



Wrap-up Discussion

12212859 Sijie Li

Summary | Motivation and Method



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SOUTHERN UNIVERSITY OF SCIENCE AND TECHNOLOGY

生物医学工程系
Department of Biomedical Engineering

Single-cell Technologies

Motivation

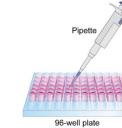


RNA sequencing: 2 Major Techniques

Separate → Label → Sequence

Well-based

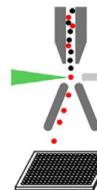
Smart-Seq



Full length transcripts
→ detect isoform



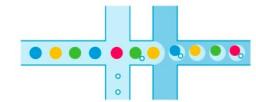
Capture **every cell**
→ capture small & rare cell



High sensitivity
→ Detect more genes in one cell
6,000 ~ 10,000 genes/cell (well-based)
1,000 ~ 4,000 genes/cell (drop-based)

Drop-based

10X Chromium



One end transcripts
→ 3' or 5' bias



Capture rate depends
on cell size & shape

High Throughput

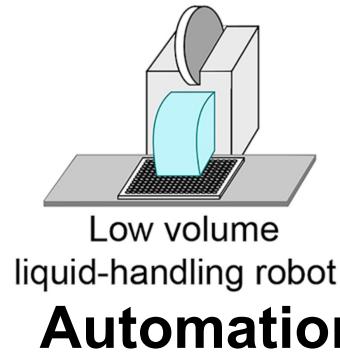
→ Detect more cells in one runs
100s cells/plate (well-based)
10,000s cells/lane (drop-based)

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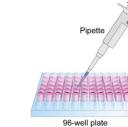


logies

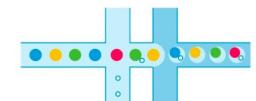
NA sequencing: 2 Major Techniques

Separate → Label → Sequence

Well-based
Smart-Seq



Drop-based
10X Chromium



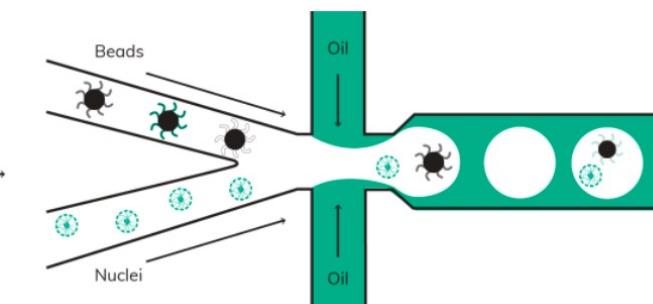
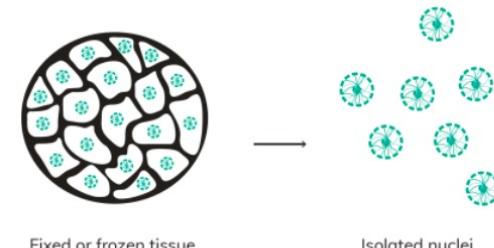
Do not fragment → Nanopore/PacBio Sequencing

One end transcripts
→ 3' or 5' bias



Single-nucleus RNA-seq

Better reflect cell composition of sample



Capture rate depends
on cell size & shape

High Throughput

→ Detect more cells in one runs
100s cells/plate (well-based)
10,000s cells/lane (drop-based)

Beyond These 2 Techniques



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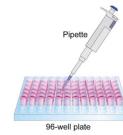
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Single-cell Technologies

