Mahidol University
Wisdom of the Land

Single-cell and Spatial Omics in Human Health and Diseases

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Department of Microbiology, Faculty of Science

Integrative Computational BioScience (ICBS) Center

SCBM 373 Bioinformatics

10/04/2026

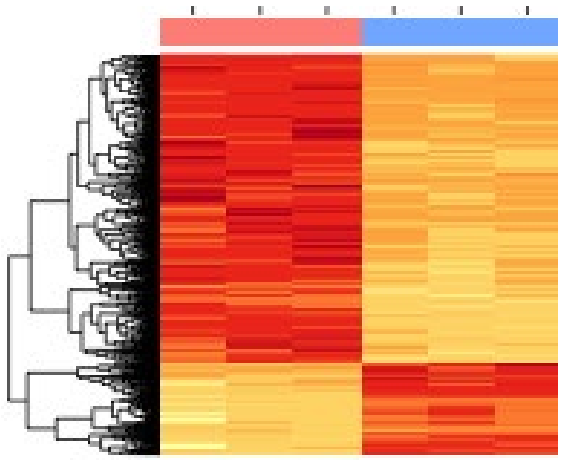


Take-home messages from the previous lecture

- Cellular phenotype can be reflected by gene expression i.e. mRNA expression
- Transcriptomics – comprehensive info on gene expression
- **Transcriptomics ~ RNA-seq**
- From RNA-seq, you get **reads** from a sequencer (most likely Illumina) you would then need to **align/map** them to **reference** sequences
- **Differential gene expression (DGE)** tells you how different gene expression is in 2 different sample groups
- Functional **gene ontology (GO)** or **gene set enrichment analysis (GSEA)** tells you what DE genes might represent pathway-wise
- Special considerations needed for bacterial cells or viral RNA

Differential gene expression in diseased vs healthy conditions

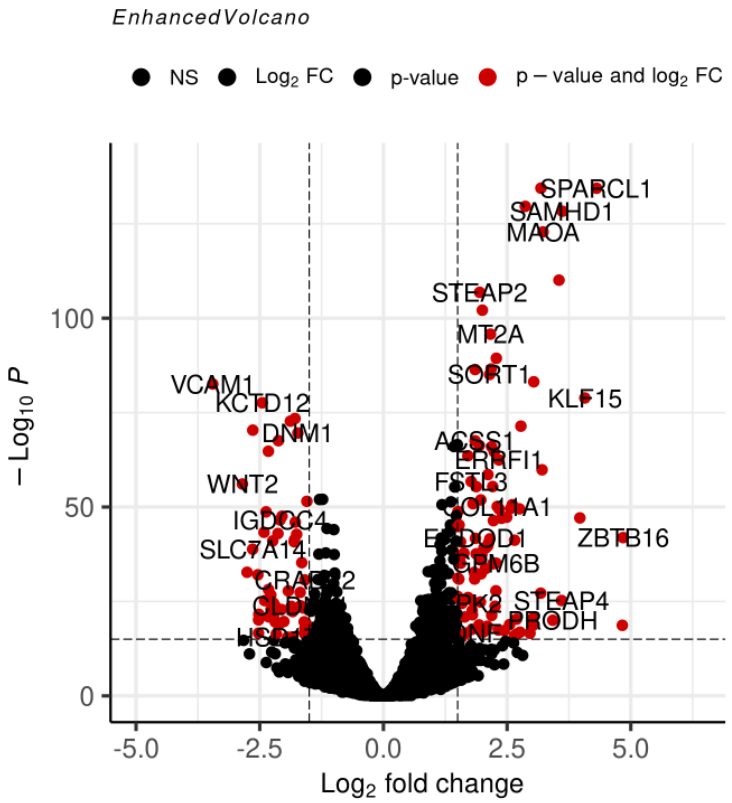
Heatmap



Diseased Healthy

Volcano plot

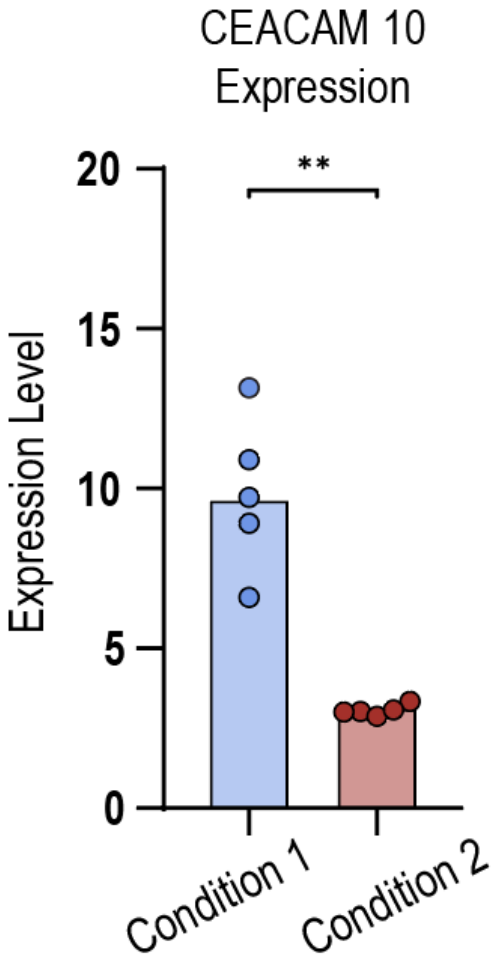
Diseased vs. healthy

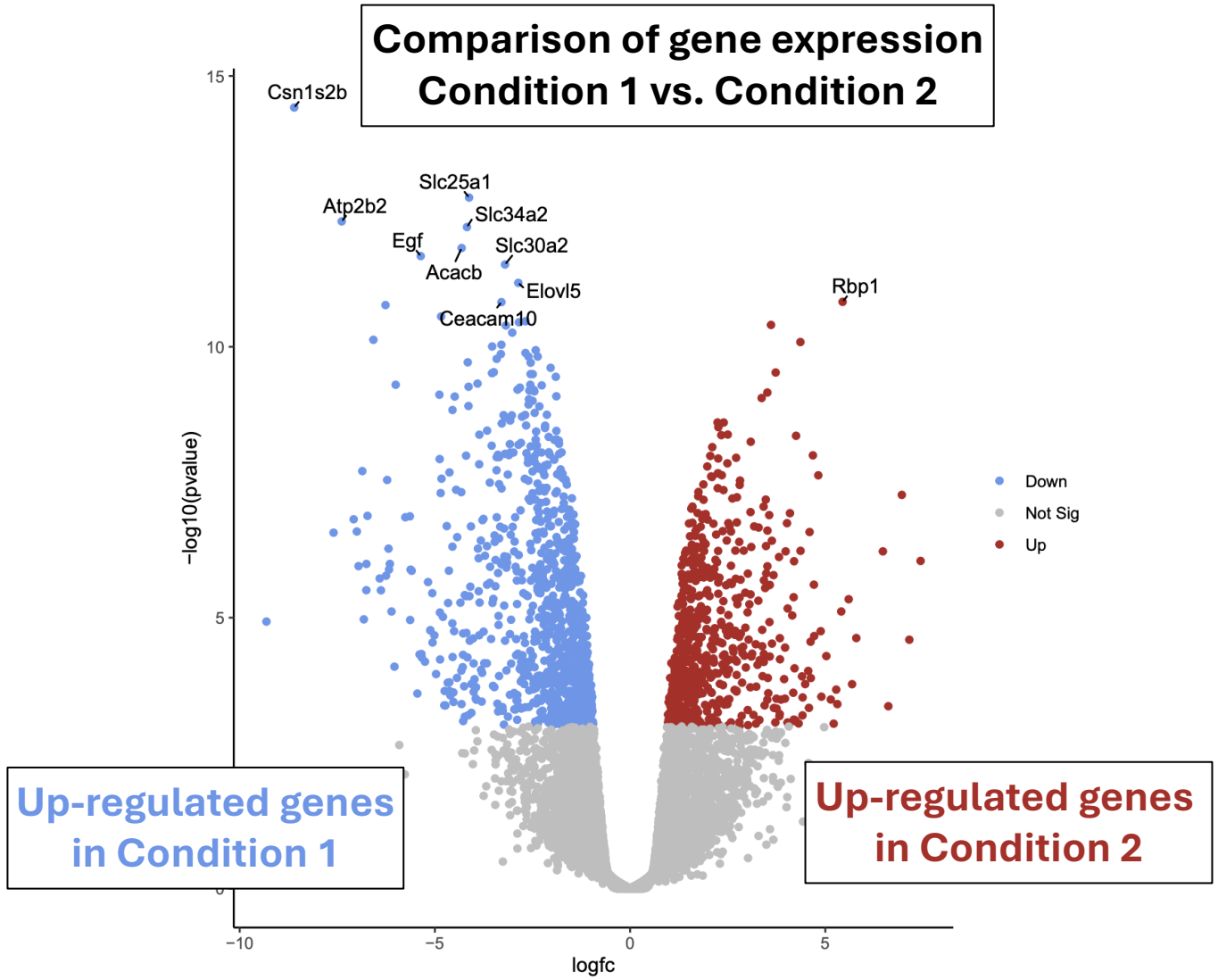


What could transcriptomics do?

- Systemic phenotyping: providing an overview of information on phenotypic changes at the mRNA level in experimental settings (e.g. healthy vs. diseased states)

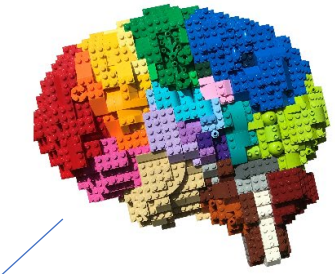
Single-gene level vs. transcriptomic level detection





So far, transcriptomics was done at the tissue/organ samples

organ/tissue



Tissue dissection/processing



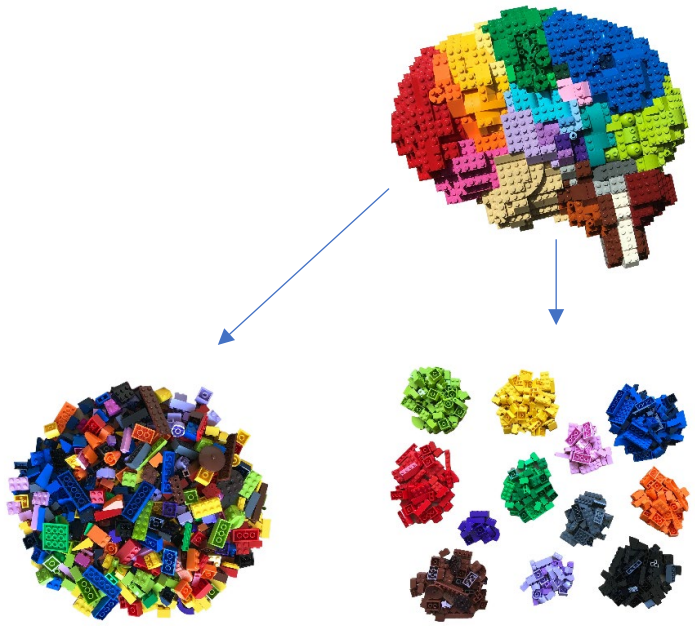
RNA-seq

What could be problematic about this way of investigation?

What if we look at all of the genes expressed at once and use “transcriptomes” to identify individual cells and investigate their changes in diseased states?

Transcriptomics at single-cell level

organ/tissue

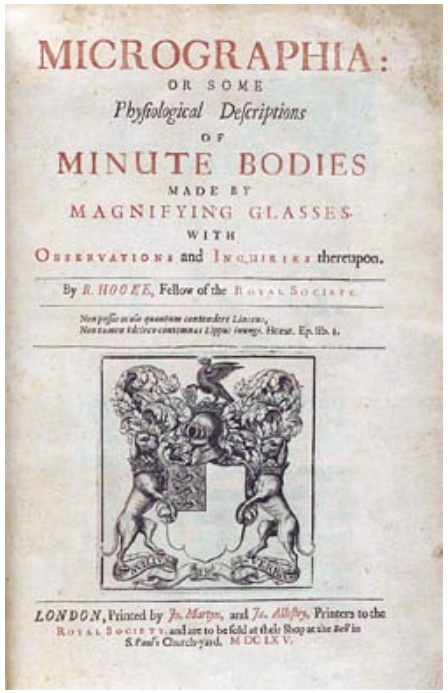


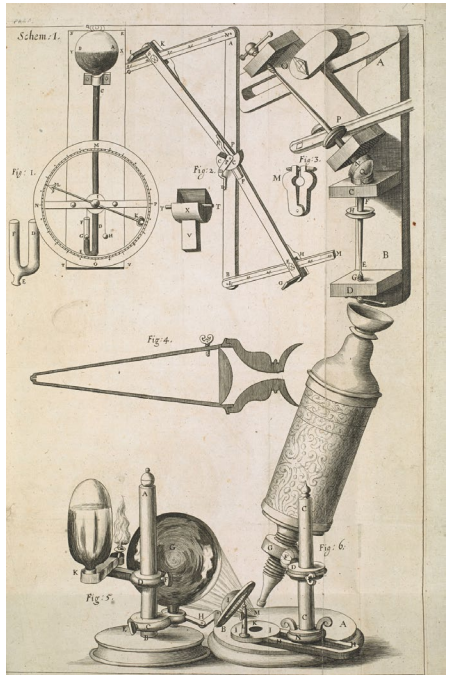
Mixed population

Individual cells

Description of “cells” dated back to 1665




MICROGRAPHIA:
OR SOME
Physiological Descriptions
OF
MINUTE BODIES
MADE BY
MAGNIFYING GLASSES
WITH
OBSERVATIONS and INQUIRIES thereupon.
By R. HOOKE, Fellow of the ROYAL SOCIETY.
LONDON, Printed by Jo. Martyn, and Ja. Allestry, Printers to the ROYAL SOCIETY, and are to be sold at their Shop at the Bell in S. Paul's Church-yard. MDCLXV.





Robert Hooke’s microscope (Photo Credit : public domain/Wikimedia Commons)

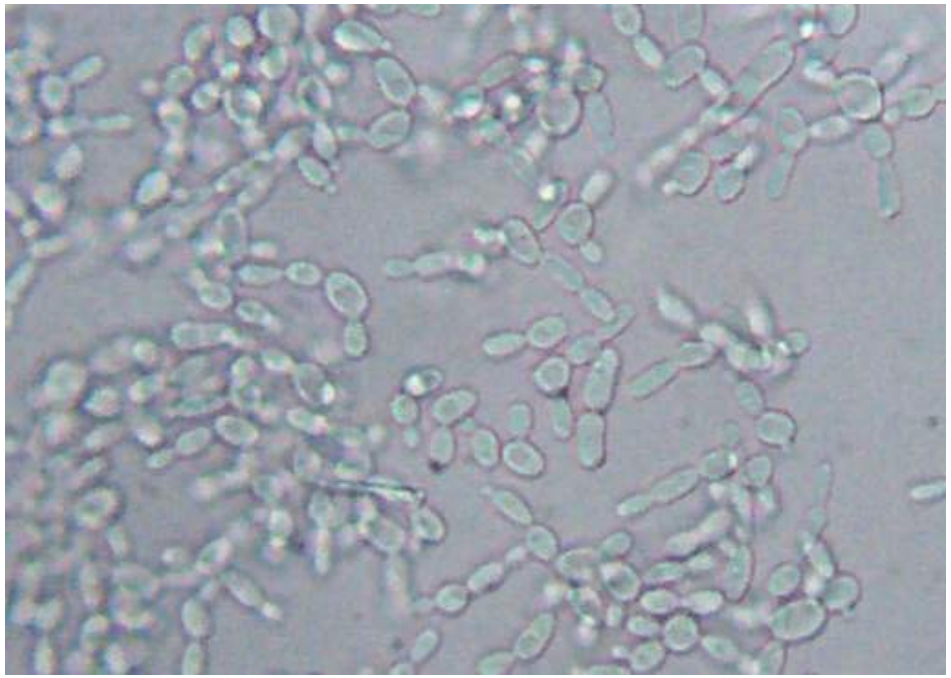
Micrographia: or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses. With Observations and Inquiries Thereupon.



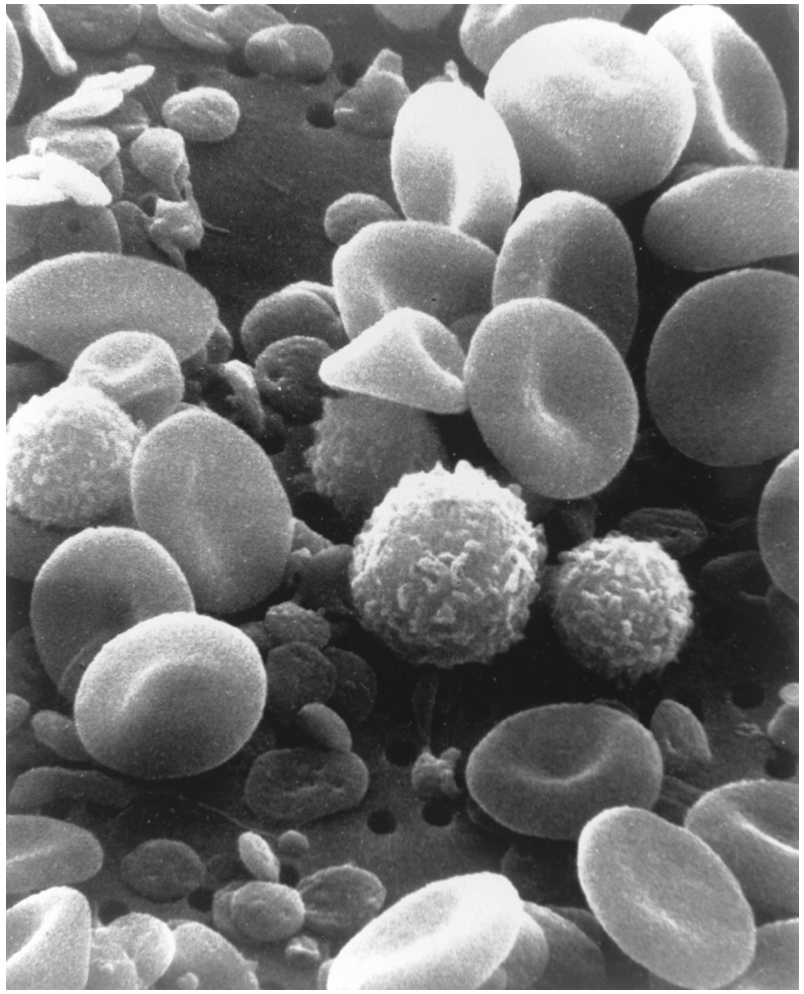
“I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular... these pores, or ***cells****,...were indeed the first microscopical pores I ever saw, and perhaps, that were ever seen, for I had not met with any Writer or Person, that had made any mention of them before this...”*

Robert Hooke, Biographia, 1665

Microscopy: a powerful tool to study cells



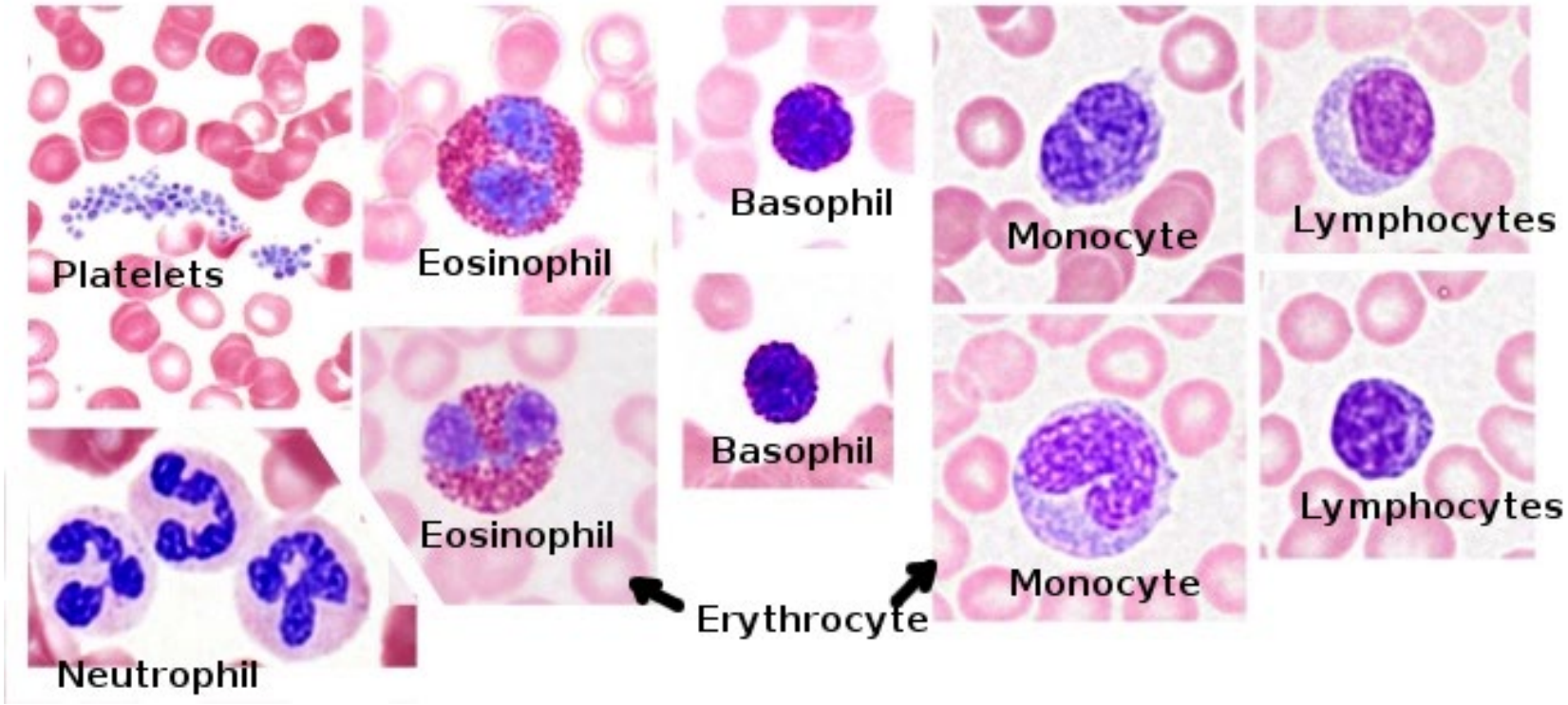
https://commons.wikimedia.org/wiki/File:Candida_wet_mount.jpg



https://visualsonline.cancer.gov/details.cfm?imageid=2129

Microscopy: a powerful tool to study cells

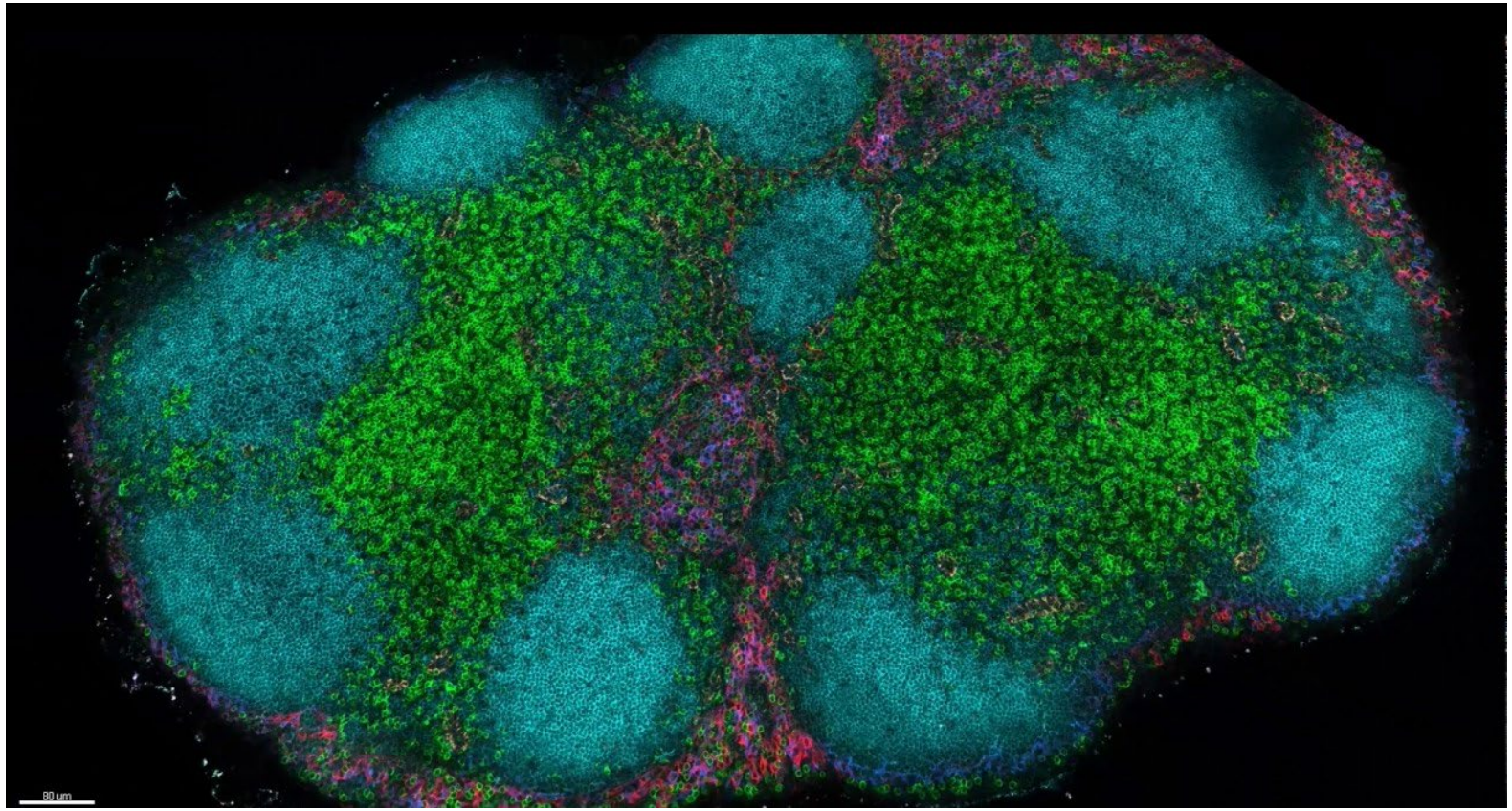
Using dyes to stain specific components/structures



Source: https://mmegias.webs.uvigo.es/02-english/guiada_a_sanguineo.php

Microscopy: a powerful tool to study cells

Immunofluorescence: using fluorescence-tagged antibodies to stain specific cell surface markers



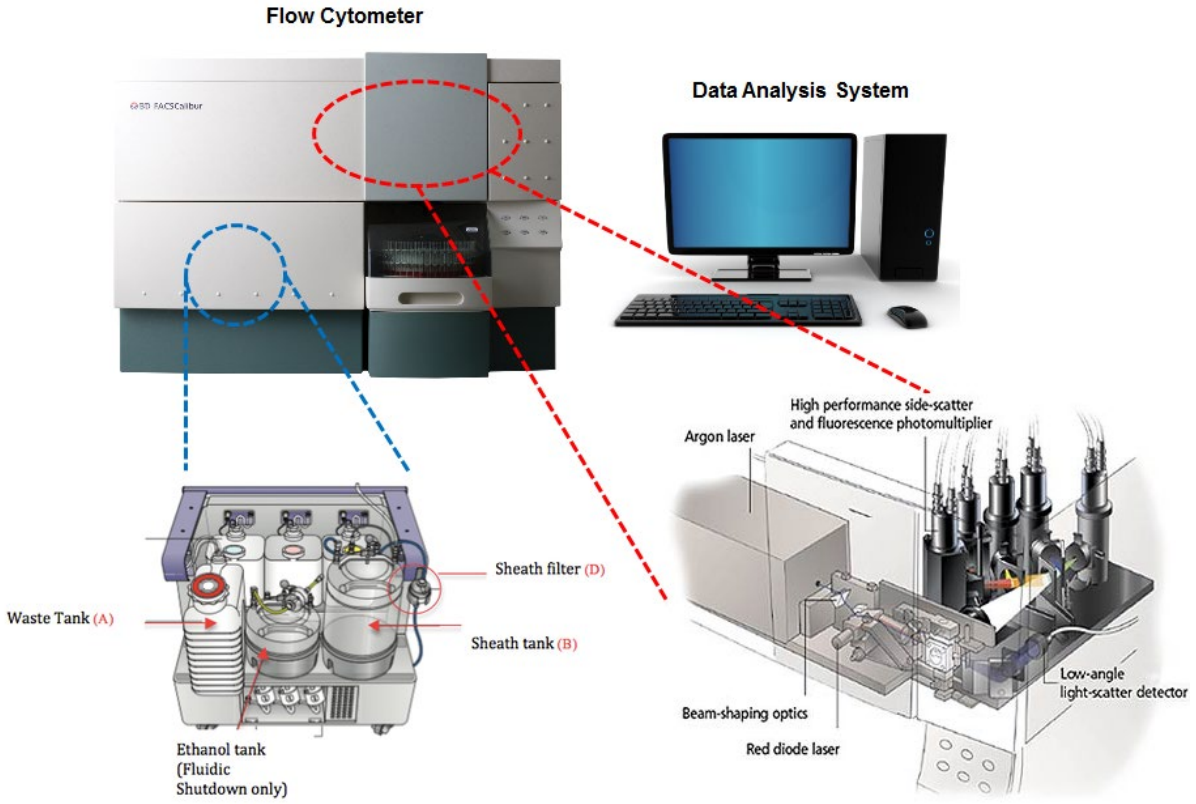
B cells

T cells

Macrophages

https://www.niaid.nih.gov/research/ronald-n-germain-md-phd

Flow cytometry: a low-throughput single-cell protein detection that revolutionizes cell phenotyping



