tagged

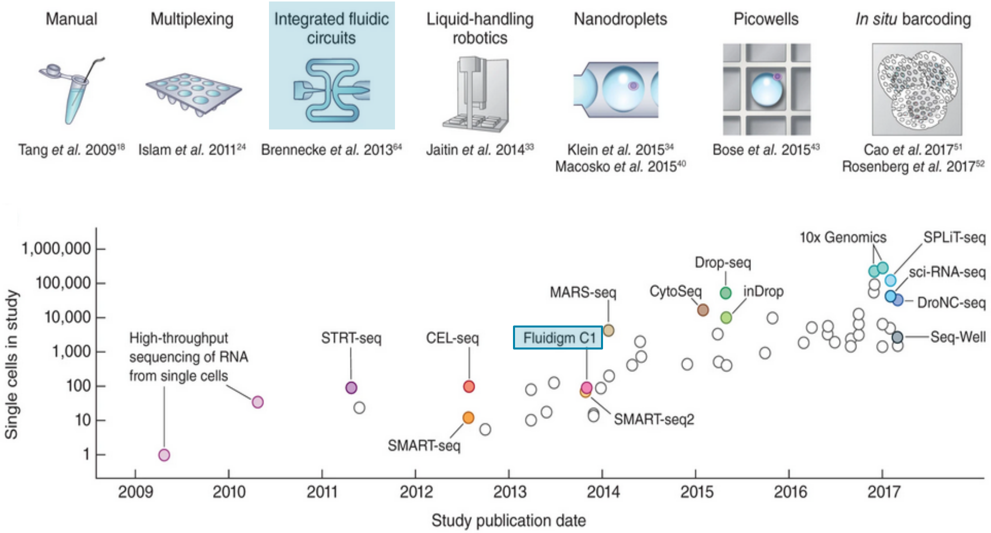
Publications :

- 2011: STRT-seq, no UMI 92 cells
- 2014: UMI, Islam et al., capture efficiency = 48%
- 2015: UMI, Zeisel et al, capture efficiency = 22%



Single-cell transcriptomics

Fluidigm C1 microfluidics

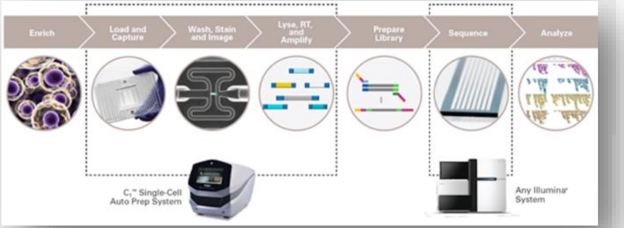


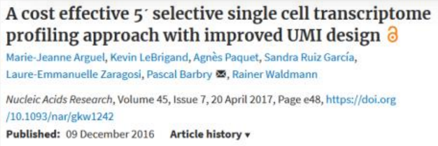
Exponential scaling of single-cell RNA-seq in the past decade
Svensson et al., *Nature Protocols*, 2018

Arguel et al., 2016

Fluidigm C1

- 96 / 800 cell chips,
- protocols for **SMARTer, CEL-seq, STRT-seq**, …
- Limiting factor: capture chamber size and doublets

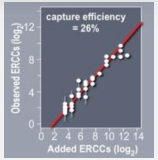



A cost effective 5′ selective single cell transcriptome profiling approach with improved UMI design

Marie-Jeanne Arguel, Kevin LeBrigand, Agnès Paquet, Sandra Ruiz García, Laure-Emmanuelle Zaragosi, Pascal Barbry ✉, Rainer Waldmann

Nucleic Acids Research, Volume 45, Issue 7, 20 April 2017, Page e48, https://doi.org/10.1093/nar/gkw1242

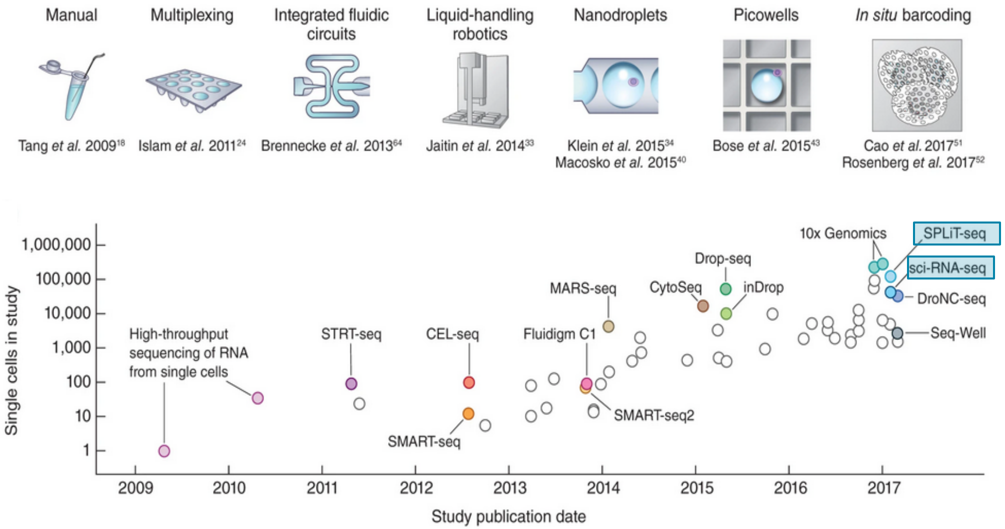
Published: 09 December 2016 **Article history** ▾



capture efficiency = **26%**

Single-cell transcriptomics

Combinatorial indexing



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

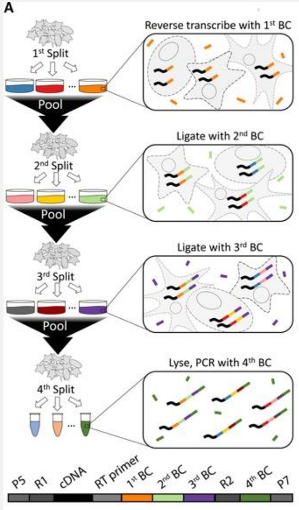
sci-RNA-seq, Cao et al, 2017
SPLIT-seq, Rosenberg et al., 2018

sci-RNA-seq (2017)

- 50,000 cells nematode *Caenorhabditis elegans*

SPLIT-seq (2018)

- 156,049 single-nucleus P22 and P11 mouse brain



Parse Biosciences Evercode kits (2021)

WT Mini

10K Cells | 1-12 Samples

The Evercode™ WT Mini is perfectly sized to begin exploring single cell transcriptomics and experience the quality of results possible with Parse.

Learn more about WT Mini

WT

10K-100K Cells | 1-48 Samples

Evercode™ Whole Transcriptome products are enabling scientists to push scRNA-seq past the limitations of previous technologies.

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100K-1M Cells | 1-96 Samples

Run up to 1 million cells and 96 biological samples or conditions in a single experiment. Scale exponentially with Evercode combinatorial barcoding.

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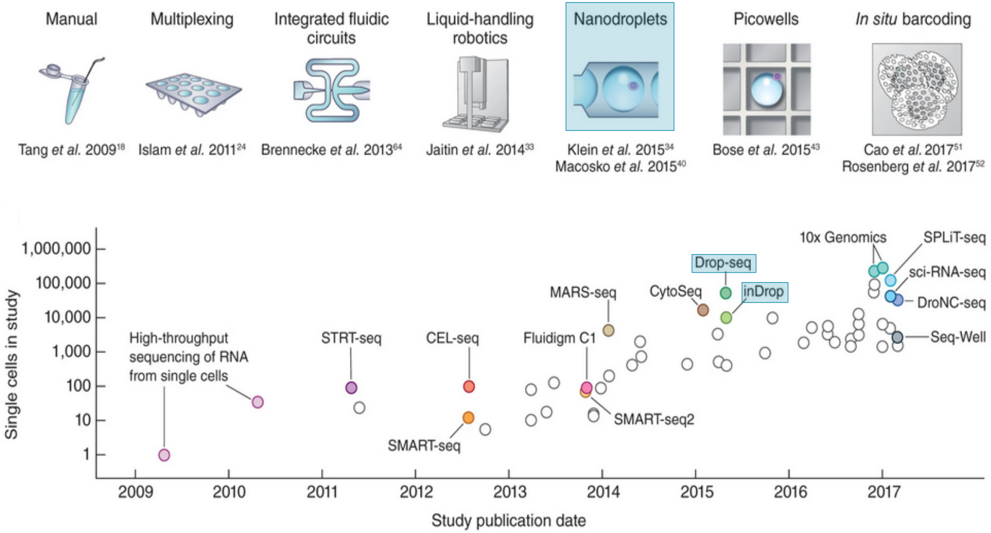
Single-cell transcriptomics

Droplet-based

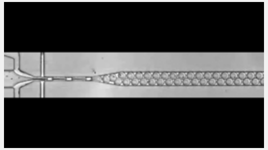
InDrop, Klein et al, 2015
Drop-seq, Macosko et al, 2015

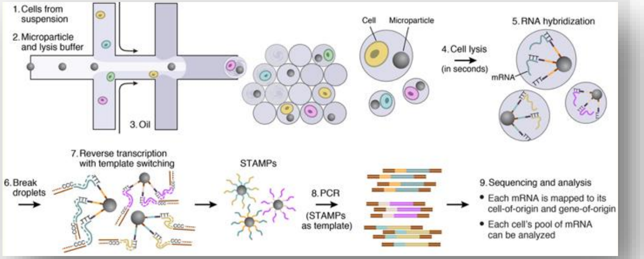


Exponential scaling of single-cell RNA-seq in the past decade
Svensson et al., *Nature Protocols*, 2018

Droplets-based protocols

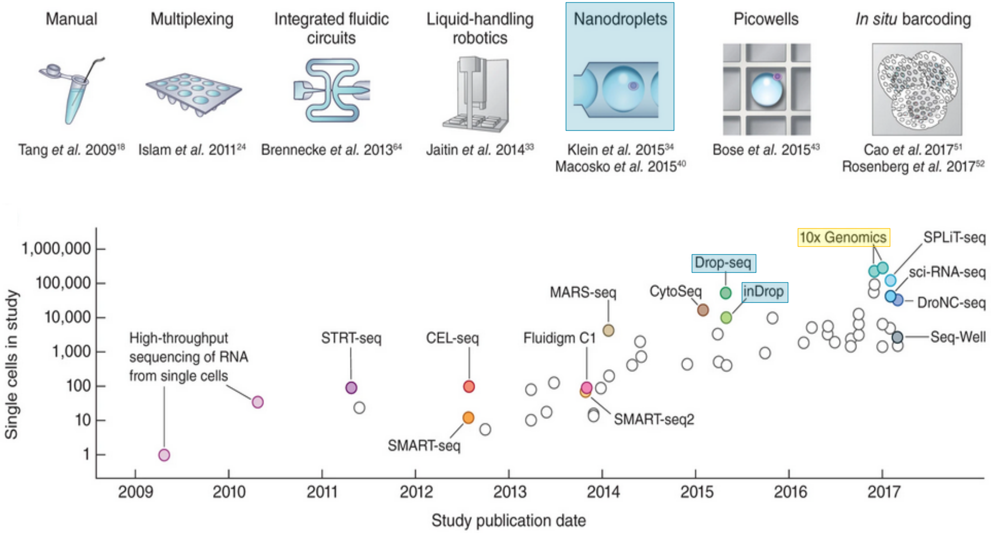
- cells +barcoded beads encapsulation
- **3' end cDNA tagged**
- 12 bp cell BC and 8bp UMI
- Capture efficiency **12,5%**
- 44,808 mouse retinal cells (39 cell types)





Single-cell transcriptomics

Droplet-based

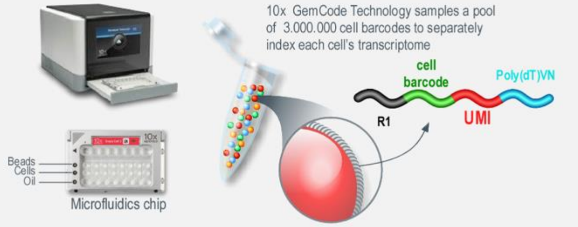


Exponential scaling of single-cell RNA-seq in the past decade
Svensson et al., *Nature Protocols*, 2018

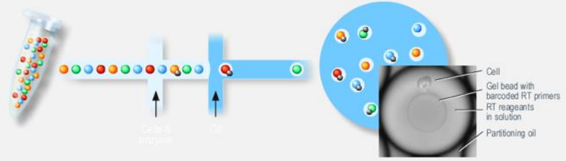
10x Genomics, Zheng et al, 2016

10x Genomics Chromium single cell controler (2016)