RNA sequencing

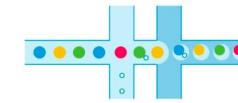
Well-based

Smart-Seq



Drop-based

10X Chromium



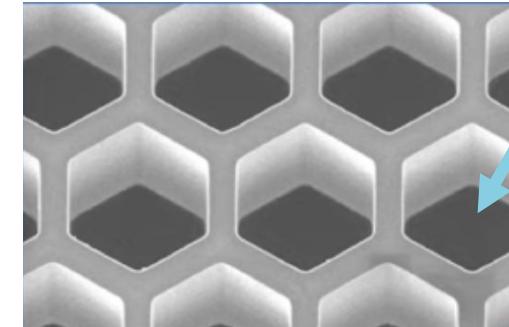
- Blue: Beads
- Red: Cells

Microwell-based

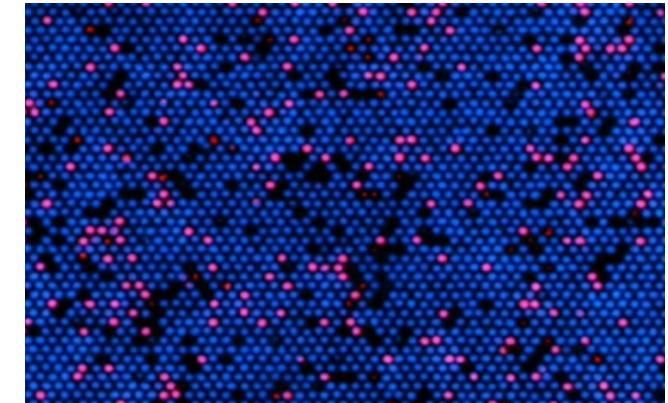
Seqwell, Celsee



$d = 10\text{s}\sim100\text{s } \mu\text{m}$



Beads with
Barcode and
reaction
agents



Beyond These 2 Techniques

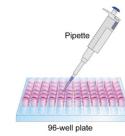


Single-cell Technologies

RNA sequencing

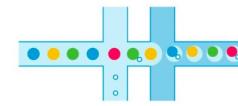
Well-based

Smart-Seq



Drop-based

10X Chromium



Microwell-based

Seqwell, Celsee

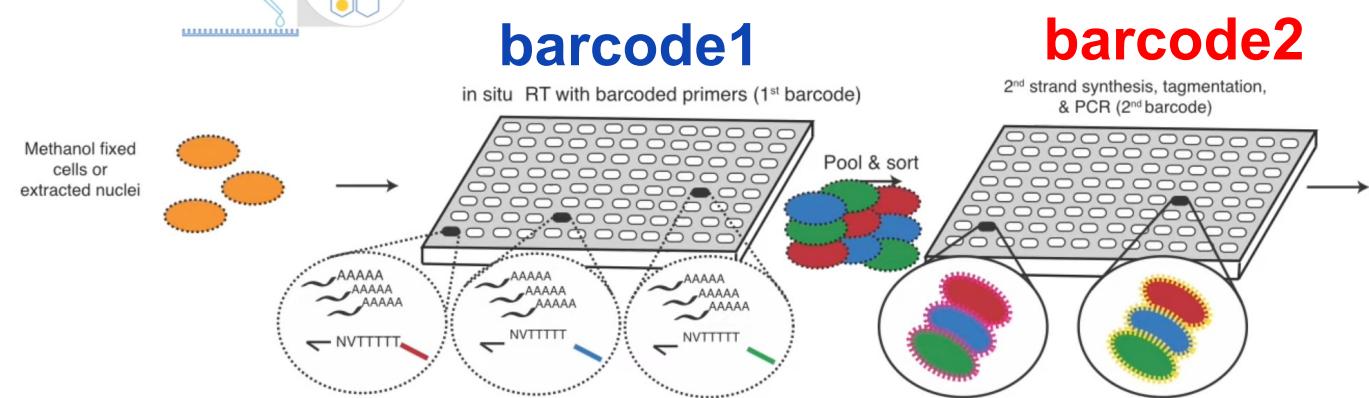


👍 Use different ways to separate cells → Lower hardware (microfluidic) barrier and cost...