Fluidics

BD FACSCalibur Optical Bench

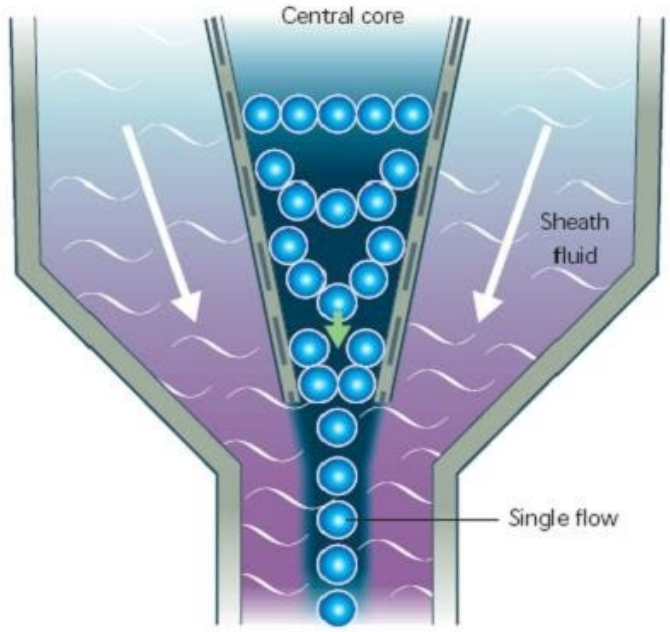
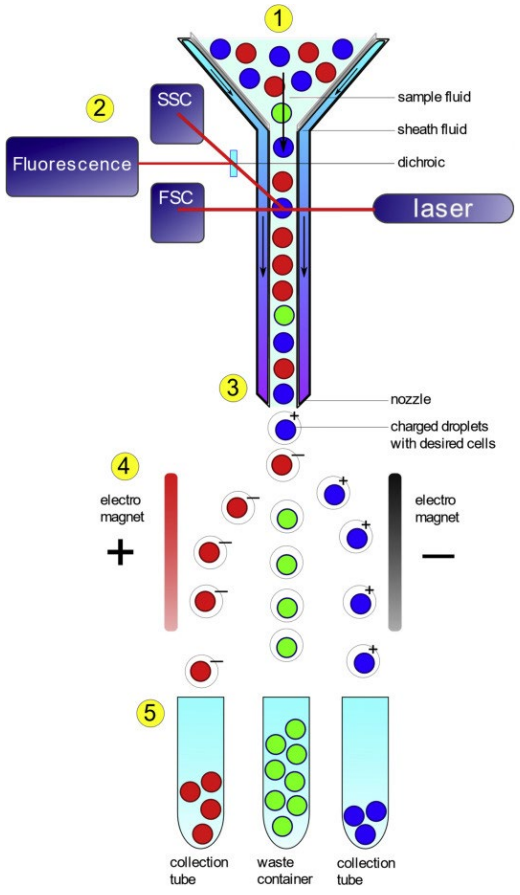
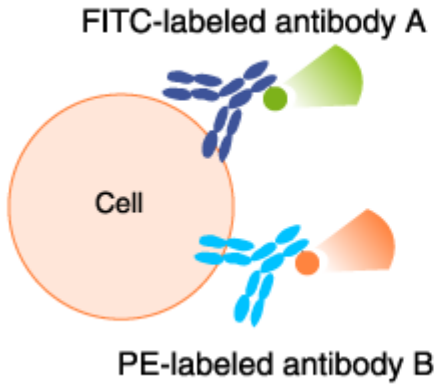
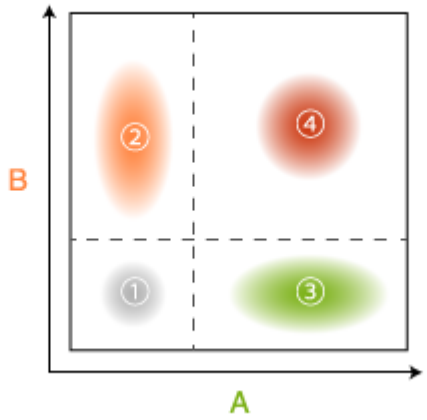


FIGURE 1 Hydrodynamic focusing produces a single stream of particles

Flow cytometry: a low-throughput single-cell protein detection that revolutionizes cell phenotyping

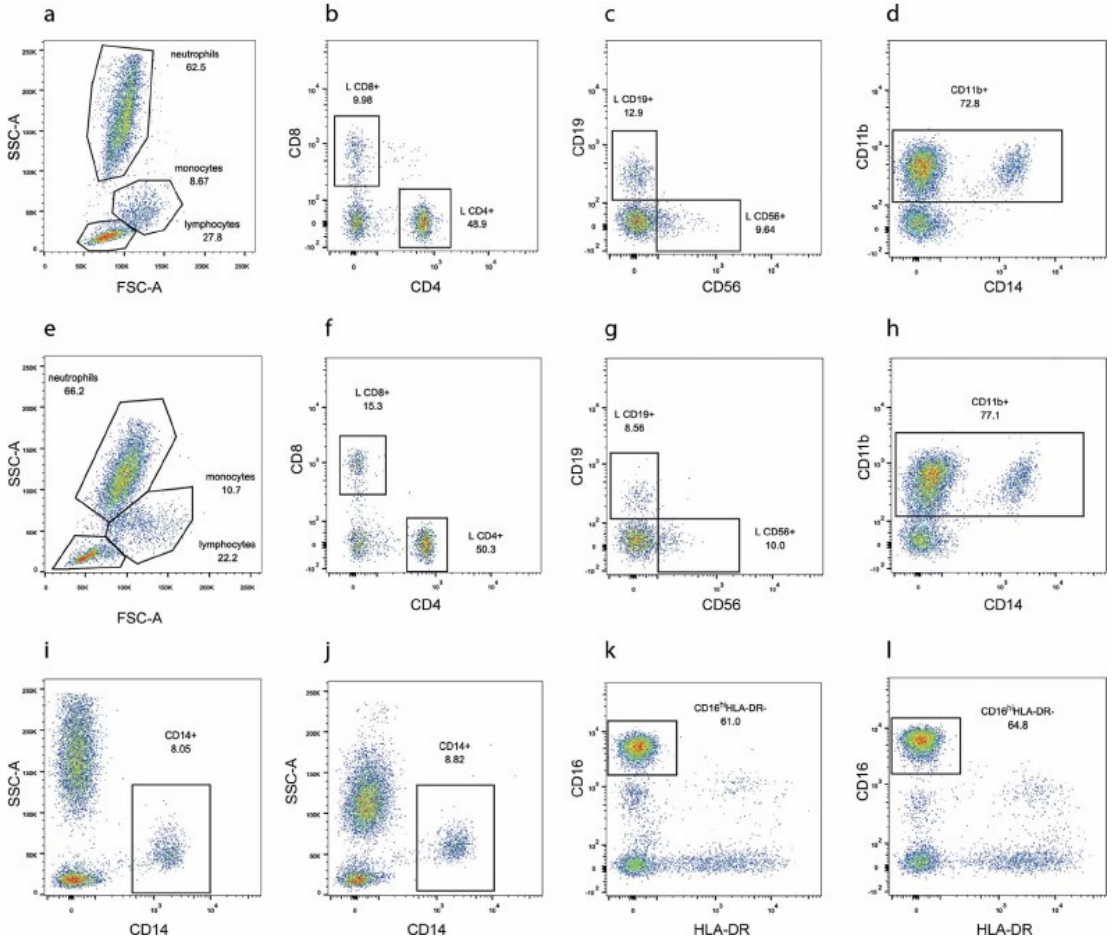






1) Cells not expressing either A or B
2) Cells expressing only B
3) Cells expressing only A
4) Cells expressing both A and B

Flow cytometry: a low-throughput single-cell protein detection that revolutionizes cell phenotyping



Typically, 8-12 markers are detected at a time. Up to 18 markers.

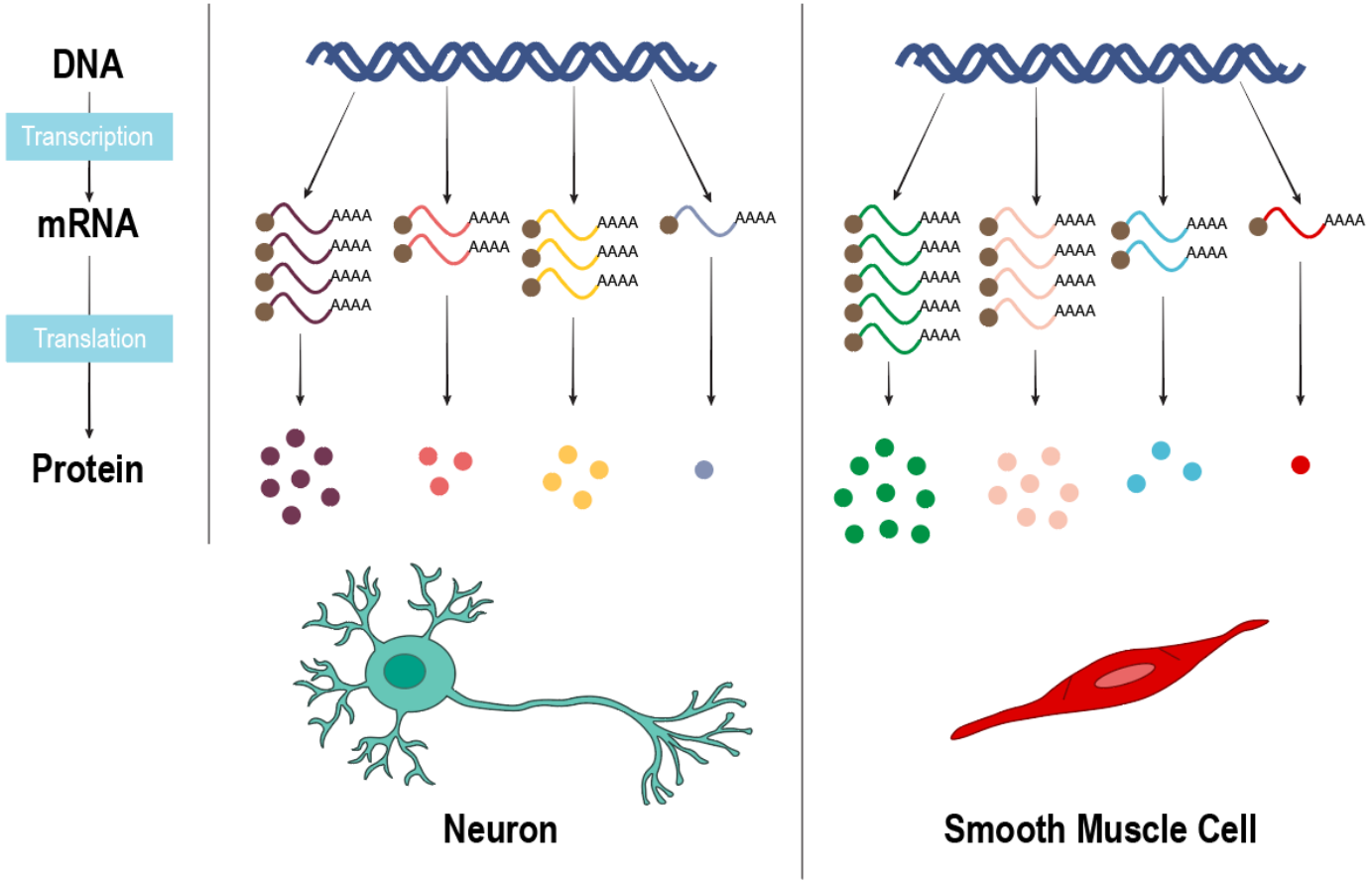
More multiplexing approaches possible thought not easily accomplished

Limitations of the prior cell type identification approaches

- **Direct microscopy**: reliance on morphology-based classification
  - What if different cells have similar shapes/structures?
- **Targeted, markers-based approaches** (e.g. immunoflourescence, flow cytometry)
  - Which markers to use? Is the cell type/state identification biased by the marker selection?
  - Limited number of markers to be used in a single experiment
    - Typically, 3-4 for IF; 8-12 for flow cytometry. If more, complicated experimental optimizations are required

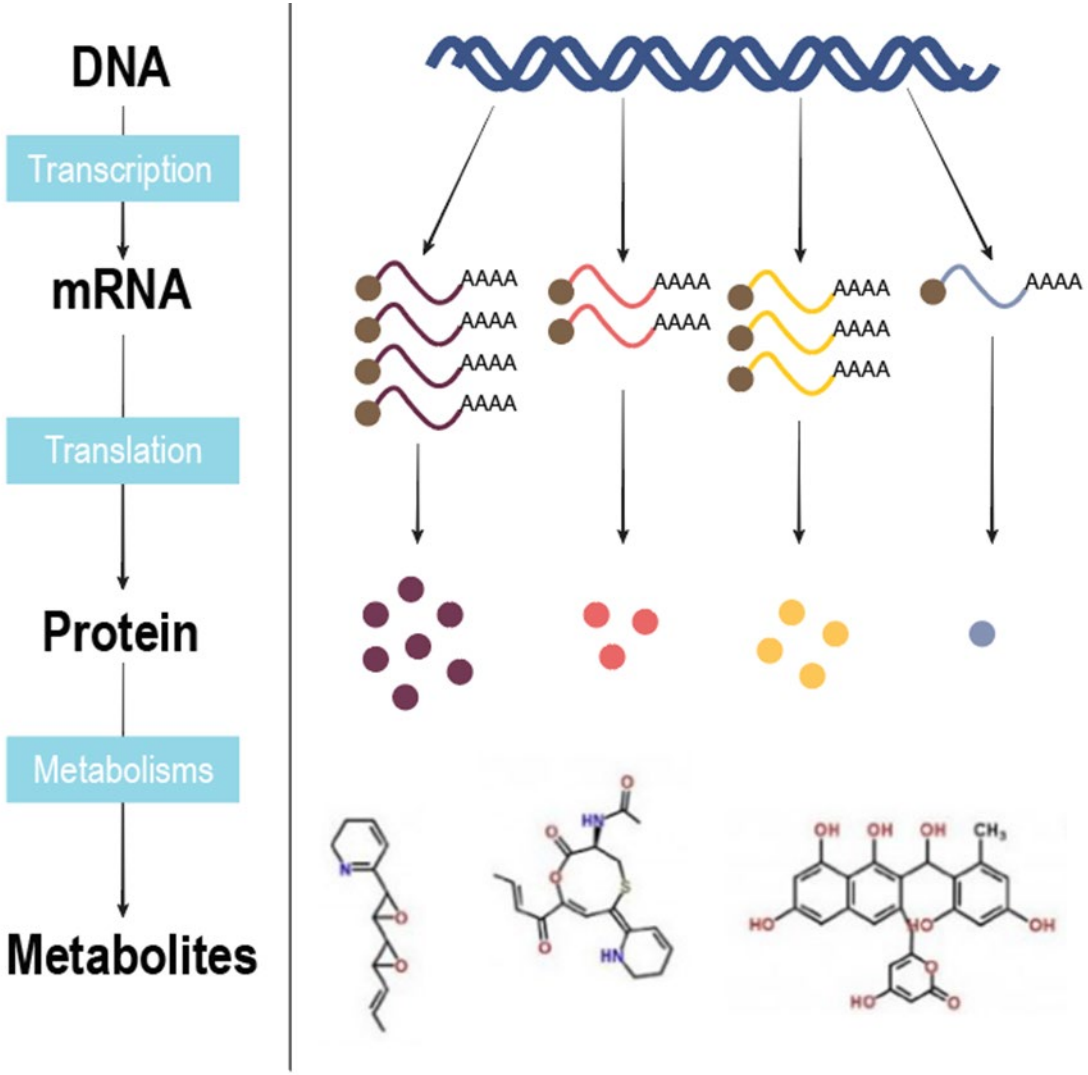
What if we look at all of the genes expressed at once and use “transcriptomes” (or “proteomes”) to identify cells?

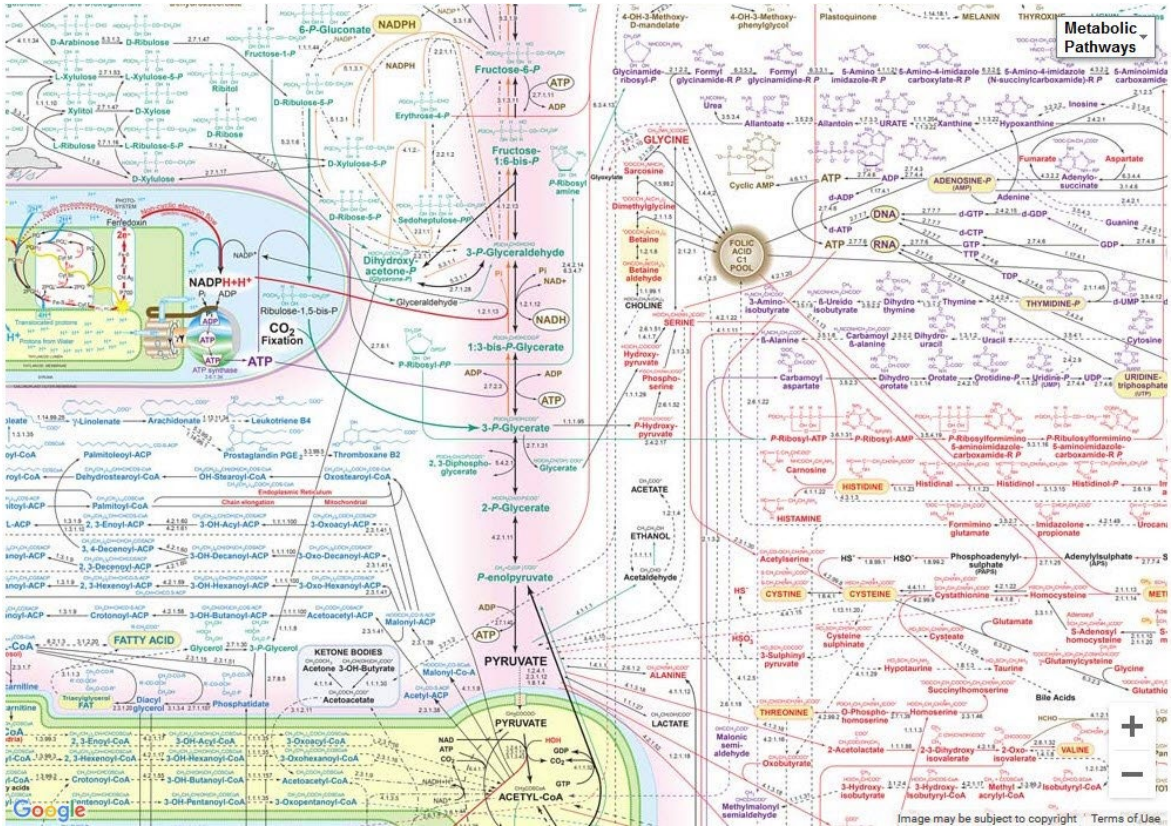
Transcriptomes/proteomes dictate cell identities and functions



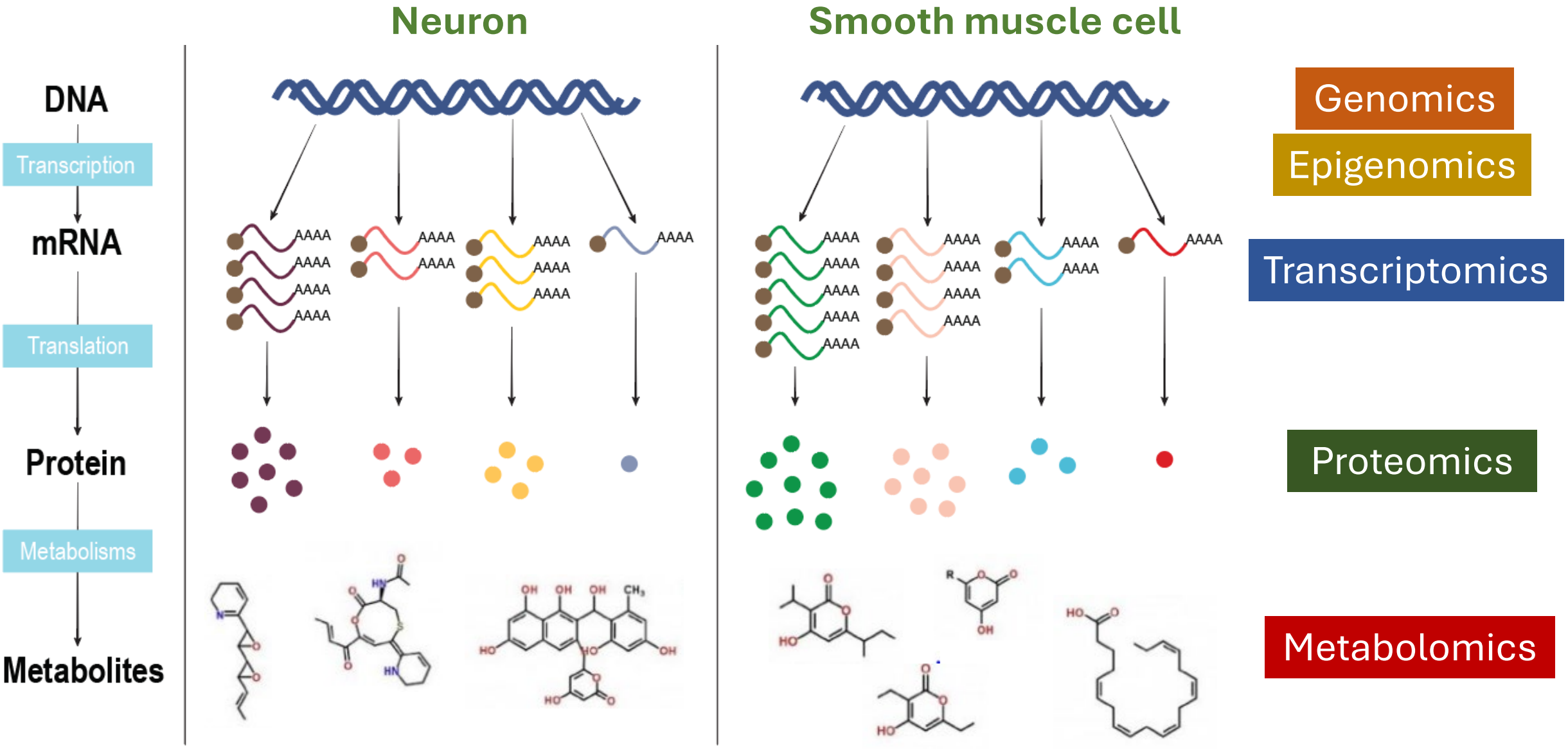
Created by Waradon Sungnak

The central dogma+





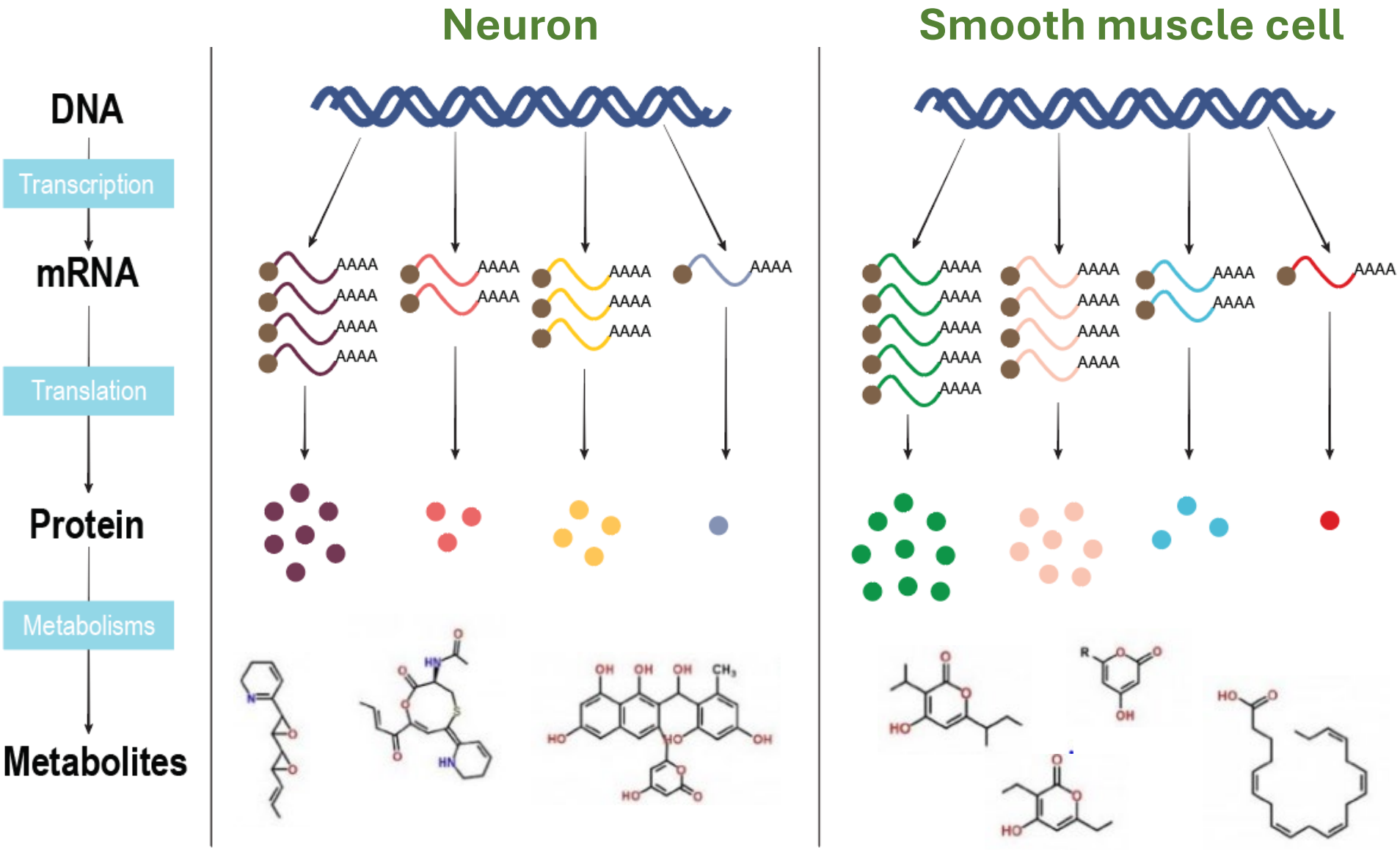
Comprehensive studies of the central dogma at different levels



Why transcriptomics?

- Scalability and sensitivity of NGS compared to mass spectrometry
- PCR-based amplification of cDNA, enabling robust profiling from extremely low-input samples

Comprehensive studies of the central dogma at different levels



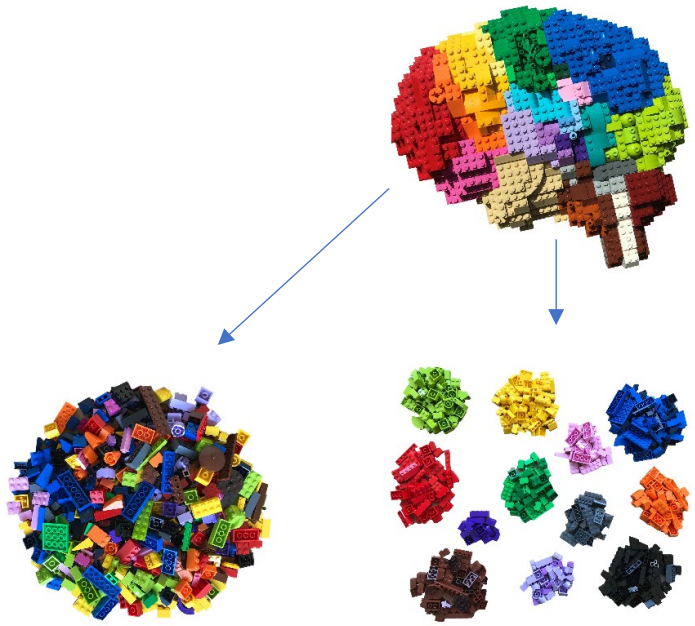
Next-generation sequencing

Mass spectrometry

What if we can check transcriptomes at a single-cell resolution?

Transcriptomics at single-cell level

organ/tissue



Mixed population

Individual cells

What could single-cell transcriptomics do?

- Cell atlas: identifying cell types/states in a given environment in a less biased manner (i.e. used transcriptomes instead of selected markers to define cell types/states)
- Systemic phenotyping in a cell type-specific manner: providing an overview of information on phenotypic changes at the mRNA level in experimental settings (e.g. cell type X in healthy vs diseased states)

Why was studying transcriptomes in single cells really difficult in the past?

Working with RNA is technically demanding!

- Chemical stability of RNA is low
- RNases are everywhere in the environment!

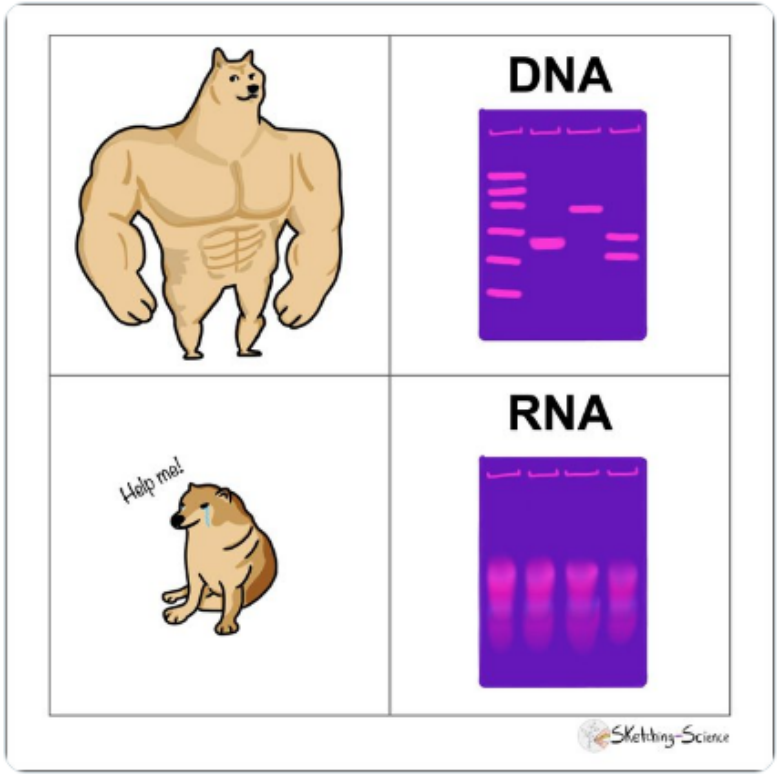


← Tweet

c0ncOrdance
@c0ncOrdance

The only solution to RNase contamination:

1. Take a giant bathtub.
2. Fill it with RNaseZAP or similar RNase removal solution.
3. Put everything in the lab in the bathtub.
4. Get in the bathtub.
5. You live & work there now.