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

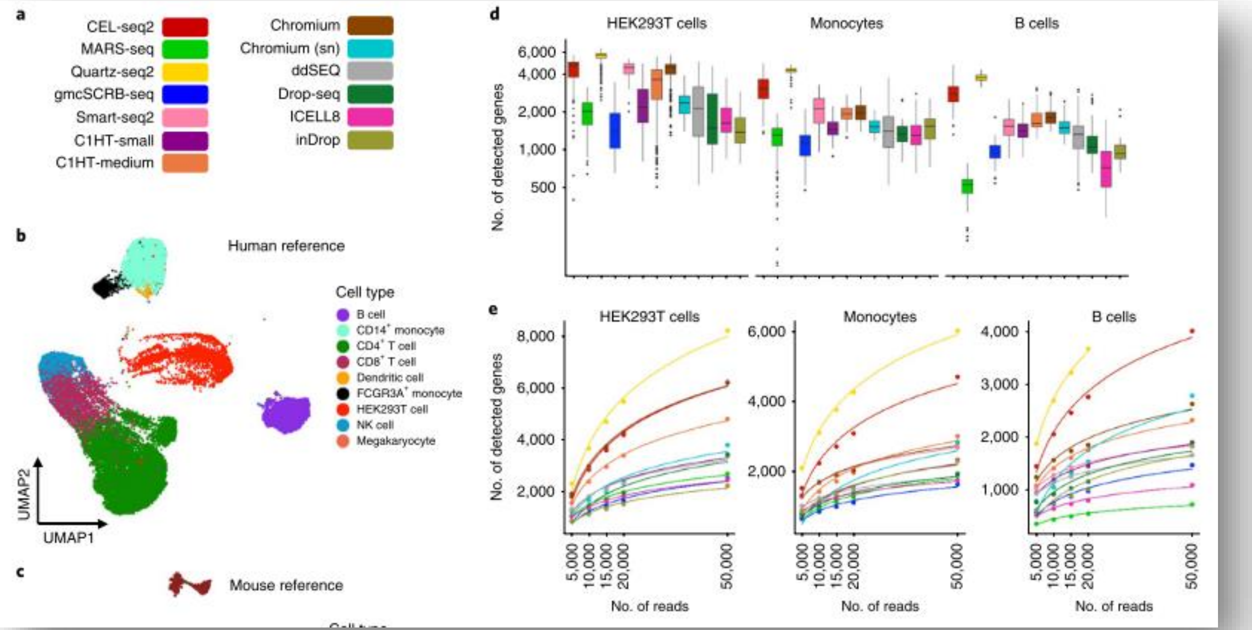

10x GemCode Technology samples a pool of 3.000.000 cell barcodes to separately index each cell's transcriptome

10x Barcoded Gel Beads are mixed with cells, enzyme and oil to create single cell GEMs (Gel bead in emulsion)



Single-cell transcriptomics

Comparative studies



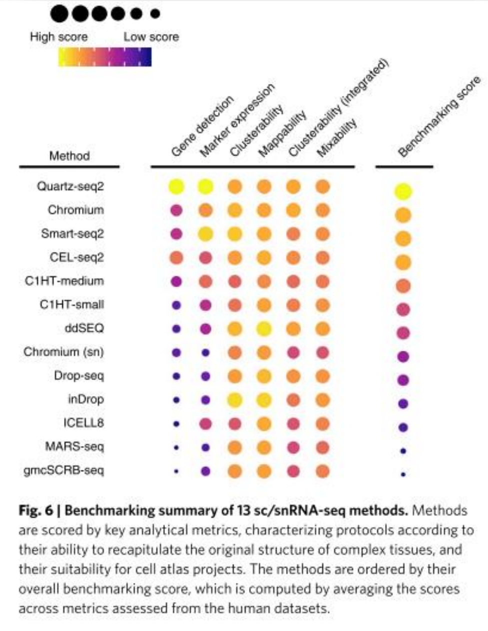


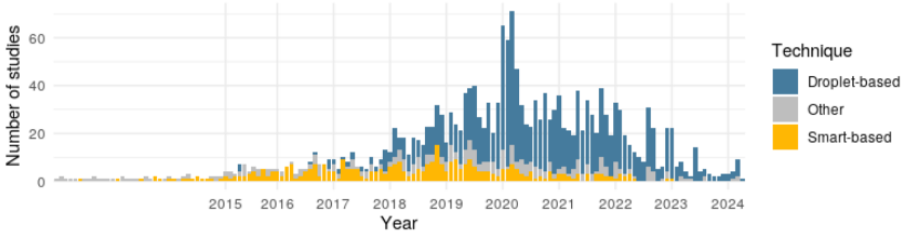
Fig. 6 | Benchmarking summary of 13 sc/snRNA-seq methods. Methods are scored by key analytical metrics, characterizing protocols according to their ability to recapitulate the original structure of complex tissues, and their suitability for cell atlas projects. The methods are ordered by their overall benchmarking score, which is computed by averaging the scores across metrics assessed from the human datasets.

Benchmarking single-cell RNA-sequencing protocols for cell atlas projects
Mereu et al., Nat.Biotech, 2020

What do you want to study ?
→ more cells for statistics
→ more genes for subsequent biology
→ sequencing cost you can support
→ 5p, 3p of full length protocols

Single-cell transcriptomics

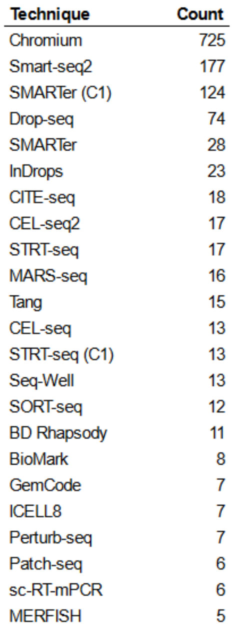
Single-cell approaches in publications



https://doi.org/10.1093/database/baaa073

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

- Huge amount of single-cell studies in the past 10 years,
- Droplet-based approaches = 61% (Chromium: 47%)

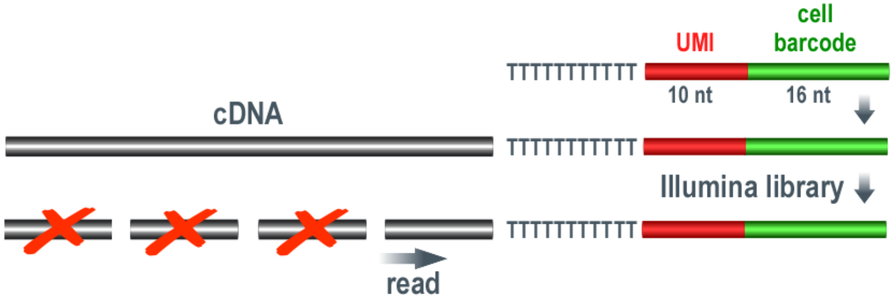


Technique	Count
Chromium	725
Smart-seq2	177
SMARTer (C1)	124
Drop-seq	74
SMARTer	28
InDrops	23
CITE-seq	18
CEL-seq2	17
STRT-seq	17
MARS-seq	16
Tang	15
CEL-seq	13
STRT-seq (C1)	13
Seq-Well	13
SORT-seq	12
BD Rhapsody	11
BioMark	8
GemCode	7
ICELL8	7
Perturb-seq	7
Patch-seq	6
sc-RT-mPCR	6
MERFISH	5

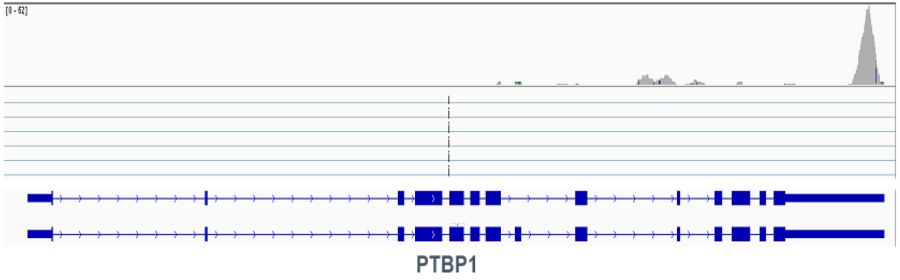
Droplet-based approaches
- Digital Gene Expression (UMI)
- High cell number throughput
- Limited capture efficiency (<10%)
- 3p or 5p signal (SAGE-like)

Single-cell Transcriptomics

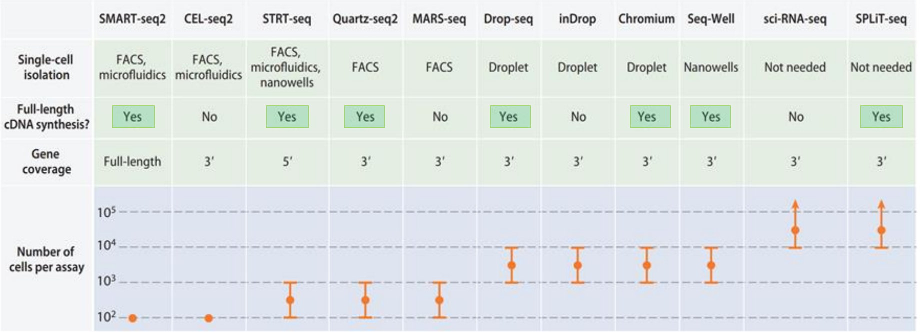
Single-cell droplets-based rely on short reads



Illumina Nextseq500



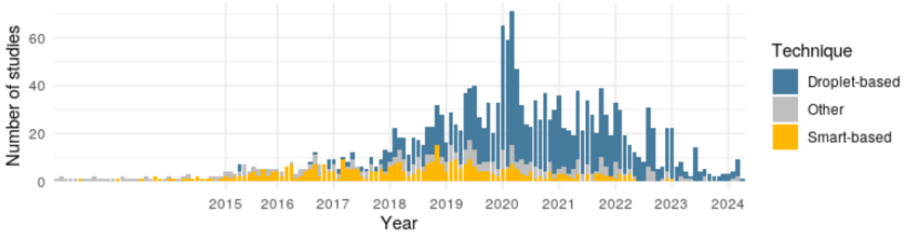
Short-read sequencing yields just a short read close to 3'end



	SMART-seq2	CEL-seq2	STRT-seq	Quartz-seq2	MARS-seq	Drop-seq	inDrop	Chromium	Seq-Well	sci-RNA-seq	SPLiT-seq
Single-cell isolation	FACS, microfluidics	FACS, microfluidics	FACS, microfluidics, nanowells	FACS	FACS	Droplet	Droplet	Droplet	Nanowells	Not needed	Not needed
Full-length cDNA synthesis?	Yes	No	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes
Gene coverage	Full-length	3′	5′	3′	3′	3′	3′	3′	3′	3′	3′
Number of cells per assay											

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 single-cell 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
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

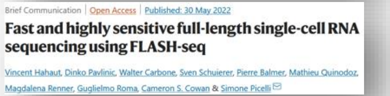
Smart-based approach

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


Article | Open Access | Published: 30 May 2022

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

Michael Hagemann-Jensen, Christoph Ziegenhain & Rickard Sandberg


Brief Communication | Open Access | Published: 30 May 2022

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

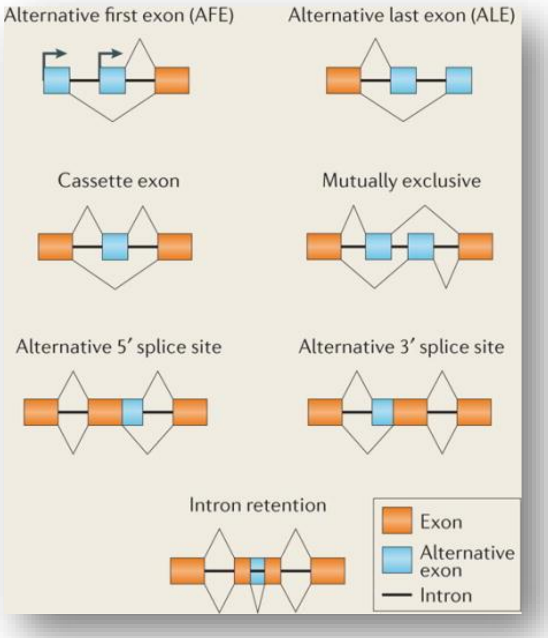
Vincent Hahaut, Dinko Pavlinic, Walter Carbone, Sven Schuierer, Pierre Balmer, Mathieu Quinodoz, Magdalena Renner, Guglielmo Roma, Cameron S. Cowan & Simone Picelli

UMIs detected in HEK293 cells
Droplets 10x: 30k (50k reads)
Plate-based : 60k (200k reads)
Smart-seq3: 150k (750k reads)

Mantis Microdispenser

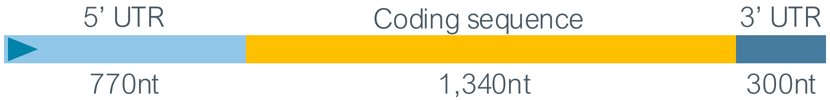
Transcriptomics

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

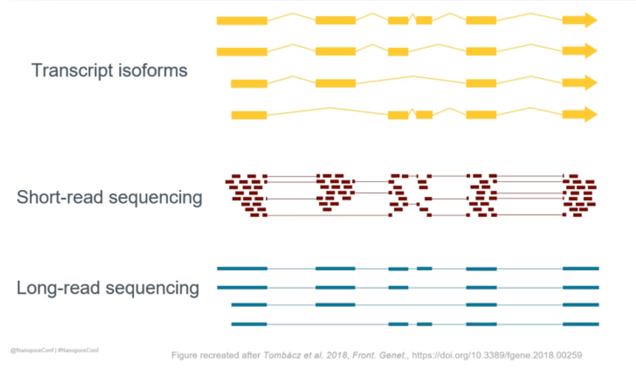


Figure recreated after *Tombácz et al. 2018, Front. Genet.*, https://doi.org/10.3389/fgene.2018.00259