Combinatorial Indexing

SPLiT-seq

Unique combination of
(barcode1,barcode2)



Cao et al., Science 2017

Beyond These 2 Techniques



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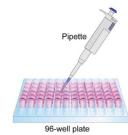
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Single-cell Technologies

RNA sequencing

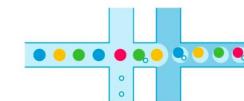
Well-based

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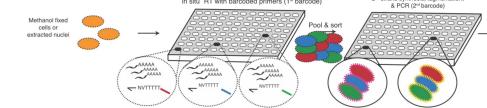
Microwell-based

Seqwell, Celsee



Combinatorial Indexing

SPLiT-seq



Not only scRNA

scProtein Quantifying

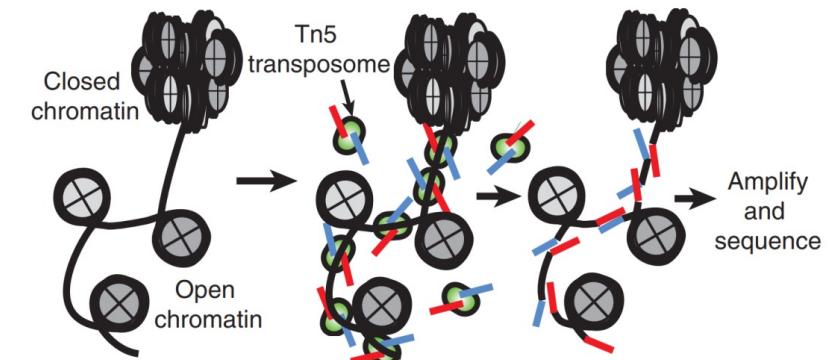
CITE-seq (RNA+protein: multi-omic)



Stoeckius et al., Nature Methods 2017

scChromatin accessibility

scATAC-seq



Beyond These 2 Techniques



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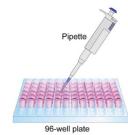
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Single-cell Technologies

RNA sequencing

Well-based

Smart-Seq



Drop-based

10X Chromium



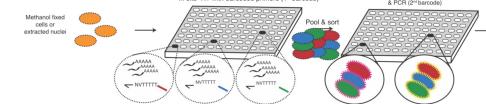
Microwell-based

Seqwell, Celsee



Combinatorial Indexing

SPLiT-seq



Not only scRNA

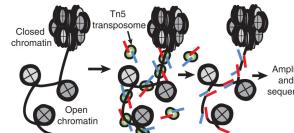
scProtein Quantifying

CITE-seq



scChromatin accessibility

scATAC-seq

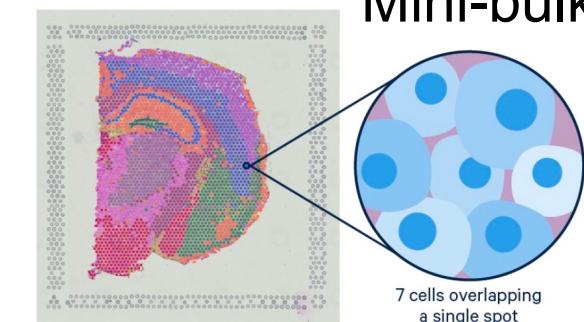
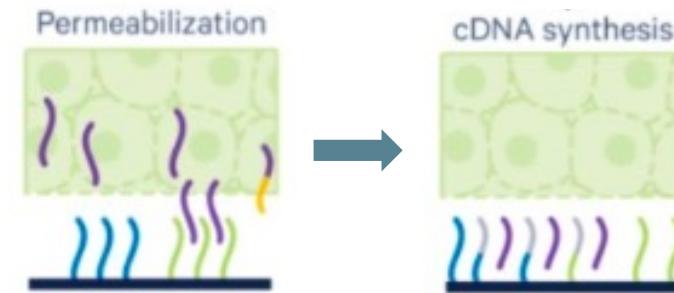


