# Single-cell and Spatial isoform Transcriptomics

Kévin Lebrigand
Computational Biology Core
IPMC, CNRS, Côte d'Azur University, France
✉ lebrigand@ipmc.cnrs.fr
🐦 @kevinlebrigand

Overview

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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

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

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Published: 17 March 2023   **Article history** ▾

01

Single-cell transcriptomics

Single-cell Transcriptomics
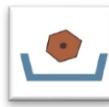
Context

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

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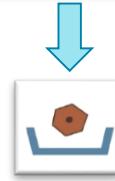
Single-cell Transcriptomics

Unique Molecular Identifiers (UMI)
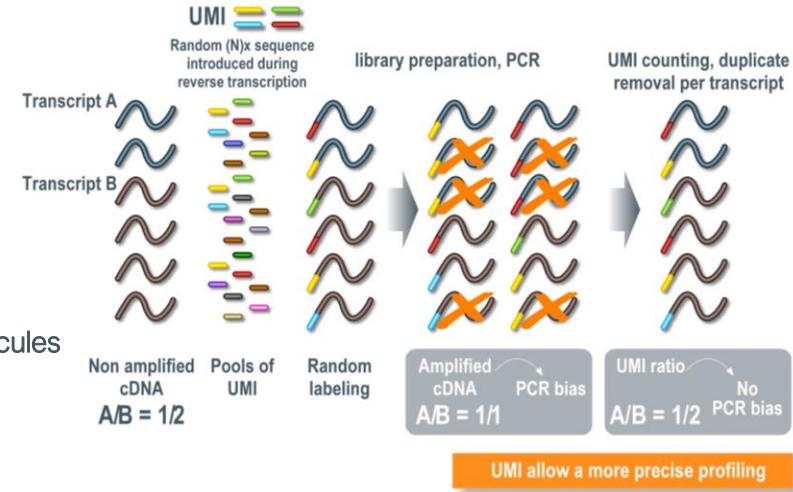
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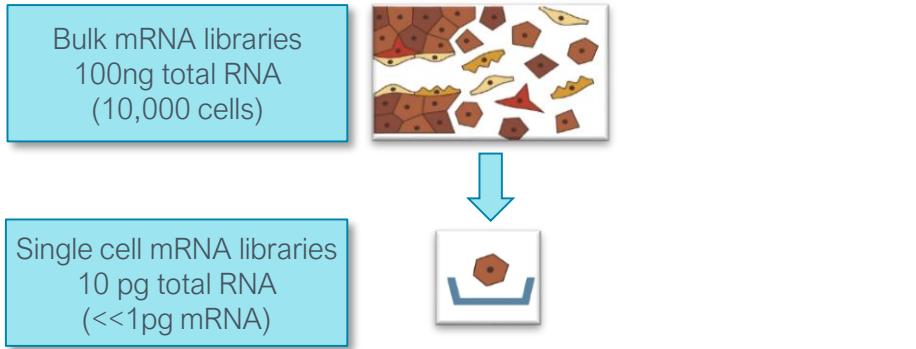
- Elimination of PCR amplification bias and artefacts

- 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

UMI

Random (N)x sequence introduced during reverse transcription

library preparation, PCR

UMI counting, duplicate removal per transcript

Transcript A

Transcript B

Non amplified cDNA
A/B = 1/2

Pools of UMI

Random labeling

Amplified cDNA   PCR bias
A/B = 1/1

UMI ratio
A/B = 1/2   No PCR bias

UMI allow a more precise profiling

Single-cell Transcriptomics
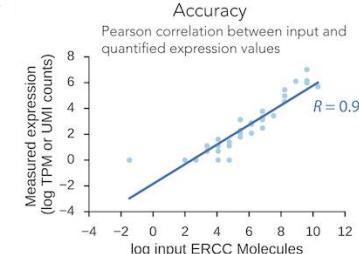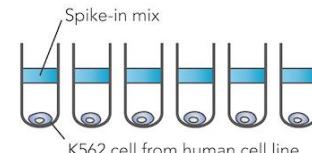
ERCC spike-in allow capture efficiency evaluation

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

$R = 0.91$

Spike-in mix

K562 cell from human cell line

Measured expression (log TPM or UMI counts)

log input ERCC Molecules

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
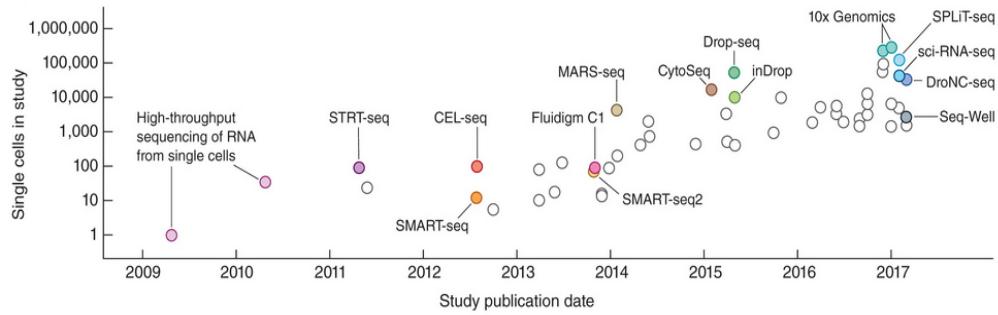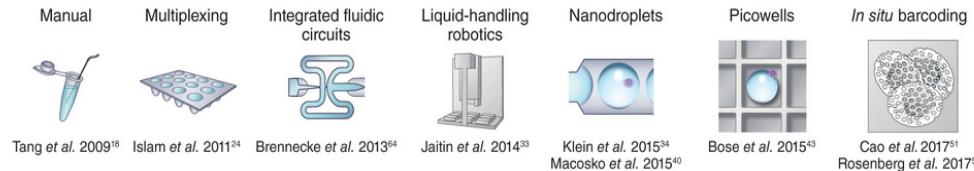
# Single-cell transcriptomics
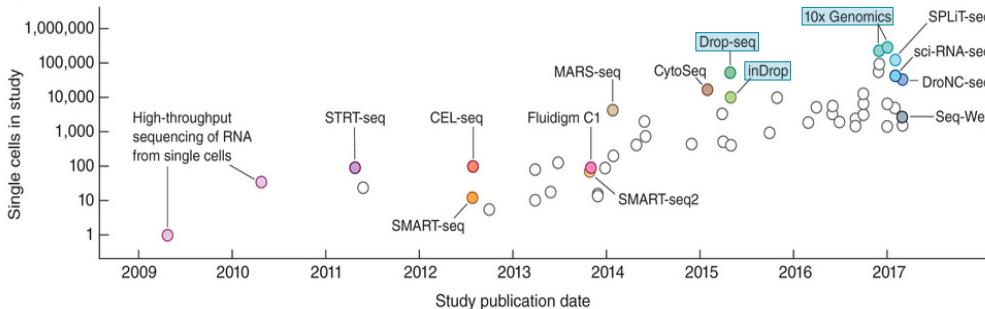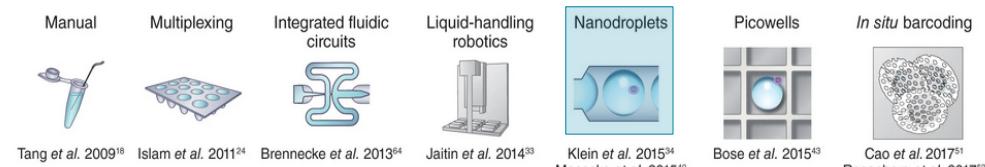
Evolution of isolation techniques and throughput

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

# Single-cell transcriptomics

Droplet-based approaches
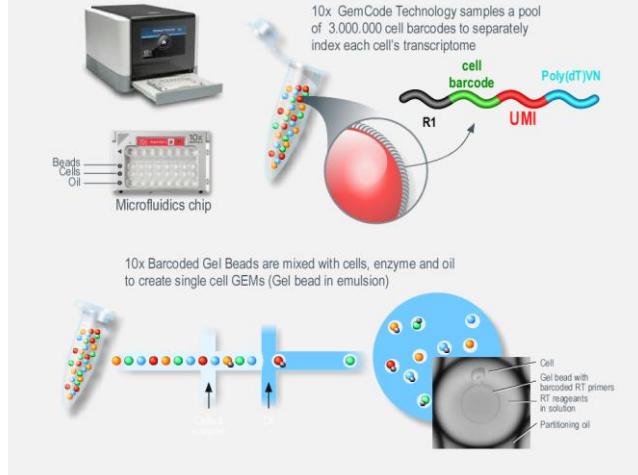
Exponential scaling of single-cell RNA-seq in the past decade
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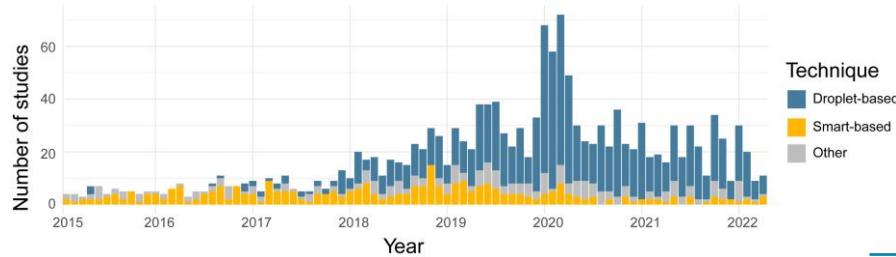
10x Genomics Chromium single cell controler (2016)
- Easy-to-set-up and robust workflow
- High scalability (1,3M cells dataset)

Single-cell transcriptomics

Single cell approaches in publications

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

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

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

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

PTBP1

Single-cell transcriptomics
Single cell approaches in publications

A curated database reveals trends in single cell transcriptomics
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- 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
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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 ✉
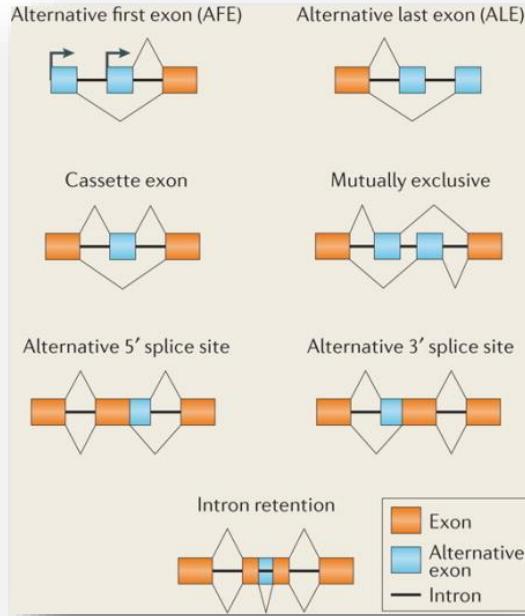
Brief Communication | Open Access | Published: 30 May 2022
Fast and highly sensitive full-length single-cell RNA sequencing using FLASH-seq
Vincent Hahaut, Dinko Pavlinic, Walter Carbone, Sven Schuierer, Pierre Balmer, Mathieu Quinodoz, Magdalena Renner, Guglielmo Roma, Cameron S. Cowan & Simone Picelli ✉

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** :

5' UTR	Coding sequence	3' UTR
770nt	1,340nt	300nt

Alternative splicing and disease
Tazi et al., 2008

02
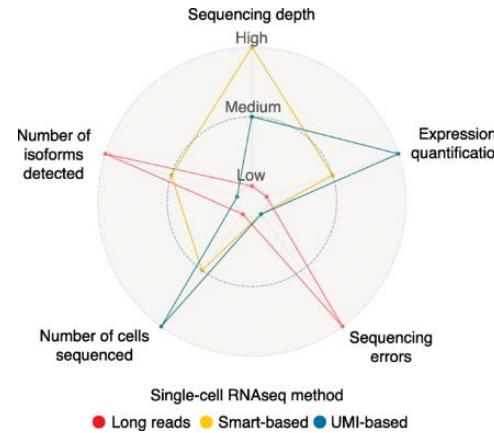
Single-cell isoform transcriptomics

Requirement for single-cell full-length transcriptomics

Arzalluz-Luque et al., 2018

Four required conditions :

- **full-length** transcript sequencing using long reads,
- **low sequencing errors** and artefacts,
- **high number of cells** sequenced,
- **high capture efficiency** and sequencing depth

Single-cell RNAseq method
● Long reads ● Smart-based ● UMI-based

Main approaches :

- Smart-based methods, produce short reads across the entire transcript length, cannot accommodate UMIs, short reads difficult to assign unambiguously to isoform.