Inference required

Direct full exonic layout
One read is one molecule

Nature Method of the Year 2022

Single-cell long-read transcriptomics

Droplets-based approach short reads vs long reads

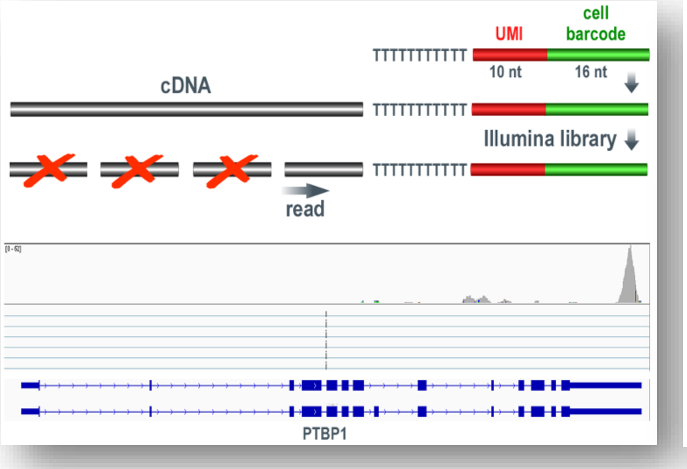
Thursday

10x GENOMICS

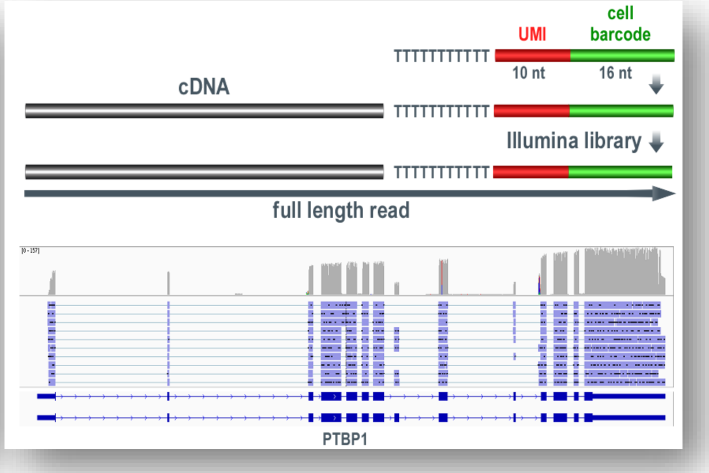
Standard short-read sequencing

Long-read full-length sequencing

illumina



Gene-level matrix



Oxford NANOPORE Technologies

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

Thursday

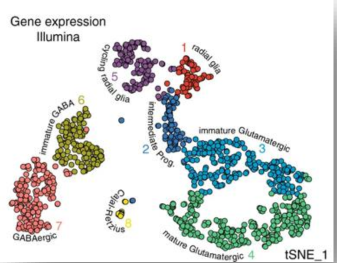
E18 C57BL/6 mouse
hippocampus, cortex,
and ventricular zone

1.200 cells

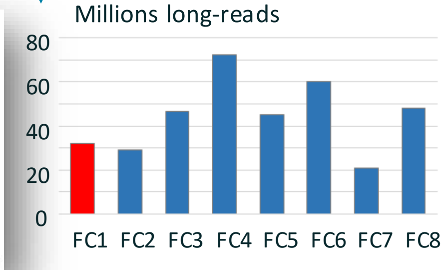
split emulsion before RT

15%
190 cells

85%
951 cells

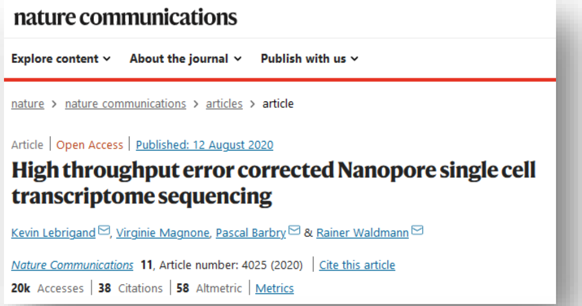


standard workflow



190 cells: 32M
951 cells: 322M

R9.4, LSK-109 chemistry (2018)



nature communications

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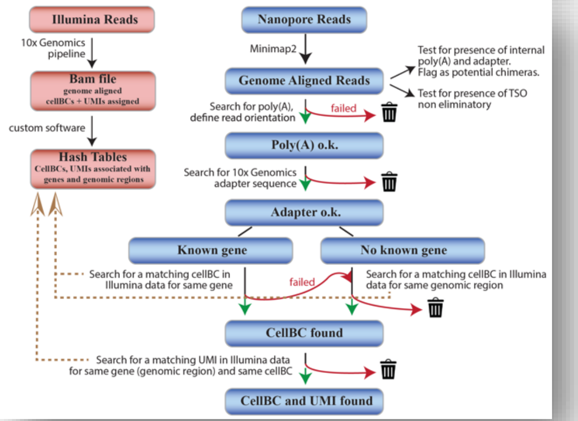
Article | Open Access | Published: 12 August 2020

High throughput error corrected Nanopore single cell transcriptome sequencing

Kevin Lebrigand, Virginie Magnone, Pascal Barbry & 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

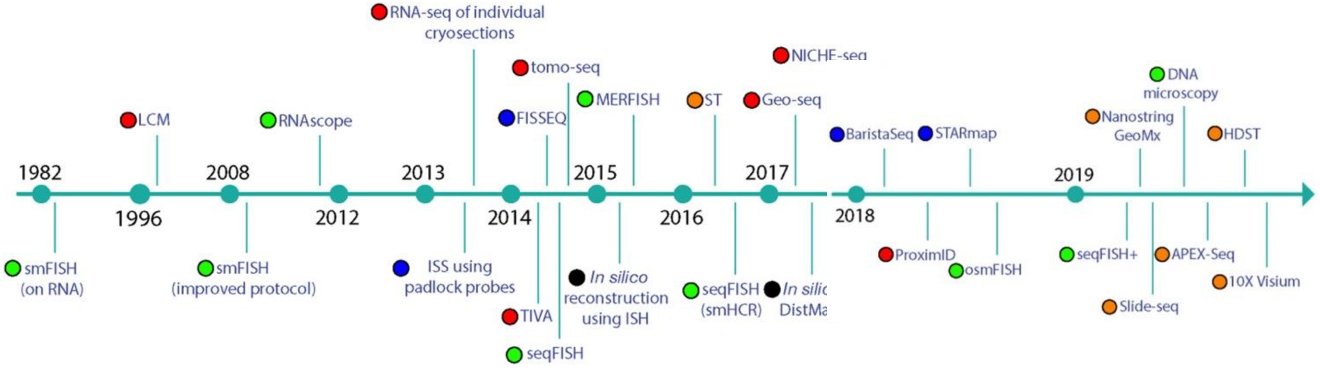
02

Spatial in-situ capture transcriptomics

Spatial Transcriptomics approaches

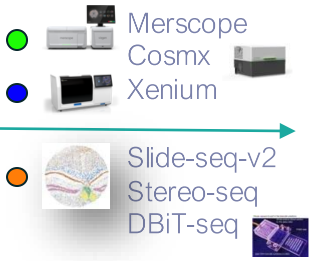
Historical timeline

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



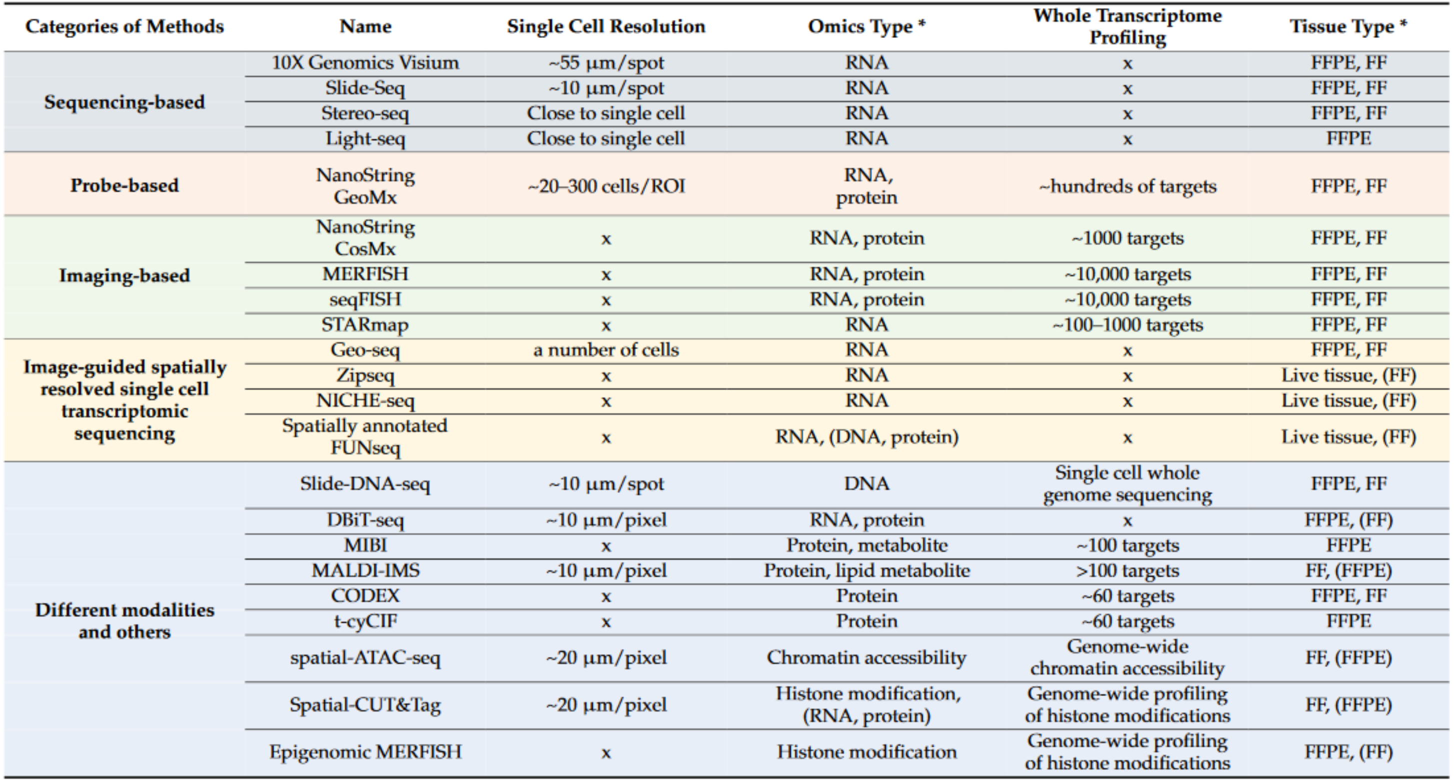
Spatially Resolved Transcriptomes Next Generation Tools for Tissue Exploration
Asp et al., BioEssays, 2020

🔴 Section 1. Technologies based on microdissected gene expression
🟢 Section 2. *In situ* hybridization technologies
🔵 Section 3. *In situ* sequencing technologies
🟠 Section 4. *In situ* capturing technologies
⚫ Section 5. *In silico* reconstruction of spatial data