7:25 AM · Dec 9, 2020 · Twitter Web App

How many cells do we need to run a conventional RNA-seq experiment?



Illumina Stranded mRNA Prep (as an example)

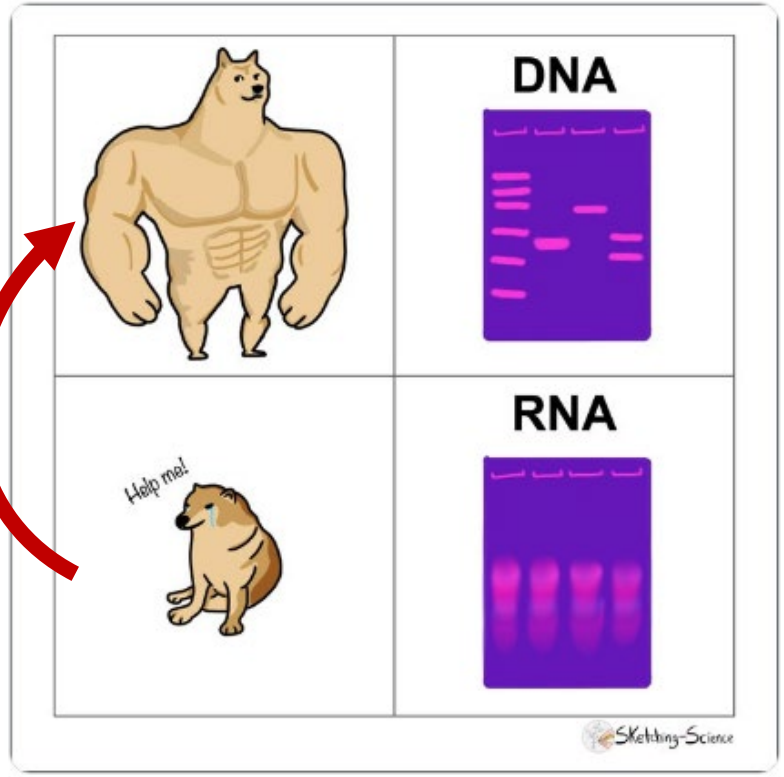
- Required input: at least **25 ng** standard-quality total RNA from a sample
- The amount of RNA per human cell is around **10–30 pg**
- You need **~2,500 cells**. Accounting for cell/RNA loss during preparation, I'd say at least **5,000 cells**
- In practice, having at least **10,000 cells** per sample is a comfortable number!

Is it possible to sequence RNA from a single cell?

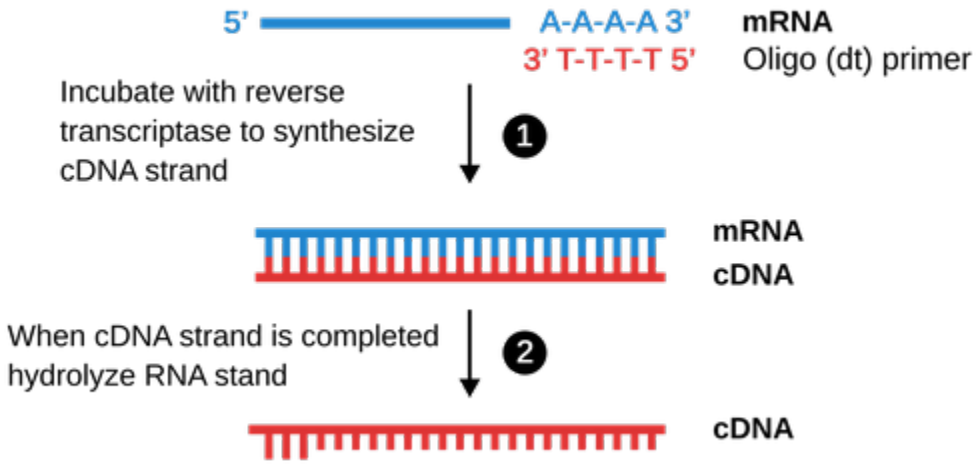
- Required input: at least **25 ng** standard-quality total RNA from a sample
- The amount of RNA per human cell is around **10–30 pg**

At least 1000x more sensitivity is needed!

Reverse transcription (RT) is a key step for most of the RNA-based studies



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Using Oligo (dt) primer targeting poly(A) tails of mRNA allows reverse transcription of all mRNA in the sample

The Nobel Prize in Physiology or Medicine 1975 | Summary

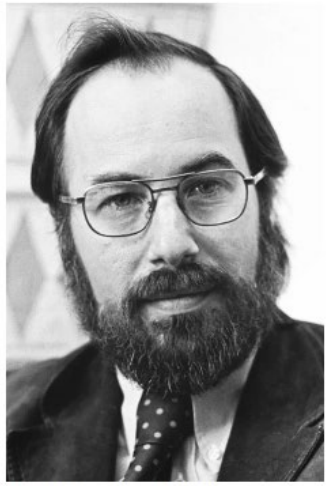


Photo from the Nobel Foundation archive.
David Baltimore
Prize share: 1/3



Photo from the Nobel Foundation archive.
Renato Dulbecco
Prize share: 1/3



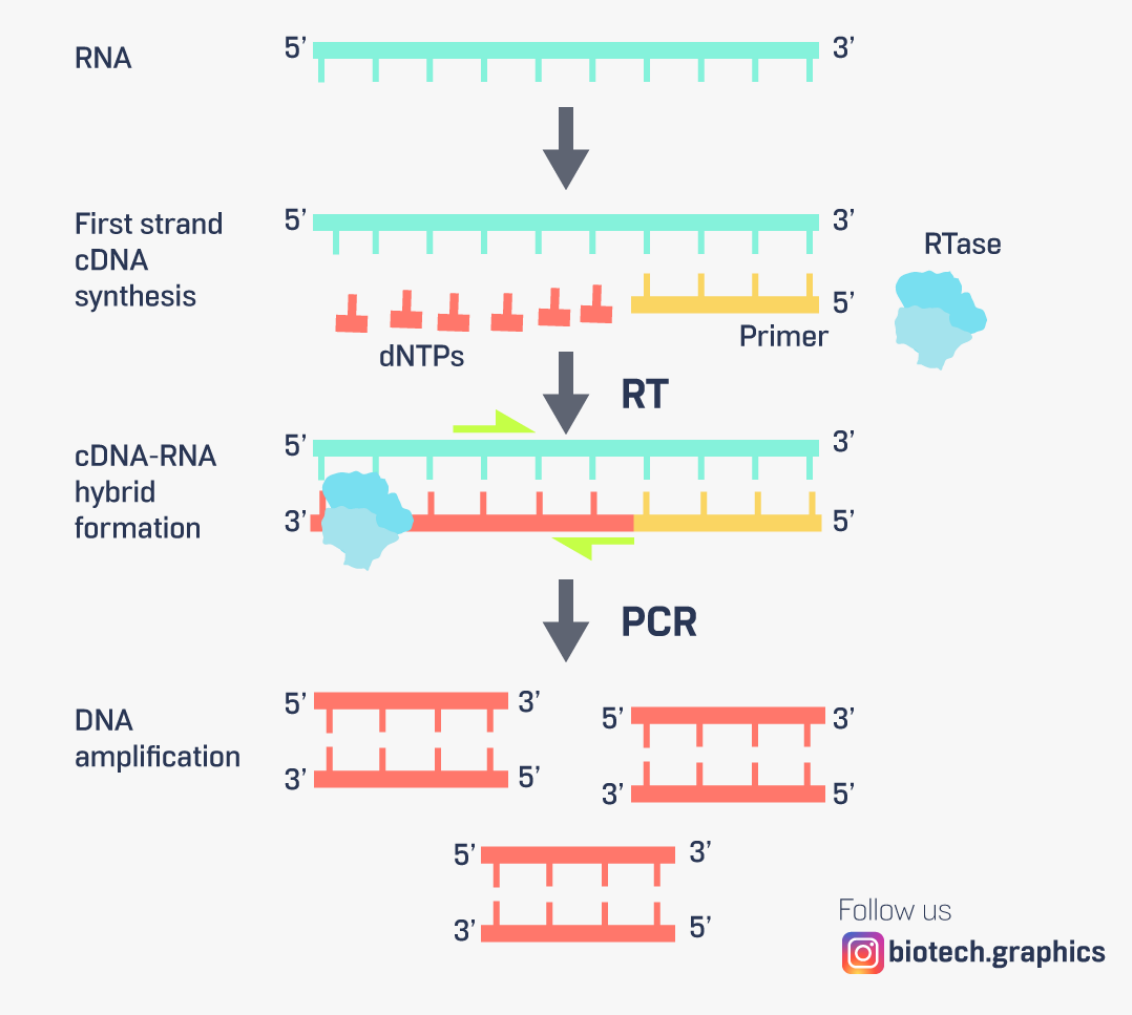
Photo from the Nobel Foundation archive.
Howard Martin Temin
Prize share: 1/3

The Nobel Prize in Physiology or Medicine 1975 was awarded jointly to David Baltimore, Renato Dulbecco and Howard Martin Temin "for their discoveries concerning the interaction between tumour viruses and the genetic material of the cell"

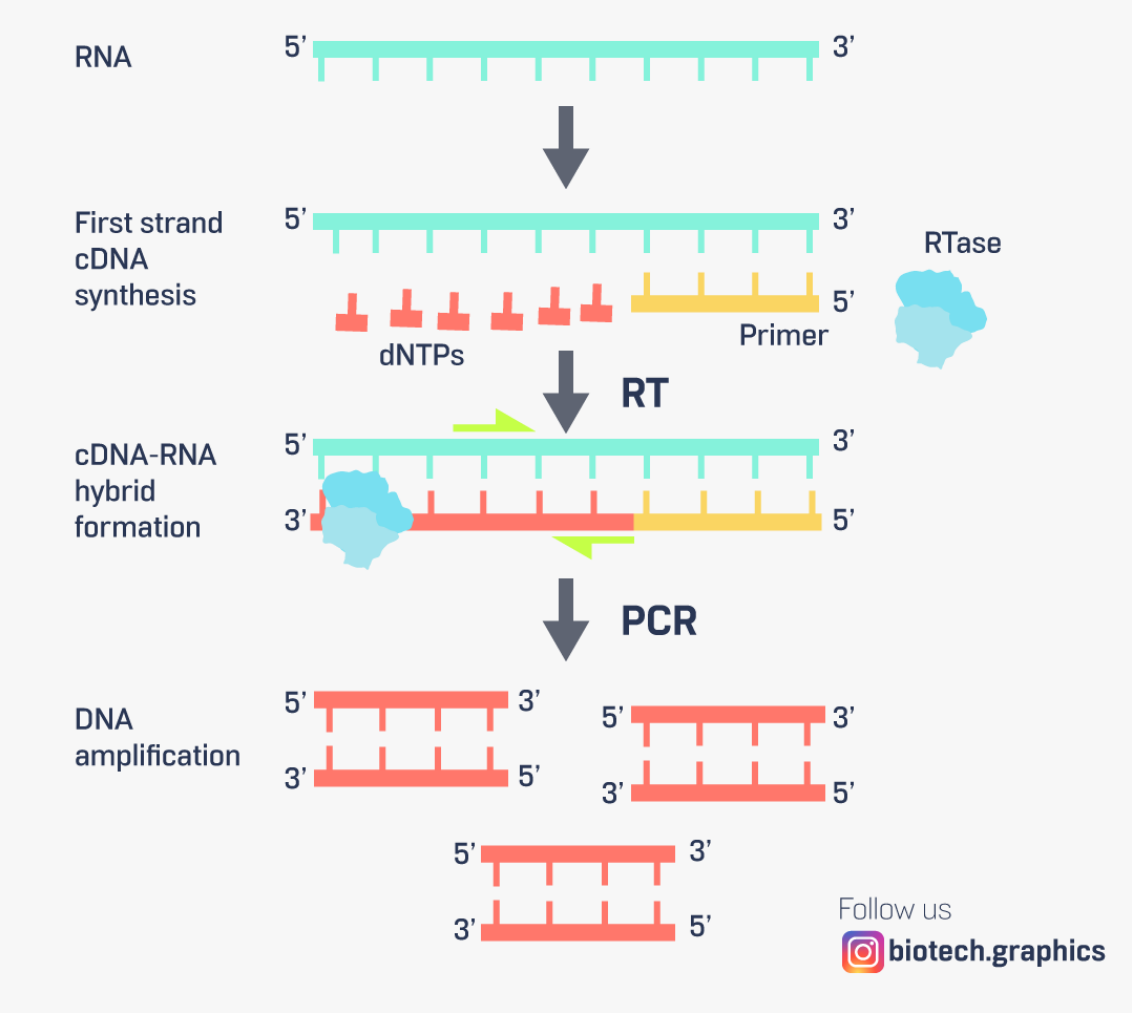
The simultaneous discovery in 1970 of reverse transcriptase in virions of retroviruses by **Howard Temin** and **David Baltimore**

Nobel Prize in 1975

RT is a one-to-one reaction. This is why it's typically coupled with a subsequent PCR step with specific primers targeting genes of interest...



What if we'd like to amplify all of the cDNA molecules (of all genes in a sample) by PCR to improve sensitivity for transcriptomic studies?



We need to use primer(s) that target all of the cDNA. How would we do it?

Different approaches to prepare RNA-derived DNA libraries with low RNA input: keys to single-cell RNA-seq

- **Template-switching*****
- *In vitro* transcription
- Random priming
- Direct ligation
- Poly(A) tailing

Two widely used RTs in molecular biology research

- Avian Myeloblastosis Virus Reverse Transcriptase (**AMV** RT)
- Moloney Murine Leukemia Virus Reverse Transcriptase (**MMLV** RT)

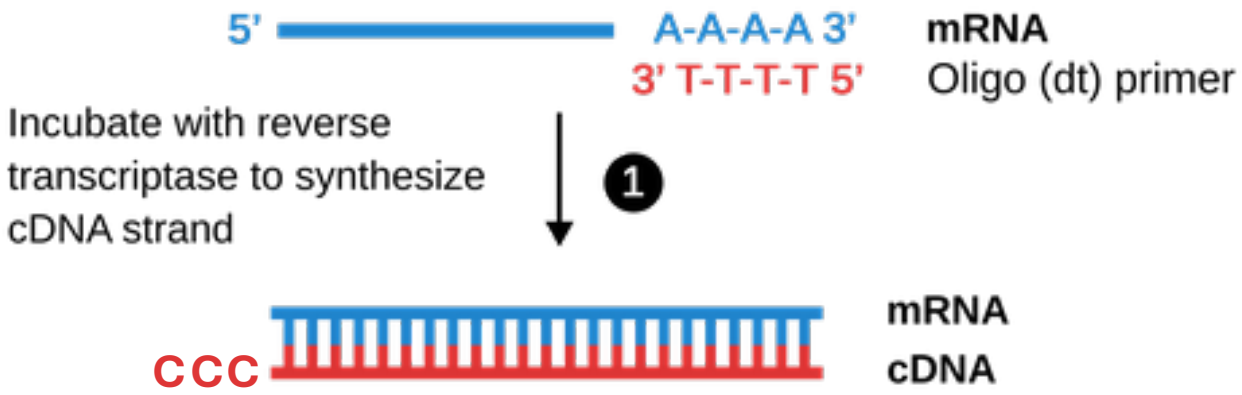
What is template switching?

- Template switching is a phenomenon where certain reverse transcriptase enzymes are able to **“switch” the RNA templates** when making cDNA

- Example: reverse transcriptase enzymes from Moloney murine leukemia virus (**MMLV**), which have been extensively engineered genetically into multiple versions

- So **template switching ~ MMLV reverse transcriptase**

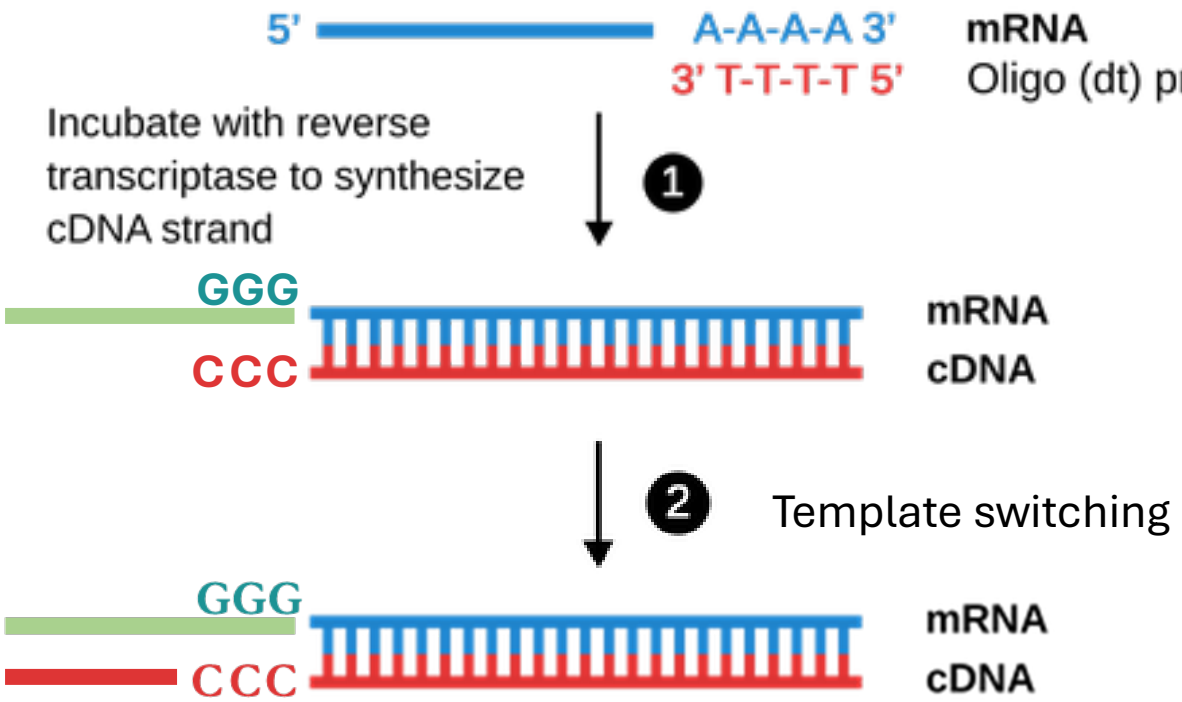
What's special about MMLV reverse transcriptase?



- MMLV reverse transcriptase has a weird behavior...
- It adds oligo-cytidines (typically CCC) to the end of first-strand cDNA once it reaches the end of the RNA template

If no additional trick is done, this phenomenon will have little to no impact on your typical experiments

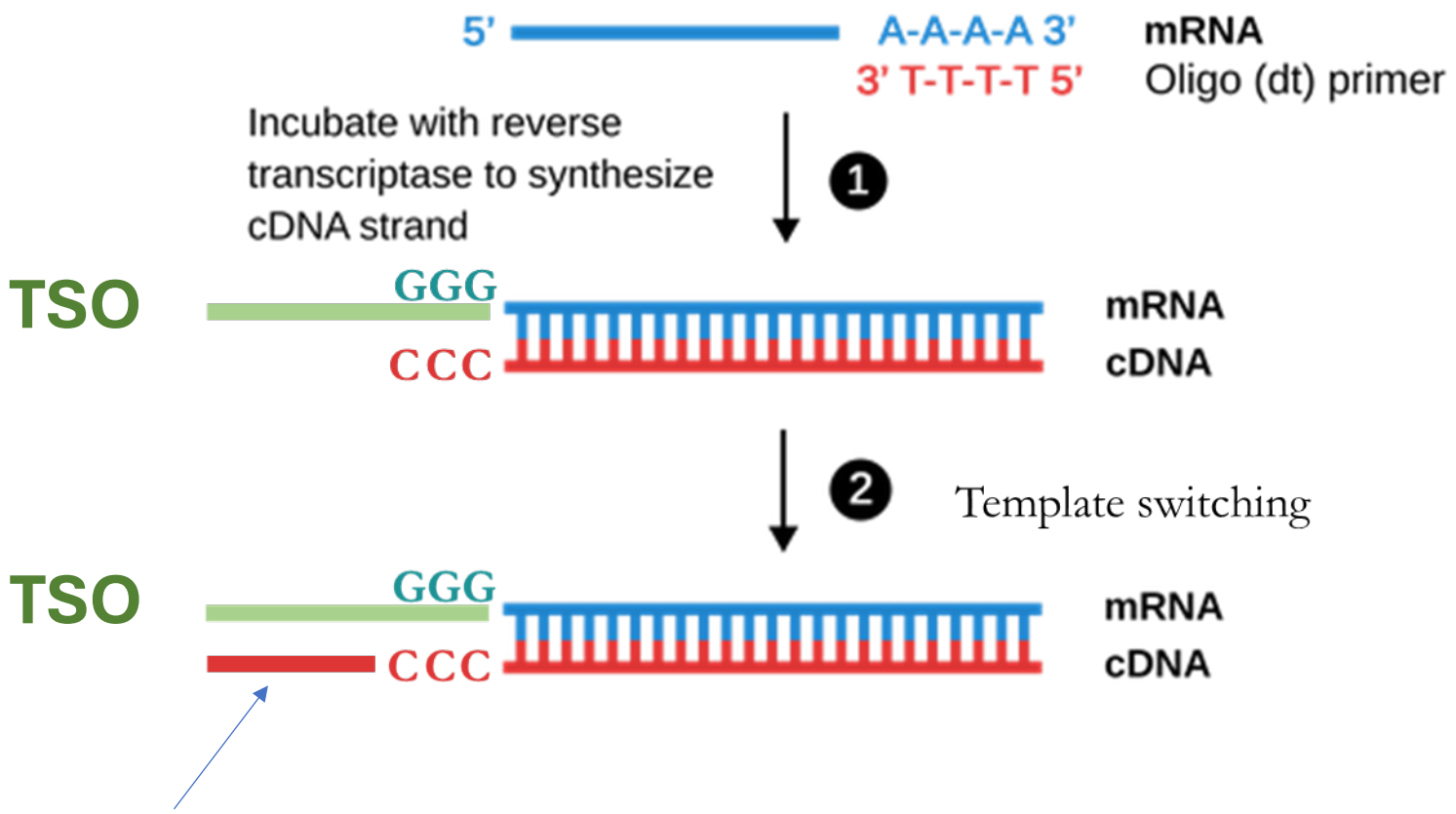
What's special about MMLV reverse transcriptase?



- If there is another oligonucleotide with GGG in the reaction, the oligonucleotide can bind to 3' CCC overhang
- Once it binds, MMLV RT could continue extending the cDNA using the oligonucleotide as the new template

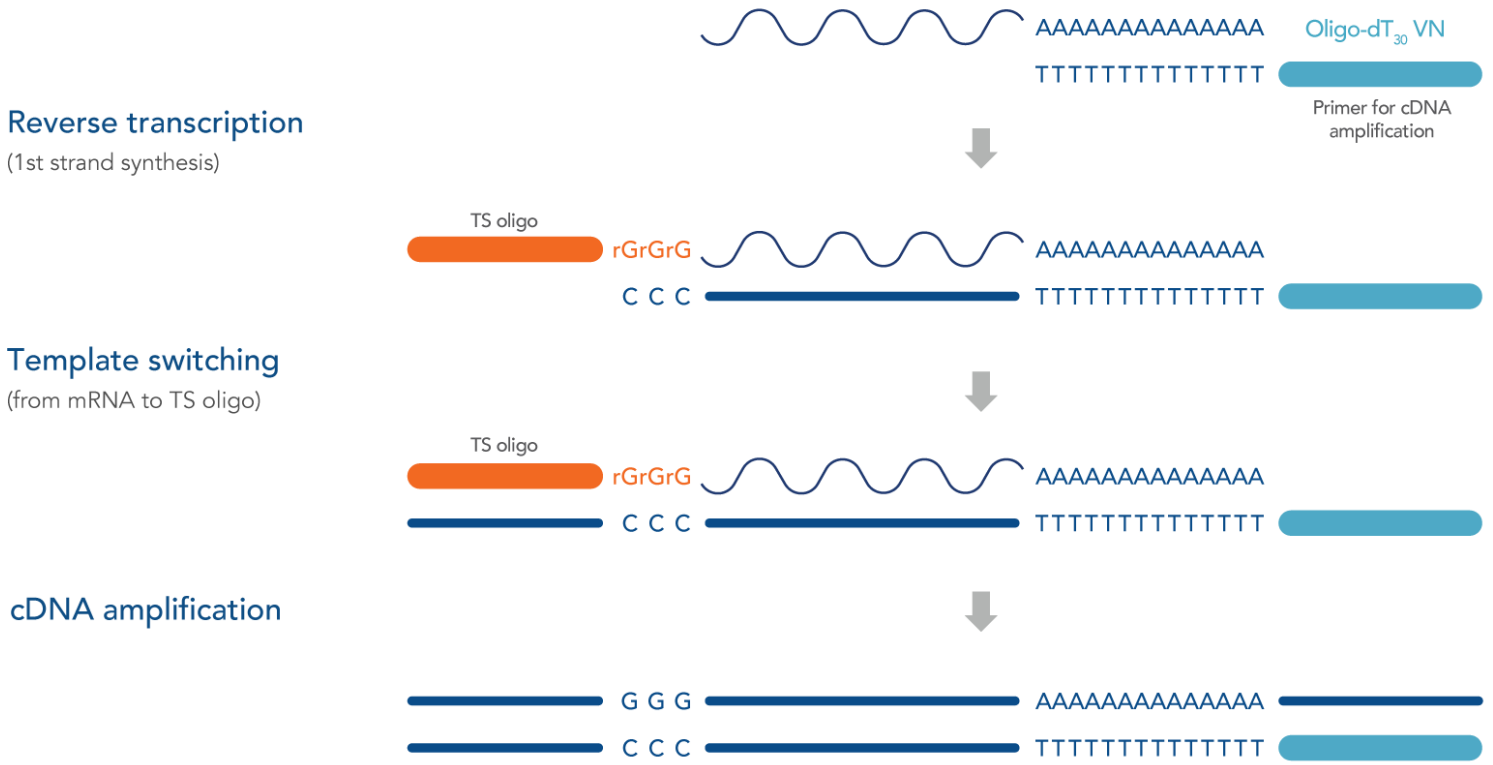
The oligo with 3' GGG is known as template-switch oligo (TSO)

The use of TSO adds a universal sequence for subsequent PCR...



