ZipSeq and scNT-seq: Two novel methods to improve spatially and temporally resolved single-cell transcriptomics

TJC, 08.09.2020 – PATH C22, Breuer Eva, Clavien Lab

ZipSeq: barcoding for real-time mapping of single cell transcriptomes

Kenneth H. Hu[1], John P. Eichorst[1,2], Chris S. McGinnis[3], David M. Patterson[3], Eric D. Chow[4], Kelly Kersten[1], Stephen C. Jameson[5], Zev J. Gartner[3], Arjun A. Rao[1,2] and Matthew F. Krummel[1,2] ✉

nature methods
August 2020

Review

Uncovering an Organ's Molecular Architecture at Single-Cell Resolution by Spatially Resolved Transcriptomics

Jie Liao,[1,3] Xiaoyan Lu,[1,3] Xin Shao,[1] Ling Zhu,[2] and Xiaohui Fan,[1,2,*]

CellPress REVIEWS
June 2020

Massively parallel and time-resolved RNA sequencing in single cells with scNT-seq

Qi Qiu[1,2,3,7], Peng Hu[1,2,3,7], Xiaojie Qiu[4,6], Kiya W. Govek[1,5], Pablo G. Cámara[1,5] and Hao Wu[1,2,3] ✉

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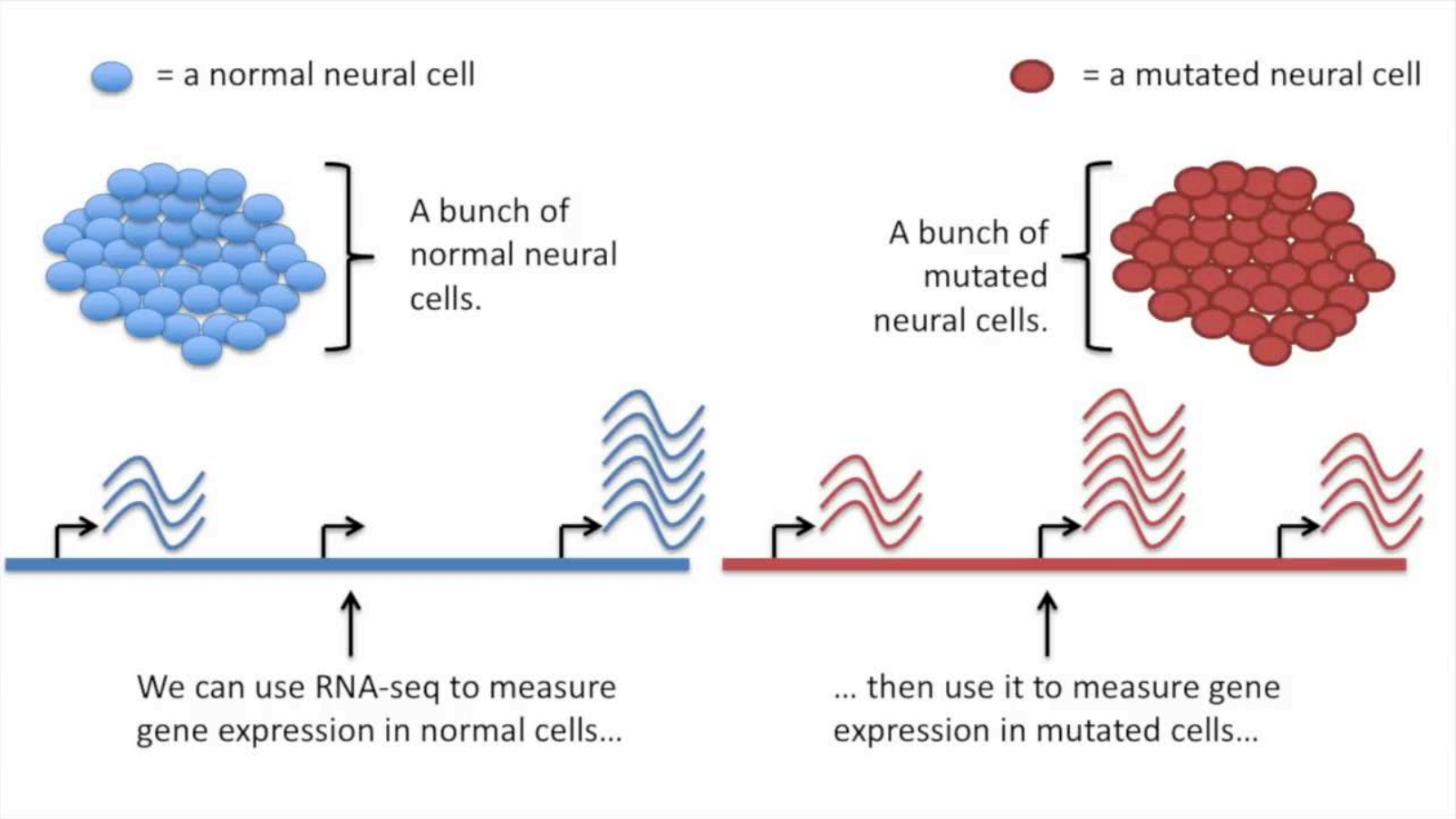
single-cell RNA sequencing (scRNA-seq)