Spatial Transcriptomics approaches

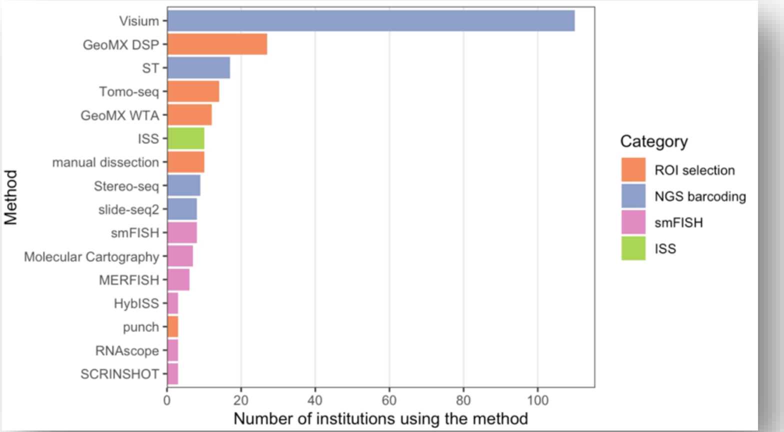
A lot of different options

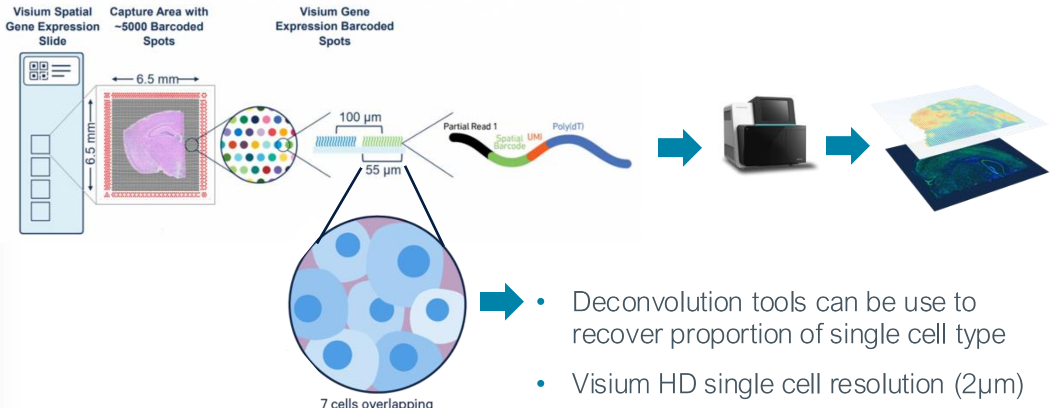


Categories of Methods	Name	Single Cell Resolution	Omics Type *	Whole Transcriptome Profiling	Tissue Type *
Sequencing-based	10X Genomics Visium	~55 µm/spot	RNA	x	FFPE, FF
	Slide-Seq	~10 µm/spot	RNA	x	FFPE, FF
	Stereo-seq	Close to single cell	RNA	x	FFPE, FF
	Light-seq	Close to single cell	RNA	x	FFPE
Probe-based	NanoString GeoMx	~20–300 cells/ROI	RNA, protein	~hundreds of targets	FFPE, FF
Imaging-based	NanoString CosMx	x	RNA, protein	~1000 targets	FFPE, FF
	MERFISH	x	RNA, protein	~10,000 targets	FFPE, FF
	seqFISH	x	RNA, protein	~10,000 targets	FFPE, FF
	STARmap	x	RNA	~100–1000 targets	FFPE, FF
Image-guided spatially resolved single cell transcriptomic sequencing	Geo-seq	a number of cells	RNA	x	FFPE, FF
	Zipseq	x	RNA	x	Live tissue, (FF)
	NICHE-seq	x	RNA	x	Live tissue, (FF)
	Spatially annotated FUNseq	x	RNA, (DNA, protein)	x	Live tissue, (FF)
Different modalities and others	Slide-DNA-seq	~10 µm/spot	DNA	Single cell whole genome sequencing	FFPE, FF
	DBiT-seq	~10 µm/pixel	RNA, protein	x	FFPE, (FF)
	MIBI	x	Protein, metabolite	~100 targets	FFPE
	MALDI-IMS	~10 µm/pixel	Protein, lipid metabolite	>100 targets	FF, (FFPE)
	CODEX	x	Protein	~60 targets	FFPE, FF
	t-cyCIF	x	Protein	~60 targets	FFPE
	spatial-ATAC-seq	~20 µm/pixel	Chromatin accessibility	Genome-wide chromatin accessibility	FF, (FFPE)
	Spatial-CUT&Tag	~20 µm/pixel	Histone modification, (RNA, protein)	Genome-wide profiling of histone modifications	FF, (FFPE)
	Epigenomic MERFISH	x	Histone modification	Genome-wide profiling of histone modifications	FFPE, (FF)

In-situ capture Spatial Transcriptomics (2017-2022)

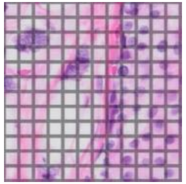
Visium (2019, ST 2017) is widely adopted by academics





7 cells overlapping
a single spot

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

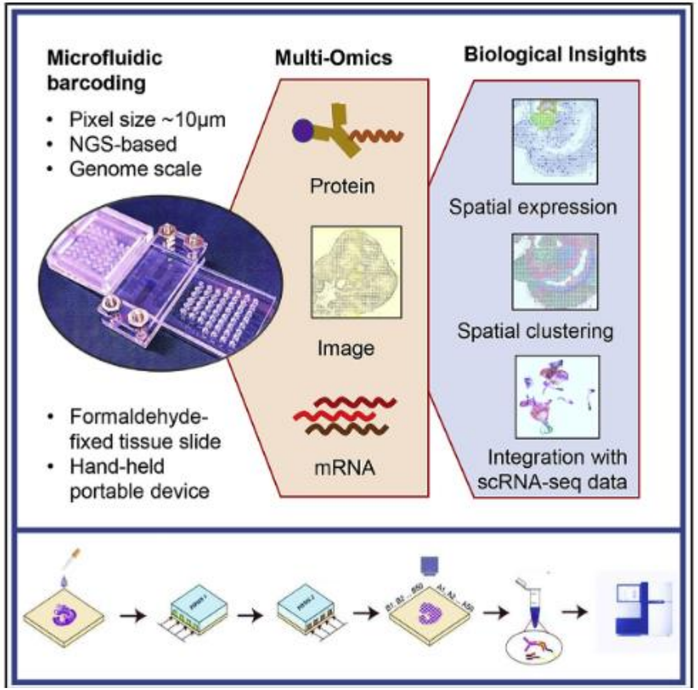


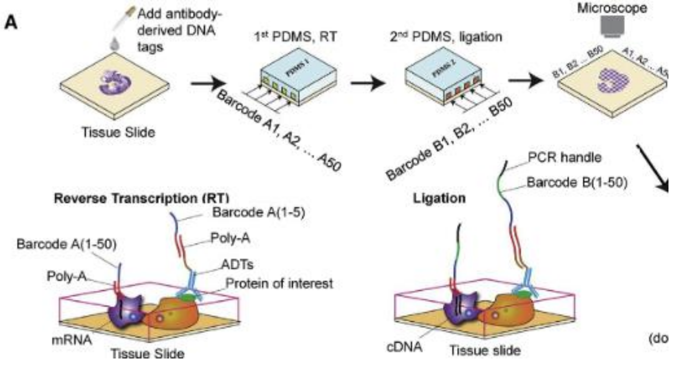
In-situ capture Spatial Transcriptomics (2017-2022)

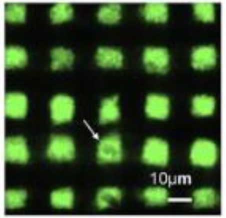
DBit-seq (2020)

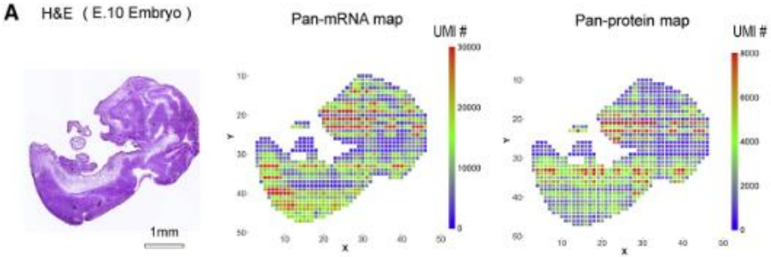
High-Spatial-Resolution Multi-Omics Sequencing via Deterministic Barcoding in Tissue

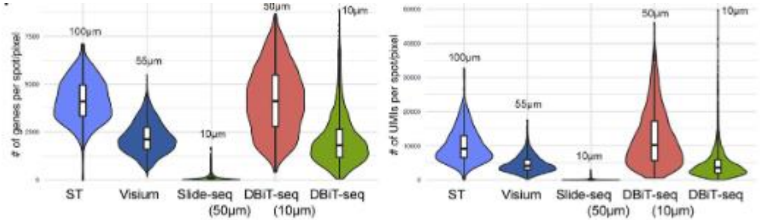
Yang Liu,[1,2,5] Mingyu Yang,[1,2,5] Yanxiang Deng,[1,2,5] Graham Su,[1,2] Archibald Enninful,[1] Cindy C. Guo,[1] Toma Tebaldi,[2,4]
Di Zhang,[1] Dongjoo Kim,[1] Zhiliang Bai,[1] Eileen Norris,[1] Alisia Pan,[1] Jiatong Li,[1] Yang Xiao,[1] Stephanie Halene,[2,4]
and Rong Fan[1,2,3,6,*]





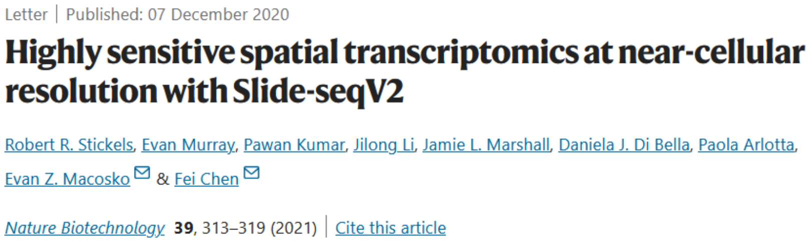






In-situ capture Spatial Transcriptomics (2017-2022)

Slide-seq v2 (2021)

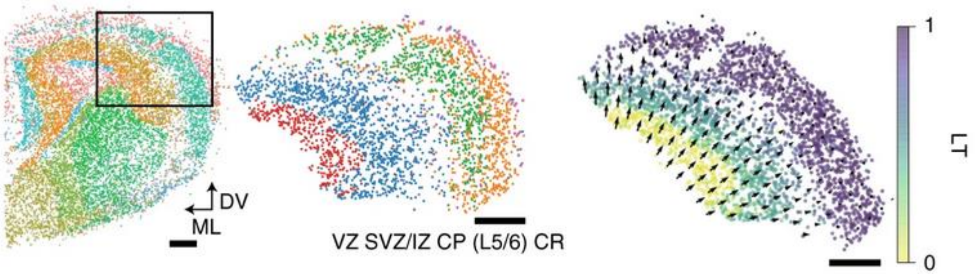


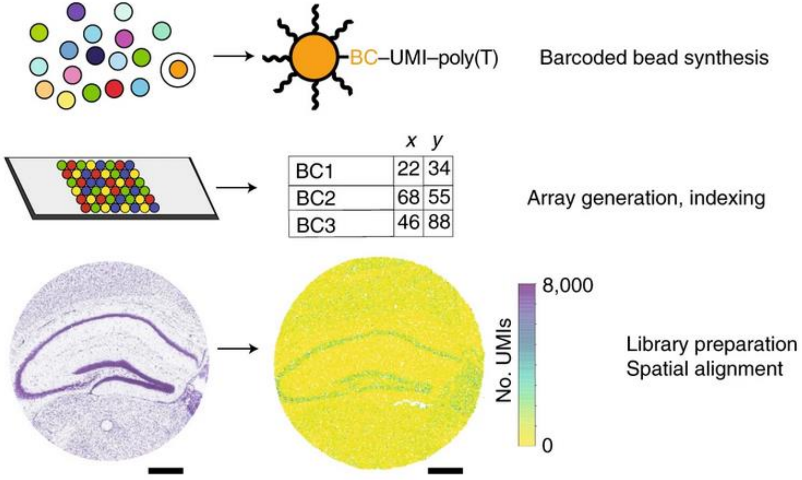
Letter | Published: 07 December 2020

Highly sensitive spatial transcriptomics at near-cellular resolution with Slide-seqV2

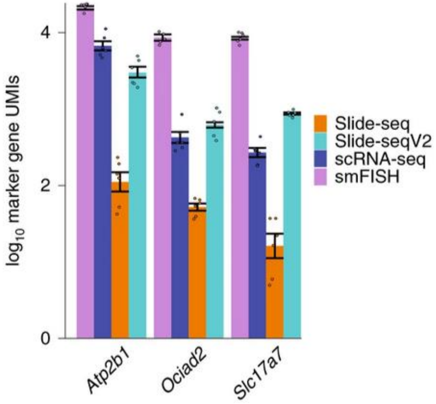
Robert R. Stickels, Evan Murray, Pawan Kumar, Jilong Li, Jamie L. Marshall, Daniela J. Di Bella, Paola Arlotta, Evan Z. Macosko ✉ & Fei Chen ✉

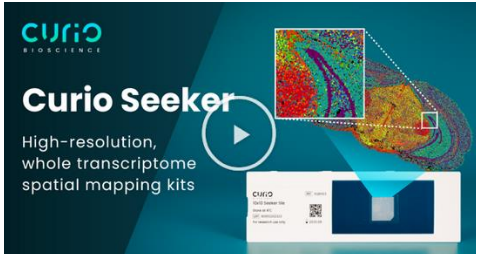
Nature Biotechnology **39**, 313–319 (2021) | Cite this article





	x	y
BC1	22	34
BC2	68	55
BC3	46	88





In-situ capture Spatial Transcriptomics (2017-2022)

Stereo-seq (2022)

Spatiotemporal transcriptomic atlas of mouse organogenesis using DNA nanoball-patterned arrays

