Spatial

o m s

c i

Literature talk

January 24th, 2025

Tae Kim

MacMillan Group

Spatial omics

Literature talk

January 24th, 2025

Tae Kim

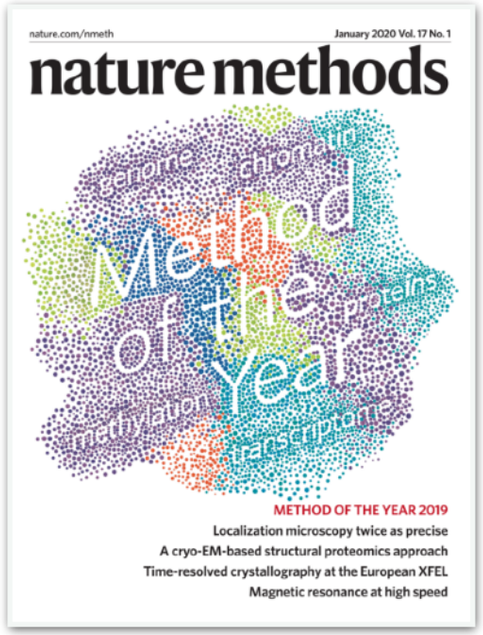
MacMillan Group

Nature Methods — Method of the Year

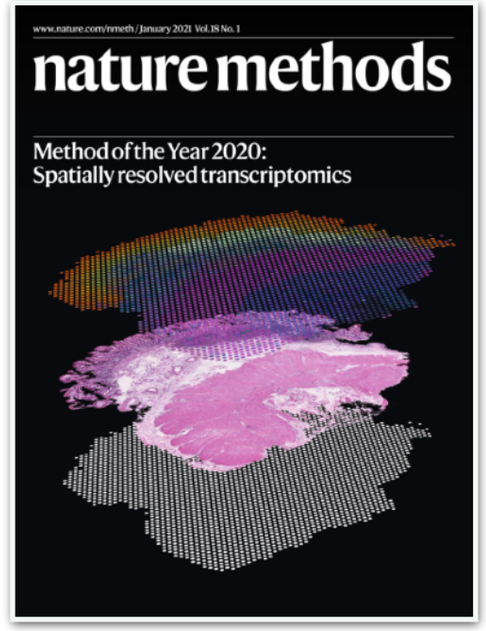
2017

2018

2019

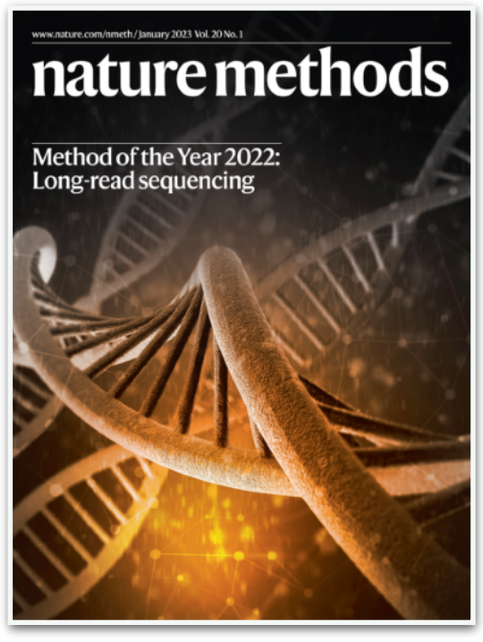


2020

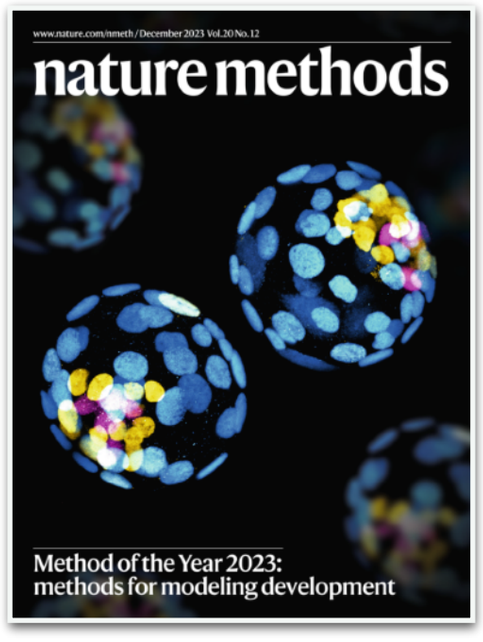


2021

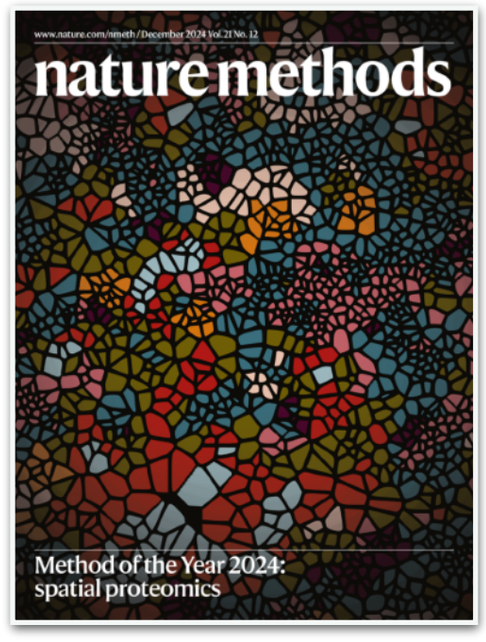
2022



2023

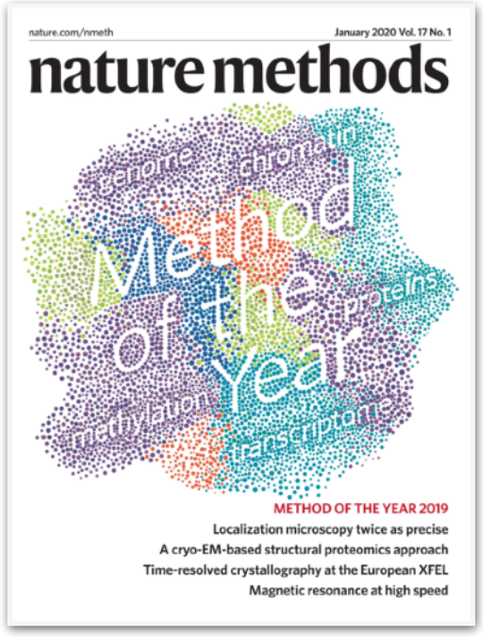


2024



Nature Methods — Method of the Year

2019



Single-cell multimodal **omics**

2020

Spatially resolved transcript**omics**

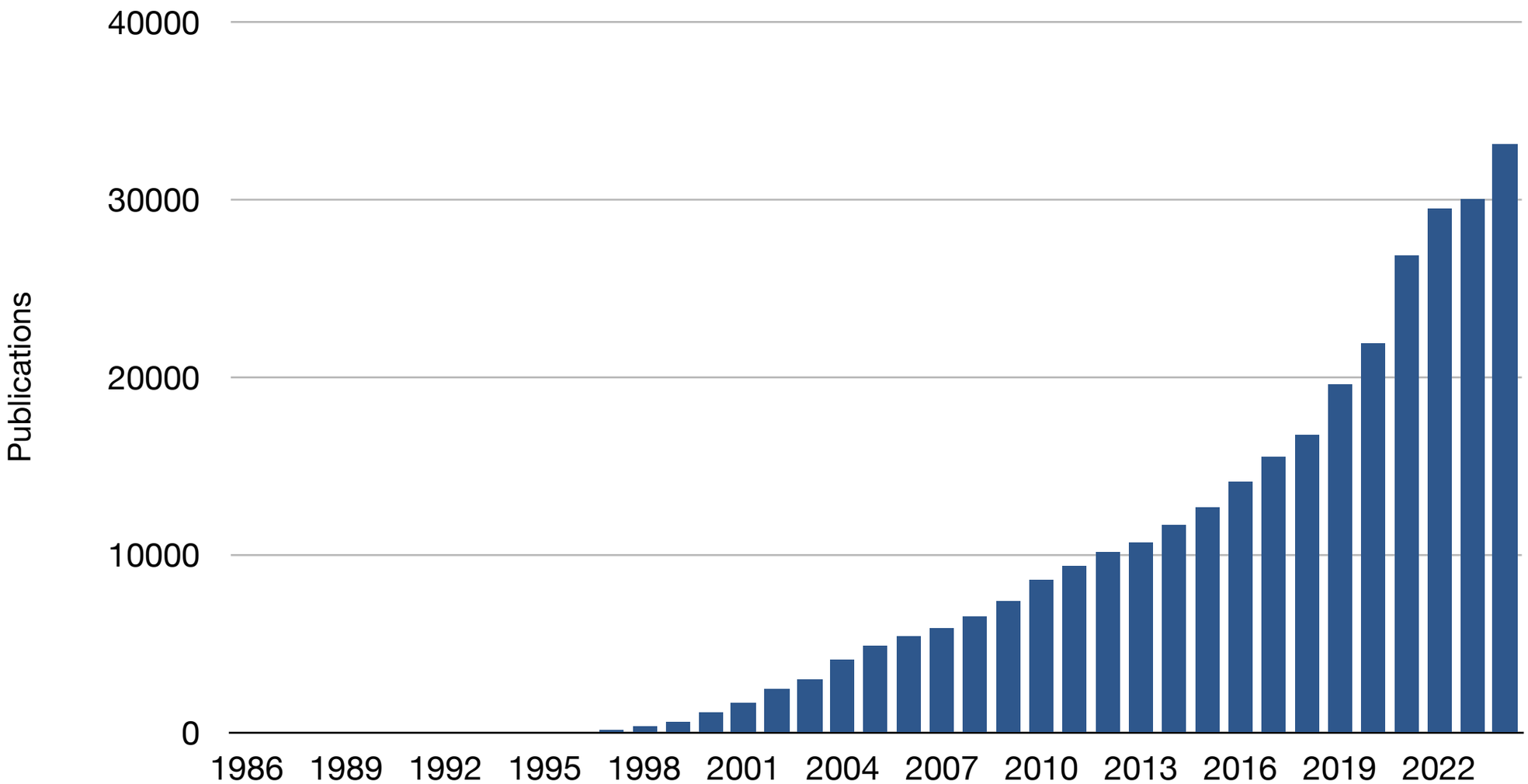
2022

Long-read **sequencing**

2024

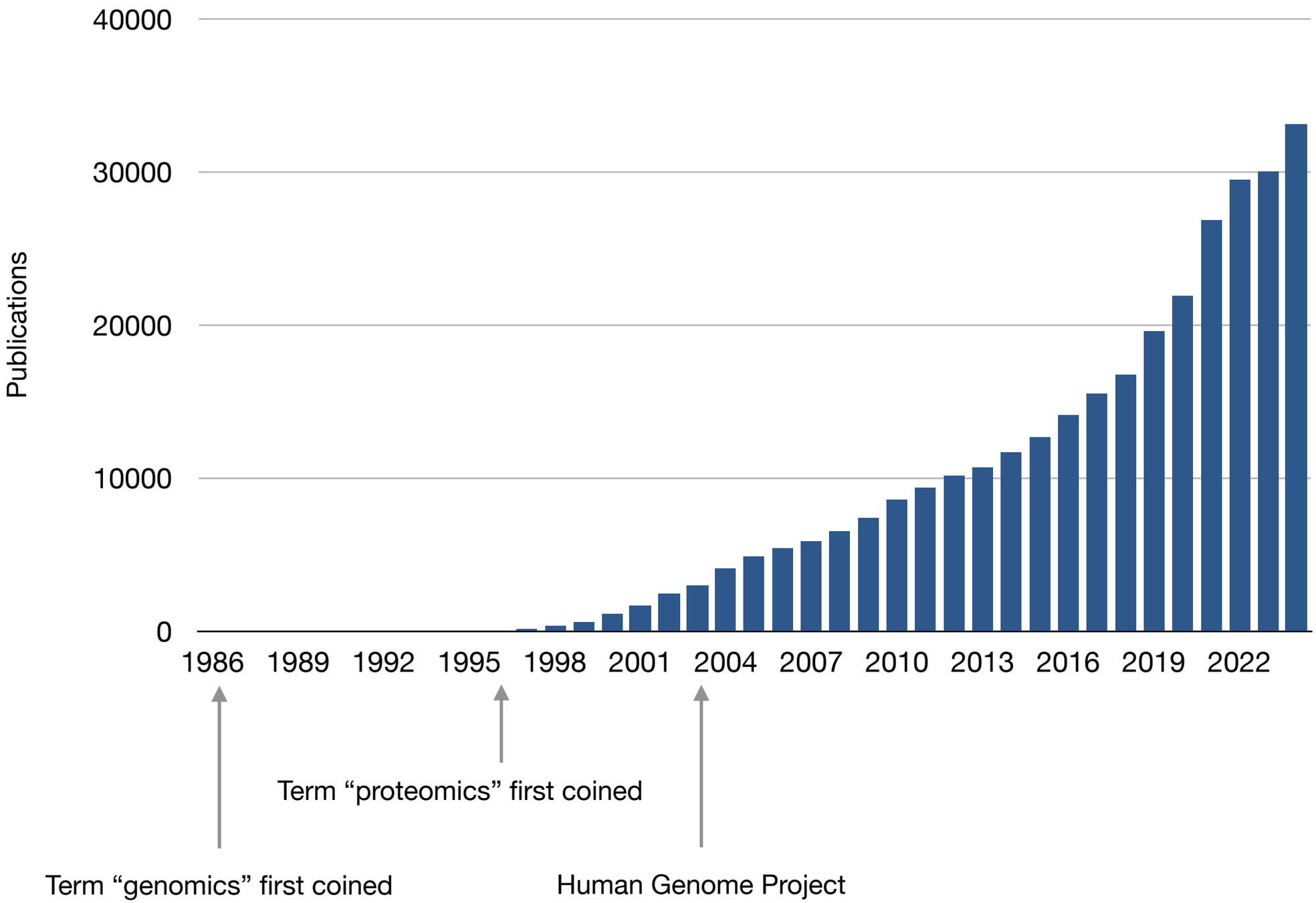
Spatial prote**omics**

We are living in the OMICS era…



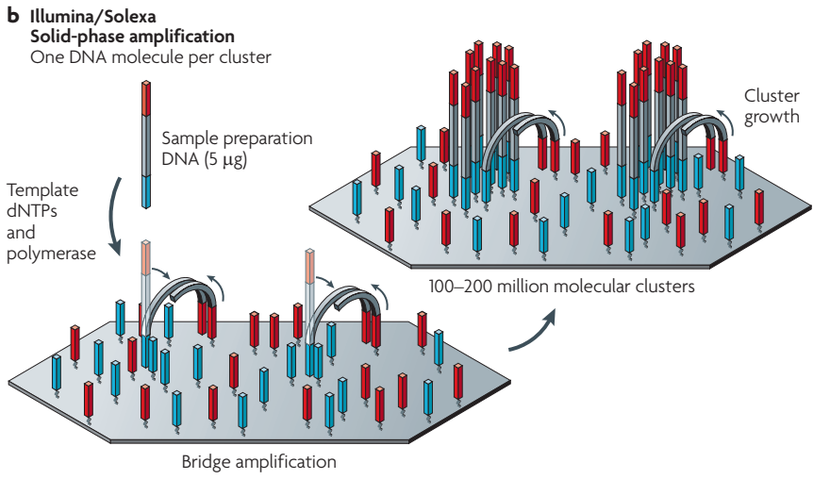
Web of Science database search (Jan 2025) of papers containing keywords: omics, genomics, transcriptomics, proteomics, metabolomics

We are living in the OMICS era…

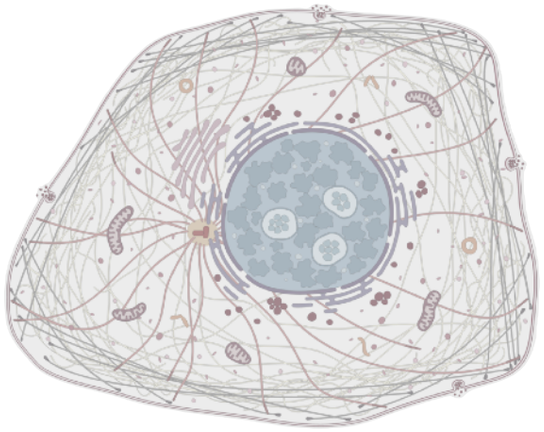


Web of Science database search (Jan 2025) of papers containing keywords: omics, genomics, transcriptomics, proteomics, metabolomics

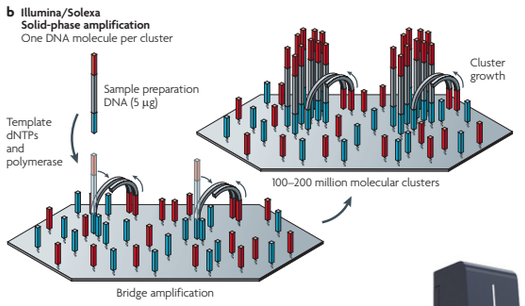
Sequencing and MS technologies revolutionized omics



Spatial information is often lost in omics methods

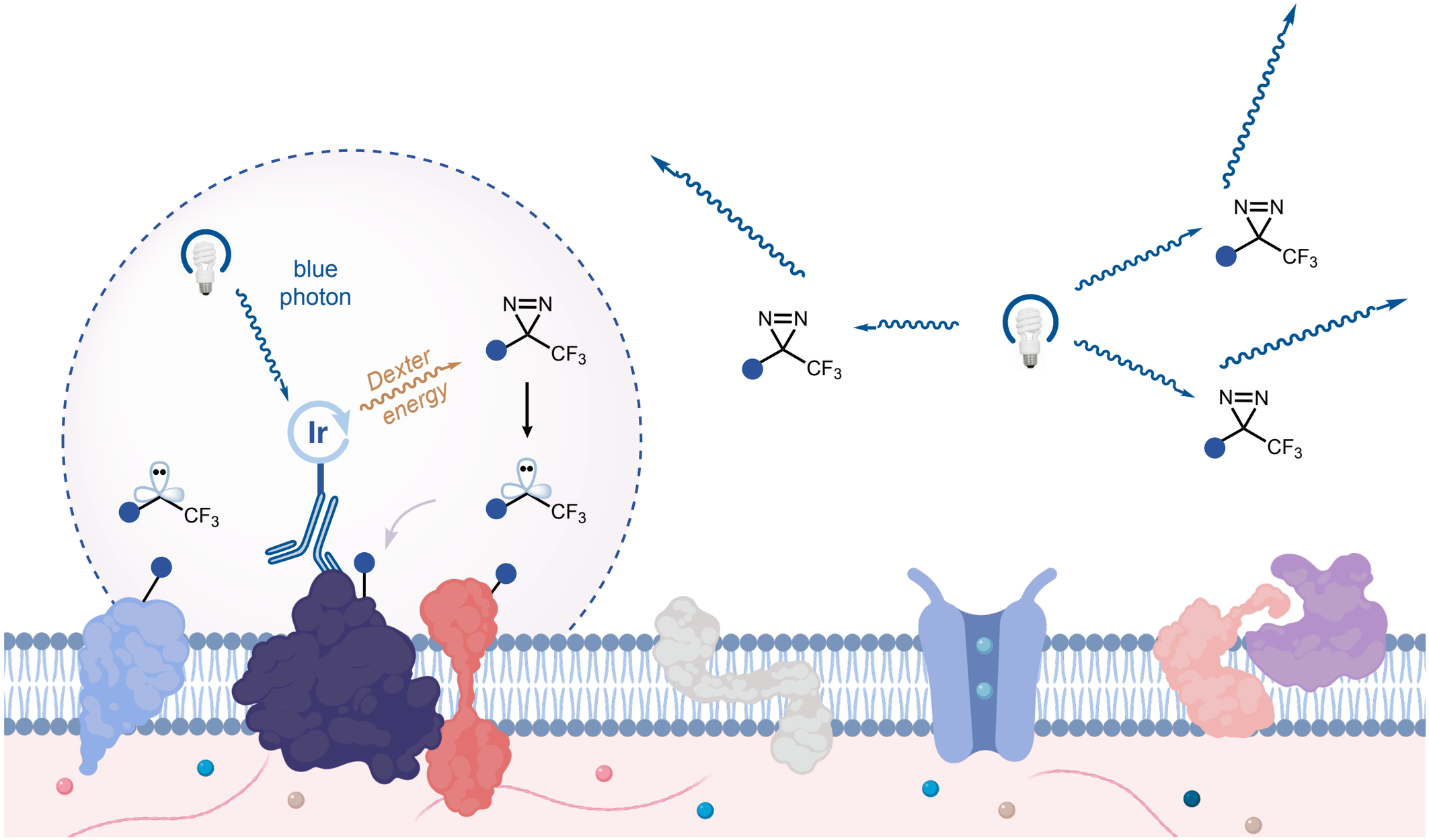


Cell lysis
Tissue homogenization



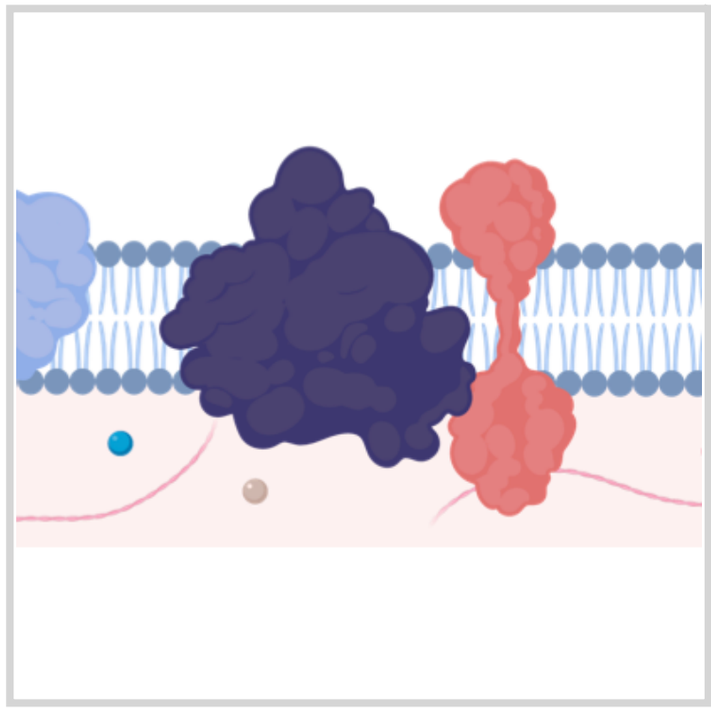
Spatial information is often lost in omics methods

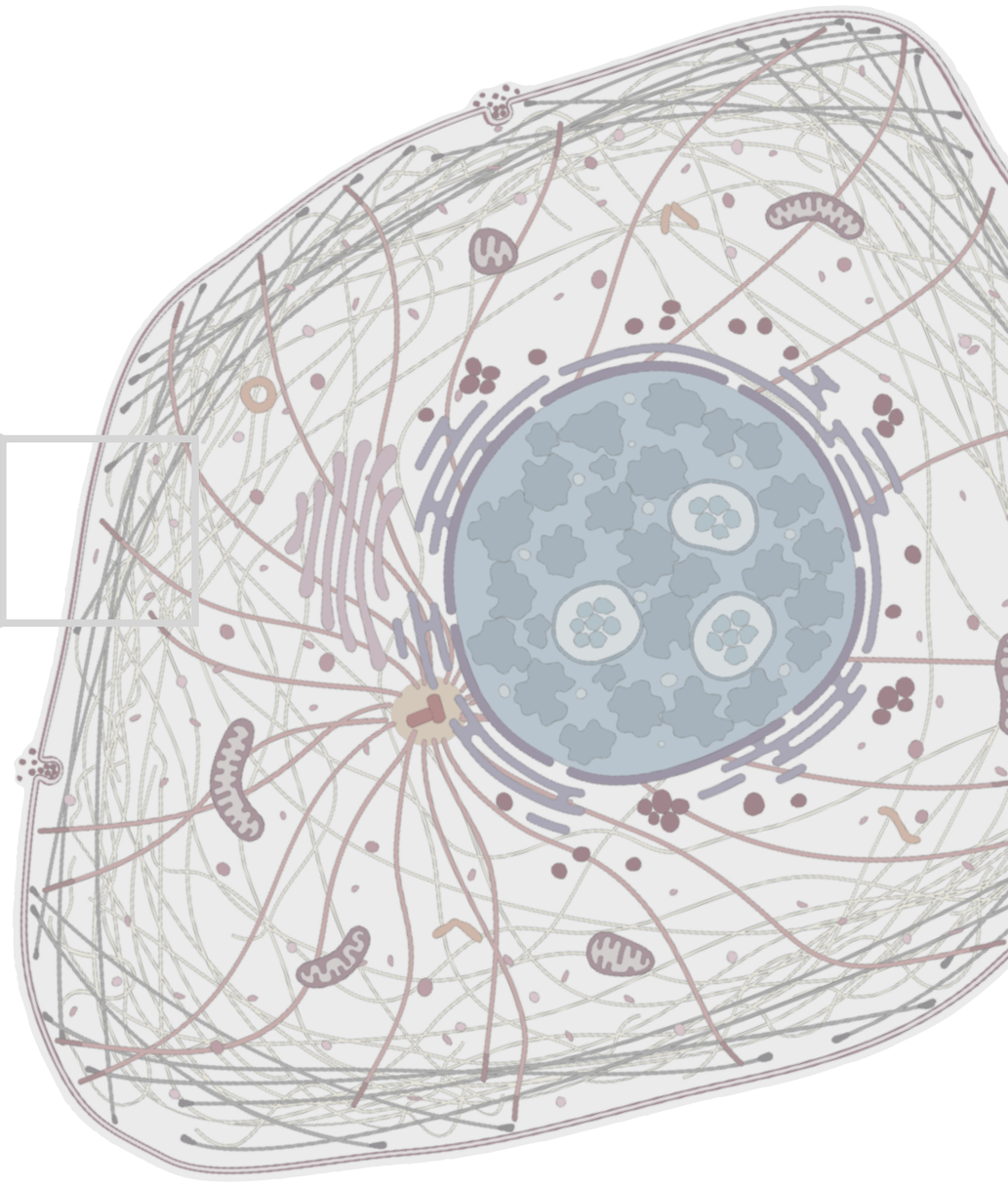
Proximity labeling is one way to preserve spatial information…



Proximity labeling is one way to preserve spatial information…

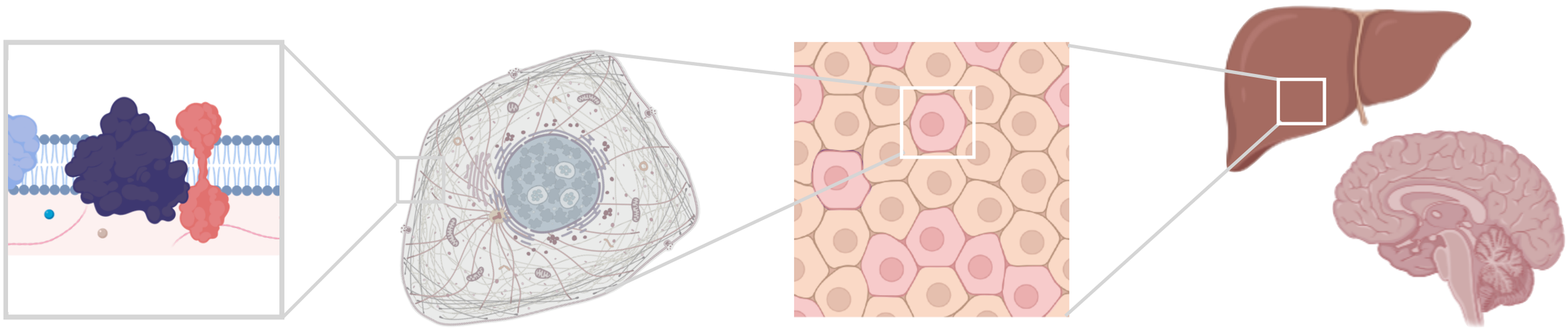
…but on sub-cellular scales





Protein-protein interactions
Protein-RNA interactions
Protein-DNA interactions
Organelle-organelle interactions
Cell-cell interactions
…

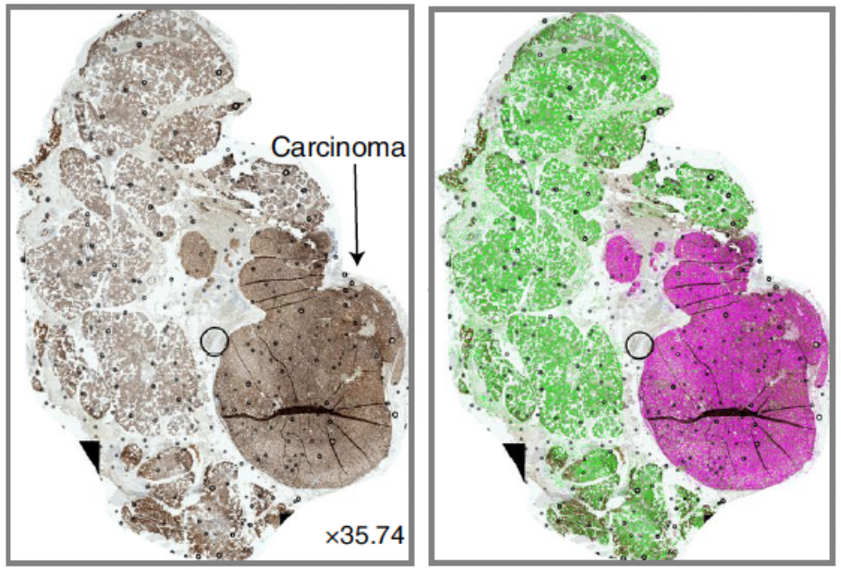
Spatial omics



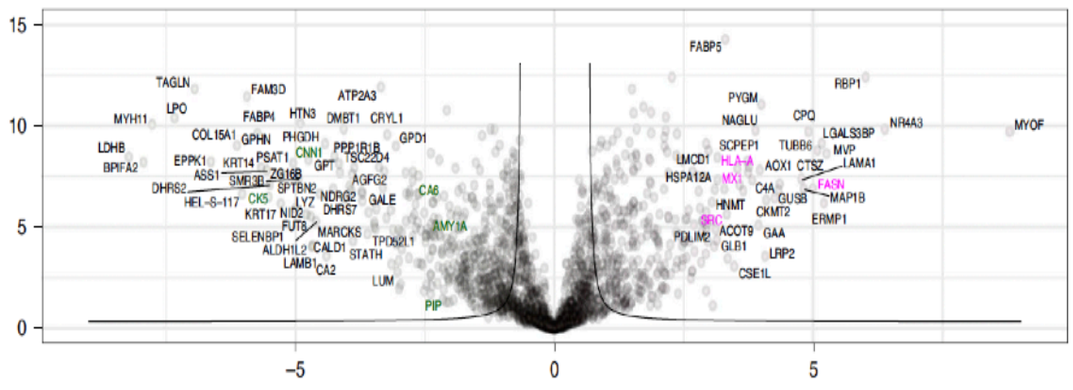
biomolecule *cell* *tissue* *organ*

**What are the transcriptomic/proteomic profiles of *each cell*
in the context of a complex cell network?**

Spatial omics

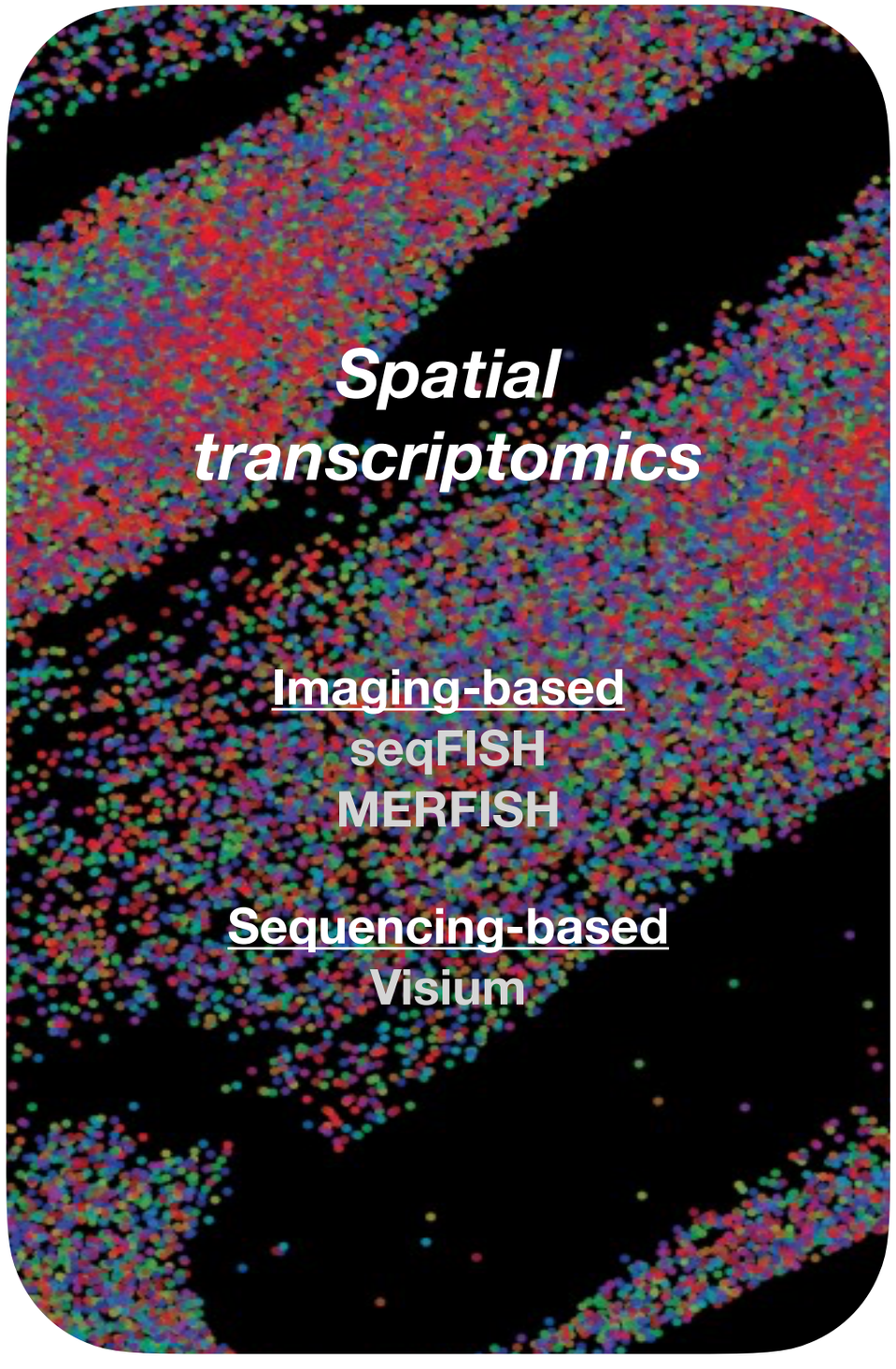


What are the transcriptomic/proteomic profiles of *each cell* in the context of a complex cell network?



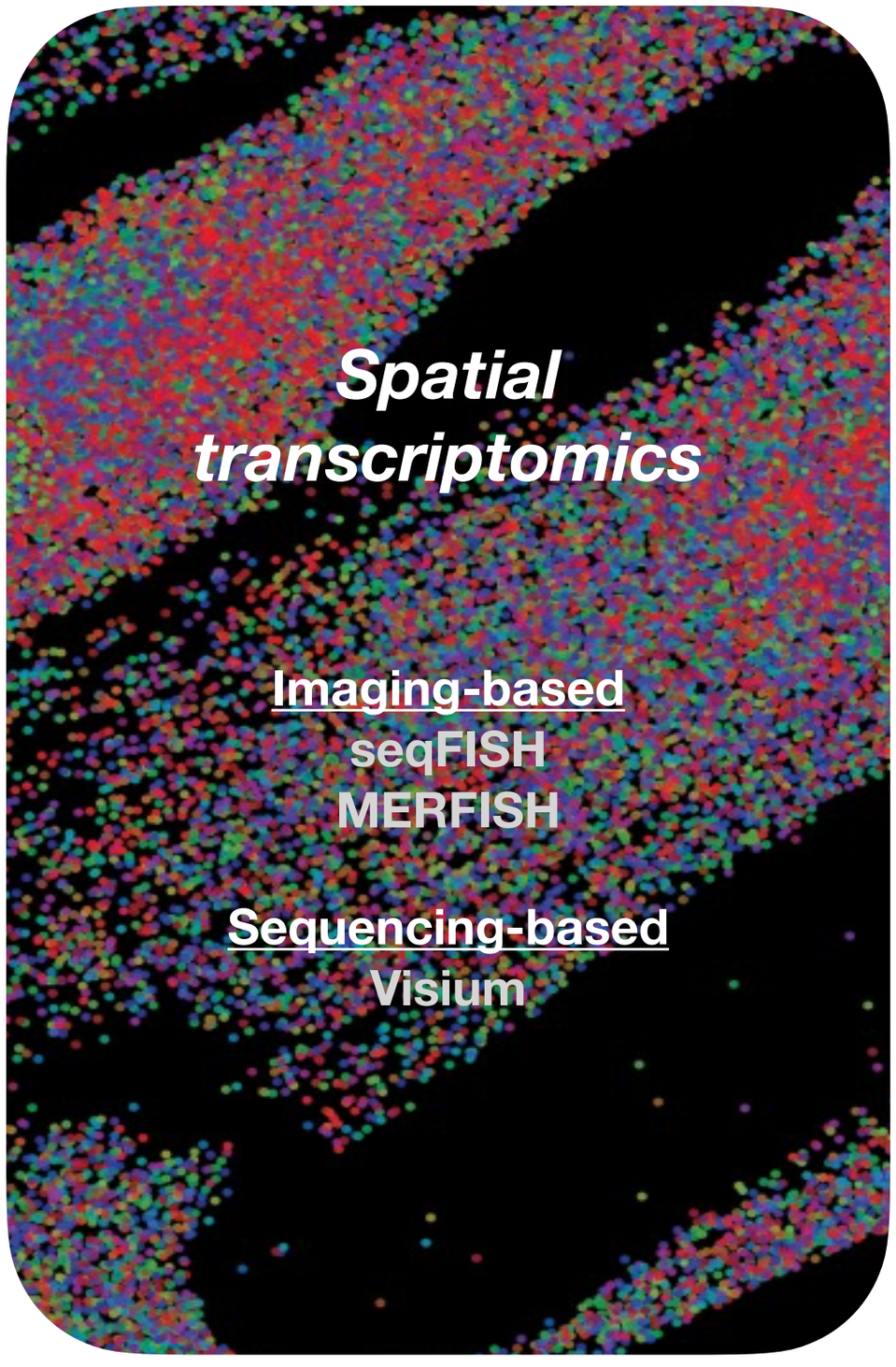
Mund, A. … Horvath, P.; Mann, M. *Nat. Biotech.* **2022**, *40*, 1231.

Outline


Spatial transcriptomics
Imaging-based
seqFISH
MERFISH
Sequencing-based
Visium


Spatial proteomics
Multiplexed antibody-based
4i
MS-based
DVP

Outline



Spatial transcriptomics

Imaging-based
seqFISH
MERFISH

Sequencing-based
Visium

Spatial proteomics

Multiplexed antibody-based
4i

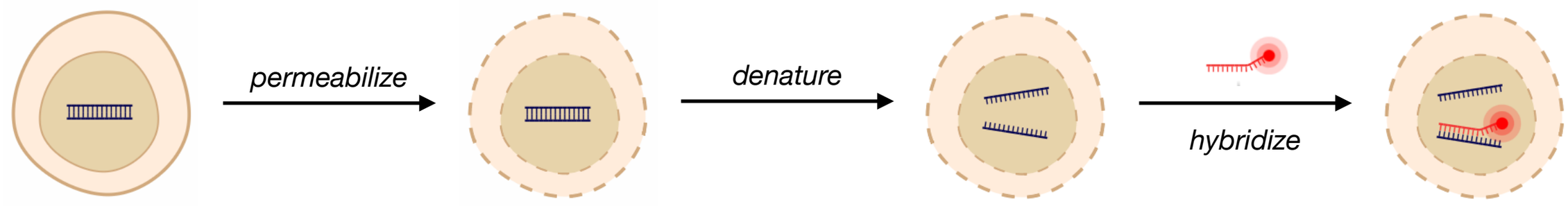
MS-based
DVP

Imaging-based spatial transcriptomics

***I**n-**S**itu **H**ybridization (ISH)*

Imaging-based spatial transcriptomics

***I**n-**S**itu **H**ybridization (ISH)*

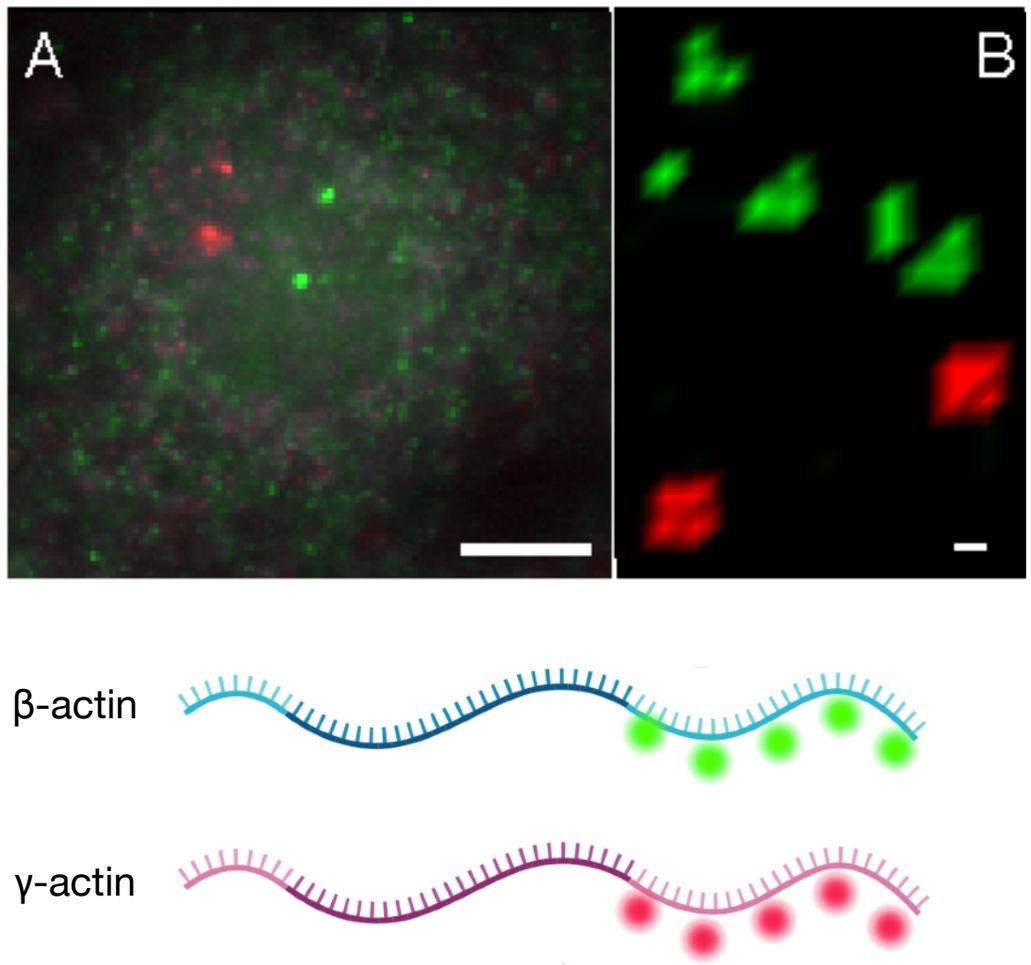


- rRNA and rDNA in *Xenopus laevis* oocytes visualized in first radioactive ISH in 1969
- Visualize DNA or RNA with radiolabeled/biotinylated/fluorescent complementary strands
- No need for denaturation for RNA visualization

Purdue, M. L.; Gall, J. G. *Proc. Natl. Acad. Sci.* **1969**, *64*, 600.; *Review*: Levsky, J.M.; Singer, R.H. *J. Cell Sci.* **2003**, *116*, 2833.

Imaging-based spatial transcriptomics

***S**ingle **m**olecule **F**luorescence **I**n-**S**itu **H**ybridization (smFISH)*



Femino, A. M.; Fay, F. S.; Fogarty, K.; Singer, R. H. *Science* **1998**, *280*, 585.