- UMI-based methods, limited to sequencing of the 3′ (or 5′ end), well suited to accurate gene-level expression quantification.

- Long-reads (i.e. single molecule) allows a sequencing of each transcript molecule as a single read providing full isoform connectivity, but suffers from a high prevalence of sequencing errors.

Requirement for single-cell full-length transcriptomics

Arzalluz-Luque et al., 2018

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scNaUMI-seq protocol

Single-cell long-read transcriptomics

Droplets-based approach short reads vs long reads

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

is lost

remain accessible

Single-cell long-read transcriptomics

SiCeLoRe, bioinformatics for Single Cell Long Read

1.200 cells

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

split emulsion before RT

15%
190 cells

85%
951 cells

Millions long-reads

190 cells: 32M
951 cells: 322M

standard workflow

R9.4, LSK-109 chemistry (2018)

Rainer Waldmann

https://github.com/ucagenomix/sicelore

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Illumina-guided demultiplexing strategy

Barcodes assignment accuracy

Efficiency of cell barcode / UMI assignment

30% of the Nanopore reads were assigned to a cell barcode and UMI

Reasons for losses
- Low QV reads
- PCR artefacts : TSO or poly(A) on both sides
- Out-of-cell barcodes (empty droplet RNA)
- UMI not in Illumina dataset (low sequencing saturation)

Accuracy of cell barcode / UMI assignment

Single-cell long-read transcriptomics reveals diversity
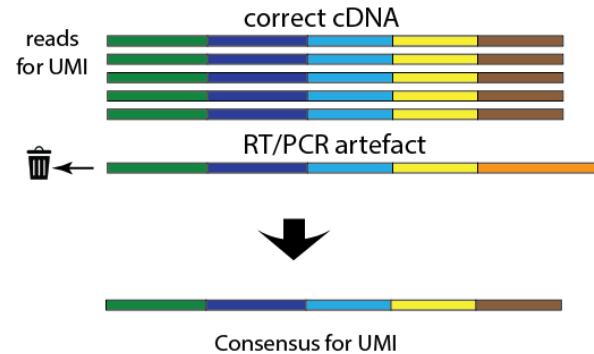
76 isoform-switching genes along neuronal maturation

Single-cell long-read transcriptomics reveals sequence heterogeneity
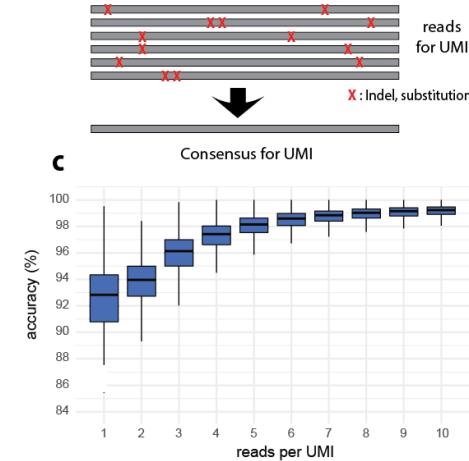
Consensus sequence computation per UMI

UMIs enable elimination of PCR artifacts

Crucial for accurate novel isoform discovery

UMIs enable correction of sequencing errors
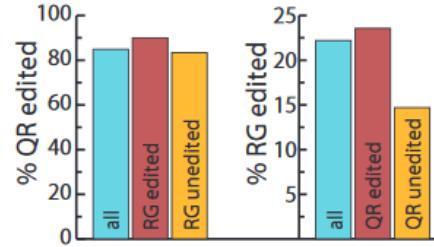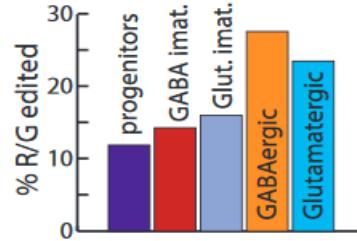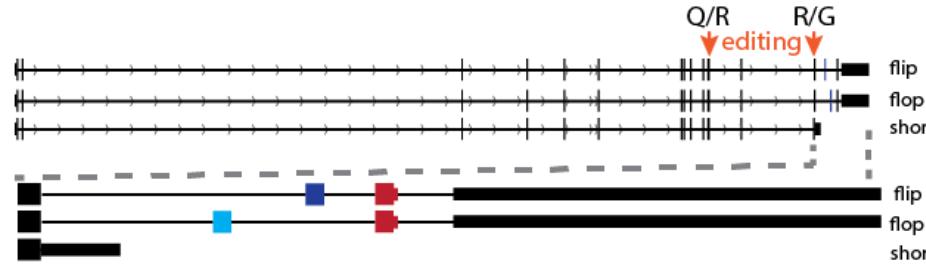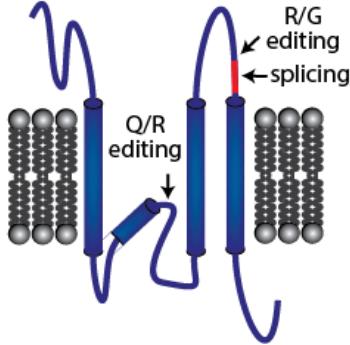
High accuracy for Single Nucleotide Variation call

Q/R site regulates AMPA receptor Ca^{2+}-permeability
R/G site is involved in desensitization and recovery of the receptor
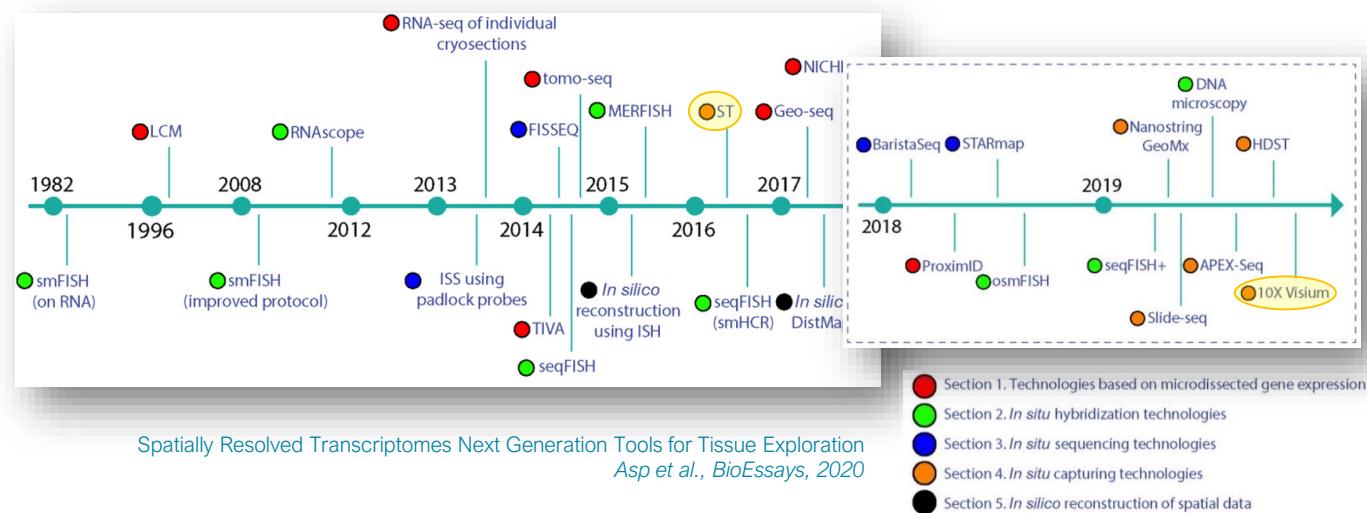
03

Spatial isoform transcriptomics

Spatial Transcriptomics approaches
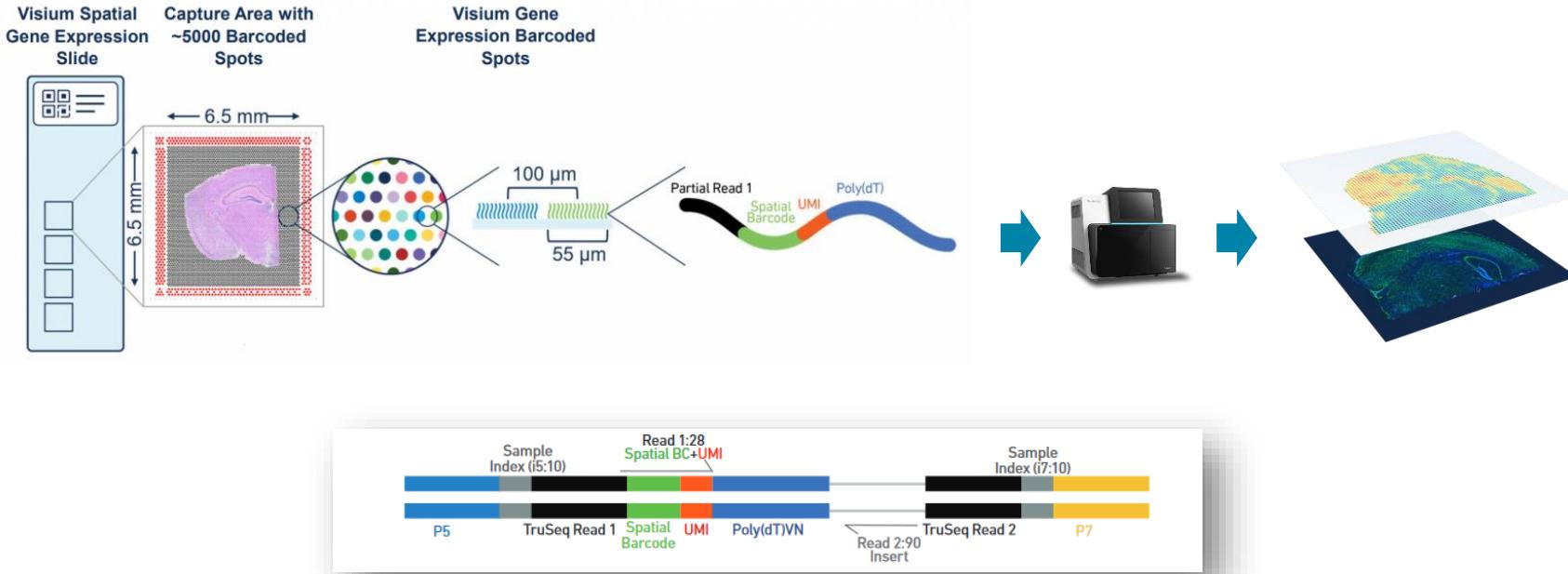
Historical timeline