Universal sequence **"handle"** for PCR

SMART: **s**witching **m**echanism **a**t the 5' end of the **R**NA **t**ranscript



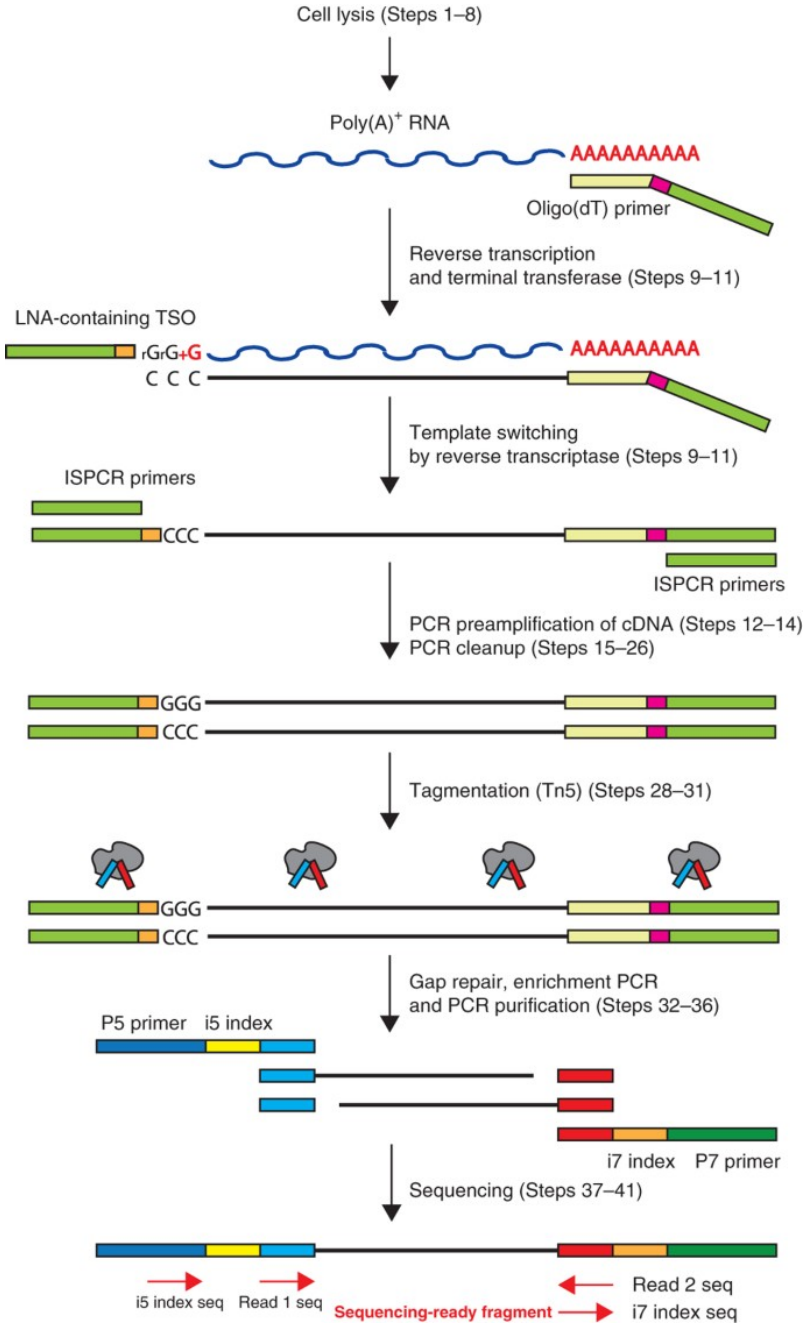
https://sg.idtdna.com/pages/education/decoded/article/use-of-template-switching-oligos-(ts-oligos-tsos)-for-efficient-cdna-library-construction#references



Rickard Sandberg



Simone Picelli

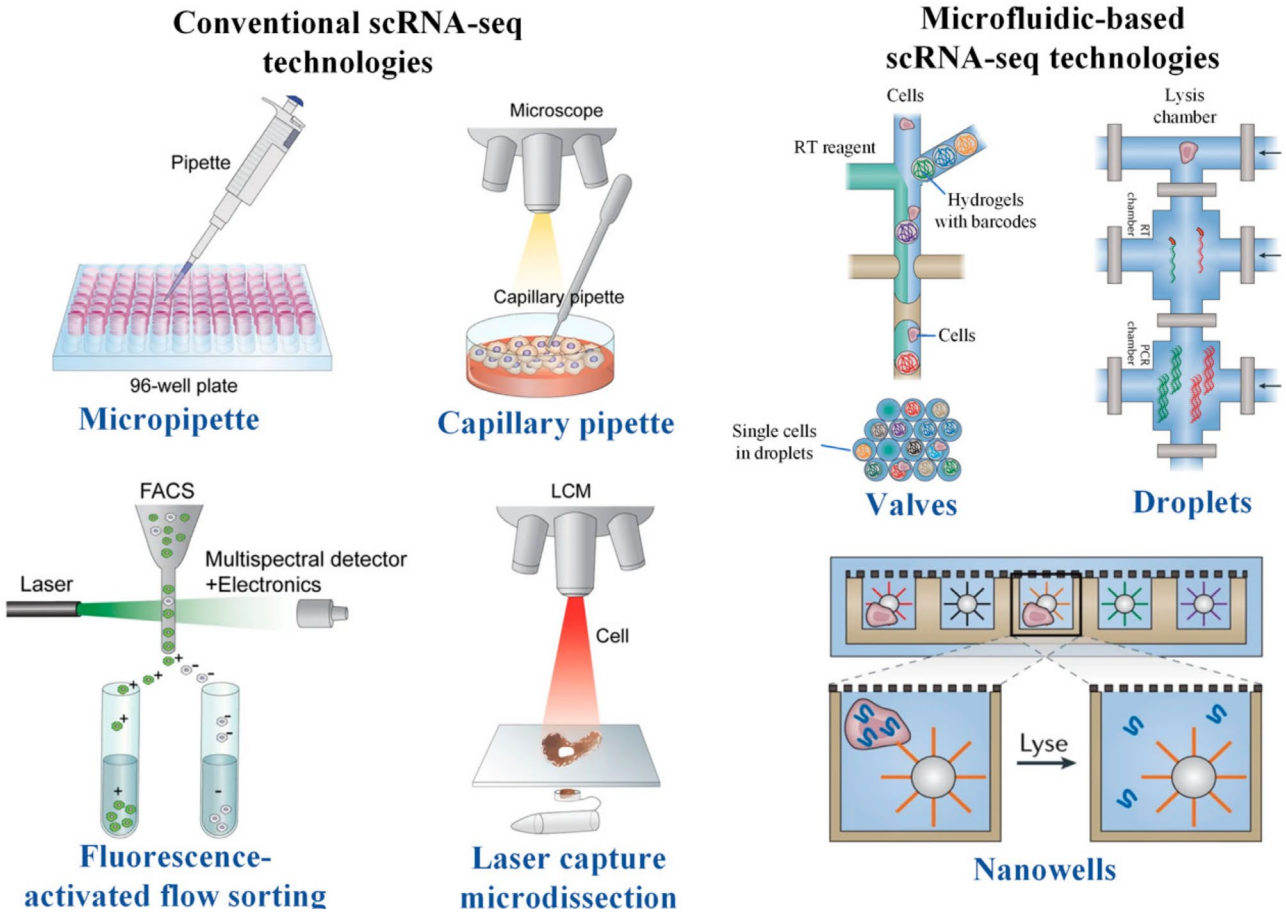


SMART-seq2

Picelli et al. *Nat. Methods*. 2013

Picelli et al. *Nat. Protoc*. 2014

Single-cell separation is instrumental to scRNA-seq



Choi et al. *Cells*. 2020

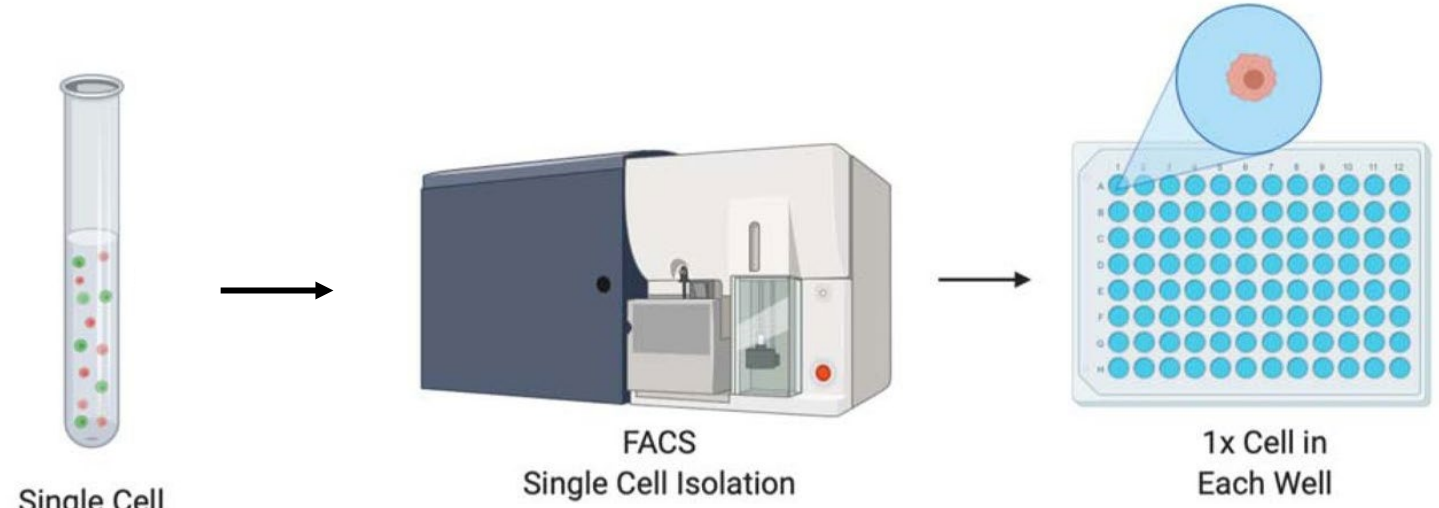
SMART-seq2



Rickard Sandberg

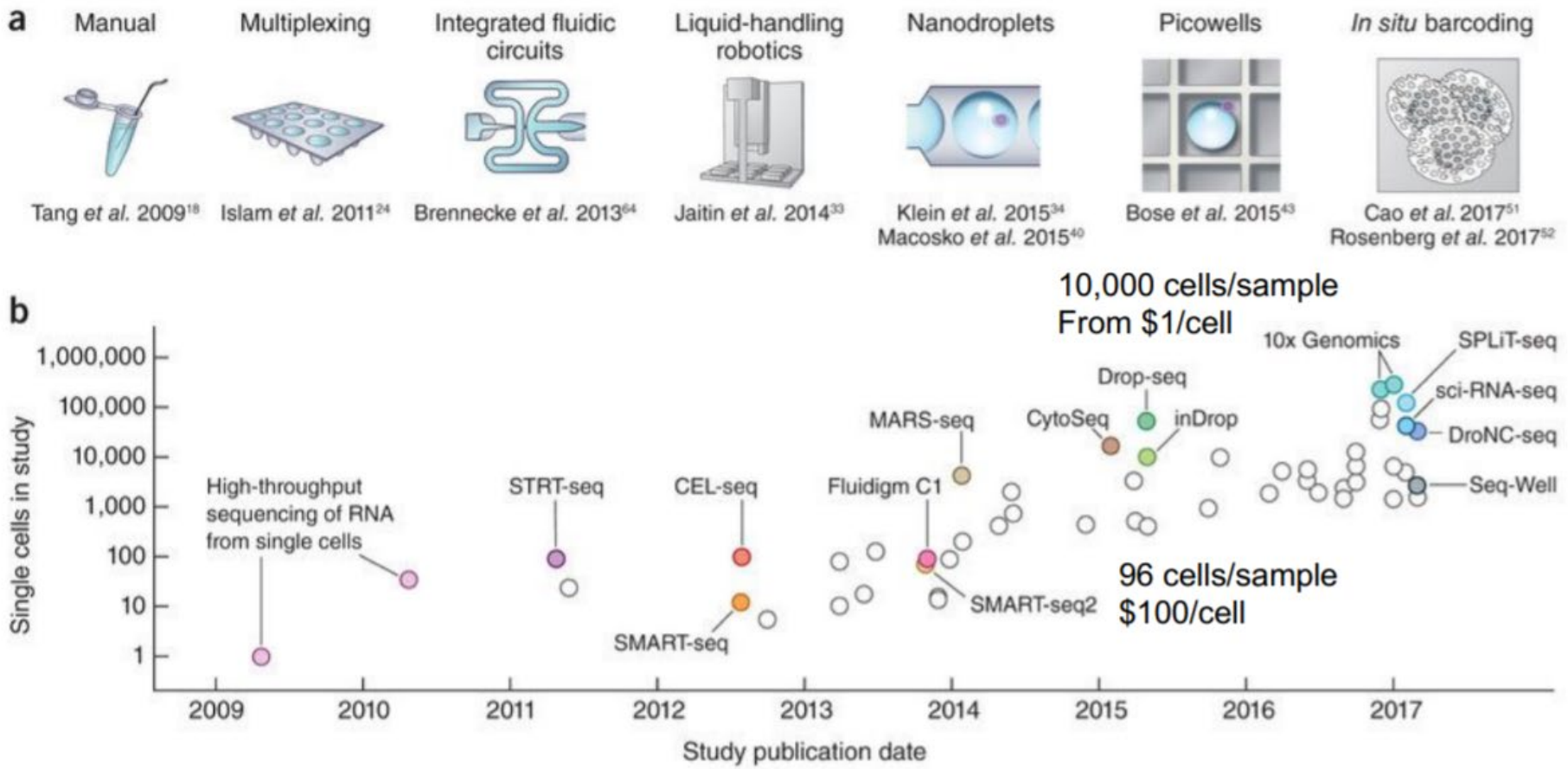


Simone Picelli



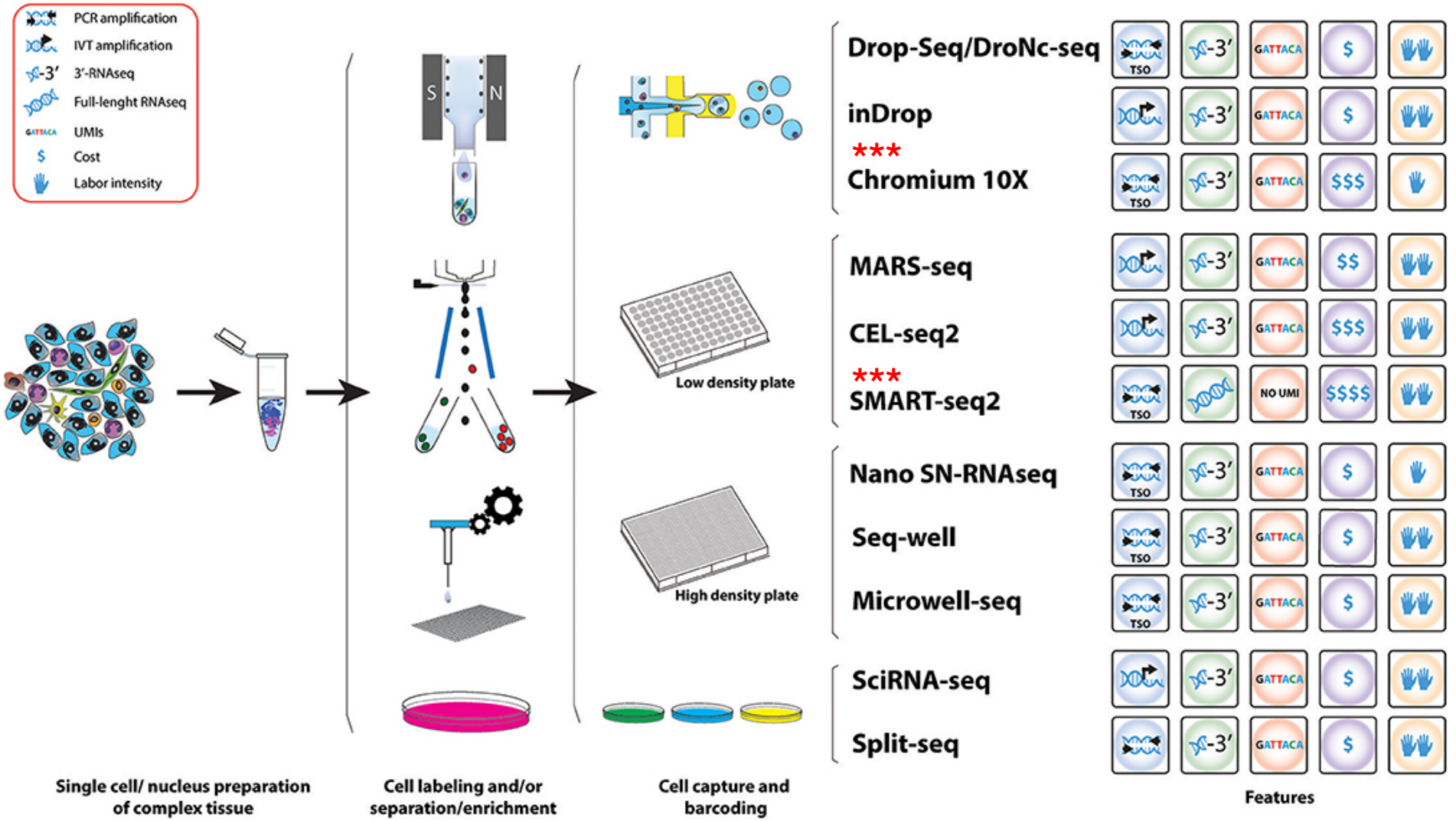
Picelli et al. *Nat. Methods*. 2013
Picelli et al. *Nat. Protoc*. 2014

Development of scRNA-seq technology



Svensson et al., *Nature Methods*, 2017

A variety of scRNA-seq technology platforms



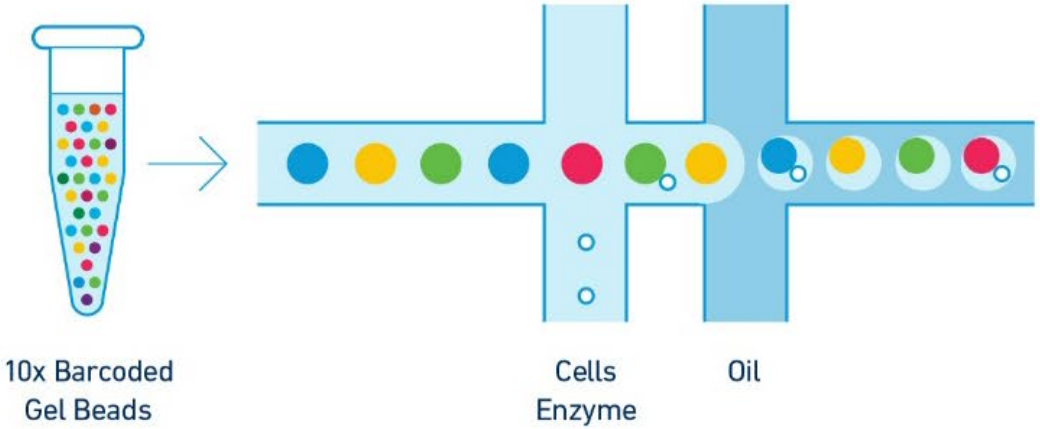
Valdes-Mora et al., *Front. Immunol.*, 2018

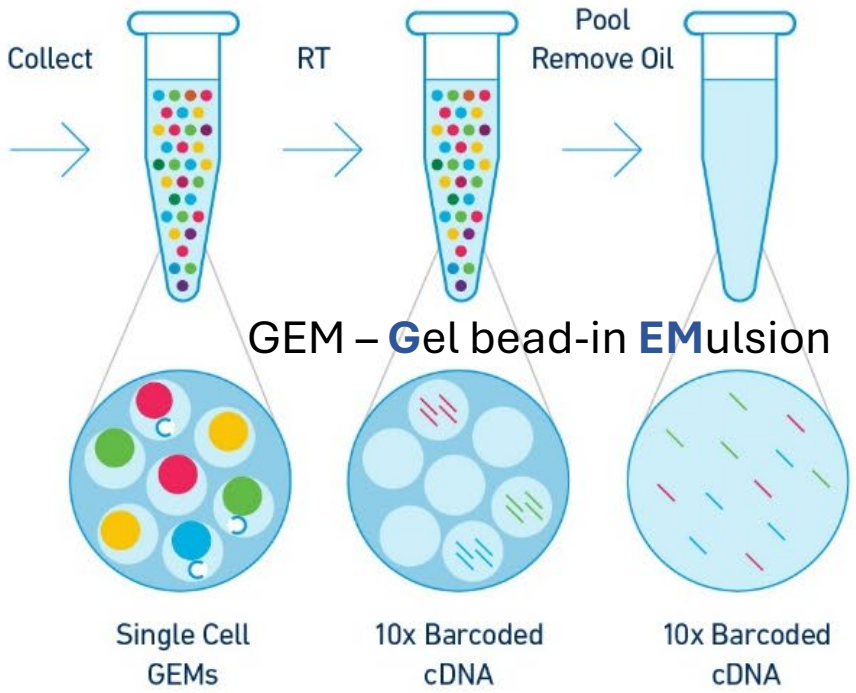
In 2016, The release of a scRNA-seq platform from 10X Genomics rocked the world. Their platform is still the most widely used one until now.



10X Chromium

10X Chromium relies on microfluidics to generate single-cell emulsion droplets that allow single-cell RT reaction





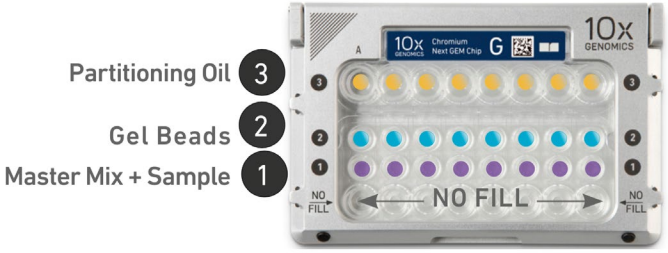


Image credit: 10X genomics

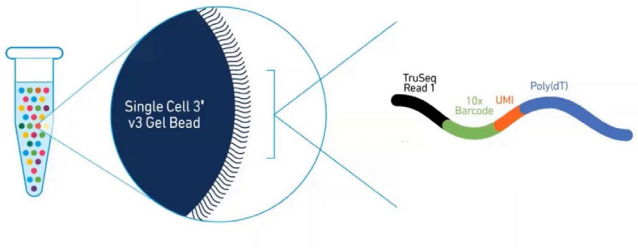
...to identify cellular subtypes and rare cells in the sample



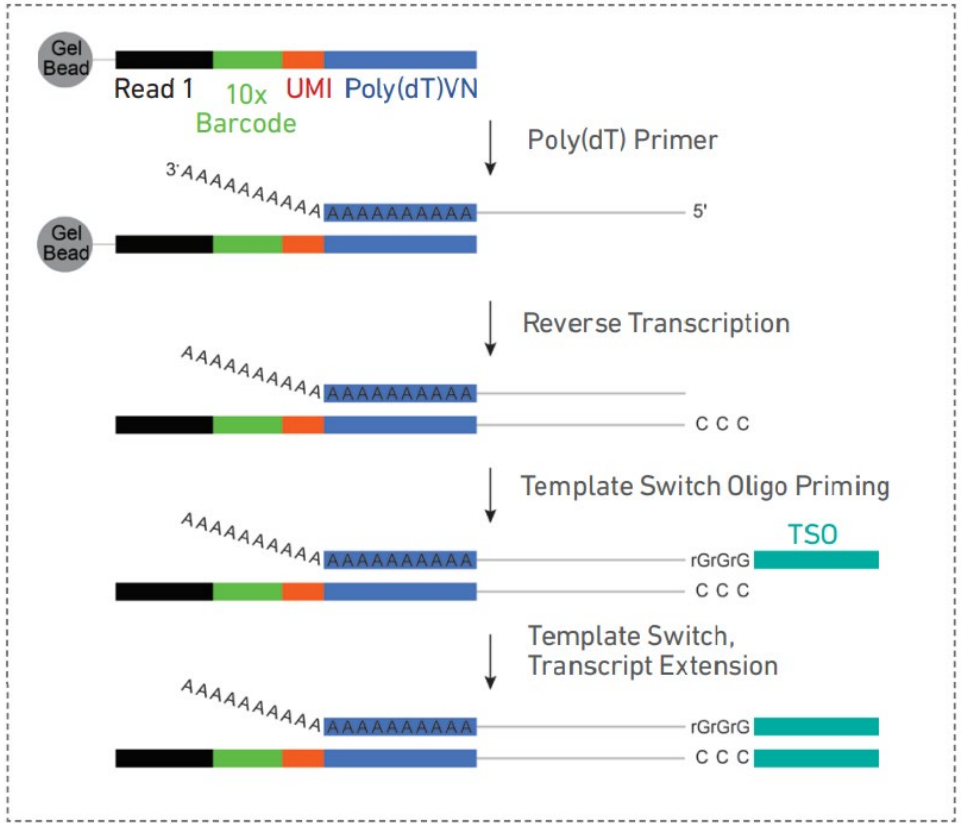
Beads carry unique barcodes that allow unique tagging.

Amplification is based on template switching…

Single Cell 3’ v3 Gel Beads



Inside individual GEMs



Pooled cDNA amplification

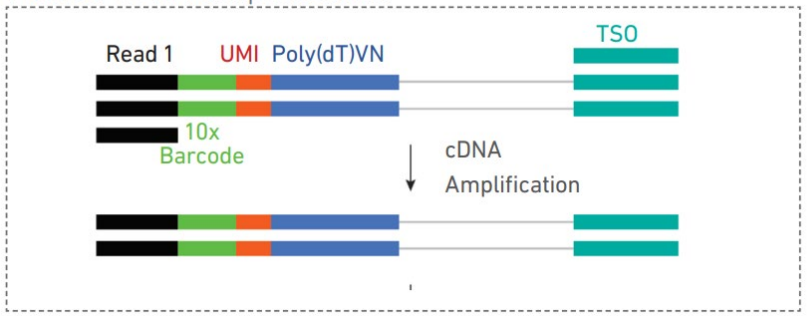


Image credit: 10X genomics

10X barcode: 16nt-DNA sequences unique to each bead: allowing you to track which cell a given RNA is from later

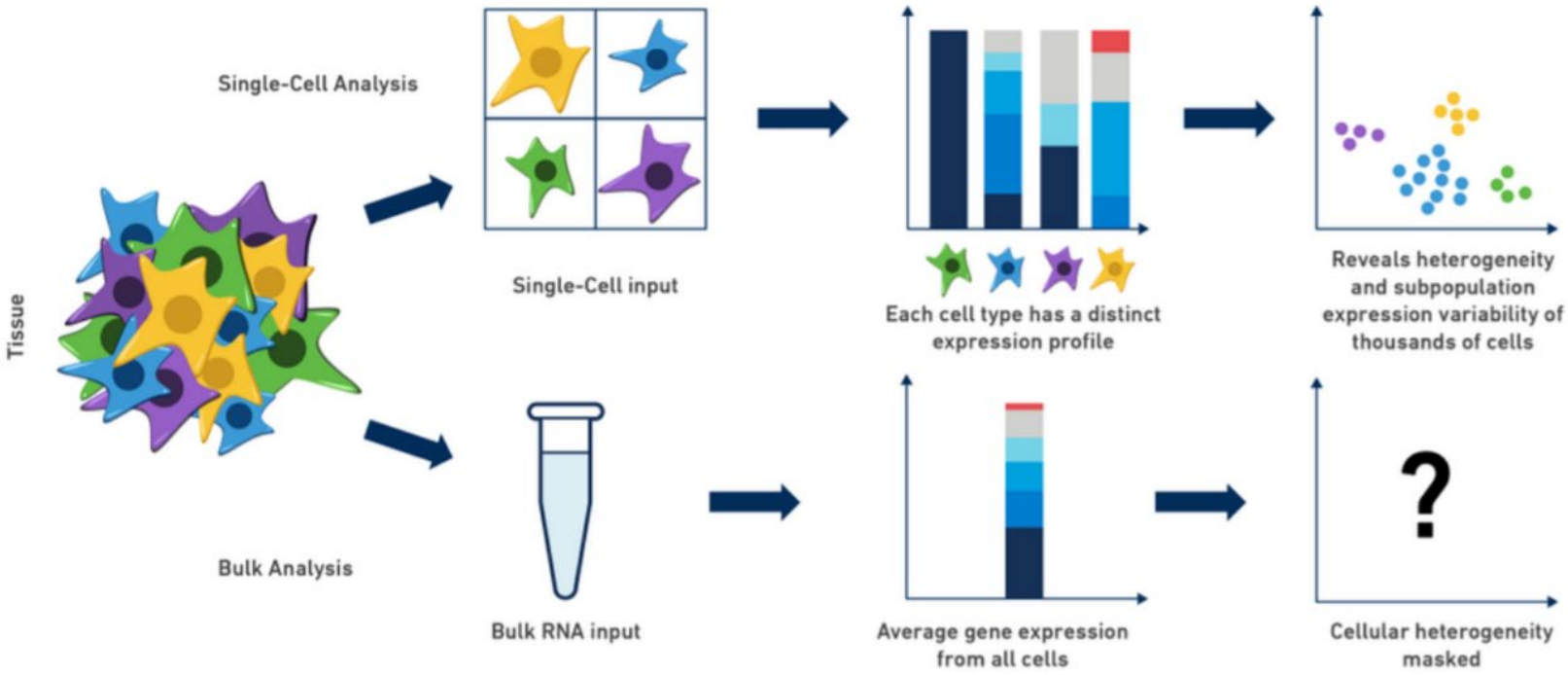
Before the age of scRNA-seq, you needed RNA from at least ~10,000 cells to be able to perform RNAseq

Bulk RNA-sequencing

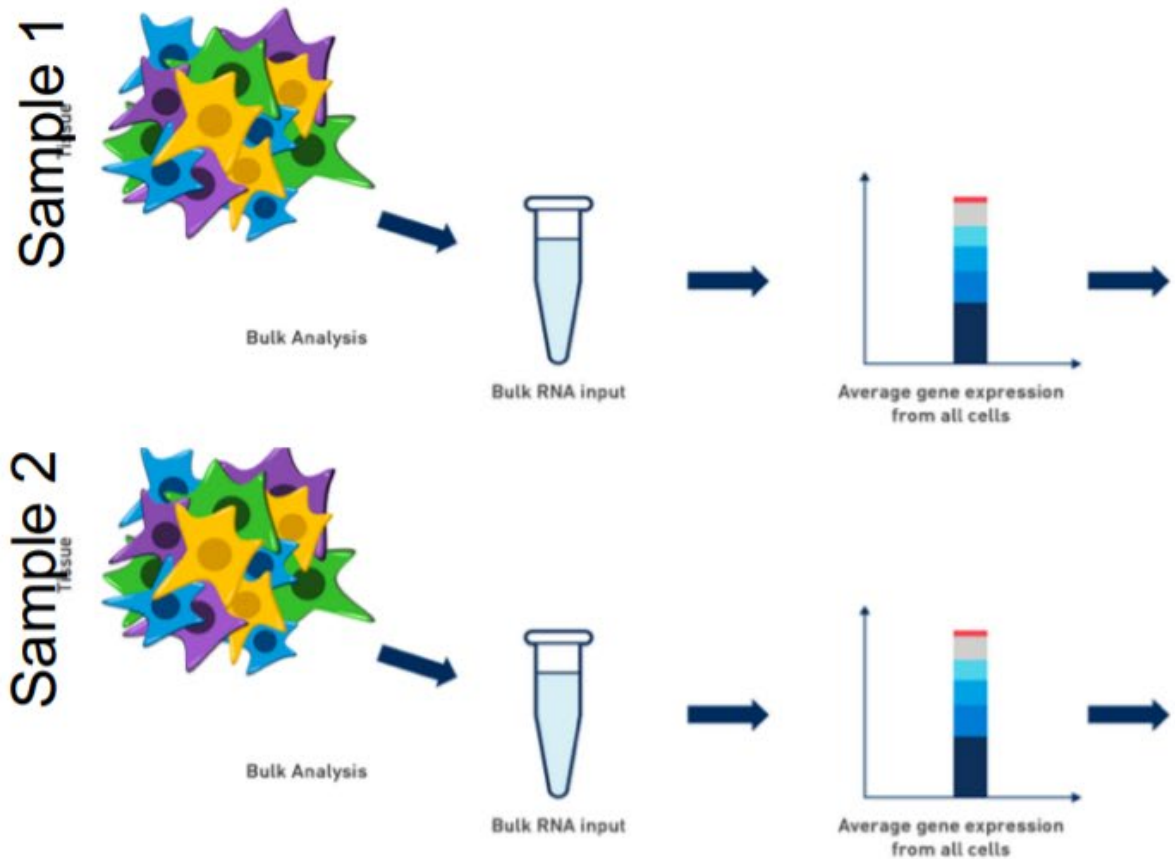
Average expression level for each gene across a large population of input cells