Imaging-based spatial transcriptomics

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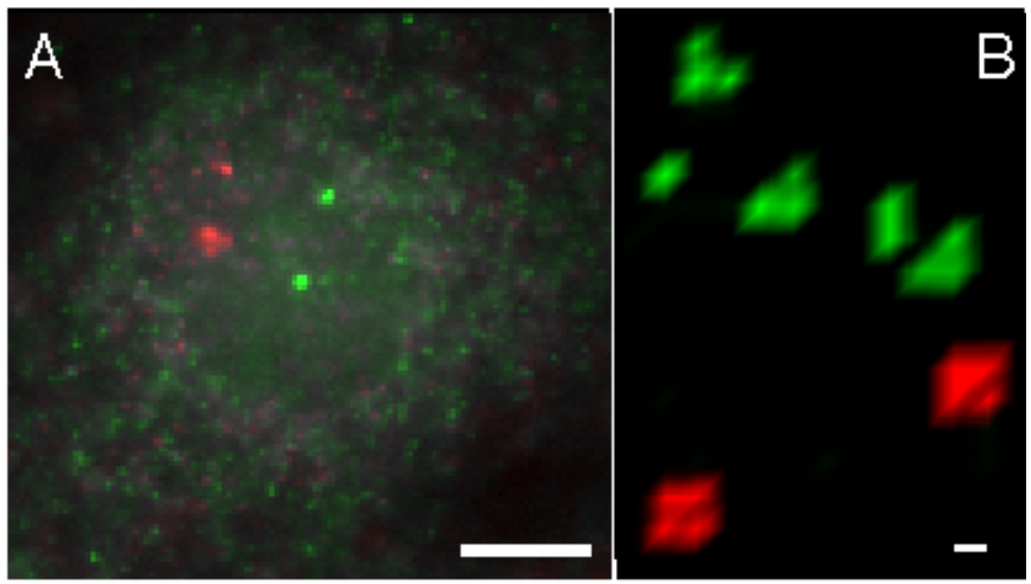

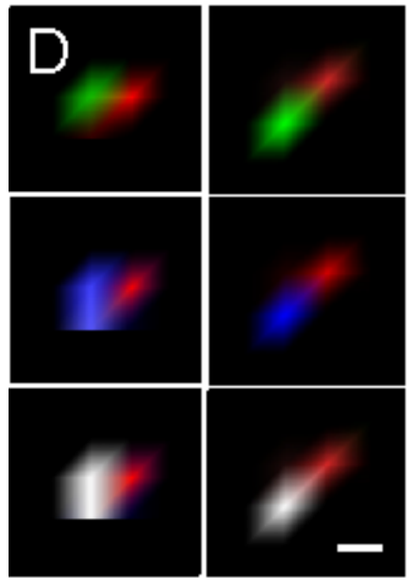

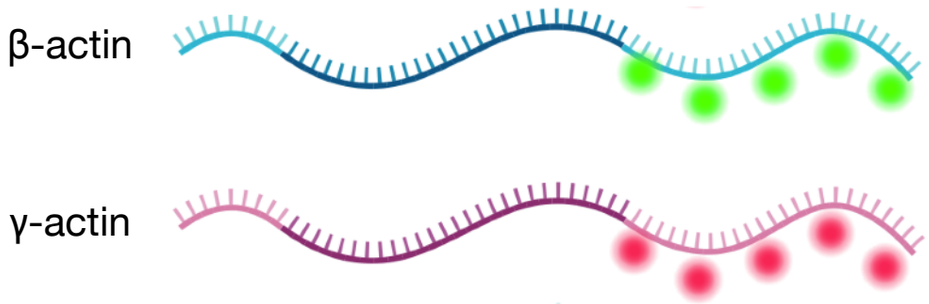

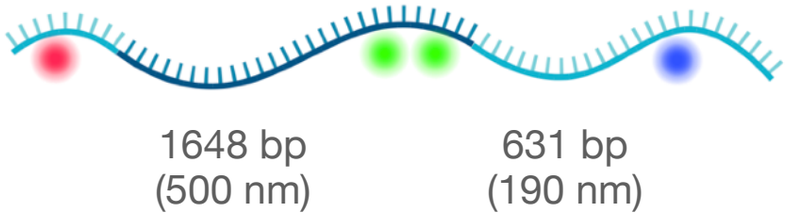

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Imaging-based spatial transcriptomics

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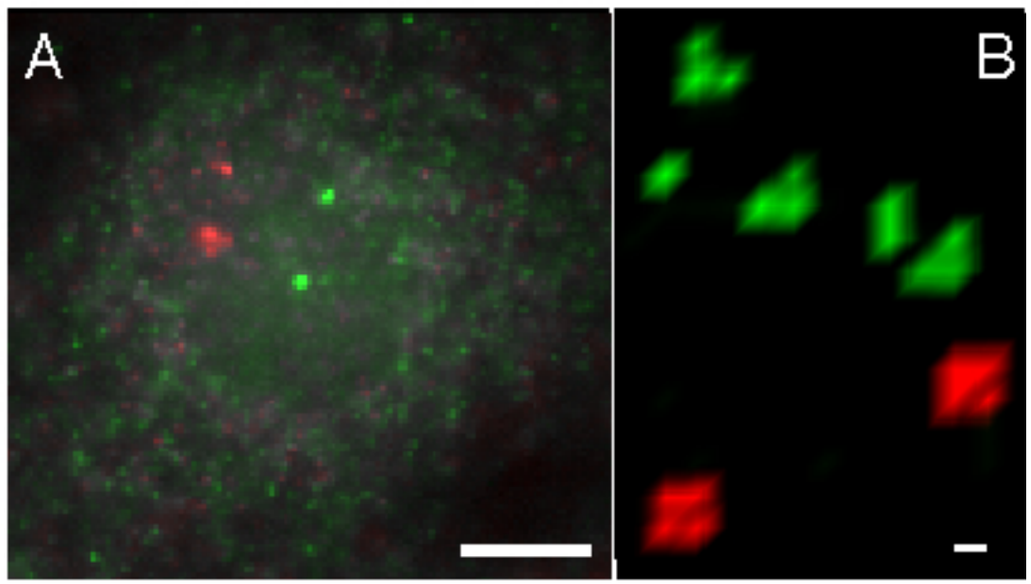
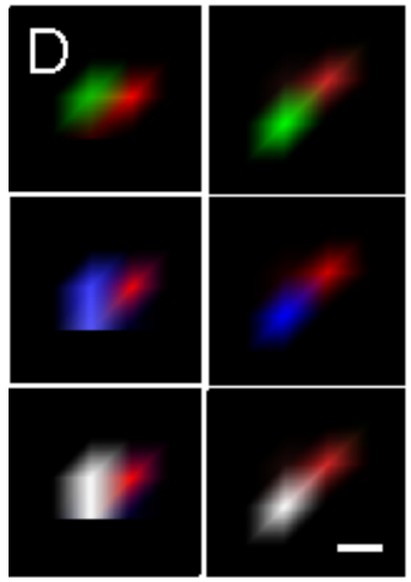
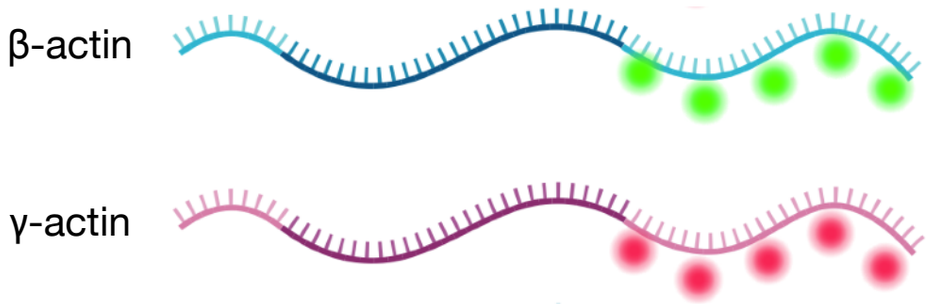
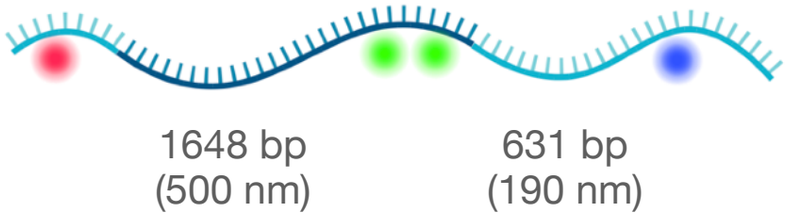






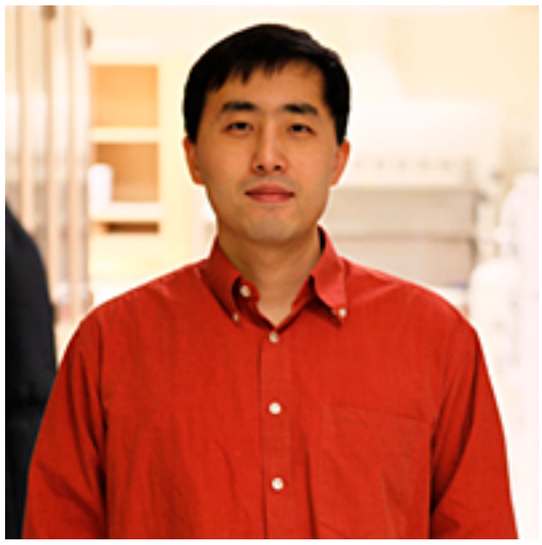


- Individual mRNA molecules resolved
- Quantitative measure of transcripts

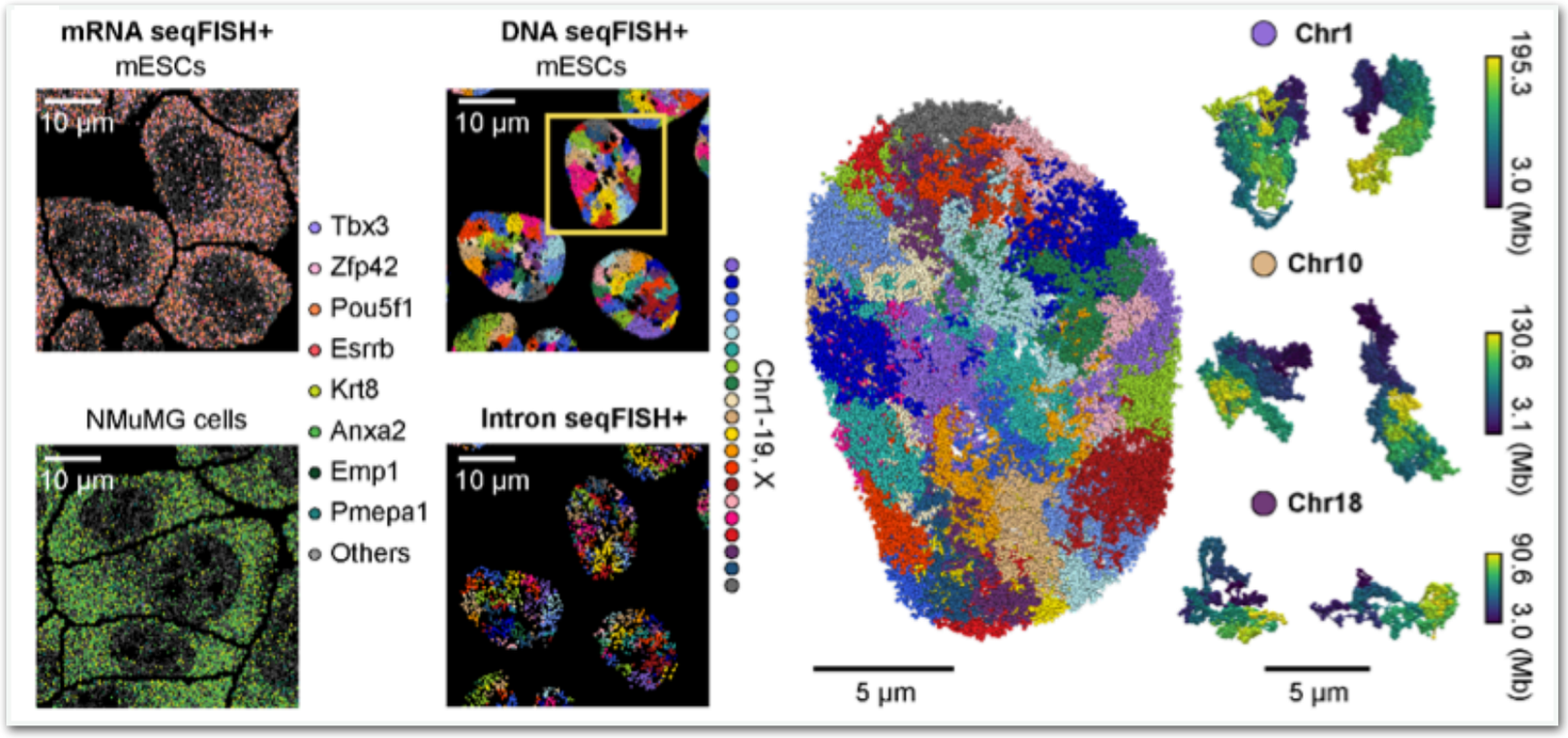
Femino, A. M.; Fay, F. S.; Fogarty, K.; Singer, R. H. *Science* **1998**, *280*, 585.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*



Long Cai
Caltech



Takei, Y.; Yang, Y. … Cai, L. *biorXiv* **2023**

Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*

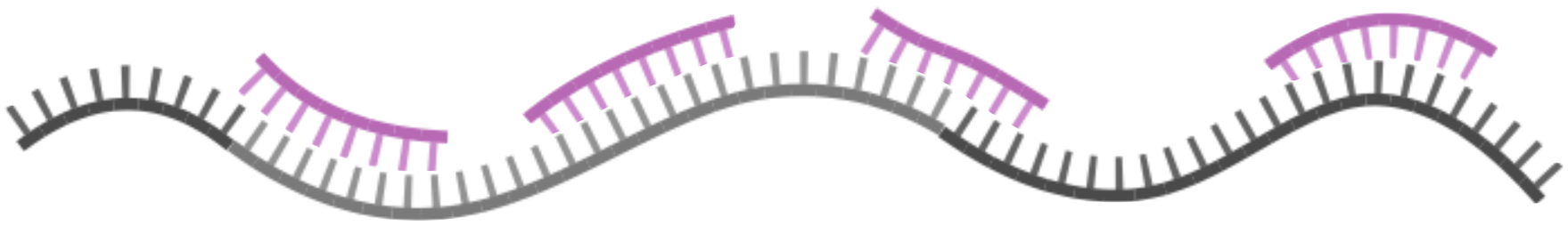
Gene 1

Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*

A set of 20-30 FISH probes specific to each gene



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Imaging-based spatial transcriptomics

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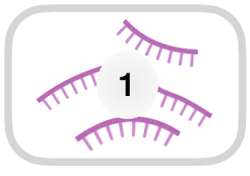

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Imaging-based spatial transcriptomics

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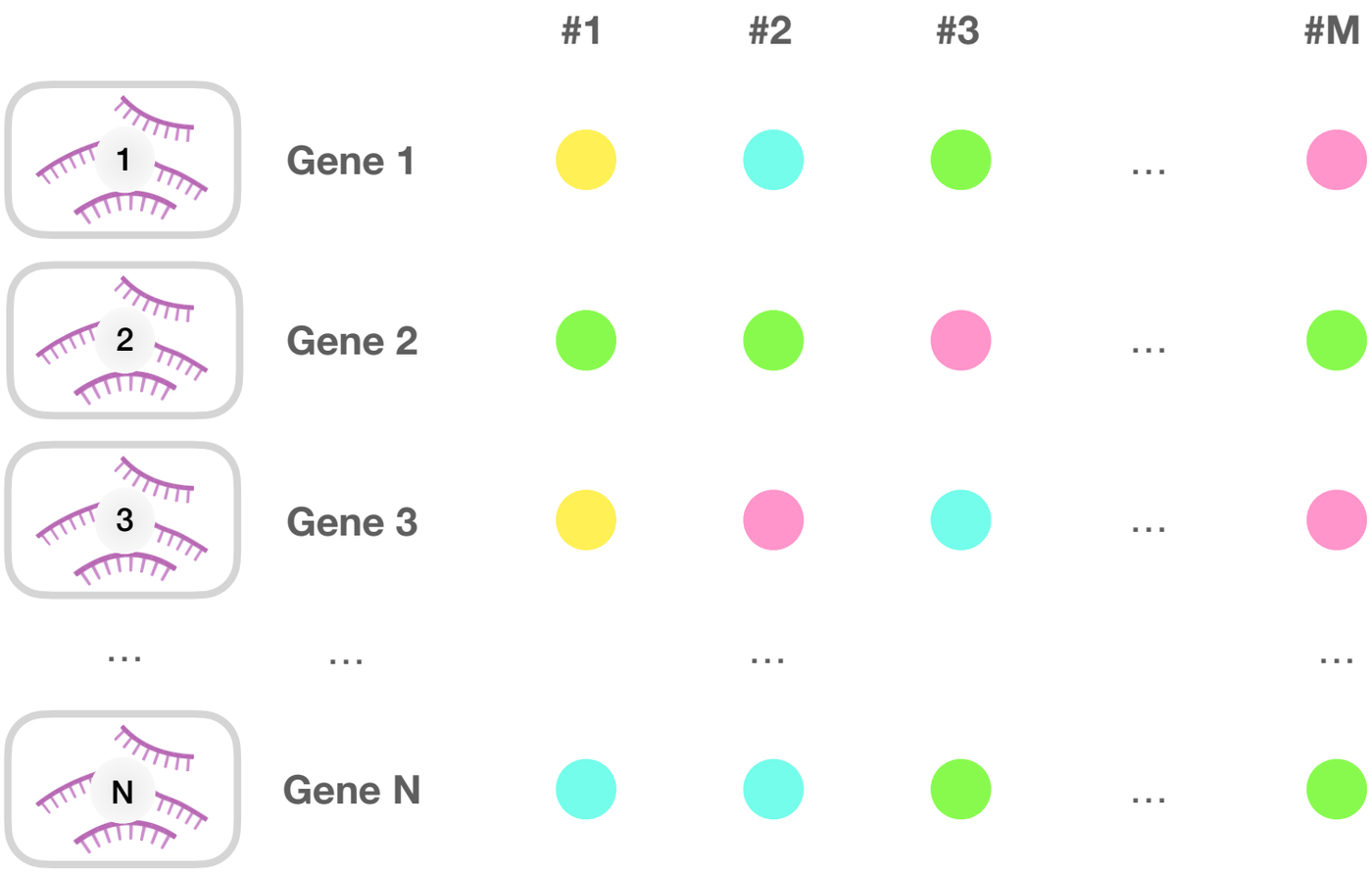

- Combinatorial labeling with color-based barcodes and sequential hybridization

Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

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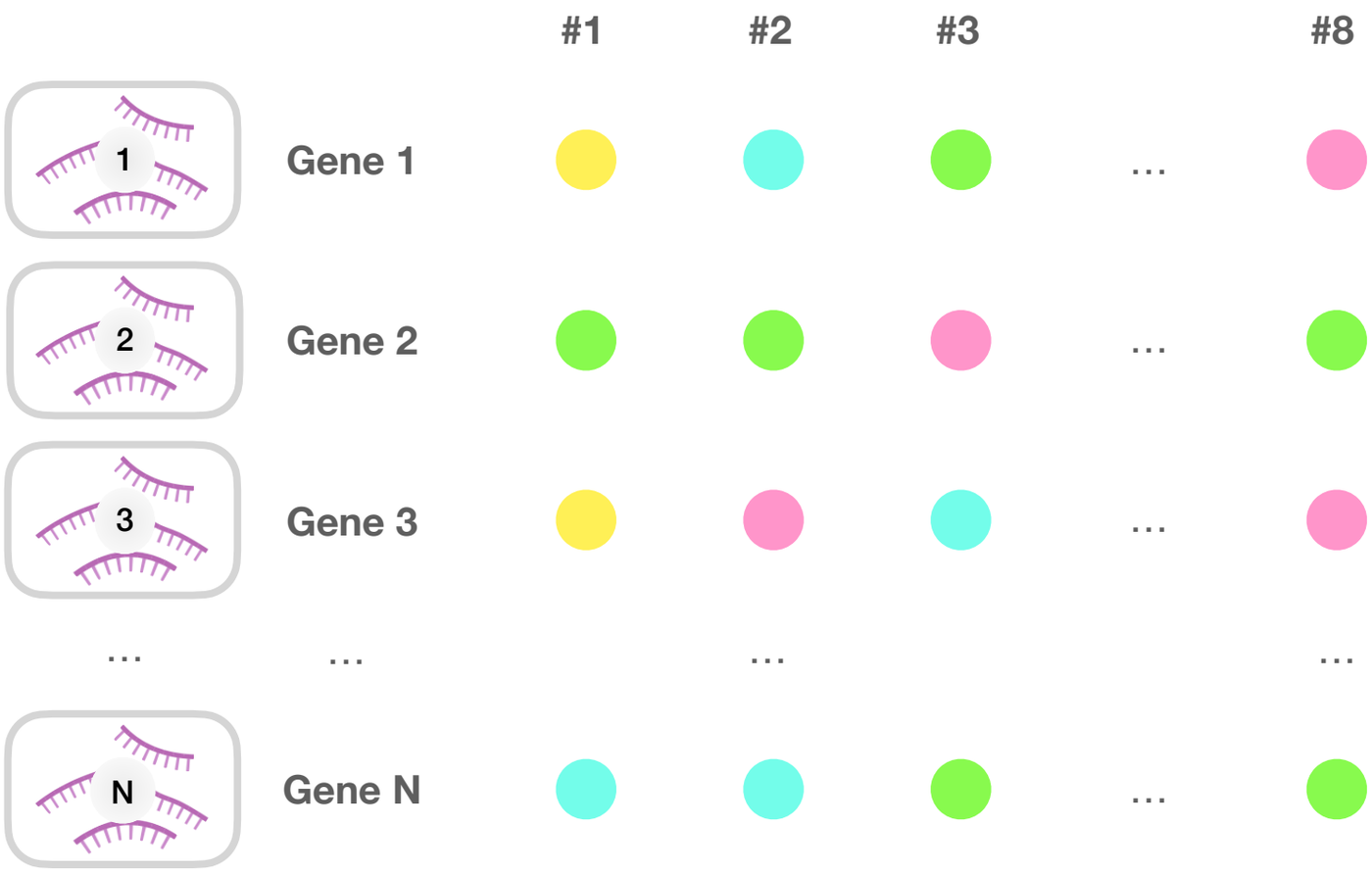

- Combinatorial labeling with color-based barcodes and sequential hybridization
- In theory, four colors & eight rounds can cover entire genome (4^8=65536)

Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

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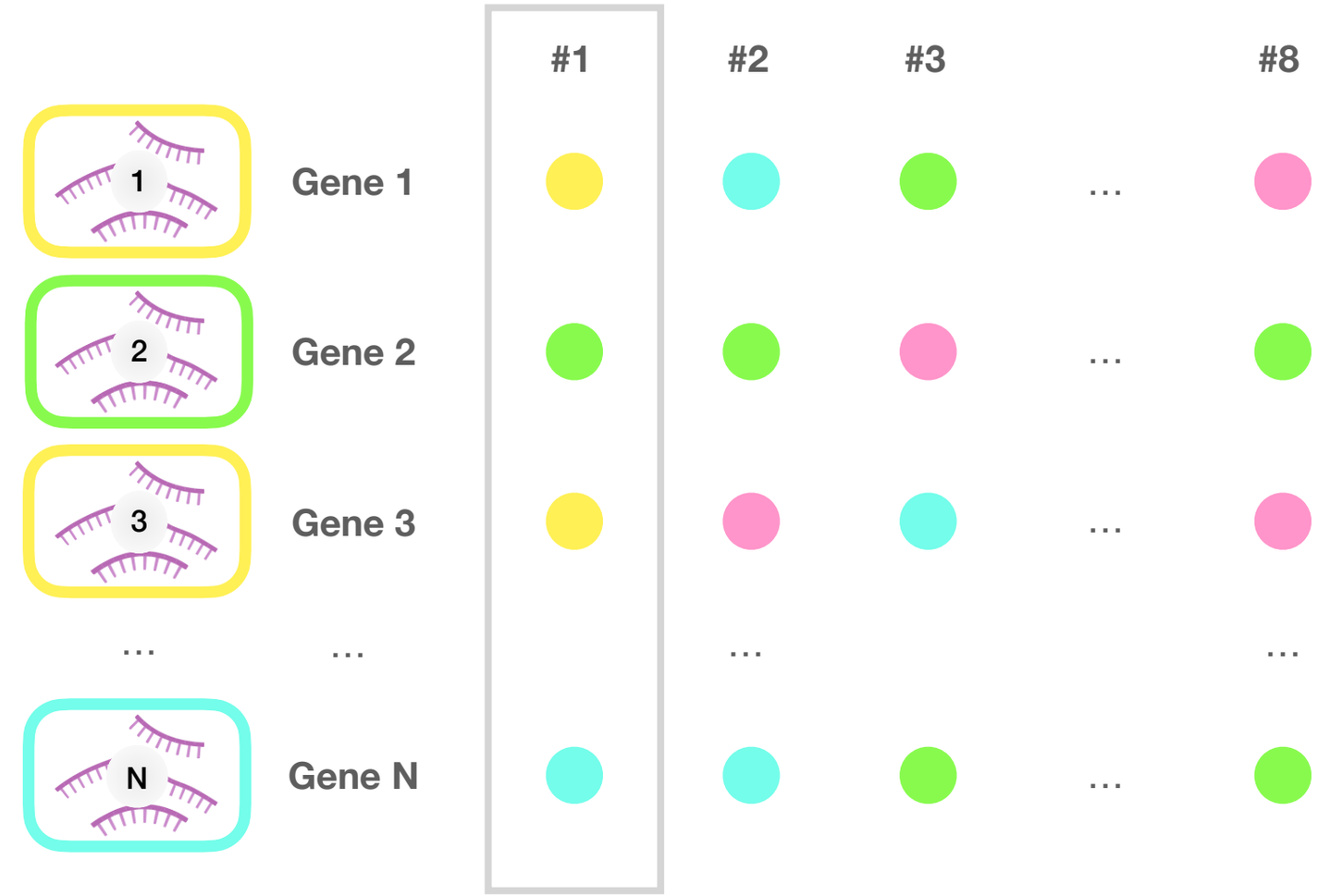

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Imaging-based spatial transcriptomics

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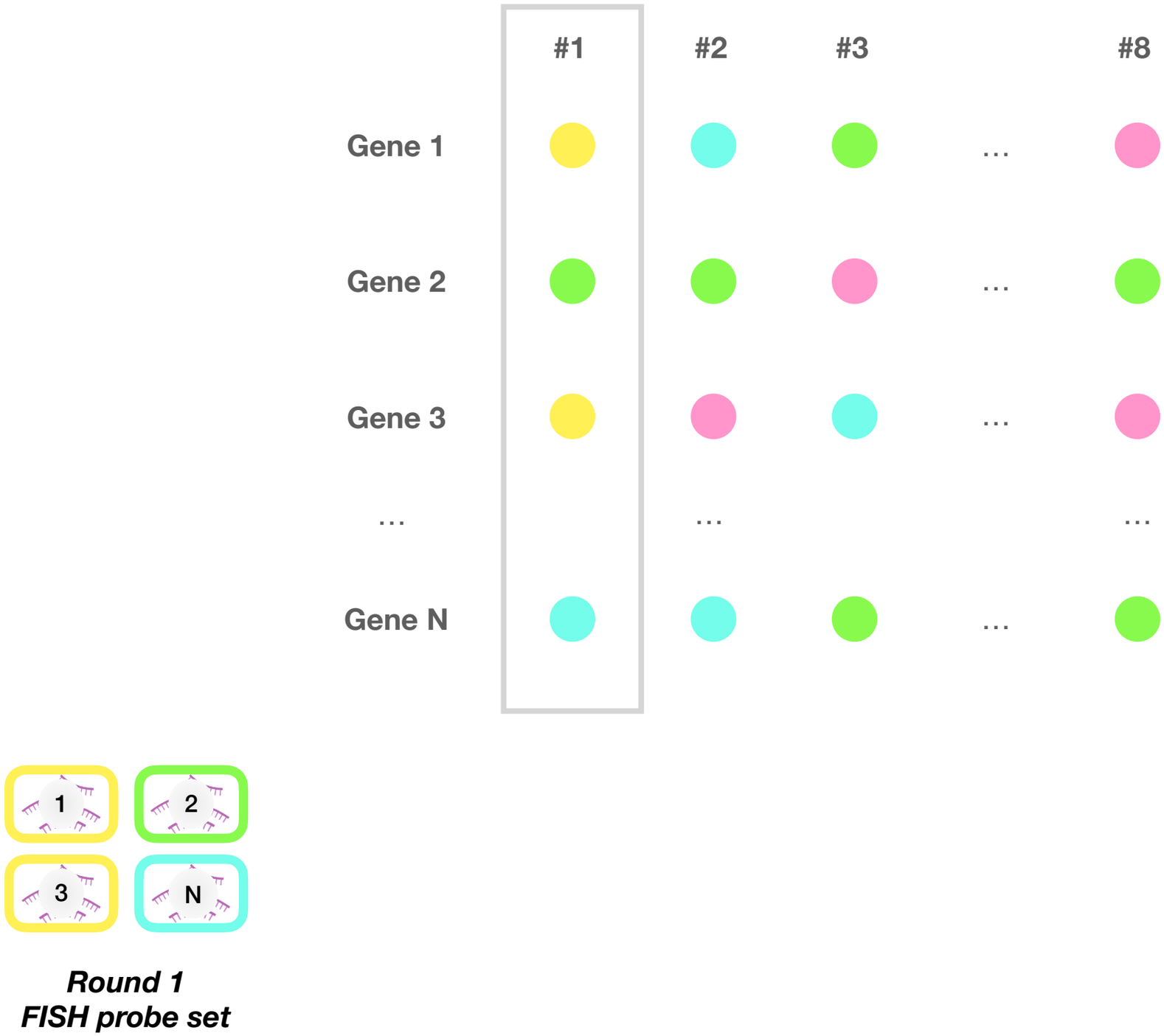

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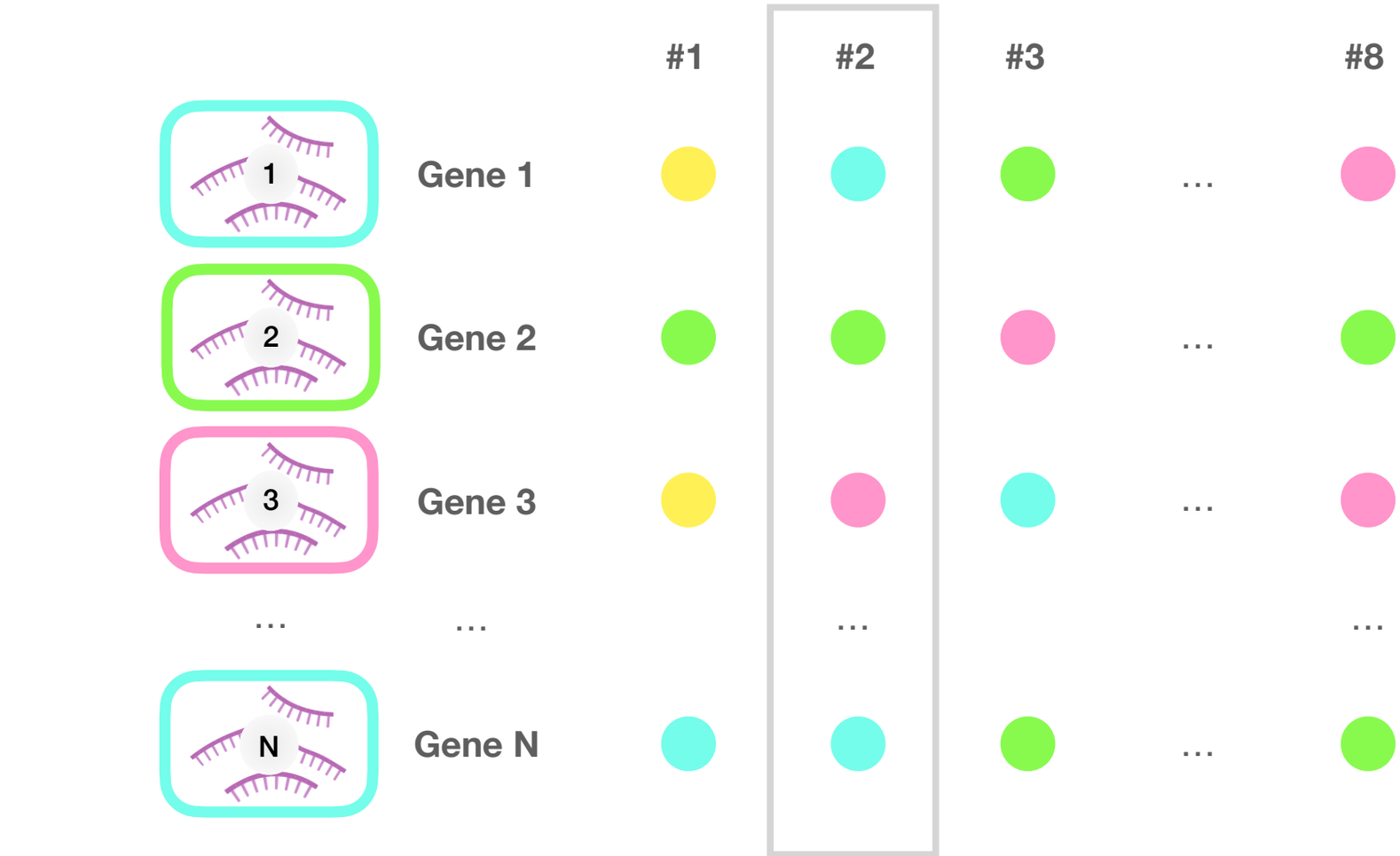
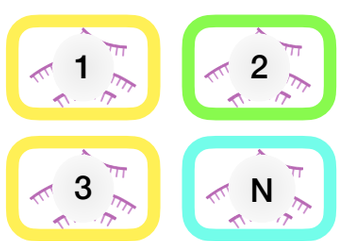


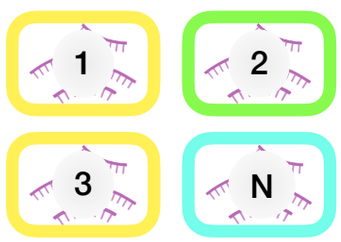


Round 1
FISH probe set

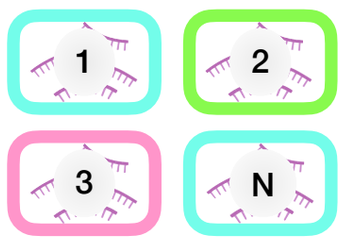
Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*



Round 1 FISH probe set

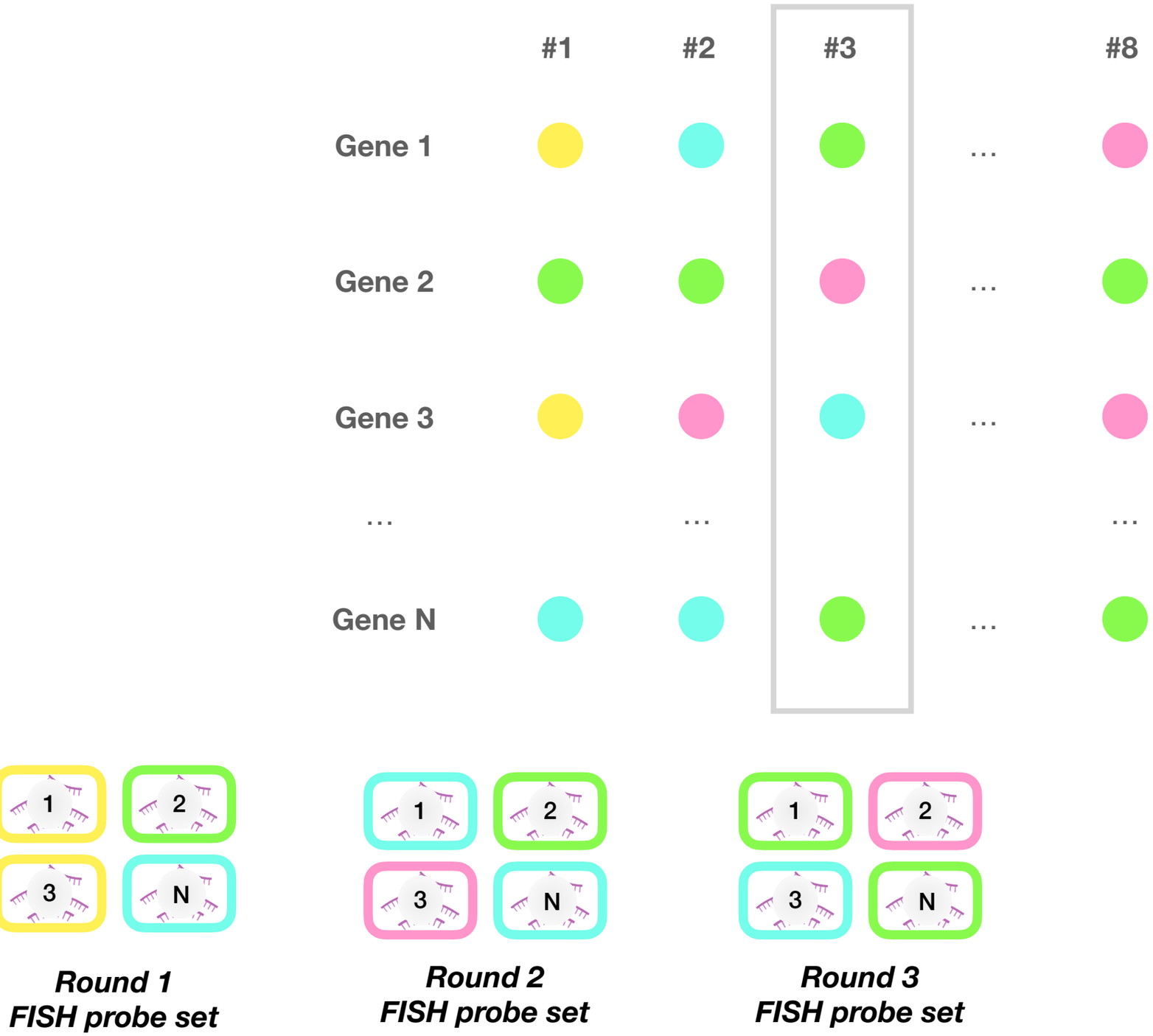


Round 2 FISH probe set

Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*



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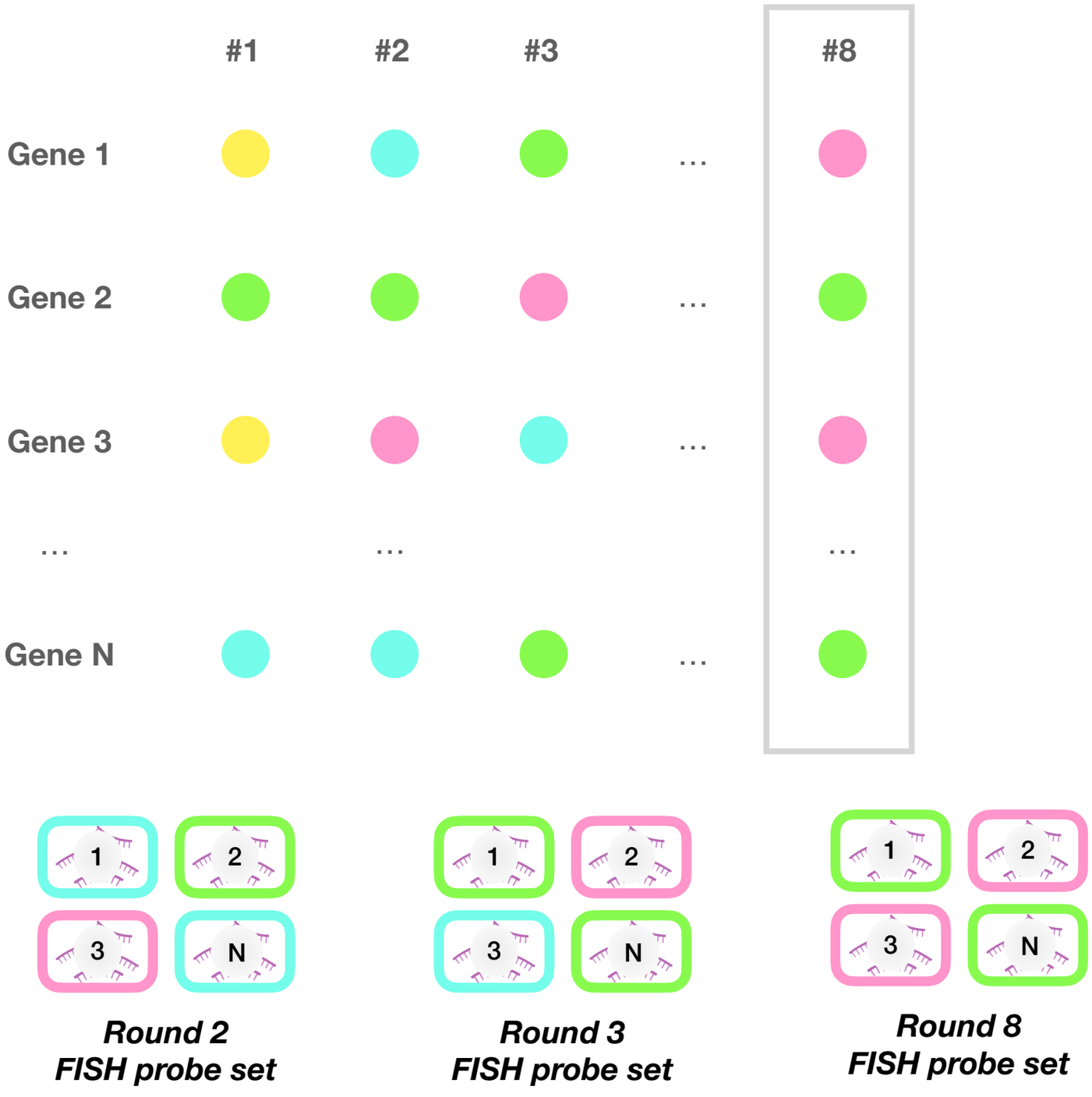

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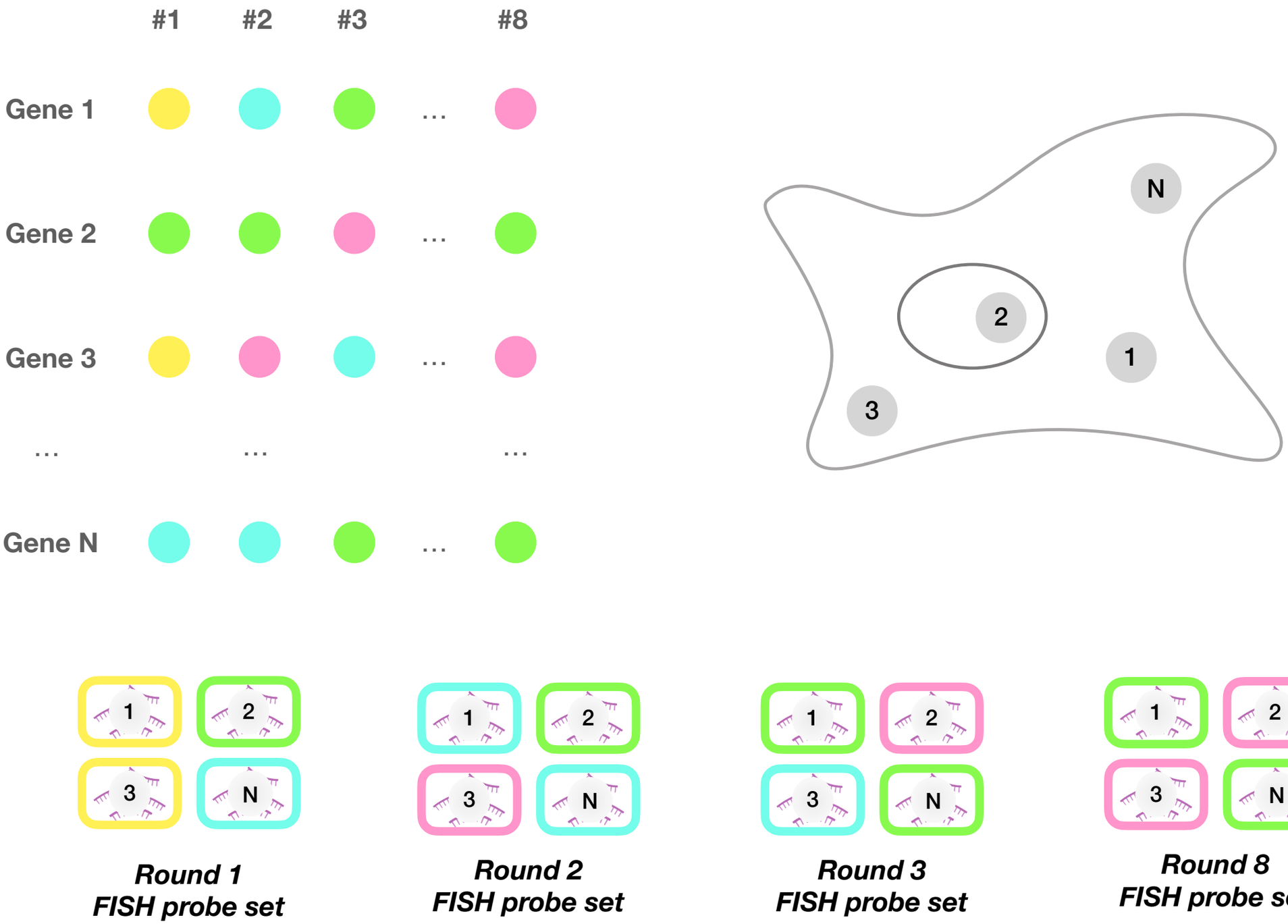