Not only substance profile

Spatial transcriptome

10X Visium, Stereo-seq (BGI genomics)

Mini-bulk



Beyond These 2 Techniques



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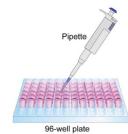
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Single-cell Technologies

RNA sequencing

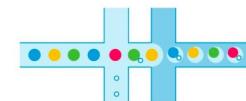
Well-based

Smart-Seq



Drop-based

10X Chromium



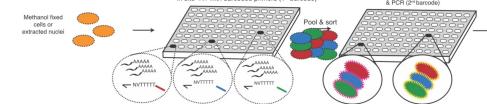
Microwell-based

Seqwell, Celsee



Combinatorial Indexing

SPLiT-seq



Not only scRNA

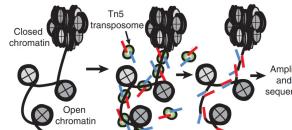
scProtein Quantifying

CITE-seq

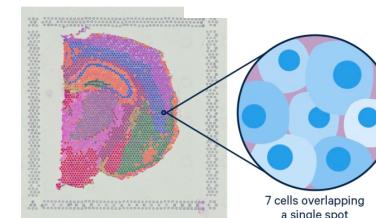
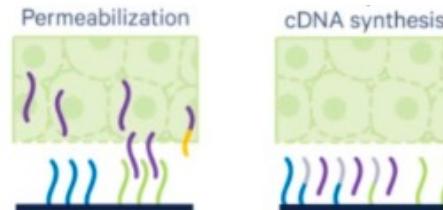