StatQuest with Josh Starmer: A gentle introduction to RNA-seq. Source: University of North Carolina at Chapel Hill: https://www.youtube.com/watch?v=tlf6wYJrwKY&t=589s

single-cell RNA sequencing (scRNA-seq)

Preparing an RNA-seq library

Step 1: Isolate the RNA

StatQuest with Josh Starmer: A gentle introduction to RNA-seq. Source: University of North Carolina at Chapel Hill: https://www.youtube.com/watch?v=tlf6wYJrwKY&t=589s

single-cell RNA sequencing (scRNA-seq)

Genome:

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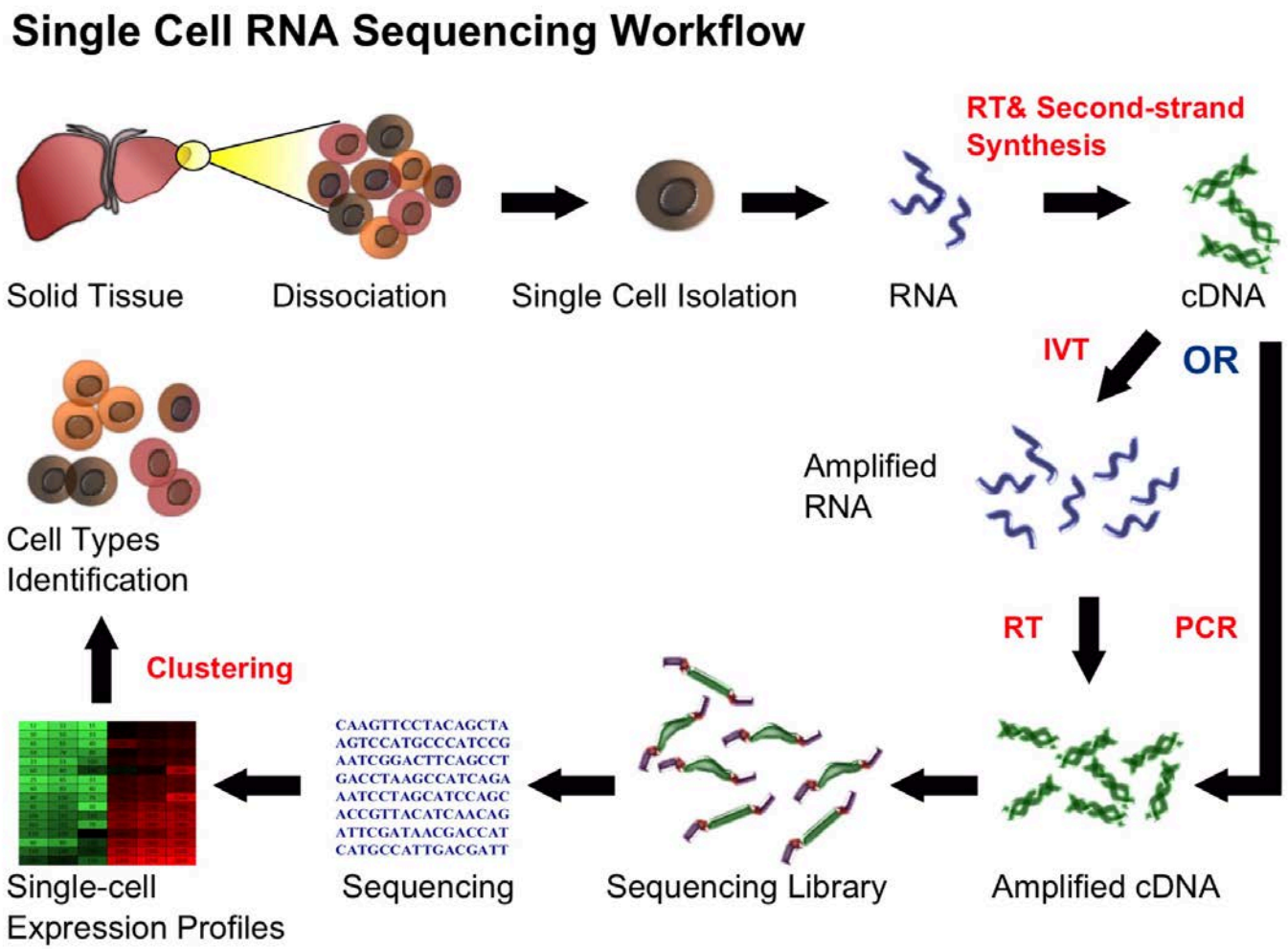
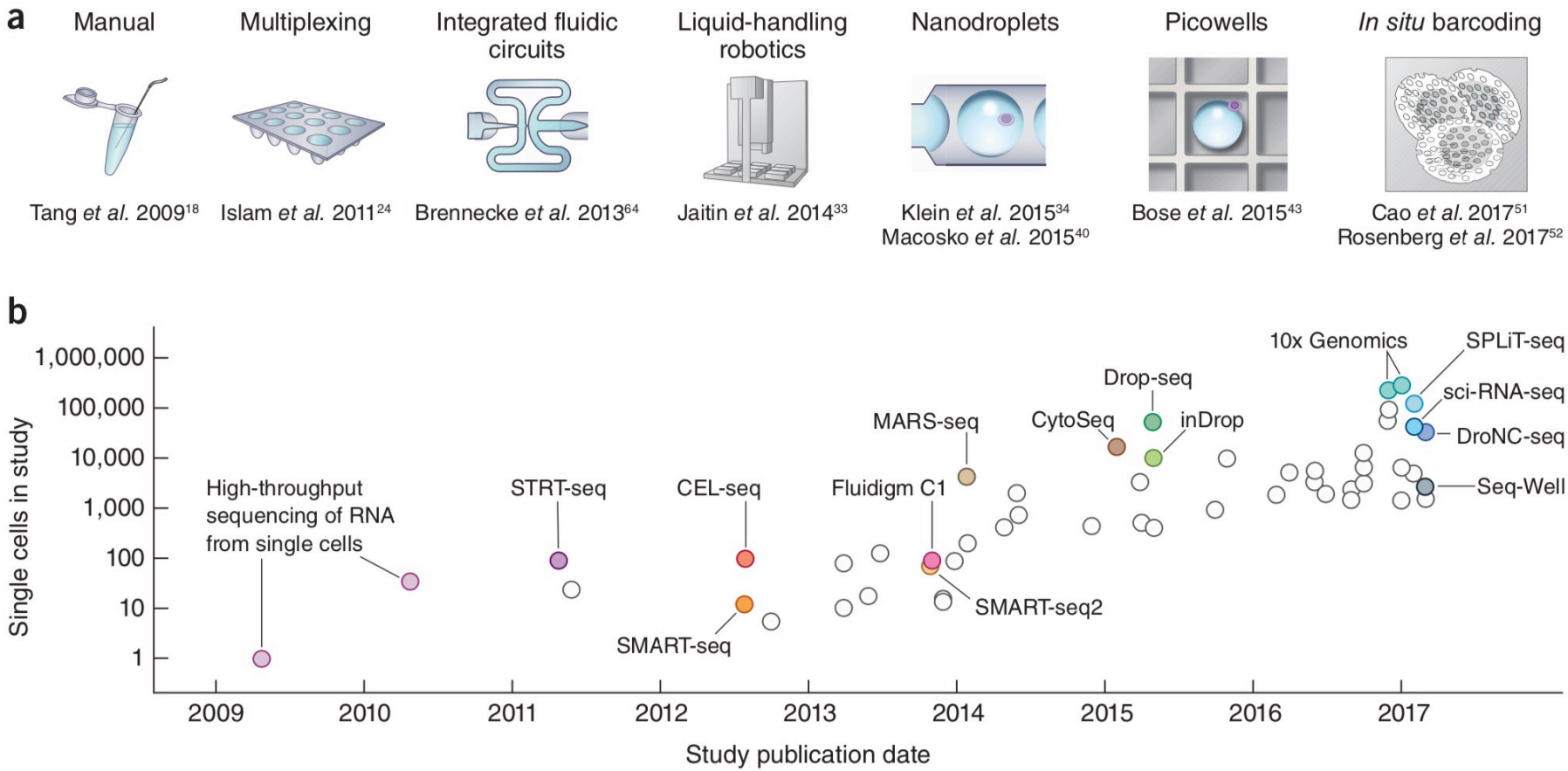
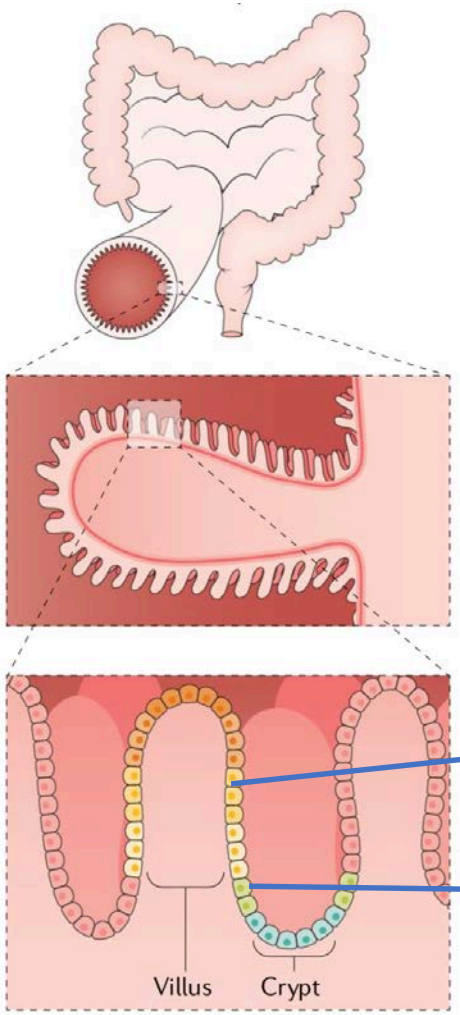