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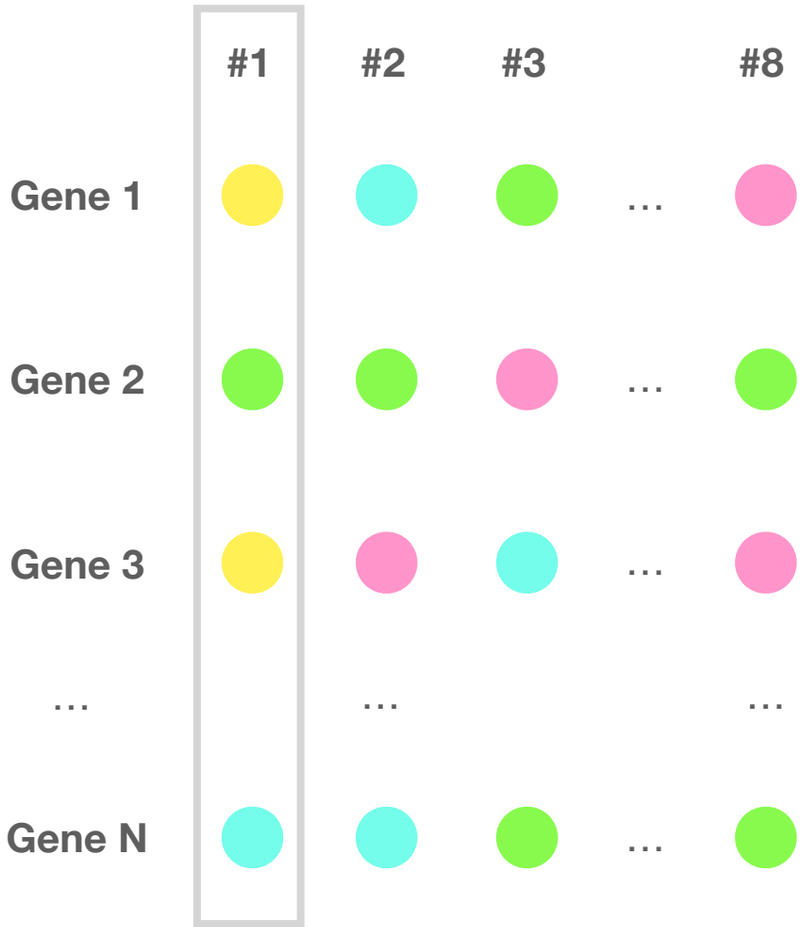
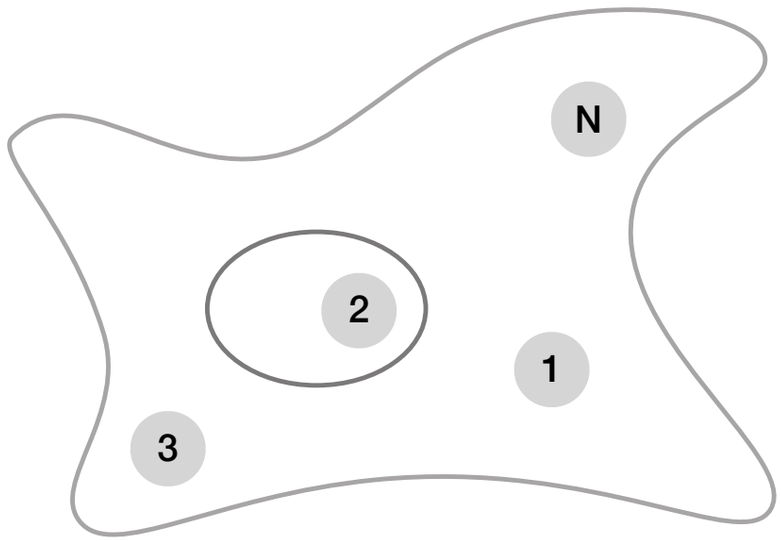
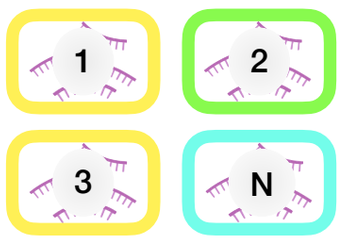




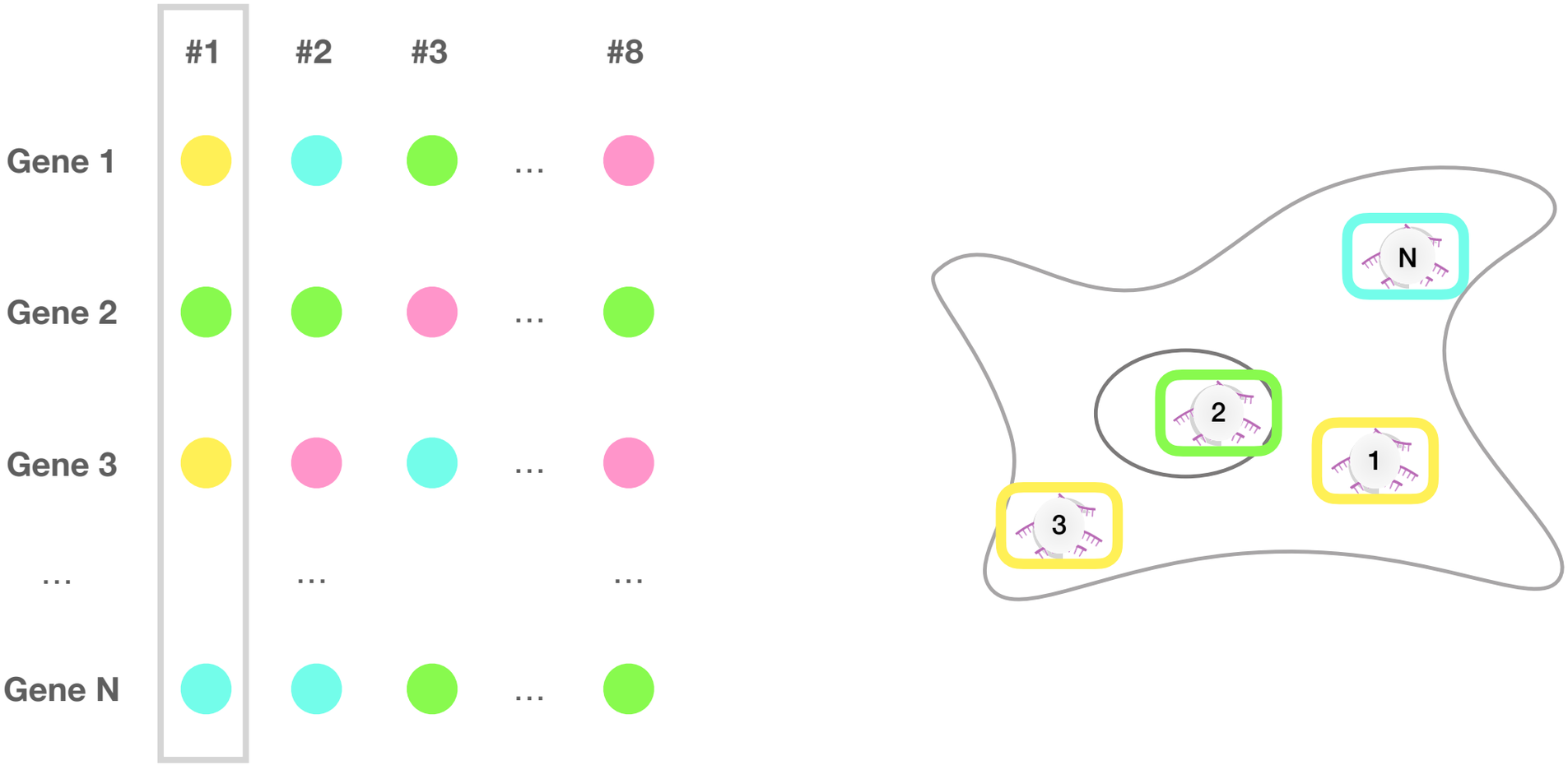


Round 1
FISH probe set

Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

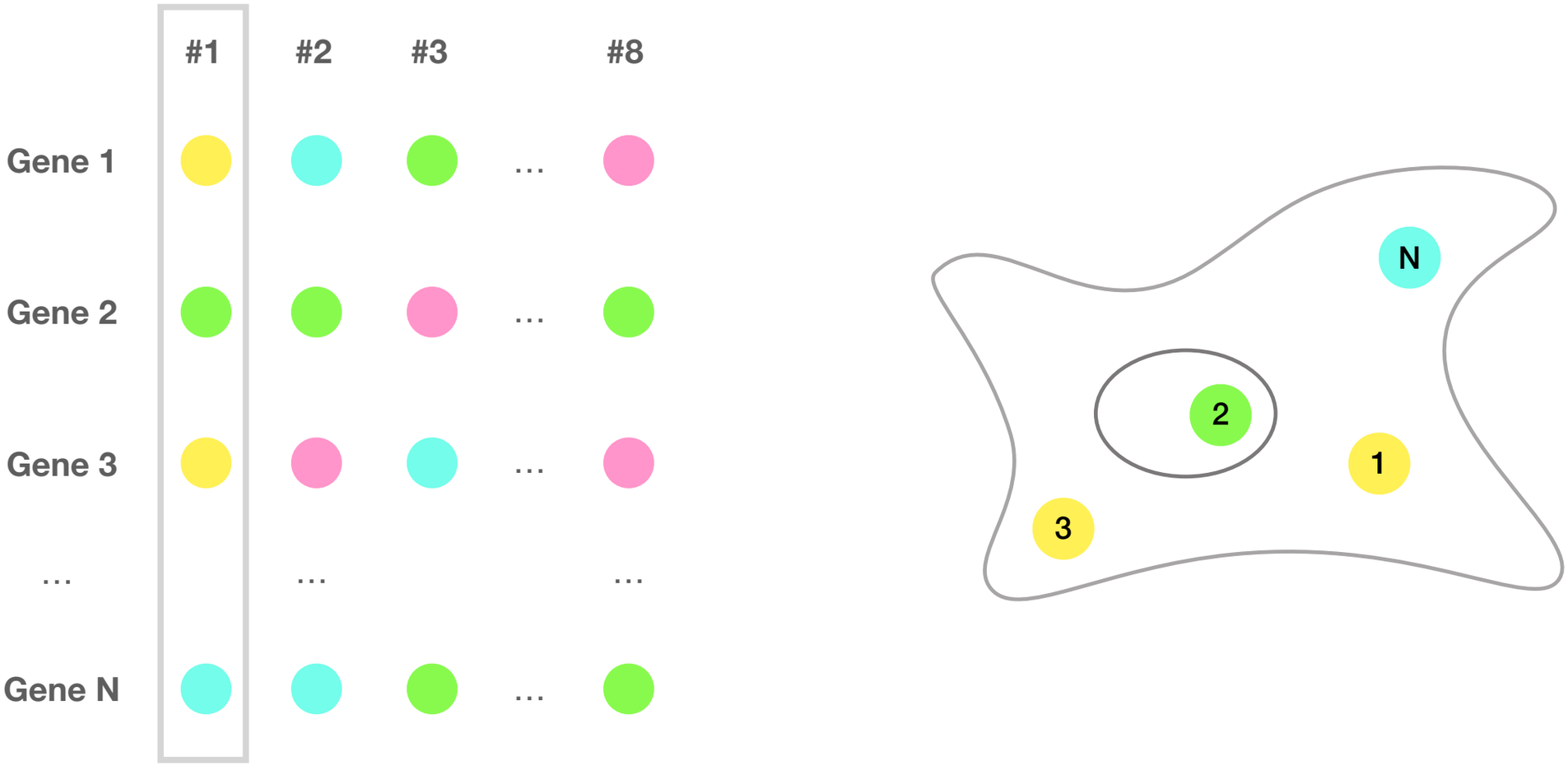
***seq**uential FISH (seqFISH)*



Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

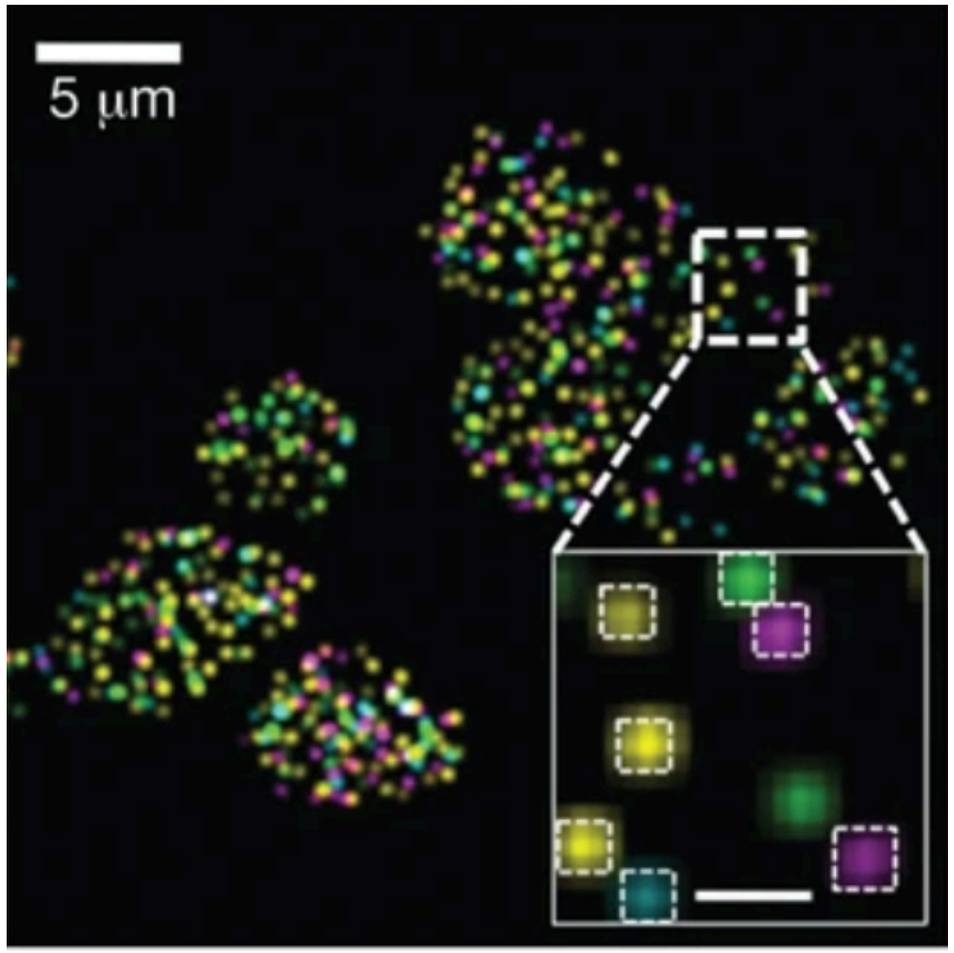
***seq**uential FISH (seqFISH)*

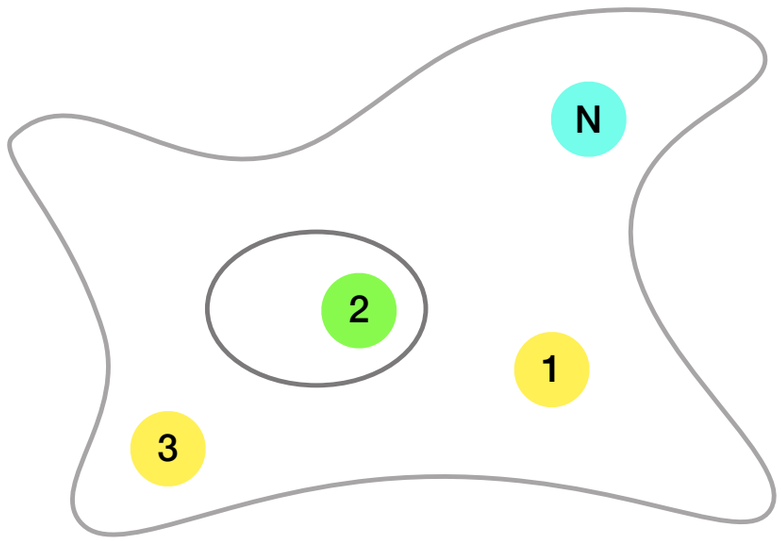


Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*

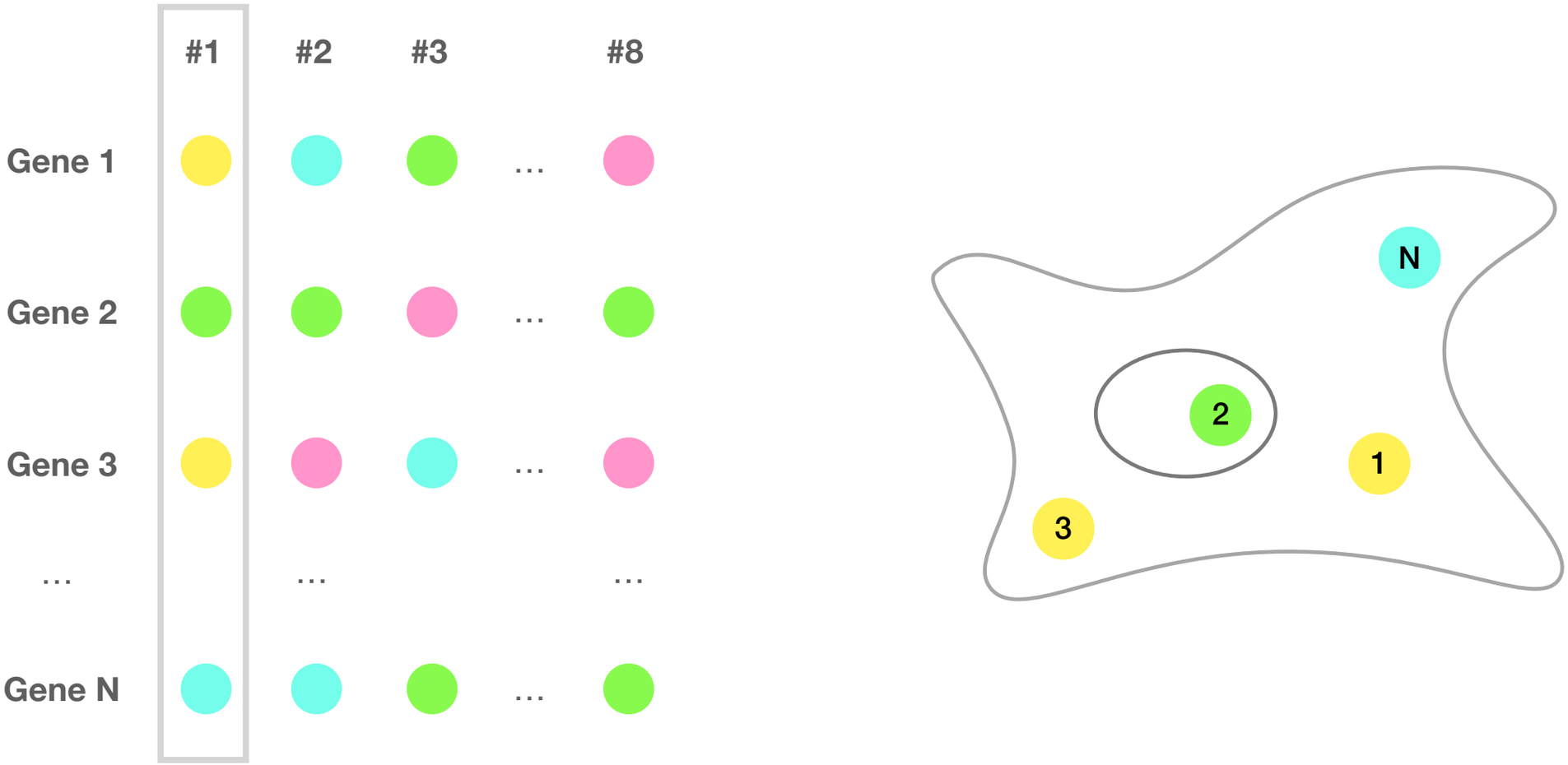




Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

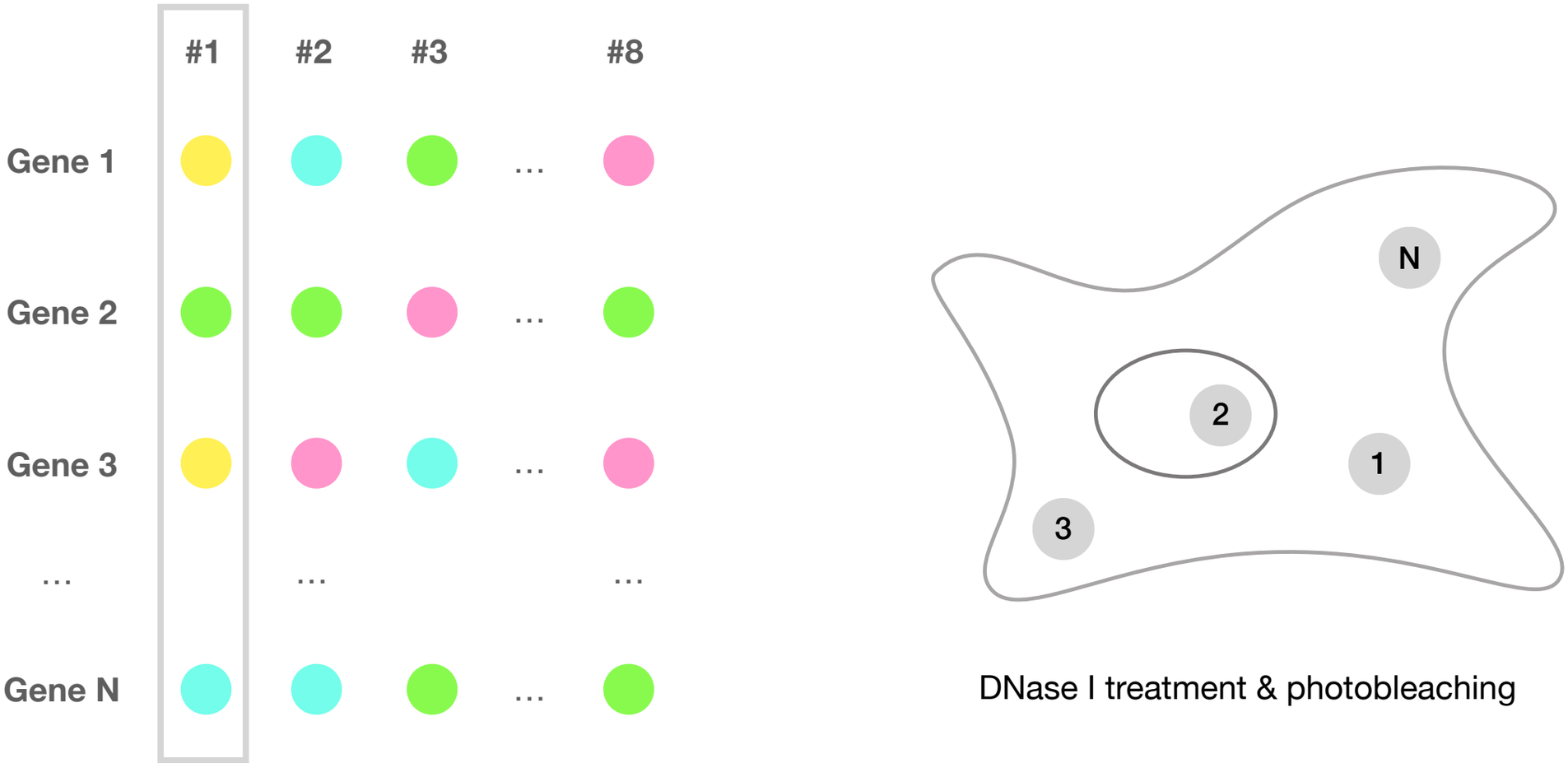
***seq**uential FISH (seqFISH)*



Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

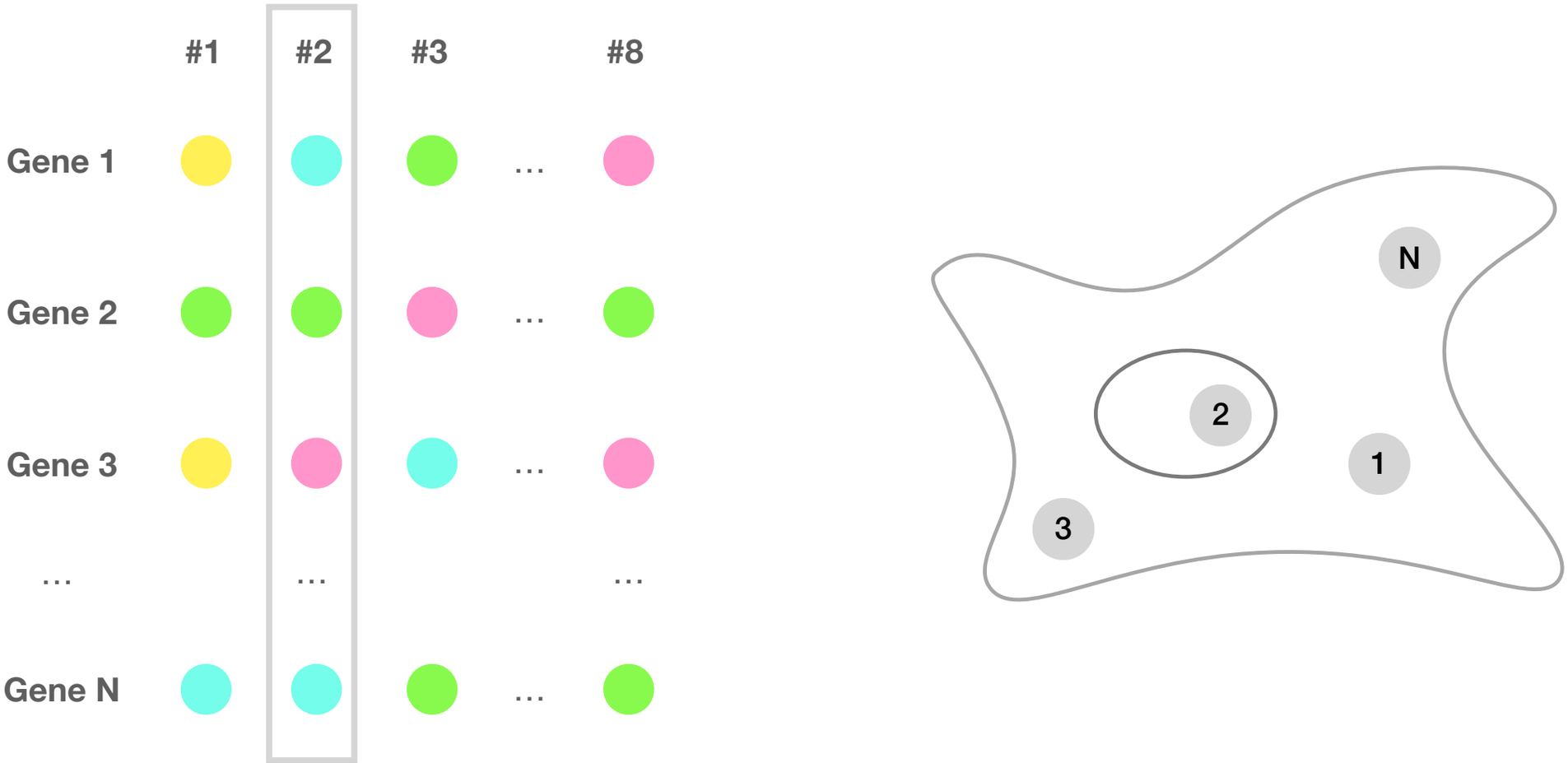
***seq**uential FISH (seqFISH)*

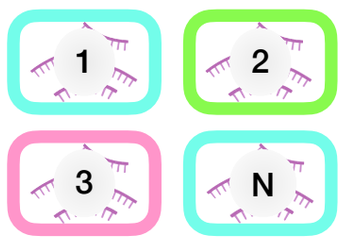


Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*



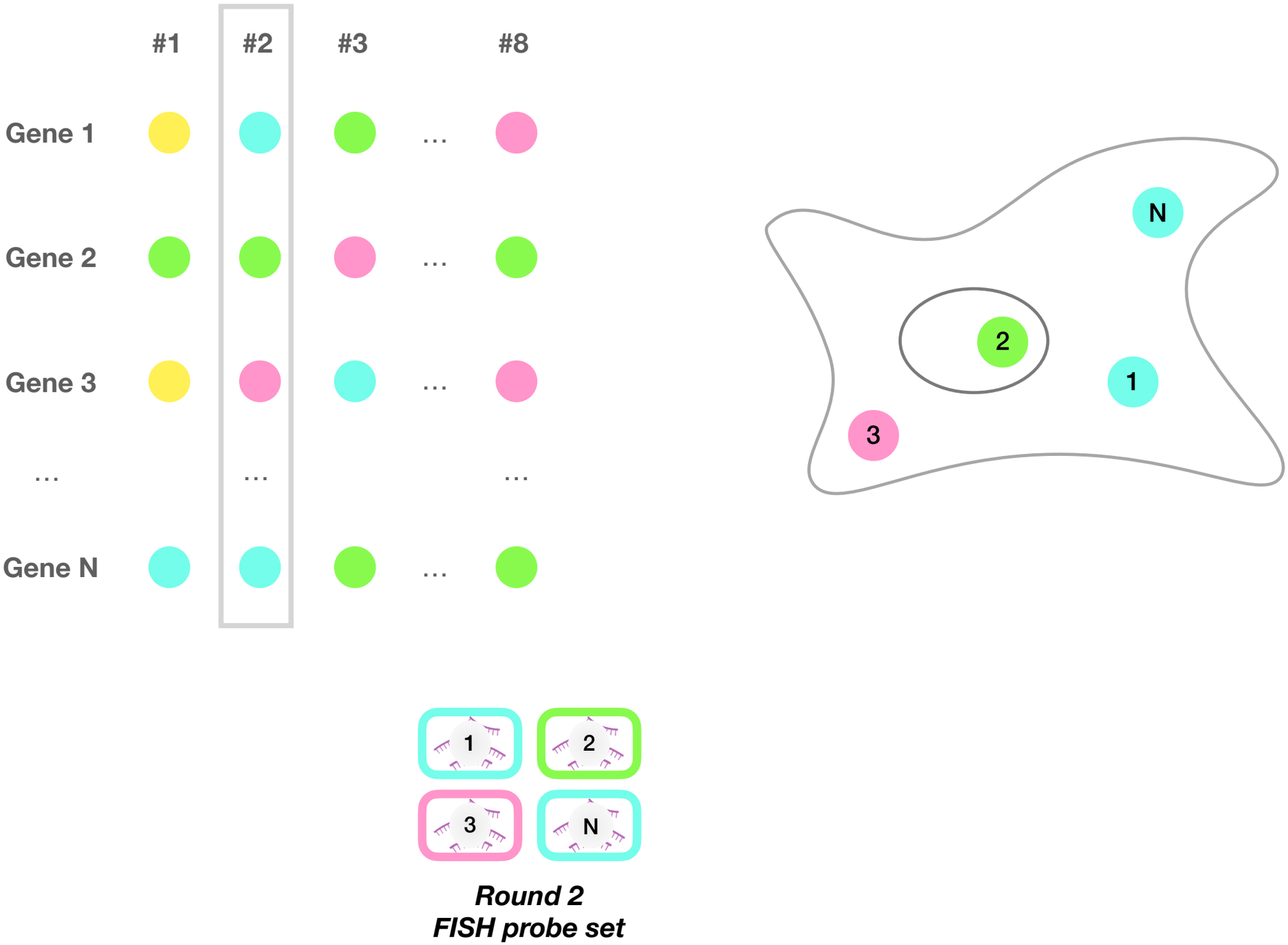


Round 2
FISH probe set

Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

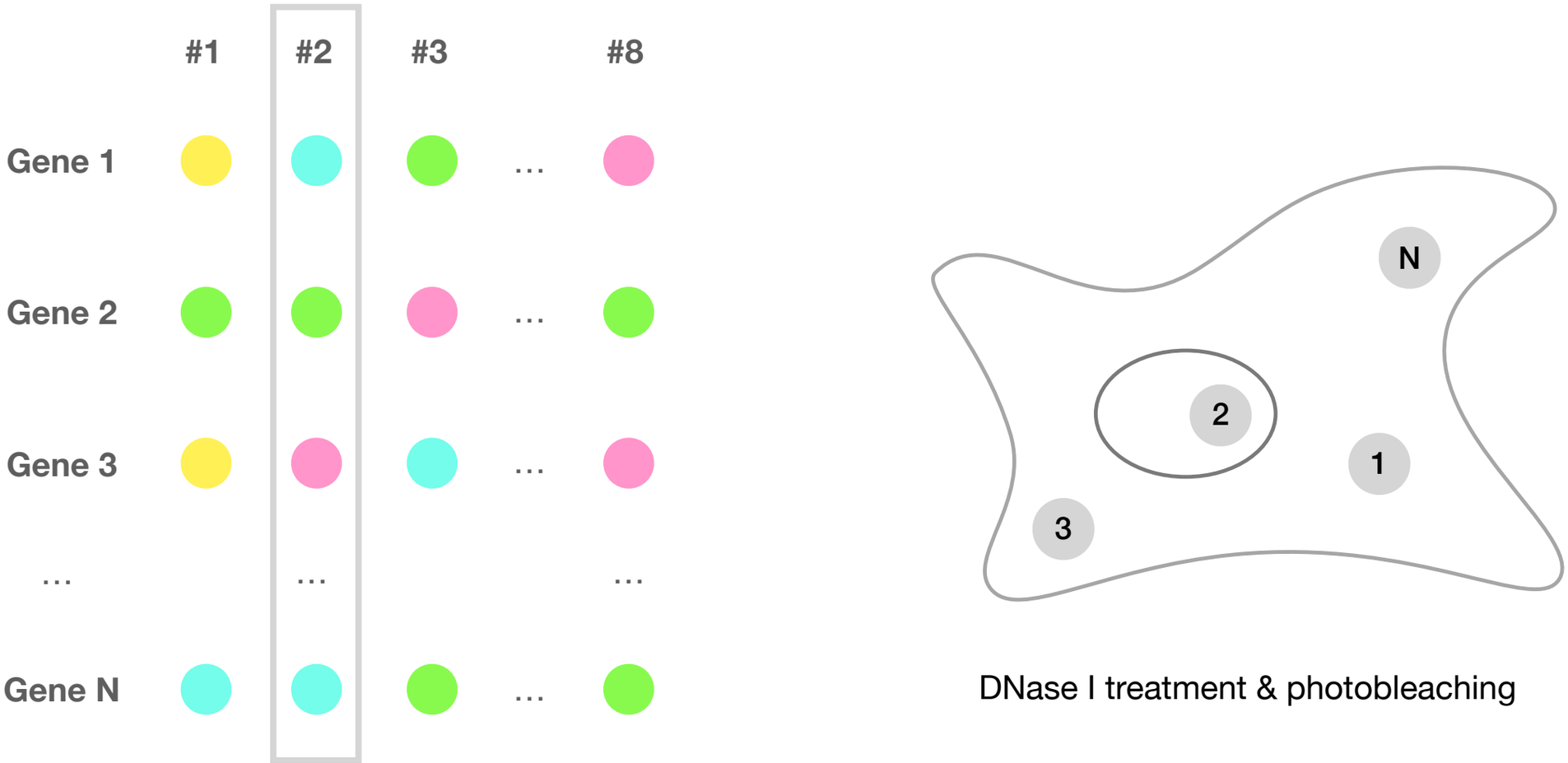
***seq**uential FISH (seqFISH)*



Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

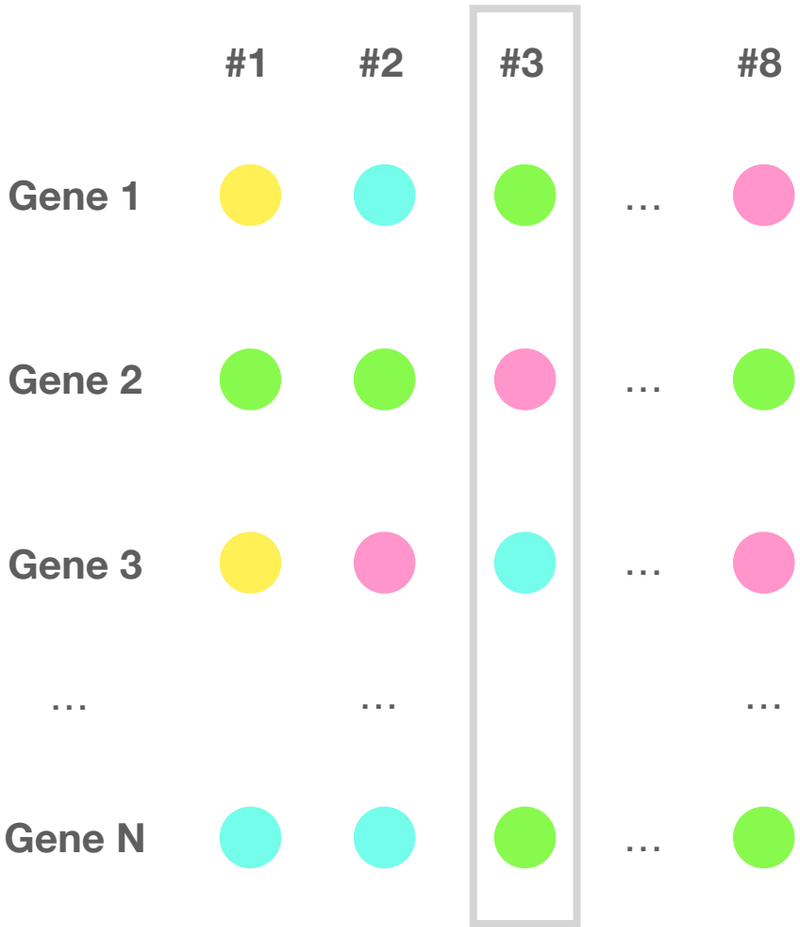
***seq**uential FISH (seqFISH)*

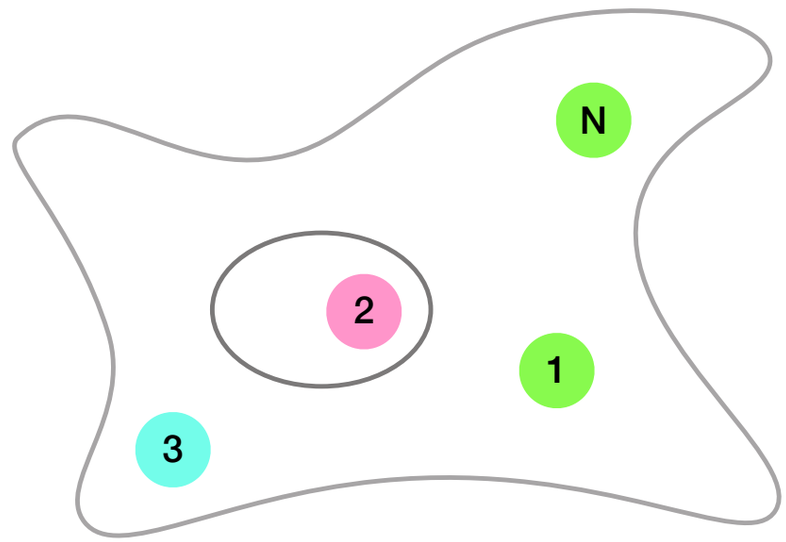


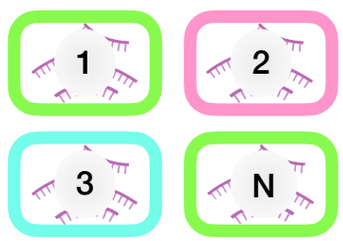
Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*





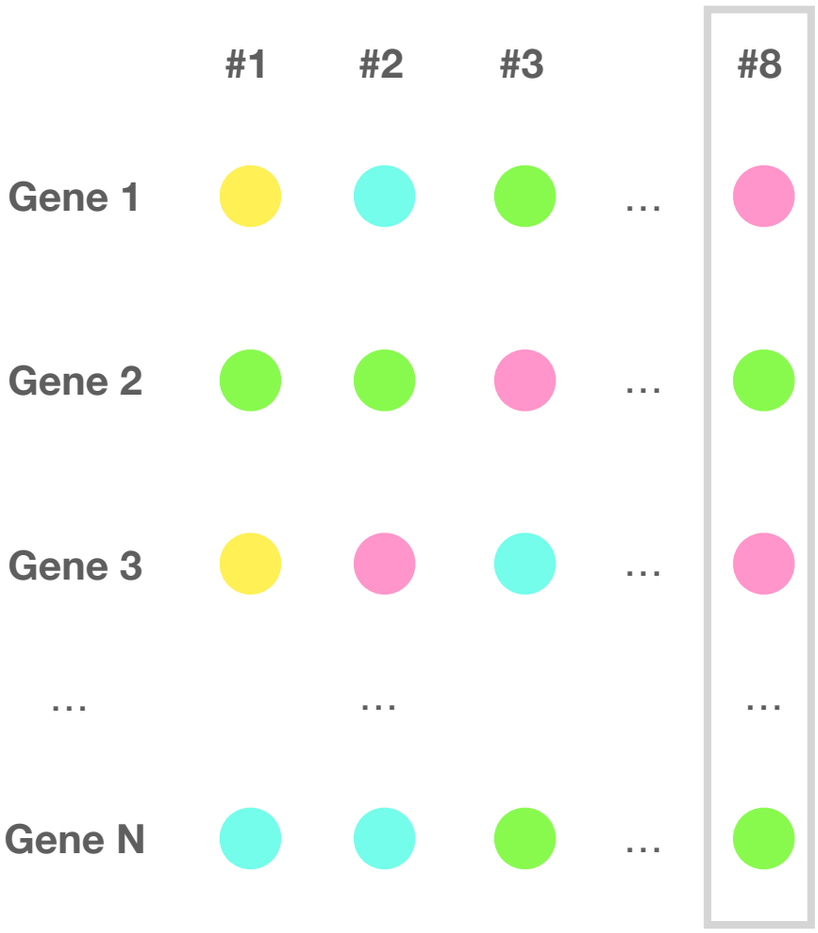


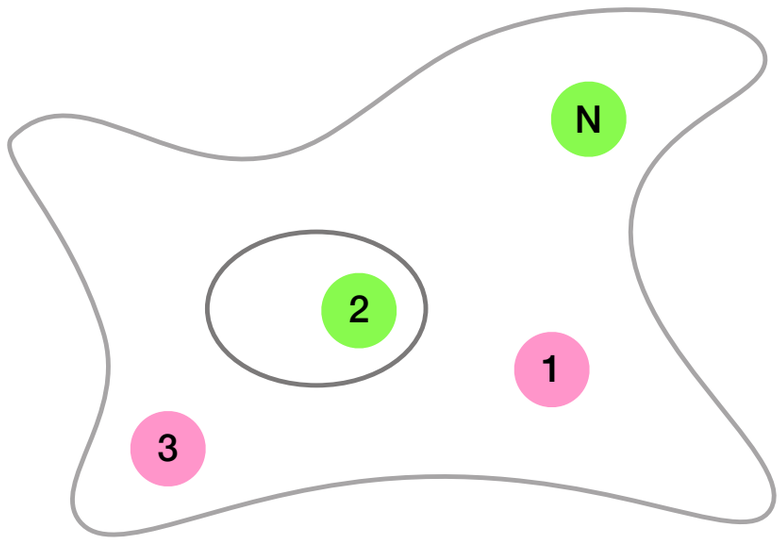
Round 3
FISH probe set

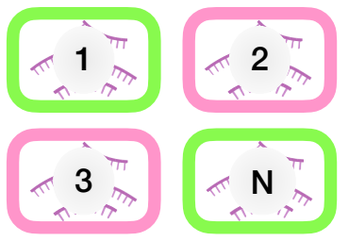
Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*





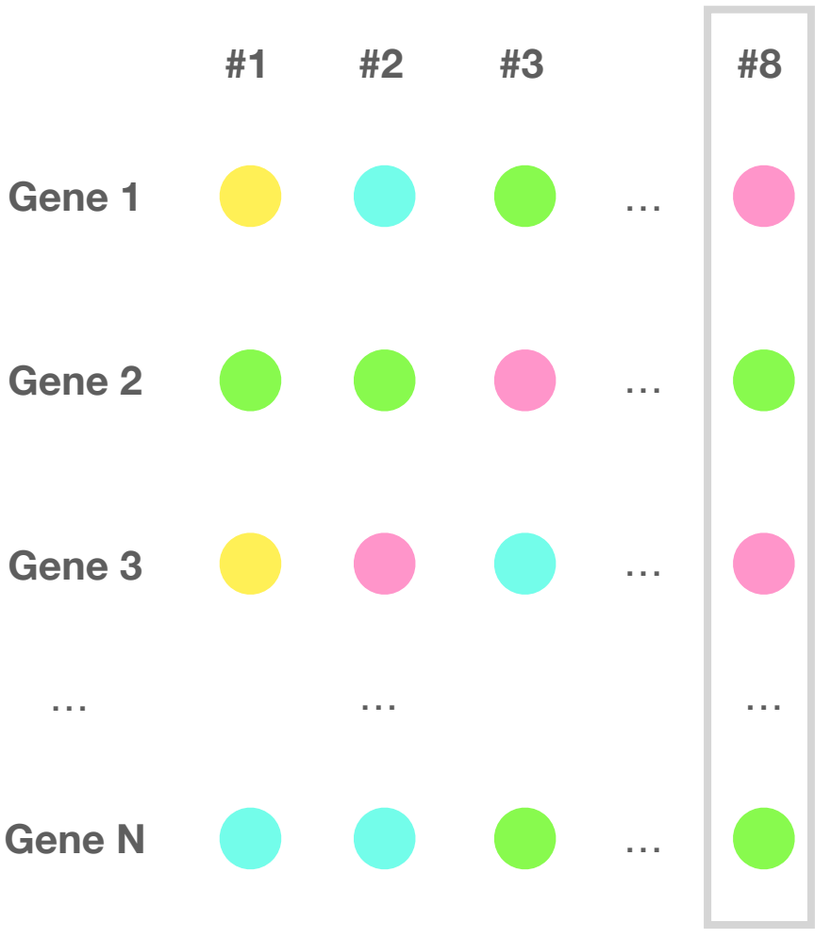


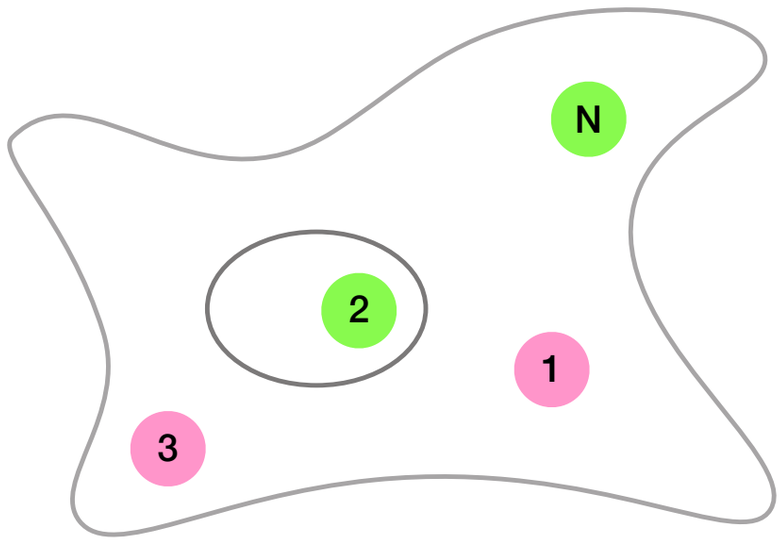
Round 8
FISH probe set

Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*





Decoding of barcode reveals identity of single mRNA molecule

Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

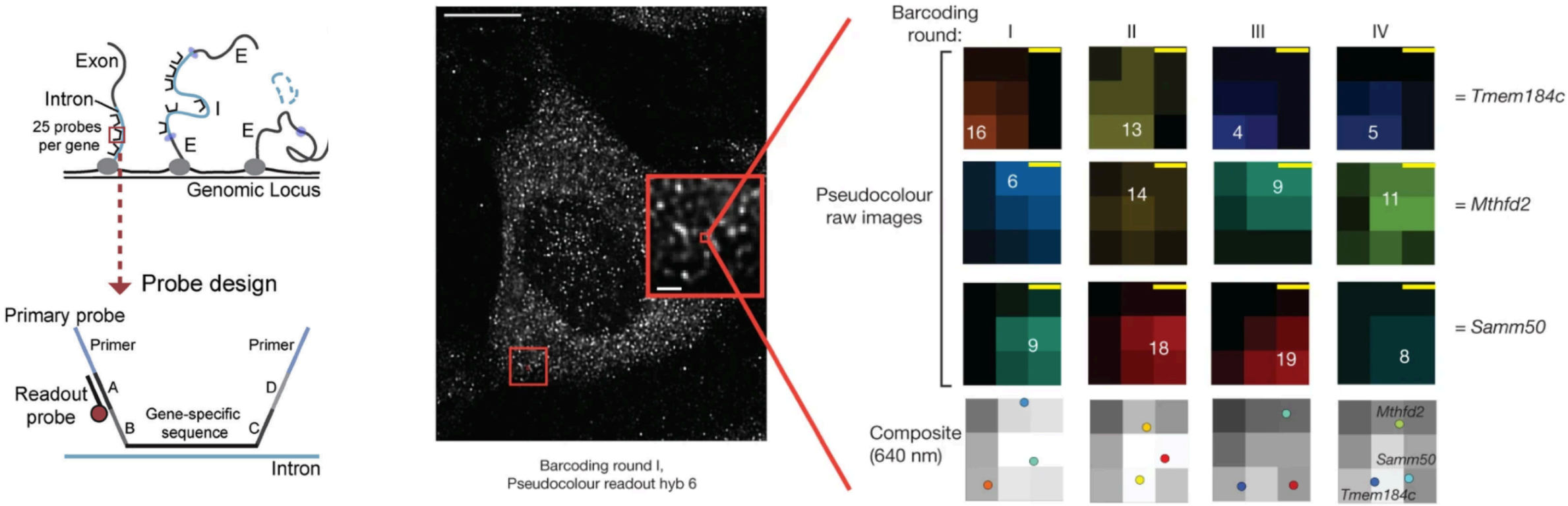
Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*

Lubeck, E.; Coskun, A. F.; Zhiyentayev, T.; Ahmad, M.; Cai, L. *Nat. Methods* **2014**, *11*, 360.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*

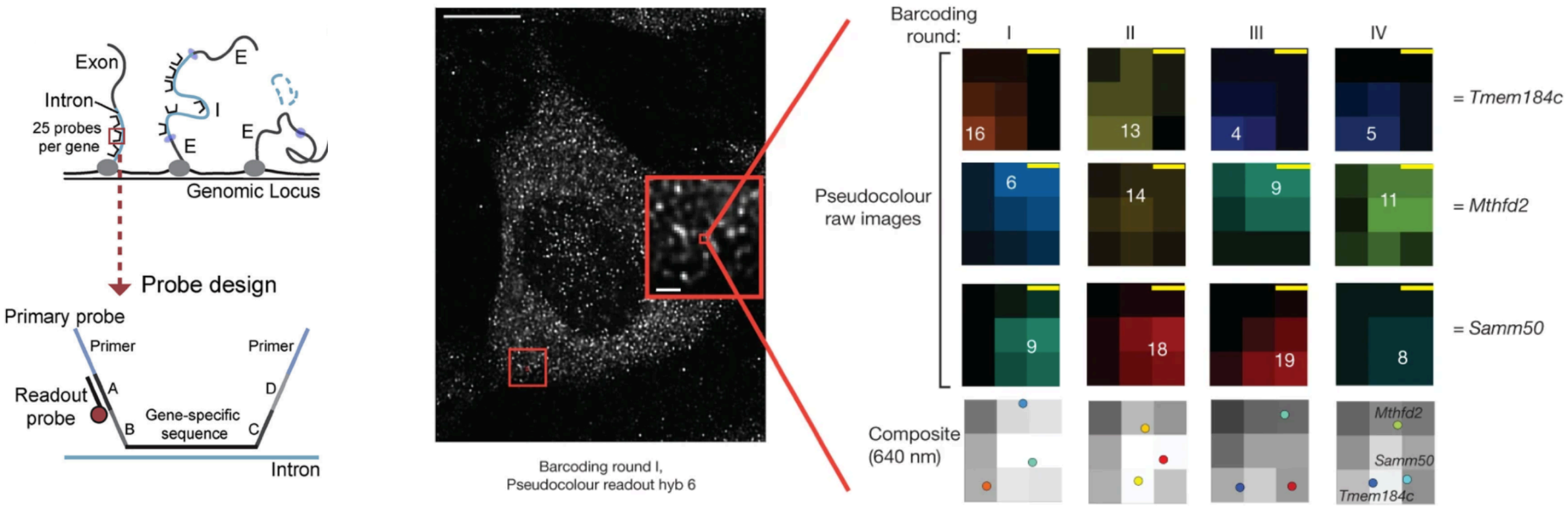


- Meticulous probe design and use of pseudocolors improved efficiency/resolution/specificity

Eng, L. C. -H.; Lawson, M. … Cai, L. *Nature* **2019**, *568*, 235.