Single-cell RNA-sequencing (scRNA-seq)

Gene expression profile of individual cells are measured



Bulk RNA sequencing

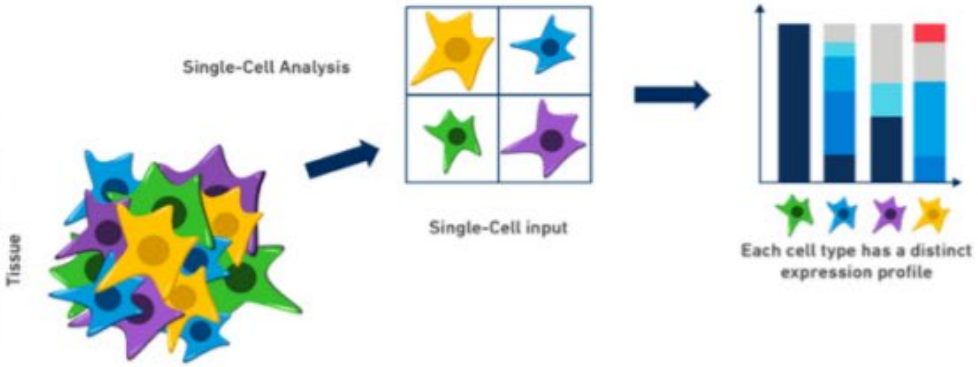


	Sample 1	Sample 2
Gene A	0	10
Gene B	20	1
…	…	…
Gene N	5	100

Sample > Library Prep > Sequencing > Raw reads > Alignment > Count matrix

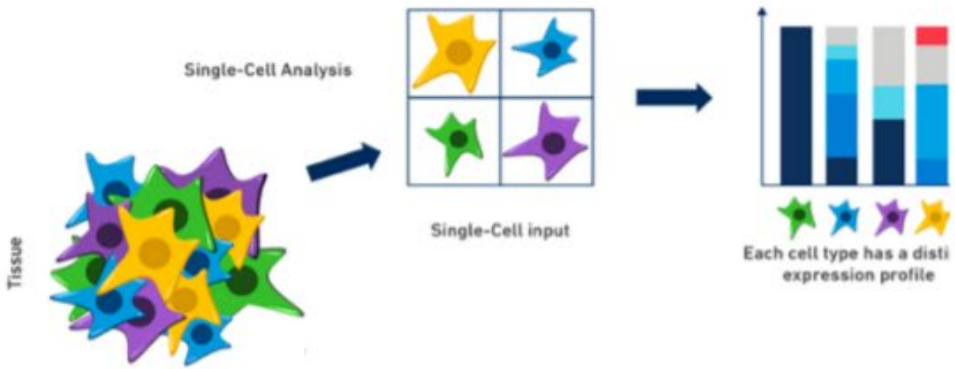
Single-Cell RNA sequencing

Sample 1



	Cell 1	Cell 2
Gene A	0	10
Gene B	20	1
…	…	…
Gene N	5	100

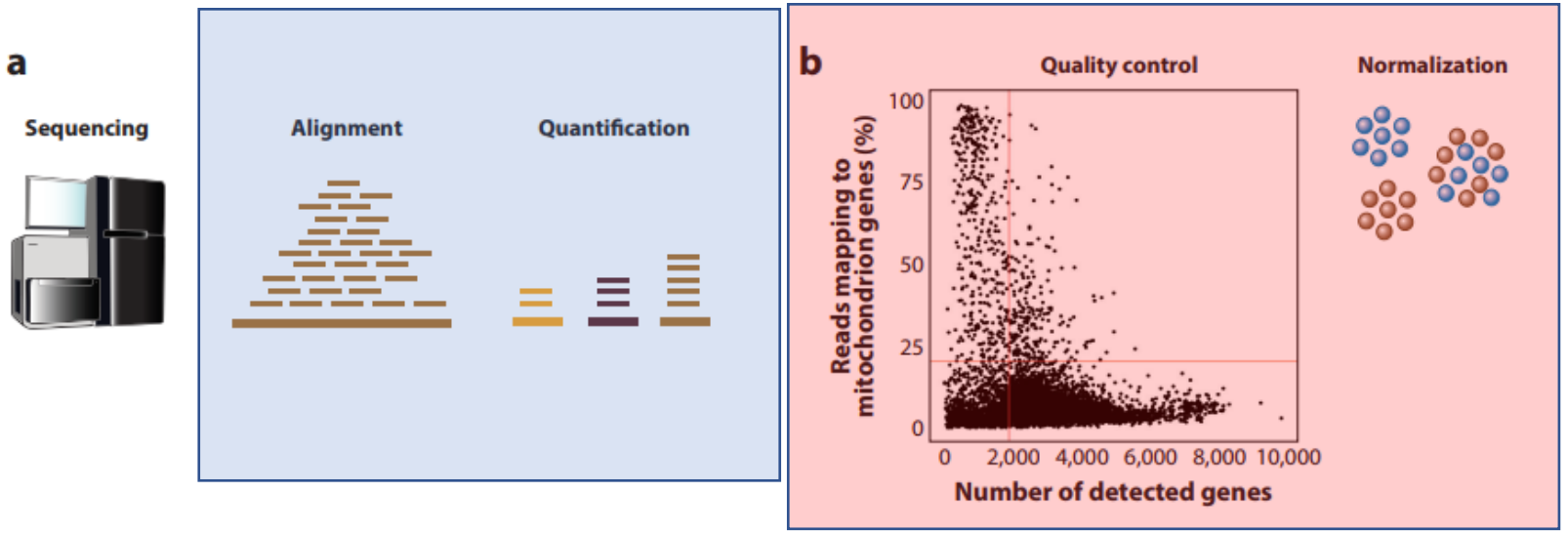
Sample 2

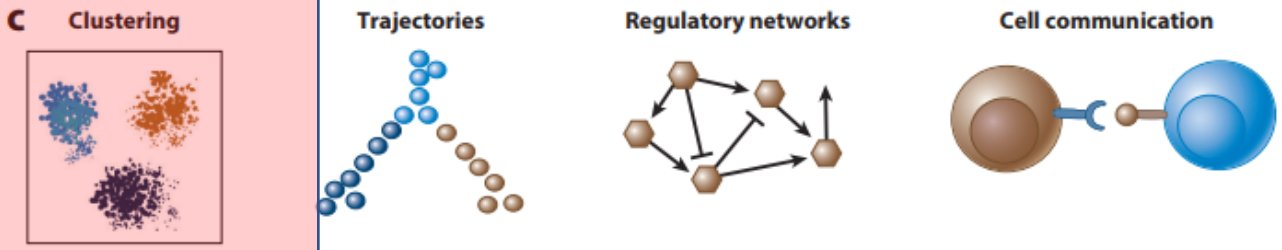


	Cell 1	Cell 2
Gene A	0	10
Gene B	20	1
…	…	…
Gene N	5	100

- Per sample (sample barcoding)
- Per cell (10X cell barcoding)
- Per transcript (Unique molecular identifier - UMI)

Single-cell RNA-seq: common analysis pipeline





- dimensionality reduction
- clustering
- marker findings/DGE
- cell type annotation

Alignment/Quantification
- Cellranger

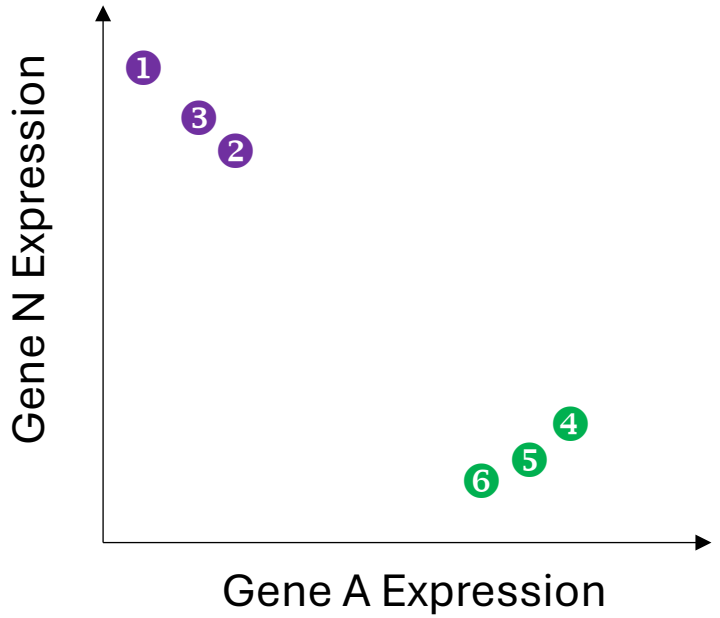
Common analysis packages
- Seurat (R-based)
- Scanpy (Python-based)
- SingleCellExperiment (R-based)

Efremova et al., *Nat. Rev. Immunol.*, 2020

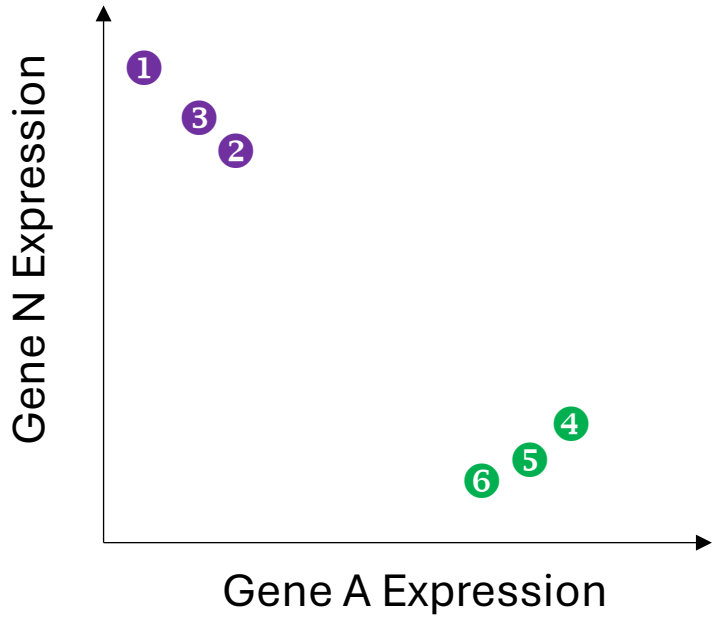
How can we tell different cell types apart based on single-cell transcriptomes?

If you have multiple cells/samples and you would like to see how similar the samples are based on gene expression, what would you do?

	Neuron			Smooth Muscle Cell		
	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6
Gene A	0	2	1	100	85	72
Gene B	43	52	36	29	72	35
…	…	…	…	…	…	…
Gene N	98	73	65	12	9	4



If you have multiple cells/samples and you would like to see how similar the samples are based on gene expression, what would you do?

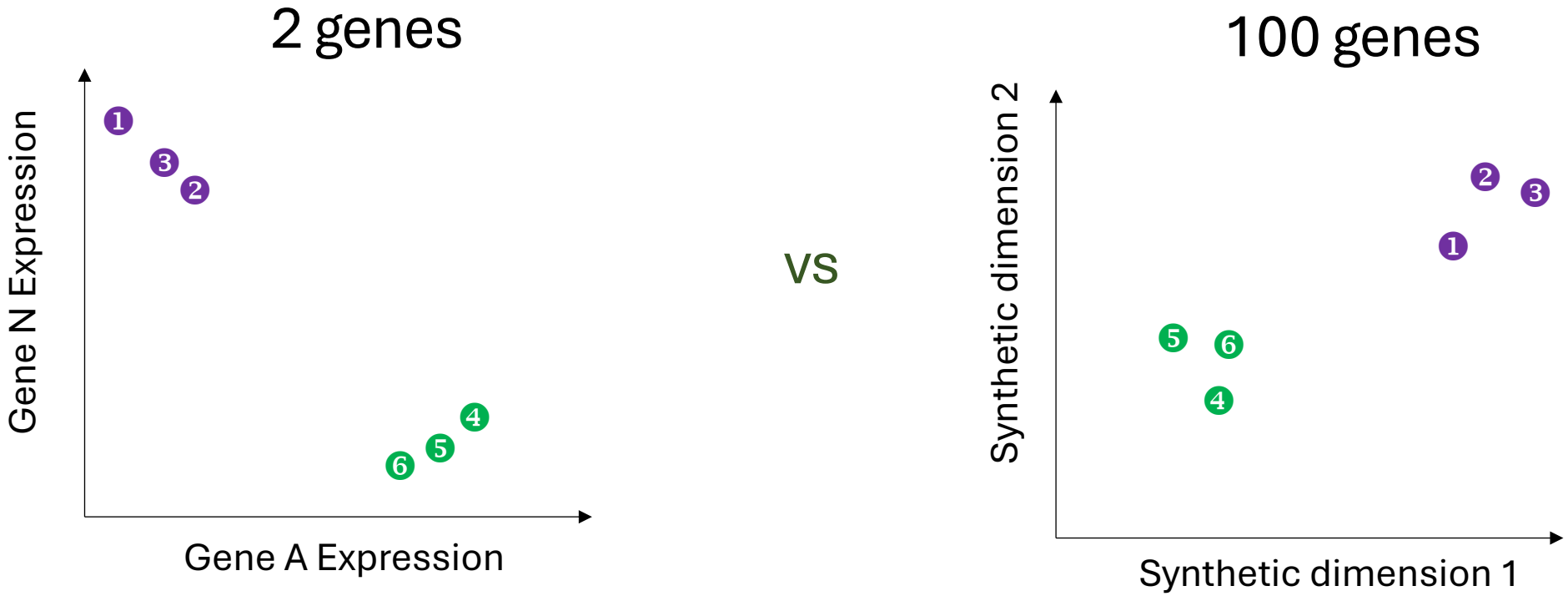


When you consider the expression of 2 genes, you can represent the results in a 2D plot. But what if you would like to see the similarity of samples based on 100 DE genes?

100D plot?!?

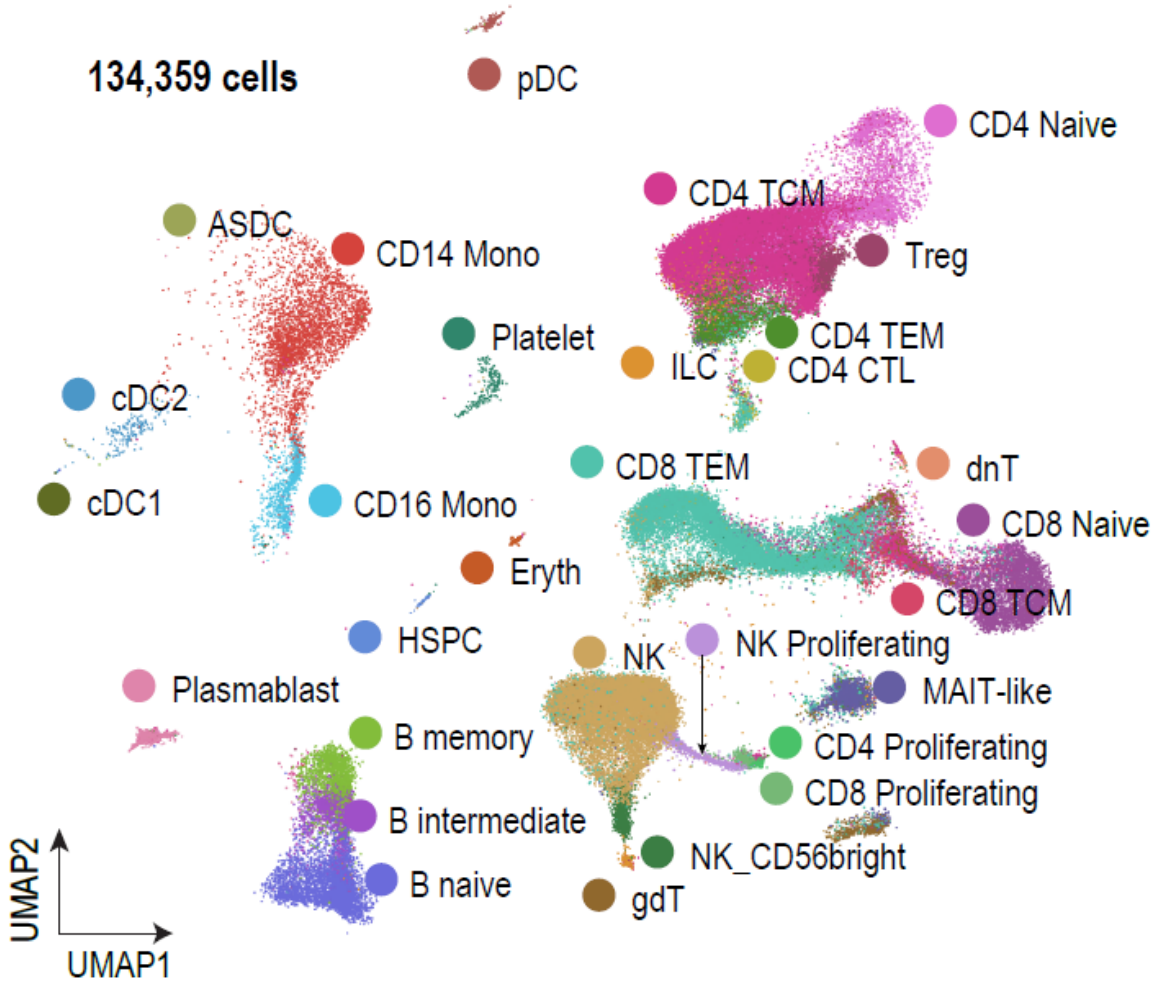
Dimensionality reduction

You can use mathematical algorithms to collapse 100 dimensions into 2...



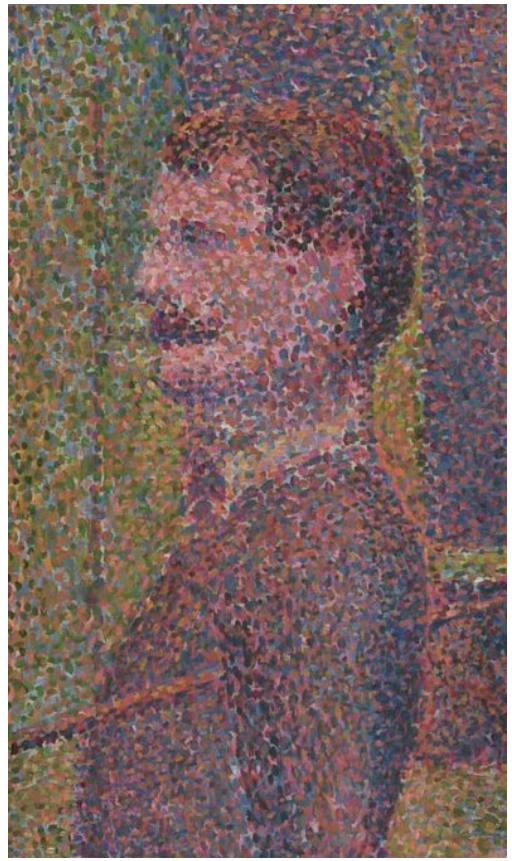
Commonly used approaches: **PCA**, NMF, t-SNE, **UMAP**

UMAP plot illustrating human peripheral blood mononuclear cells (PBMCs)







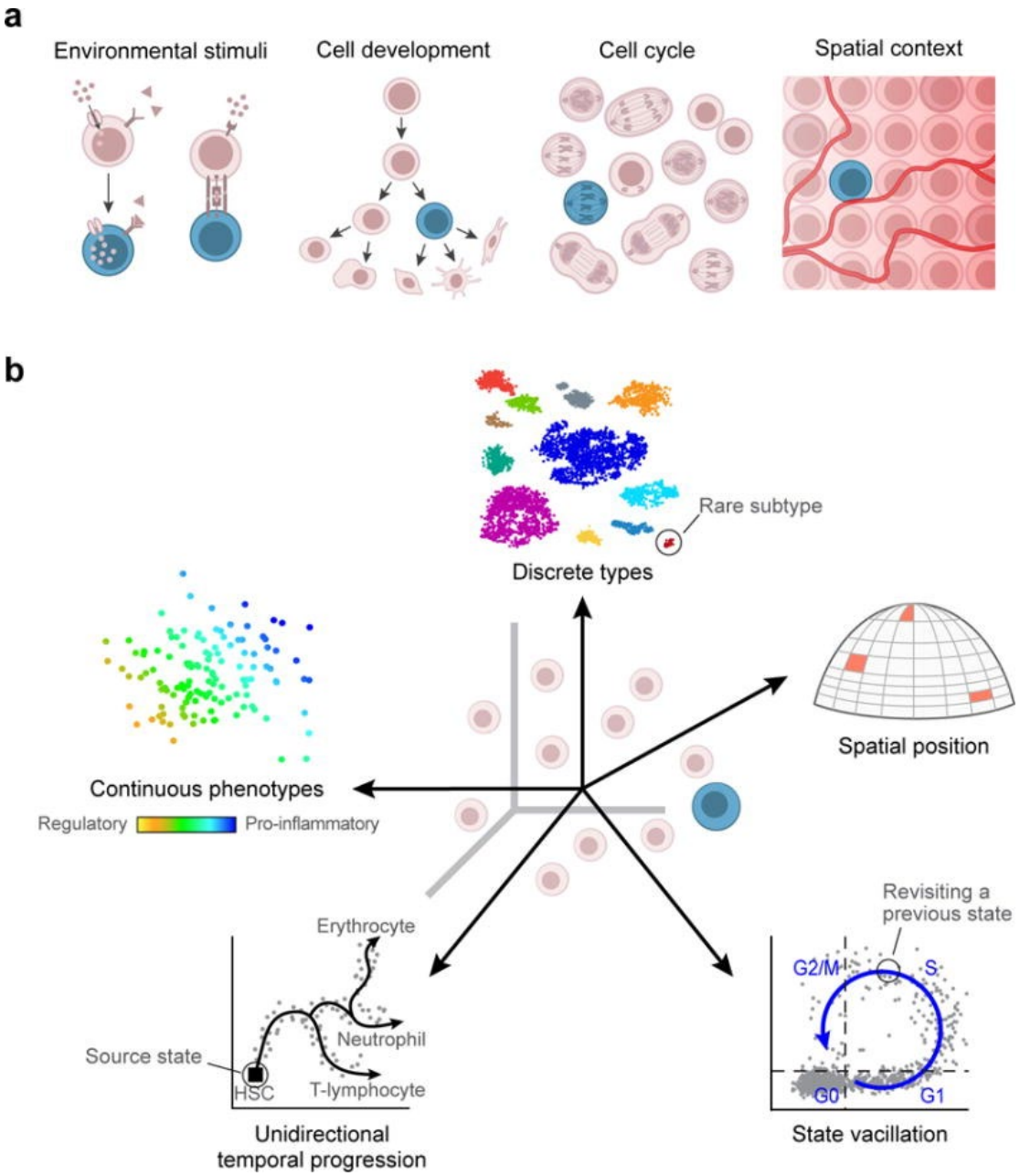


A popular single-cell analysis package is known as **Seurat**, which was named after a 19th century French artist **Georges Seurat** known for his "**pointillism**" style



What could single-cell transcriptomics do?

- Cell atlas: identifying cell types/states in a given environment in a less biased manner (i.e. used transcriptomes instead of selected markers to define cell types/states)
- Systemic phenotyping in a cell type-specific manner: providing an overview of information on phenotypic changes at the mRNA level in experimental settings (e.g. cell type X in healthy vs diseased states)



Single-cell transcriptomics reveals the "vectors" of cellular identity

" To understand cells—the basic unit of life—we must not only catalog them and their molecular profiles but also determine the factors that shape them. A cell's identity, which is reflected in its molecular profile, is formed by the instantaneous intersection of multiple factors."

Wagner et al. *Nat Biotechnol*. 2016

Human Cell Atlas (HCA)

a **Comprehensive Reference Map**
of the Types and Properties of
All Human Cells
as a Basis for Understanding
Diagnosing Monitoring
and Treating Health and Disease