Exponential scaling of scRNA-seq



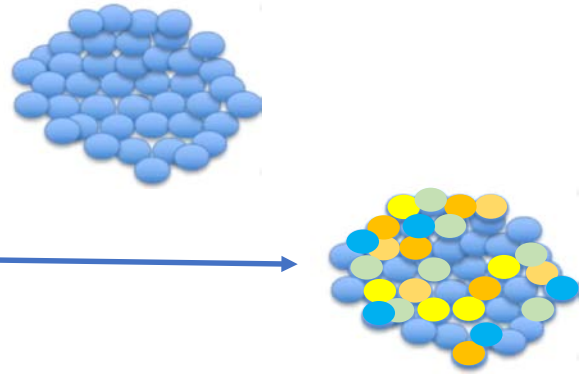
Svensson et al.: Exponential scaling of single-cell RNA-seq in the past decade. Nature Protocols, 2018.

Spatial heterogeneity of tissues/organs



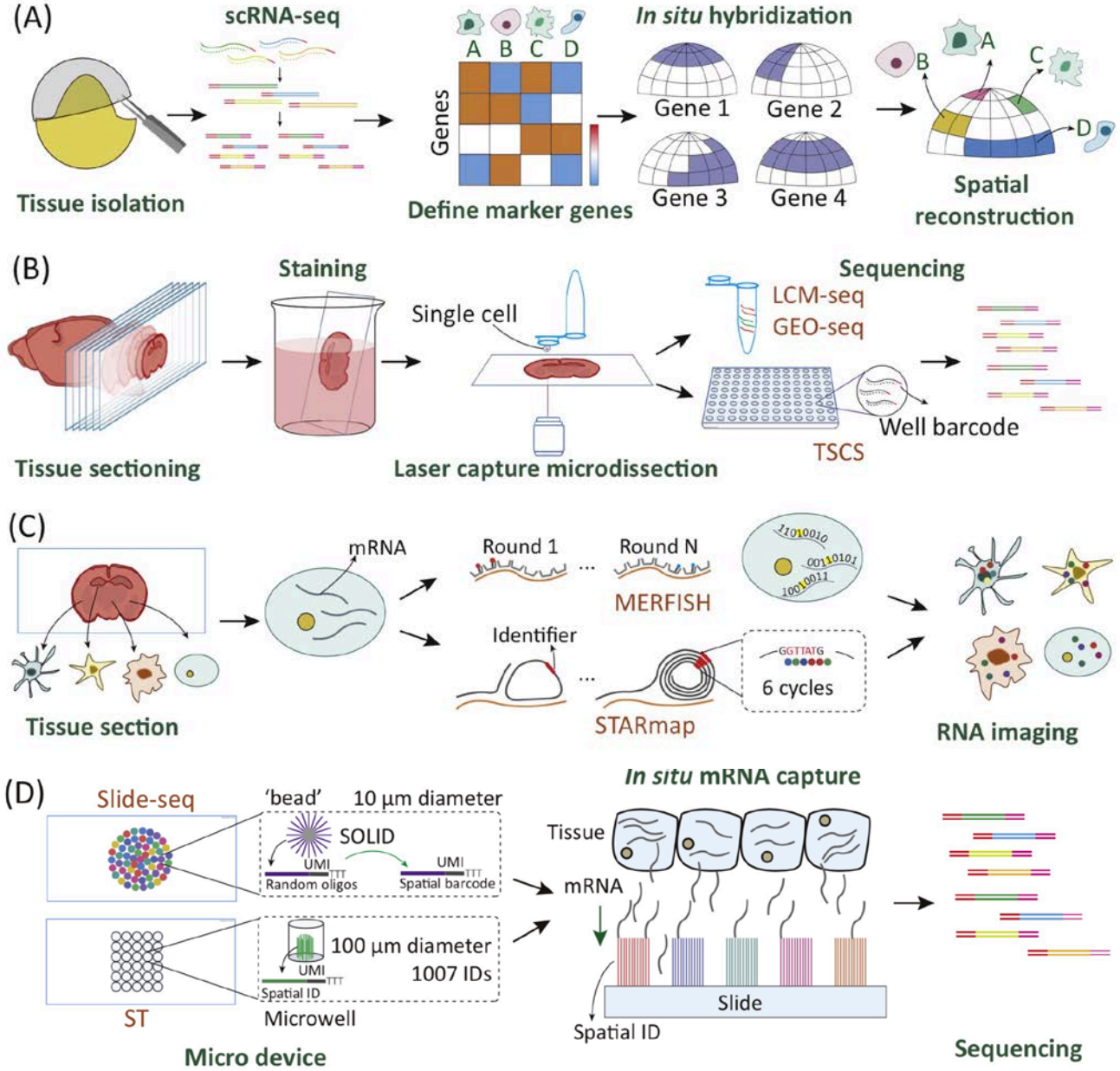
scRNAseq requires the dissociation of tissues into isolated cells → complete loss of spatial information

To identify spatial patterns of gene expression, the original tissue coordinates of the sequenced cells need to be resolved



Spatially resolved scRNAseq

4 types of spatially resolved transcriptomics



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

Combine real-time phenotyping with scRNA-seq

Problems:

In multiplexed imaging techniques (MIBI, MERFISH, SeqFish) good spatial resolution, but probes must be selected a priori.