Imaging-based spatial transcriptomics

***seq**uential FISH (seqFISH)*



- Meticulous probe design and use of pseudocolors improved efficiency/resolution/specificity
- Error-correcting rounds can be added for missing signals

Eng, L. C. -H.; Lawson, M. … Cai, L. *Nature* **2019**, *568*, 235.

Imaging-based spatial transcriptomics

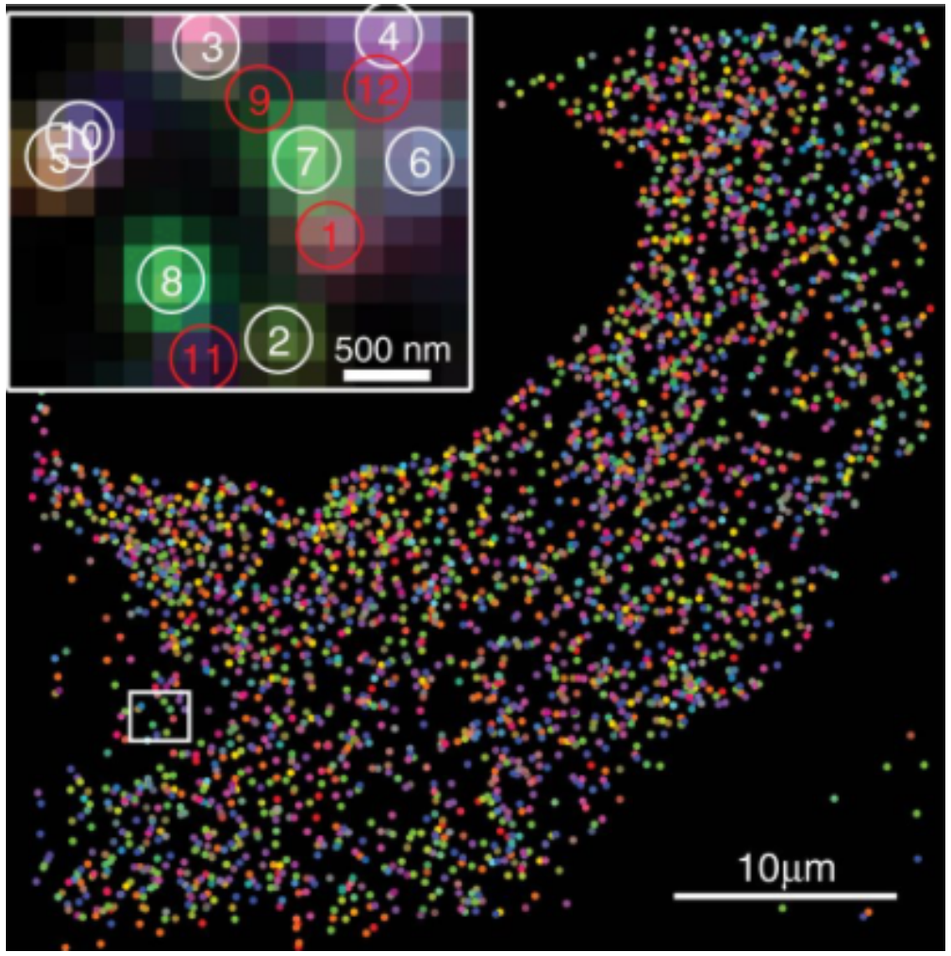
***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Xiaowei Zhuang
Harvard / HHMI

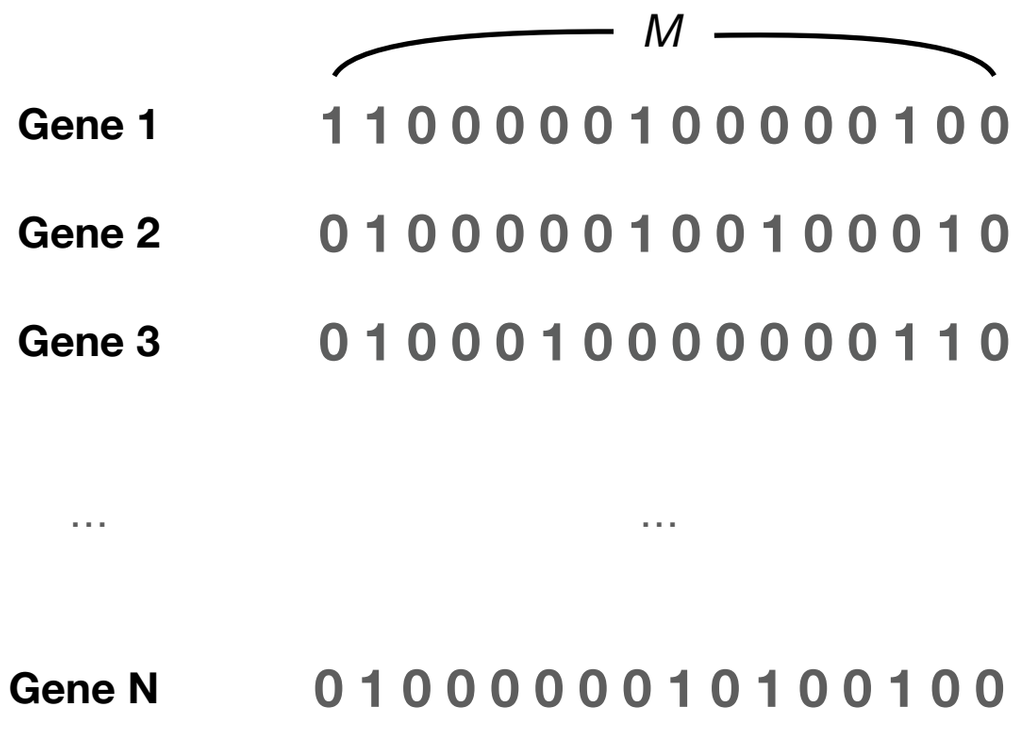


- Error-robust encoding scheme
- 140 RNA species (detect & correct errors)
- 1001 RNA species (detect but not correct errors)

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

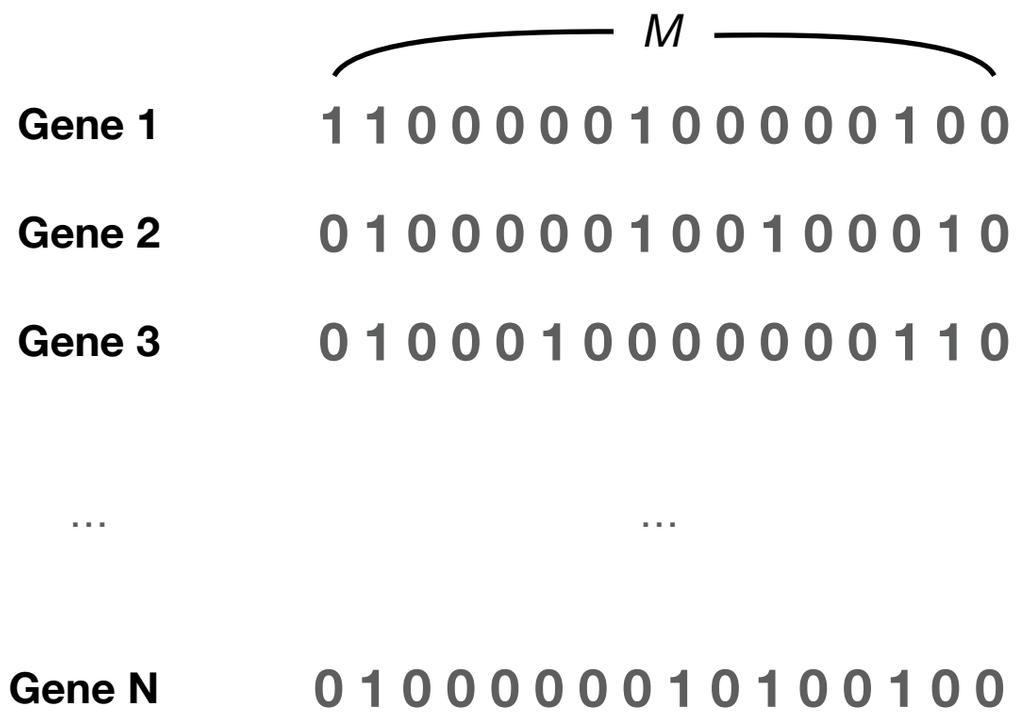
***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*



Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

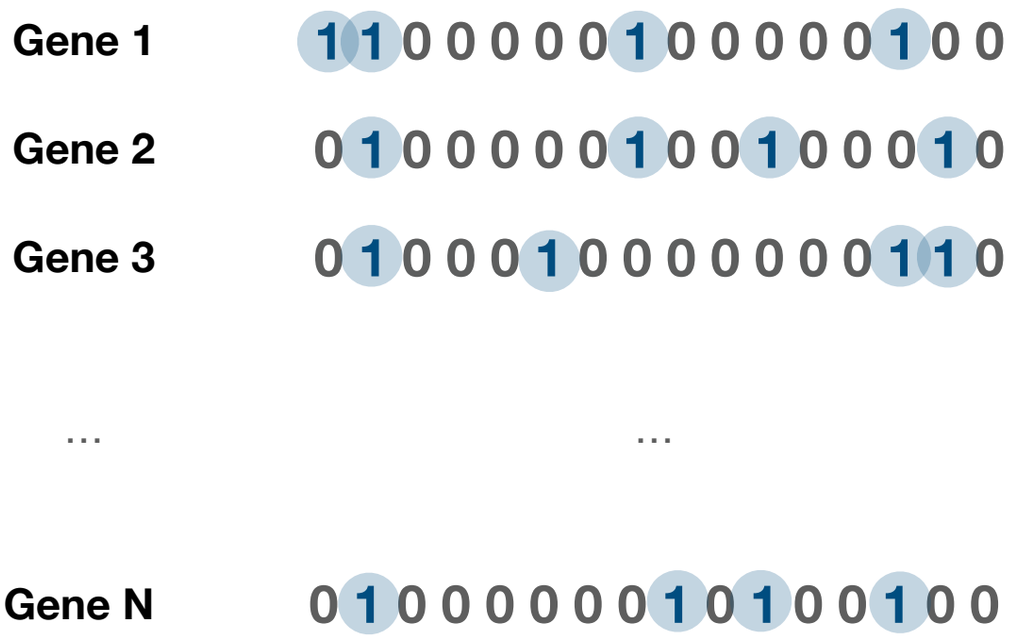


- Each gene encoded with *M*-bit binary word

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*



- Each gene encoded with *M*-bit binary word
- Each word has four ‘1’s

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Gene 1	**1** 1 0 0 0 0 0 1 0 0 **0** 0 0 **1** **0** 0
Gene 2	**0** 1 0 0 0 0 0 1 0 0 **1** 0 0 **0** **1** 0
Gene 3	0 1 0 0 0 1 0 0 0 0 0 0 0 1 1 0
…	…
Gene N	0 1 0 0 0 0 0 0 1 0 1 0 0 1 0 0

- Each gene encoded with *M*-bit binary word
- Each word has four ‘1’s
- Each word pair has a Hamming distance of 4

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Gene 1	1 1 0 0 0 0 0 1 0 0 0 0 0 1 0 0
Gene 2	0 1 0 0 0 **0** 0 **1** 0 0 **1** 0 0 **0** 1 0
Gene 3	0 1 0 0 0 **1** 0 **0** 0 0 **0** 0 0 **1** 1 0
…	…
Gene N	0 1 0 0 0 0 0 0 1 0 1 0 0 1 0 0

- Each gene encoded with *M*-bit binary word
- Each word has four ‘1’s
- Each word pair has a Hamming distance of 4

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Gene 1	1 1 0 0 0 0 0 1 0 0 0 0 0 1 0 0
Gene 2	0 1 0 0 0 0 0 **1 0** 0 1 0 0 **0 1** 0
Gene 3	0 1 0 0 0 1 0 0 0 0 0 0 0 1 1 0
...	...
Gene N	0 1 0 0 0 0 0 **0 1** 0 1 0 0 **1 0** 0

- Each gene encoded with *M*-bit binary word
- Each word has four ‘1’s
- Each word pair has a Hamming distance of 4

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Gene 1	**1 1 0 0 0 0 0 1 0 0 0 0 1 0 0**
Gene 2	**0 1 0 0 0 0 0 1 0 0 1 0 0 0 1 0**
Gene 3	**0 1 0 0 0 1 0 0 0 0 0 0 1 1 0**
…	…
Gene N	**0 1 0 0 0 0 0 0 1 0 1 0 0 1 0 0**
?	**0 0 0 0 0 0 0 0 1 0 1 0 0 1 0 0**

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

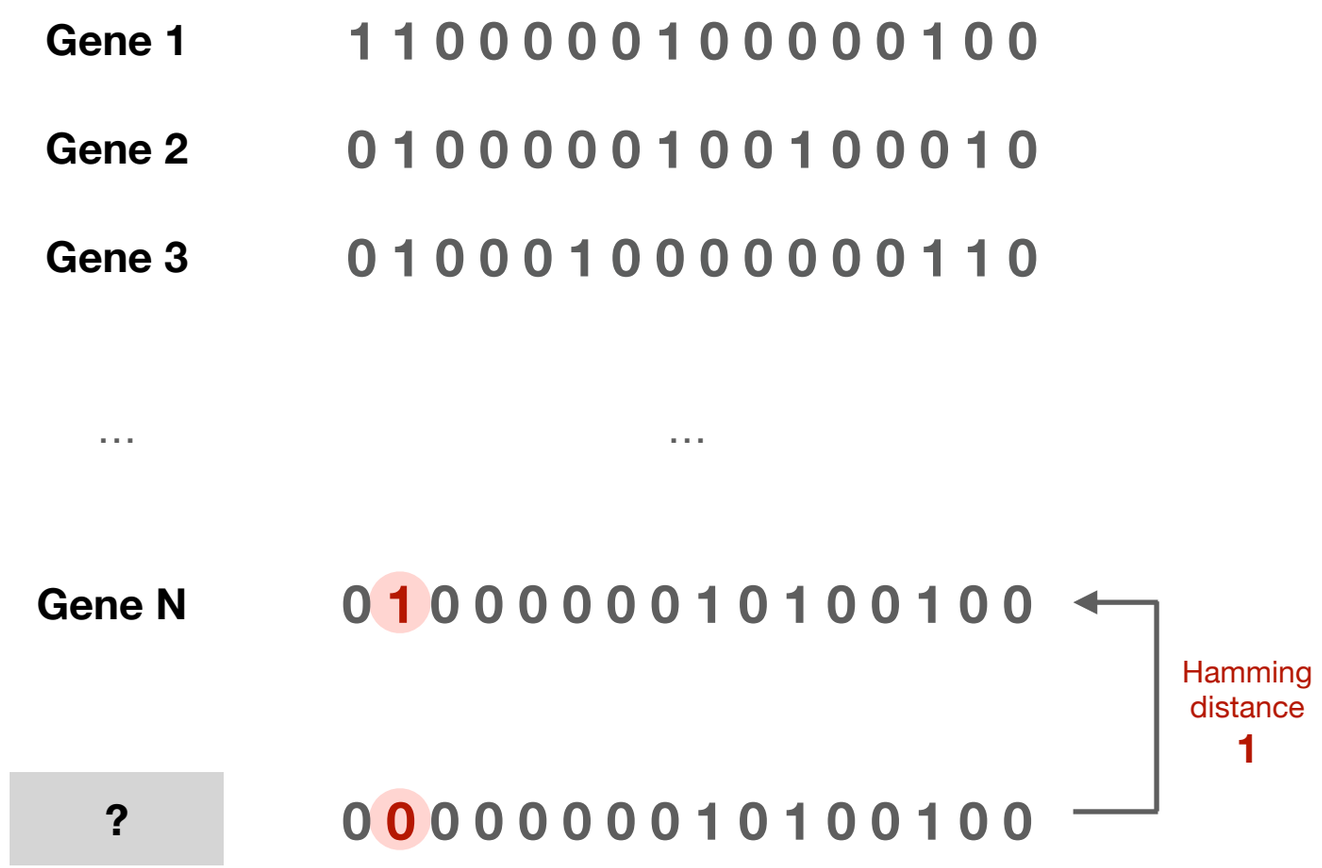
***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Gene 1	**1 1 0 0 0 0 0 1 0 0 0 0 1 0 0**
Gene 2	**0 1 0 0 0 0 0 1 0 0 1 0 0 0 1 0**
Gene 3	**0 1 0 0 0 1 0 0 0 0 0 0 1 1 0**
...	...
Gene N	**0 1 0 0 0 0 0 0 1 0 1 0 0 1 0 0**
?	**0 0 0 0 0 0 0 0 1 0 1 0 0 1 0 0**

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

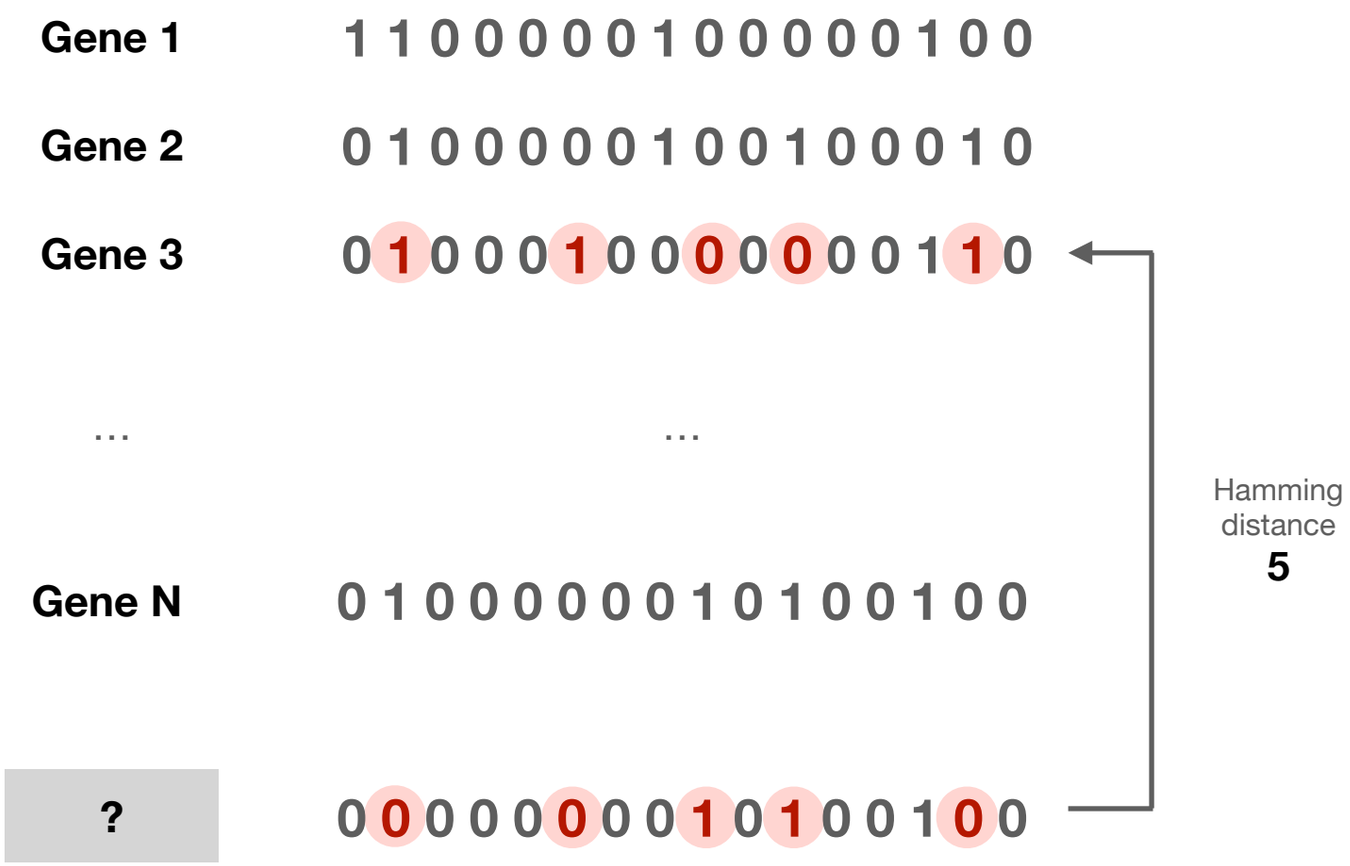


Only a single M-bit word has Hamming distance of 1

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

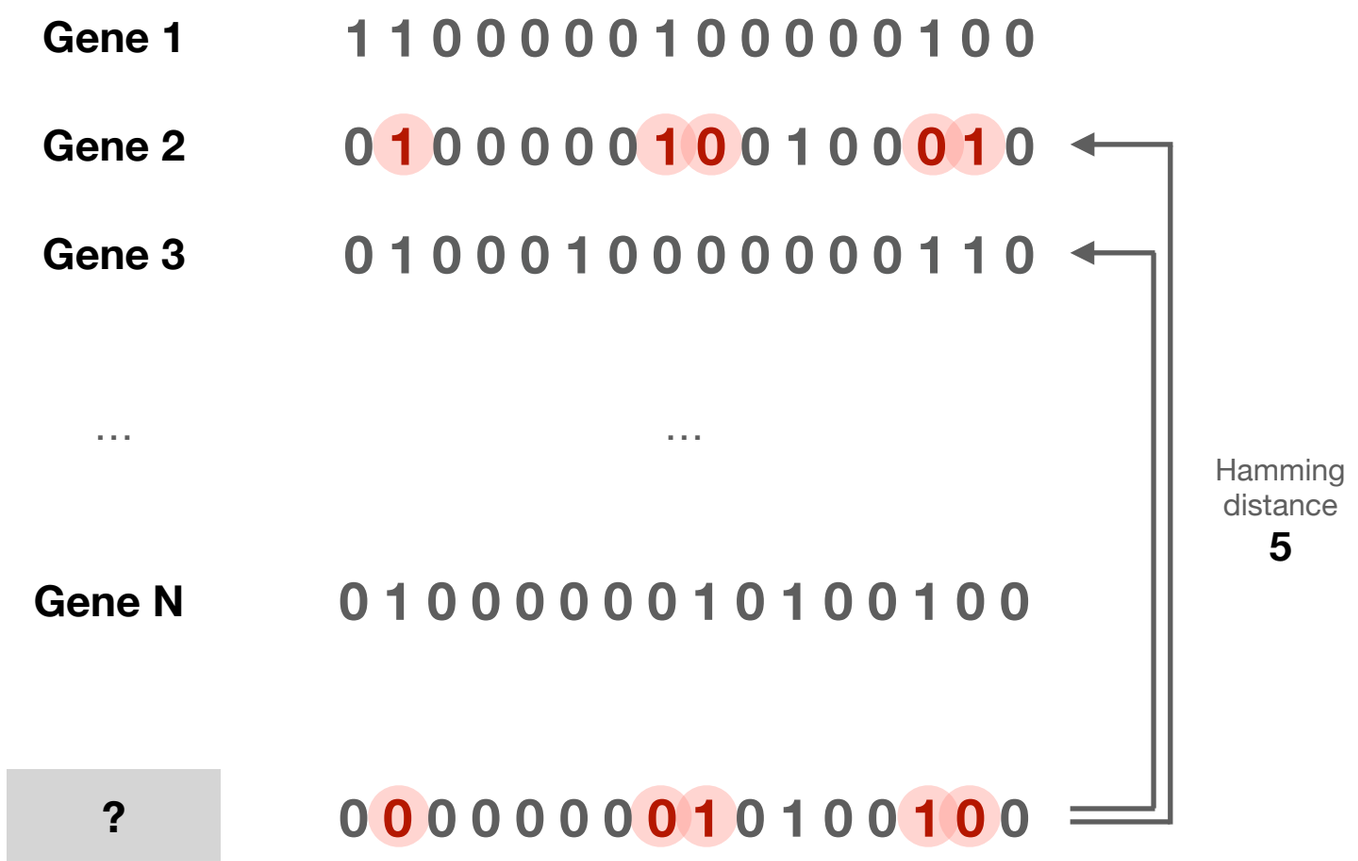


Only a single M-bit word has Hamming distance of 1

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*



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Imaging-based spatial transcriptomics

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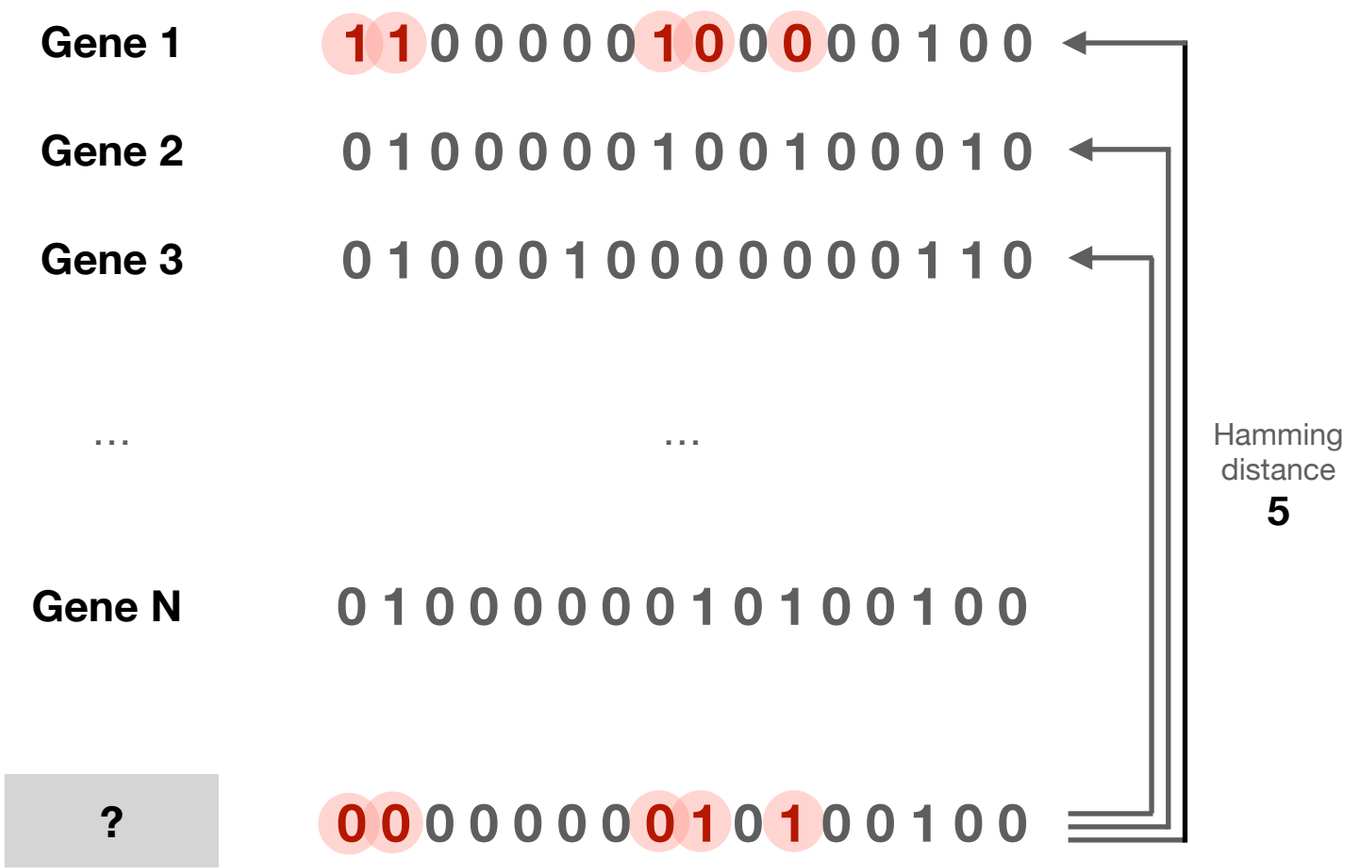
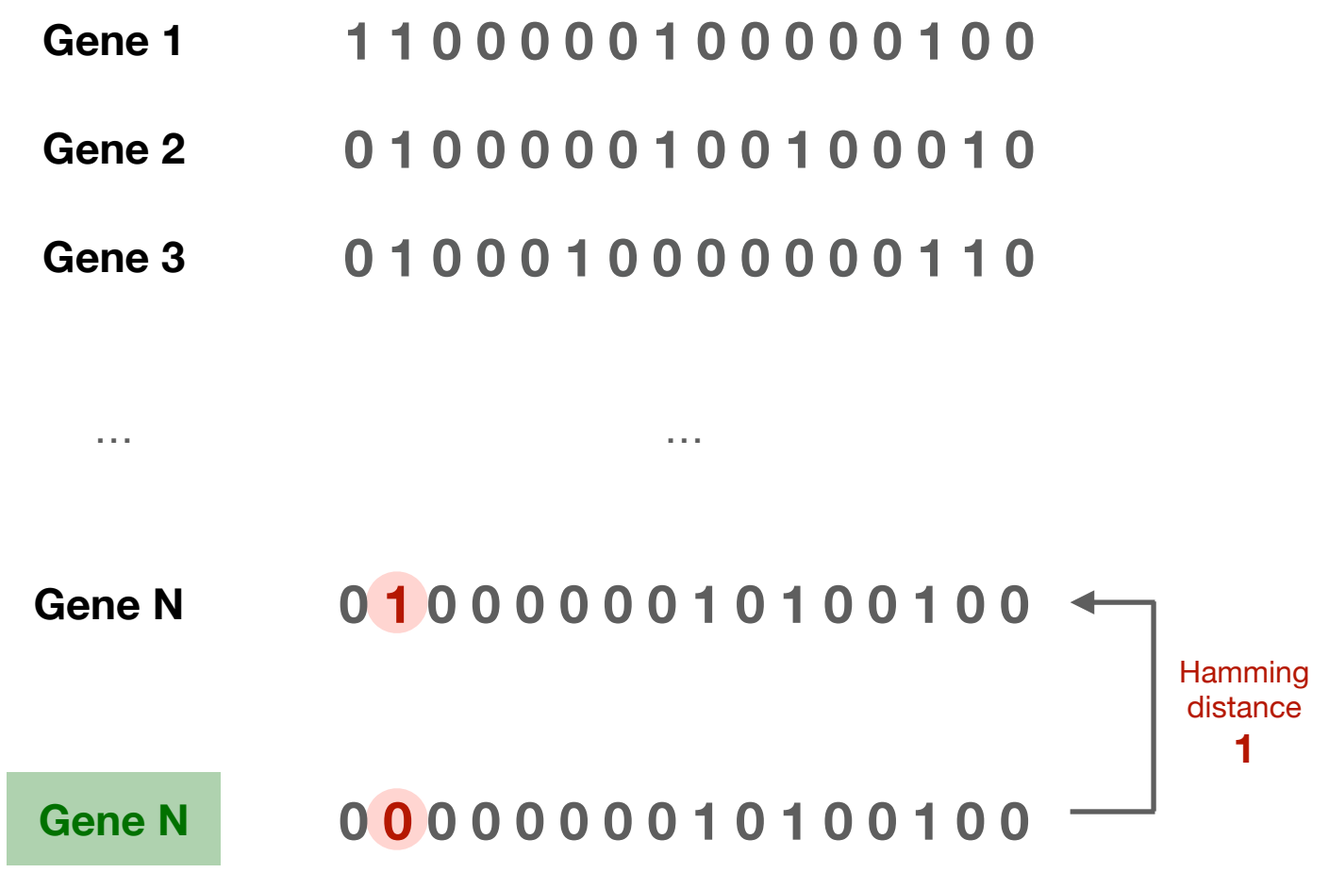


Only a single M-bit word has Hamming distance of 1

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*



Only a single M-bit word has Hamming distance of 1

Single-bit error correction possible

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Gene 1	**1 1 0 0 0 0 0 1 0 0 0 0 1 0 0**
Gene 2	**0 1 0 0 0 0 0 1 0 0 1 0 0 0 1 0**
Gene 3	**0 1 0 0 0 1 0 0 0 0 0 0 1 1 0**
…	…
Gene N	**0 1 0 0 0 0 0 0 1 0 1 0 0 1 0 0**
?	**0 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0**

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

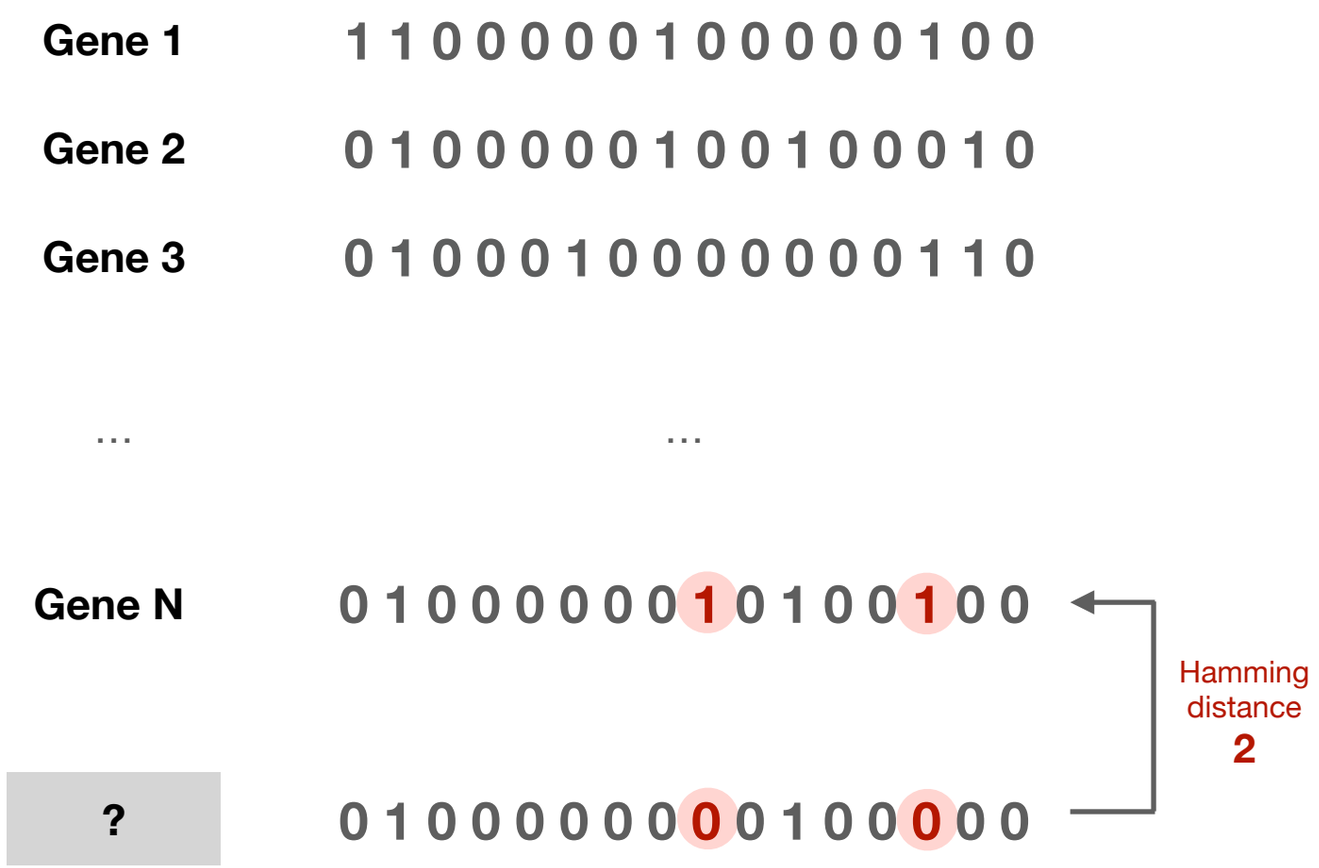
***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Gene 1	**1 1 0 0 0 0 0 1 0 0 0 0 1 0 0**
Gene 2	**0 1 0 0 0 0 0 1 0 0 1 0 0 0 1 0**
Gene 3	**0 1 0 0 0 1 0 0 0 0 0 0 1 1 0**
...	...
Gene N	**0 1 0 0 0 0 0 0 1 0 1 0 0 1 0 0**
?	**0 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0**