Ao Chen,[1,2,26] Sha Liao,[1,26] Mengnan Cheng,[1,3,26] Kailong Ma,[1,26] Liang Wu,[1,3,4,26] Yiwei Lai,[1,5,26] Xiaojie Qiu,[6,7,26] Jin Yang,[8] Jiangshan Xu,[1,3] Shijie Hao,[1,3] Xin Wang,[1] Huifang Lu,[1] Xi Chen,[1] Xing Liu,[1] Xin Huang,[1,3] Zhao Li,[1] Yan Hong,[1] Yujia Jiang,[1,9] Jian Peng,[1] Shuai Liu,[1] Mengzhe Shen,[1] Chuanyu Liu,[1,10] Quanshui Li,[1] Yue Yuan,[1] Xiaoyu Wei,[1] Huiwen Zheng,[1,9] Weimin Feng,[1,3] Zhifeng Wang,[1,4] Yang Liu,[1] Zhaohui Wang,[1] Yunzhi Yang,[1,9] Haitao Xiang,[1,3] Lei Han,[1] Baoming Qin,[5] Pengcheng Guo,[5] Guangyao Lai,[5] Pura Muñoz-Cánoves,[11,12] Patrick H. Maxwell,[13] Jean Paul Thiery,[14] Qing-Feng Wu,[15] Fuxiang Zhao,[1] Bichao Chen,[1] Mei Li,[1] Xi Dai,[1,3] Shuai Wang,[1,3] Haoyan Kuang,[1] Junhou Hui,[1] Liqun Wang,[16] Ji-Feng Fei,[16] Ou Wang,[1] Xiaofeng Wei,[17] Haorong Lu,[17] Bo Wang,[17] Shiping Liu,[1,4] Ying Gu,[1,18] Ming Ni,[8] Wenwei Zhang,[1,19] Feng Mu,[8] Ye Yin,[1,20] Huanming Yang,[1,21] Michael Lisby,[2] Richard J. Cornall,[22] Jan Mulder,[23,24] Mathias Uhlén,[23,24] Miguel A. Esteban,[1,5,25,*] Yuxiang Li,[1,*] Longqi Liu,[1,9,10,*] Xun Xu,[1,18,27,*] and Jian Wang[1,21,*]

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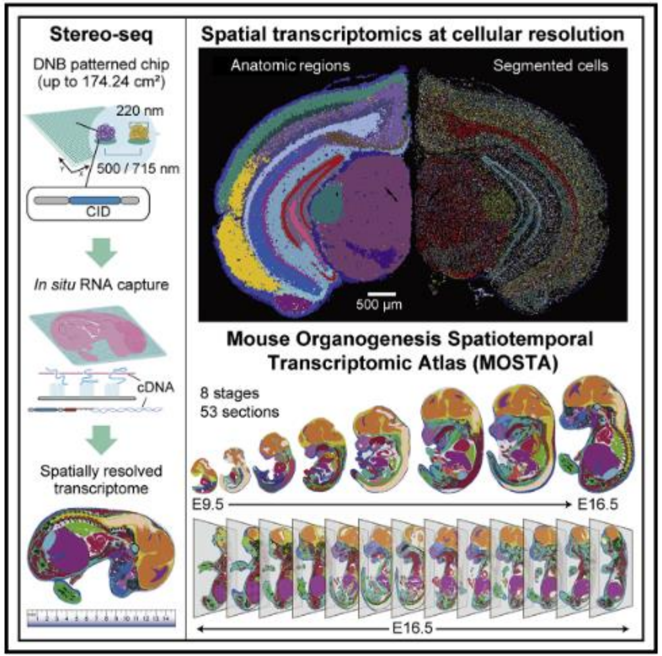
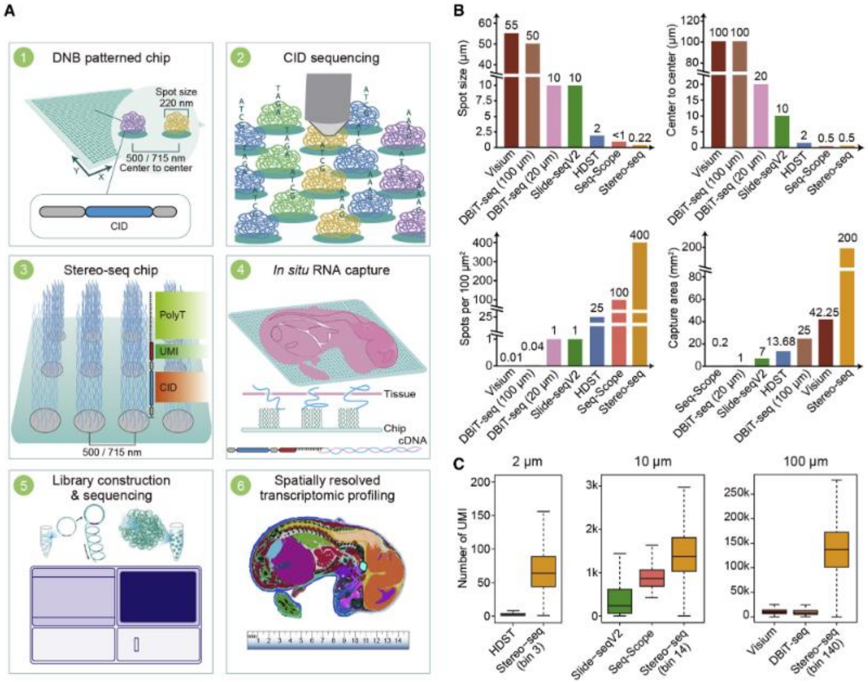




bin 50μm analysis

https://en.stomics.tech/

In-situ capture Spatial Transcriptomics (2017-2022)

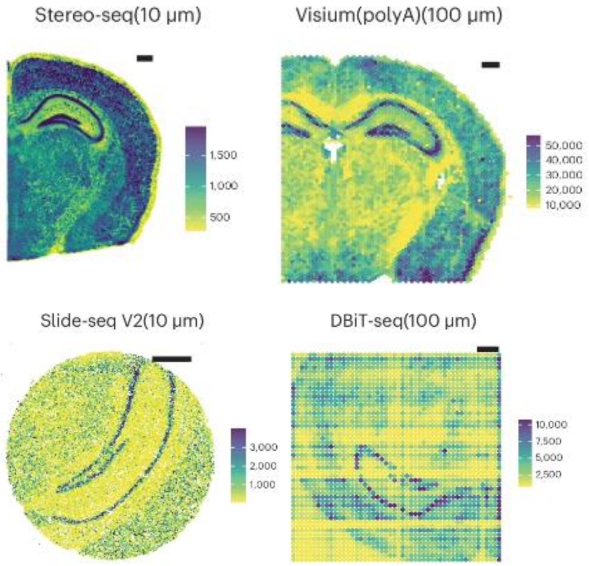
Systematic comparison

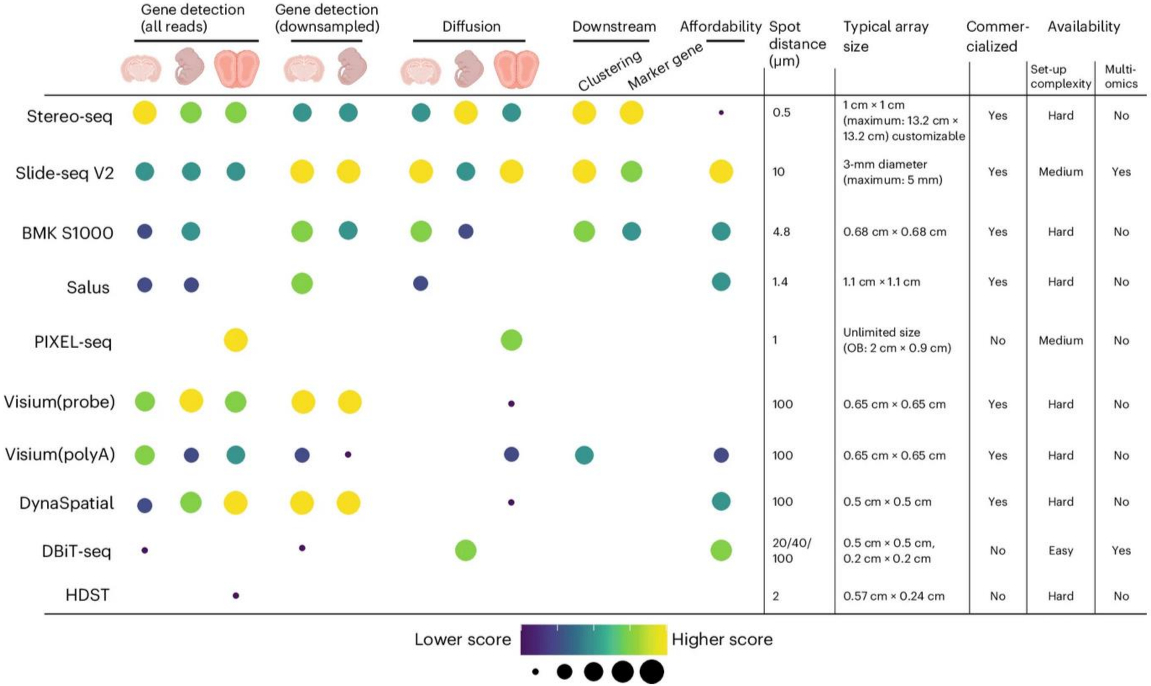
Analysis | Open access | Published: 04 July 2024

Systematic comparison of sequencing-based spatial transcriptomic methods

Yue You, Yuting Fu, Lanxiang Li, Zhongmin Zhang, Shikai Jia, Shihong Lu, Wenle Ren, Yifang Liu, Yang Xu, Xiaojing Liu, Fuqing Jiang, Guangdun Peng, Abhishek Sampath Kumar, Matthew E. Ritchie, Xiaodong Liu & Luyi Tian

Nature Methods **21**, 1743–1754 (2024) | Cite this article





	Gene detection (all reads)			Gene detection (downsampled)		Diffusion			Downstream		Affordability	Spot distance (μm)	Typical array size	Commercialized	Availability	
									Clustering	Marker gene					Set-up complexity	Multi-omics
Stereo-seq	●	●	●	●	●	●	●	●	●	●	•	0.5	1 cm × 1 cm (maximum: 13.2 cm × 13.2 cm) customizable	Yes	Hard	No
Slide-seq V2	●	●	●	●	●	●	●	●	●	●	●	10	3-mm diameter (maximum: 5 mm)	Yes	Medium	Yes
BMK S1000	●	●		●	●	●	●		●	●	●	4.8	0.68 cm × 0.68 cm	Yes	Hard	No
Salus	●	●		●		●					●	1.4	1.1 cm × 1.1 cm	Yes	Hard	No
PIXEL-seq			●					●				1	Unlimited size (OB: 2 cm × 0.9 cm)	No	Medium	No
Visium(probe)	●	●	●	●	●			•				100	0.65 cm × 0.65 cm	Yes	Hard	No
Visium(polyA)	●	●	●	●	•			●	●		●	100	0.65 cm × 0.65 cm	Yes	Hard	No
DynaSpatial	●	●	●	●	●			•			●	100	0.5 cm × 0.5 cm	Yes	Hard	No
DBiT-seq	•			•			●				●	20/40/100	0.5 cm × 0.5 cm, 0.2 cm × 0.2 cm	No	Easy	Yes
HDST			•									2	0.57 cm × 0.24 cm	No	Hard	No

Spatial isoform Transcriptomics (SiT)

Nucleic Acids Research, 2023

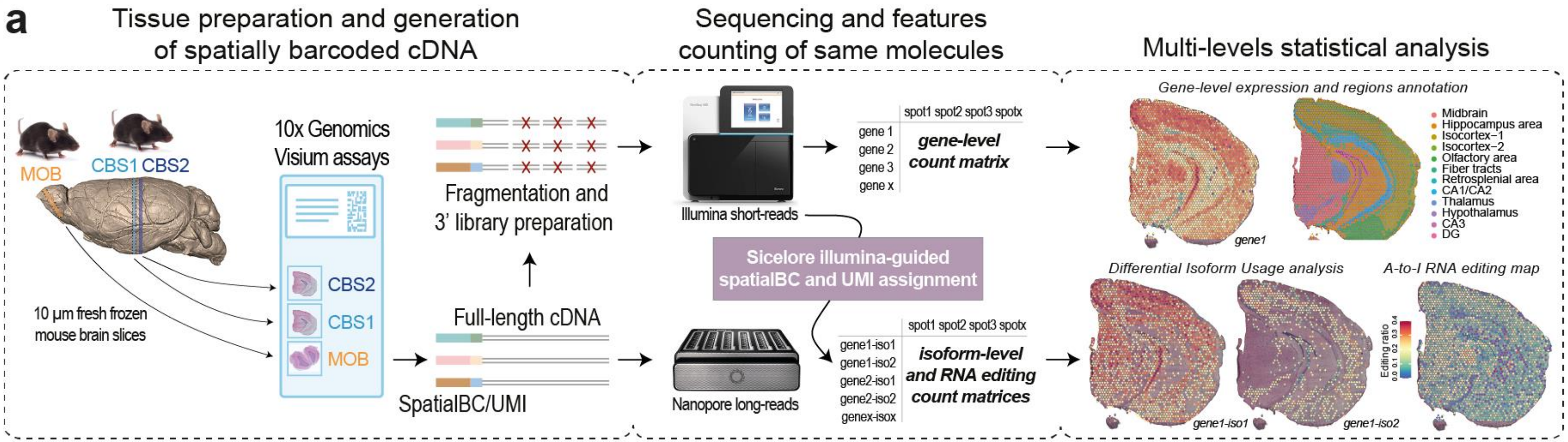
Thursday

The spatial landscape of gene expression isoforms in tissue sections

Kevin Lebrigand, Joseph Bergenstråhle, Kim Thrane, Annelie Mollbrink, Konstantinos Meletis, Pascal Barbry ✉, 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** ▾