Grid-based approaches with good spatial resolution, but the ROI are defined before imaging on fixed tissue sections.

Question:

How to link high-dimensional scRNA-seq to the in-vivo spatial dimensions and real-time phenotypical analyses from microscopy?

ZipSeq: barcoding for real-time mapping of single cell transcriptomes

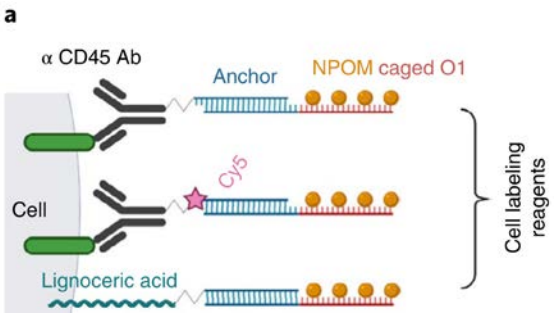
Kenneth H. Hu[1], John P. Eichorst[1,2], Chris S. McGinnis[3], David M. Patterson[3], Eric D. Chow[4], Kelly Kersten[1], Stephen C. Jameson[5], Zev J. Gartner[3], Arjun A. Rao[1,2] and Matthew F. Krummel[1,2]

Solution:

"Printing" a DNA barcode onto live cells in a spatially defined manner
→ which can be read out during typical scRNA-seq workflow

Design of ZipSeq oligonucleotides & workflow

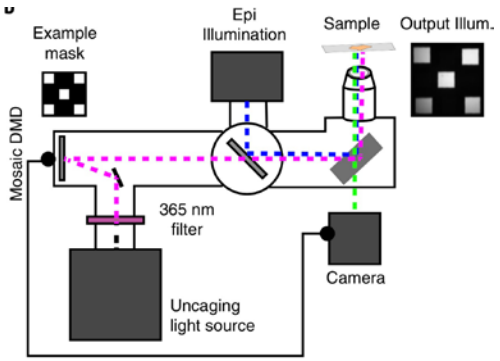
„anchor“



Ab/lipid covalently conjugated to „anchor“ sequence

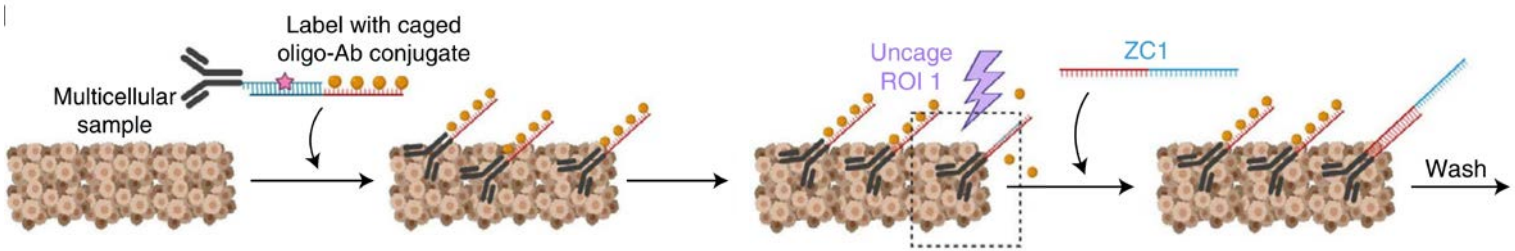
Zipcode

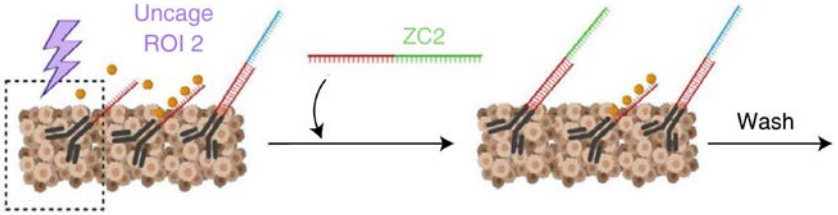
light source



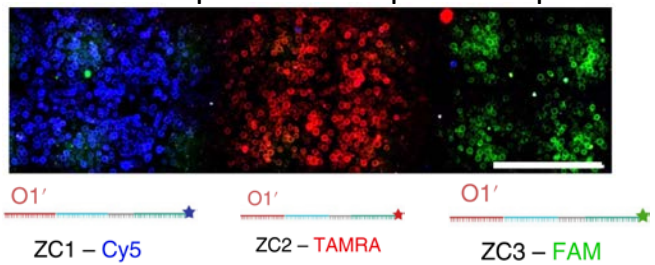
Imaging and photo-uncaging of sample with 365nm

workflow





Proof of concept: labeled zipcode sequences



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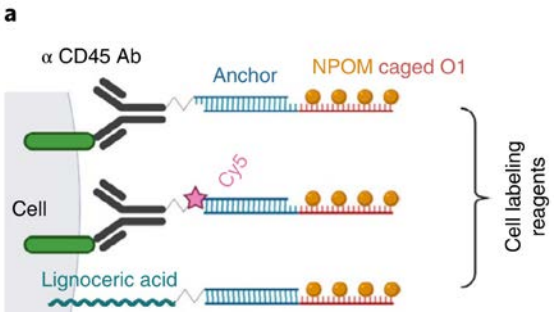