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

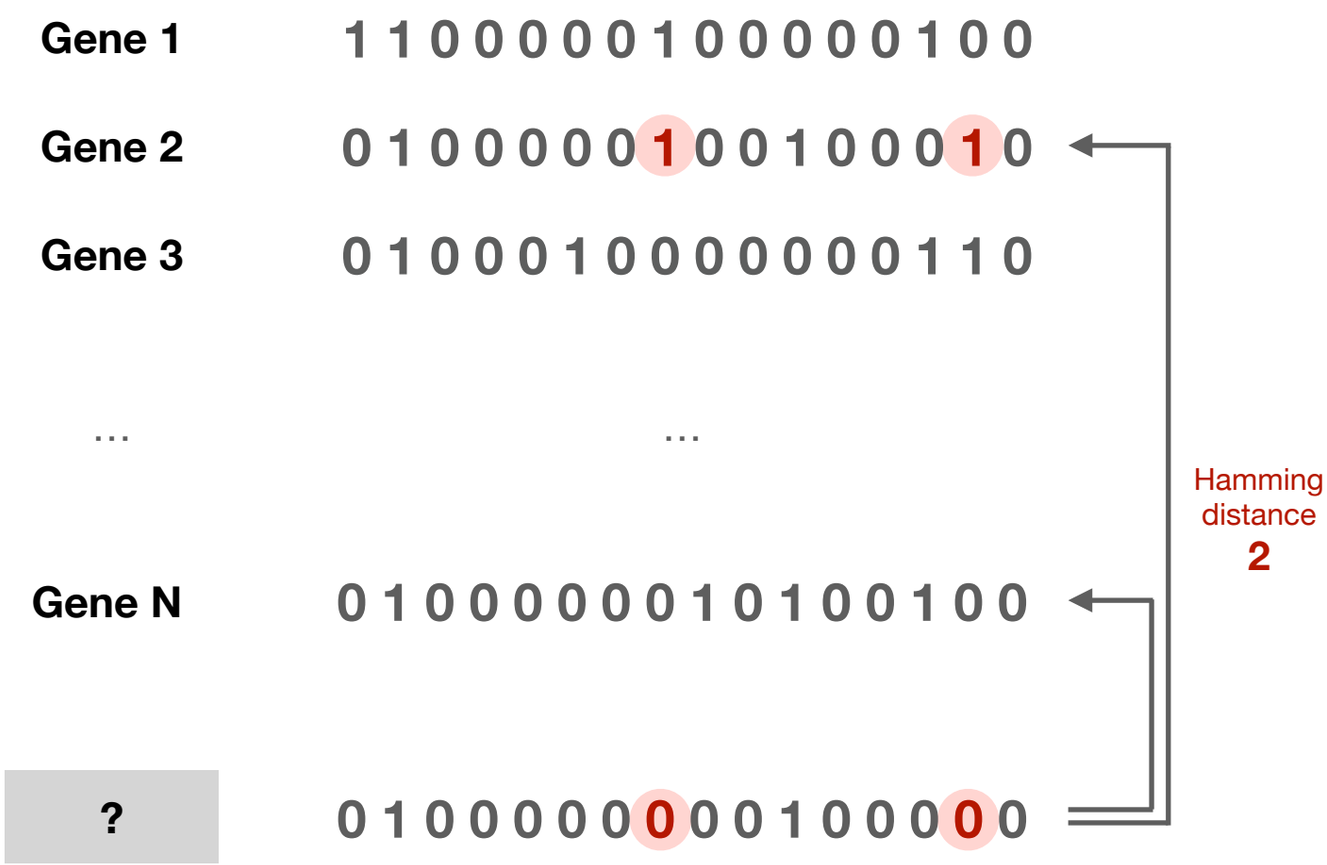


Multiple M-bit words can have Hamming distance of 2

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

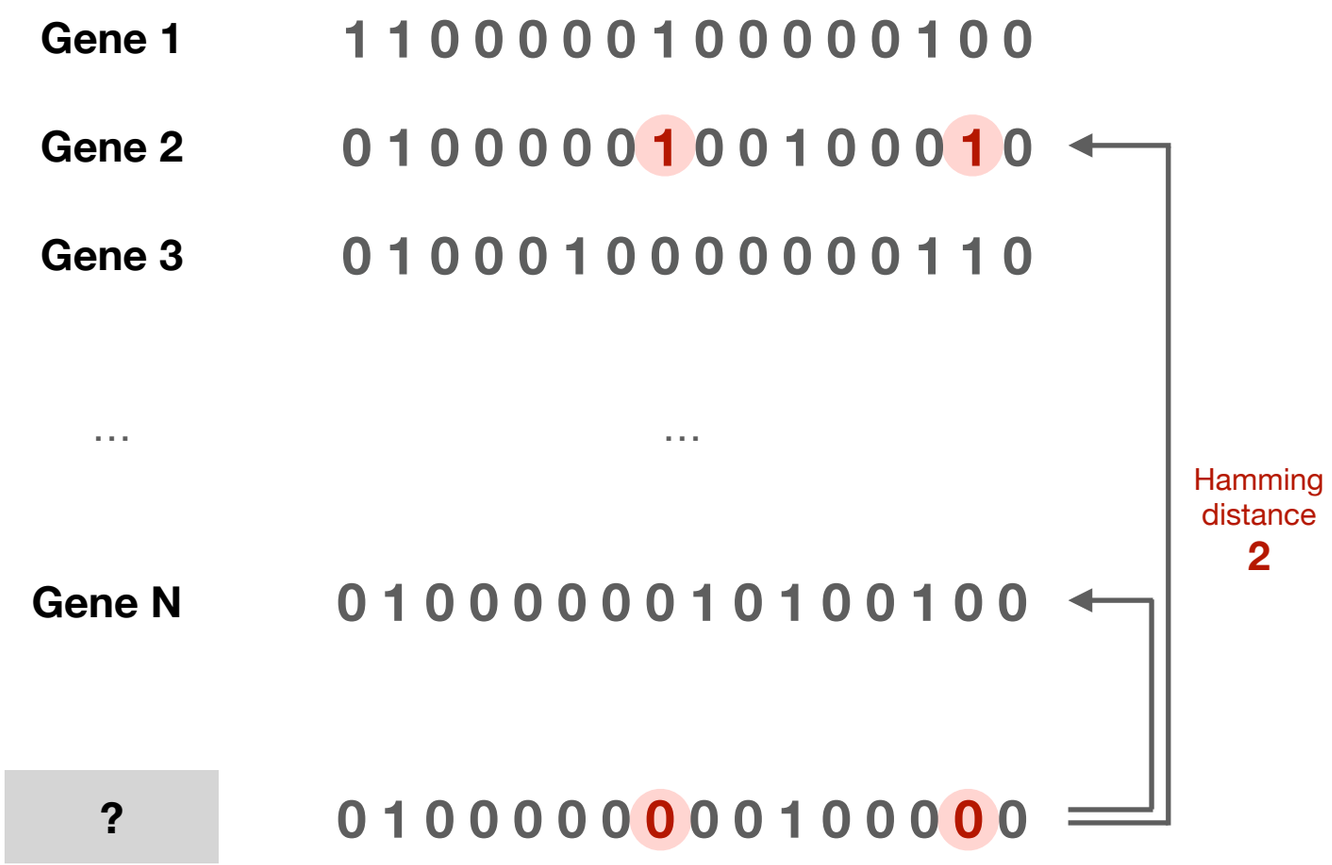


Multiple M-bit words can have Hamming distance of 2

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*



Multiple M-bit words can have Hamming distance of 2

Cannot correct double-bit errors

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Single-bit error correction possible

Cannot correct double-bit errors

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Imaging-based spatial transcriptomics

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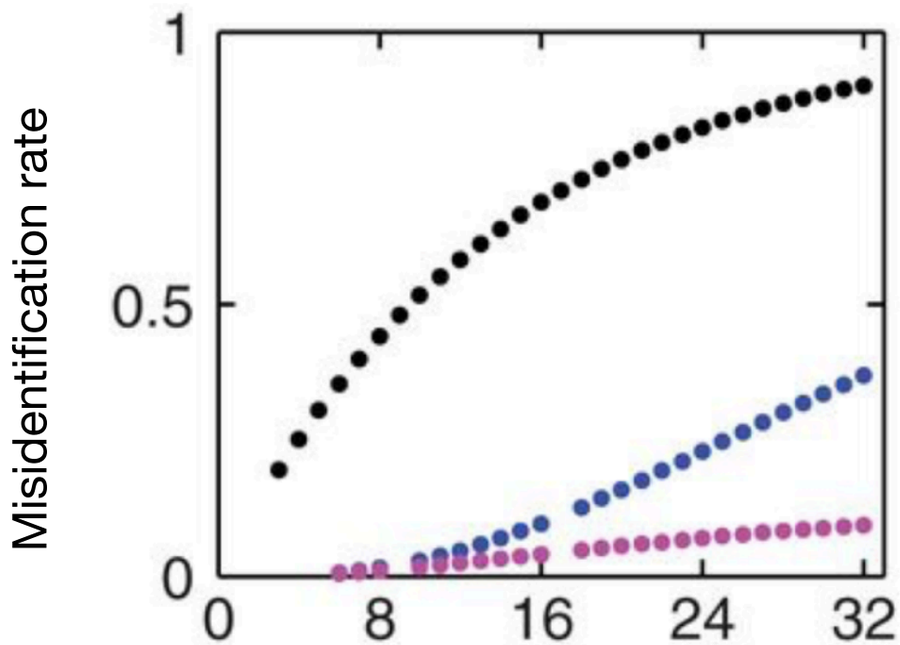

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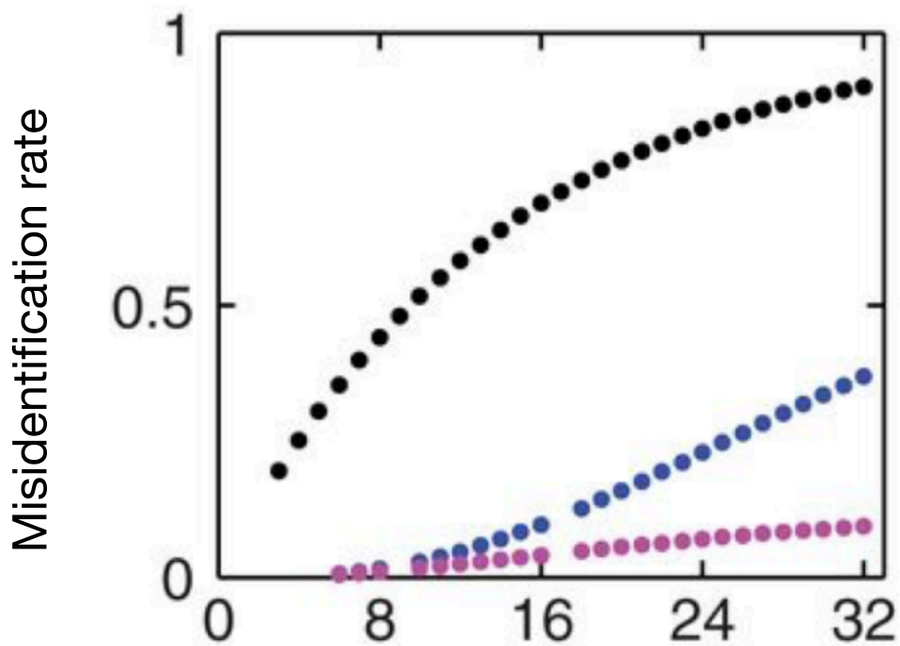

No “vocabulary”

Single-bit error correction possible

Cannot correct double-bit errors

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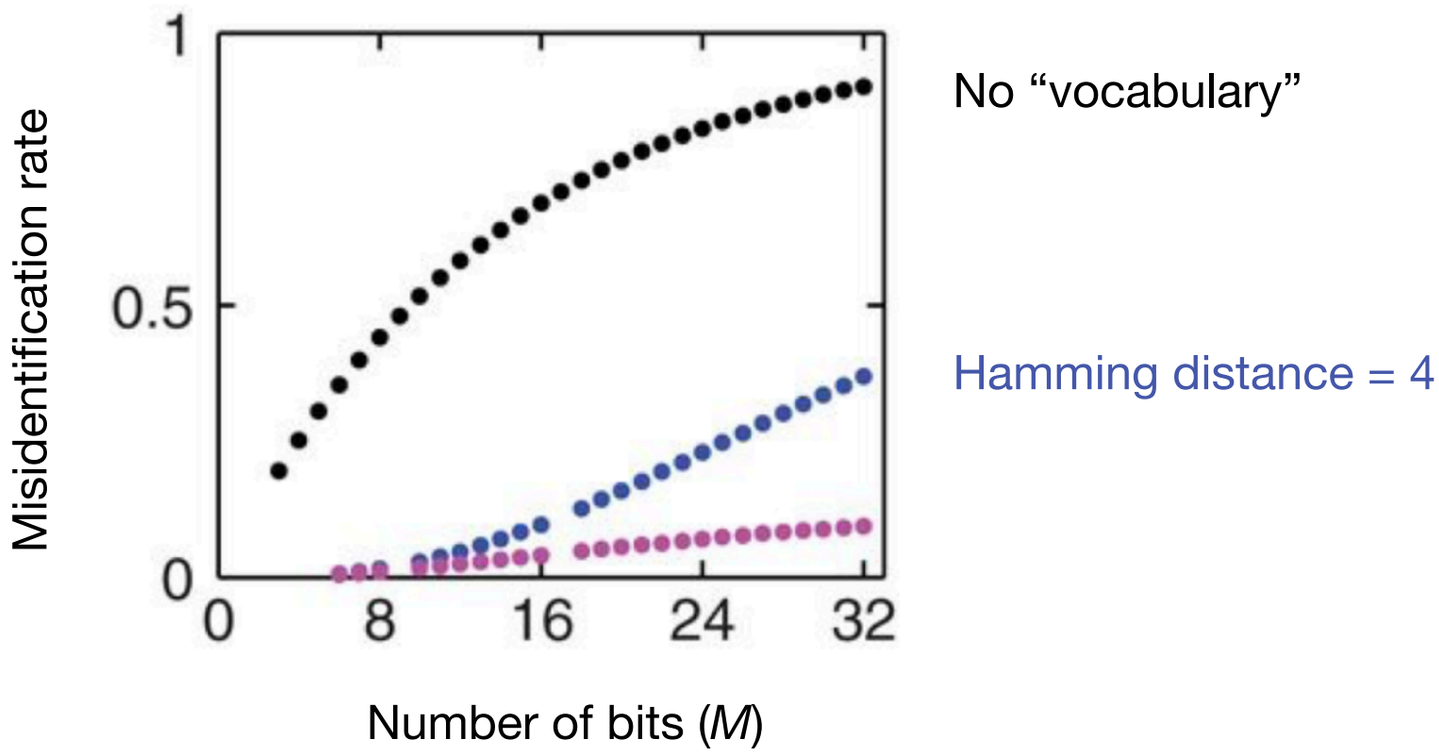

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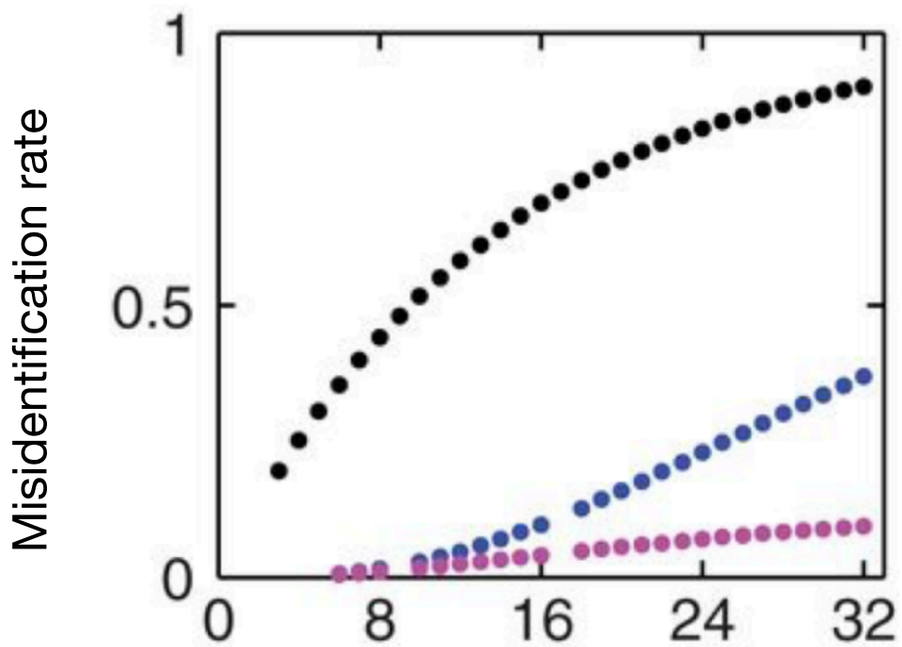

No “vocabulary”

Hamming distance = 4

Hamming distance = 4
and # of ‘1’s = 4

Single-bit error correction possible

Cannot correct double-bit errors

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Imaging-based spatial transcriptomics

***M**ultiplexed **E**rror-**R**obust FISH (MERFISH)*

Gene 1	**1 1 0 0 0 0 0 1 0 0 0 0 1 0 0**	
Gene 2	**0 1 0 0 0 0 0 1 0 0 1 0 0 0 1 0**	
Gene 3	**0 1 0 0 0 1 0 0 0 0 0 0 1 1 0**	
...	...	...
Gene N	**0 1 0 0 0 0 0 0 1 0 1 0 0 1 0 0**	

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

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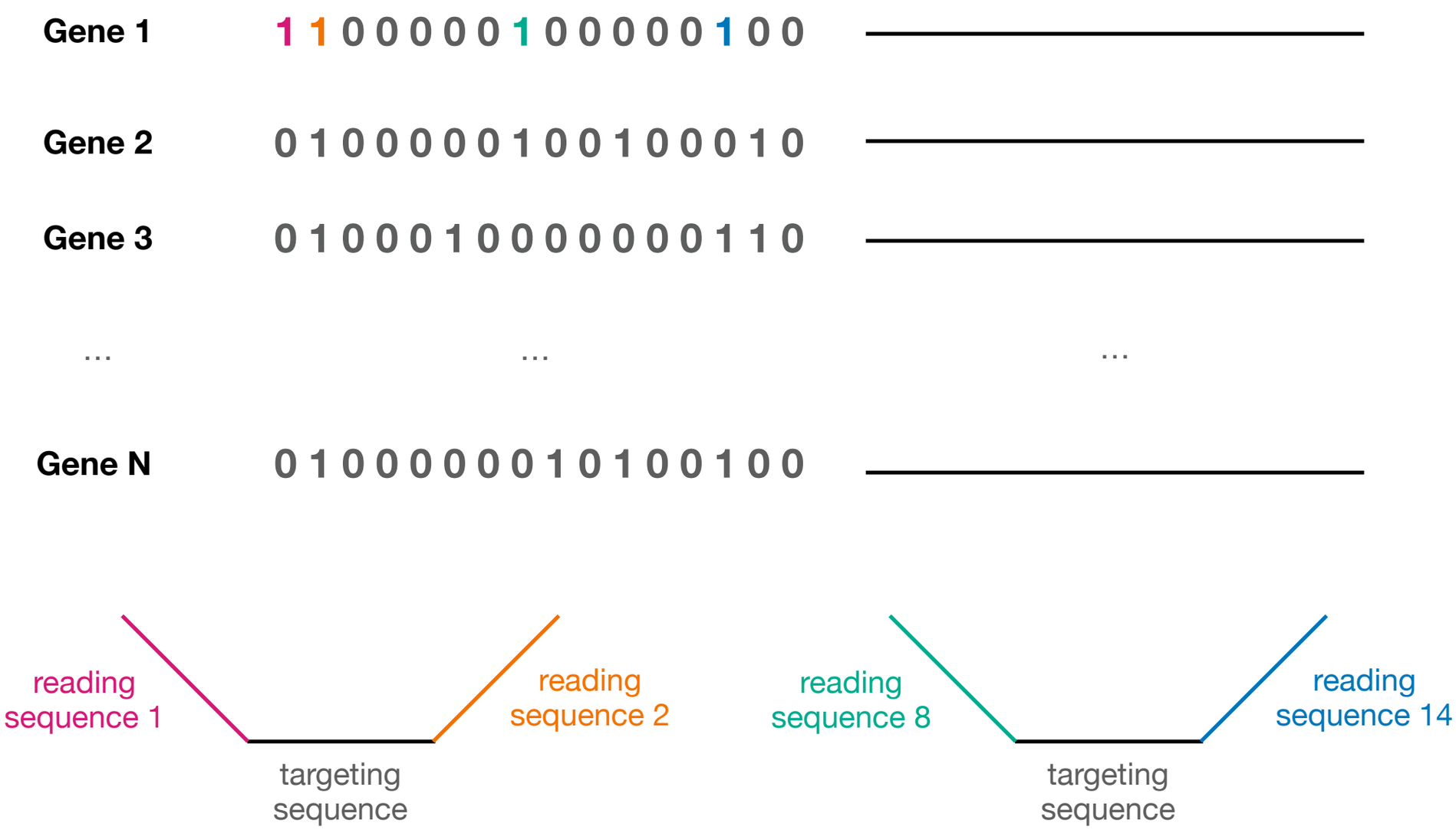

- Two encoding probes, four readout sequences in total per mRNA

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

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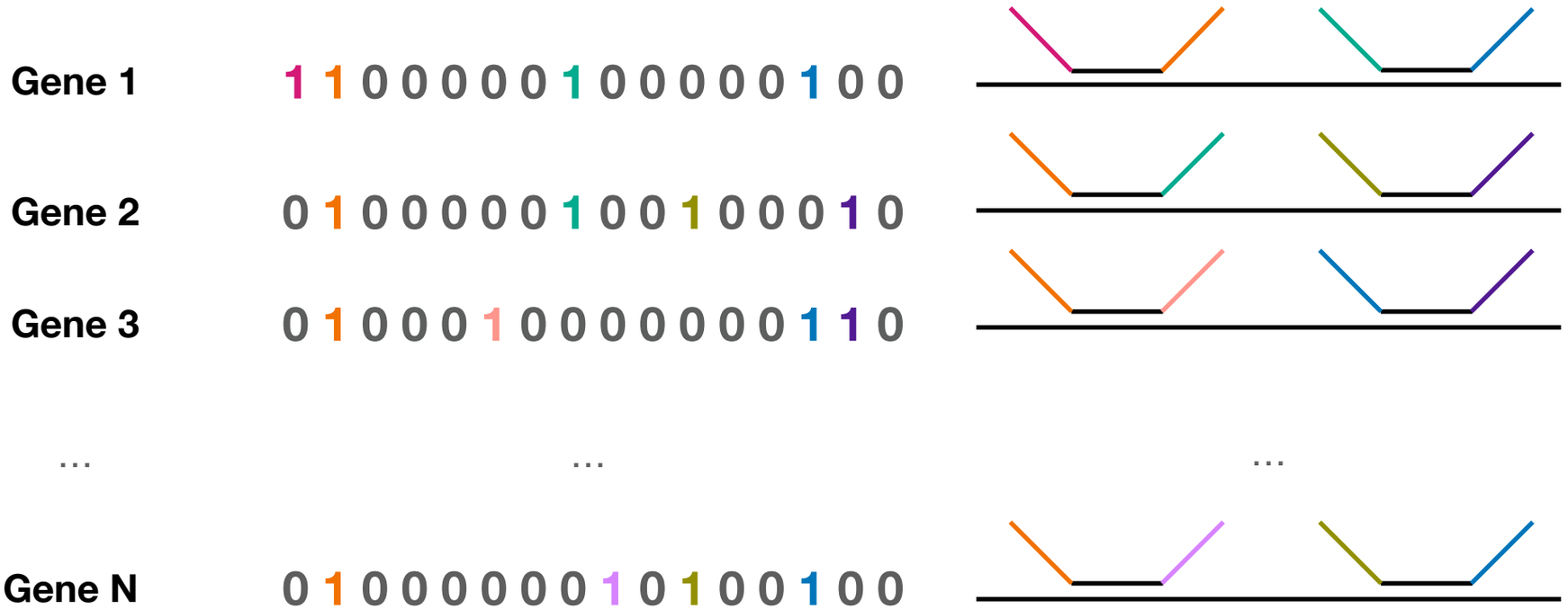

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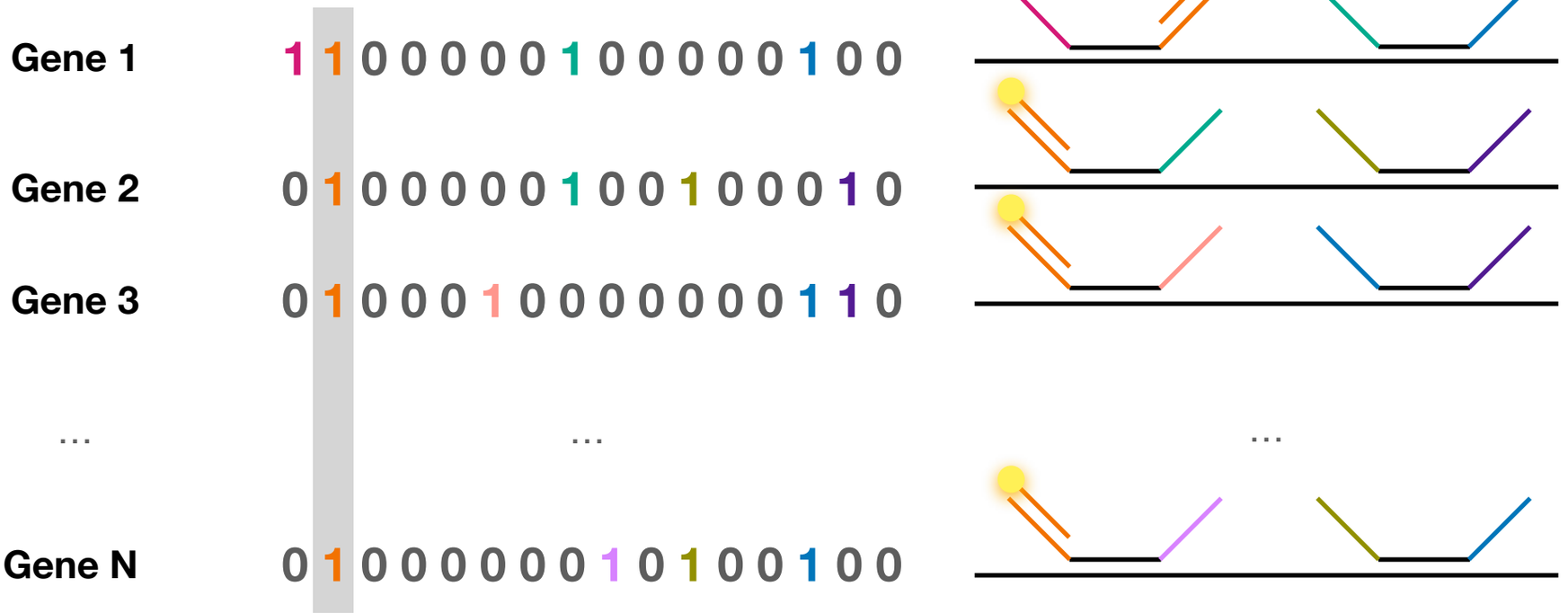

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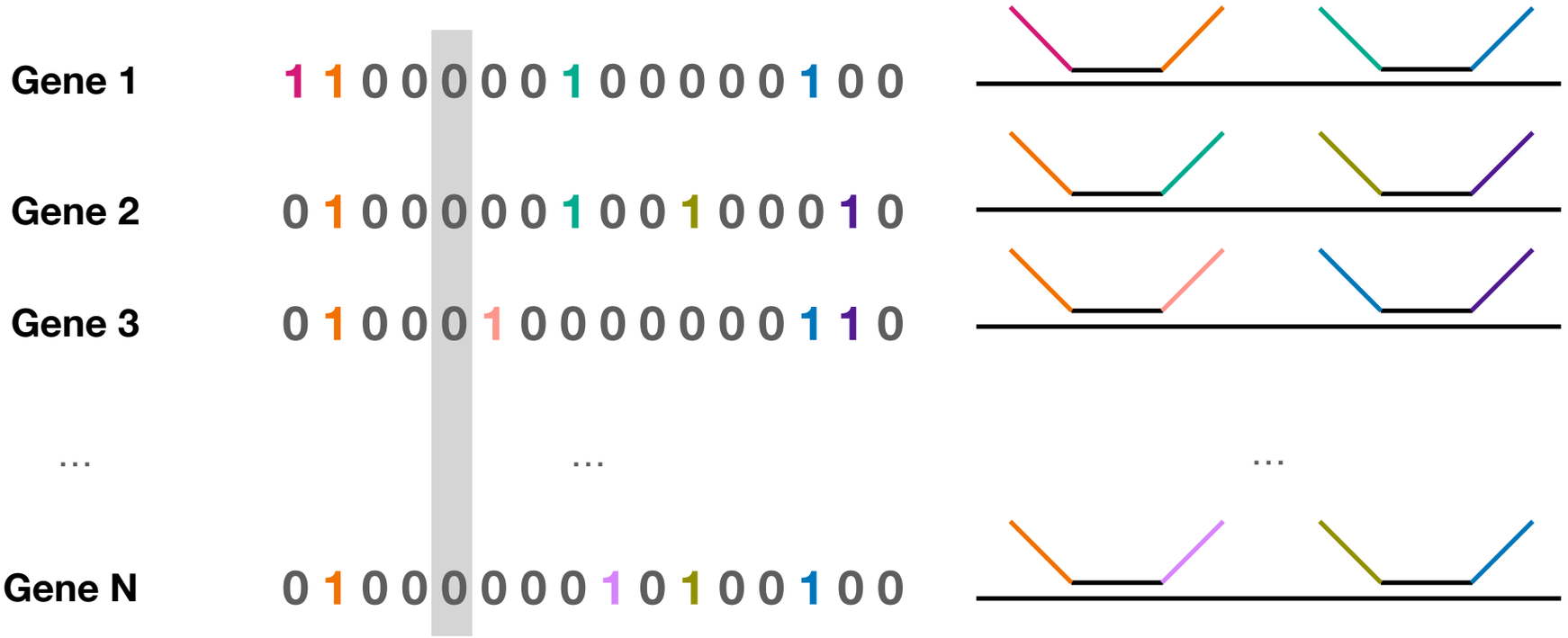

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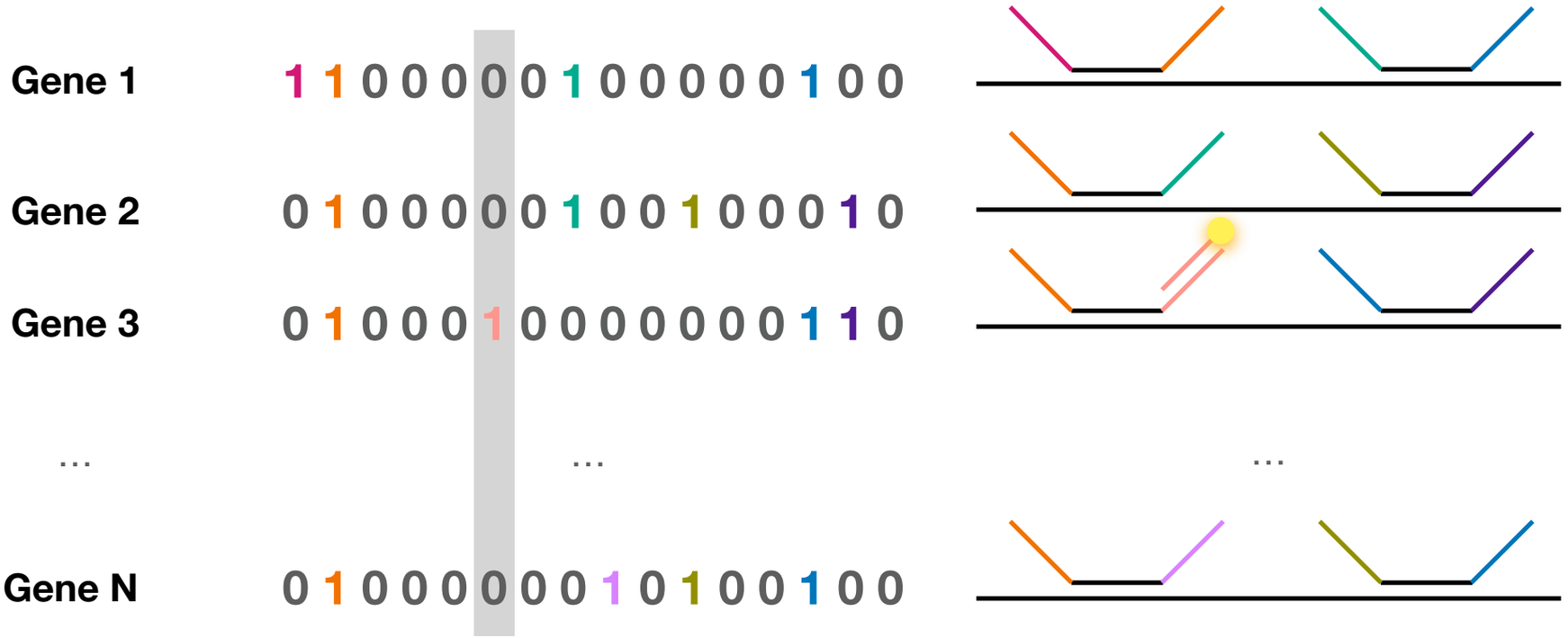

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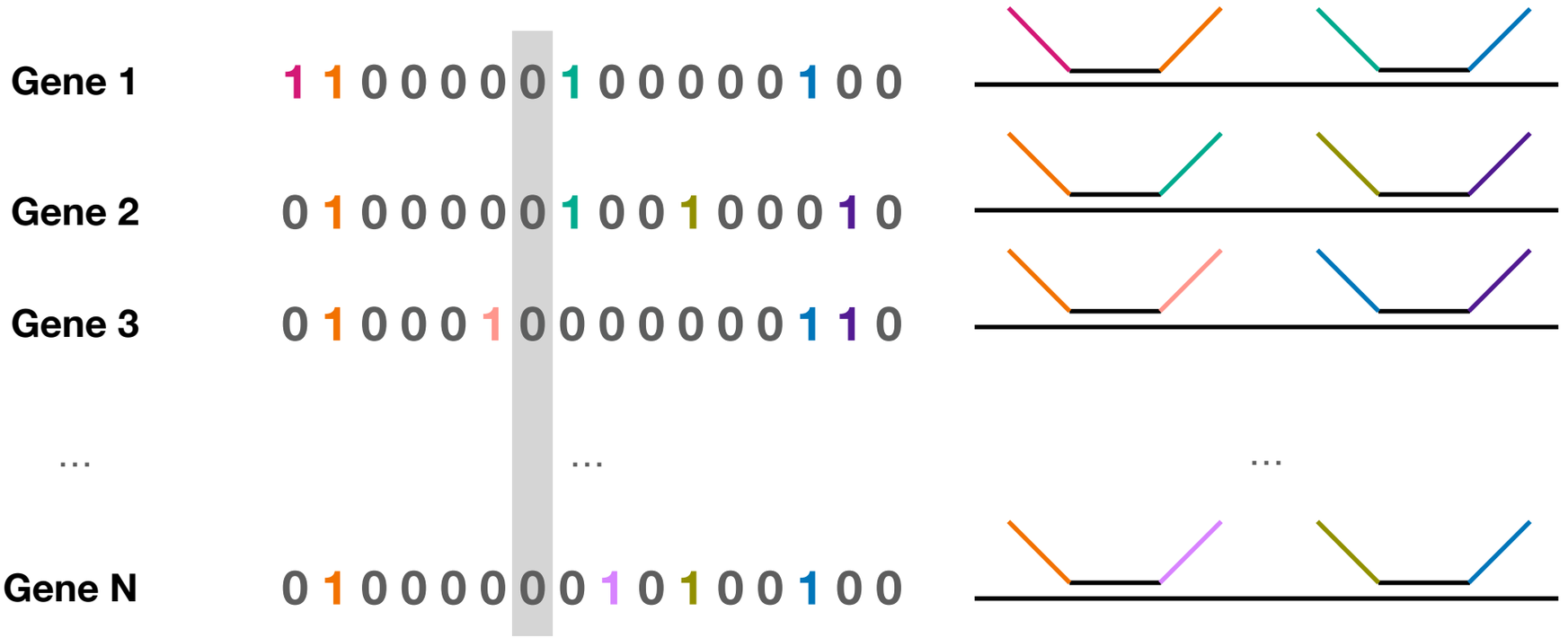

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Imaging-based spatial transcriptomics

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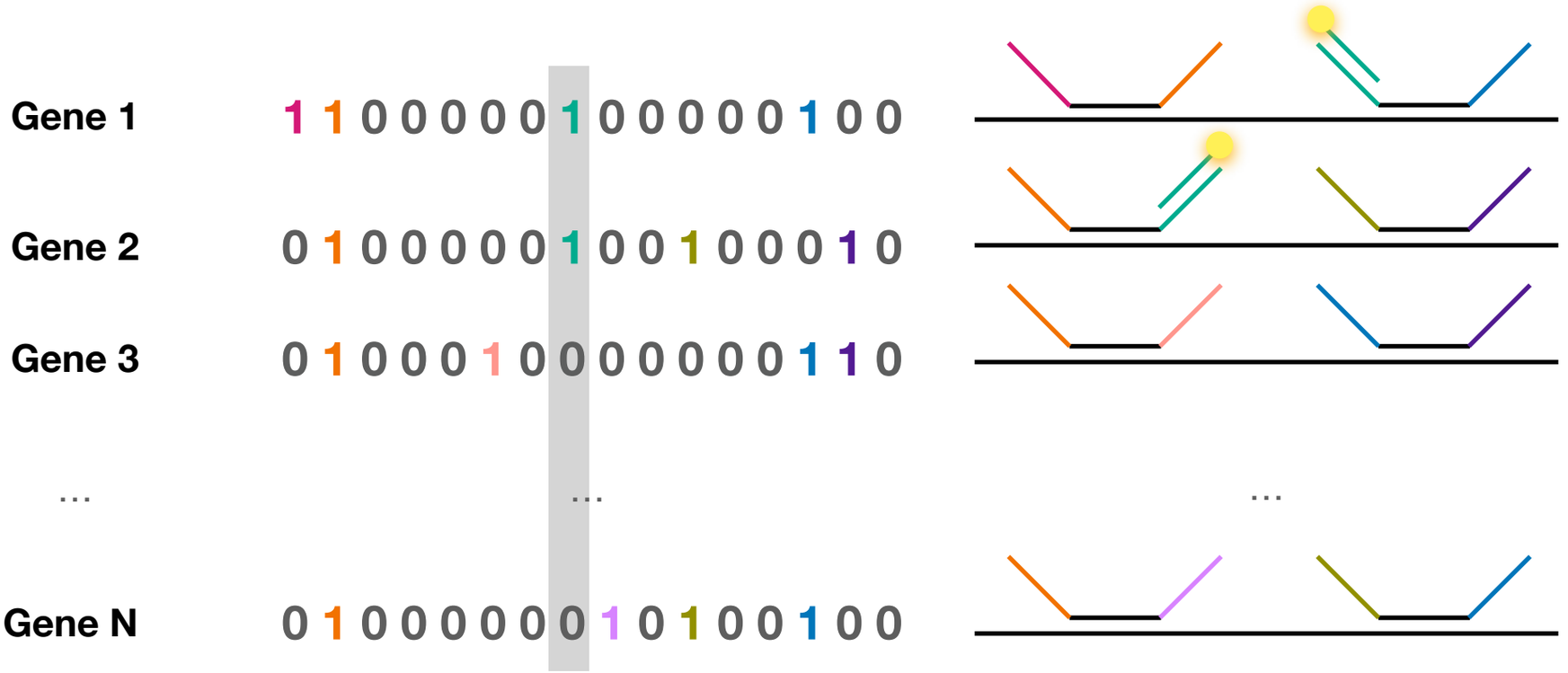

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Imaging-based spatial transcriptomics

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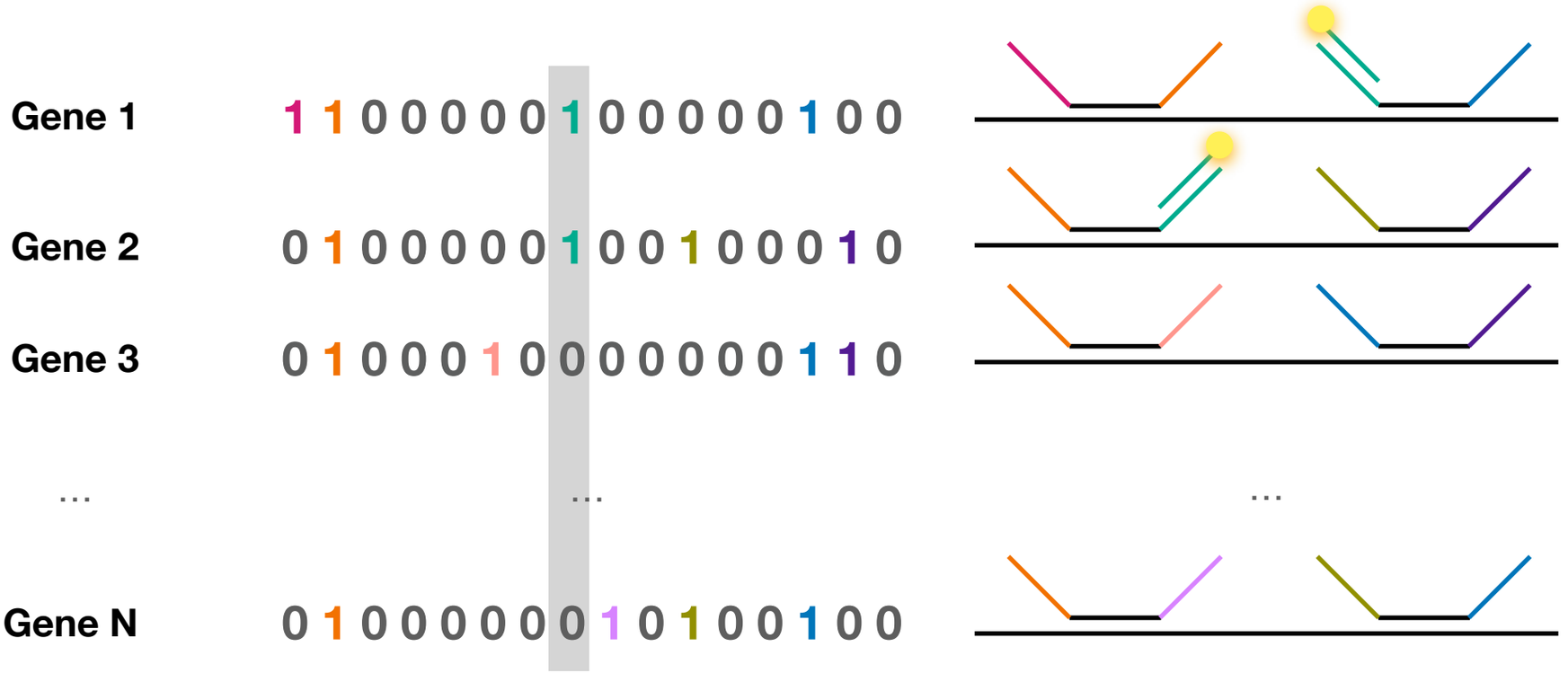

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Imaging-based spatial transcriptomics

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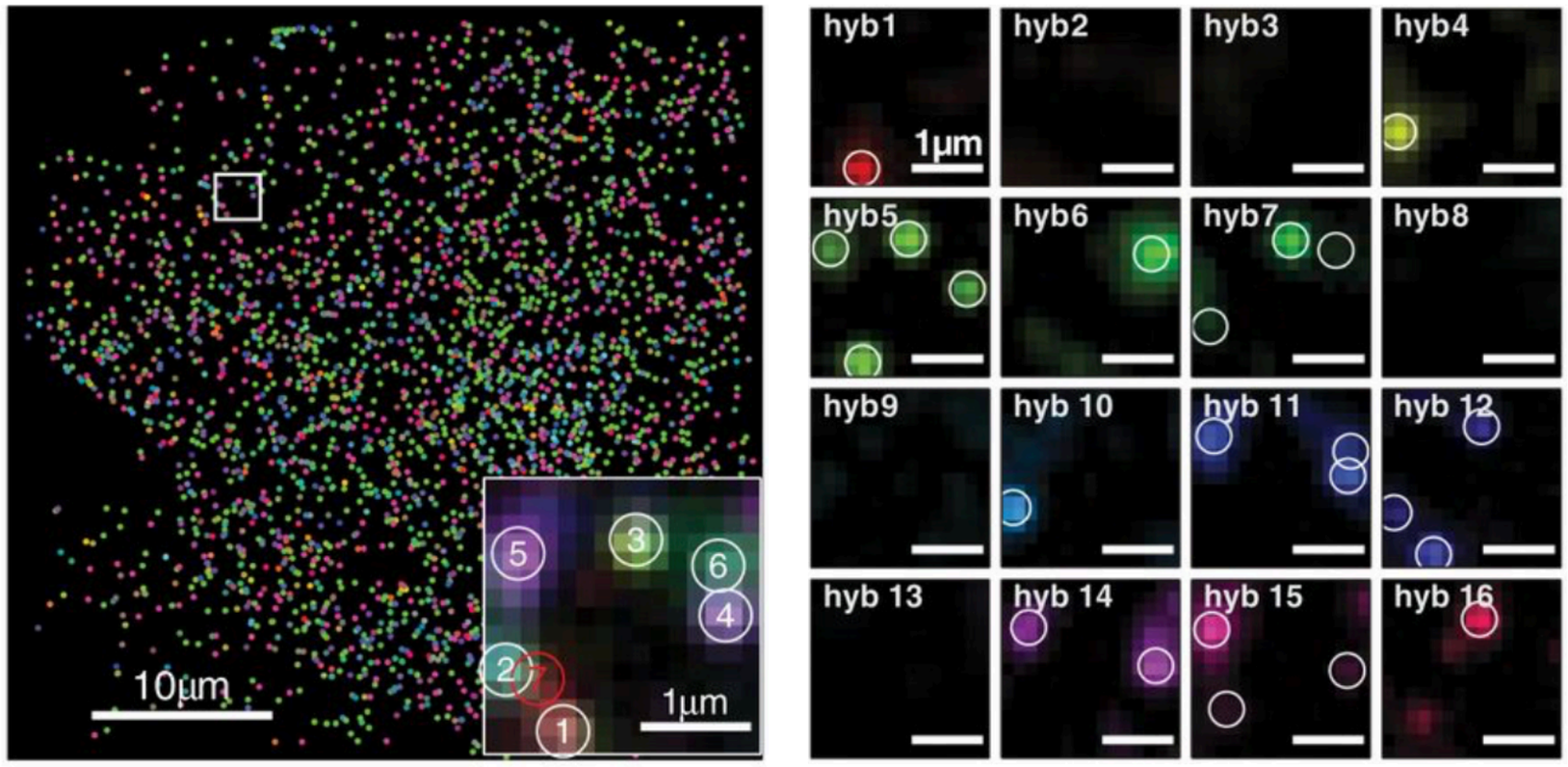

- 140 RNA species, 16 hybridization rounds
- Errors detected and corrected

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Imaging-based spatial transcriptomics

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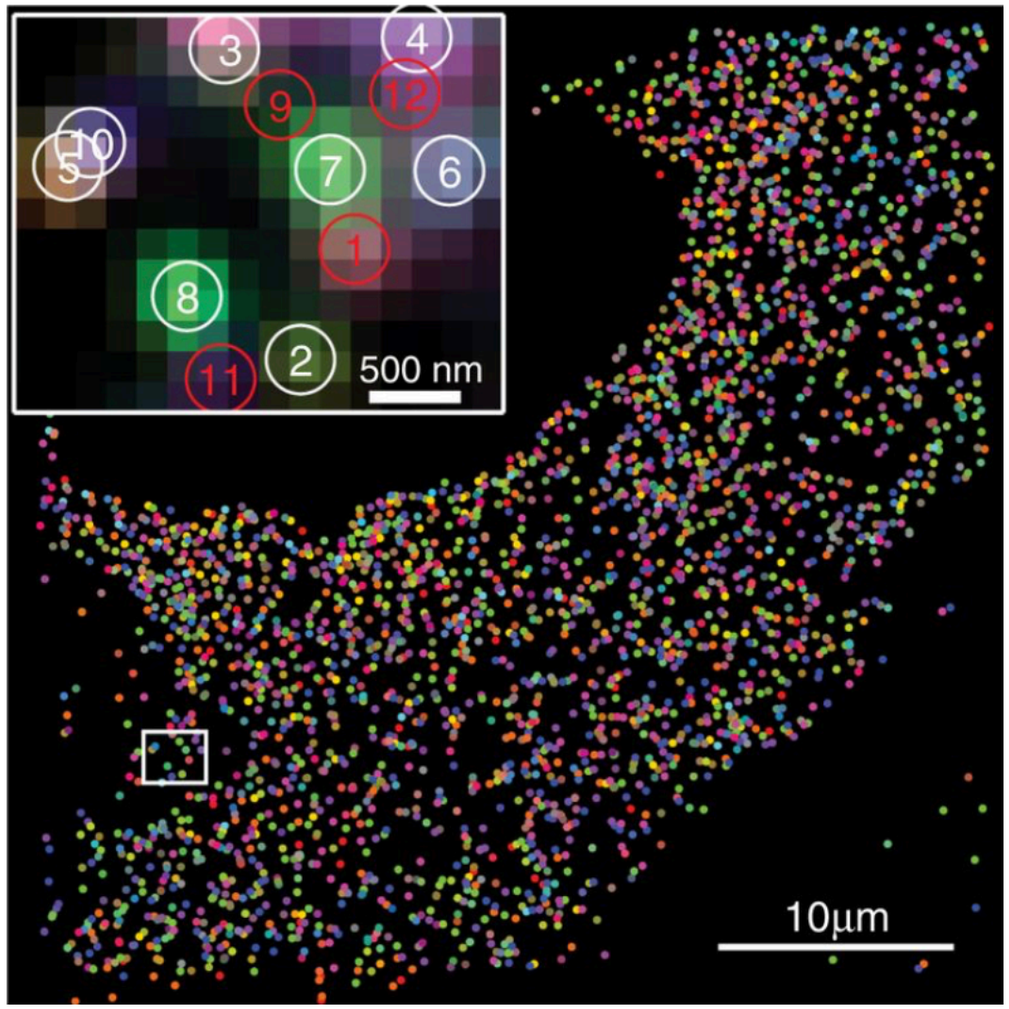
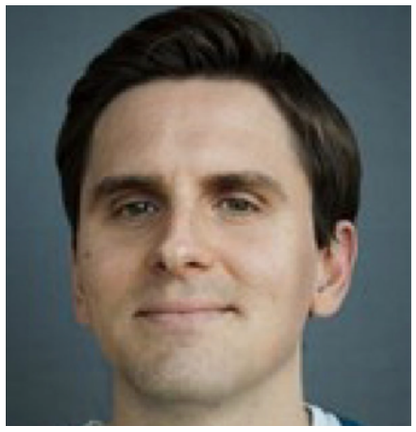


- 1001 RNA species, 14 hybridization rounds
- No error correction (modified Hamming distance 2)

Chen, K. H.; Boettiger, A. N.; Moffitt, J. R.; Wang, S.; Zhuang, X. *Science* **2015**, *348*, aaa6090.