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

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

In-situ capture spatial transcriptomics

10x Genomics Visium (2019)

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

# Spatial isoform Transcriptomics (SiT)

Nucleic Acids Research, 2023

Nanopore promethION long-read sequencing

Provides isoform-level spatial transcriptomics

*Sequencing saturation curves per samples*

Transcripts full-length coverage

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

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

# SiT reveals specific splicing pattern across MOB regions
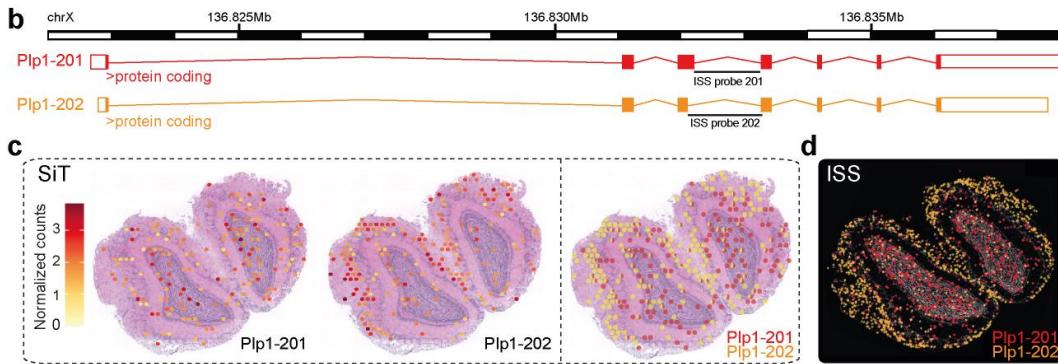
Plp1 Differential Transcript Usage (DTU)

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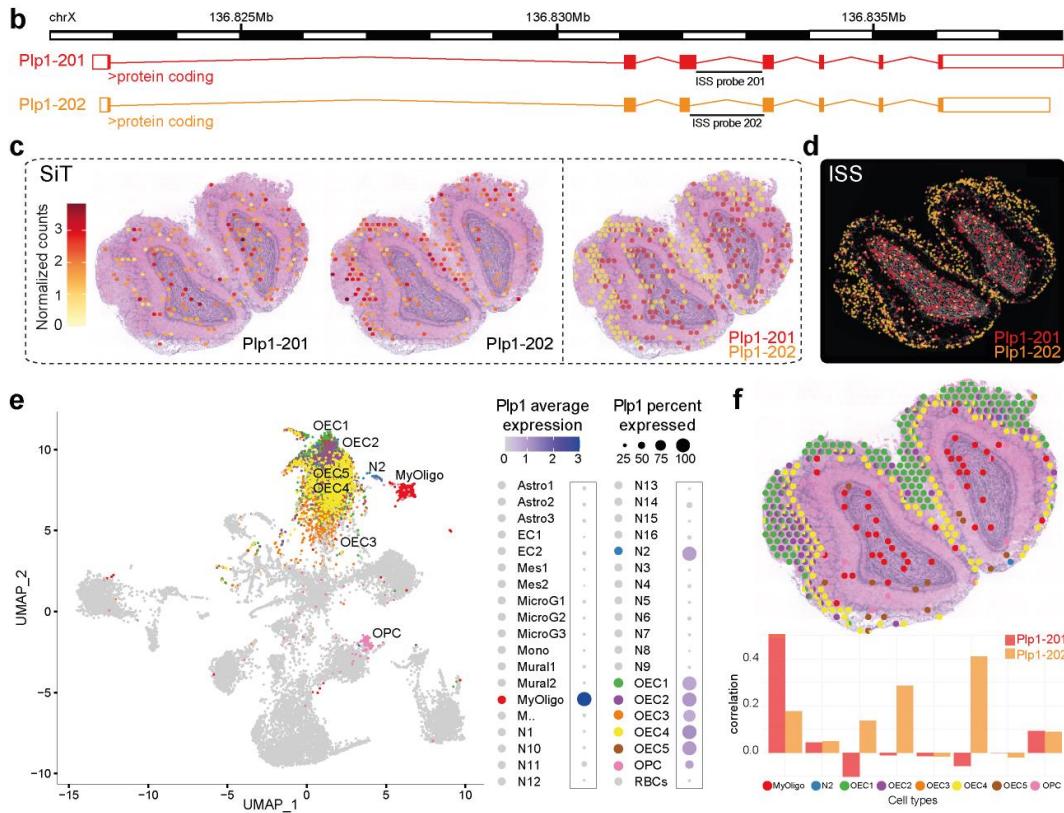
Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination

In Situ Sequencing Data

SiT reveals specific splicing pattern across MOB regions

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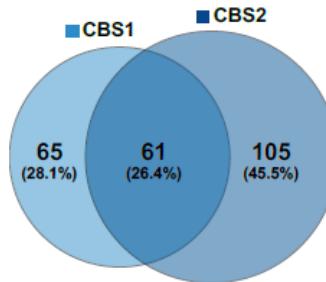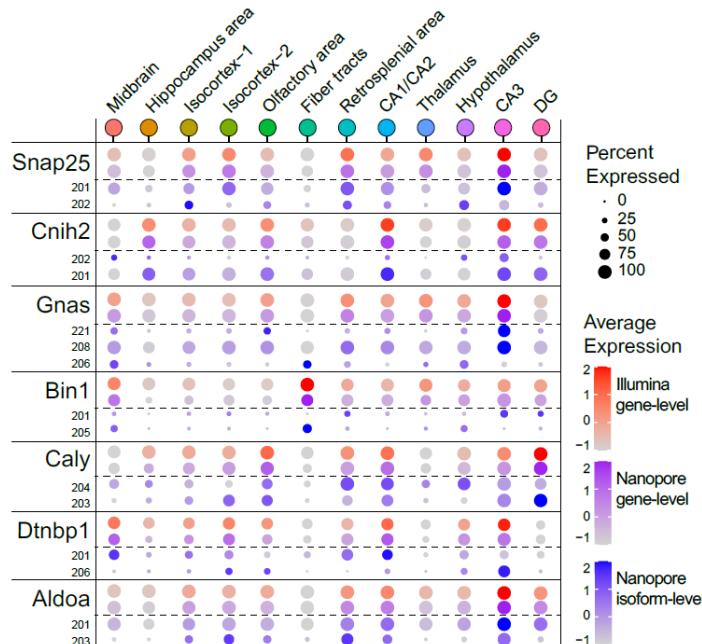

Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination

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.

Differential Transcript Usage (DTU) across CBS regions
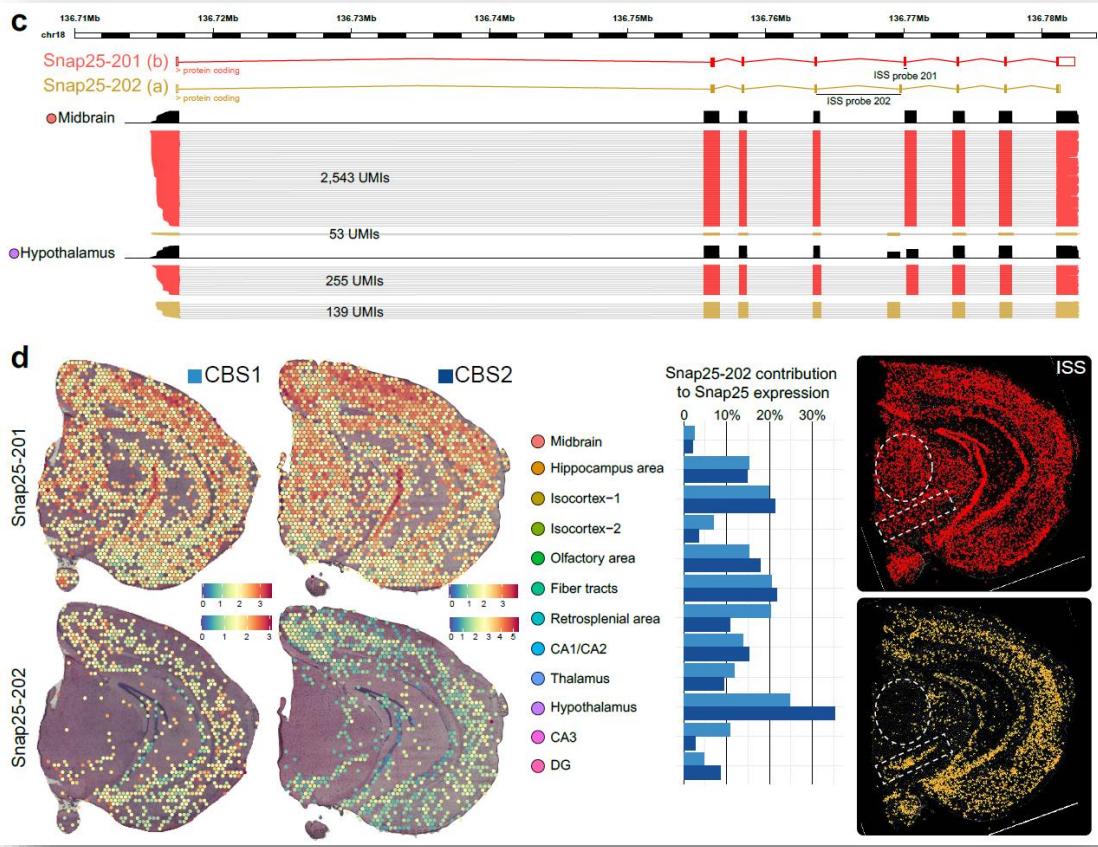
61 common switching genes detected