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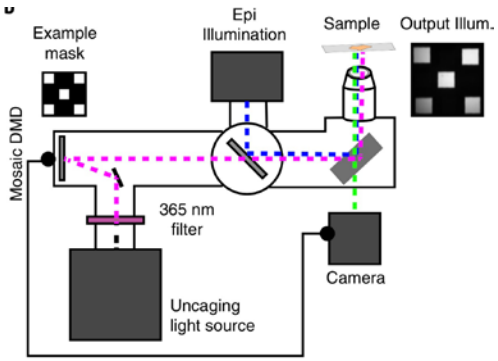
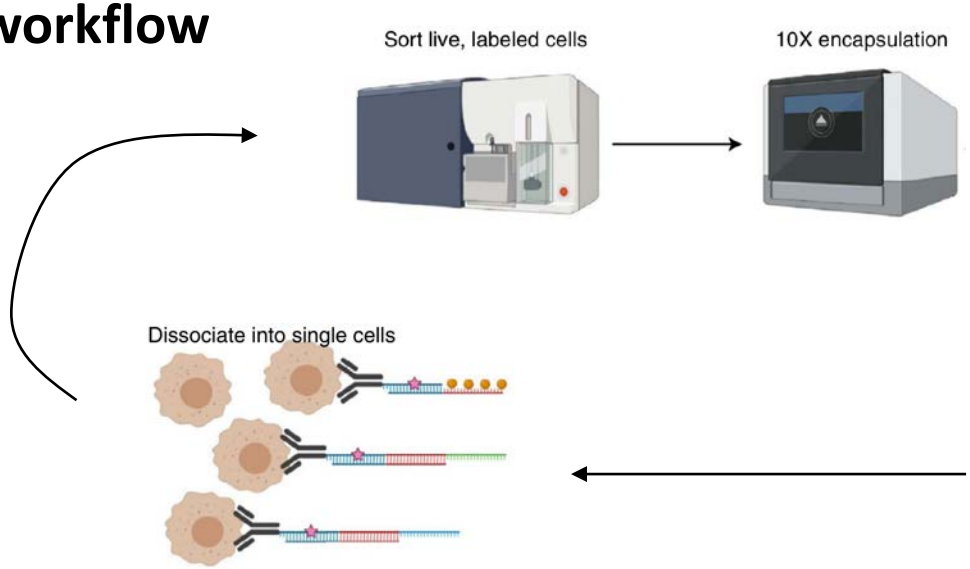
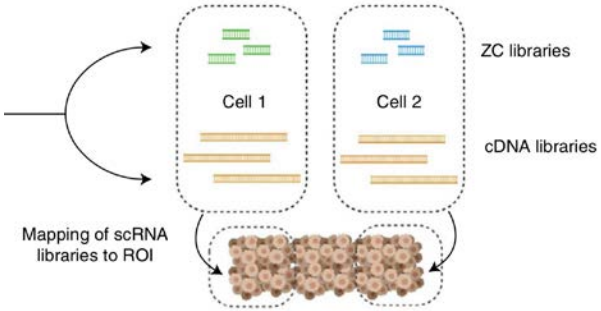


Imaging and photo-uncaging of sample

workflow





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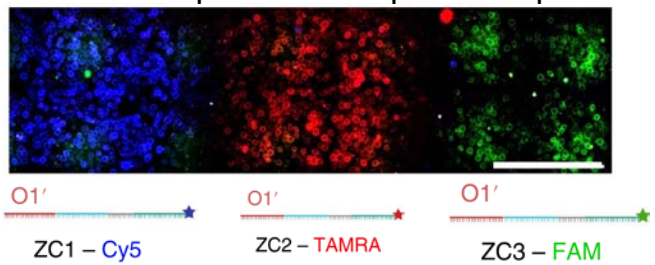
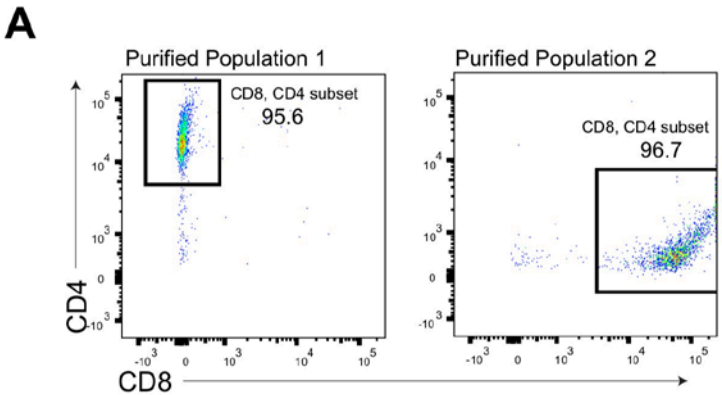
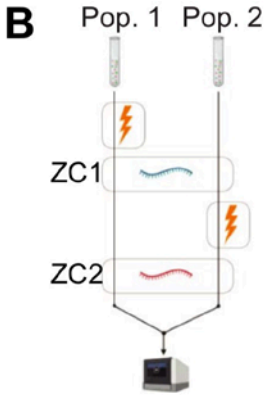