Sequencing-based spatial transcriptomics

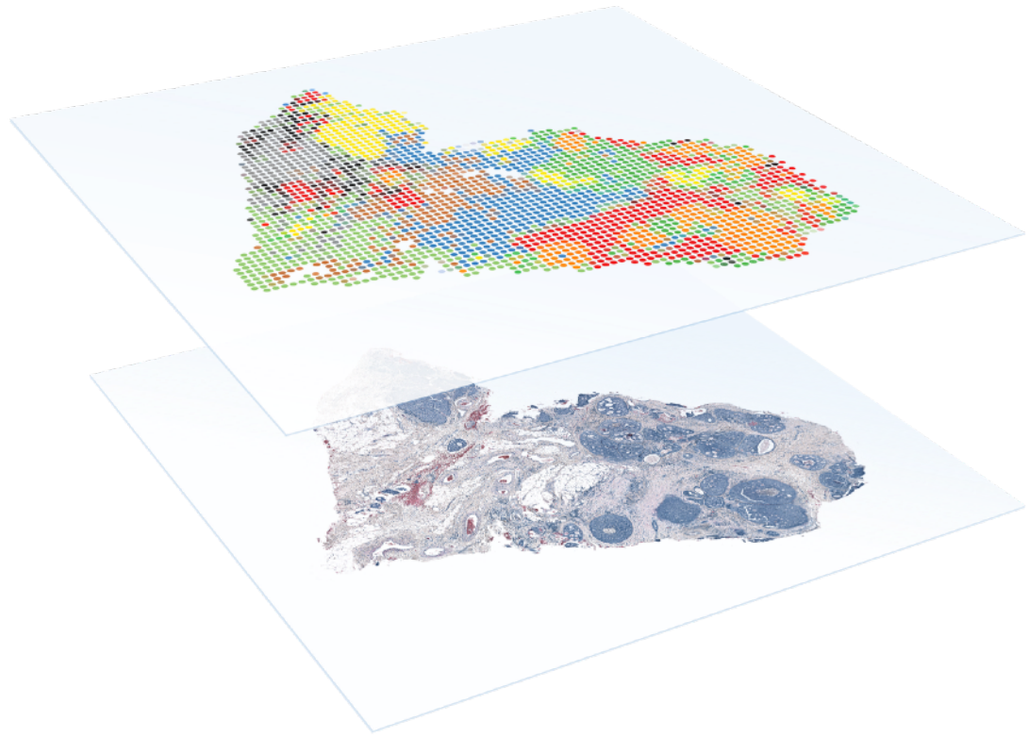
Spatial transcriptomics / Visium



Jonas Frisén
Karolinska Institutet

Joakim Lundeberg
KTH Royal Institute of Technology

Patrick L. Ståhl



First used term ***“spatial transcriptomics”***

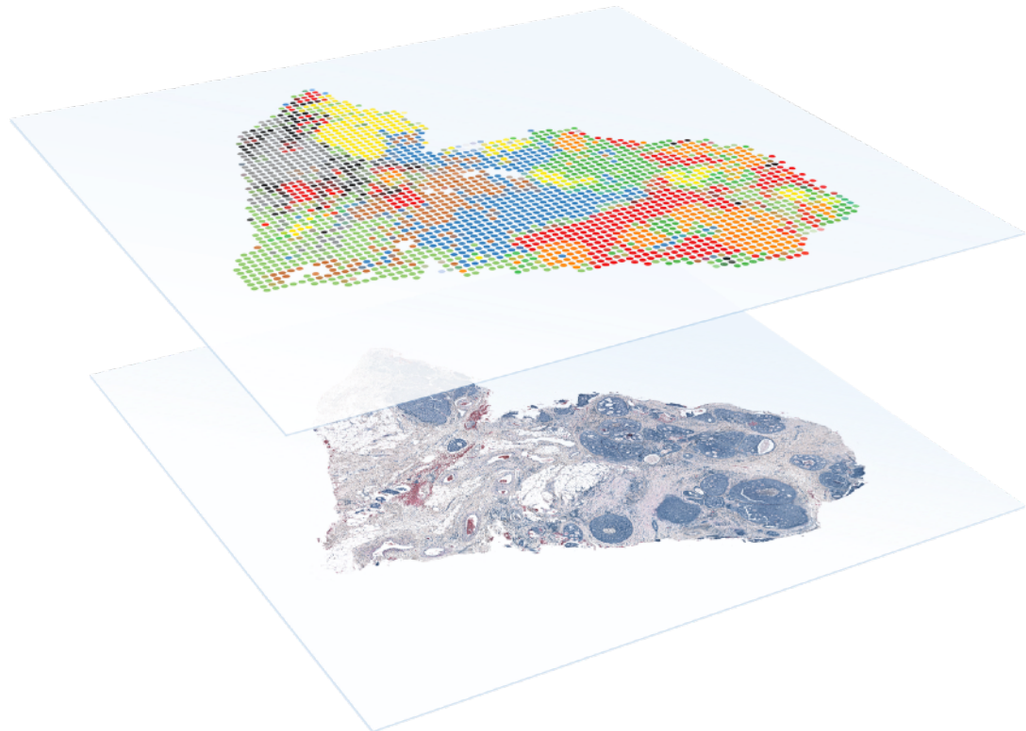
Ståhl, P. L.; Salmén, F.; … Lundeberg, J.; Frisén, J. *Science* **2016**, *353*, 78.

Sequencing-based spatial transcriptomics

Spatial transcriptomics / Visium

10x GENOMICS®

Acquired 2019, developed and commercialized into “Visium”



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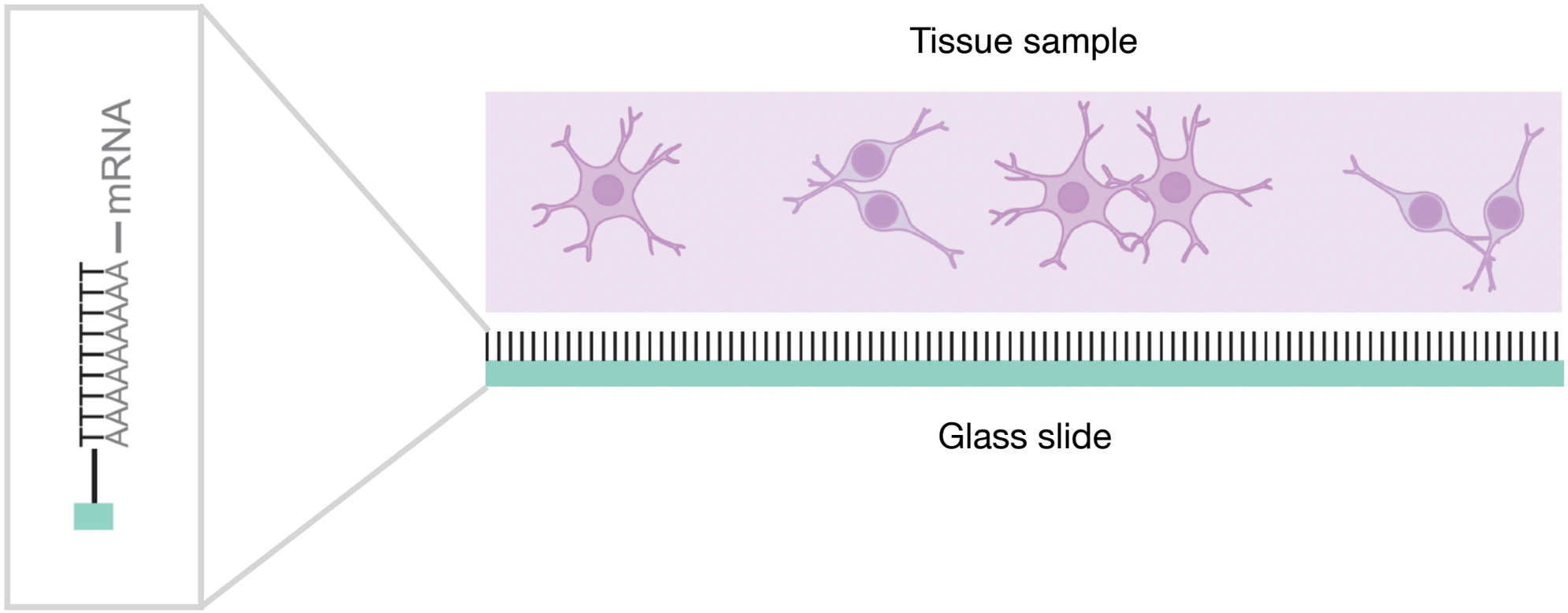
Spatial transcriptomics / Visium



- oligo-dT primers on glass slide to capture mRNA

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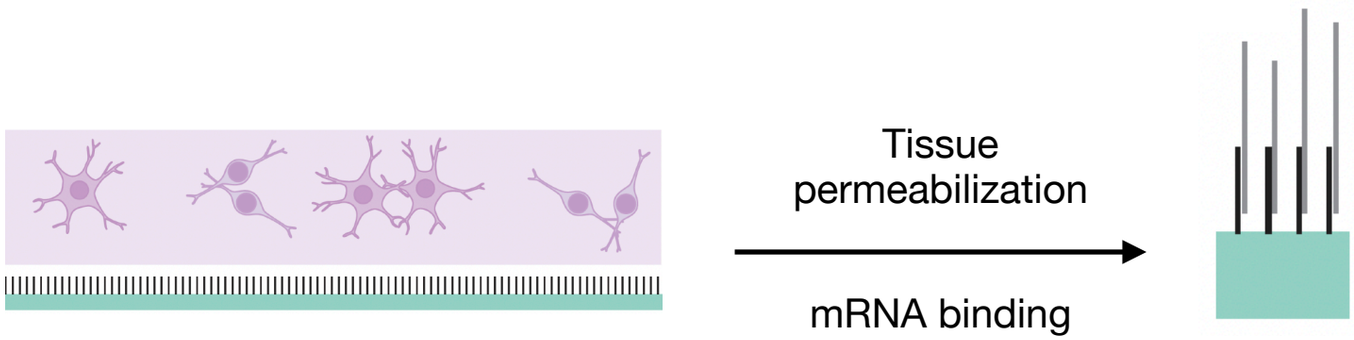
Sequencing-based spatial transcriptomics

Spatial transcriptomics / Visium



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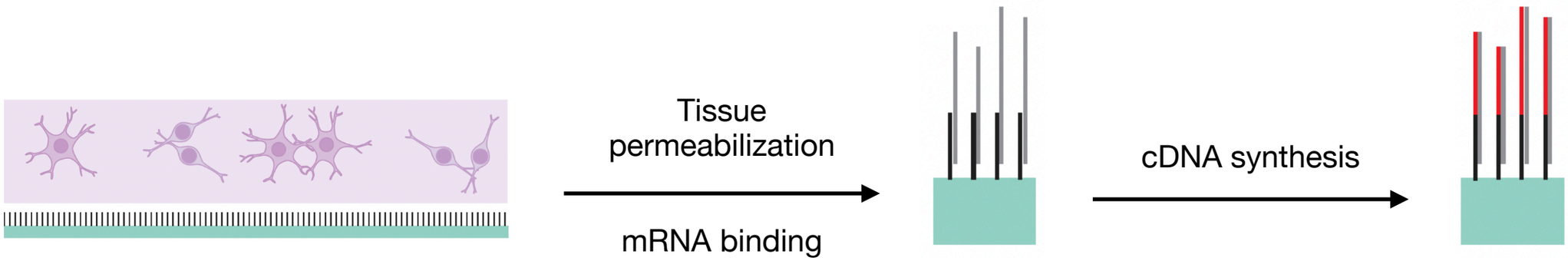
Spatial transcriptomics / Visium



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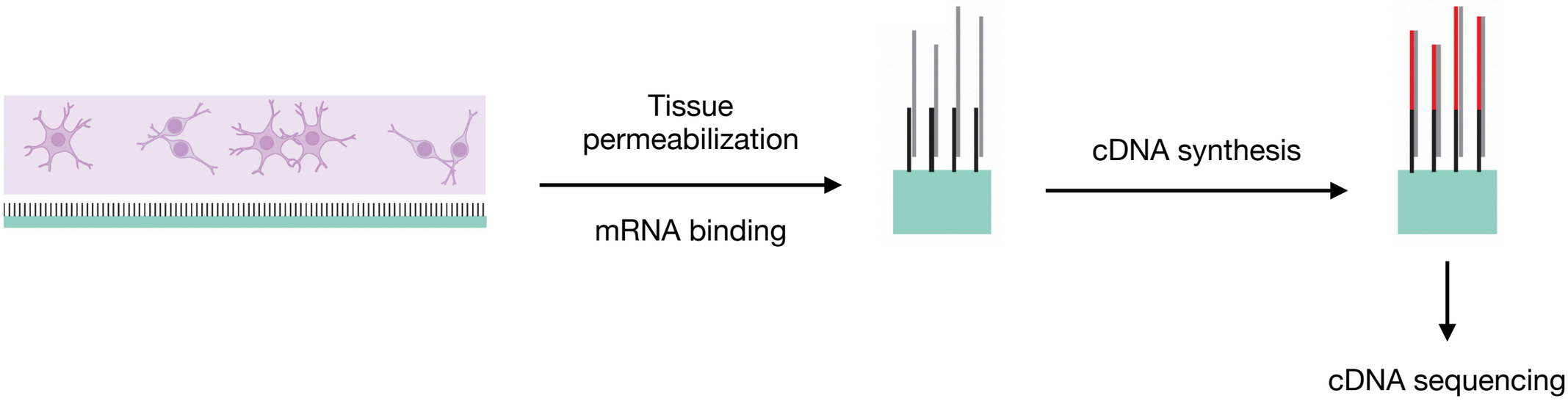
Spatial transcriptomics / Visium



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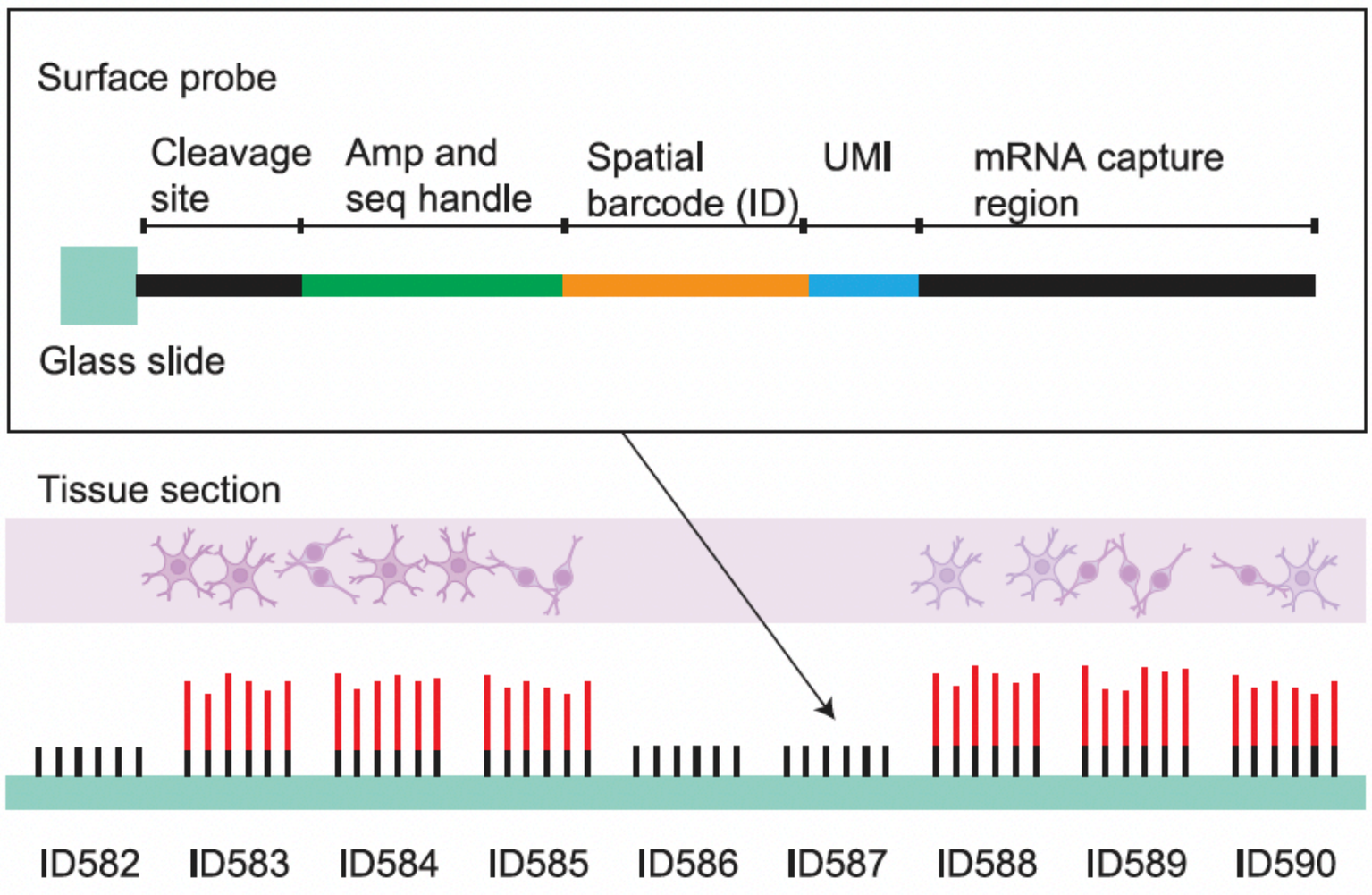
Spatial transcriptomics / Visium

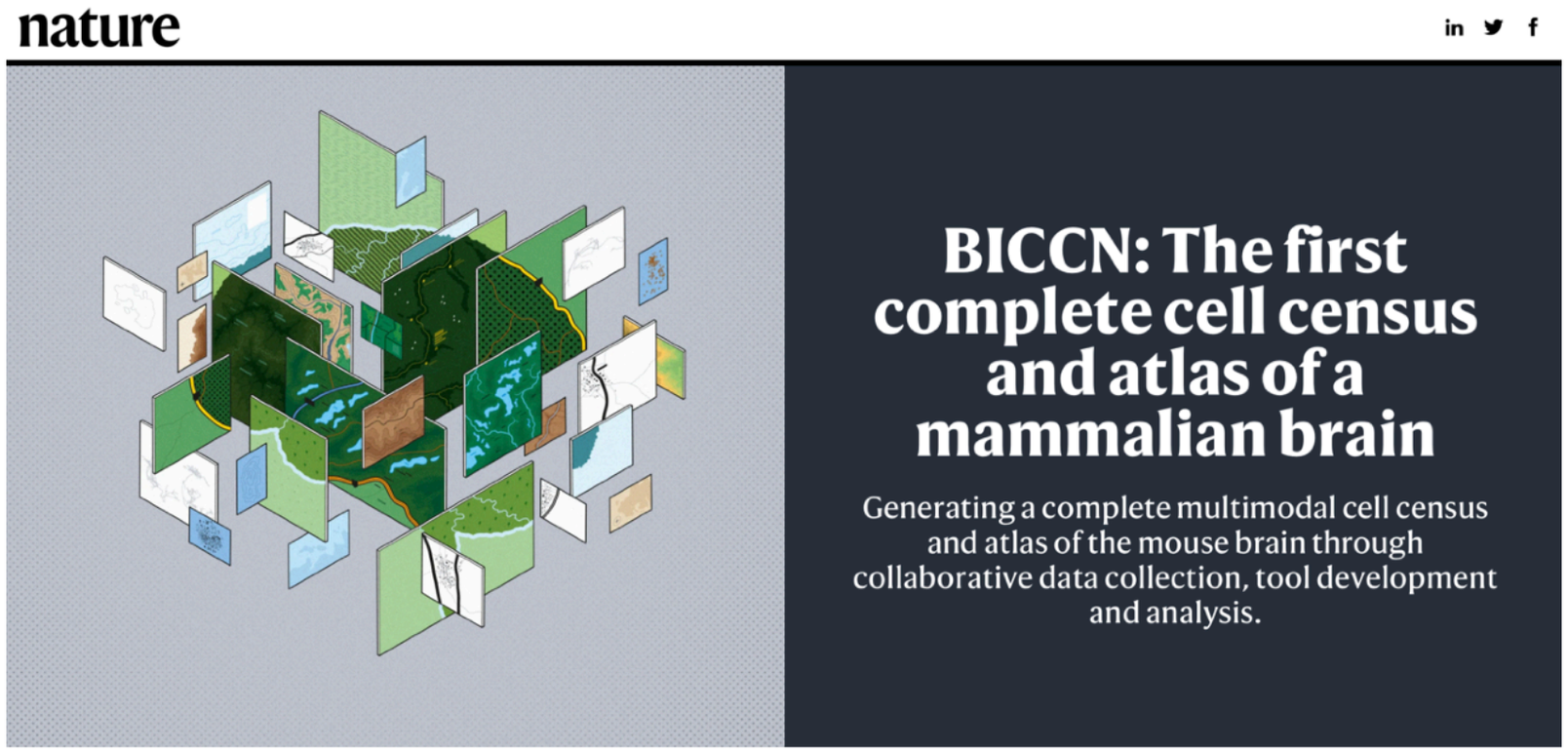


- oligo-dT primers on glass slide to capture mRNA
- spatial barcode to preserve spatial information of each transcript
- unique molecular identifier (UMI) for barcoding each transcript

Ståhl, P. L.; Salmén, F.; … Lundeberg, J.; Frisén, J. *Science* **2016**, *353*, 78.

Spatial transcriptomics

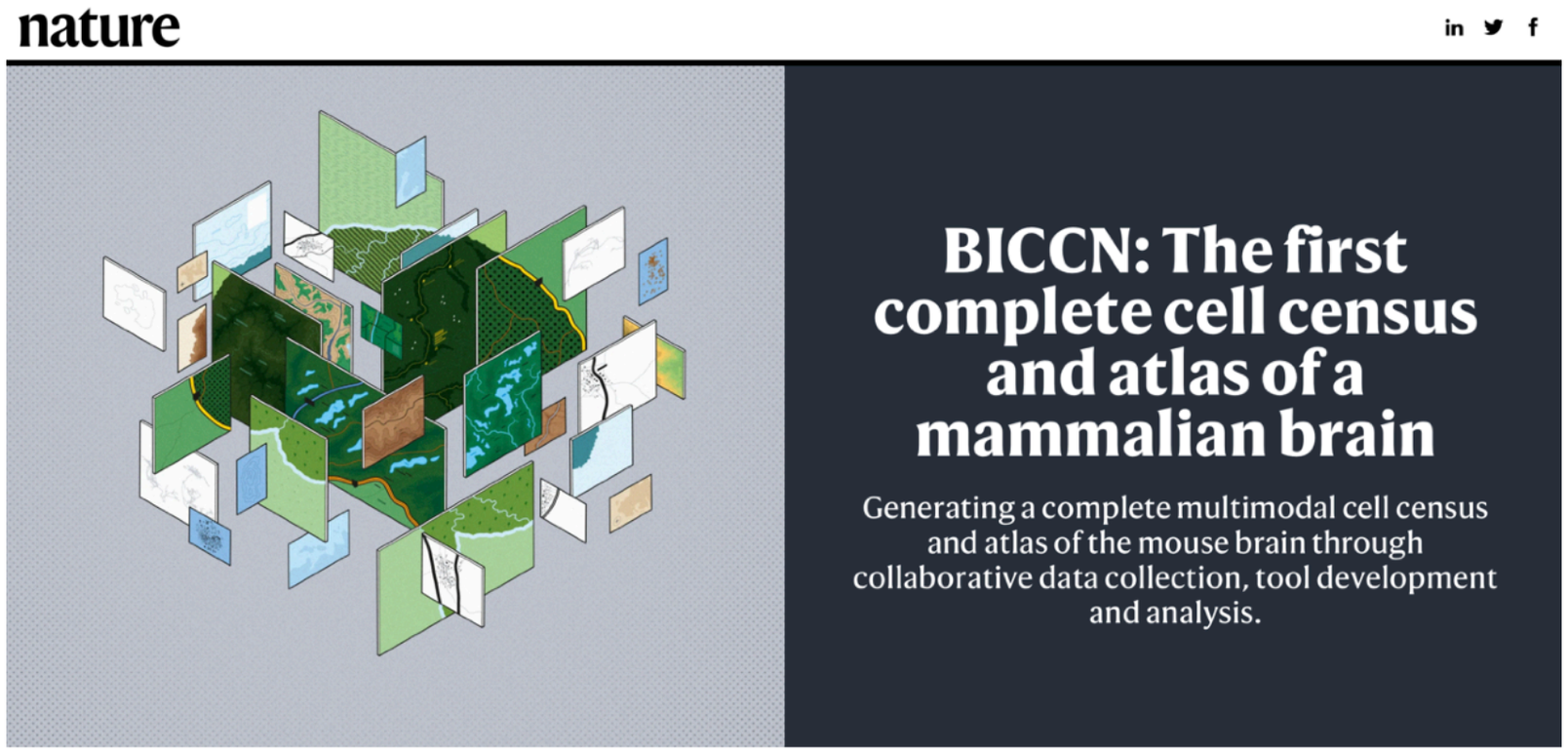
Applications: Brain Initiative Cell Census Network



Nature **2023**, *624*, 317.

Spatial transcriptomics

Applications: Brain Initiative Cell Census Network



Article | Open access | Published: 13 December 2023

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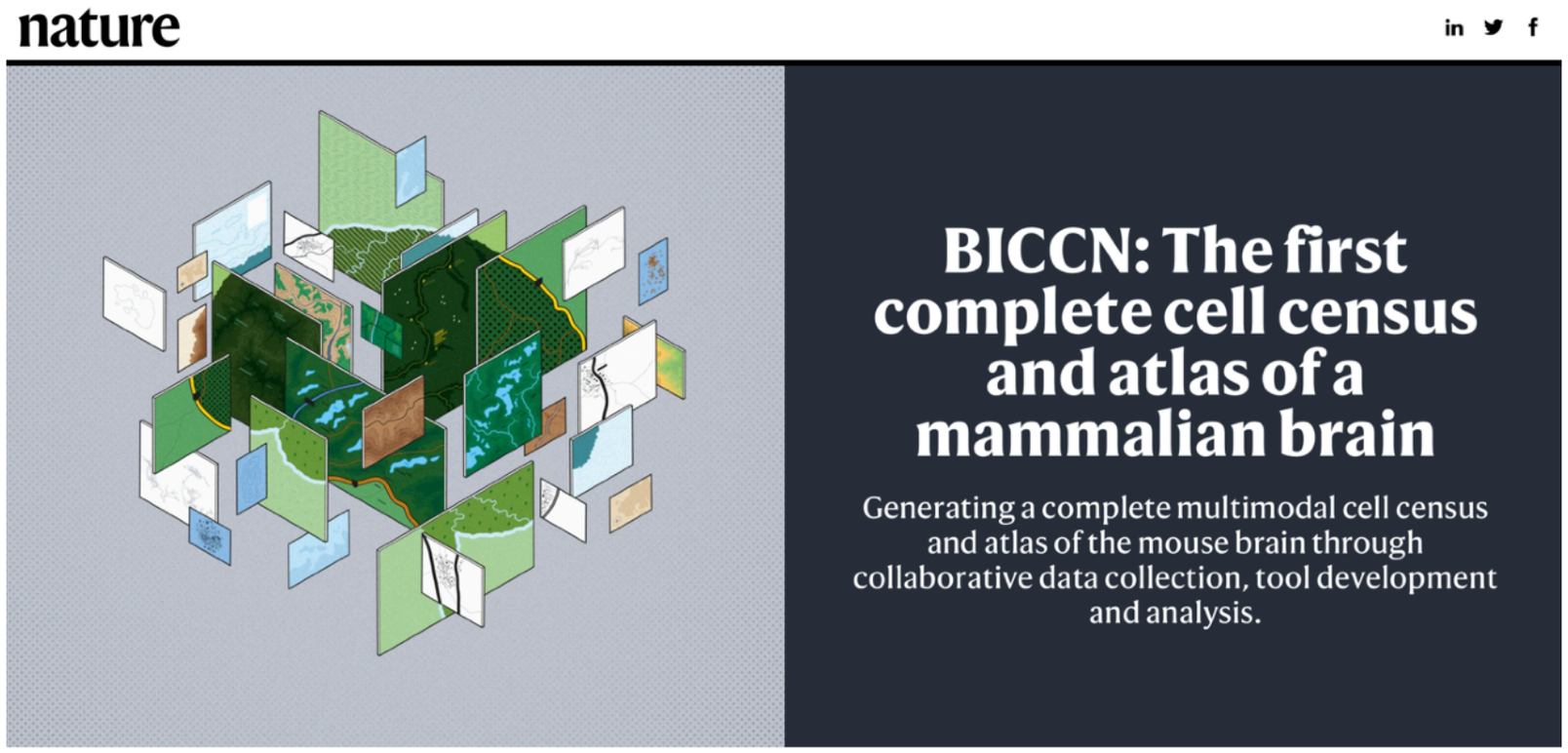
Zizhen Yao, Cindy T. J. van Velthoven, Michael Kunst, Meng Zhang, Delissa McMillen, Changkyu Lee, Won Jung, Jeff Goldy, Aliya Abdelhak, Matthew Aitken, Katherine Baker, Pamela Baker, Eliza Barkan, Darren Bertagnolli, Ashwin Bhandiwad, Cameron Bielstein, Prajal Bishwakarma, Jazmin Campos, Daniel Carey, Tamara Casper, Anish Bhaswanth Chakka, Rushil Chakrabarty, Sakshi Chavan, Min Chen, ...

Hongkui Zeng + Show authors

Nature **2023**, *624*, 317.

Spatial transcriptomics

Applications: Brain Initiative Cell Census Network



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Molecularly defined and spatially resolved cell atlas of the whole mouse brain

Meng Zhang, Xingjie Pan, Won Jung, Aaron R. Halpern, Stephen W. Eichhorn, Zhiyun Lei, Limor Cohen, Kimberly A. Smith, Bosiljka Tasic, Zizhen Yao, Hongkui Zeng & Xiaowei Zhuang

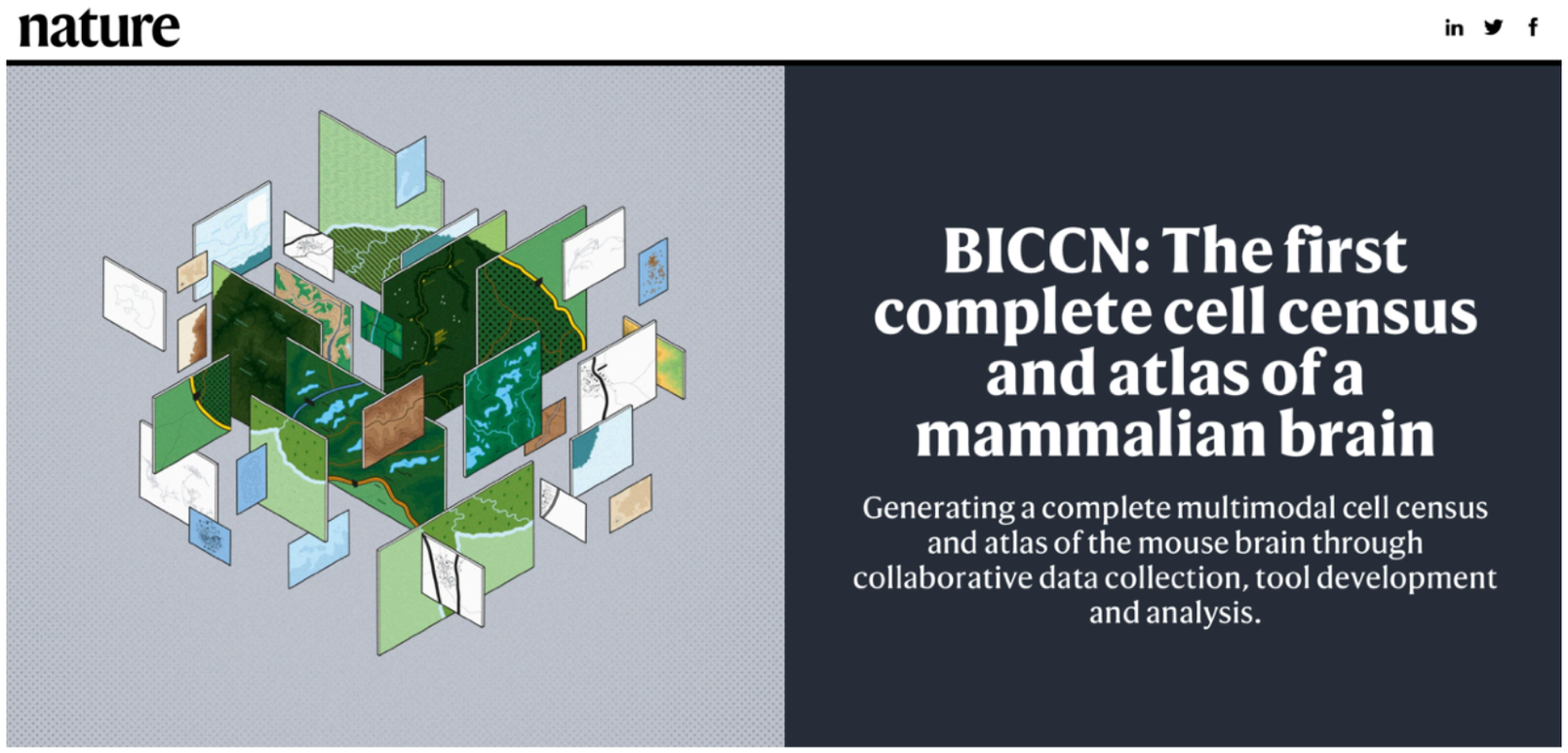
Nature **624**, 343–354 (2023) | Cite this article

58k Accesses | **77** Citations | **650** Altmetric | Metrics

Nature **2023**, *624*, 317.

Spatial transcriptomics

Applications: Brain Initiative Cell Census Network



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The molecular cytoarchitecture of the adult mouse brain

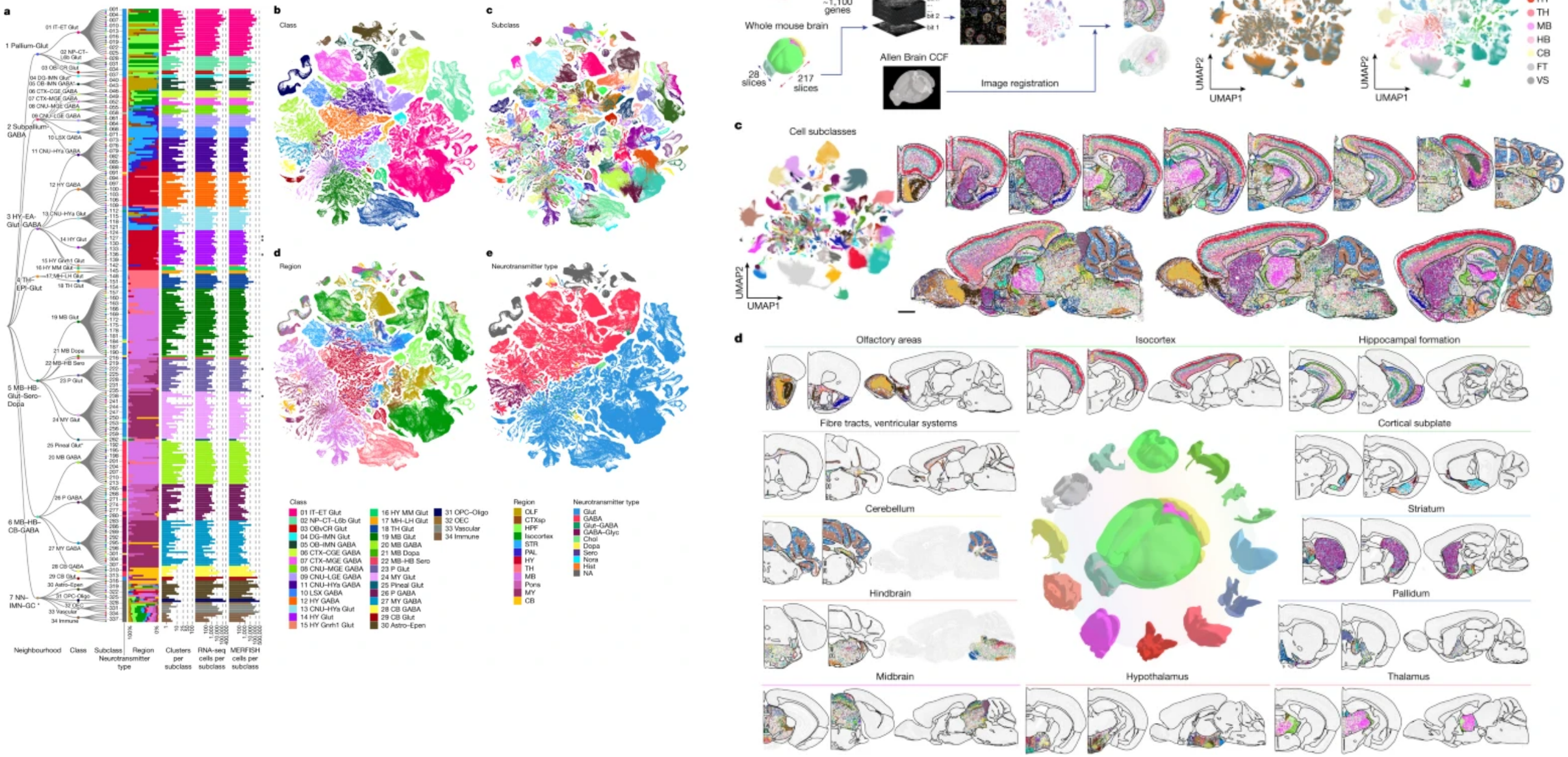
Jonah Langlieb, Nina S. Sachdev, Karol S. Balderrama, Naeem M. Nadaf, Mukund Raj, Evan Murray, James T. Webber, Charles Vanderburg, Vahid Gazestani, Daniel Tward, Chris Mezias, Xu Li, Katelyn Flowers, Dylan M. Cable, Tabitha Norton, Partha Mitra, Fei Chen & Evan Z. Macosko

Nature **624**, 333–342 (2023) | Cite this article

Nature **2023**, *624*, 317.

Spatial transcriptomics

Applications: Brain Initiative Cell Census Network



Yao, Z.; Velthoven, C. T. J.; … Zeng, H. *Nature* **2023**, *624*, 317.; Zhang, M.; Pan, X.; … Zhuang, X. *Nature* **2023**, *624*, 343.

Outline

Spatial transcriptomics

Imaging-based
seqFISH
MERFISH

Sequencing-based
Visium

Spatial proteomics

Multiplexed antibody-based
4i

MS-based
DVP

Challenges in spatial proteomics

Transcriptomics | ***Proteomics***

Nat. Methods **2023**, *20*, 317.

Challenges in spatial proteomics

Transcriptomics	***Proteomics***
Easily amplified by PCR	Cannot be amplified (picograms of total protein)

Nat. Methods **2023**, *20*, 317.

Challenges in spatial proteomics

Transcriptomics	***Proteomics***
Easily amplified by PCR	Cannot be amplified (picograms of total protein)
Well established sequencing technologies	Limited by MS sensitivity and throughput

Nat. Methods **2023**, *20*, 317.

Challenges in spatial proteomics

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$3x10^5$ mRNA molecules per HeLa cell	$1x10^9$ protein molecules per HeLa cell

Nat. Methods **2023**, *20*, 317.

Challenges in spatial proteomics

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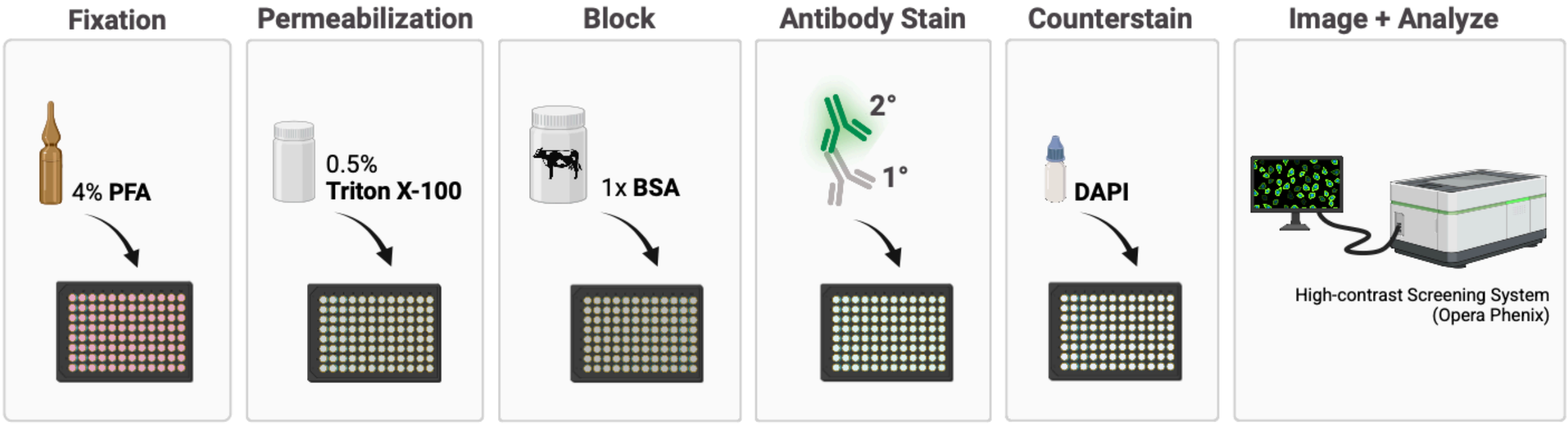
Nat. Methods **2023**, *20*, 317.