Snap25 DTU across CBS regions
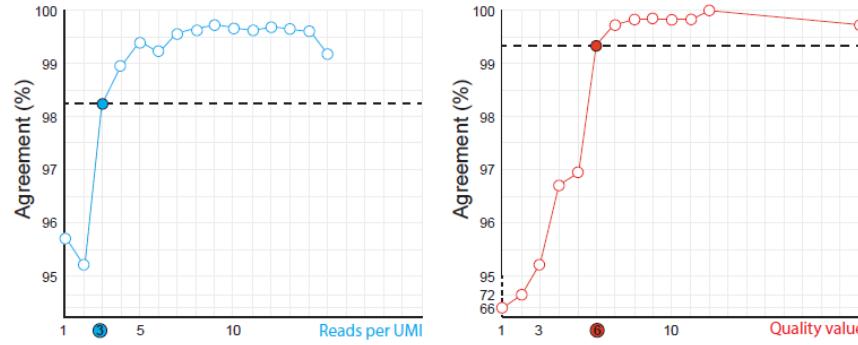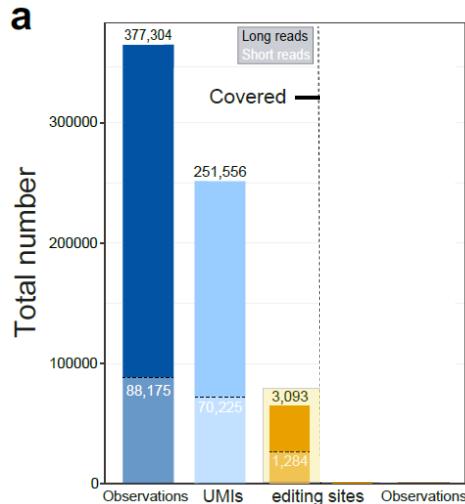
Presynaptic plasma membrane protein involved in the regulation of neurotransmitter release

SiT reveals full-length sequence heterogeneity (CBS)

High confidence (>99%) SNV call calibration using short-read

- Exploration of 5,817 A-to-I RNA editing sites described in the literature (Ramaswami et al., 2013 (RADAR), Licht et al., 2019)
- Long read high confidence call thresholding, looking at agreement between long and short read base calls for 88,175 shared UMIs
  - number of reads per UMI >= 3
  - consensus Phred score QV >= 6
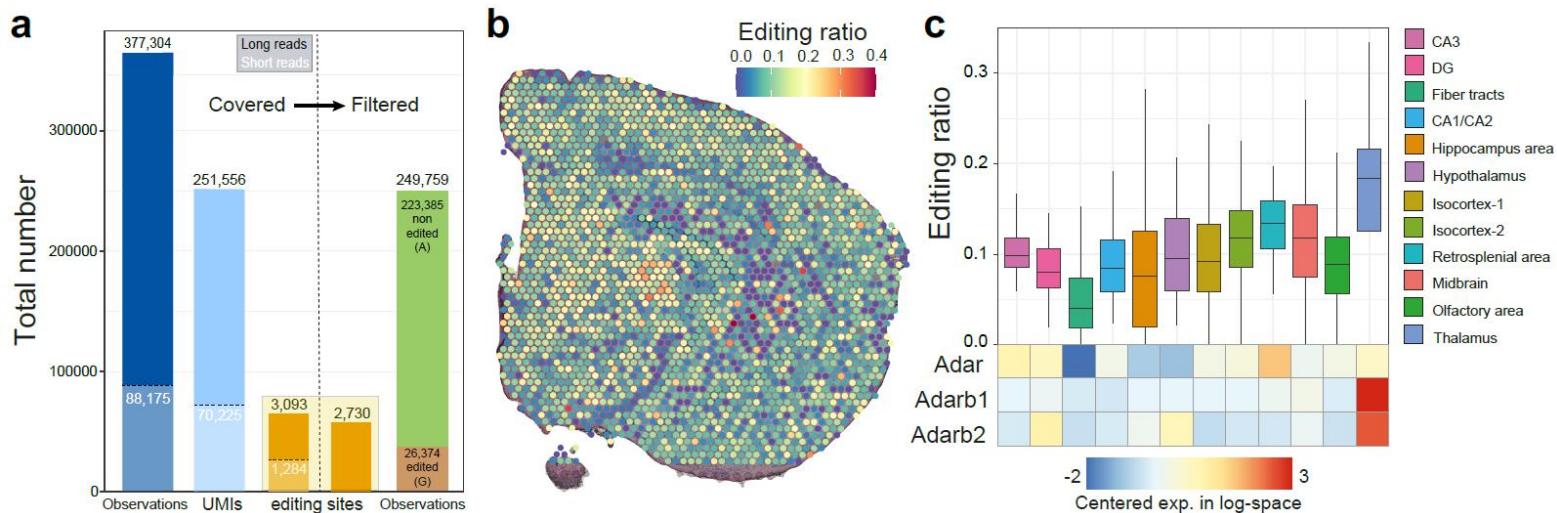
Supplementary Figure 11. Agreement of editing rates between long-read and short-read data for the genomic regions that are detected by both approaches. The percentage of agreement between the two sequencing approaches is plotted as a function of nanopore read numbers per UMI (left plot) and nanopore consensus base quality value (right plot). The highlighted thresholds were used for editing site calling with nanopore reads.

SiT reveals full-length sequence heterogeneity (CBS)
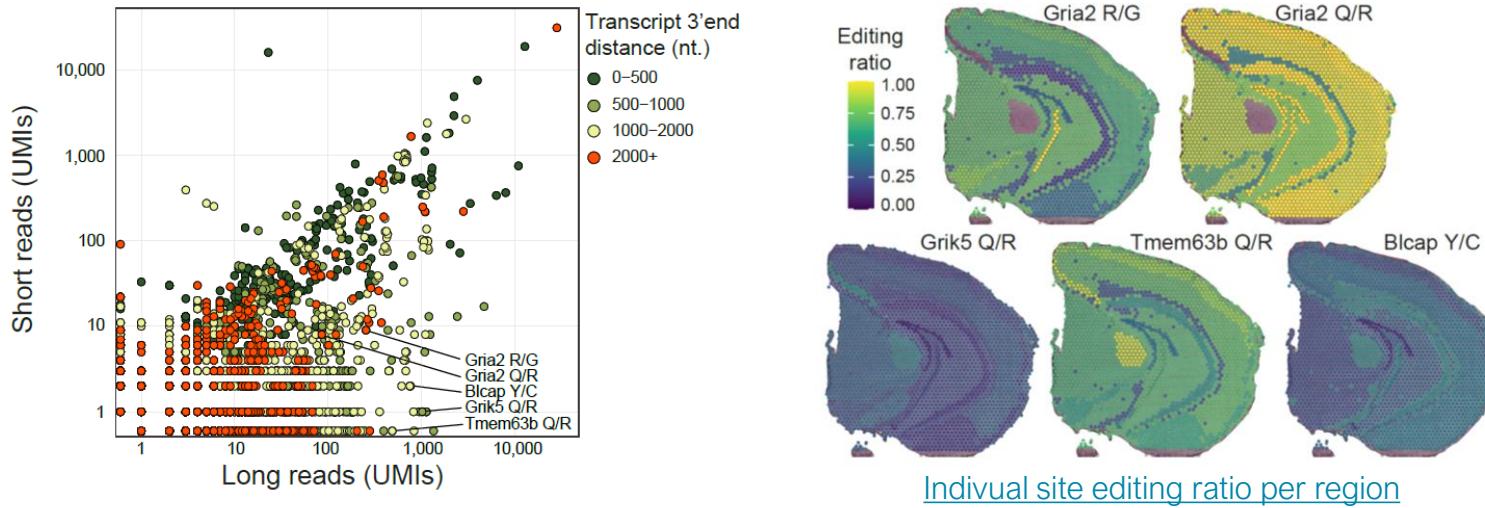
Global A-to-I RNA editing spatial map

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- 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 A-to-I RNA editing specificity in the mouse brain
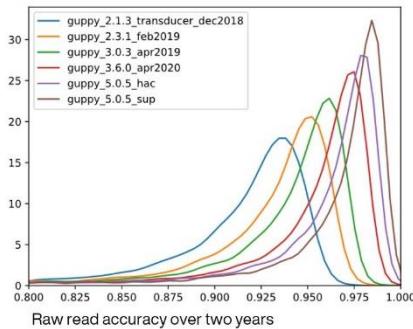
Short vs Long -read editing site calls

Indivual site editing ratio per region

Single cell and Spatial isoform transcriptomics

Summary

- Accurate single-cell and spatial transcriptomics using Nanopore long-read sequencing is feasible

- Long reads sequencing reveals 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 achieve

- Sicelore-2.1 : we don't need short reads anymore

Raw read accuracy over two years

Nanopore PromethION sequencing

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

2022: 150M reads/FC, 98% raw read accuracy
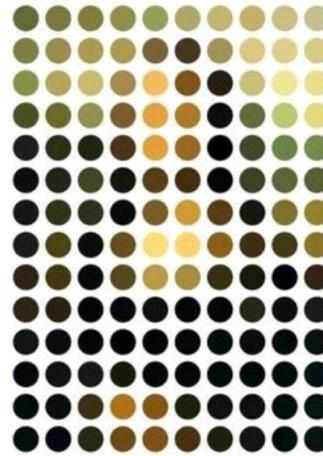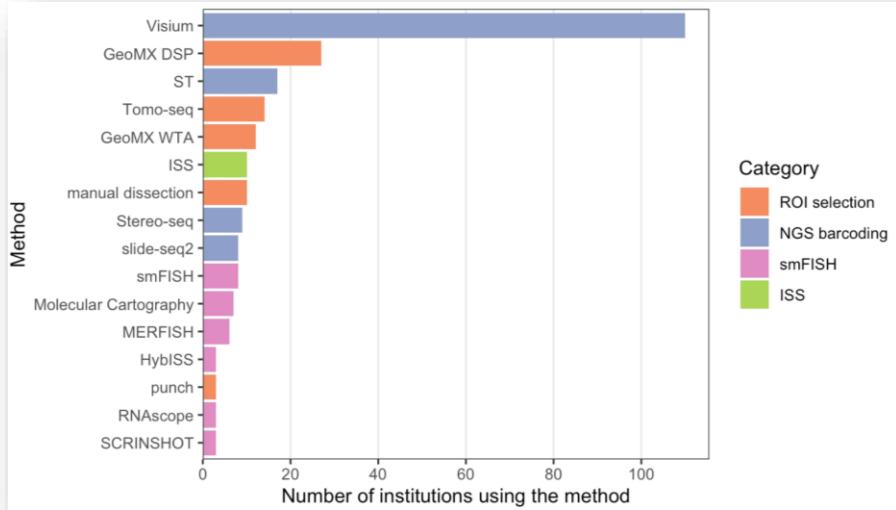
https://github.com/ucagenomix/sicelore-2.1
- Visium and single-cell 3' and 5' libraries
- Illumina-free profiling available

04

Spatial single-cell 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)
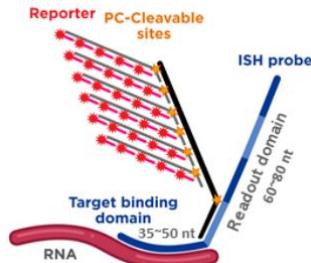
Spatial imaging technologies (2023)

No more sequencing for direct single-cell resolution
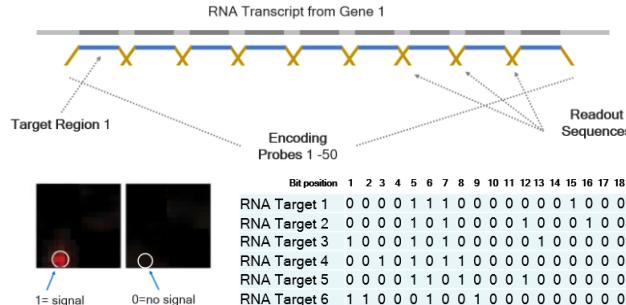
Nanostring CosMx
ISH-based