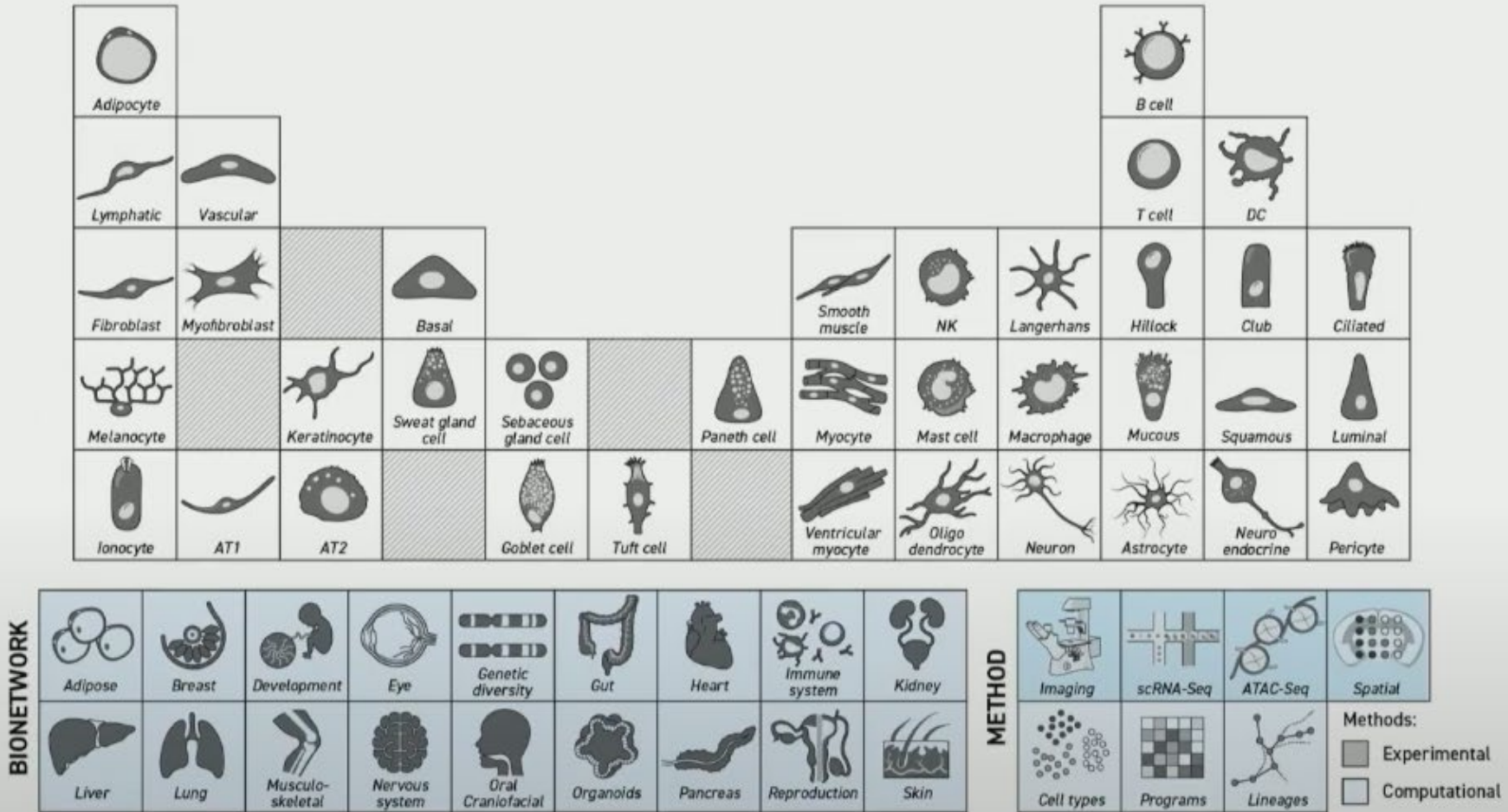


37 trillion cells
in the human body

History of the HUMAN CELL ATLAS Partners since Jan 2016



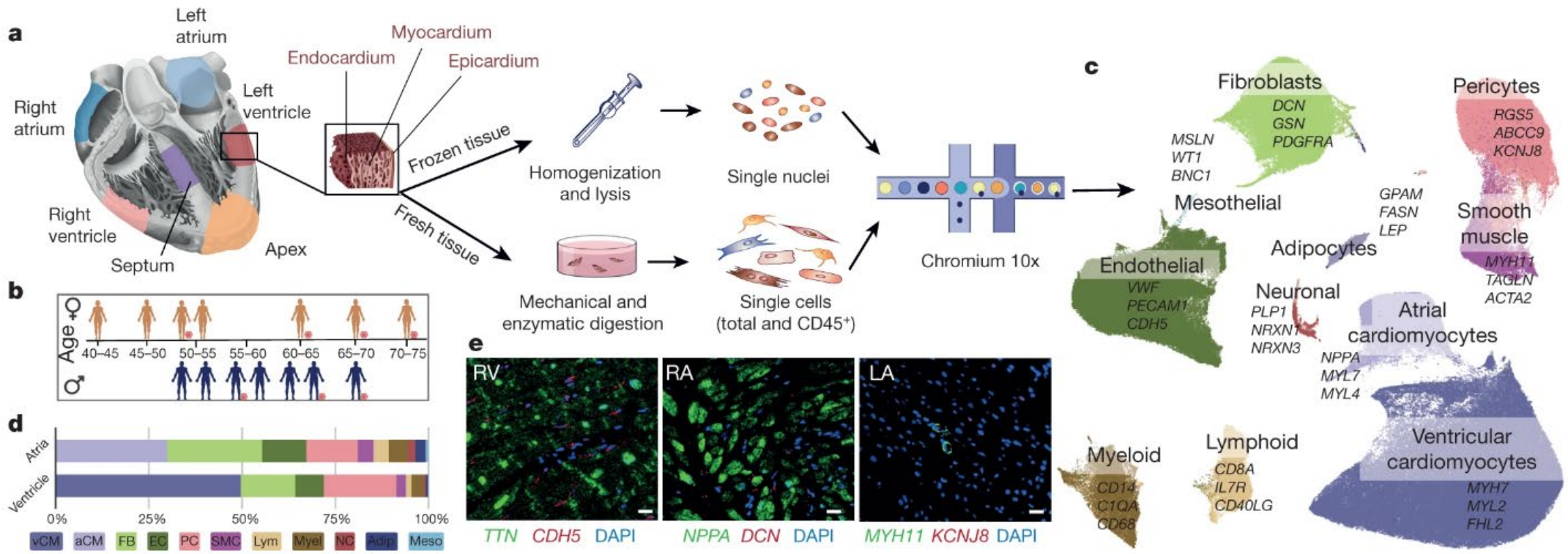
HCA: A periodic table of our cells



A rapidly growing global and open scientific initiative

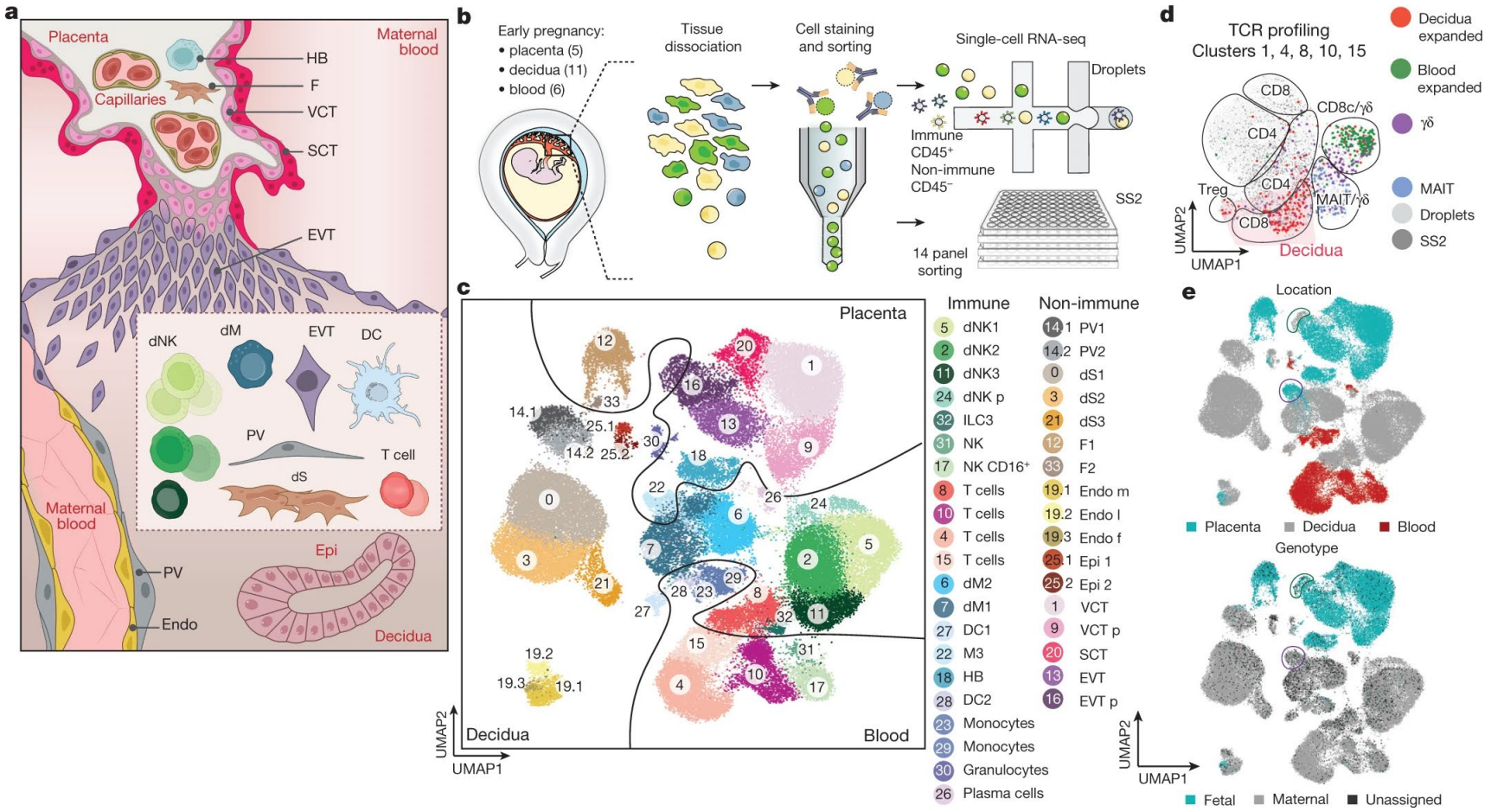


Example: atlas of human hearts

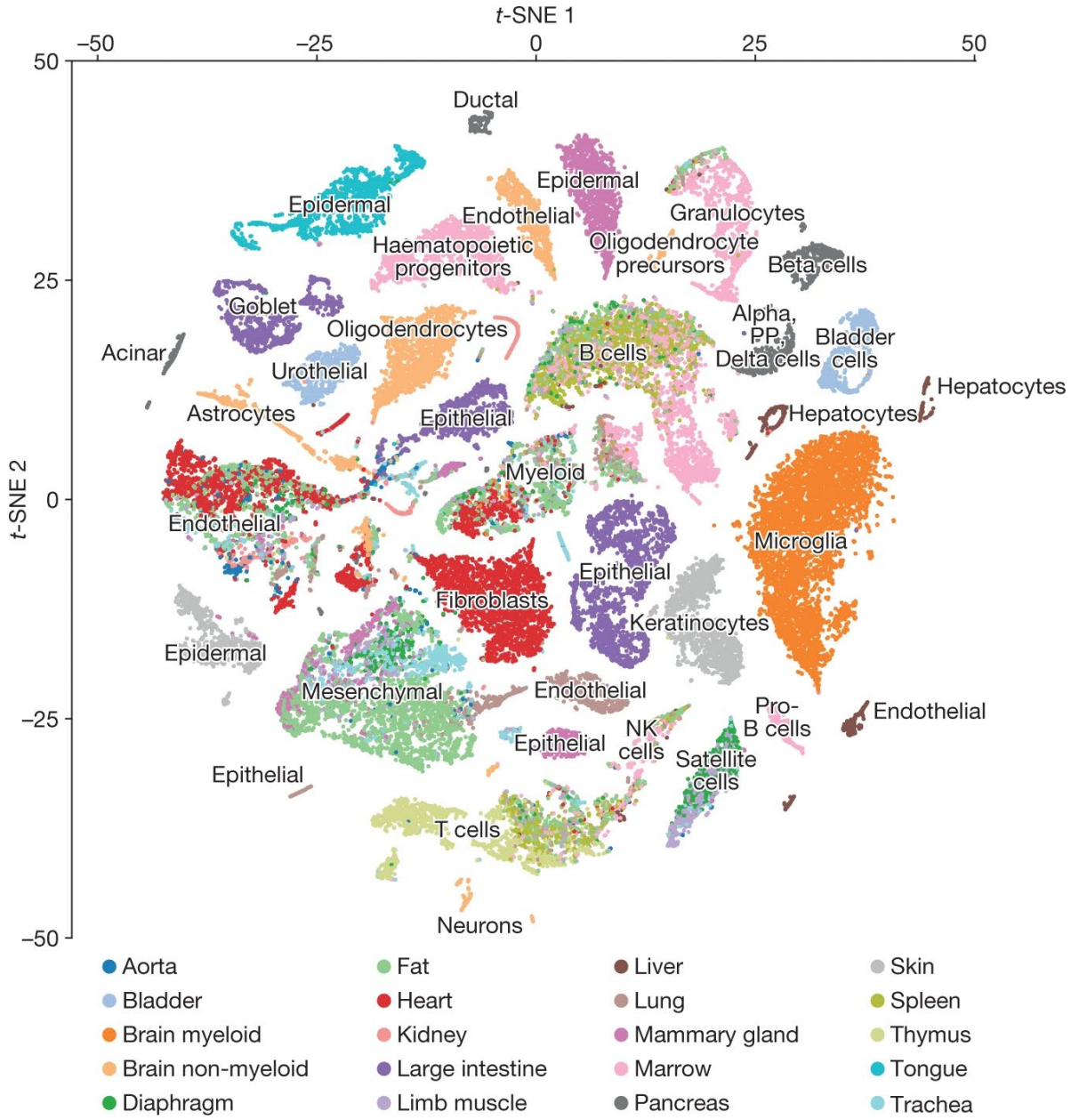


Litviňuková et al., *Nature*, 2020

Example: atlas of early fetal-maternal interface



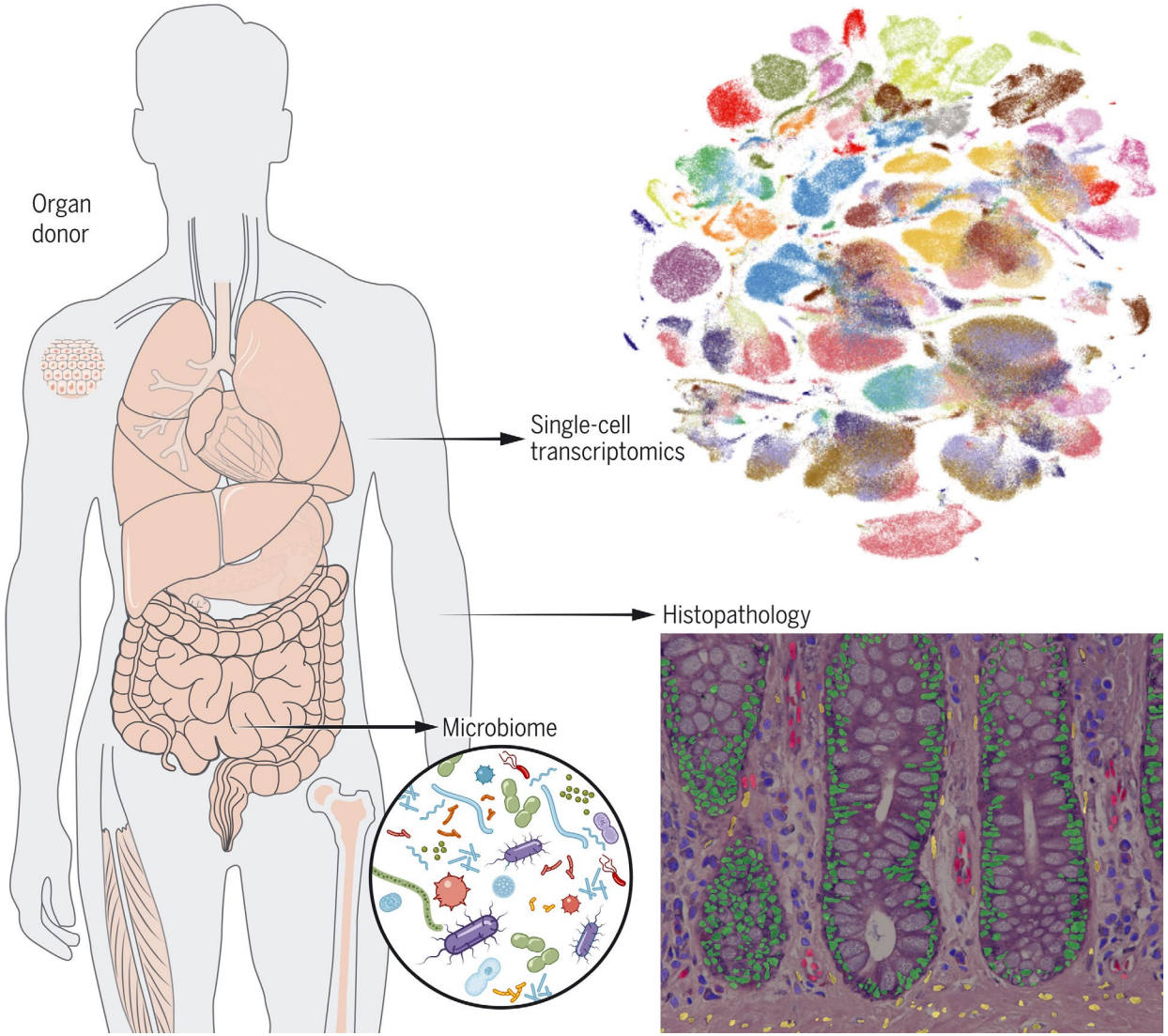
Vento-Tormo et al., *Nature*, 2018



Tabula Muris

Single-cell transcriptomic profiles of 20 mouse organs

The Tabula Muris Consortium. *Nature*. 2018



Tabula Sapiens

500,000 cells from 24 different tissues and organs, many from the same donor - >400 cell types identified

The Tabula Sapiens Consortium. *Science*. 202

Over 100M cells profiled across the human body to date

RESEARCH AREAS	18
INDIVIDUALS	9,285
SAMPLES	13,208
CELLS	109,393,296



How unconscious biases can skew forensic science p. 686

Hydrodynamics emerges in spin chains pp. 699, 716, & 720

Global shifts in coastal wetlands p. 744

Science

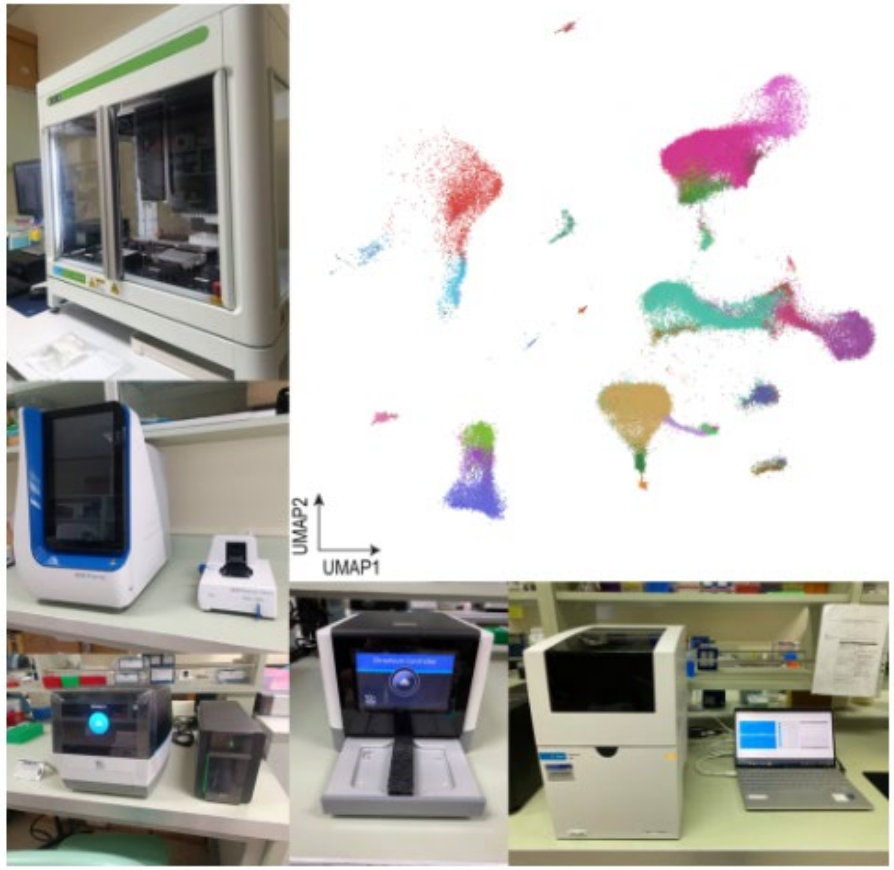
$15
13 MAY 2022
science.org
AAAS



HUMAN CELL ATLAS

Mapping cell identity across tissues and organs pp. 695 & 711–713

Access to



Single Cell Genomics Facility

In association with Mahidol University's Integrative Computational BioScience Center (ICBS) and the Faculty of Science's Systems Biology of Disease (SyBiD) research unit, we are among the pioneers who established Thailand's first single-cell genomics facility, with an extensive international collaborative network, including:

- Wellcome Sanger Institute, UK (www.sanger.ac.uk)
- The Human Cell Atlas Consortium (www.humancellatlas.org)

Our personnel, primarily from the Department of Microbiology and the Department of Biochemistry, are capable of performing sample processing and computational data analysis. We are able to run plate-based single-cell RNA sequencing (SMART-seq2), with an option for automation by Zephyr liquid robotic handler. We can also run droplet-based single-cell RNA sequencing with one 10X Chromium Controller (intended for handling BSL-3 samples) and one 10X Chromium X (capable of running high-throughput experiments).

Current capabilities that we have access to include:

- Single Cell Gene Expression (3' whole transcriptome gene expression)
- Single Cell Immune Profiling (5' whole transcriptome gene expression with paired full-length TCR/BCR sequencing)
- Single Cell ATAC (chromatin accessibility profiling)
- Single Cell Multiome ATAC + Gene Expression
- Extended capabilities in conjunction with single-cell gene expression: sample multiplexing, cell surface protein detection, antigen specificity assay, and CRISPR screening

We can also currently run microwell-based single-cell RNA and proteomic analysis with the BD Rhapsody Express System.

Prospectively, we aim to further our capability to perform spatial transcriptomics and *in situ* analysis technology.

We have Thailand's first single-cell genomics facility here at MUSC...

Mahidol University
Wisdom of the Land

Integrative Computational Bioscience Center

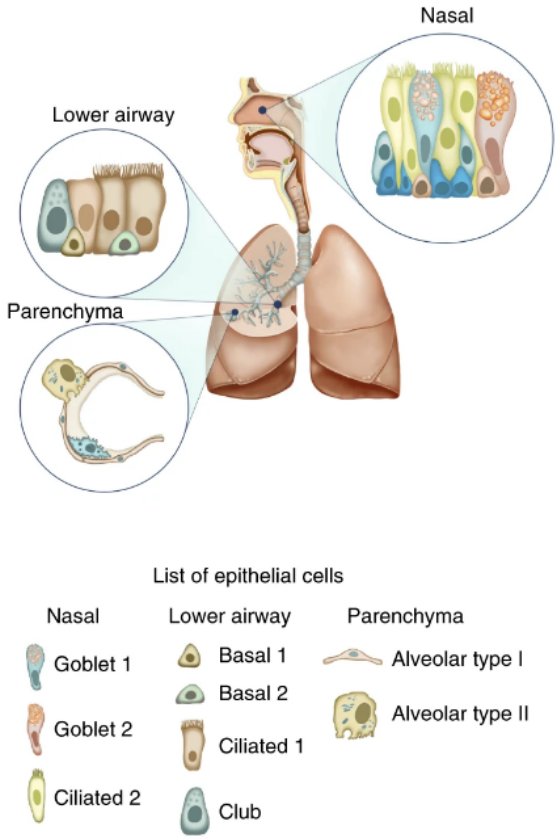
SYBID MUSC
Systemic Biology of Diseases Research Unit

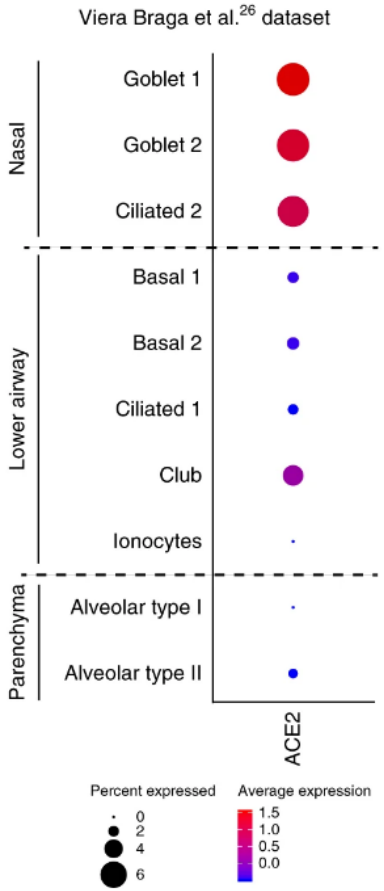
Can we use single-cell biology to better understand diseases?

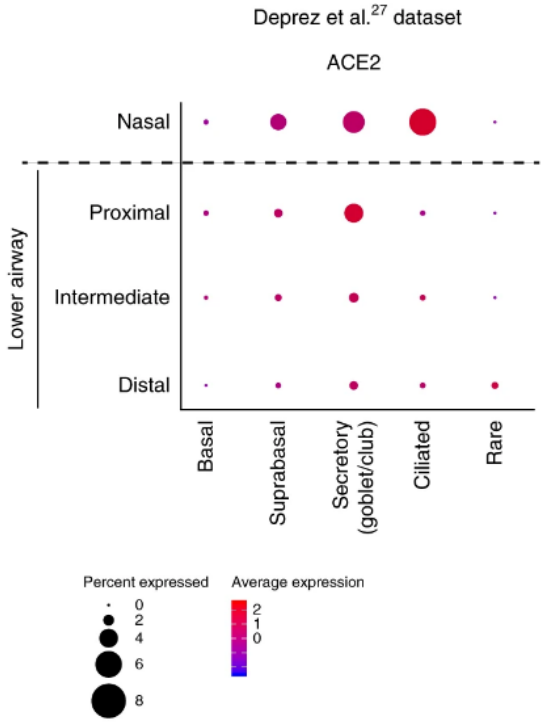
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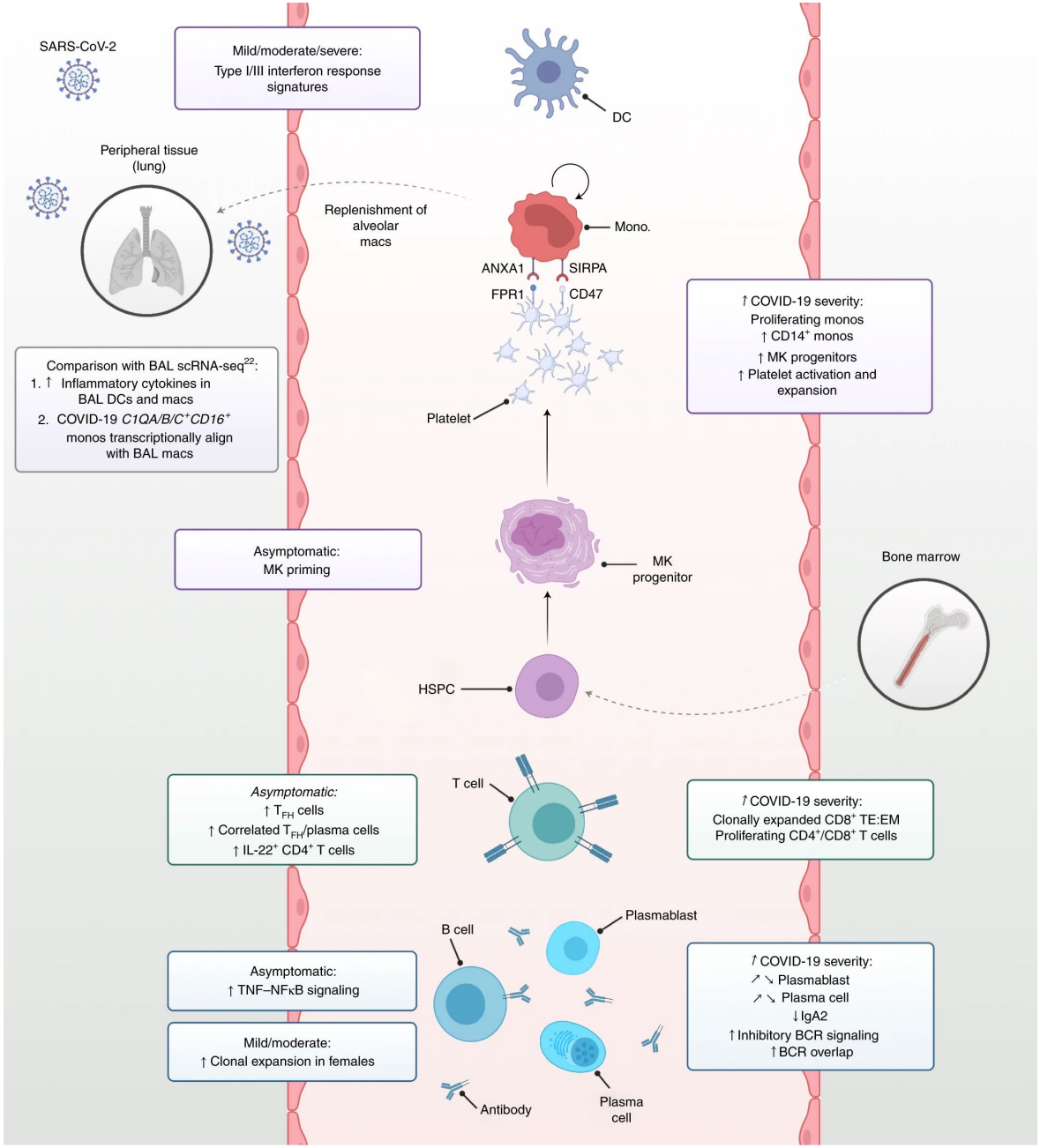
What could we learn about COVID-19 from the existing Human Cell Atlas datasets?







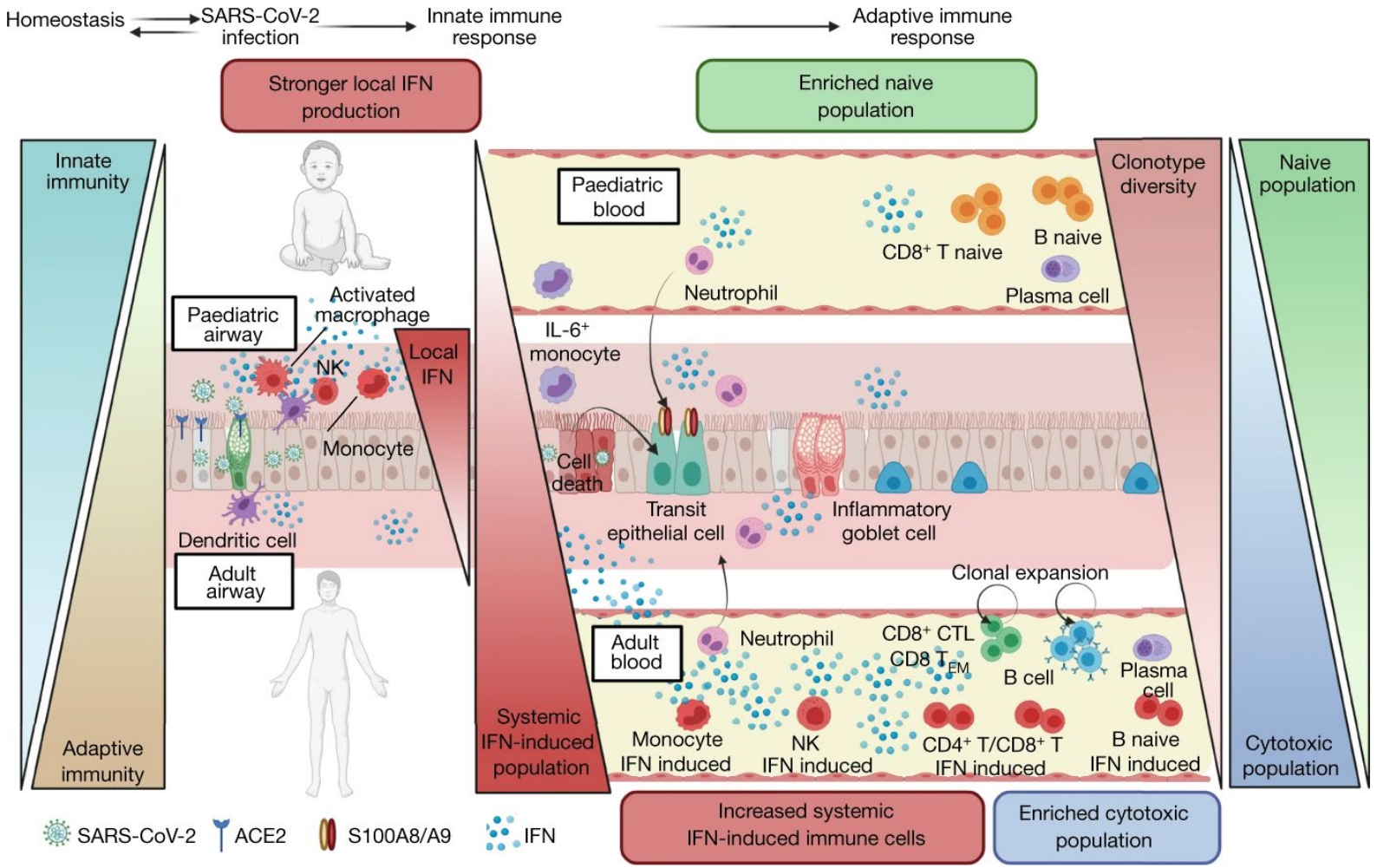
Sungnak et al. *Nature Medicine*. 2020



What are the systemic immune responses during acute SARS-CoV-2 infection across disease severity?