scChromatin accessibility

scATAC-seq



Spatial transcriptome



Not only sequencing

Microscopy-based

MERFISH, 10X Xenium

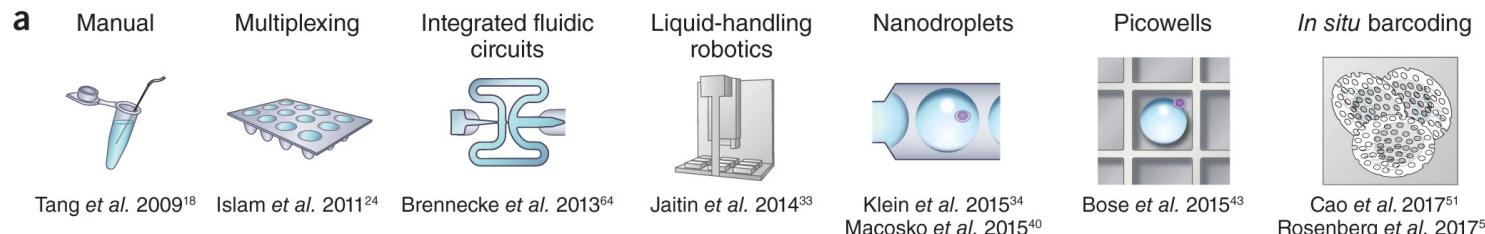
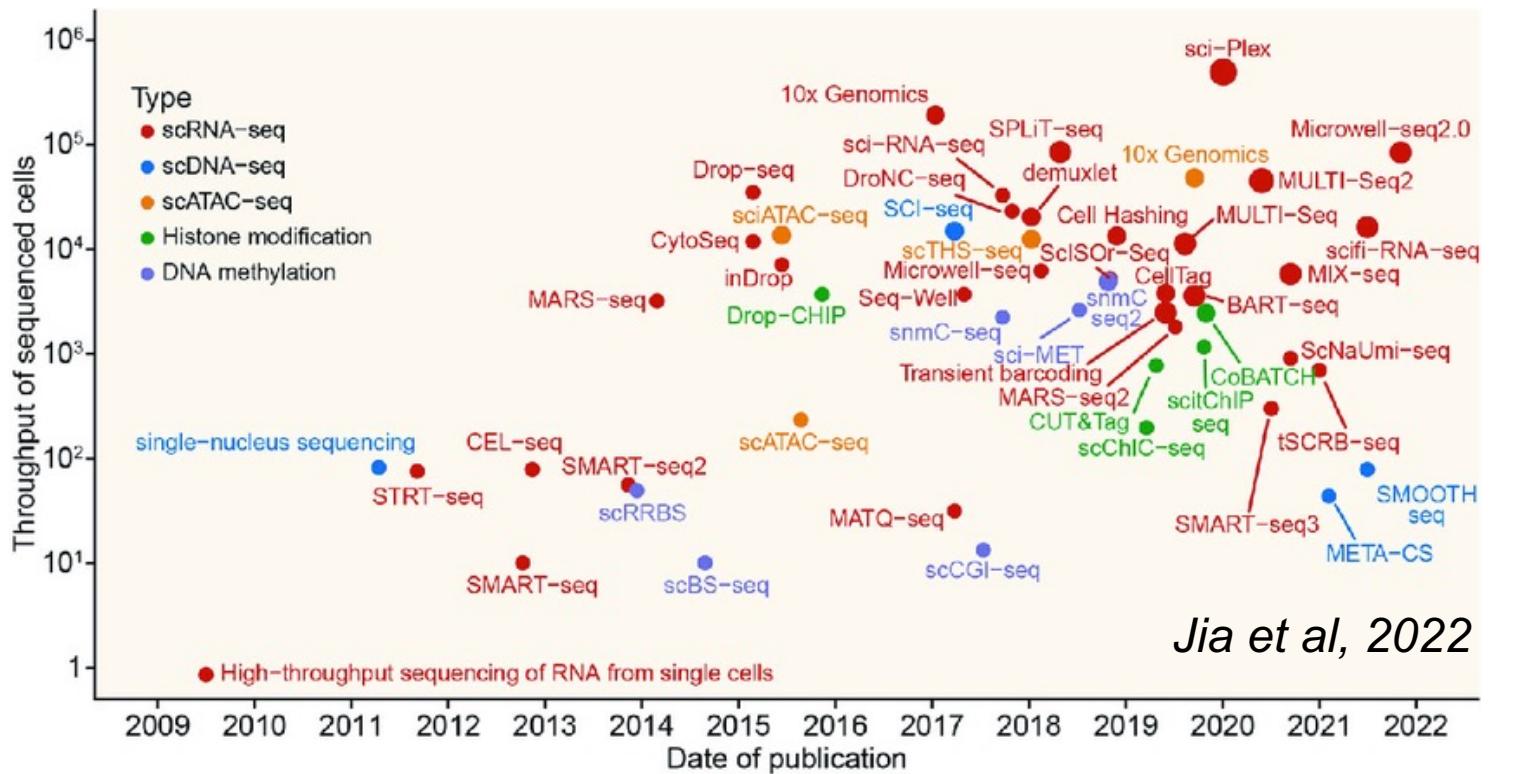


Prospect | Technologies



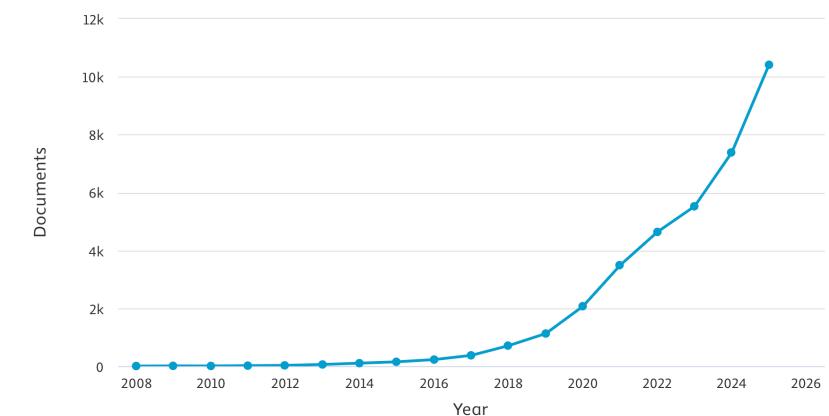
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Svensson et al, *Nature Protocols* 2018

Keyword: single-cell RNA sequencing
Scopus documents



- ↑ Throughput
- ↑ Information dimensions
- ↓ Cost

Benefit from the BME technology development: automation, miniaturization (MEMS), ...