- 960 targets
- Sensitivity : < 30-80%
- Resolution: 200 nm
- Imaging area: 16 mm2

Vizgen Merscope
Merfish

- 500 targets (1,000 soon)
- Sensitivity : 30-80%
- Resolution: 100 nm
- Imaging area: 100 mm2

10xGenomics Xenium
Cartana ISS, padlock probes / RCA

- 400 targets
- Sensitivity : 5-30%
- Resolution: 200 nm
- Imaging area: 25 mm2
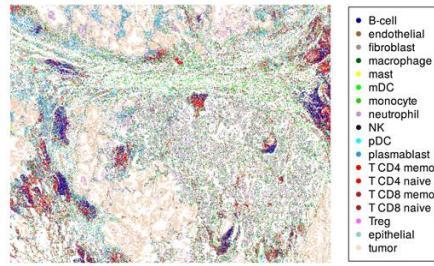
Spatial imaging technologies comparison

Compare available datasets

Vizgen Merscope
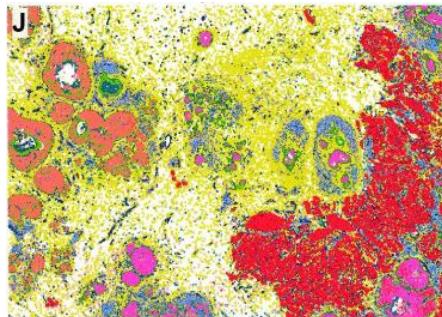
- Xiaowei Zhuang's lab merfish publications
  — Chen et al., Science (2015)
  — Moffitt et al., PNAS (2016), Science (2018)
 — Emanuel G et al., Nature Methods (2017)
  — Xia C. et al., PNAS (2019, Scientific Reports (2019)
  — Zhang M. et al., Nature (2021)
  — …

- Internal data release program
  — Human Immuno-oncology (**breast**, colon, **lung**, liver, skin, prostate, uterine and ovarian) 500 genes, >4 billion transcripts, 9 million cells
  — Mouse Liver Map (347genes)
  — Mouse brain Receptor Map (483 genes)

- External labs publications
  — Dixon E. et al., Kidney Int. (2022): Kidney
  — Wang et al., Nat. Neuro. (2022): Mouse olfactory Glomerular map
  — Stogsdill et al., Nature (2022): Neocortex microglia
  — …

Nanostring CosMx

- Release date: 11/2021
- FFPE Human NSCLC (**Lung**)
- 960 gene targets
- 8 sections for 800k cells
- Imaging area: 8 x 16 mm^2
- 259,604,214 transcripts
- Mean transcripts/cell: 265

10xGenomics Xenium

- Release date: 10/2022
- FFPE Human **Breast** cancer
- 313 gene targets
- 167,885 cells,
- 36,944,521 transcripts
- Imaging area: 40 mm^2
- Mean transcripts/cell: 193

Spatial imaging technologies comparison
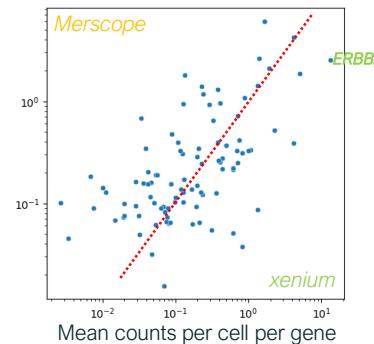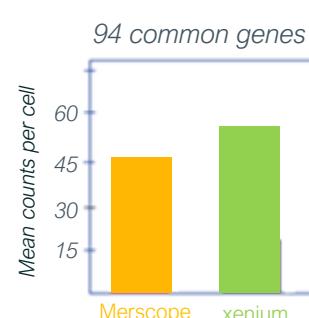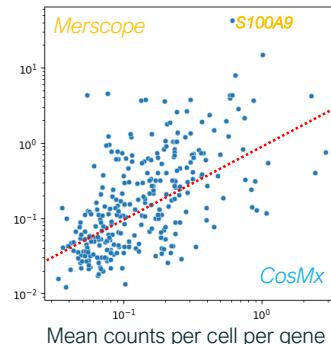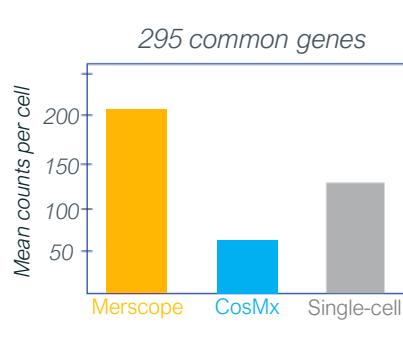
Compare available datasets: Lung and Breast cancer samples

FFPE Human Lung Cancer	Merscope	CosMx
Total cells	353 k (x4)	92 k
Detected transcripts	107 M (x4)	26 M
Gene targets	500	960 (x2)
Total RPKM	9,204	61,680 (x6)
Mean transcripts/cell	302	284

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

295 common genes

94 common genes
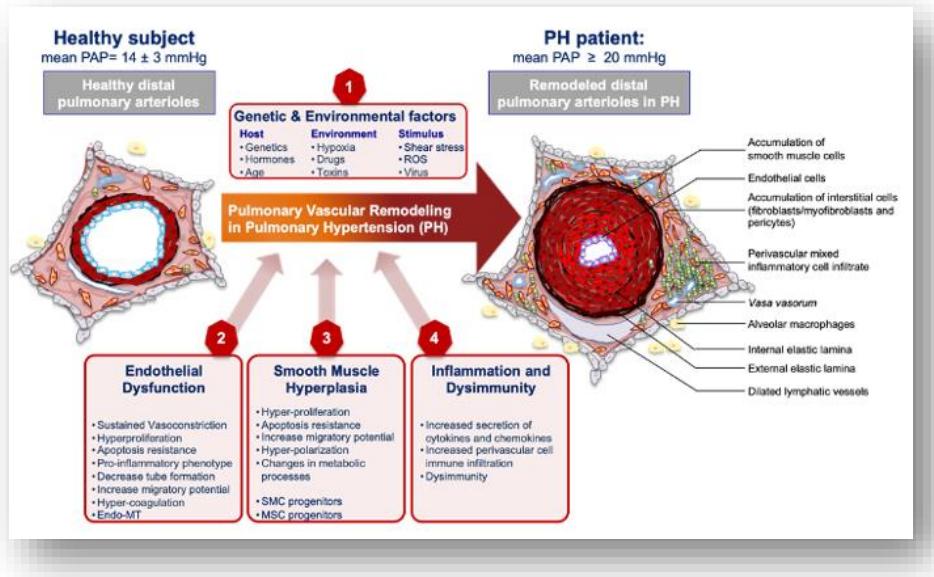
PAH : Pulmonary Arterial Hypertension

A rare vascular disorder

Characterized by the presence of occluded pulmonary arterioles resulting from the proliferation of pulmonary artery endothelial cells (PAECs), pulmonary artery smooth muscle cells (PASMCs) and fibroblasts, which leads to right heart hypertrophy and eventual cardiac failure
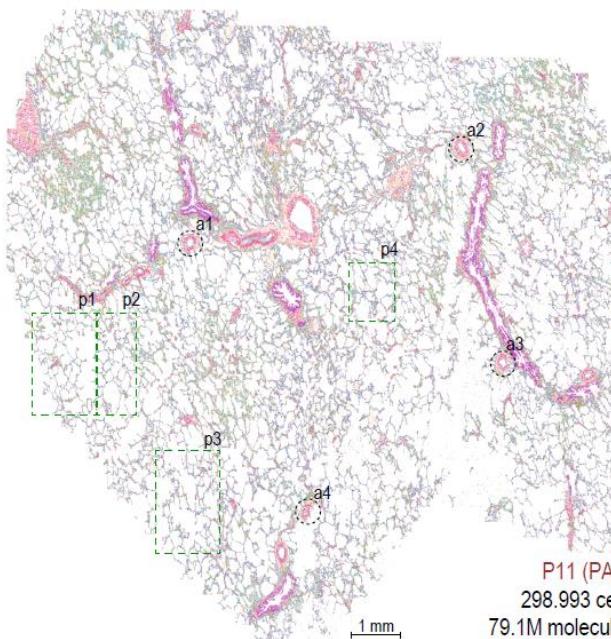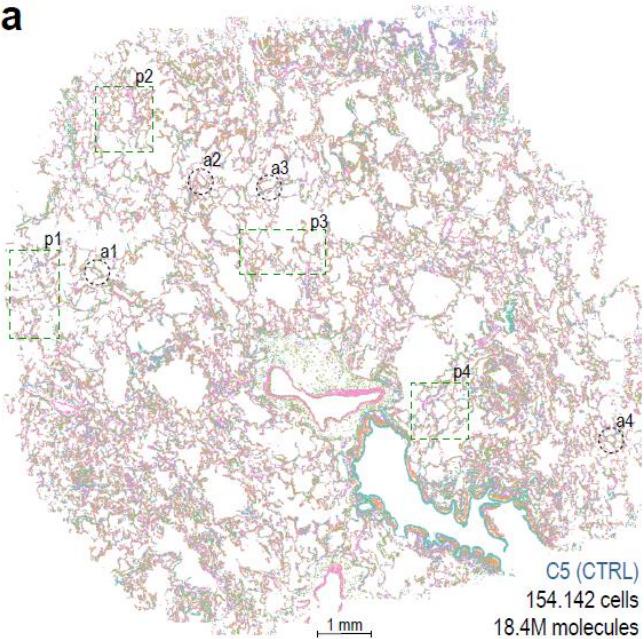
- Defined by a mean pulmonary arterial pressure >20 mmHg

- More frequent in women to men (2:1 to 4:1)

- Different origins:
  — IPAH (idiopathic or sporadic cases),
  — HPAH (heritable case family history) 6-10% monogenic autosomal-dominant - 14%♂,42%♀
  — APAH (associated forms), anorexigens / liver / congenital heart / connective tissue disease