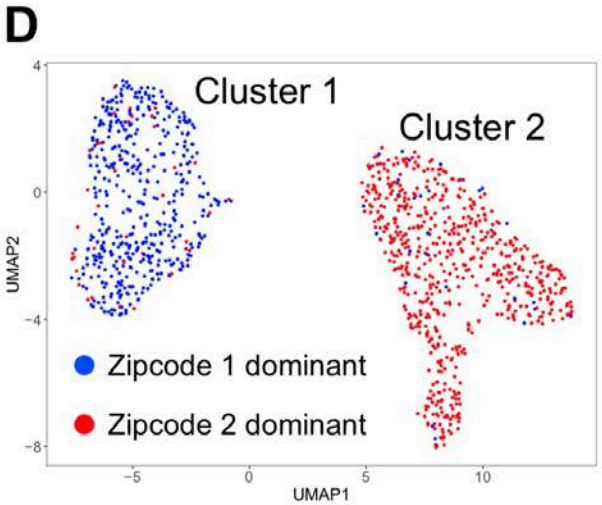
POC: distinguish 2 known populations



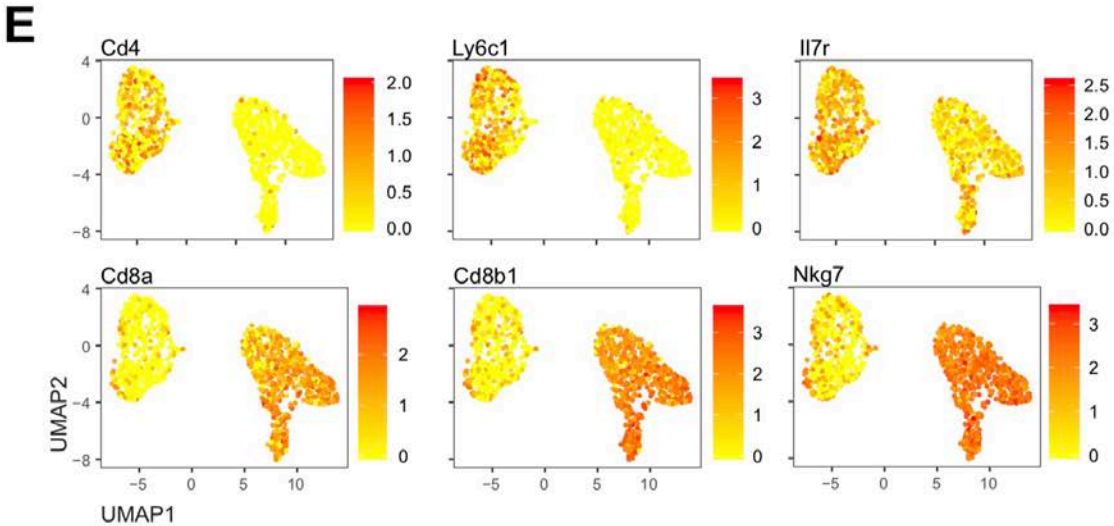
1. Separate CD4 from CD8 T-cells



2. Label both populations with ZipSeq



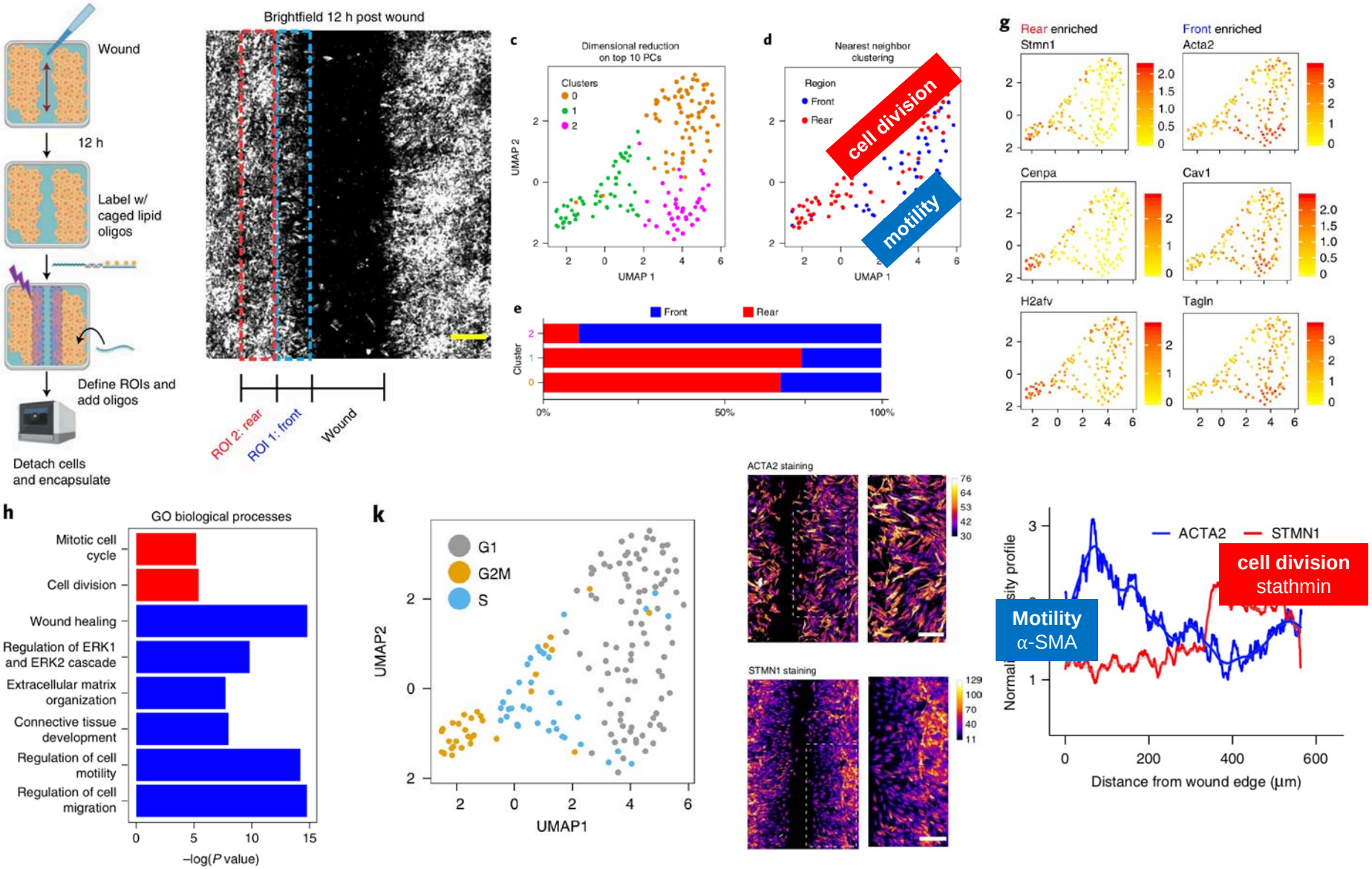
3. Clustering of labeled populations