Spatial profiling of proteins

***I**mmuno**f**luorescence (IF)*

Spatial profiling of proteins

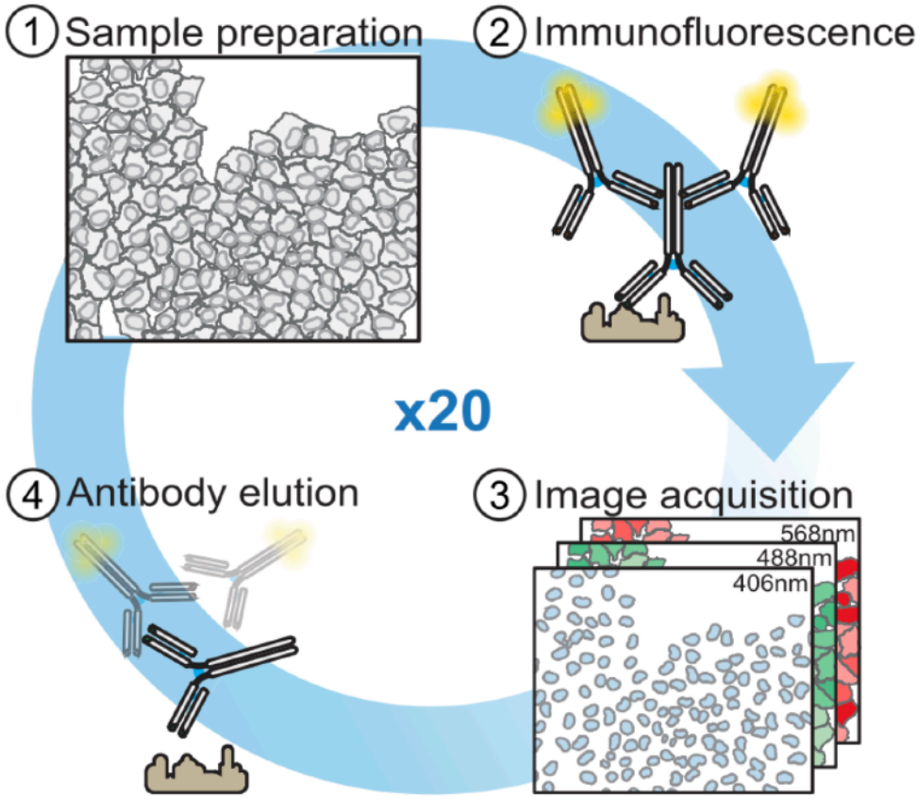
Immunofluorescence (IF)



- probing protein localization in cells heavily rely on antibody-based imaging methods

Multiplexed antibody-based spatial proteomics

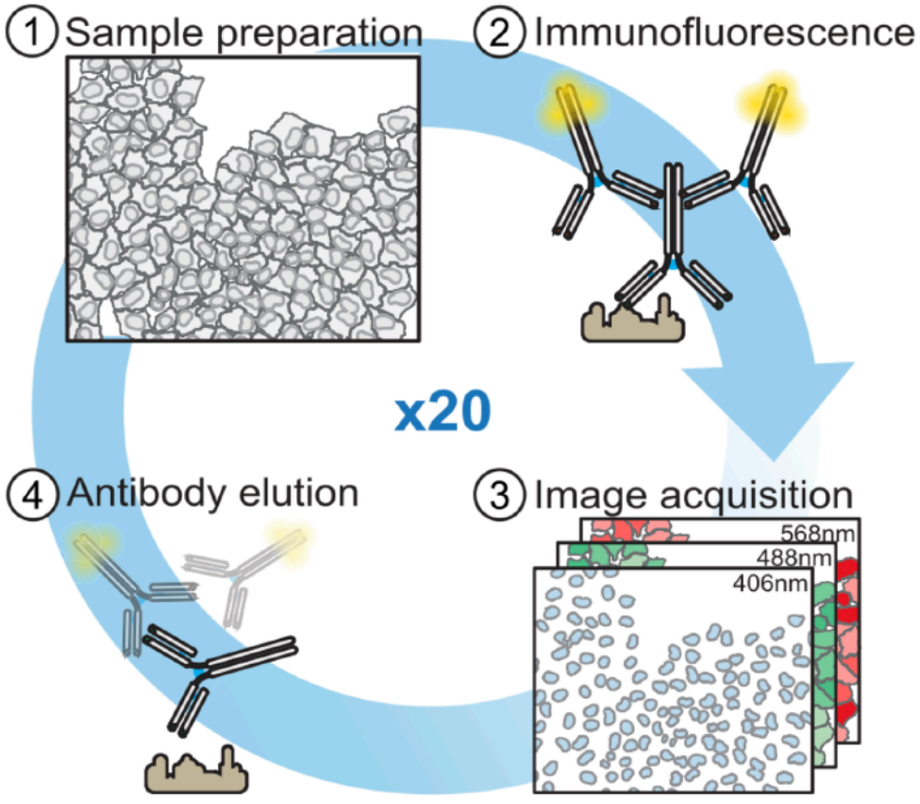
***I**terative **I**ndirect **I**mmunofluorescence **I**maging (4i)*



Gut, G.; Herrmann, M. D.; Pelkmans, L. *Science* **2018**, *361,* eaar7042.

Multiplexed antibody-based spatial proteomics

***I**terative **I**ndirect **I**mmunofluorescence **I**maging (4i)*

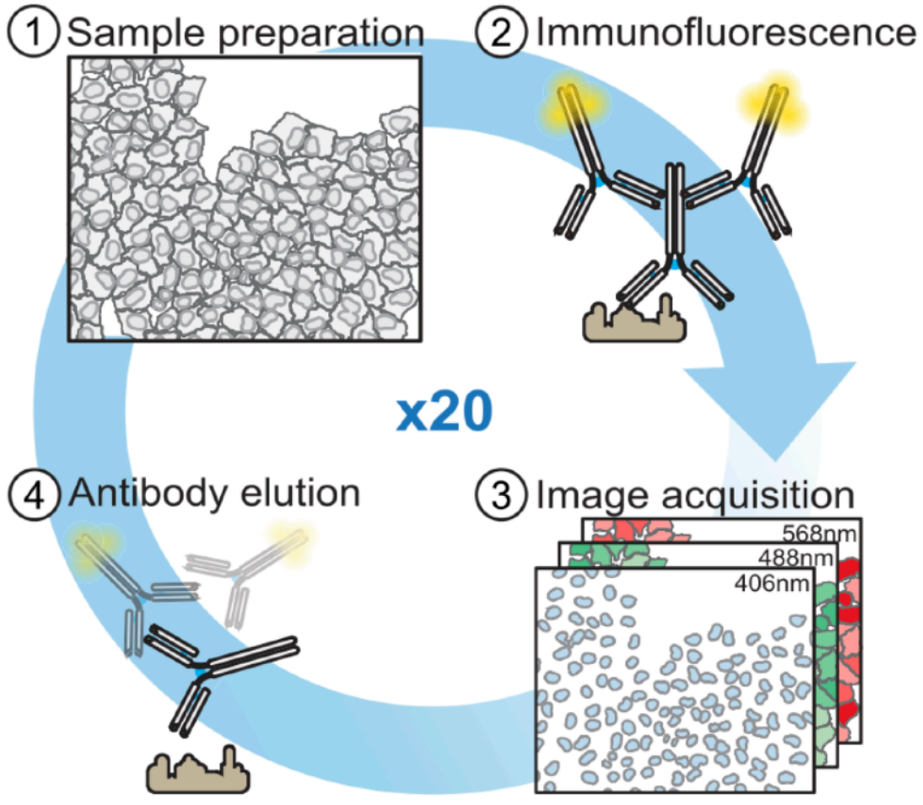


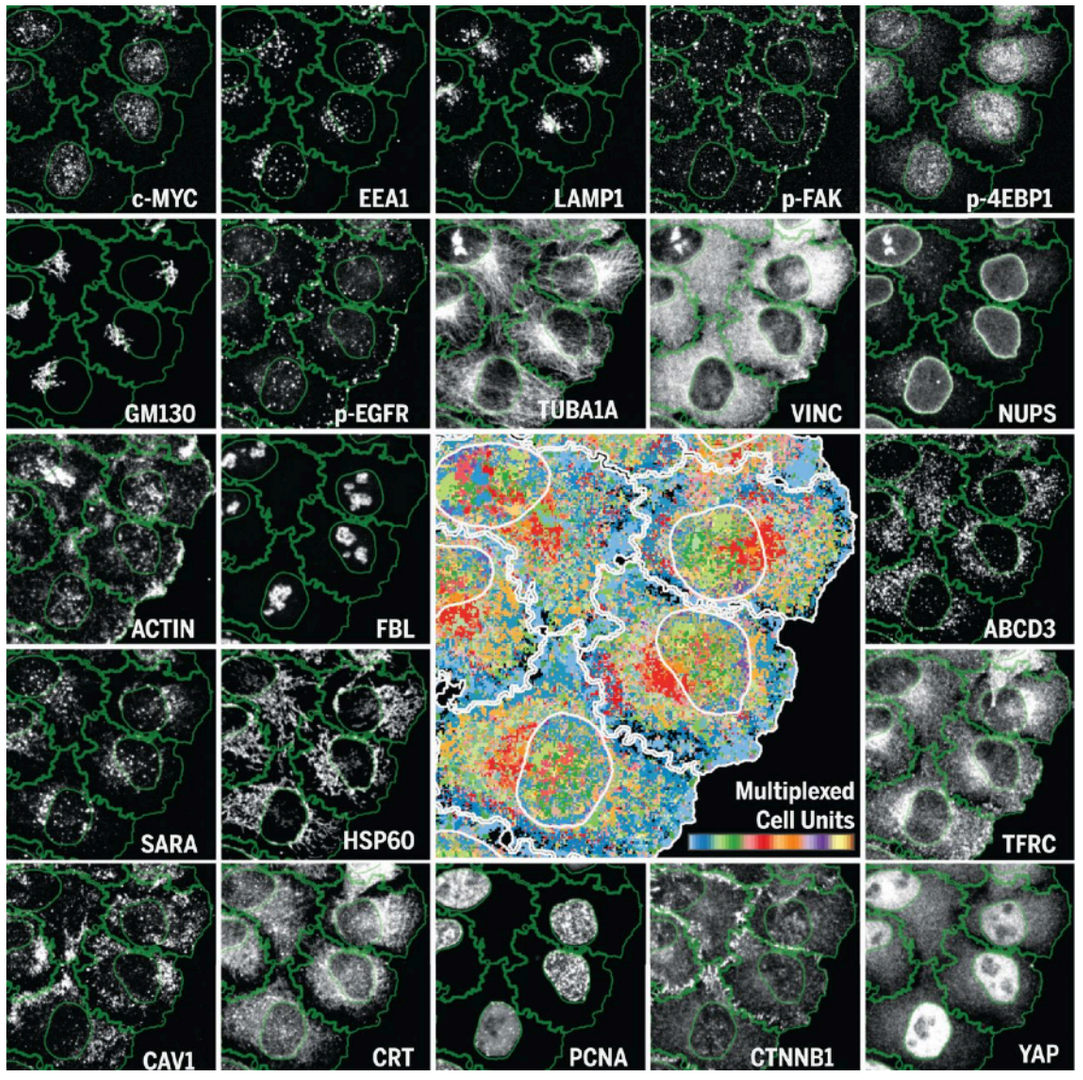
- Iterative immunofluorescence; use of specialized elution and imaging buffers

Gut, G.; Herrmann, M. D.; Pelkmans, L. *Science* **2018**, *361,* eaar7042.

Multiplexed antibody-based spatial proteomics

Iterative Indirect Immunofluorescence Imaging (4i)





- Iterative immunofluorescence; use of specialized elution and imaging buffers

Gut, G.; Herrmann, M. D.; Pelkmans, L. *Science* **2018**, *361,* eaar7042.

Multiplexed antibody-based spatial proteomics

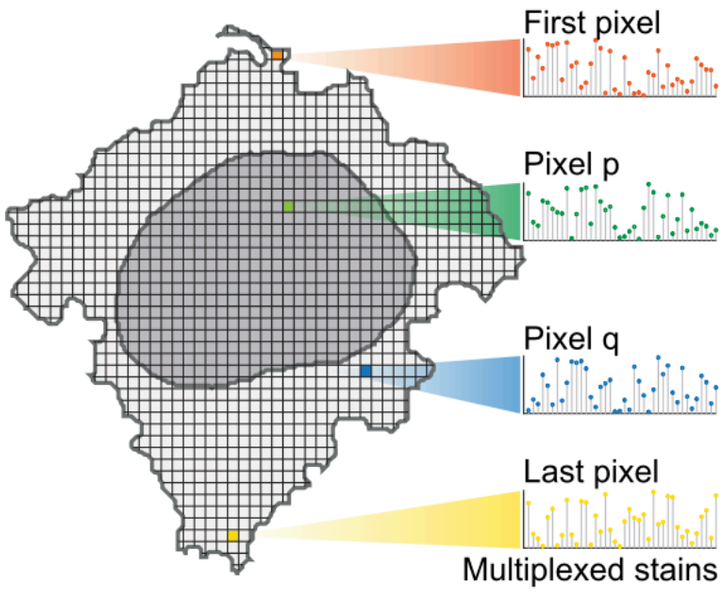
***I**terative **I**ndirect **I**mmunofluorescence **I**maging (4i)*

- Iterative immunofluorescence; use of specialized elution and imaging buffers
- Intracellular protein composition can be characterized

Gut, G.; Herrmann, M. D.; Pelkmans, L. *Science* **2018**, *361,* eaar7042.

Multiplexed antibody-based spatial proteomics

***I**terative **I**ndirect **I**mmunofluorescence **I**maging (4i)*



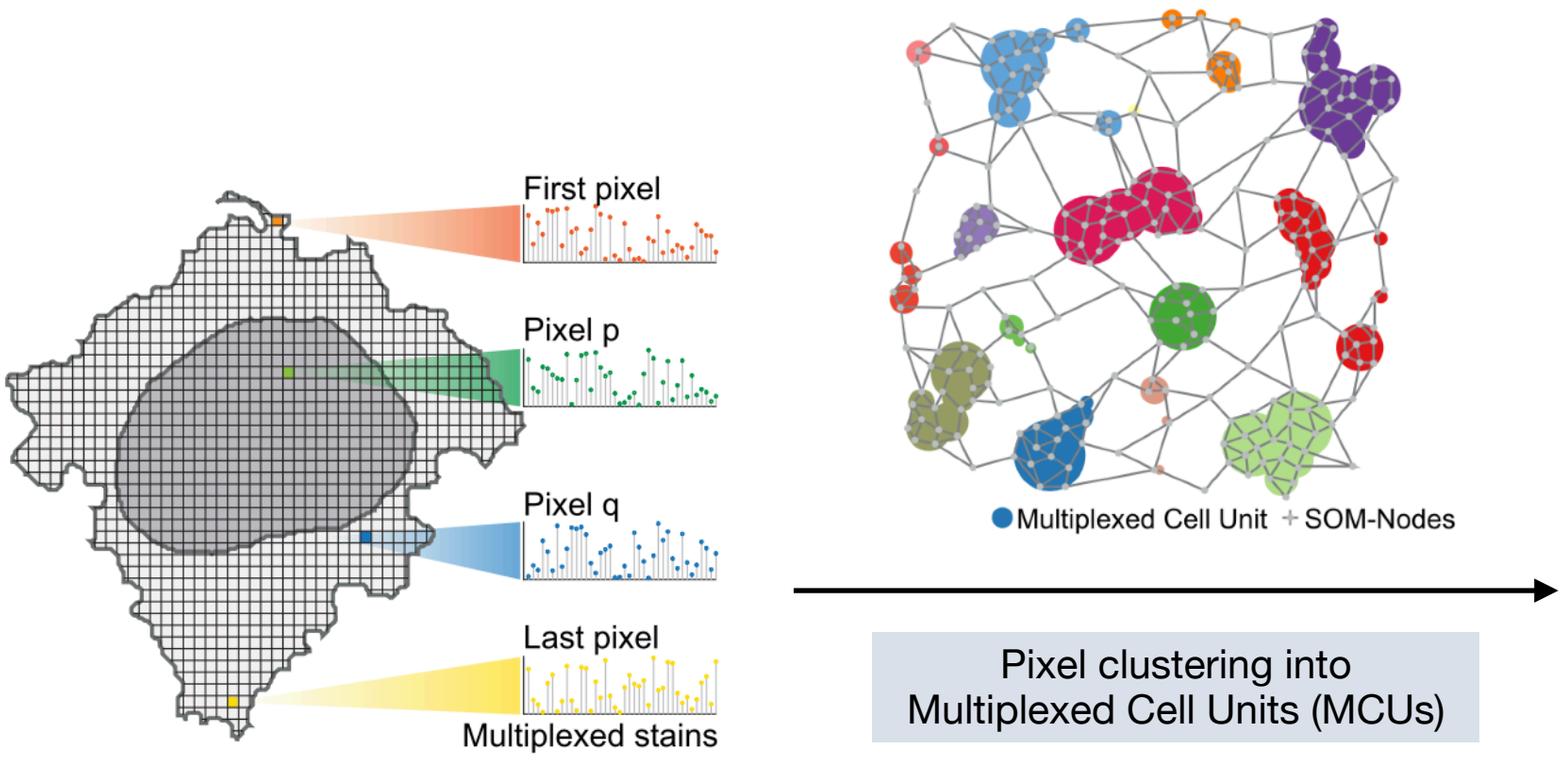
Multipixel intensity extraction

- Iterative immunofluorescence; use of specialized elution and imaging buffers
- Intracellular protein composition can be characterized

Gut, G.; Herrmann, M. D.; Pelkmans, L. *Science* **2018**, *361,* eaar7042.

Multiplexed antibody-based spatial proteomics

***I**terative **I**ndirect **I**mmunofluorescence **I**maging (4i)*



- Iterative immunofluorescence; use of specialized elution and imaging buffers
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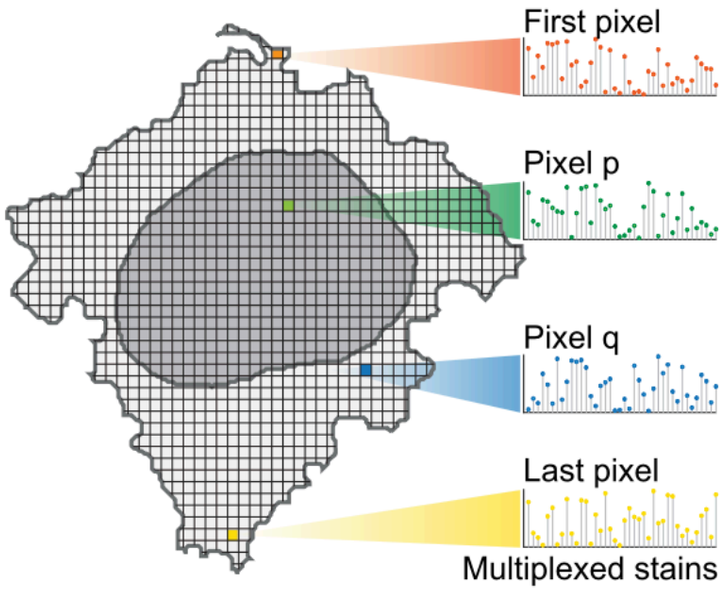
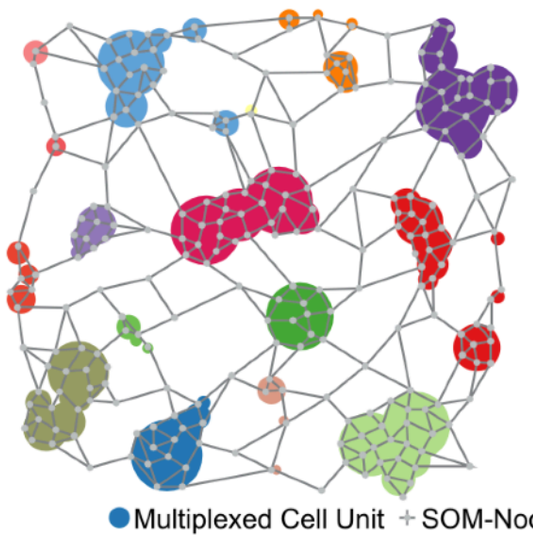
Multiplexed antibody-based spatial proteomics

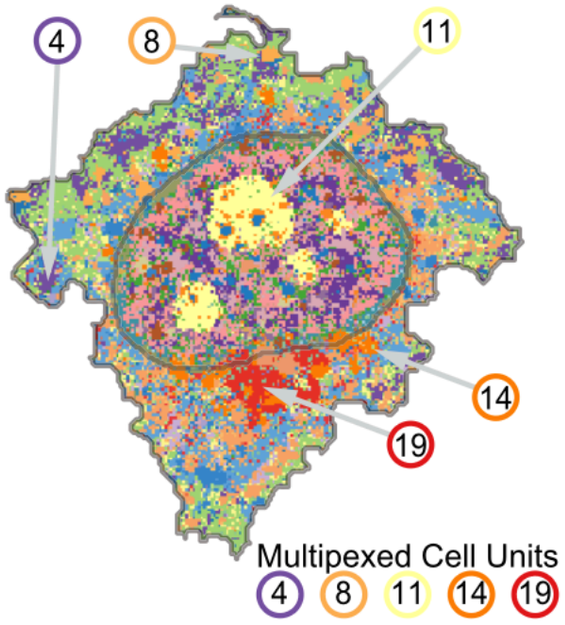
***I**terative **I**ndirect **I**mmunofluorescence **I**maging (4i)*



Multipixel intensity extraction



Pixel clustering into
Multiplexed Cell Units (MCUs)



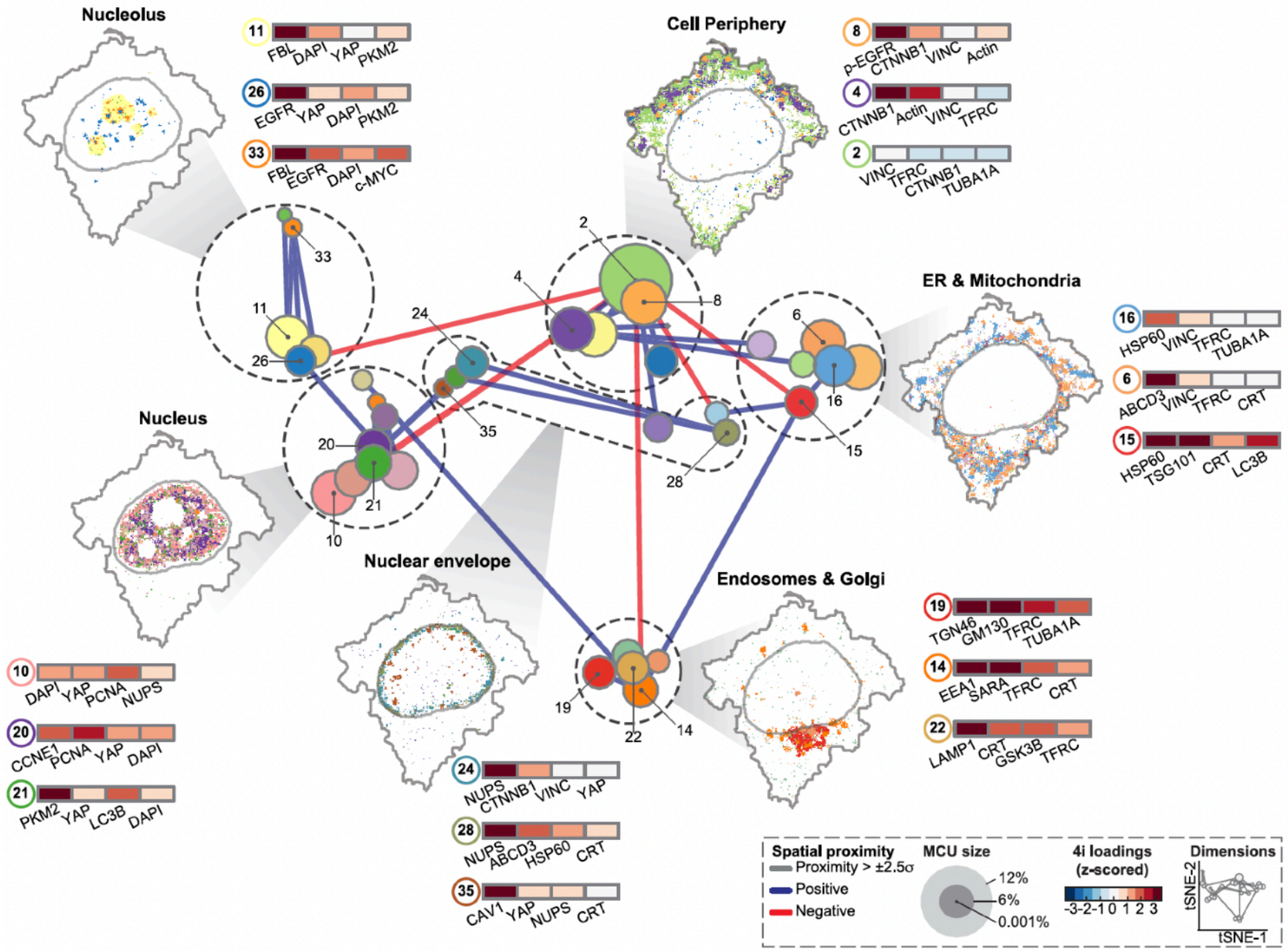
Identification of MCUs

- Iterative immunofluorescence; use of specialized elution and imaging buffers
- Intracellular protein composition can be characterized

Gut, G.; Herrmann, M. D.; Pelkmans, L. *Science* **2018**, *361,* eaar7042.

Multiplexed antibody-based spatial proteomics

***I**terative **I**ndirect **I**mmunofluorescence **I**maging (4i)*

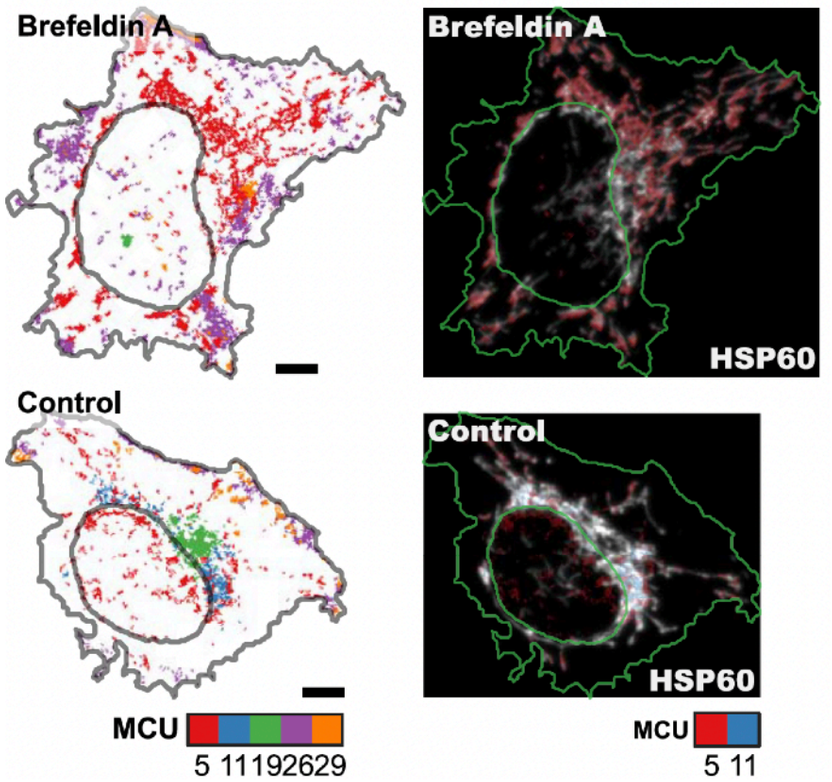


Each MCU contain generalizable information on protein subcompartmentalization

Gut, G.; Herrmann, M. D.; Pelkmans, L. *Science* **2018**, *361,* eaar7042.

Multiplexed antibody-based spatial proteomics

***I**terative **I**ndirect **I**mmunofluorescence **I**maging (4i)*

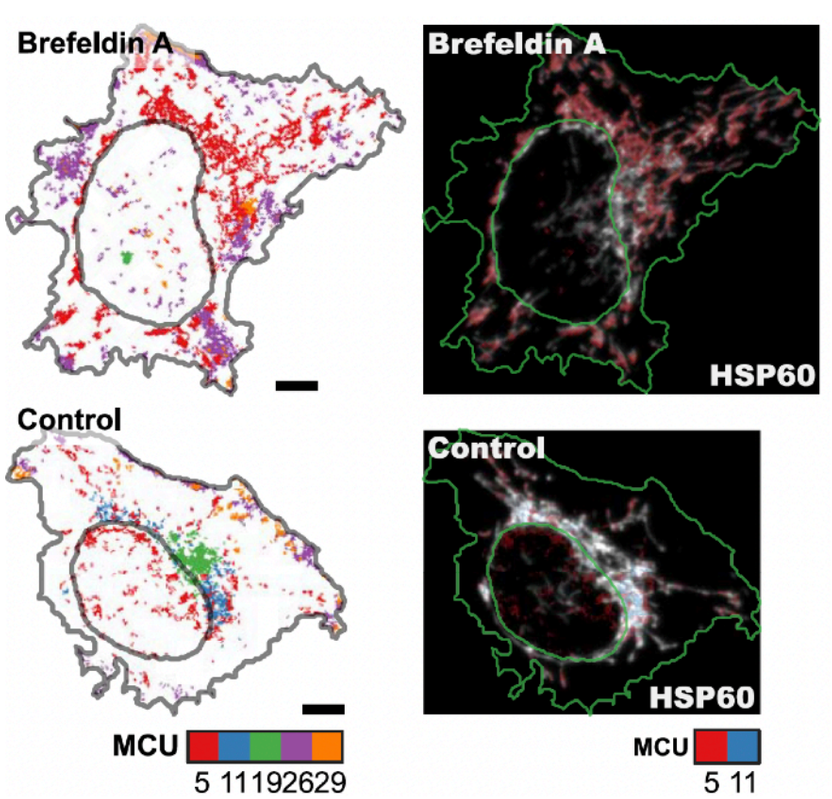


Proteome reorganization upon drug intake

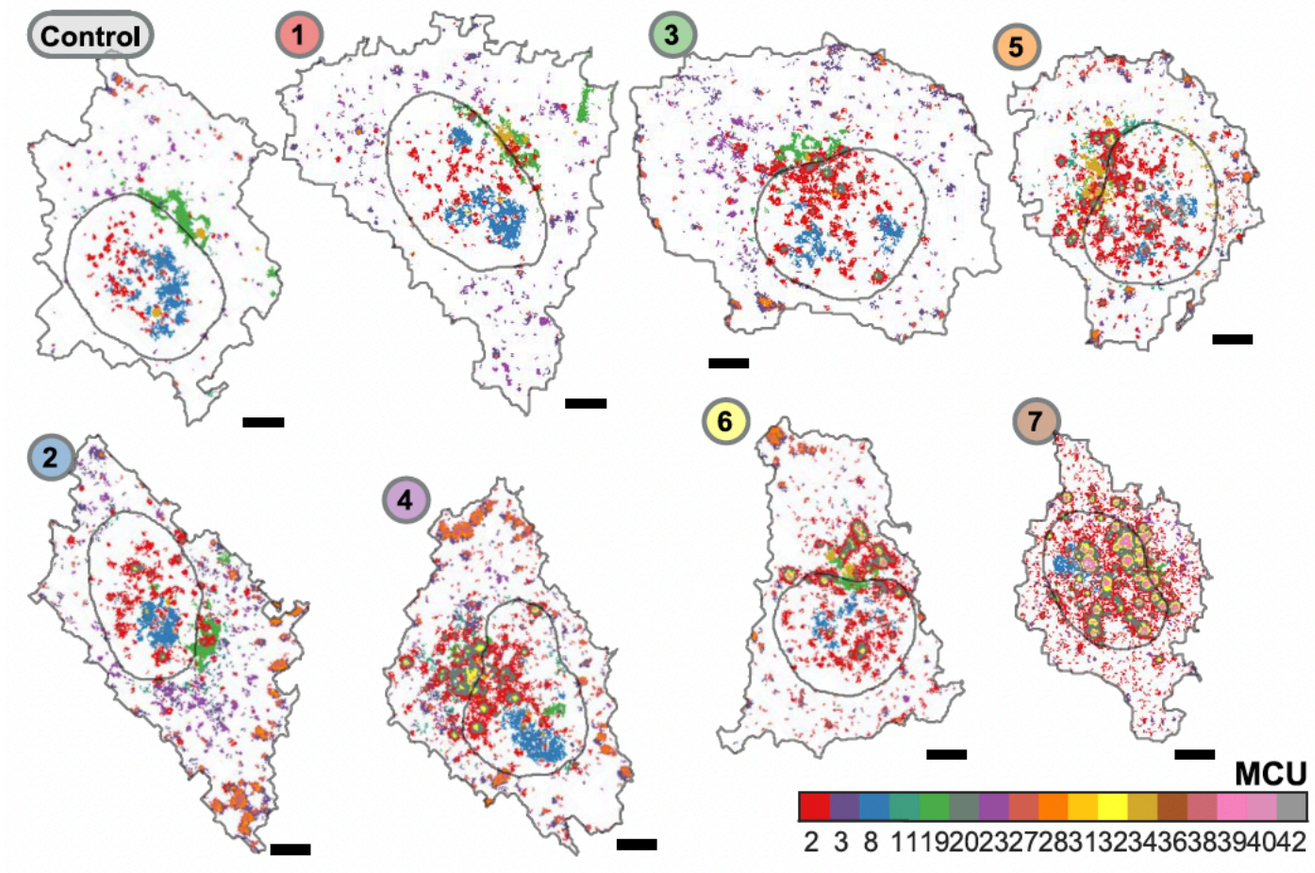
Gut, G.; Herrmann, M. D.; Pelkmans, L. *Science* **2018**, *361,* eaar7042.

Multiplexed antibody-based spatial proteomics

***I**terative **I**ndirect **I**mmunofluorescence **I**maging (4i)*



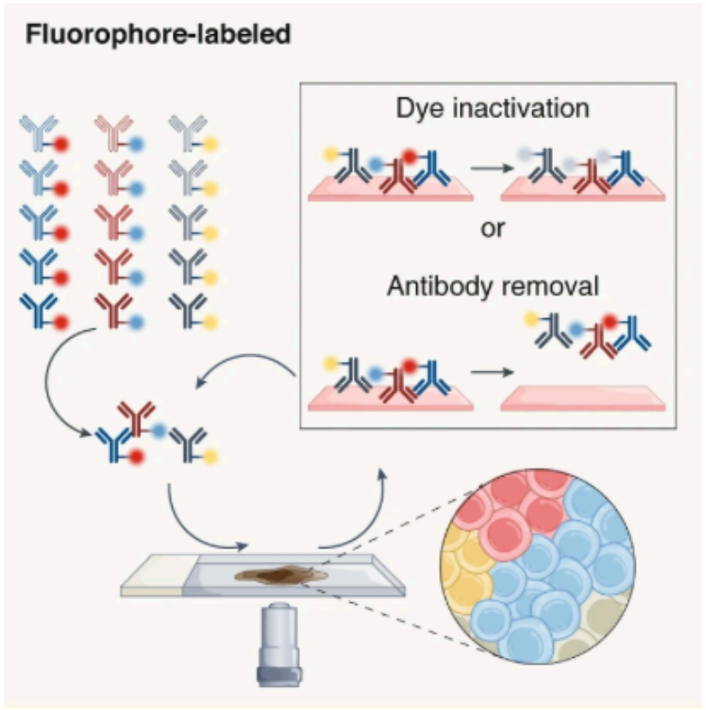
Proteome reorganization upon drug intake



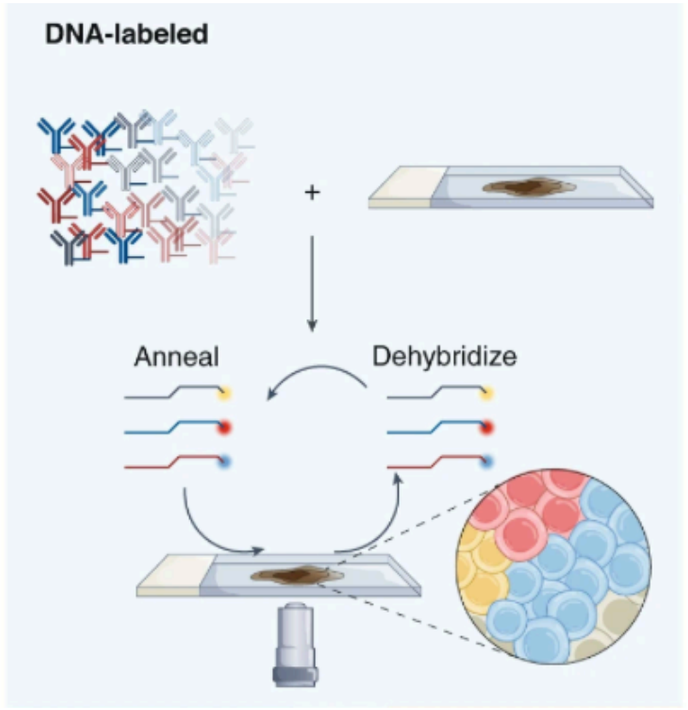
Cell heterogeneity based on protein relocalization upon external stimuli

Gut, G.; Herrmann, M. D.; Pelkmans, L. *Science* **2018**, *361,* eaar7042.

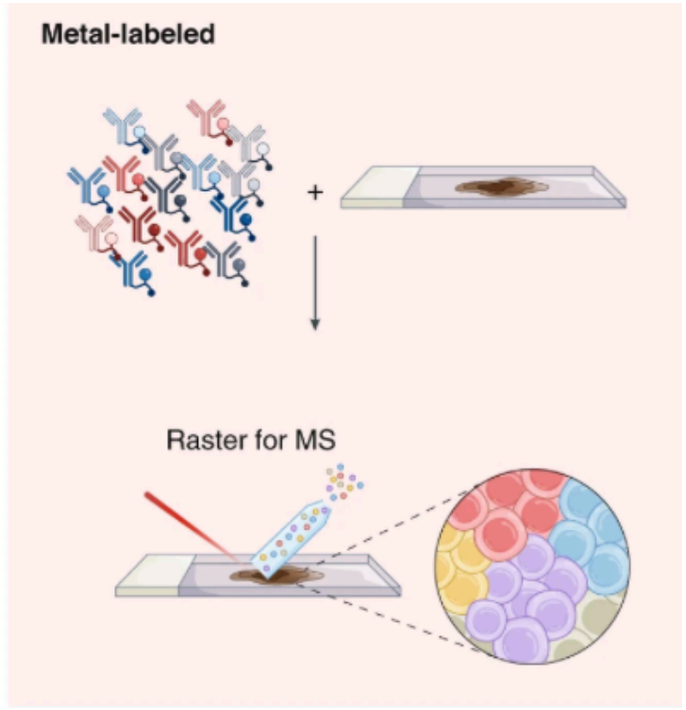
Multiplexed antibody-based spatial proteomics



CellDIVE, 4i, CyCIF
IBEX, MAP, SHIELD
…



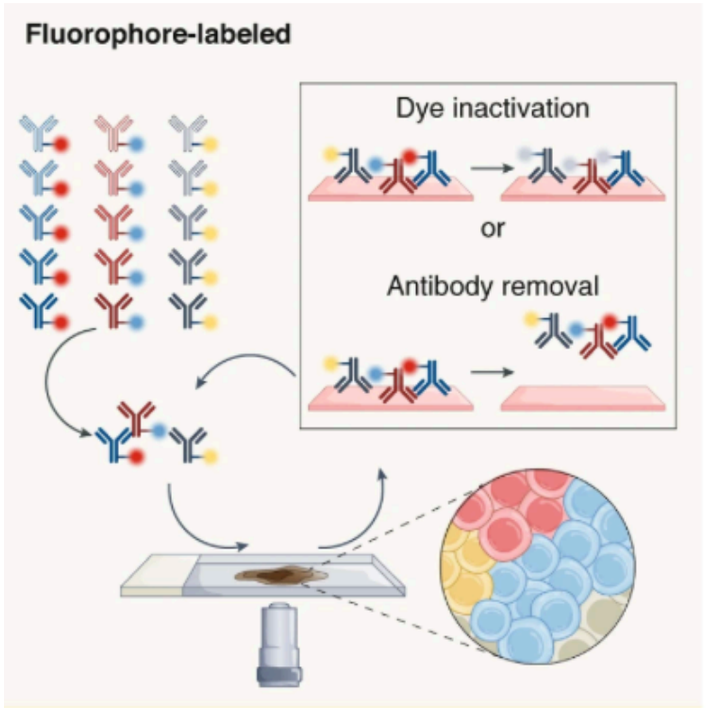
CODEX, DEI,
Immuno-SABER
…



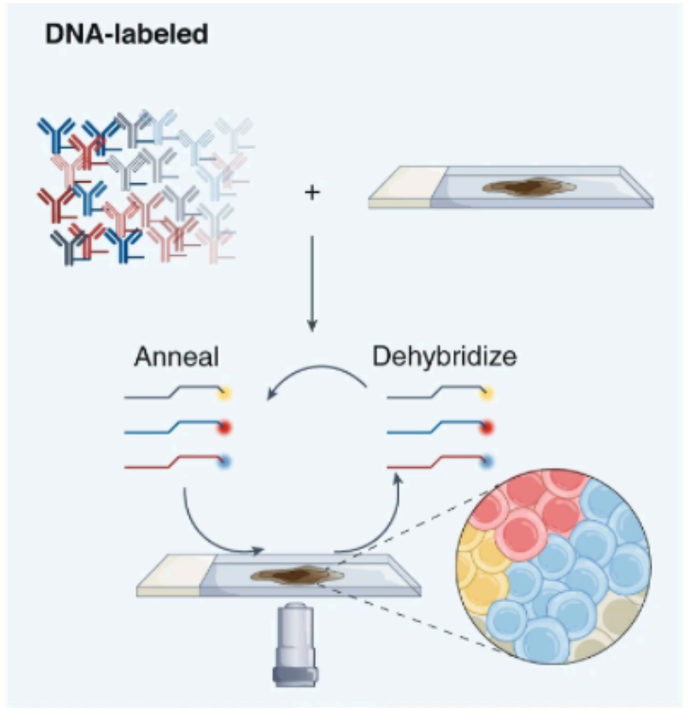
IMC, MIBI-TOF
…

Hickey, J. W.; Neumann, E. K.; … Saka, S. K. *Nat. Methods* **2022,** *19,* 284.

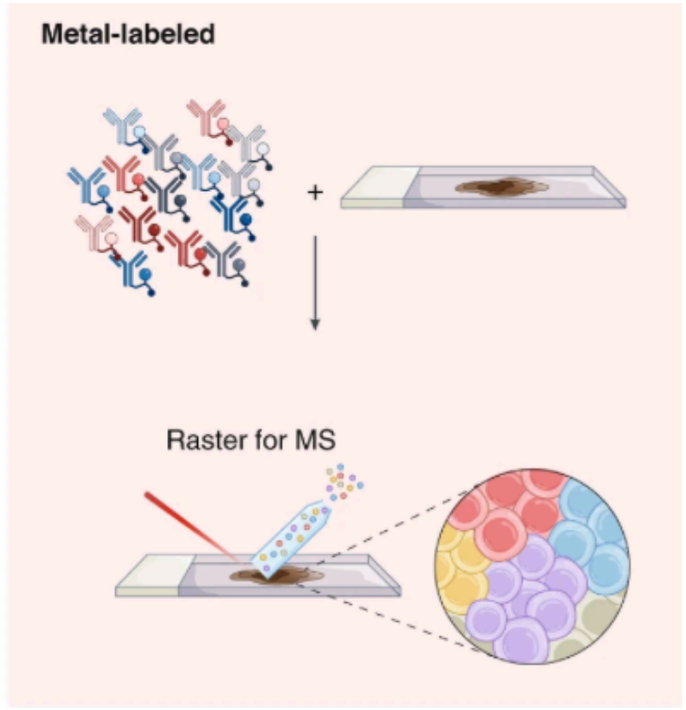
Multiplexed antibody-based spatial proteomics



CellDIVE, 4i, CyCIF
IBEX, MAP, SHIELD
…



CODEX, DEI,
Immuno-SABER
…



IMC, MIBI-TOF
…

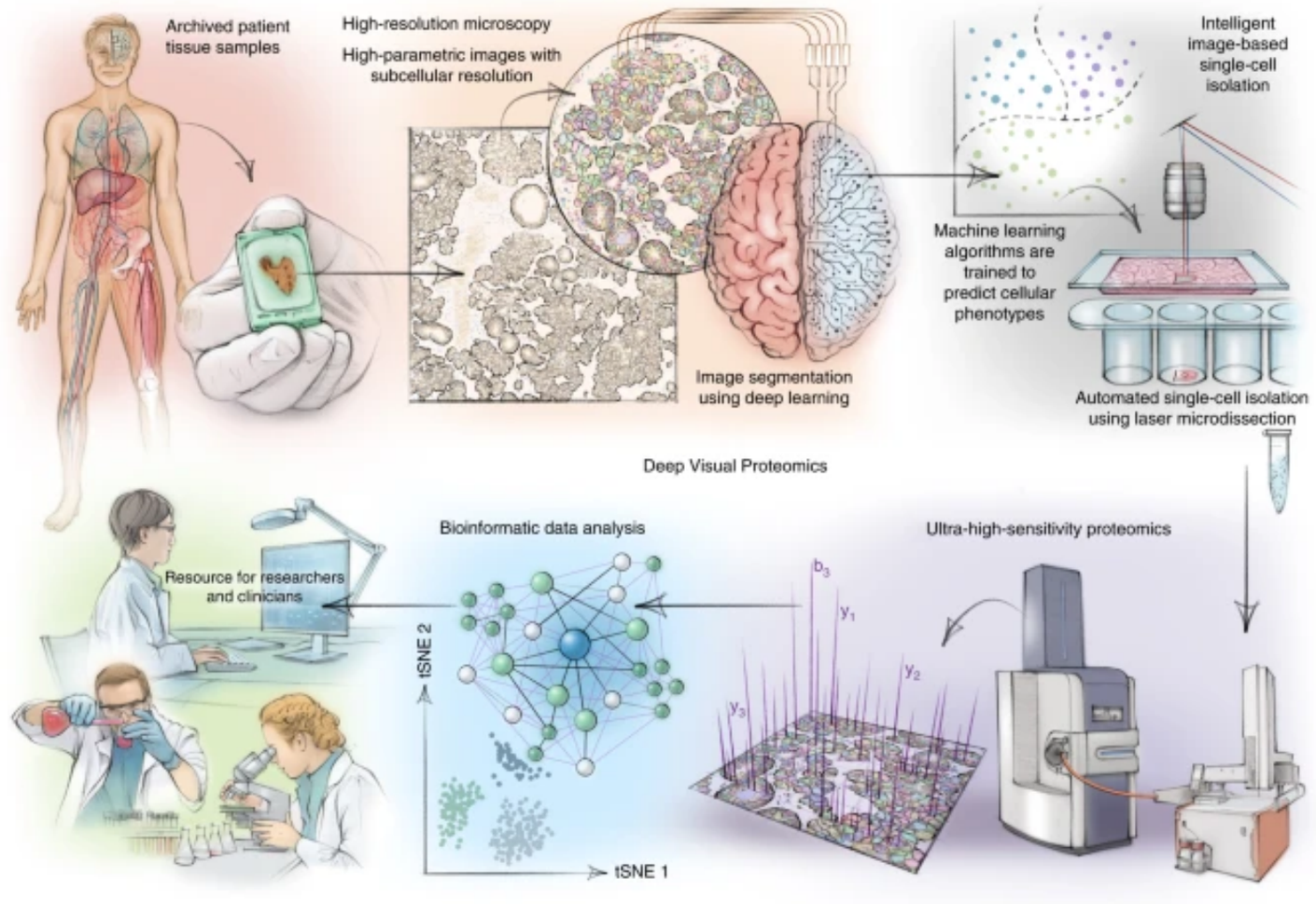
Predefined set of antibodies used… what about *unbiased* spatial proteomics?