PAH : Pulmonary Arterial Hypertension
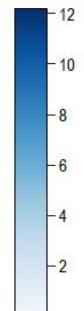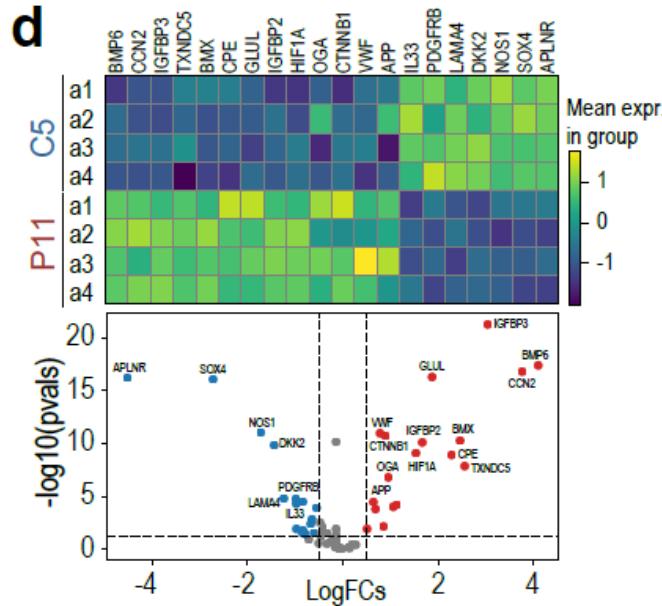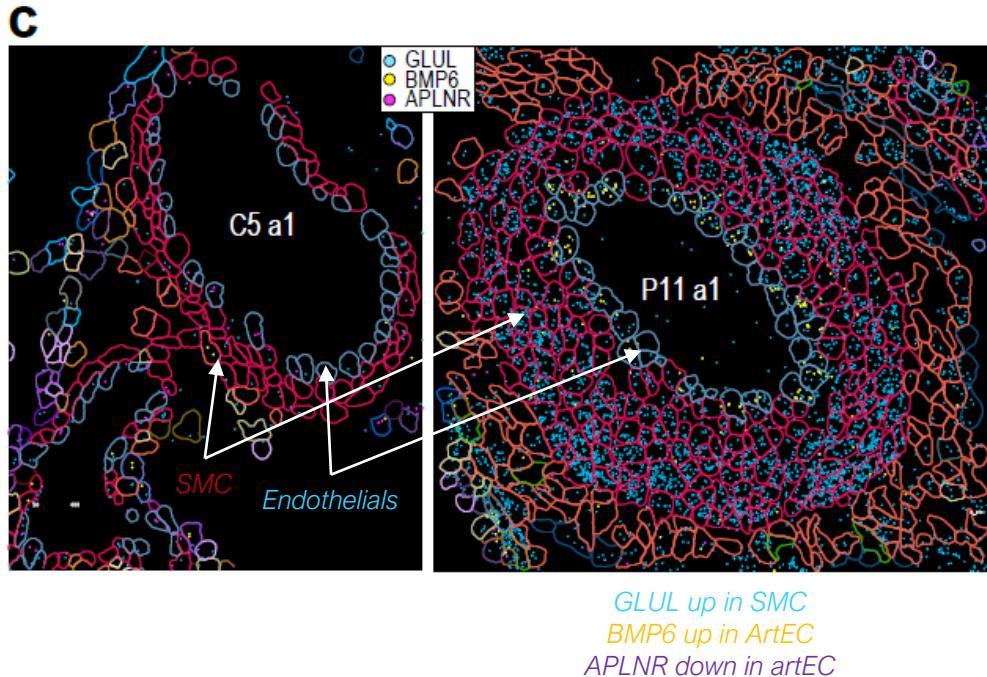
A rare vascular disorder

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

Acknowledgments

Institut de Pharmacologie Moléculaire et Cellulaire
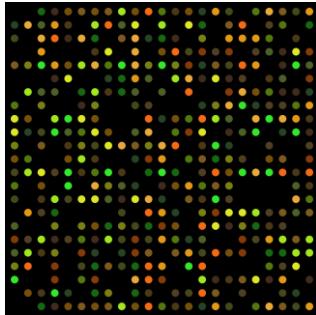
05

Supplementary

20 years of transcriptomics

Driven by microfluidics technological developments
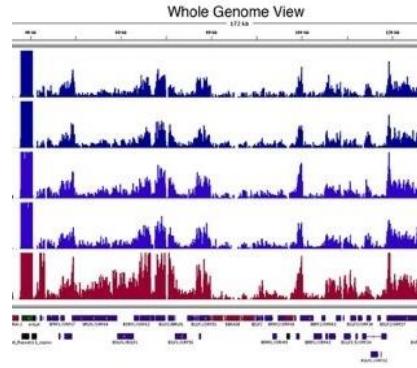
Early 2000's: DNA microarray

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

Cost : 4k€
20 samples
25k genes
0,5M matrix
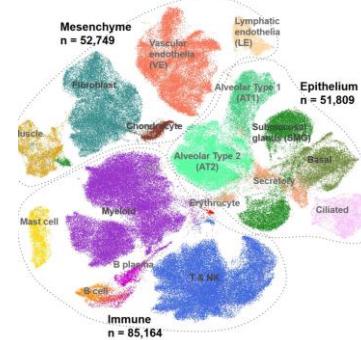
Late 2000's: RNA sequencing

Whole Genome View

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

Cost : 4k€
20 samples
50k genes
1M matrix
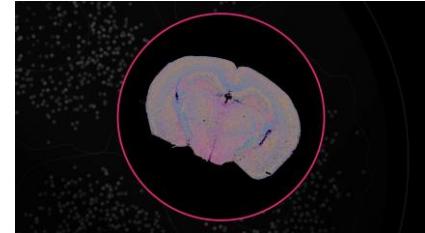
Mid 2010's: Single-cell

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

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

2020's : Spatial
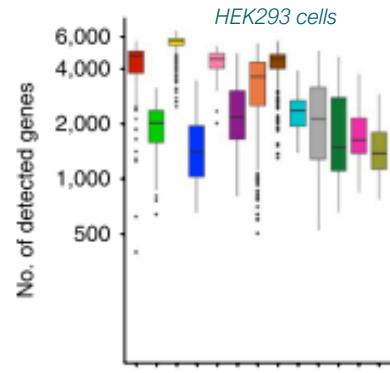
- 500-1000 gene targets
- Imaging analysis
- Multiplexing FiSH (single molecule)
- Sensitivity (30-80%)
- Sub-cellular resolution
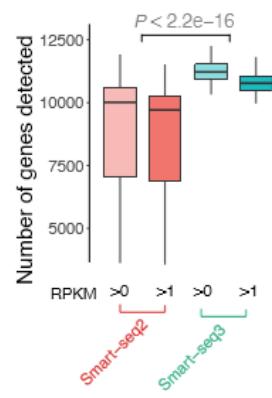
Cost : 4k€
250k cells
1k genes
250M matrix
+ Spatial dimension

Increase access to in-depth cell transcriptome
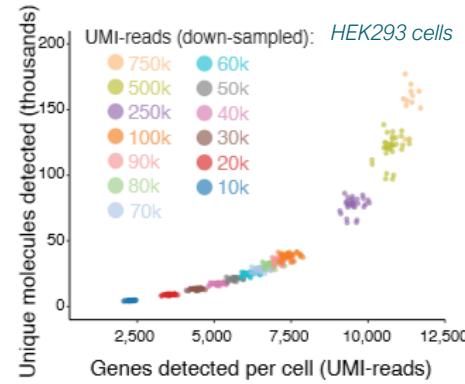
Single cell capture efficiency

HEK293 cells

CEL-seq2
MARS-seq
Quartz-seq2
gmcSCRB-seq
Smart-seq2
C1HT-small
C1HT-medium
Chromium
Chromium (sn)
ddSEQ
Drop-seq
ICELL8
inDrop

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

$P < 2.2e{-}16$

HEK293 cells

UMI-reads (down-sampled):
750k   60k
500k   50k
250k   40k
100k   30k
90k   20k
80k   10k
70k

Single-cell RNA counting at allele- and isoform-resolution using
Smart-seq3
Hagemann-Jensen et al., Nature Biotech, 2020

UMIs detected in HEK293 cells
Droplets 10x: 30k
Plate-based : 60k
Smart-seq3: 150k
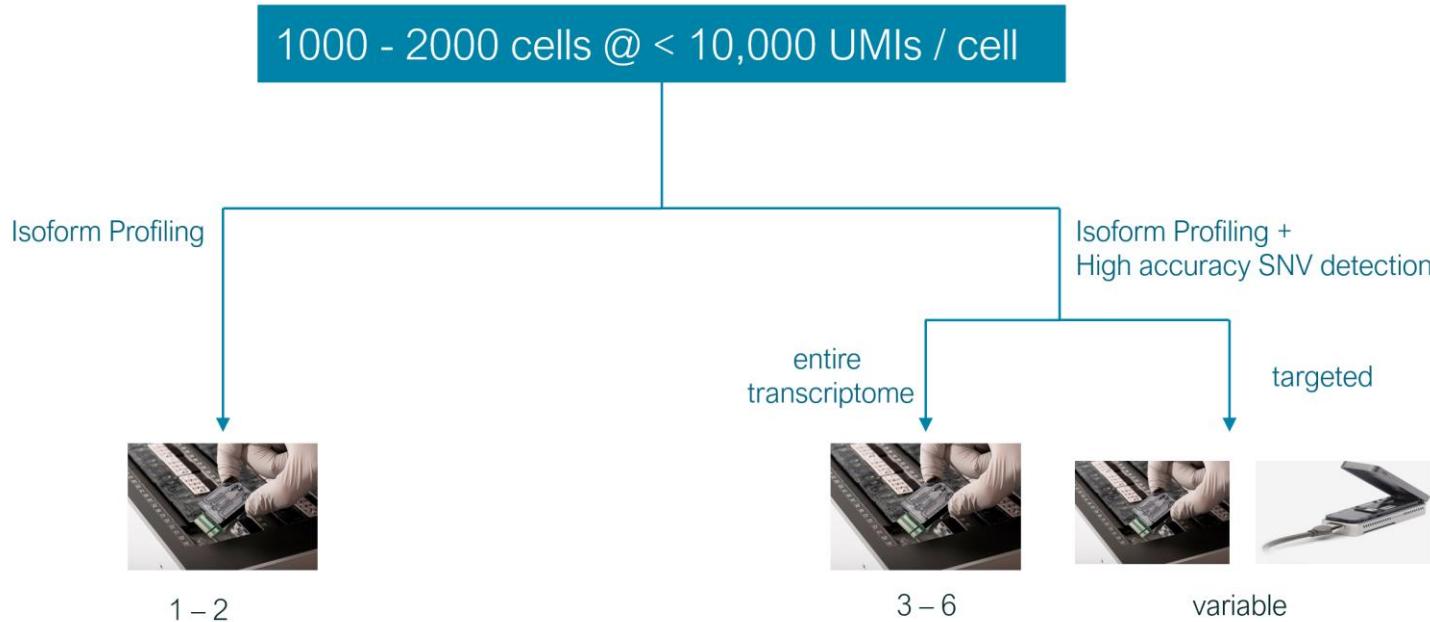
Mantis Microdispenser (Formulatrix)

How many long reads do we need ?