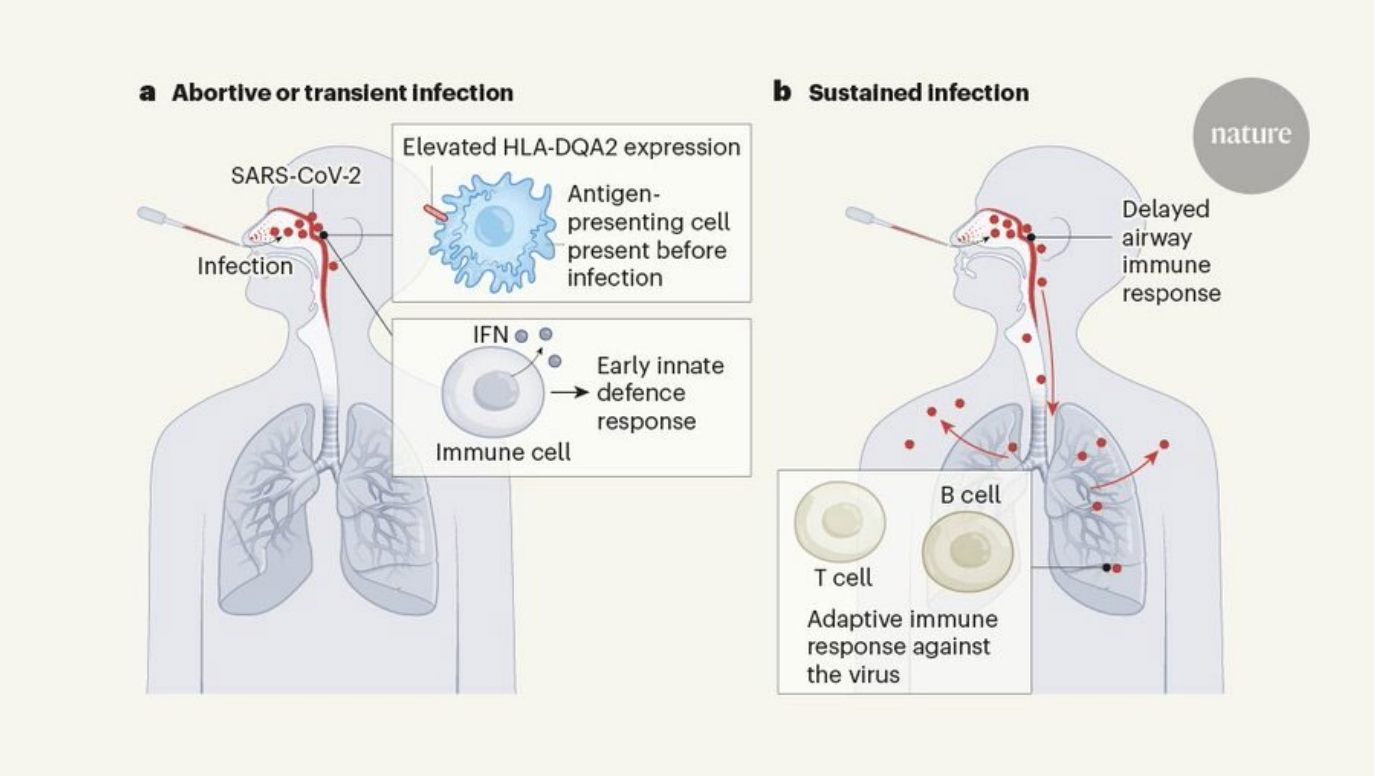
Stephenson et al. *Nature Medicine*. 2021

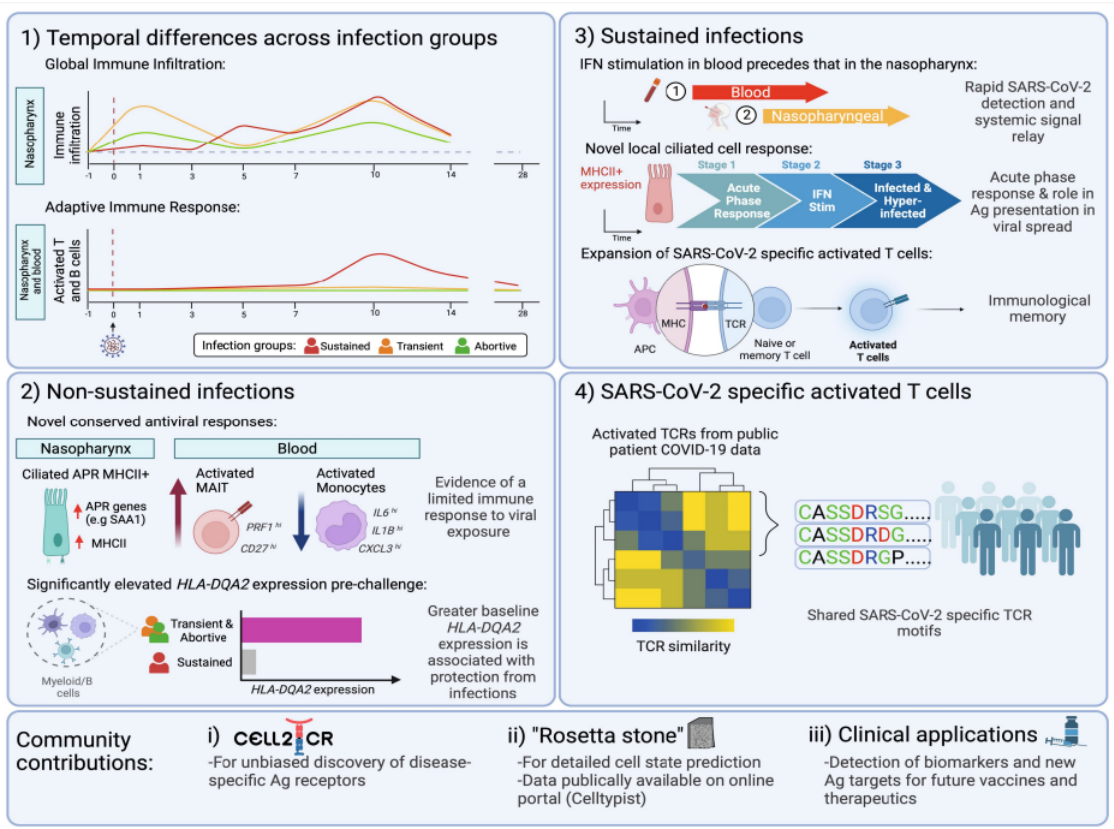
Why do kids tend to get less severe COVID-19?



Yoshida et al. *Nature*. 2022

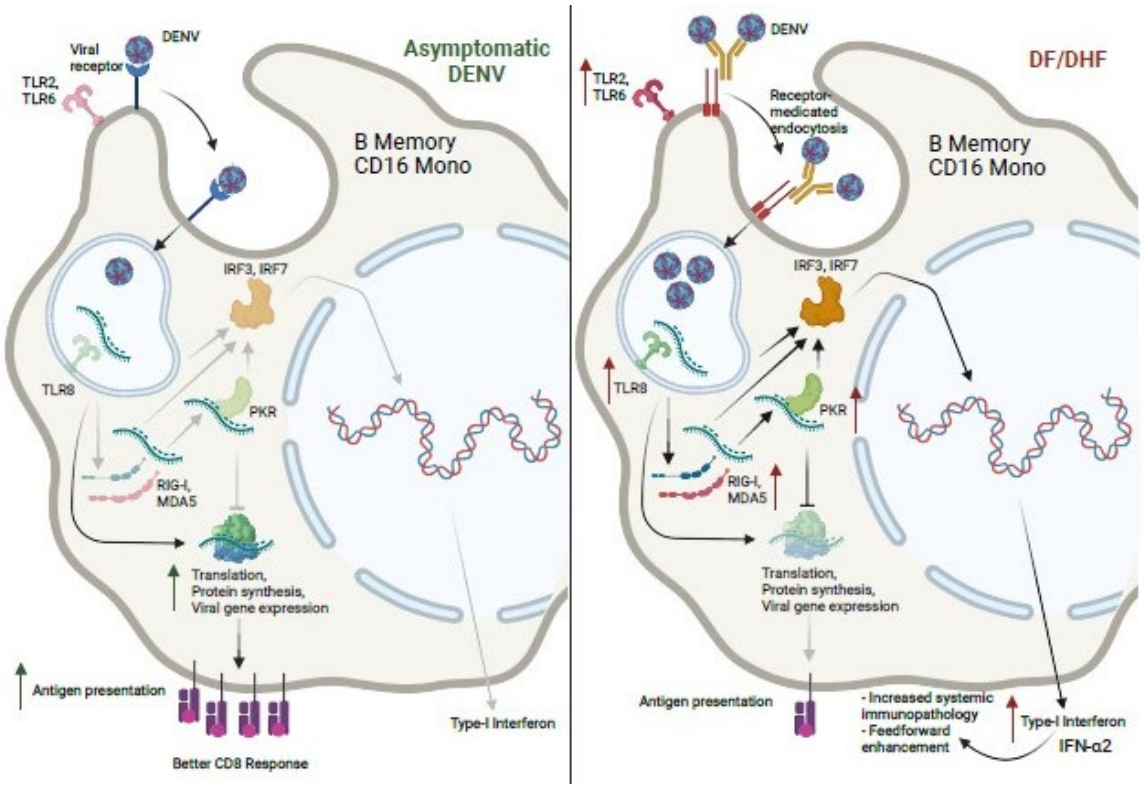
What determines whether someone gets infected or not after exposure to SARS-CoV-2?

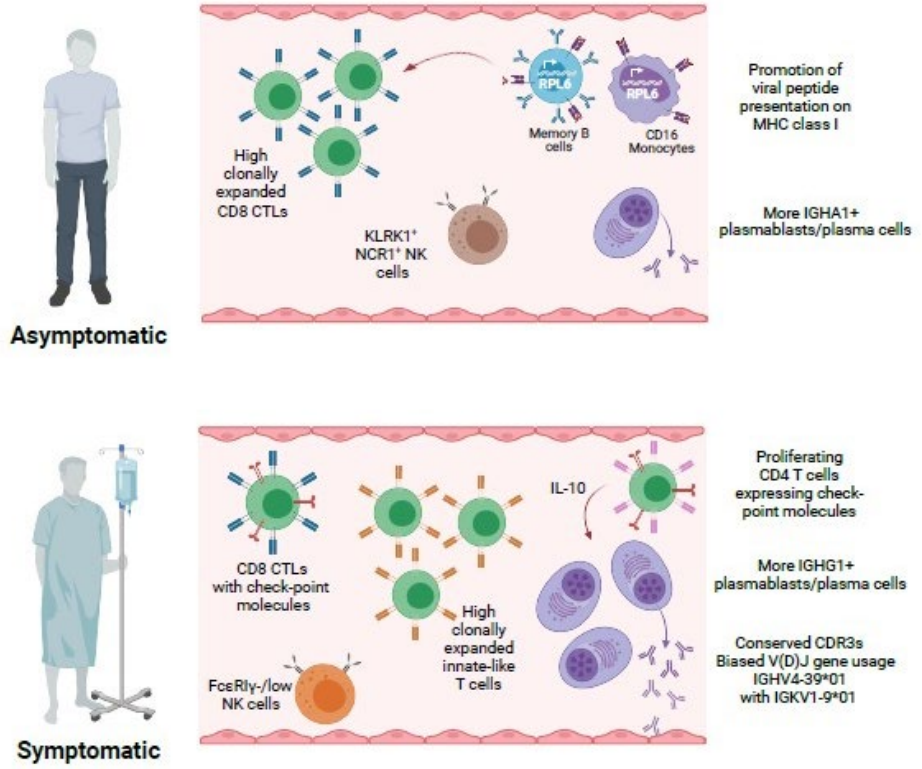




Lindeboom et al. *Nature*. 2024

What are the systemic immune responses in people with asymptomatic dengue that are distinct from those with mild/severe symptoms?



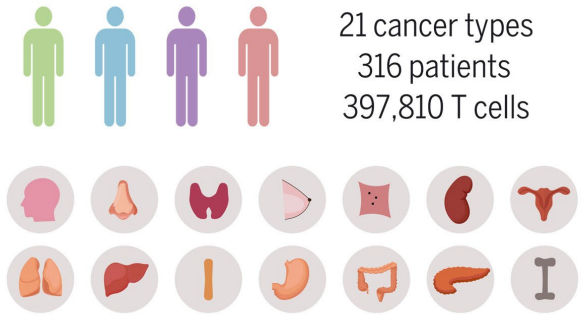


Sungnak et al. *Science Translational Medicine*. 2025

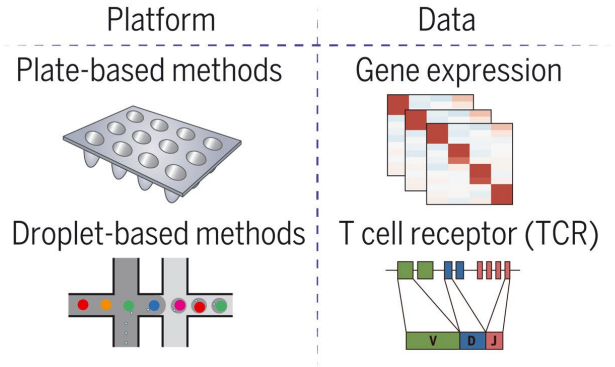
Other applications

Atlas of cancer-associated T cells

Tumors of various cancer types



Single-cell RNA-seq and TCR-seq

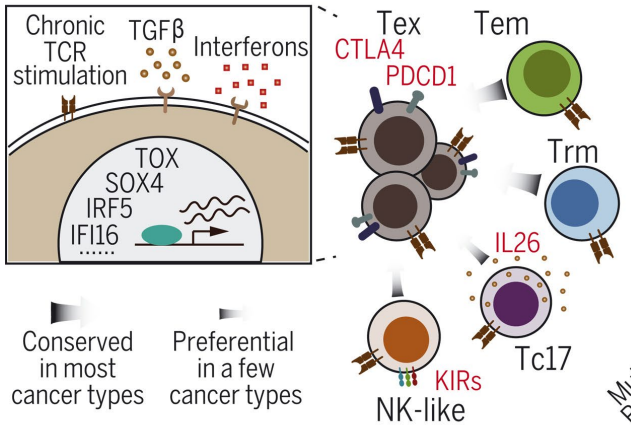


Integrated analyses

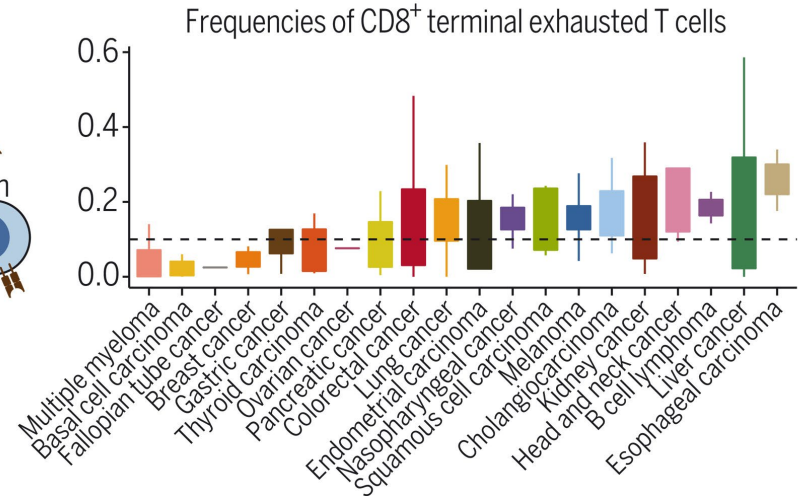
Expression characterizing and TCR tracing



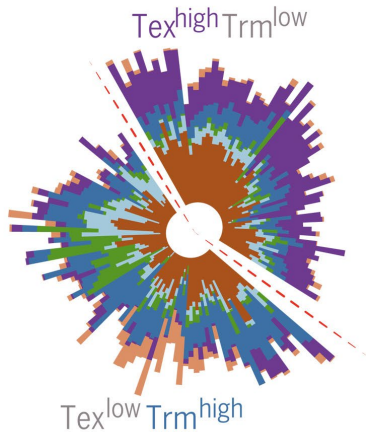
Differential usage of exhaustion paths



Comparison across cancer types

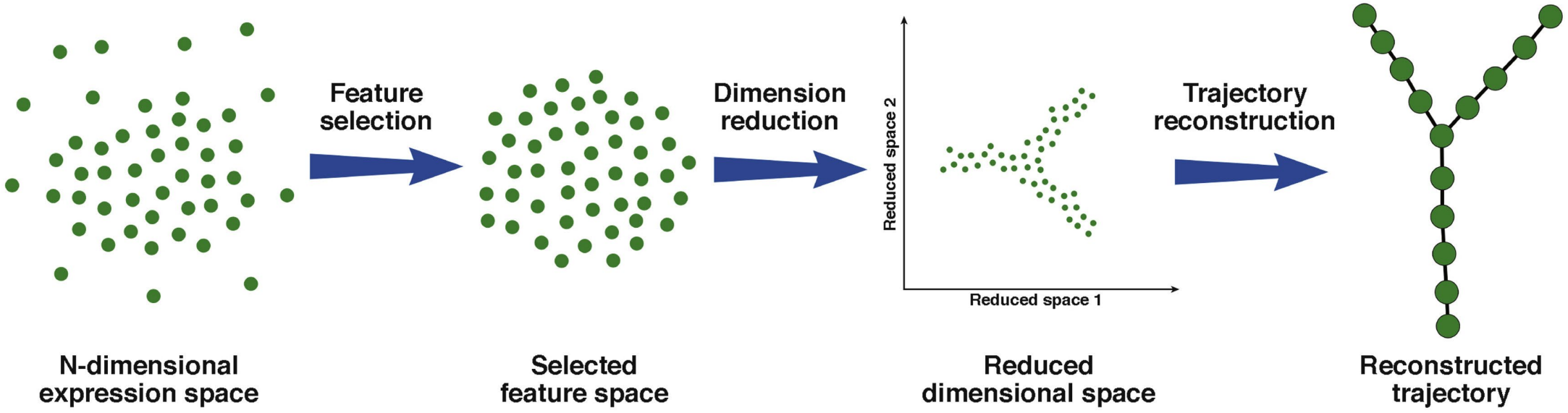


Immune-typing based on T cell compositions



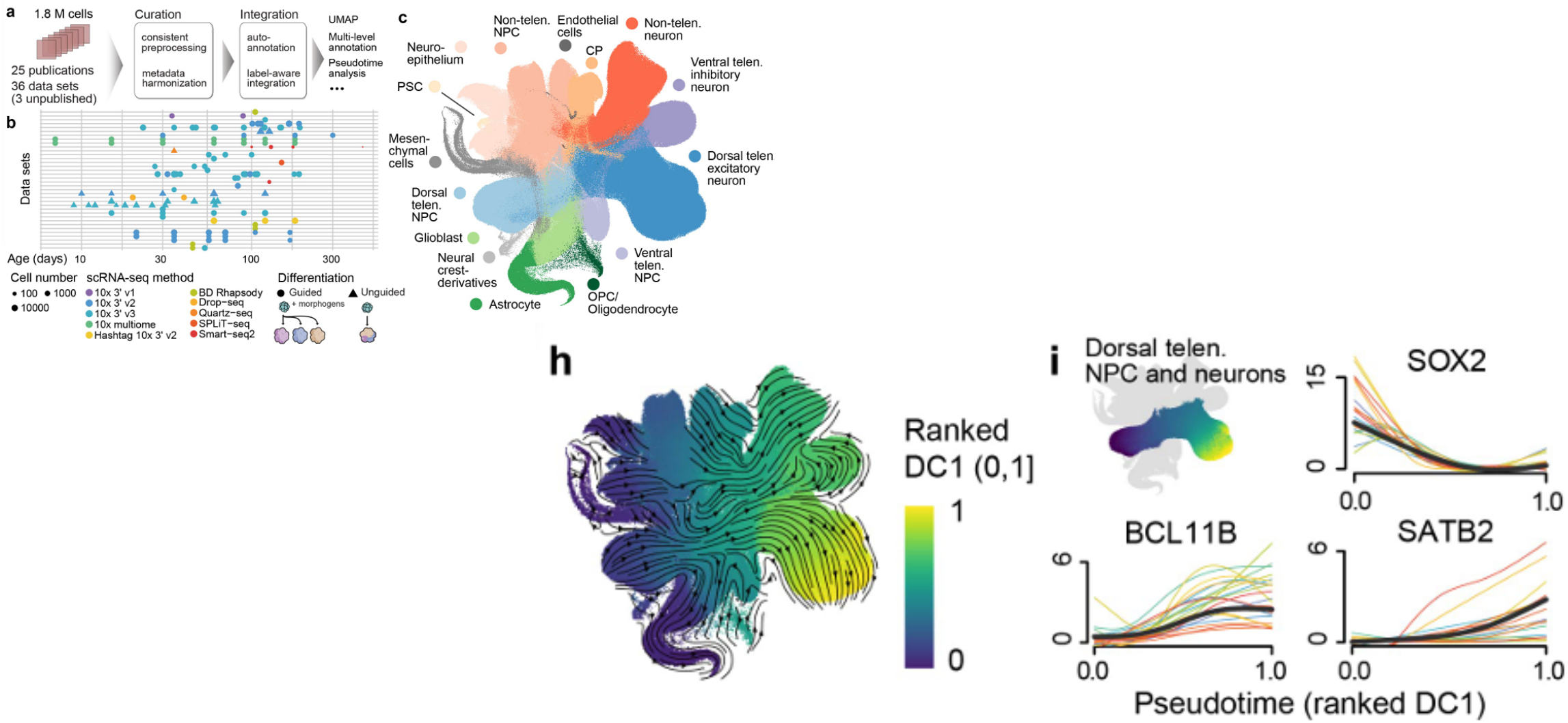
Zheng et al. *Science*. 2021

Some datasets might contain differentiating cells you can model differentiation trajectory



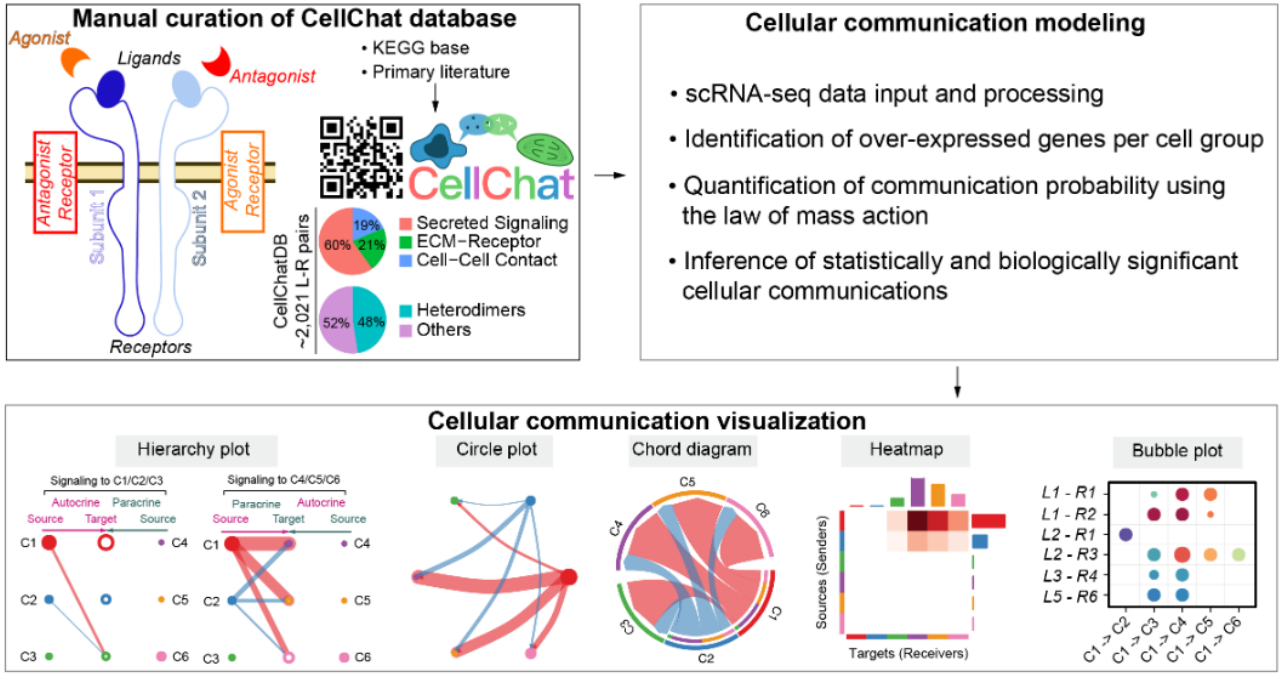
Herring et al., 2018

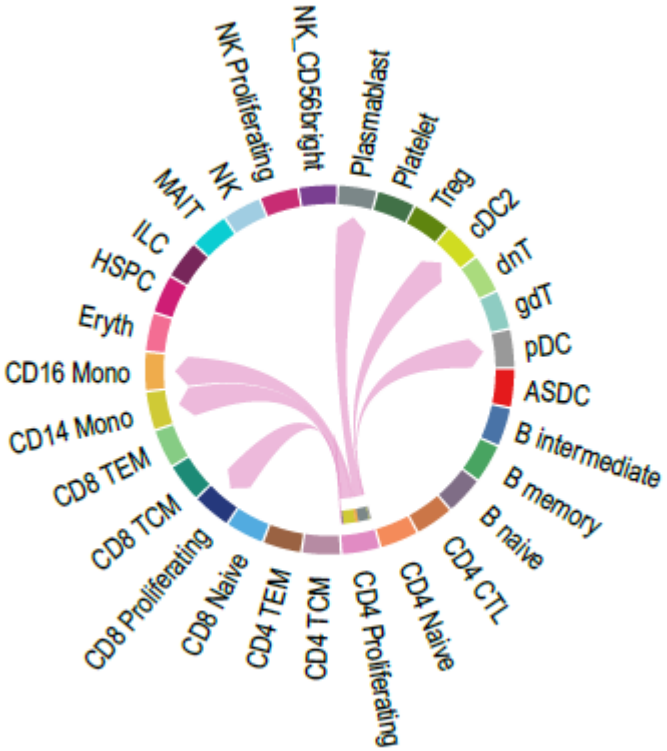
Modeling differentiation trajectory in human neural organoids



He et al., *Nature*, 2024

Modeling cell-cell communication

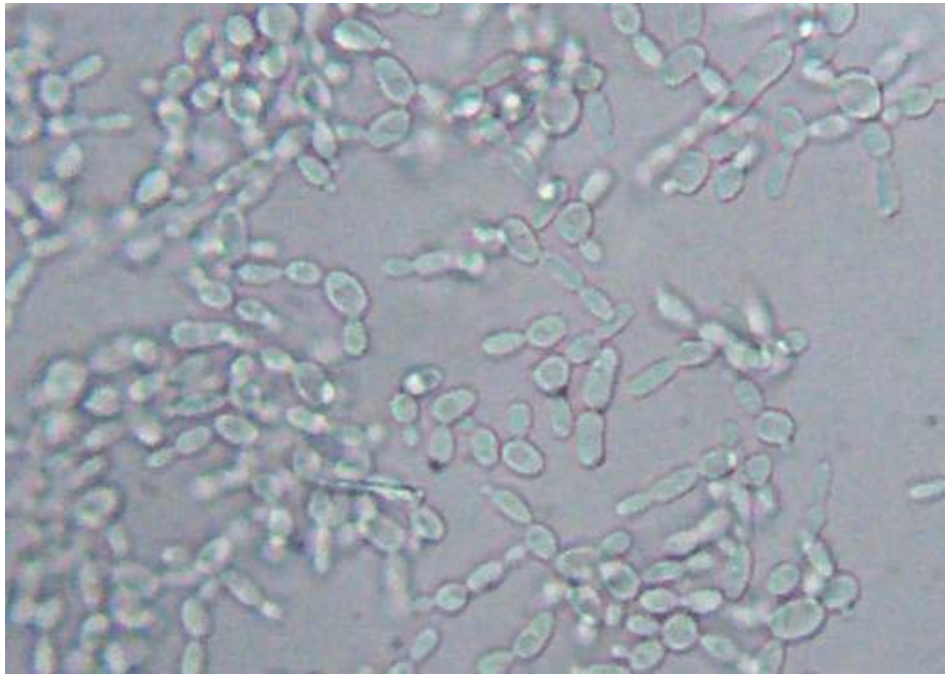




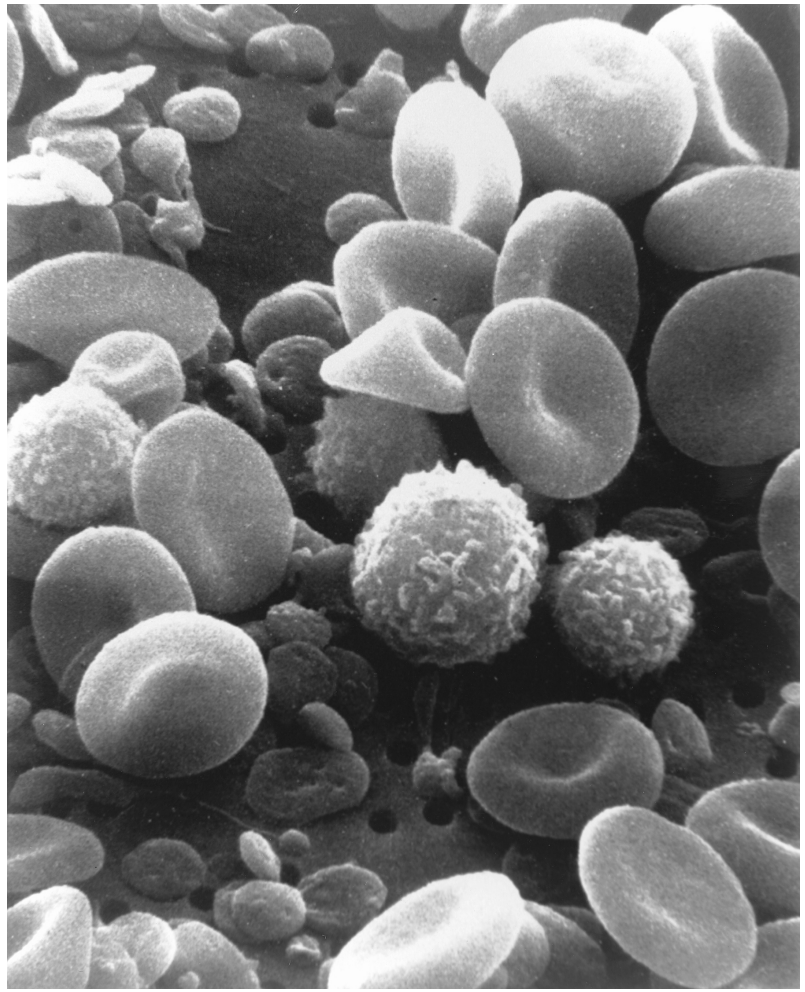
Example tools: CellChat, CellPhoneDB

Modeling IL-10/IL-10R communication during a viral infection

Microscopy: a powerful tool to study cells



https://commons.wikimedia.org/wiki/File:Candida_wet_mount.jpg

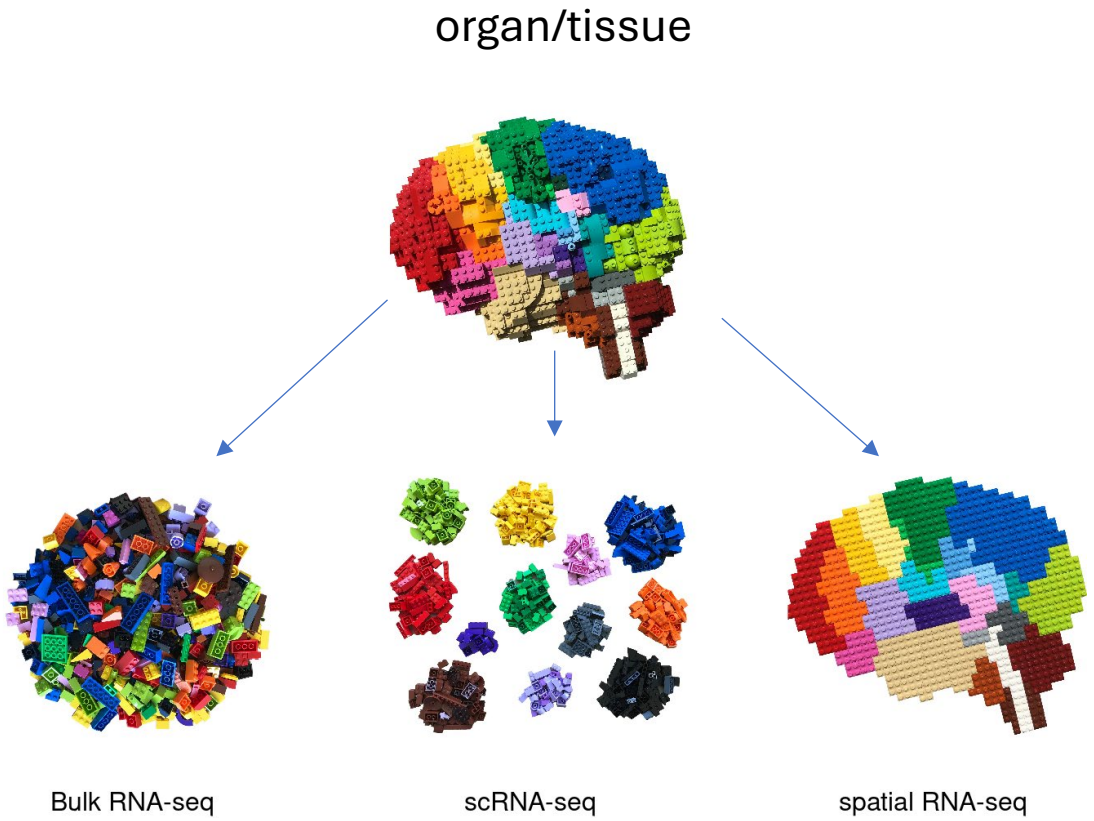


https://visualsonline.cancer.gov/details.cfm?imageid=2129

Microscopy: a powerful tool to study cells

Can we map transcriptomes with locations?