Hickey, J. W.; Neumann, E. K.; … Saka, S. K. *Nat. Methods* **2022,** *19,* 284.

MS-based spatial proteomics

***D**eep **V**isual **P**roteomics (DVP)*

Matthias Mann
Max-Planck Institute of Biochemistry



Mund, A. … Horvath, P.; Mann, M. *Nat. Biotech.* **2022**, *40*, 1231.

MS-based spatial proteomics

***D**eep **V**isual **P**roteomics (DVP)*

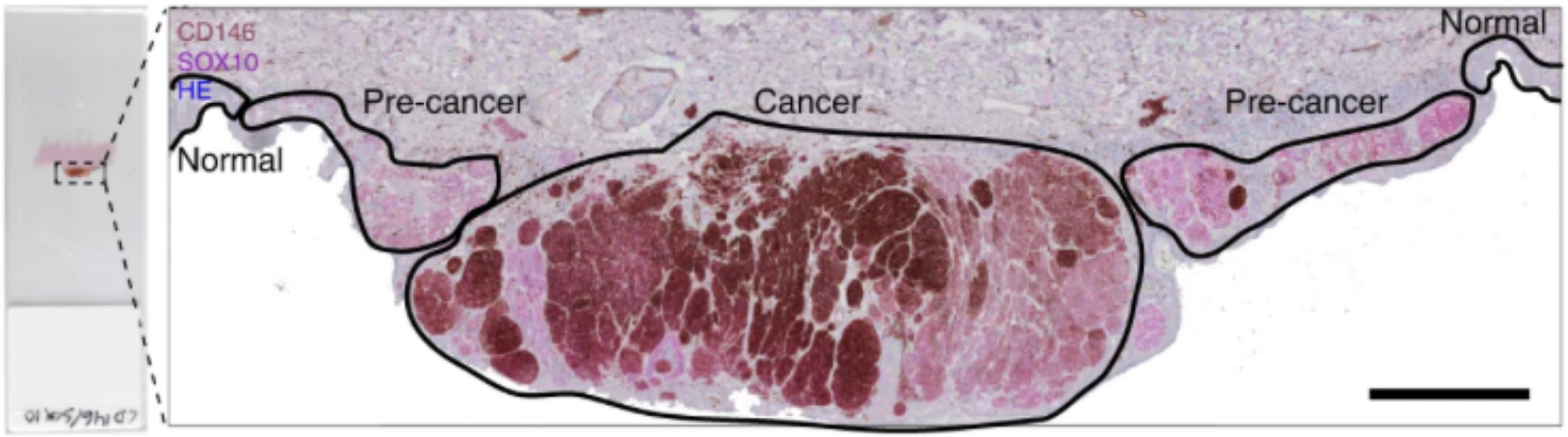
Multiplexed imaging of tissue samples	AI-based segmentation and phenotyping	Automated laser microdissection	Ultra-sensitive MS-based proteomics

Mund, A. … Horvath, P.; Mann, M. *Nat. Biotech.* **2022**, *40*, 1231.

MS-based spatial proteomics

***D**eep **V**isual **P**roteomics (DVP)*

Immunohistochemically stained melanoma tissue



CD146 (melanoma); SOX10 (melanocyte)

Multiplexed imaging of tissue samples

AI-based segmentation and phenotyping

Automated laser microdissection

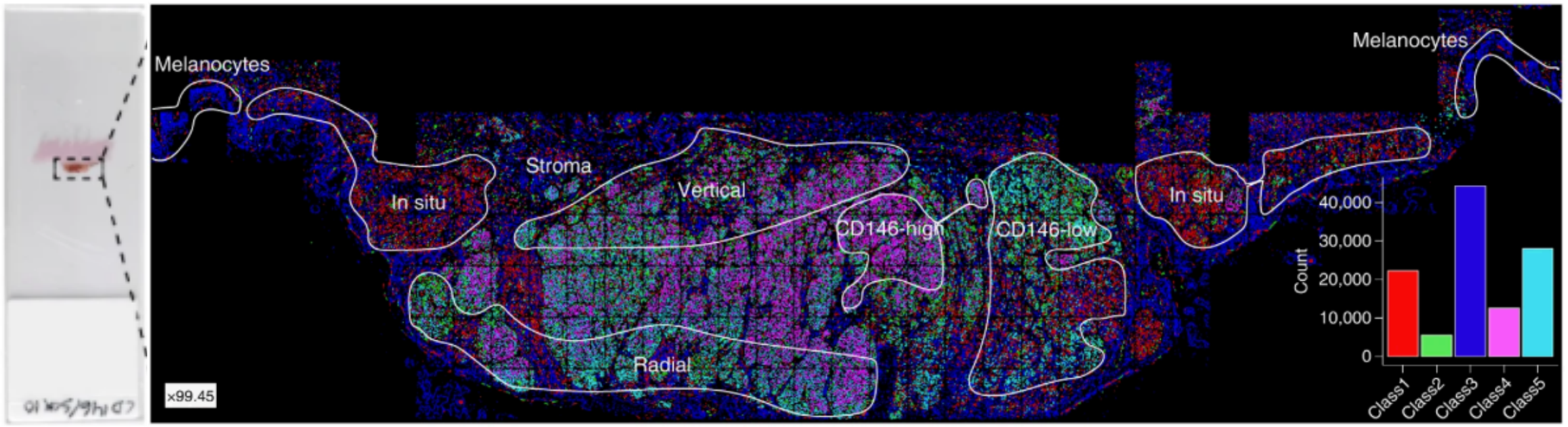
Ultra-sensitive MS-based proteomics

Mund, A. … Horvath, P.; Mann, M. *Nat. Biotech.* **2022**, *40*, 1231.

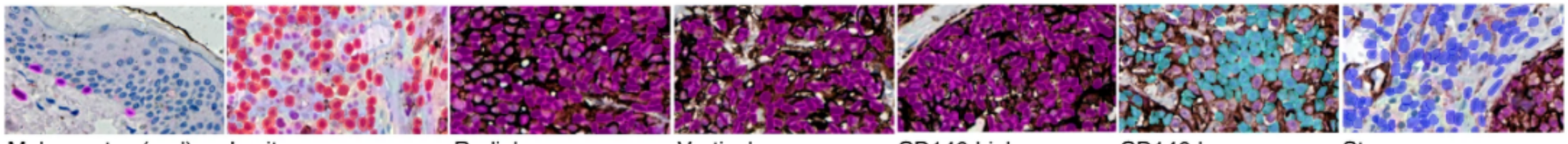
MS-based spatial proteomics

***D**eep **V**isual **P**roteomics (DVP)*

Cell classification based on CD146 and SOX10 staining intensity



CD146 (melanoma); SOX10 (melanocyte)



Multiplexed imaging of tissue samples | **AI-based segmentation and phenotyping** | Automated laser microdissection | Ultra-sensitive MS-based proteomics

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MS-based spatial proteomics

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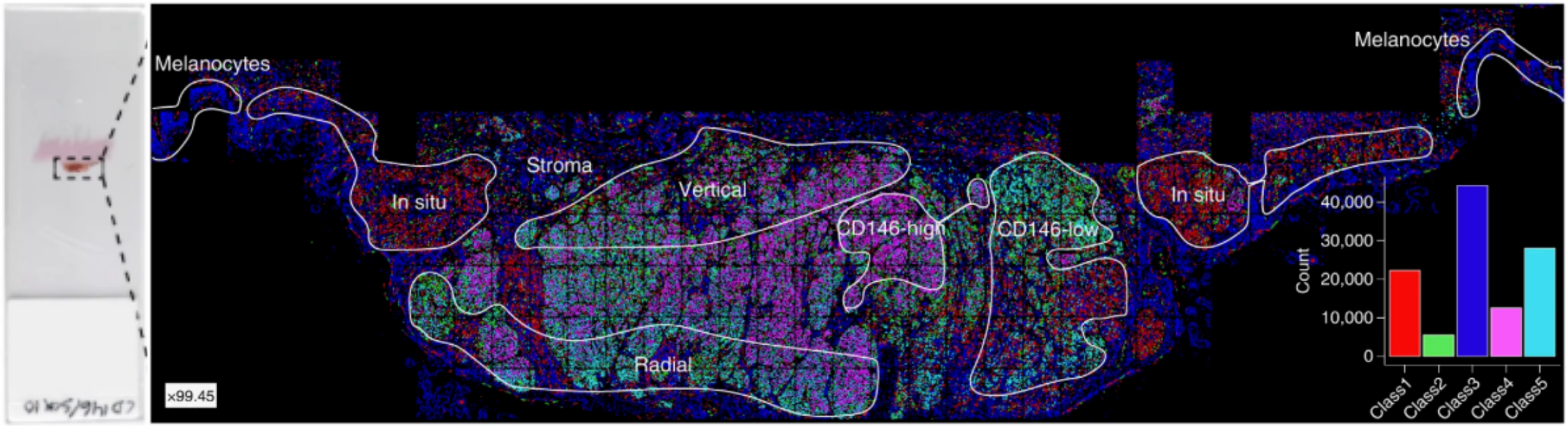
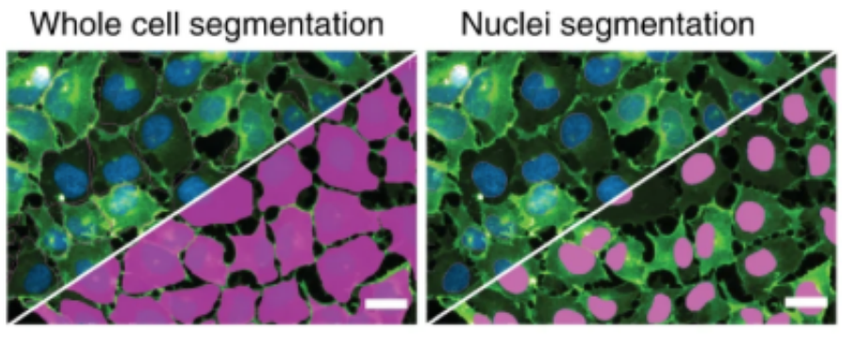


CD146 (melanoma); SOX10 (melanocyte)



Multiplexed imaging of tissue samples

AI-based segmentation and phenotyping

Automated laser microdissection

Ultra-sensitive MS-based proteomics

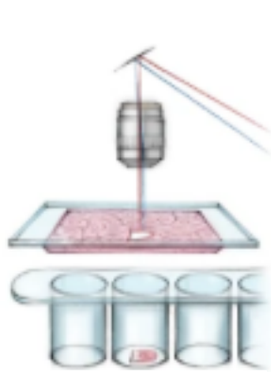
Mund, A. … Horvath, P.; Mann, M. *Nat. Biotech.* **2022**, *40*, 1231.

MS-based spatial proteomics

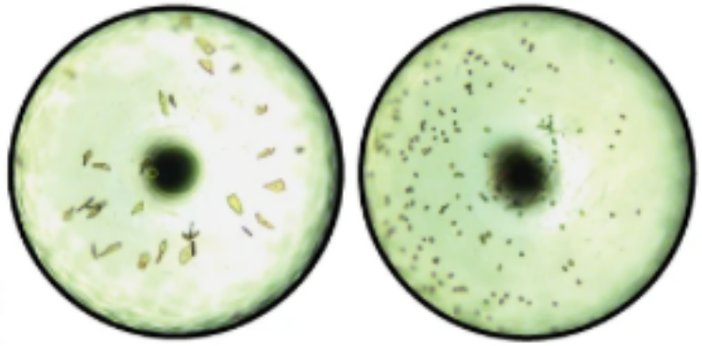
***D**eep **V**isual **P**roteomics (DVP)*

Laser microdissection (LMD)

Interfaced with image segmentation data
and single cell isolation setup



384-well



Whole cells
(50-100/well)

Nuclei enriched
(~260/well)

Multiplexed imaging
of tissue samples

AI-based segmentation
and phenotyping

**Automated
laser microdissection**

Ultra-sensitive
MS-based proteomics

Mund, A. … Horvath, P.; Mann, M. *Nat. Biotech.* **2022**, *40*, 1231.

MS-based spatial proteomics

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Laser microdissection (LMD)

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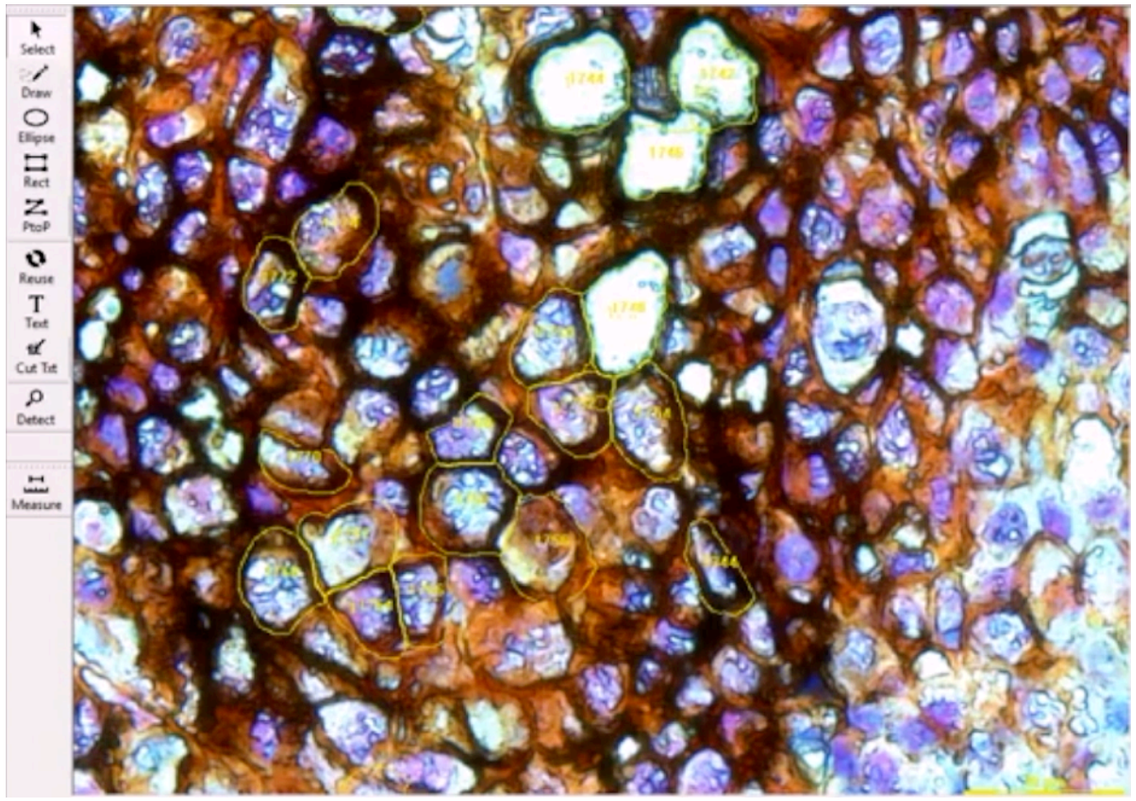

Multiplexed imaging
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Automated
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MS-based spatial proteomics

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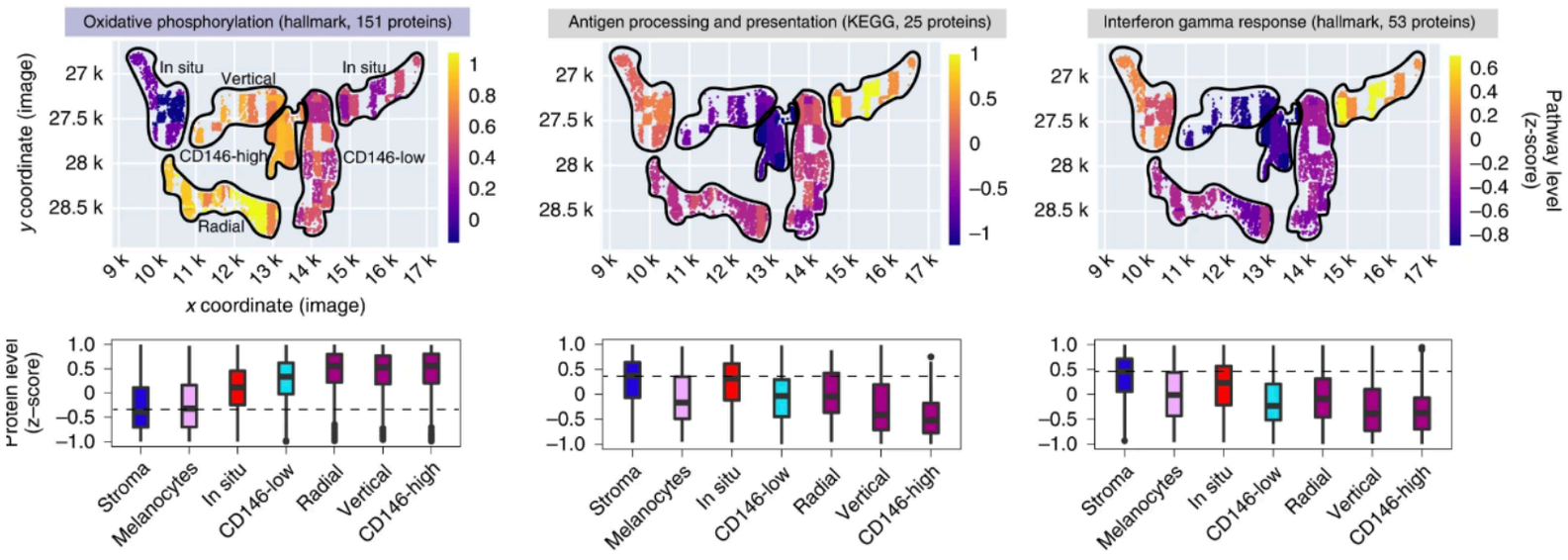


Multiplexed imaging of tissue samples

AI-based segmentation and phenotyping

Automated laser microdissection

Ultra-sensitive MS-based proteomics

Mund, A. … Horvath, P.; Mann, M. *Nat. Biotech.* **2022**, *40*, 1231.

Future outlook

Vandereyken, K.; Sifrim, A.; Thienpont, B.; Voet, T. *Nat. Rev. Genet.* **2023**, *24*, 494.

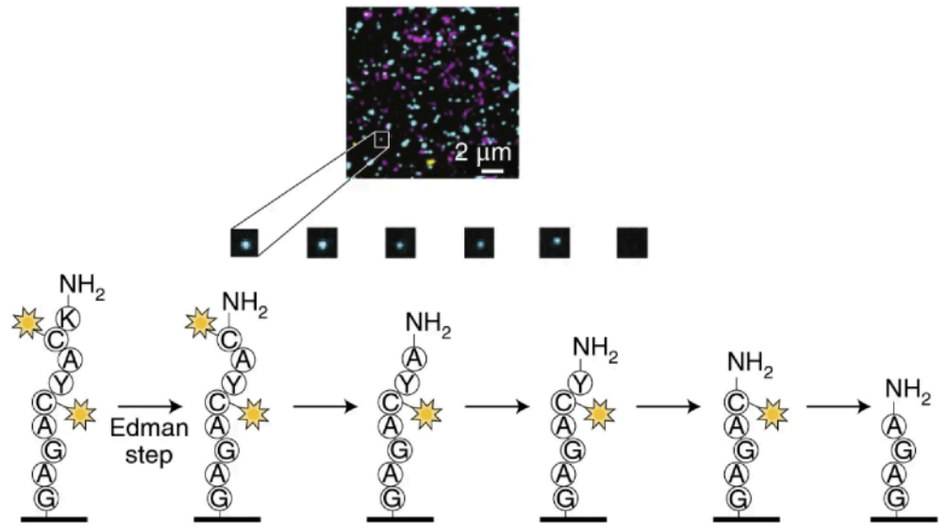
Future outlook

Improved protein sequencing methods

Alfaro, J. A.; Bohländer, P.; … Joo, C. *Nat. Methods* **2021**, *18*, 604.

Future outlook

Improved protein sequencing methods

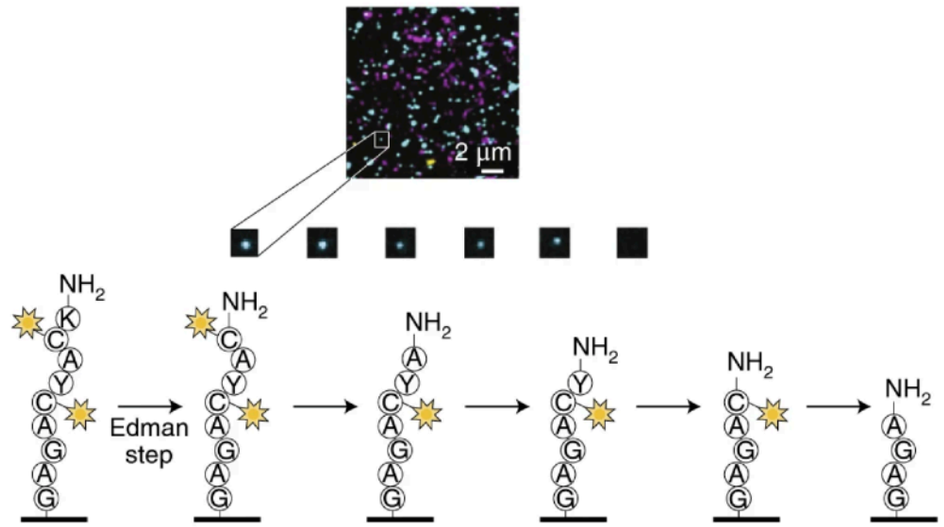


Fluorosequencing by chemical modification

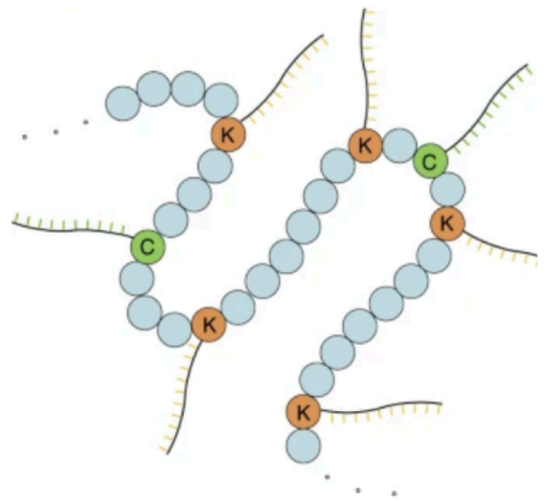
Alfaro, J. A.; Bohländer, P.; … Joo, C. *Nat. Methods* **2021**, *18*, 604.

Future outlook

Improved protein sequencing methods



Fluorosequencing by chemical modification

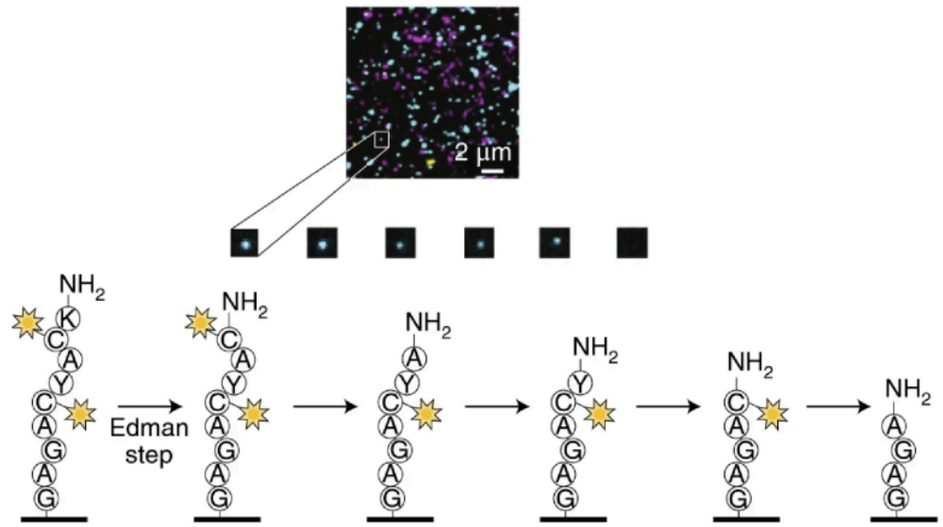


DNA-facilitated

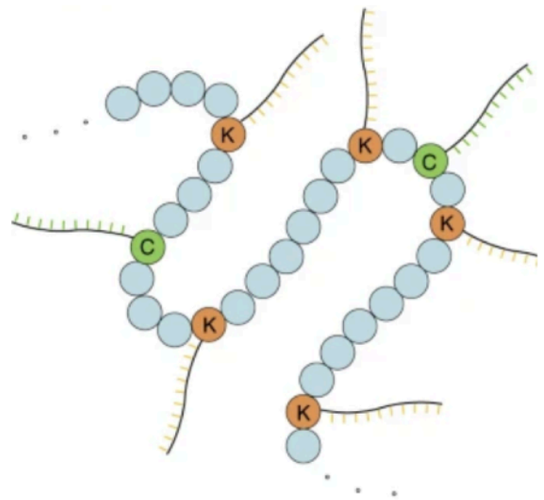
Alfaro, J. A.; Bohländer, P.; … Joo, C. *Nat. Methods* **2021**, *18*, 604.

Future outlook

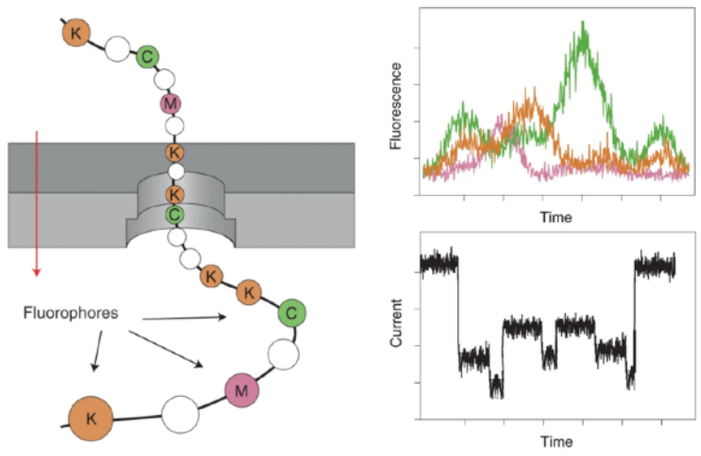
Improved protein sequencing methods



Fluorosequencing by chemical modification



DNA-facilitated

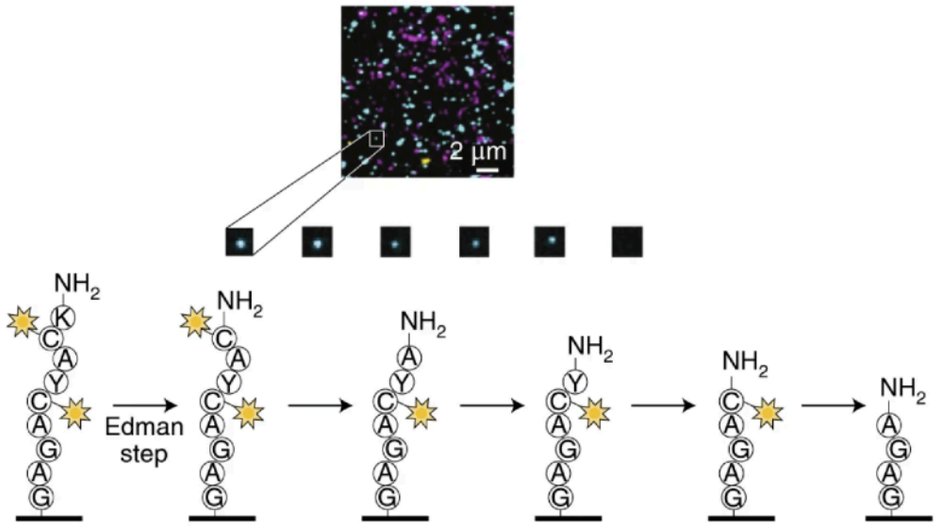


Nanopore-based fingerprinting

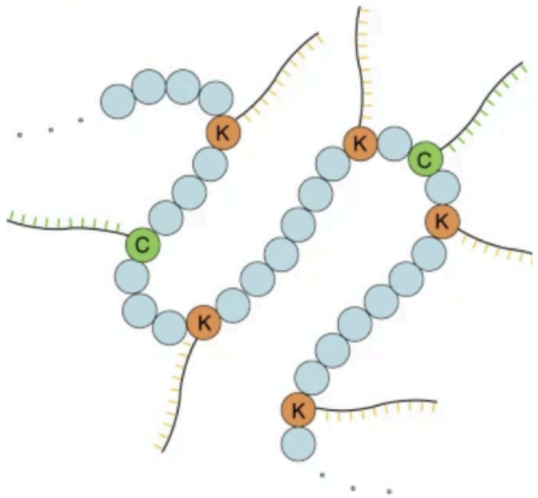
Alfaro, J. A.; Bohländer, P.; … Joo, C. *Nat. Methods* **2021**, *18*, 604.

Future outlook

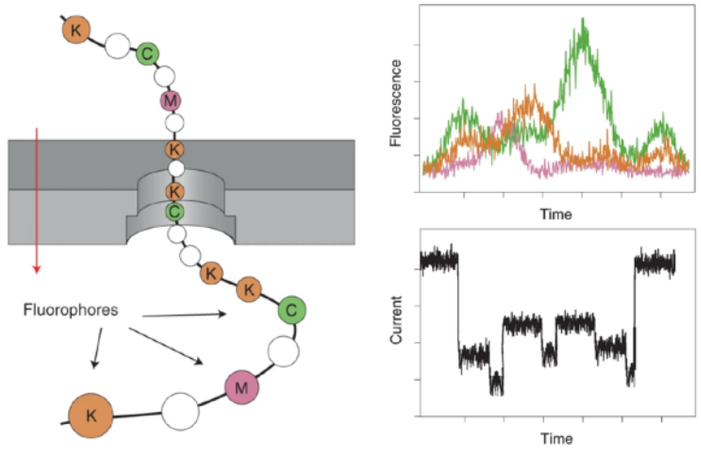
Improved protein sequencing methods



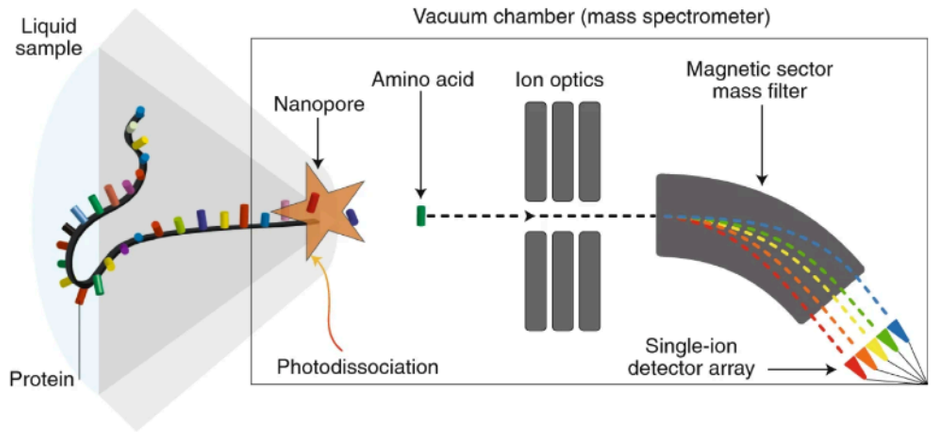
Fluorosequencing by chemical modification



DNA-facilitated



Nanopore-based fingerprinting

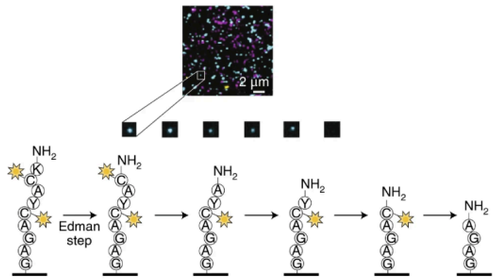


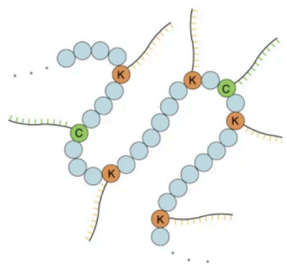
Nanopore electrospray

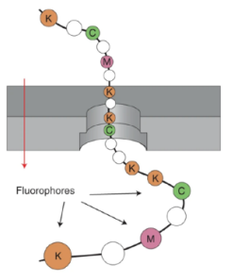
Alfaro, J. A.; Bohländer, P.; … Joo, C. *Nat. Methods* **2021**, *18*, 604.

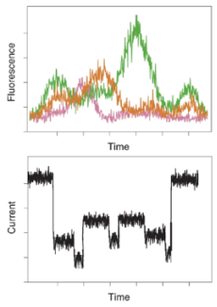
Future outlook

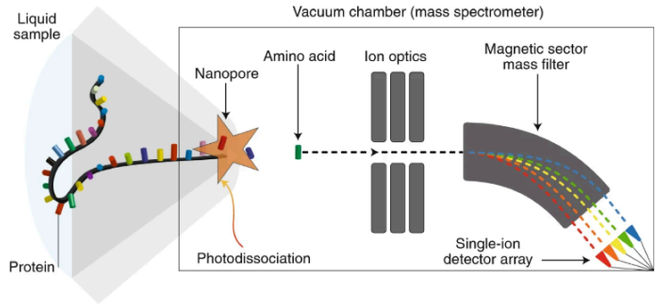
Improved protein sequencing methods



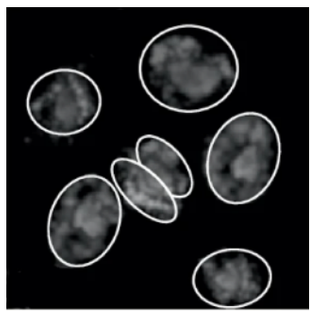




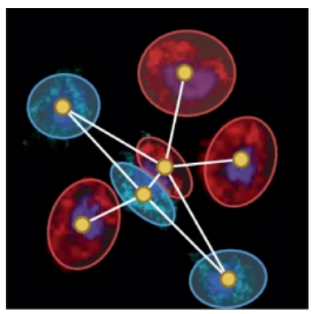




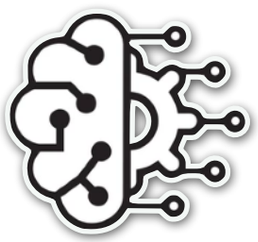
Improved data-analysis methods



Segmentation



Spatial analysis



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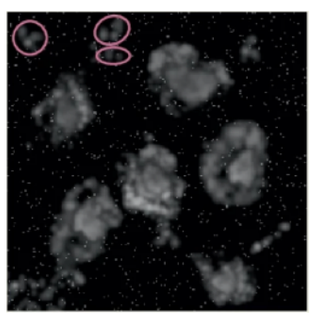
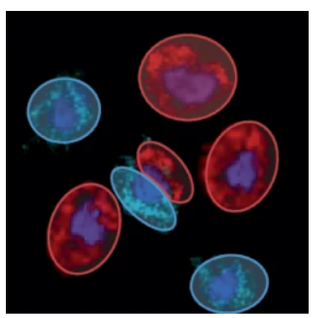


Image cleaning

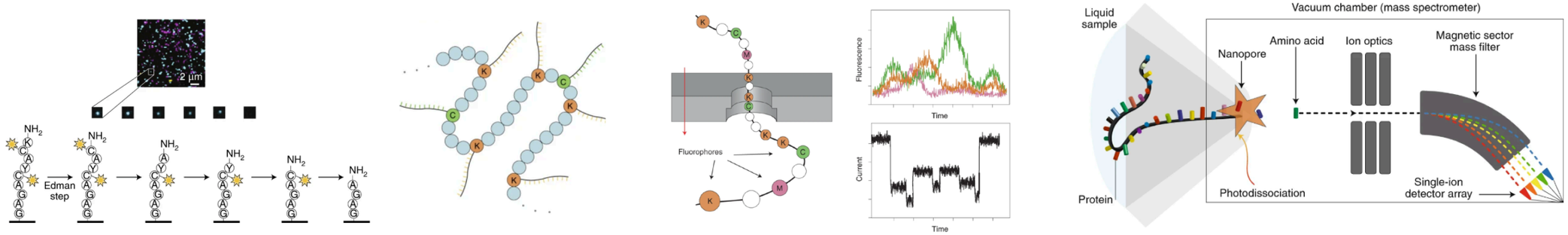


Classification

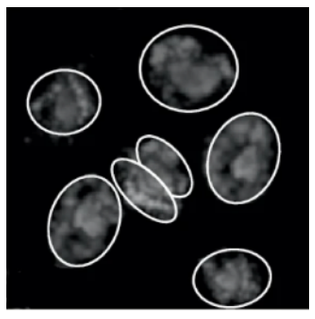
Bussi, Y.; Keren, L. *Nat. Methods* **2024**, *21,* 2212; Karin, J.; Mintz, R.; Raven, B.; Nitzan, M. *Nat. Comp. Sci*. **2024**, *4*, 941.

Future outlook

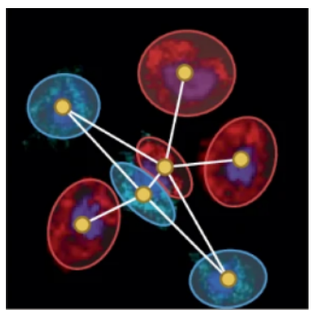
Improved protein sequencing methods



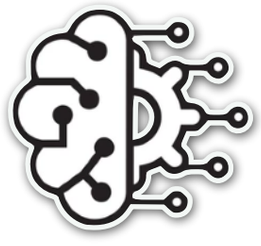
Improved data-analysis methods



Segmentation



Spatial analysis



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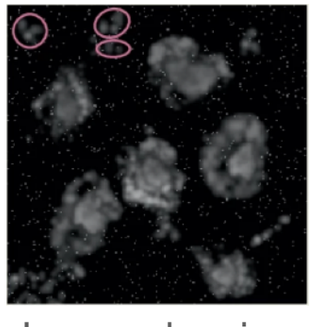
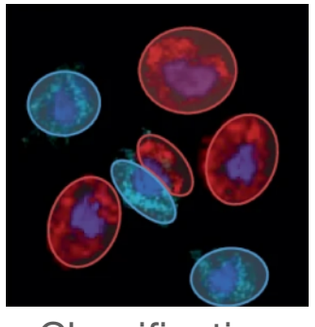
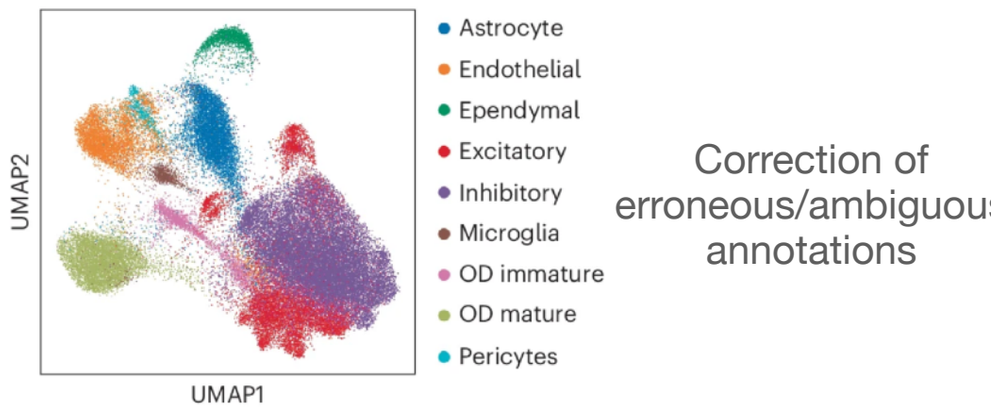


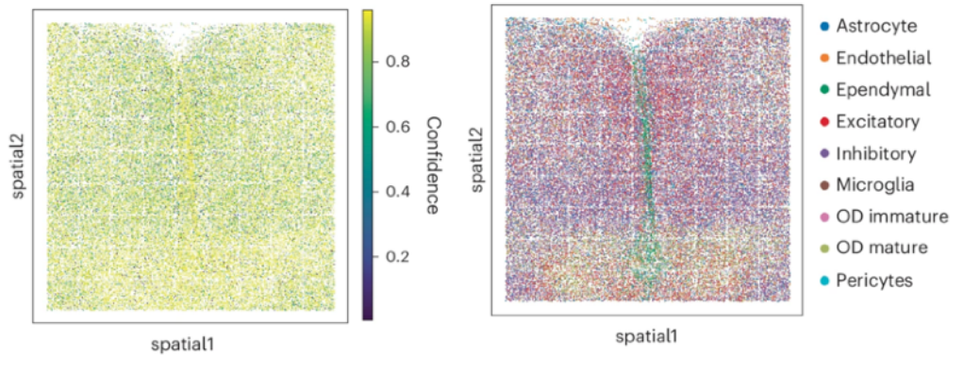
Image cleaning



Classification



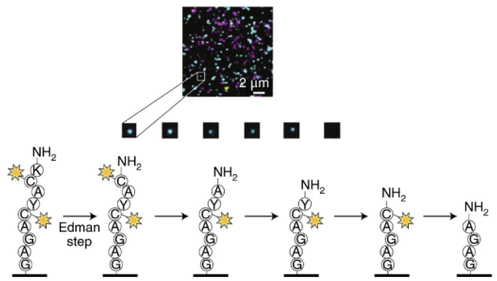
Correction of erroneous/ambiguous annotations

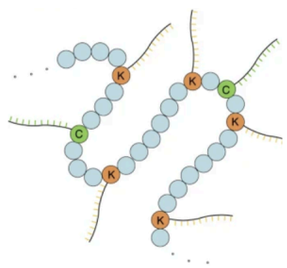


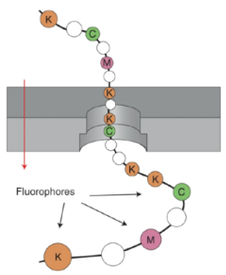
Bussi, Y.; Keren, L. *Nat. Methods* **2024**, *21,* 2212; Karin, J.; Mintz, R.; Raven, B.; Nitzan, M. *Nat. Comp. Sci*. **2024**, *4*, 941.

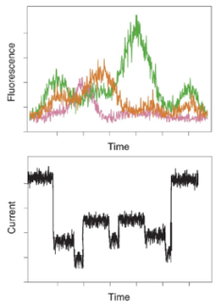
Future outlook

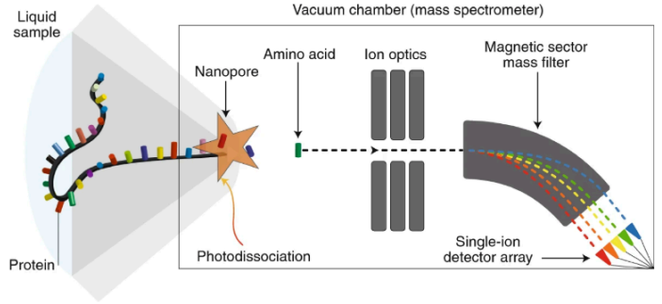
Improved protein sequencing methods



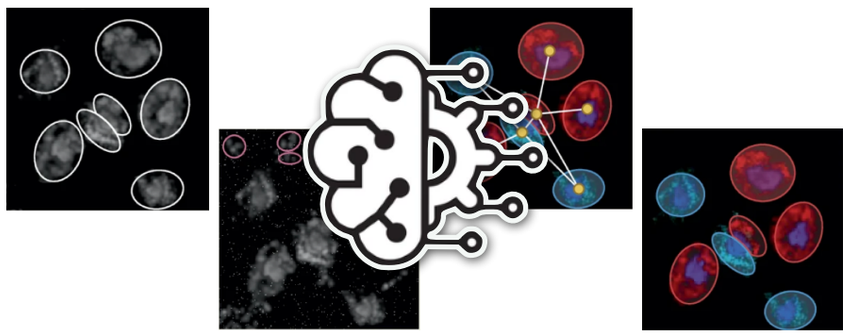








Improved data-analysis methods



Spatial multi-omics

Vandereyken, K.; Sifrim, A.; Thienpont, B.; Voet, T. *Nat. Rev. Genet.* **2023**, *24*, 494.

Thank you

Questions?