- Depends on number of cells and mRNA content of cells (complexity)

1000 - 2000 cells @ < 10,000 UMIs / cell

Isoform Profiling

Isoform Profiling +
High accuracy SNV detection

entire
transcriptome

targeted

1 – 2

3 – 6

variable

Full-length Transcriptomics at single cell and spatial resolution
Adding layers of information

Access alternative splicing events

- Huge better characterization of cell transcriptome,

- A potentially crucial layer of understanding for cell type classification,

- Perturb-seq on transcription factor and splicing factor to gain insight into this complex mechanisms

Access full-length sequence heterogeneity

- A-to-I RNA editing mechanism in-depth description,

- Abnormal cell behavior: cancer cell, access to fusion transcript, access to SNVs to better characterized dysregulated program,

- Clonality tumor exploration, drug resistance experiments,

- Allele-Specific Expression (ASE), imprinting genes landscape exploration (Slide-seq v2, 10μm)
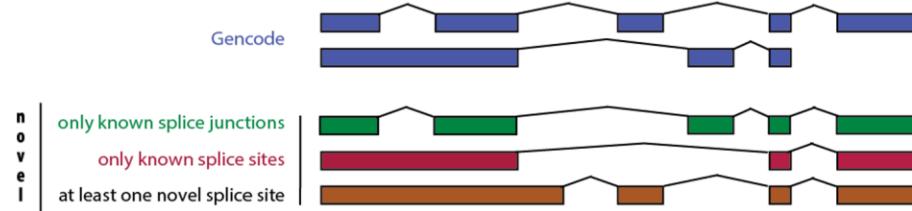
Allele-Specific Transcript Structure (ASTS) to study the effects of genetic variants on the transcriptome (sQTL). NCM2019, Dafni Glinos, Lappalainen lab , NYGC

➡ now @single cell and spatial resolution

Novel isoforms detection
E18 mouse brain single cells

Gencode

novel
- only known splice junctions
- only known splice sites
- at least one novel splice site
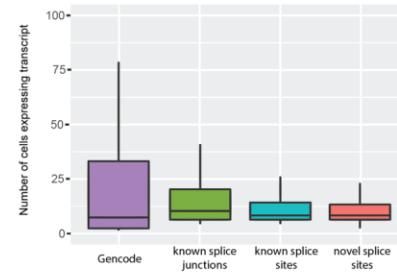
	Total	All splice junction in Illumina data	CAGE peak	polyA site	Final	% of tot
Known Gencode	33,002	33,002	20,533	14,908	11,186	34%
Novel	10,681	8,134	9,164	6,858	4,388	41%
Only known junctions	3,063	3,063	2,644	1,939	1,696	55%
Only known splice sites	2,111	1,905	1,906	1,366	1,115	53%
At least one novel splice site	5,507	3,166	4,614	3,553	1,577	29%

Novel isoforms are expressed at low level

3795 Gencode UMIs/cell
60 novel isoform UMIs/cell

Novel isoforms are expressed in fewer cells
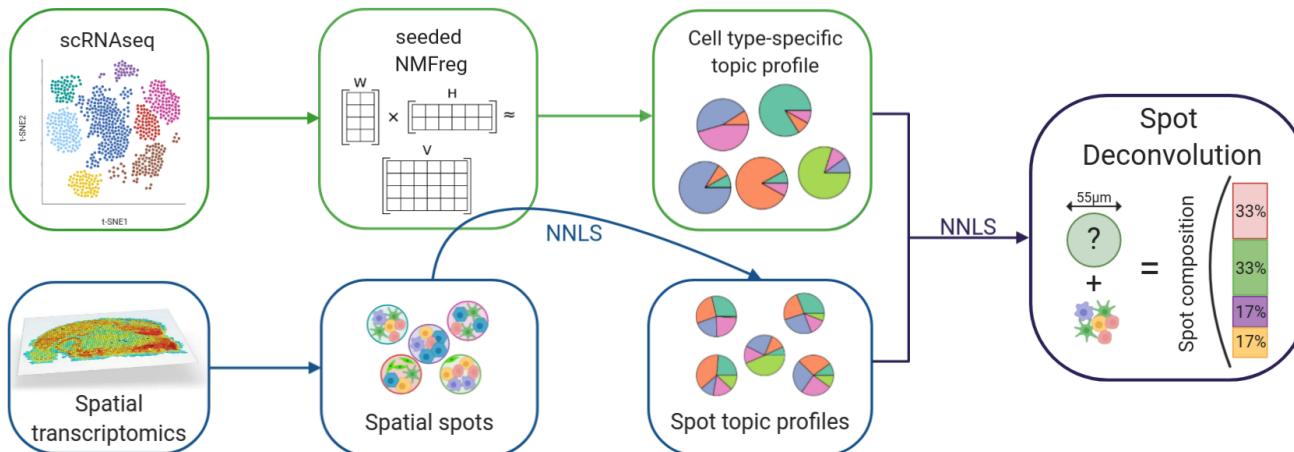
Leakiness of the splicing machinery or physiologically relevant ?

Spatial spot deconvolution
SPOTlight
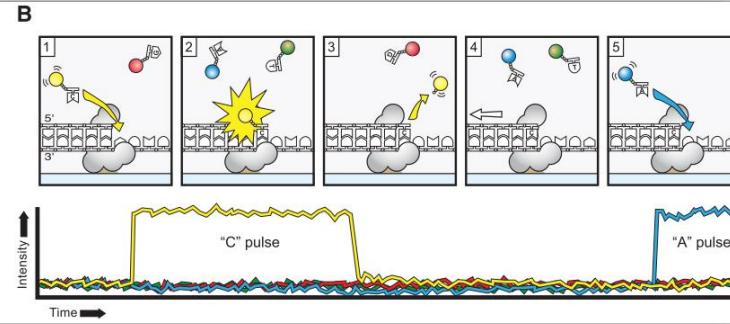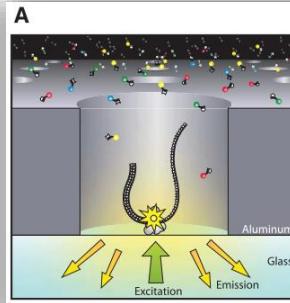
- Enables the deconvolution of cell types and cell type proportions present within each capture locations comprising mixtures of cells.

- Is based on finding topic profile signatures to identify the cell type proportions combination fits best the spot to deconvolute.

- Is centered around a seeded non-negative matrix factorization (NMF) regression, initialized using cell-type marker genes, and non-negative least squares (NNLS) to subsequently deconvolute ST capture locations (spots).

Pacific Biosciences
Single Molecule Real Time (SMRT) sequencing

(A) DNA template-DNA polymerase complex immobilized at the bottom of each optical nanostructures Zero Mode Waveguide (ZMW) illuminated by laser light from the bottom.
(B) Schematic representation of the phospholinked dNTP incorporation cycle and time trace of detected fluorescence intensity from the ZMW.

SMRTBell

circular consensus sequencing (CCS)
> 99,9% accuracy (Q30, 8 passes)
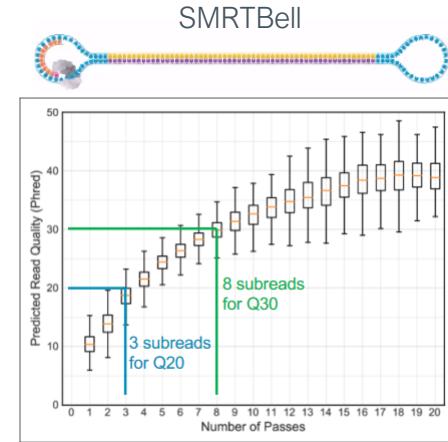
PacBio RS II (Apr.2013)
- $750,000
- 150,000 ZMWs

Sequel I (Oct.2015)
- $350,000
- 1,000,000 ZMWs
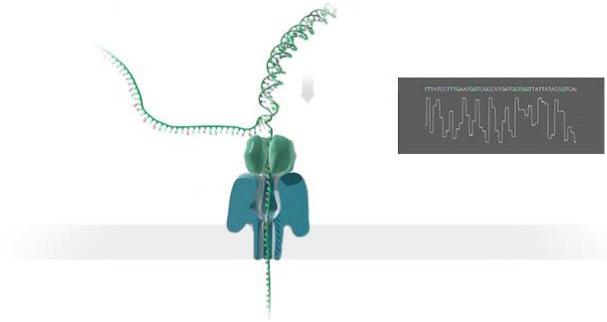
Sequel II (Apr.2019)
- $495,000
- 8,000,000 ZMWs

Oxford Nanopore Technology
Sequencing through Nanopore

A strand of DNA is passed through a Nanopore.
The current is changed as the bases G, A, T or C
pass through the pore in different combinations.