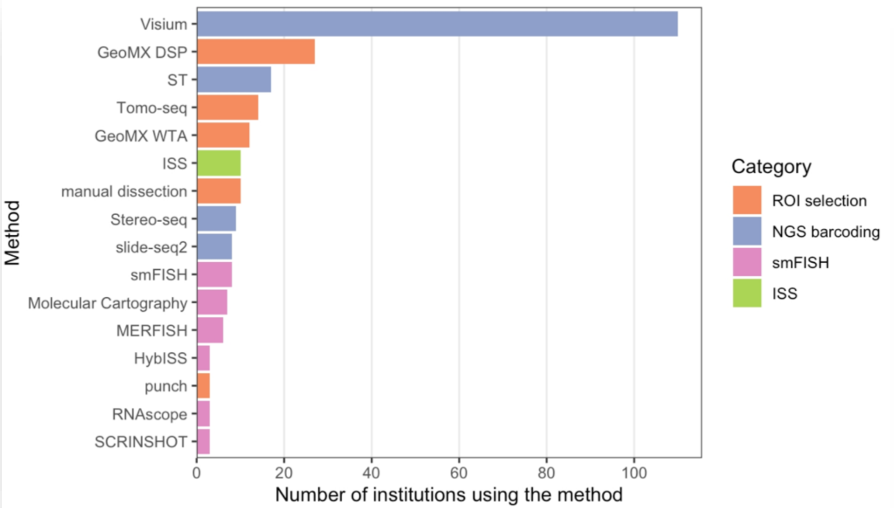


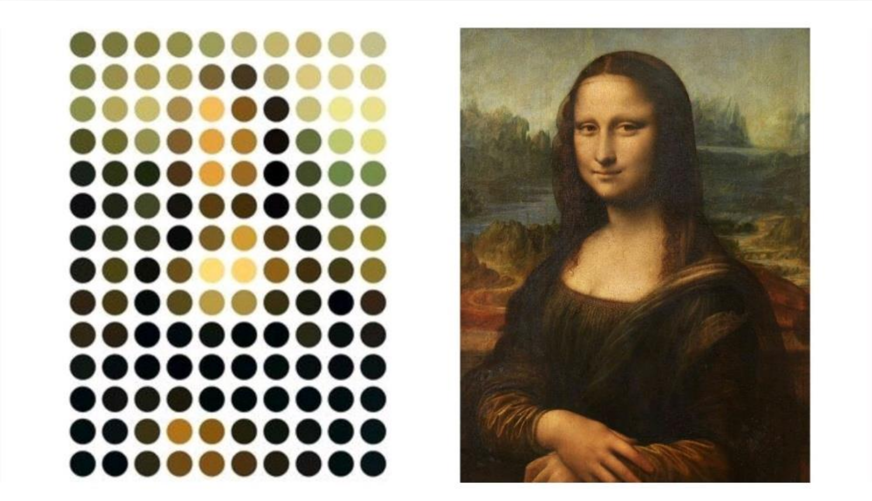
03

Spatial imaging-based transcriptomics

Spatial transcriptomics technologies (2019-2022)

Visium is widely adopted by academics





But is not the ideal readout for spatial biology

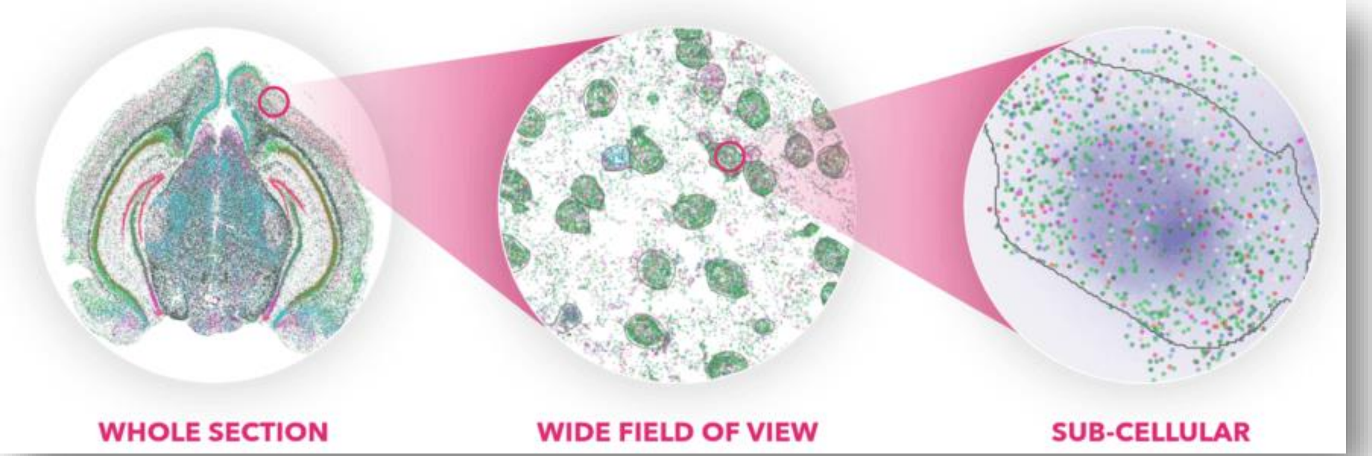
(Akoya credit rough caricature)

Imaging-based Spatial Transcriptomics (2022)

No more sequencing for direct single-cell resolution

Wednesday

- 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)

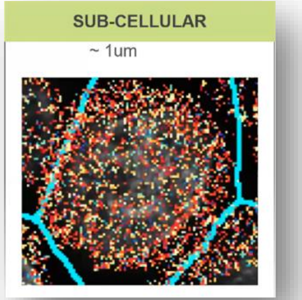


Imaging-based Spatial Transcriptomics (2022)

No more sequencing for direct single-cell resolution

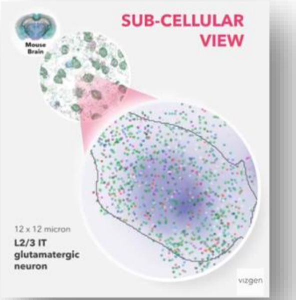
Nanostring CosMx

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



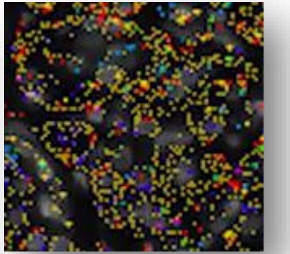
Vizgen Merscope

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



10xGenomics Xenium

- 400 - 6,000 targets
- Sensitivity : 5-30% (++)
- Imaging area: 236 mm2 (4 days)
- Resolution: 200 nm

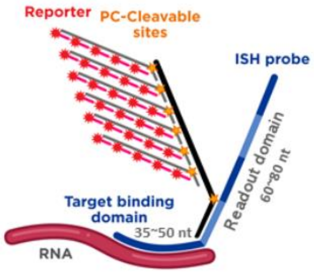


Imaging-based Spatial Transcriptomics (2022)

No more sequencing for direct single-cell resolution

Nanostring CosMx

ISH-based

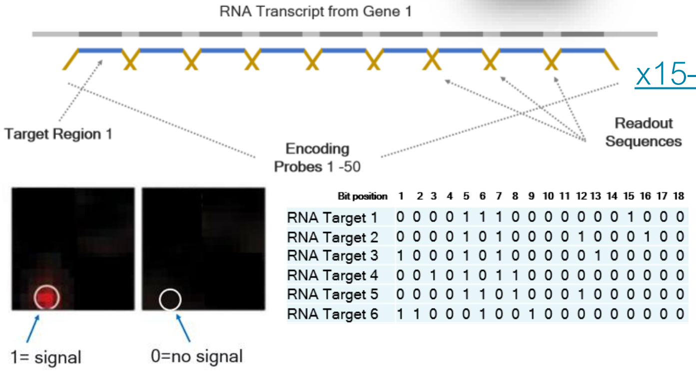


x4-8 / target gene

Vizgen Merscope

Multiplex Error-Robust FISH

Available (oct.2022)



x15-50

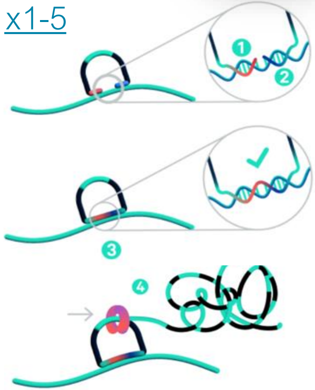
Bit position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
RNA Target 1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0
RNA Target 2	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0
RNA Target 3	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0
RNA Target 4	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
RNA Target 5	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0
RNA Target 6	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0

10xGenomics Xenium

Cartana ISS, padlock probes / RCA

Available (jan.2024)

x1-5



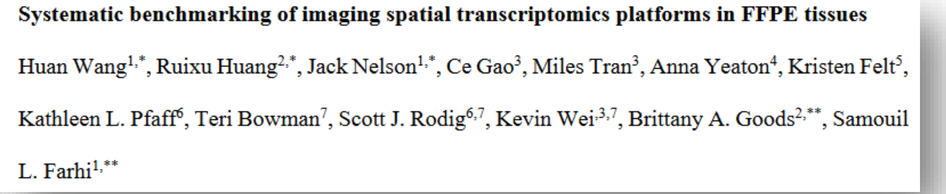
Cyclic *in situ* Hybridization Chemistries

Imaging-based Spatial Transcriptomics platforms comparison

2 recent bioRxiv comparative studies

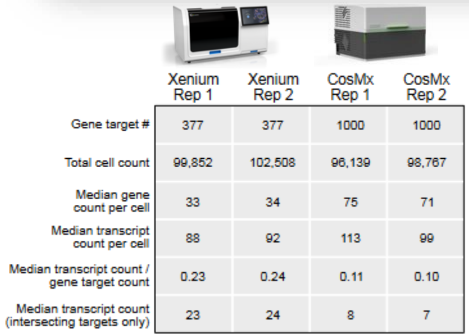
A Comparative Analysis of Imaging-Based Spatial Transcriptomics Platforms

David P. Cook[1], 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 Zamojski[9], Ioannis S. Vlachos[10,11,12,], Simon R. V. Knott[13,14], Lisa M. Butler[3,5], Jeffrey L. Wrana[1,15], Nicholas E. Banovich[16], Jasmine T. Plummer[6,7,8]*, Luciano G. Martelotto[2,3]*

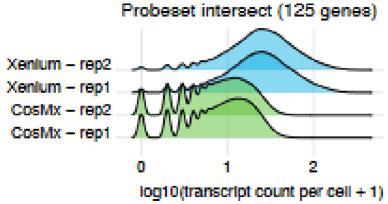


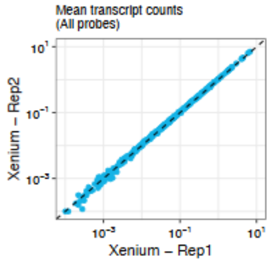
Systematic benchmarking of imaging spatial transcriptomics platforms in FFPE tissues

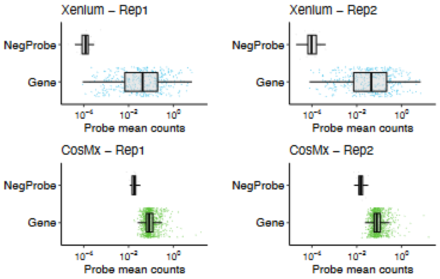
Huan Wang[1,*], Ruixu Huang[2,*], Jack Nelson[1,*], Ce Gao[3], Miles Tran[3], Anna Yeaton[4], Kristen Felt[5], Kathleen L. Pfaff[6], Teri Bowman[7], Scott J. Rodig[6,7], Kevin Wei[3,7], Brittany A. Goods[2,**], Samouil L. Farhi[1,**]

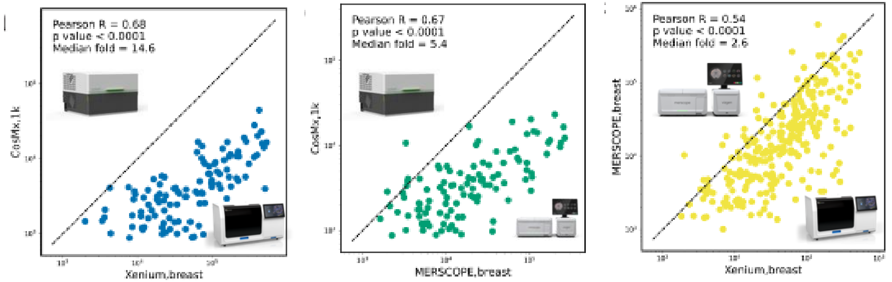


	Xenium Rep 1	Xenium Rep 2	CosMx Rep 1	CosMx Rep 2
Gene target #	377	377	1000	1000
Total cell count	99,852	102,508	96,139	98,767
Median gene count per cell	33	34	75	71
Median transcript count per cell	88	92	113	99
Median transcript count / gene target count	0.23	0.24	0.11	0.10
Median transcript count (intersecting targets only)	23	24	8	7