Prospect | Applications



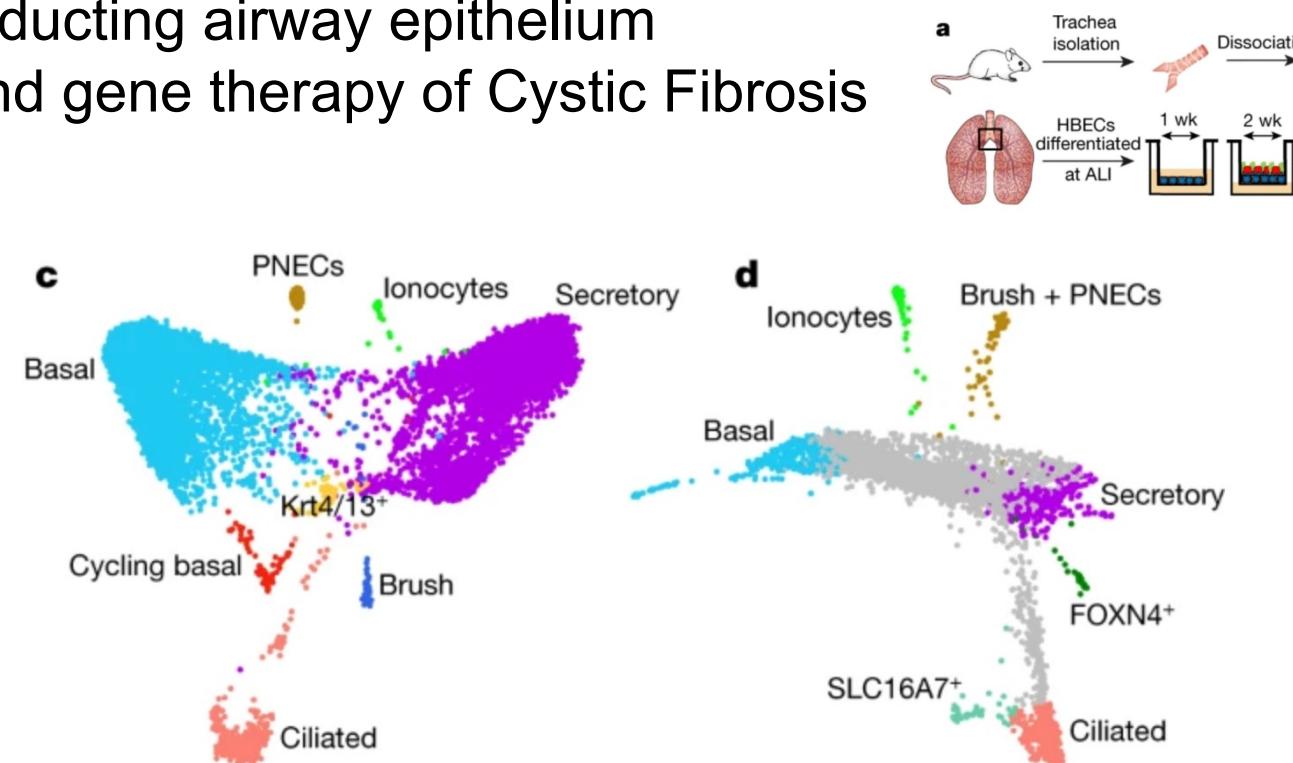
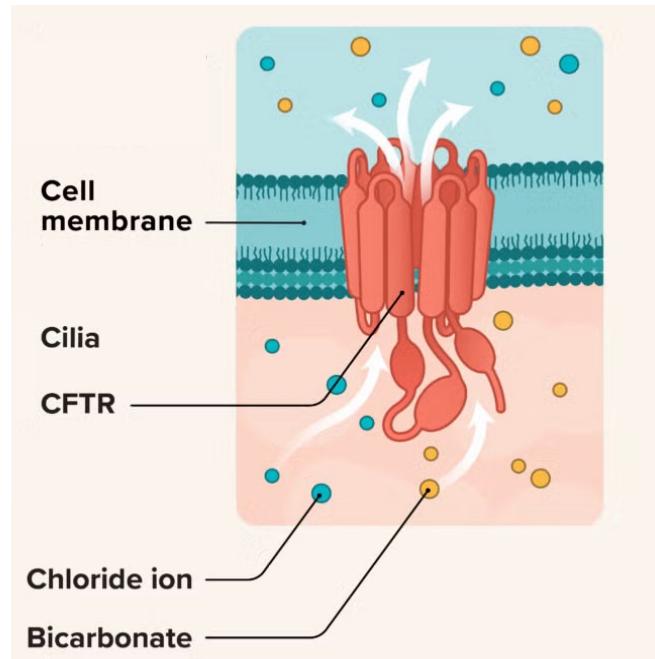
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Biological Discovery