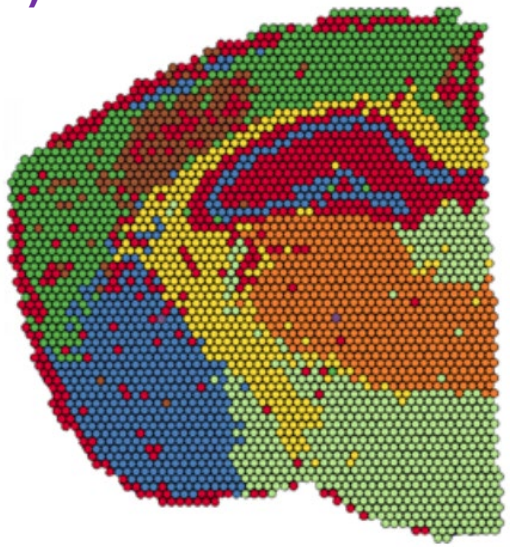
Spatial omics: omics data mapped to locations



2 Key Approaches in Spatial Transcriptomics

Sequencing-based (capture-based)

- **Unbiased/genome-wide** (no prior target selection)
- Enables discovery of **novel genes, isoforms, variants (limited)**
- Scales to **whole transcriptome across large tissue areas**

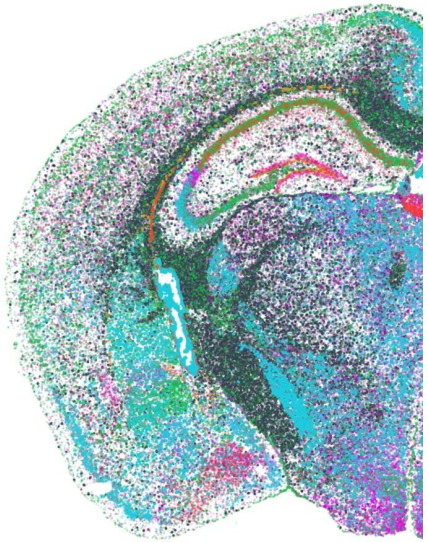


10x Visium

- Spatial resolution still **spot-based (multi-cell to near single-cell)**
- **Cell boundaries inferred computationally**, not directly measured
- Lower molecular sensitivity per transcript

Imaging-based (FISH / in situ hybridization)

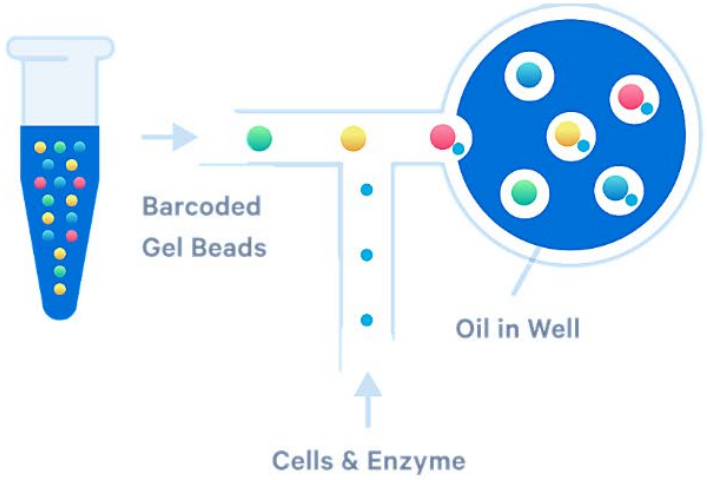
- **Single-molecule sensitivity** (counts individual transcripts)
- **Subcellular resolution** (true single-cell/intracellular localization)
- Cell boundaries defined via **segmentation**



Vizgen (MERSCOPE)

- Requires **predefined gene panel** (targeted)
- Panel size improving (100s → 1000s), but still not fully unbiased
- Optical crowding / decoding complexity
- Typically **lower throughput (area or gene space trade-off)**

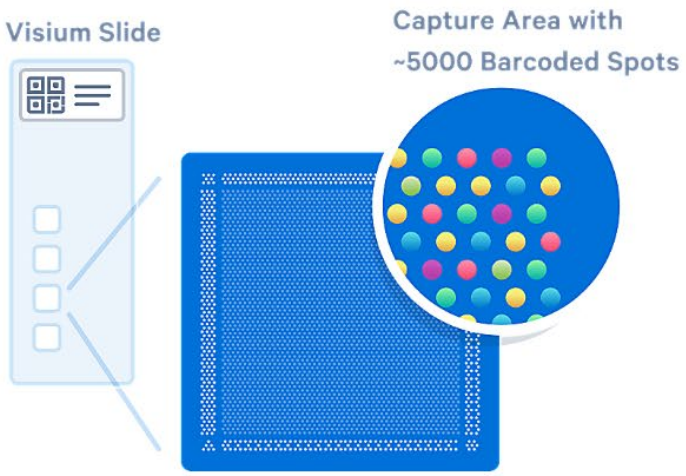
Sequencing-based Visium from 10X Genomics



Chromium

Our Next GEM technology enables analysis of individual biological components at scale.

Learn about Chromium ›



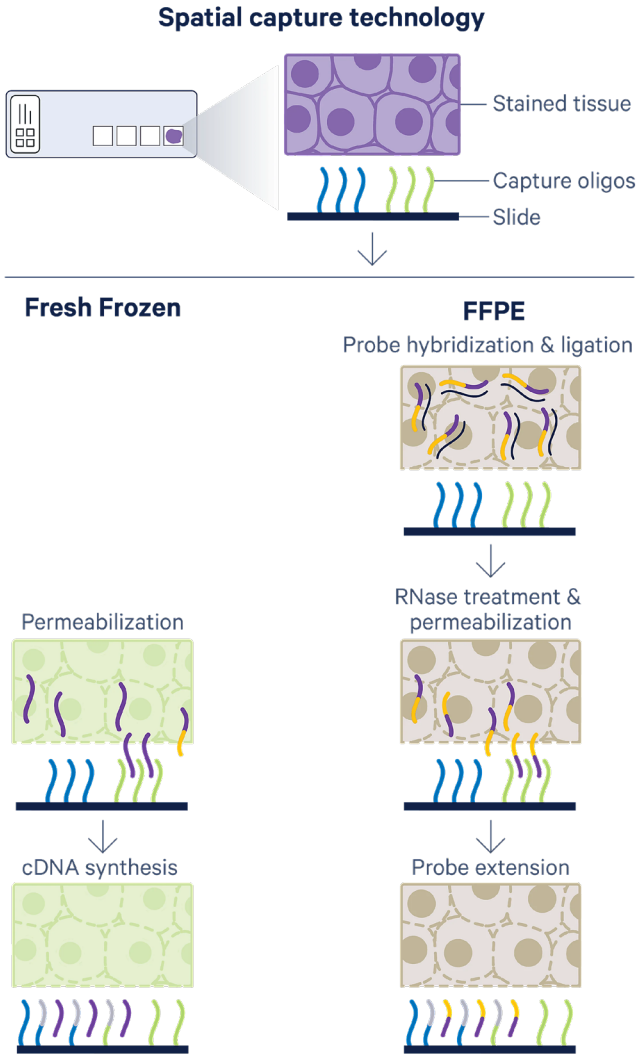
Visium

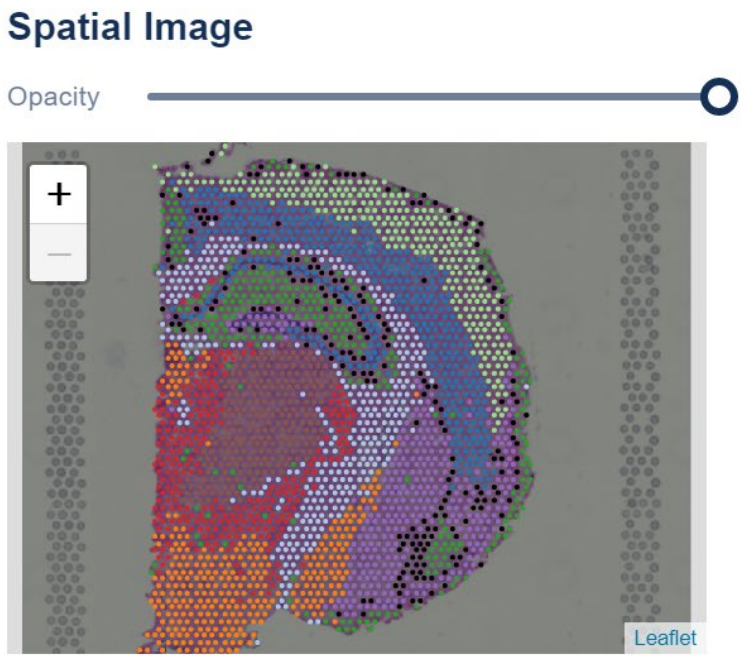
Our spatial capture technology enables analysis of mRNA molecules within their morphological context.

Learn about Visium ›

Image credit: 10X genomics

Spatial transcriptomics





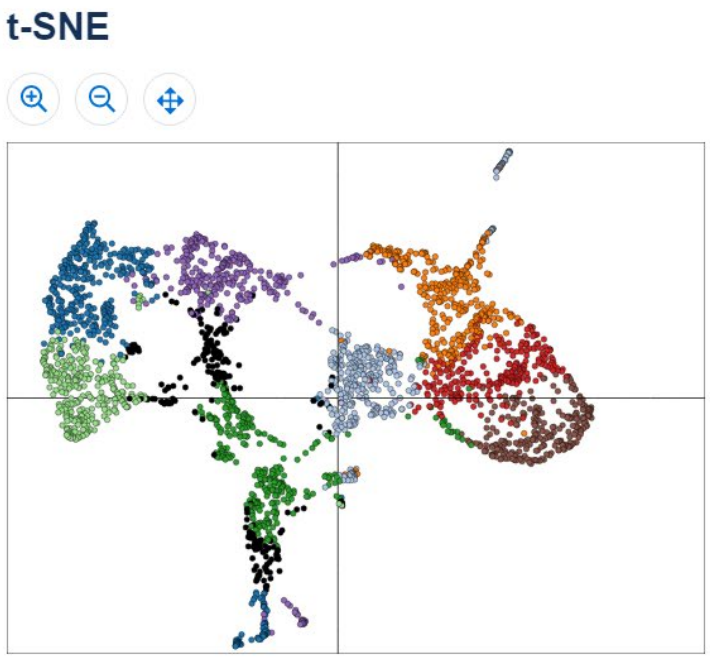


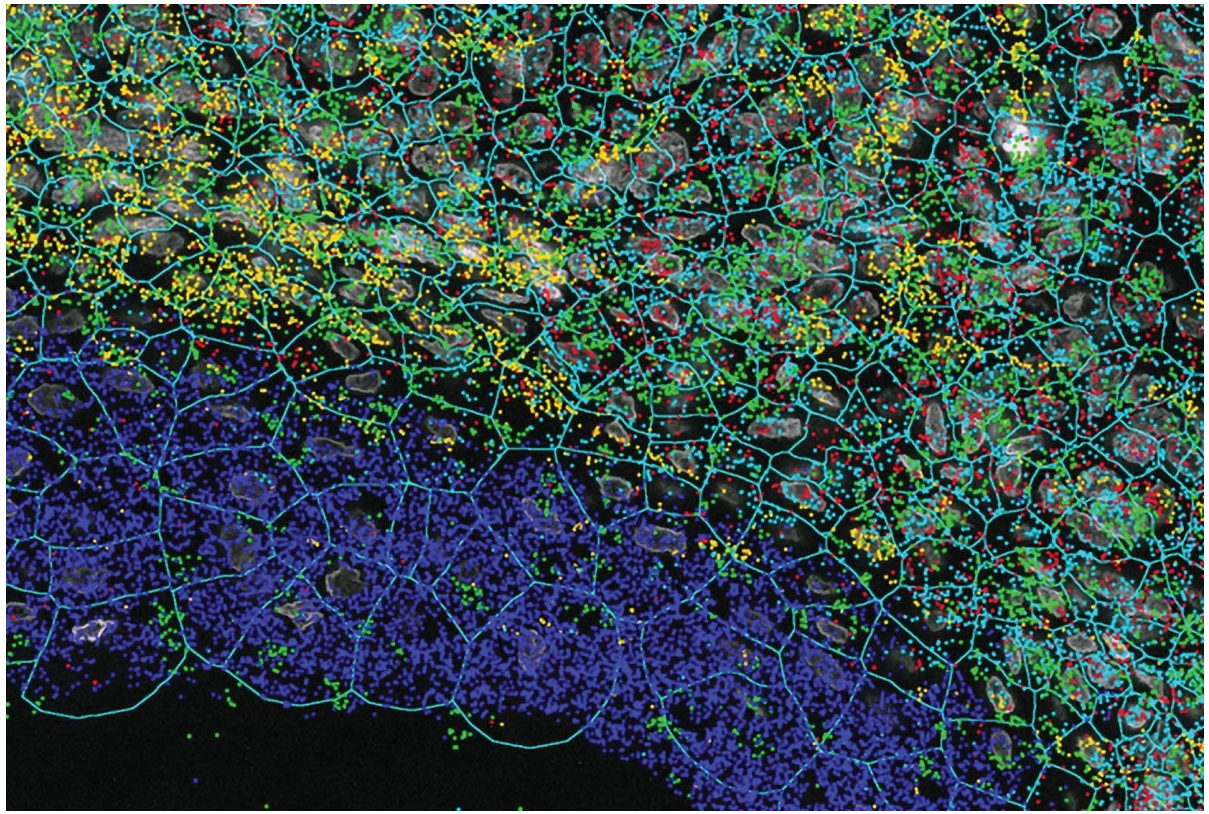
Image credit: 10X genomics

The Visium Spatial Gene Expression Solution

Unravel biological architecture in normal and diseased tissue and discover new biomarkers

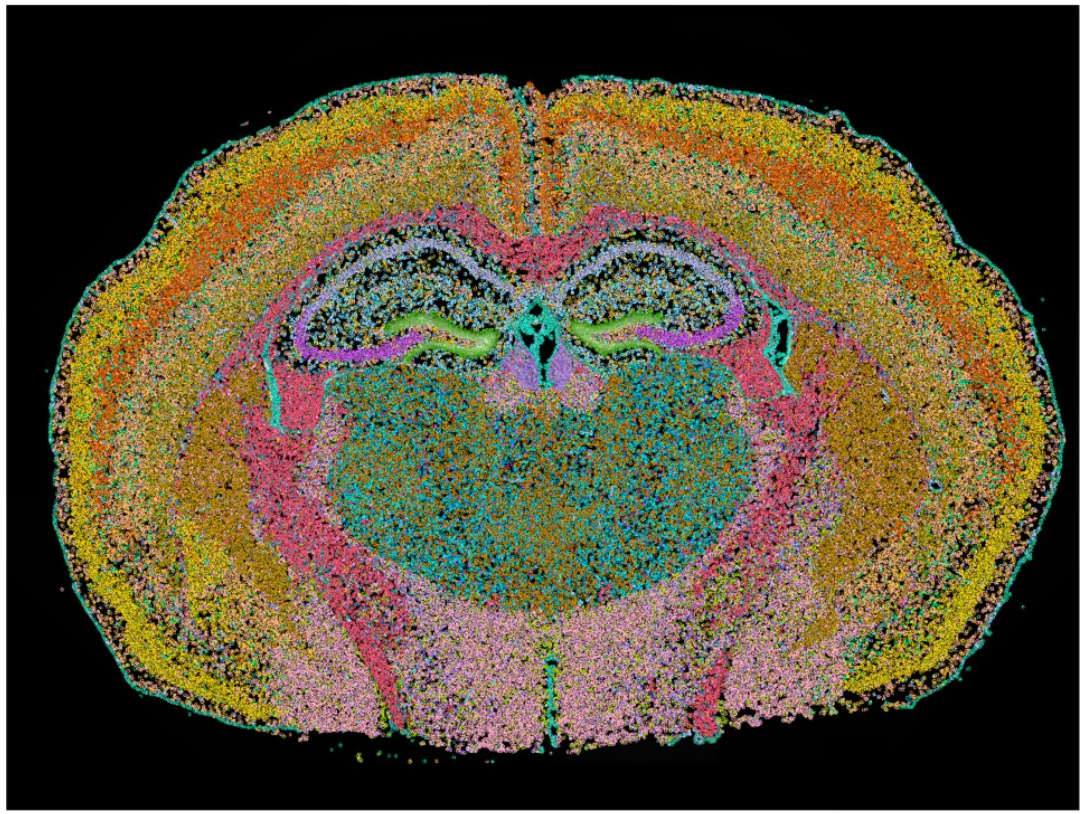


FISH-based spatial transcriptomics: down to sub-cellular profiling



CosMx

Image credit: Nanostring



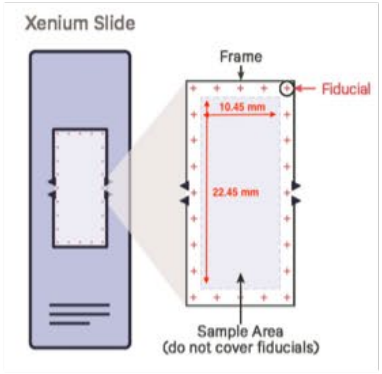
Xenium

Image credit: 10X genomics

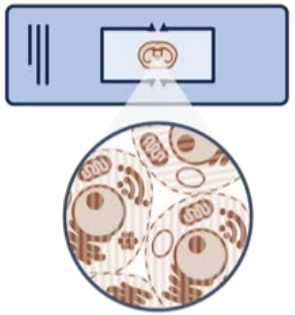
Sample Preparation

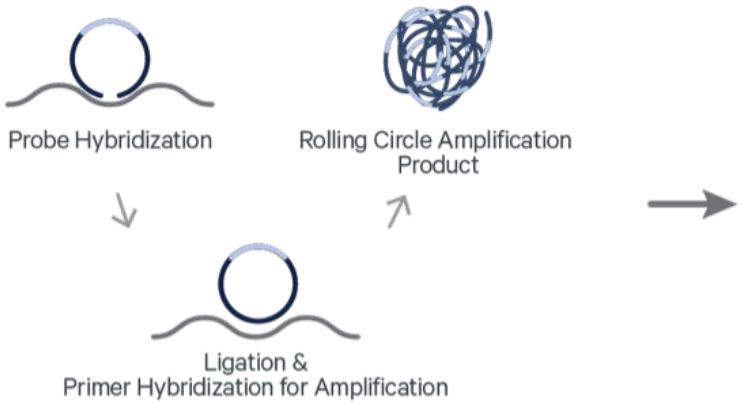
Probe Hybridization, Ligation, & Amplification

FF or FFPE Tissue Sections
on Xenium slides



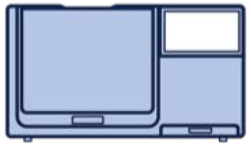
Fixation & Permeabilization (FF) or Deparaffinization & Decrosslinking (FFPE)



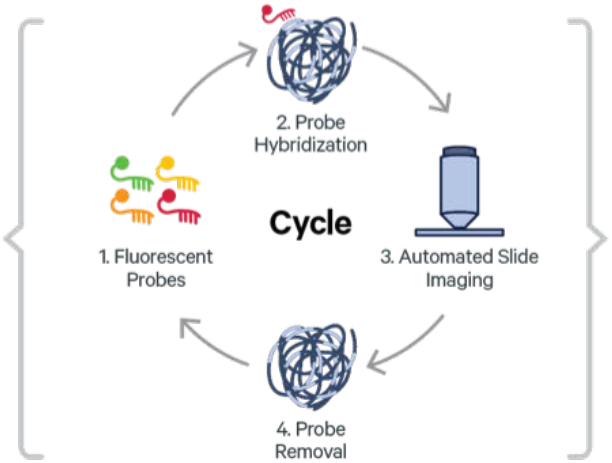


Fluorescent Probe Hybridization, Imaging, & Decoding

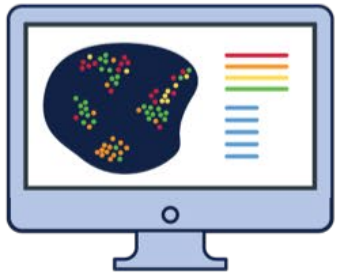
Data Visualization



Xenium Analyzer

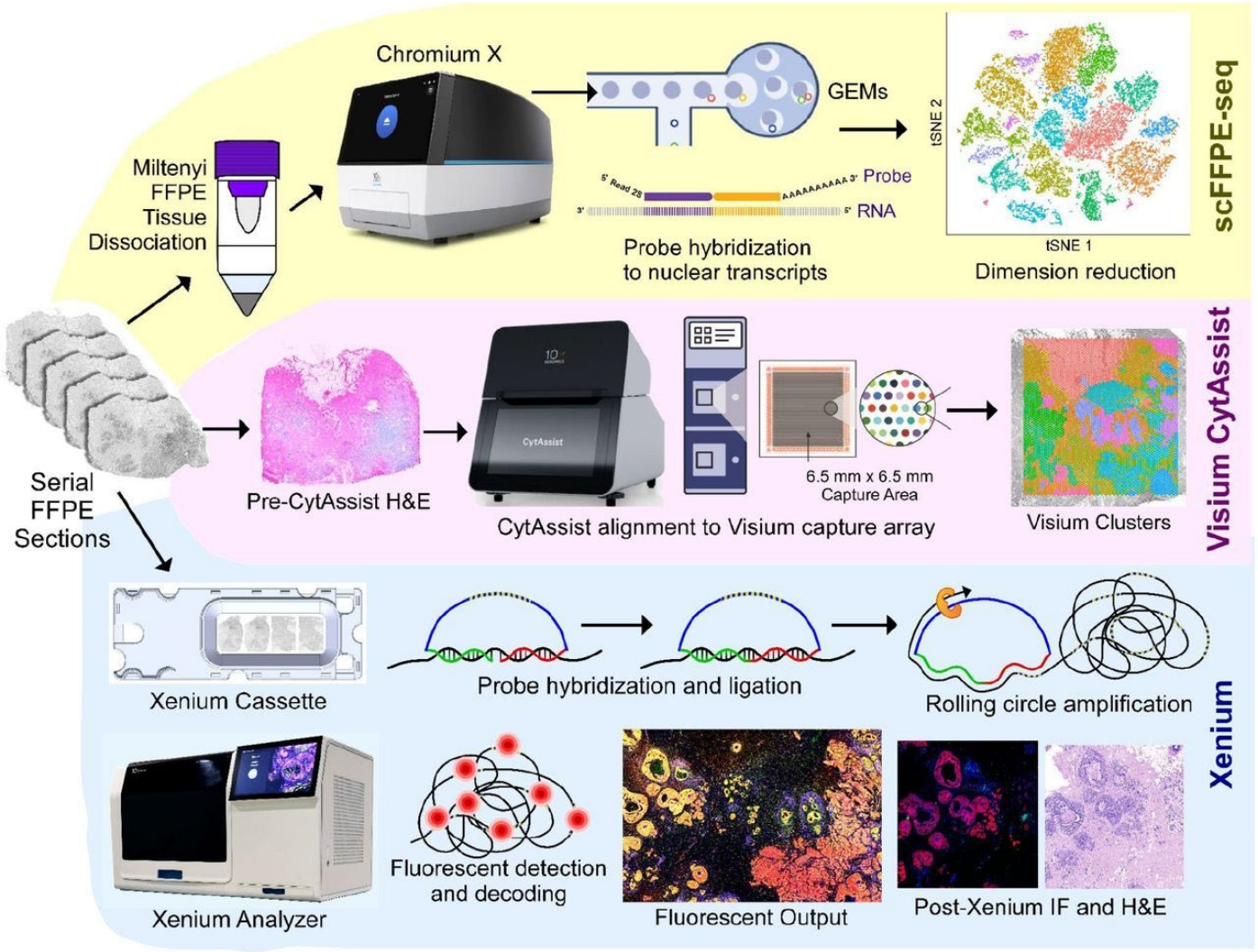








Integration with spatial omics: the new frontier – AI & image processing?

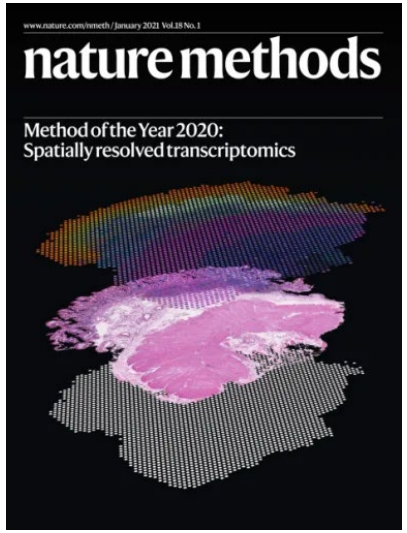


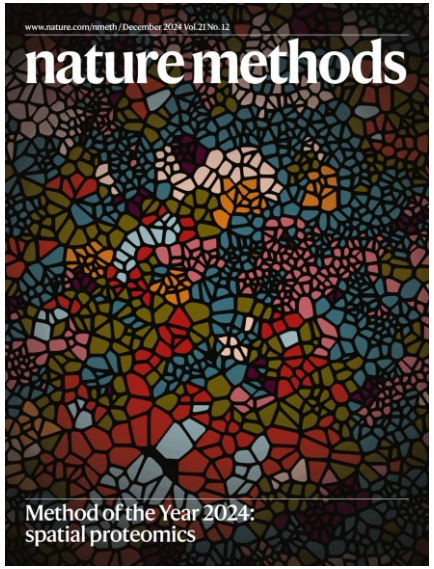
Janesick, Z. et al. *Nat Comm*. 2023

The new frontiers in omics approaches: single-cell omics and spatial omics









Nature Methods named **single-cell sequencing**, **single-cell multi-omics**, **spatial transcriptomics**, and **spatial proteomics** as methods of the year in 2013, 2019, 2020, and 2024 respectively