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

Gene targets panel design

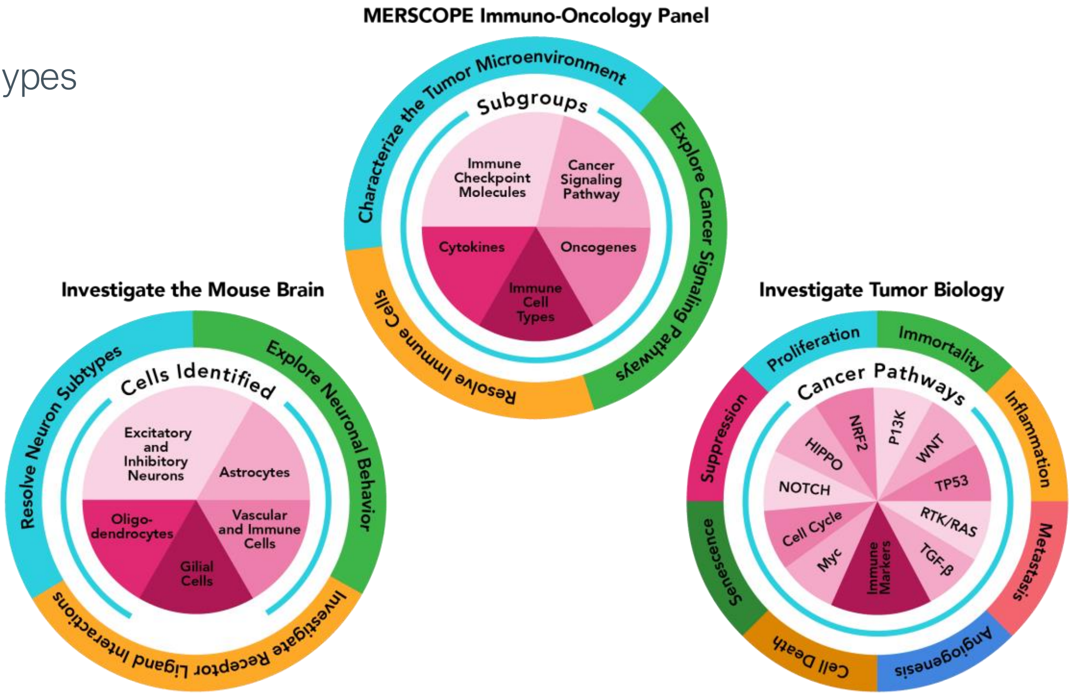
Depending on the biological question !

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

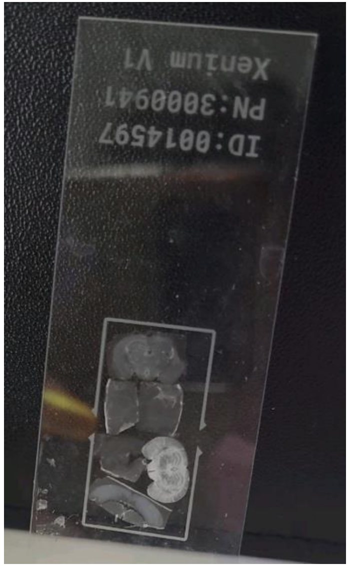
- Number of targets available
- Range of gene targets expression
- Total gene targets expression
- Budget around 15 k€ for 10 reactions



https://portal.vizgen.com/
https://cloud.10xgenomics.com/xenium-panel-designer

Experimental design

Take advantage of the large imaging area



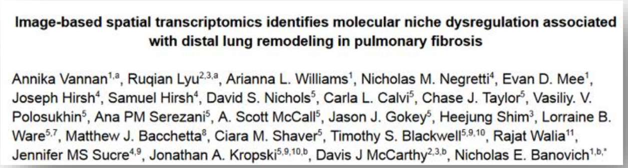
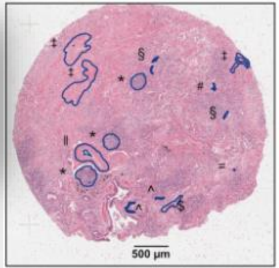
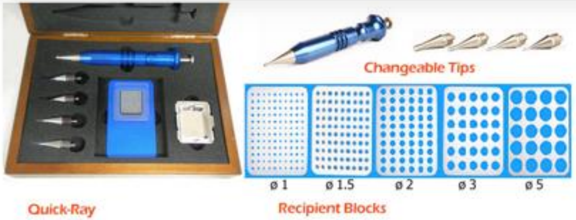


Image-based spatial transcriptomics identifies molecular niche dysregulation associated with distal lung remodeling in pulmonary fibrosis

Annika Vannan[1,a], Ruqian Lyu[2,3,a], Arianna L. Williams[1], Nicholas M. Negretti[4], Evan D. Mee[1], Joseph Hirsh[4], Samuel Hirsh[4], David S. Nichols[5], Carla L. Calvi[5], Chase J. Taylor[5], Vasiliy. V. Polosukhin[5], Ana PM Serezani[5], A. Scott McCall[5], Jason J. Gokey[5], Heejung Shim[3], Lorraine B. Ware[5,7], Matthew J. Bacchetta[8], Ciara M. Shaver[5], Timothy S. Blackwell[5,9,10], Rajat Walia[11], Jennifer MS Sucre[4,9], Jonathan A. Kropski[5,9,10,b], Davis J McCarthy[2,3,b], Nicholas E. Banovich[1,b,*]





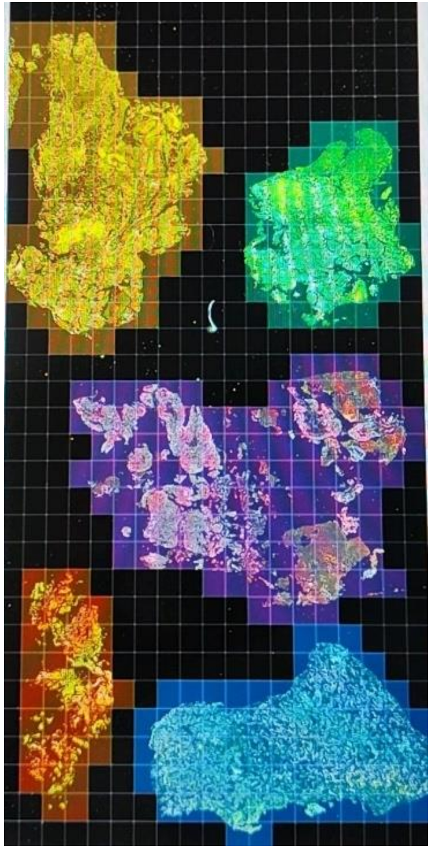
https://www.ihcworld.com/products/Quick-Ray-Mold.htm

Each slide cost around 5 k€

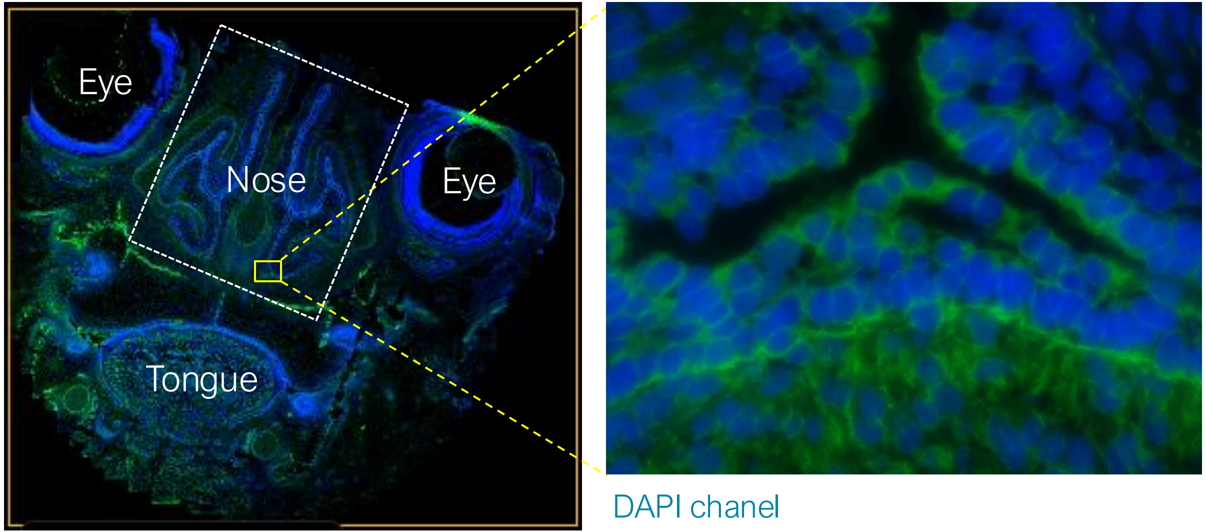
multiplexing to remove batch effect and increase replicates for robust statistical analysis

Data acquisition

DAPI and cell boundaries staining for cell segmentation



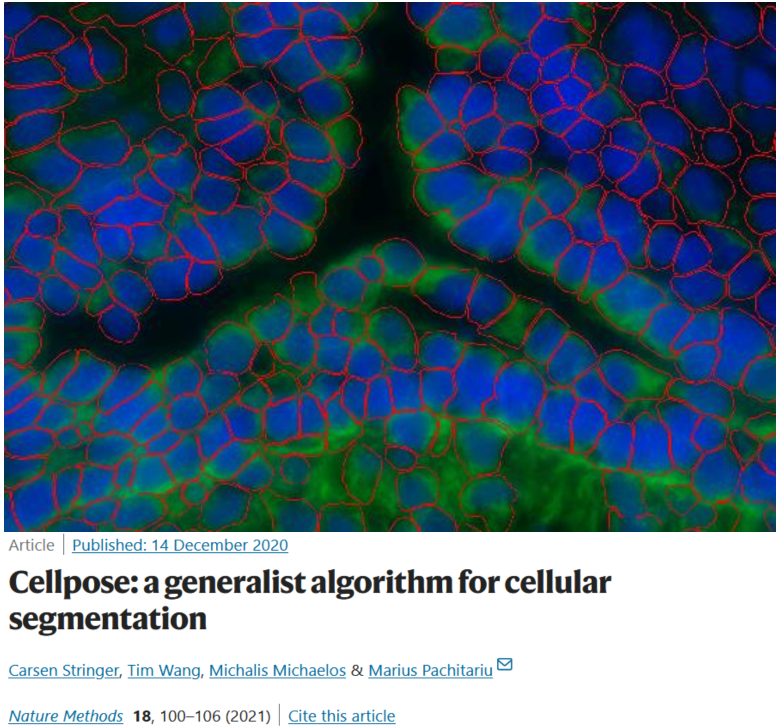
Human fetal head section (PCW9)



DAPI chanel
Cell boundaries chanel

Data acquisition

Cell segmentation



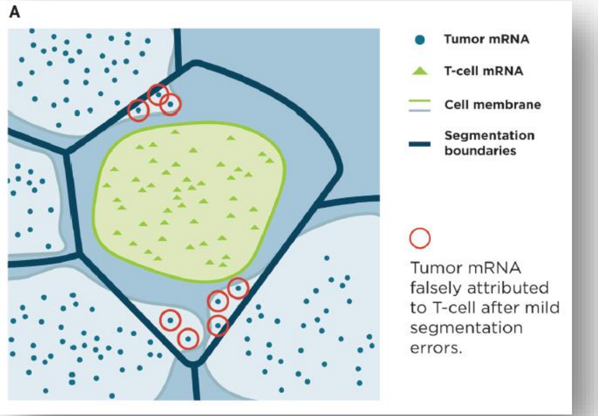
Article | Published: 14 December 2020

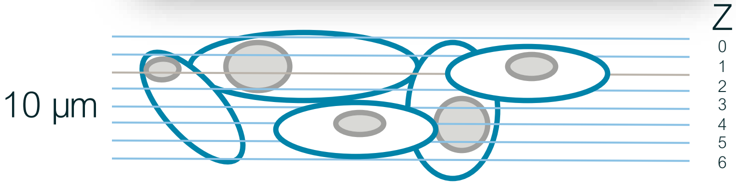
Cellpose: a generalist algorithm for cellular segmentation