E.g. New cell type discovery – **Ionocyte**

- **Major source of CFTR** (Cystic Fibrosis Transmembrane Conductance Regulator) **activity** in the conducting airway epithelium
- Insight into the mechanism and gene therapy of Cystic Fibrosis



<https://animalia-life.club>

Plasschaert et al., Nature 2018

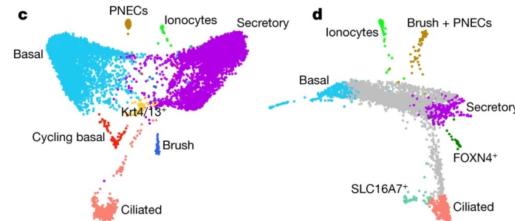
Prospect | Applications



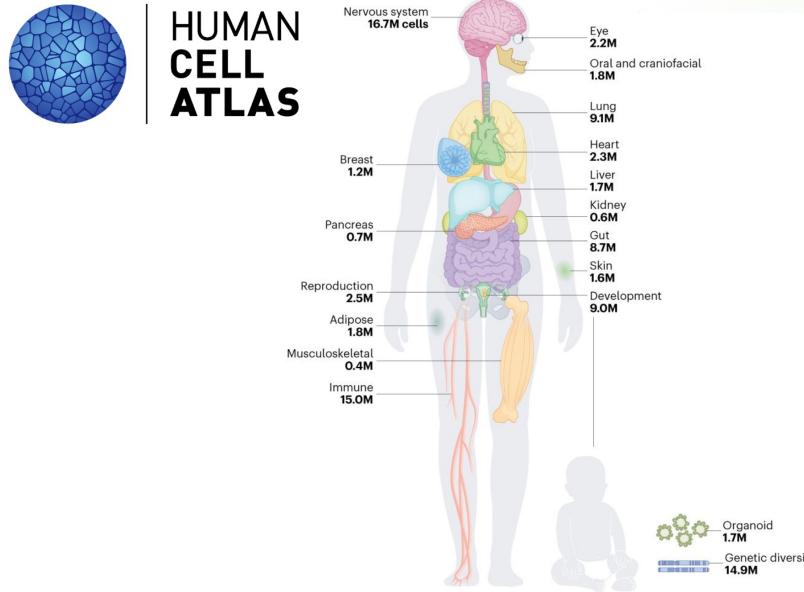
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Biological Discovery



Human Cell Atlas



With this map, we can answer:

✓ Source



✓ Celltype
✓ Location

- ✓ Development
- ✓ Interaction
- ✓ Response to perturb

Tabula Sapiens

1st draft human cell atlas of over 1.1M cells from 28 organs of 24 normal human subjects

Consortium et al., *Science* 2022



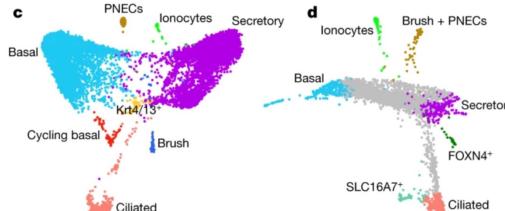
Prospect | Applications



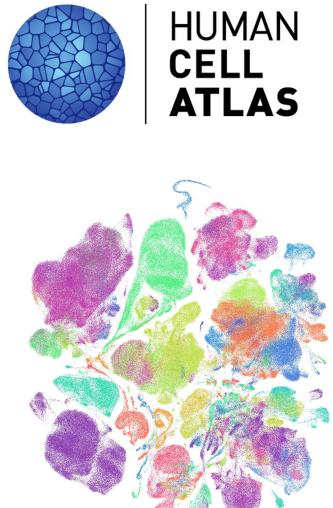
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Biological Discovery



Human Cell Atlas



HUMAN
CELL
ATLAS

Medical Application

E.g. SARS-CoV-2 entry factors

Nasal → Goblet cells and ciliated cells



Sungnak et al., *Nature Medicine* 2020



Nasal Vaccine



Nasal “mask”

Reference List



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4. Jia, Q., Chu, H., Jin, Z., Long, H., & Zhu, B. (2022). High-throughput single-cell sequencing in cancer research. *Signal Transduction and Targeted Therapy*, 7(1), 145. <https://doi.org/10.1038/s41392-022-00990-4>
5. Cao, J., Packer, J. S., Ramani, V., Cusanovich, D. A., Huynh, C., Daza, R., Qiu, X., Lee, C., Furlan, S. N., Steemers, F. J., Adey, A., Waterston, R. H., Trapnell, C., & Shendure, J. (2017). Comprehensive single-cell transcriptional profiling of a multicellular organism. *Science*, 357(6352), 661–667. <https://doi.org/10.1126/science.aam8940>
6. Plasschaert, L. W., Žilionis, R., Choo-Wing, R., Savova, V., Knehr, J., Roma, G., Klein, A. M., & Jaffe, A. B. (2018). A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature*, 560(7718), 377–381. <https://doi.org/10.1038/s41586-018-0394-6>
7. Sungnak, W., Huang, N., Bécavin, C., Berg, M., Queen, R., Litvinukova, M., Talavera-López, C., Maatz, H., Reichart, D., Sampaziotis, F., Worlock, K. B., Yoshida, M., & Barnes, J. L. (2020). SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nature Medicine*, 26(5), 681–687. <https://doi.org/10.1038/s41591-020-0868-6>
8. Stoeckius, M., Hafemeister, C., Stephenson, W., Houck-Loomis, B., Chattopadhyay, P. K., Swerdlow, H., Satija, R., & Smibert, P. (2017). Simultaneous epitope and transcriptome measurement in single cells. *Nature Methods*, 14(9), 865–868. <https://doi.org/10.1038/nmeth.4380>
9. Consortium, N. T. S., Jones, R. C., Karkanias, J., Krasnow, M. A., Pisco, A. O., Quake, S. R., Salzman, J., Yosef, N., Bulthaup, B., Brown, P., Harper, W., Hemenez, M., Ponnusamy, R., Salehi, A., Sanagavarapu, B. A., Spallino, E., Aaron, K. A., Concepcion, W., Gardner, J. M., . . . Xie, J. (2022). The Tabula Sapiens: A multiple-organ, single-cell transcriptomic atlas of humans. *Science*, 376(6594), eabl4896. <https://doi.org/10.1126/science.abl4896>