Nanopore R9.4.1 raw read accuracy
improves from **90% to 98%** in the last years
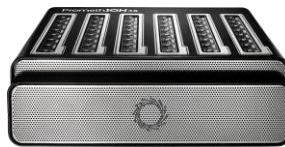
MinION
- $1,000
- 512 channels
- Up to 20 Gb / flow cell
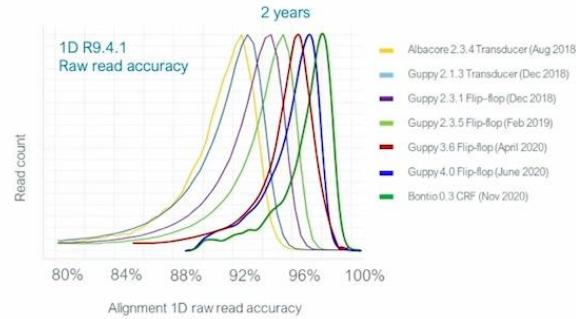
GridION
- $50,000
- 5 x 512 channels
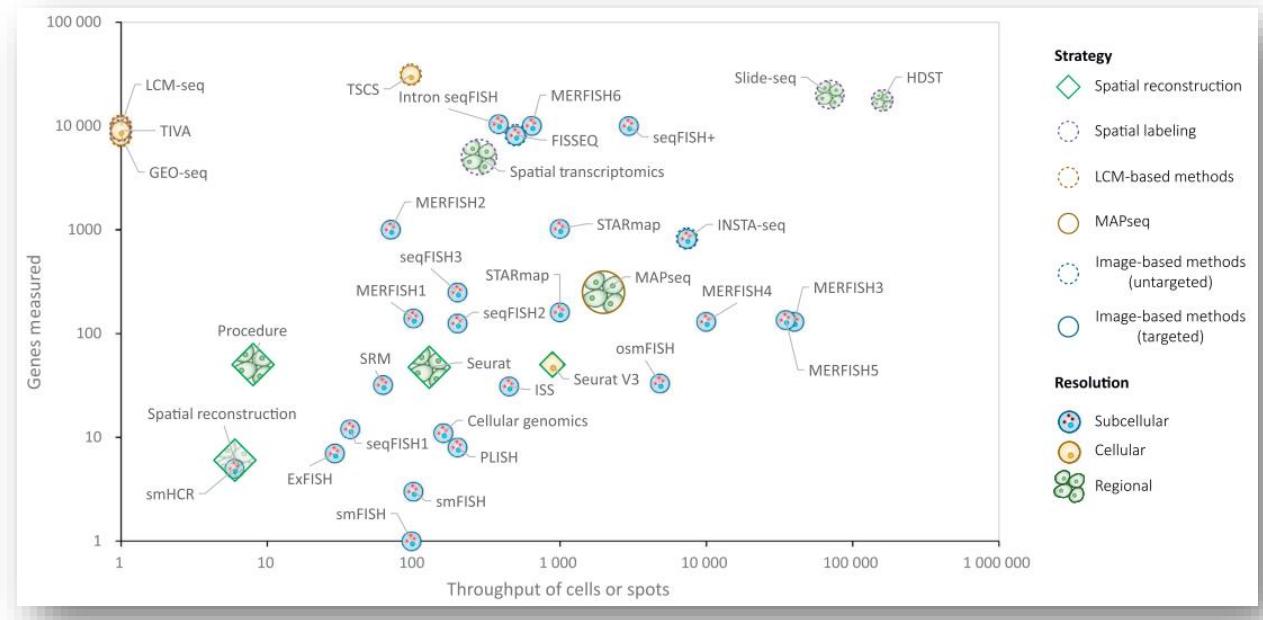
PromethION 24
- $195,000 (120 flow cells included)
- 24 x 3,000 channels
- Up to 120 Gb / flow cell
- 80.000.000 cDNA reads
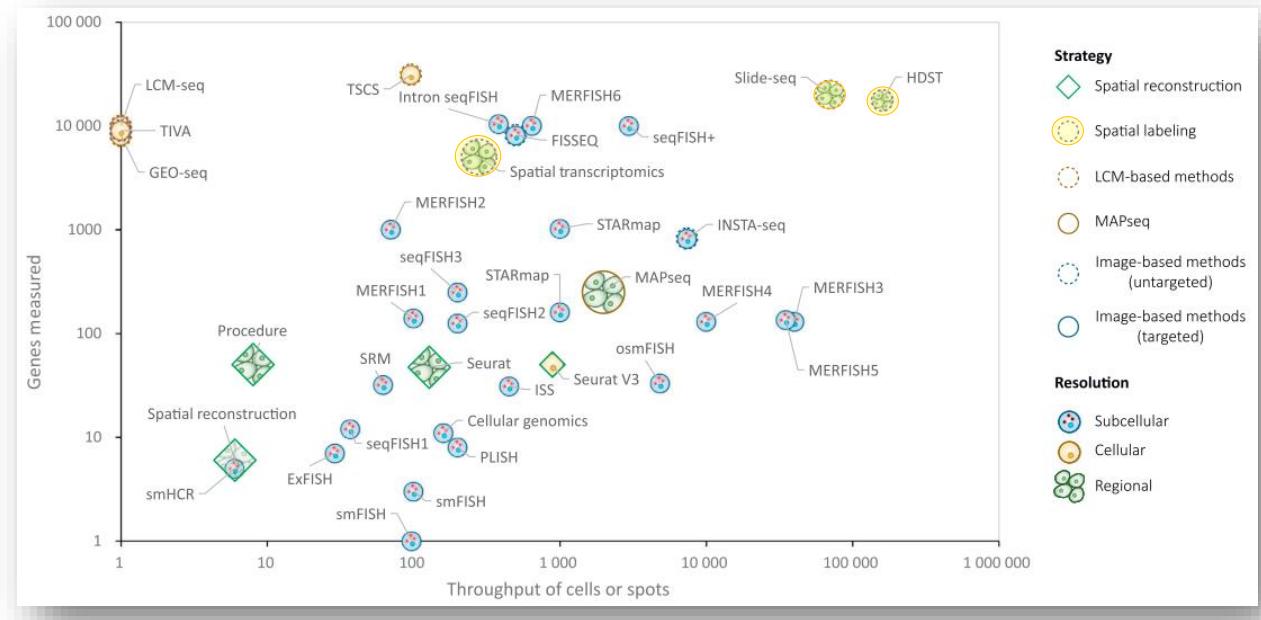
Spatial Transcriptomics approaches
Throughput of Genes and Cells

Uncovering an Organ's Molecular Architecture at Single-Cell Resolution by Spatially Resolved Transcriptomics
Liao et al., 2020
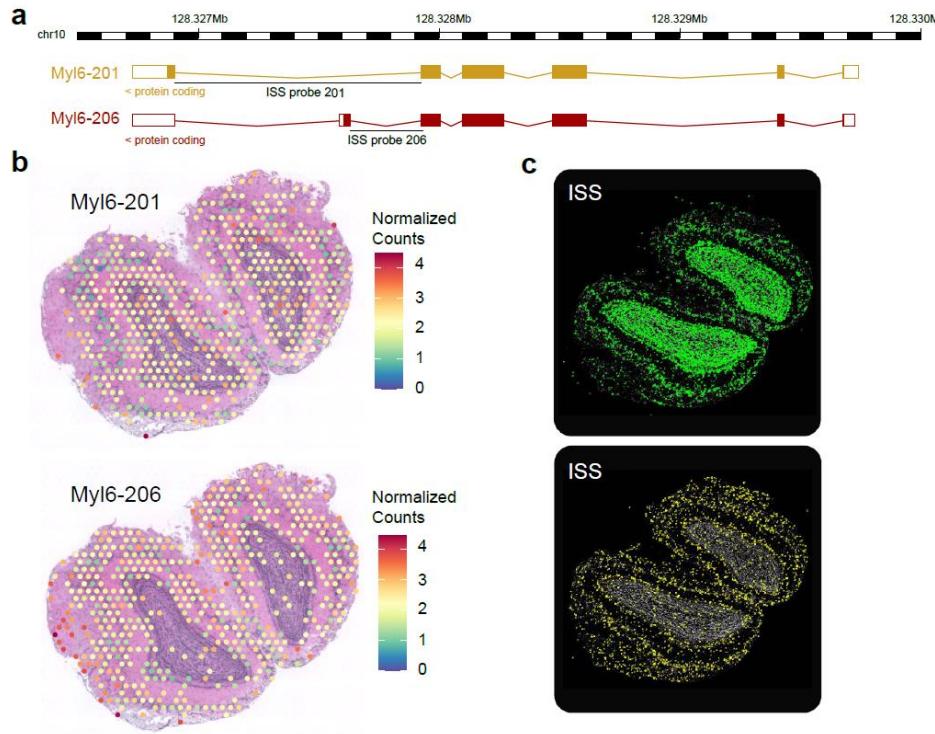
Uncovering an Organ's Molecular Architecture at Single-Cell Resolution by Spatially Resolved Transcriptomics
Liao et al., 2020

SiT reveals specific splicing pattern across regions (OB)
Myl6 Differential Transcript Usage (DTU) across regions (MOB)

Myosin Light Chain 6 (Myl6), codes for the non-phosphorylatable alkali light chain component of the hexameric Myosin motor protein, that has been shown to be involved in neuronal migration and synaptic remodeling in immature and mature neurons

Increase access to in-depth cell transcriptome
Spatial resolution

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 (2020) | Cite this article

4105 Accesses | 180 Altmetric | Metrics

- Slide-seqV2 improve library generation, bead synthesis and array indexing to reach an RNA capture efficiency ~50% that of single-cell RNA-seq data (~10-fold greater than Slide-seq), approaching the detection efficiency of droplet-based single-cell RNA-seq techniques.

- In the mouse hippocampus, the capture efficiency of Slide-seqV2 was higher than that of a recently released commercial spatial transcriptomics (ST) technology (**mean UMIs, Slide-seqV2 = 45,772 and 10x Genomics Visium = 27,952** for equal feature size (110 μm diameter binned area)
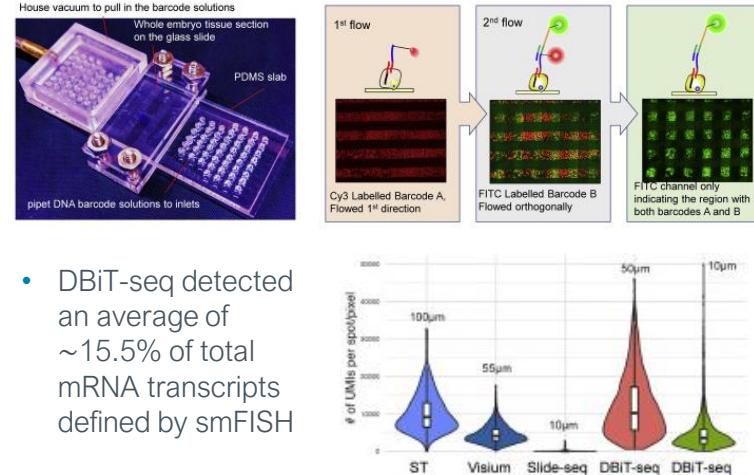

RESOURCE | VOLUME 183, ISSUE 6, P1665-1681.E18, DECEMBER 10, 2020

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

Yang Liu [5] • Mingyu Yang [5] • Yanxiang Deng [5] • ... Yang Xiao • Stephanie Halene • Rong Fan [6] ✉

Show all authors • Show footnotes

Published: November 13, 2020 • DOI: https://doi.org/10.1016/j.cell.2020.10.026
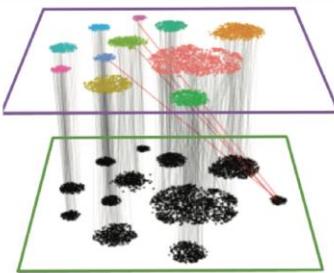
- DBiT-seq detected an average of ~15.5% of total mRNA transcripts defined by smFISH
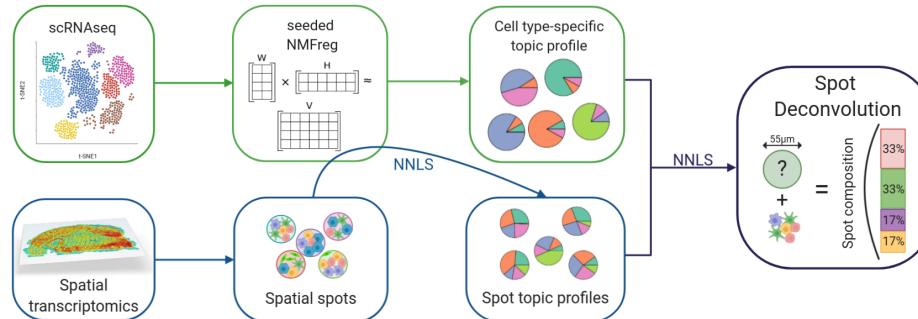
Spatial spot deconvolution / integration using scRNA-seq

Seurat integration approach
We consistently found superior performance using integration methods (as opposed to deconvolution methods), likely because of substantially different noise models that characterize spatial and single-cell datasets, and integration methods are specifically designed to be robust to these differences. We therefore apply the 'anchor'-based integration workflow that enables the probabilistic transfer of annotations from a reference to a query set.

SPOTlight enables the deconvolution of cell types and cell type proportions present within each capture locations comprising mixtures of cells. **SPOTlight** is based on finding topic profile signaturesto identify the cell type proportions combination fits best the spot to deconvolute. SPOTlight is centered around a seeded non-negative matrix factorization (NMF) regression, initialized using cell-type marker genes, and non-negative least squares (NNLS) to subsequently deconvolute ST capture locations (spots).

Tangram aligns snRNA-seq data to various spatial data collected from the same brain region, (MERFISH, STARmap, smFISH, and Visium), as well as histological images and public atlases. Tangram can map any type of sc/snRNA-seq data, including multi-modal data such as SHARE-seq data, which we used to reveal spatial patterns of chromatin accessibility. We equipped Tangram with a deep learning computer vision pipeline, which allows for automatic identification of anatomical annotations on histological images of mouse brain.