Carsen Stringer, Tim Wang, Michalis Michaelos & Marius Pachitariu

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

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

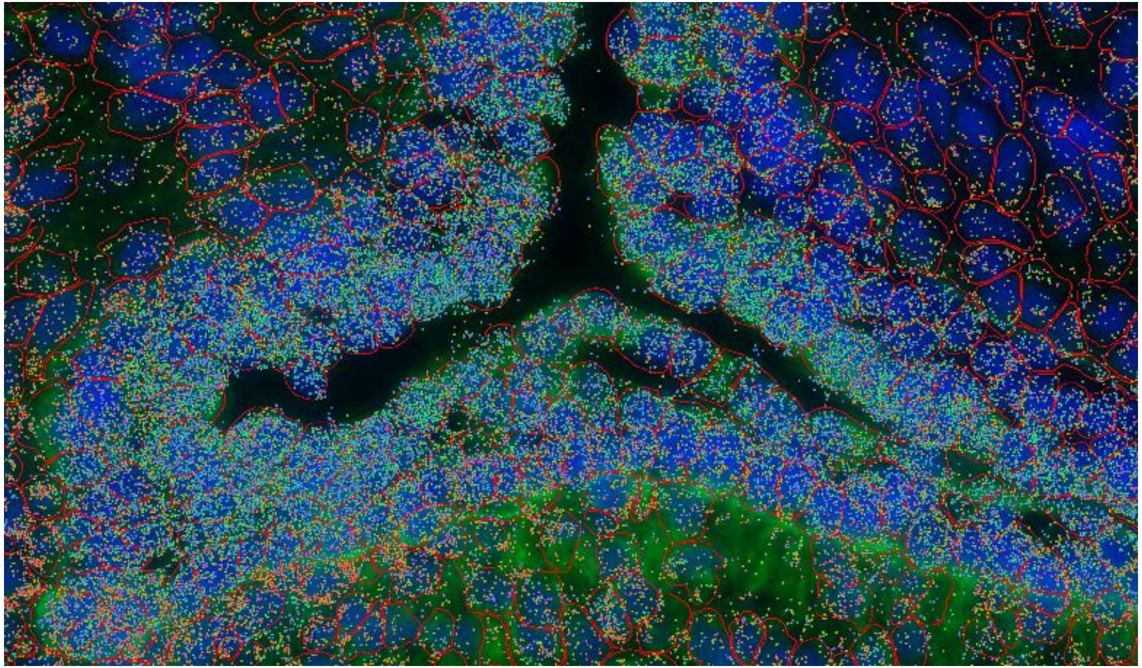




3D segmentation required, actually not used, 2D segmentation per Z then harmonizing and summing the detected transcripts for all Z into the harmonized segmentation mask (nuclei of full cell)

Raw data

Cell x gene matrix

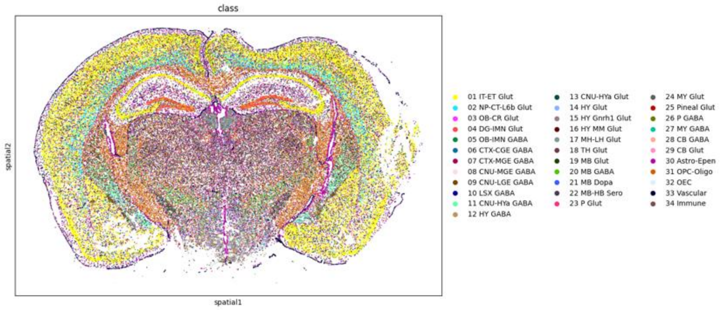


Gene-level matrix

100k’s cells

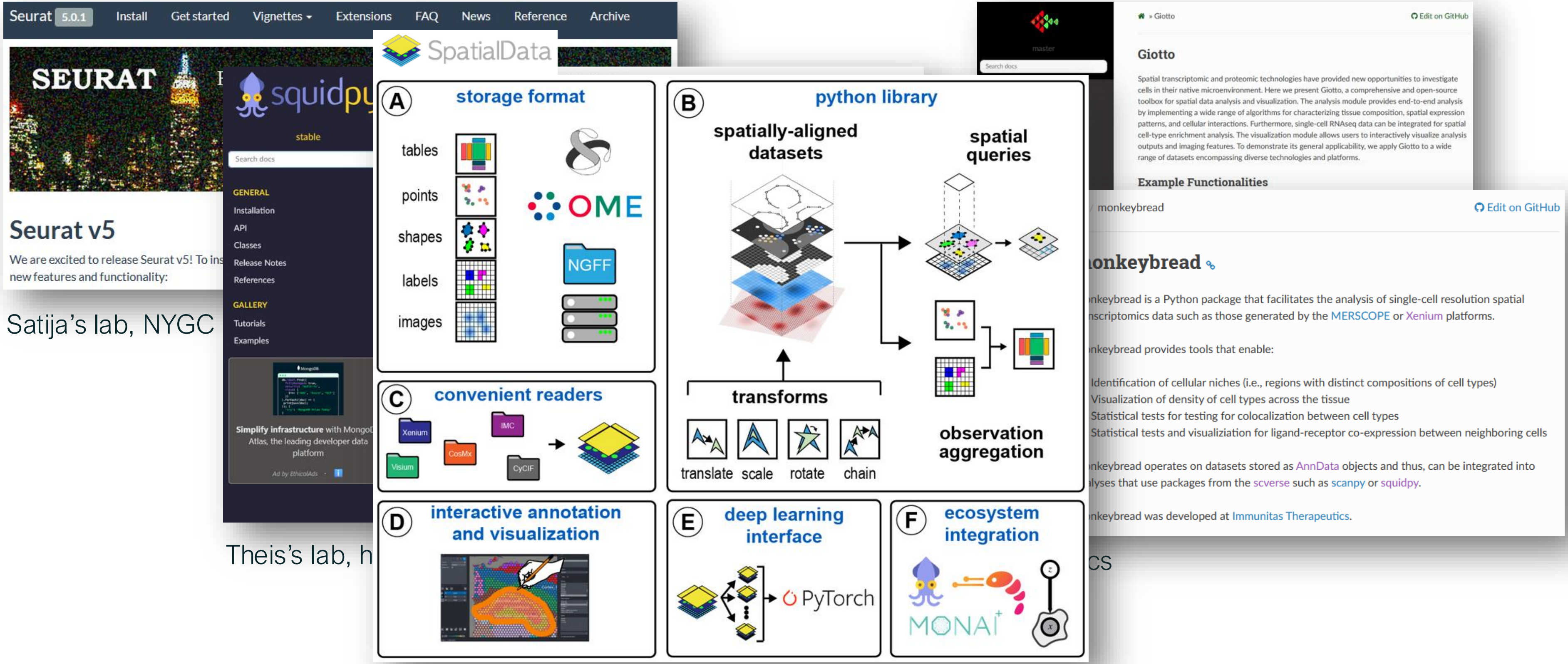
```
Clta  5  4 6 7  1 4  3  9  5  5 . 1 4 3  6 4 . 7  5 2  4
Myl6  5  2 5 1  2 4 13  2  5  2 4 4 1 4  8 4 2 3  3 1  1
Pkm   3  2 1 .  . 8  3  9  4 10 5 1 . 4 12 5 1 3  9 1  7
Tecr  3  1 5 2  4 6  5  9  6  3 2 2 1 4  7 1 1 1  3 2  8
Meis2 8 29 3 . 24 6  6 21 25 16 6 1 . 2 29 . . 6 18 . 10
 ...
```

1.000 Genes



Statistical data analysis

Standardized workflows + packages development



Seurat v5

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

Satija's lab, NYGC

Theis's lab, h

Giotto

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

Example Functionalities

onkeybread

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

nkeybread provides tools that enable:

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

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

nkeybread was developed at Immunitas Therapeutics.

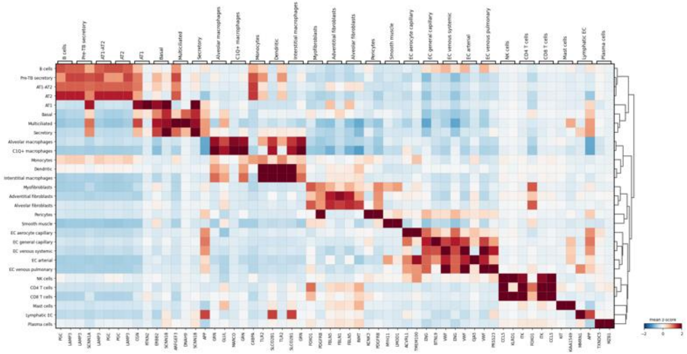
cs

Scverse ecosystem, Oliver Stegle & Fabian J. Theis

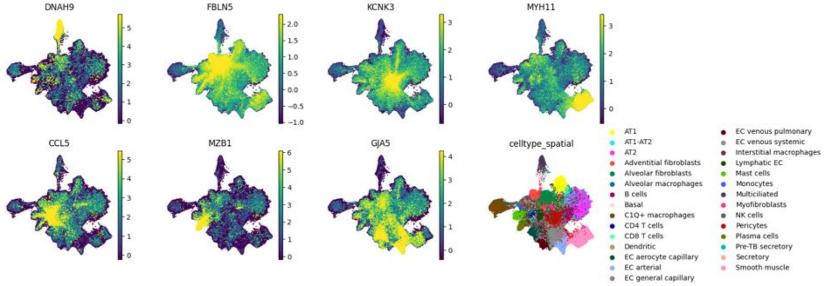
Single-cell data analysis

Scanpy and Squidpy toolkits

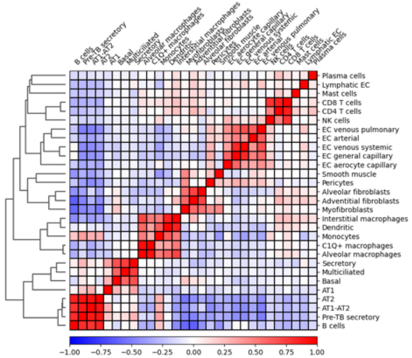
Gene marker detection, manual or automatic cell type identification



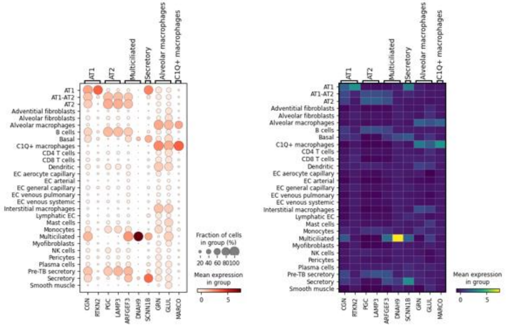
Batch effect correction, sample integration, cell type labeling transfer from single-cell references dataset



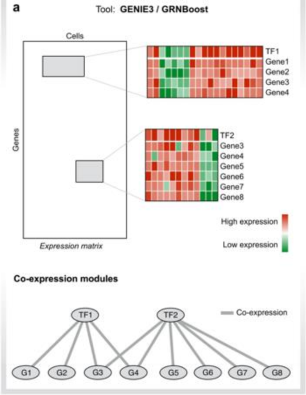
Cell type correlation



Differential expression analysis
Gene set functional enrichment

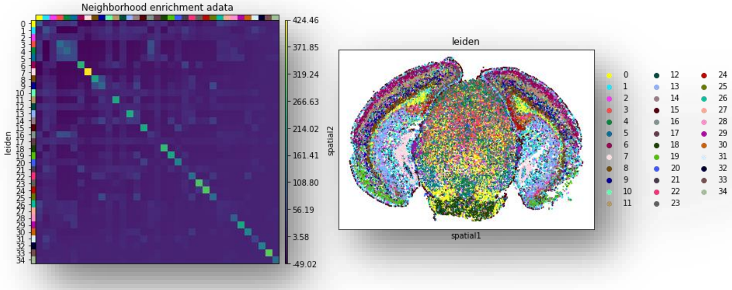


Transcription Regulatory Network



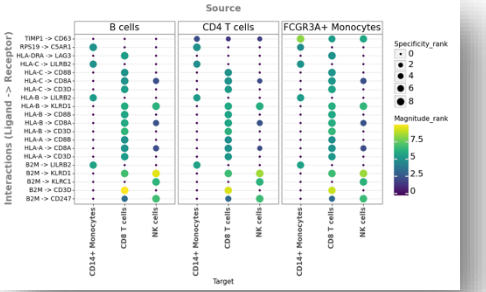
Single-cell data analysis including spatial resolution

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



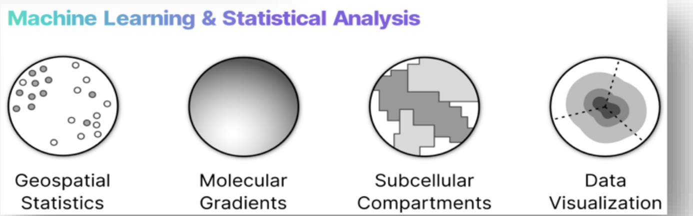
Neighbors enrichment analysis

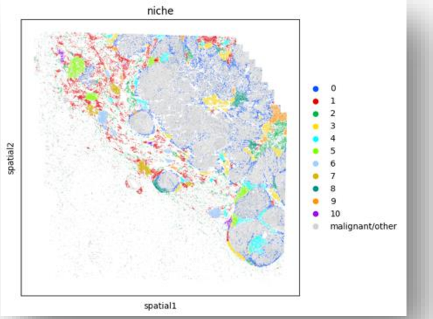
Test if cells belonging to 2 clusters are close to each other more often than expected (co-occurrence probability)



Cell-cell communication Ligand-Receptor analysis

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



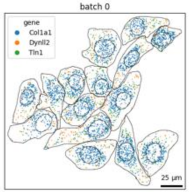


Cellular niches analysis

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”.

Sub-cellular exploration

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



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