4. Overlay of normalized gene expression levels for selected genes that define CD4 and CD8 T cell populations

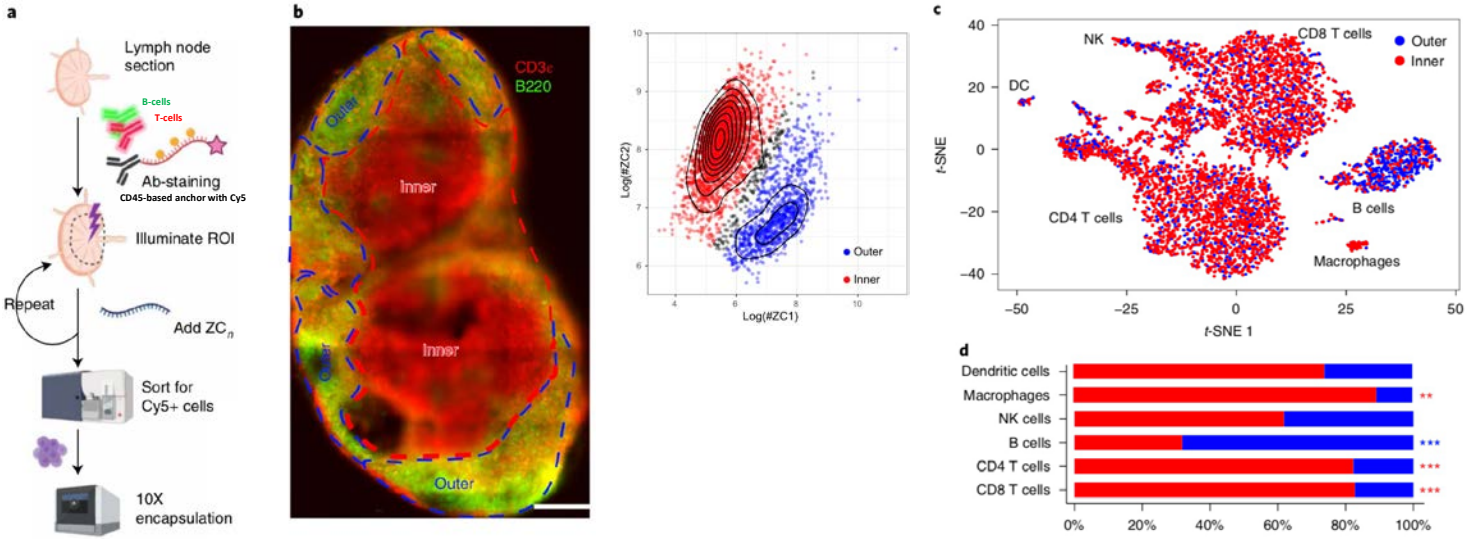
Applications of ZipSeq 1/4: wound healing

Defining spatially segregated motility and cell division in wound healing:



Applications of ZipSeq 2/4: LN-mapping

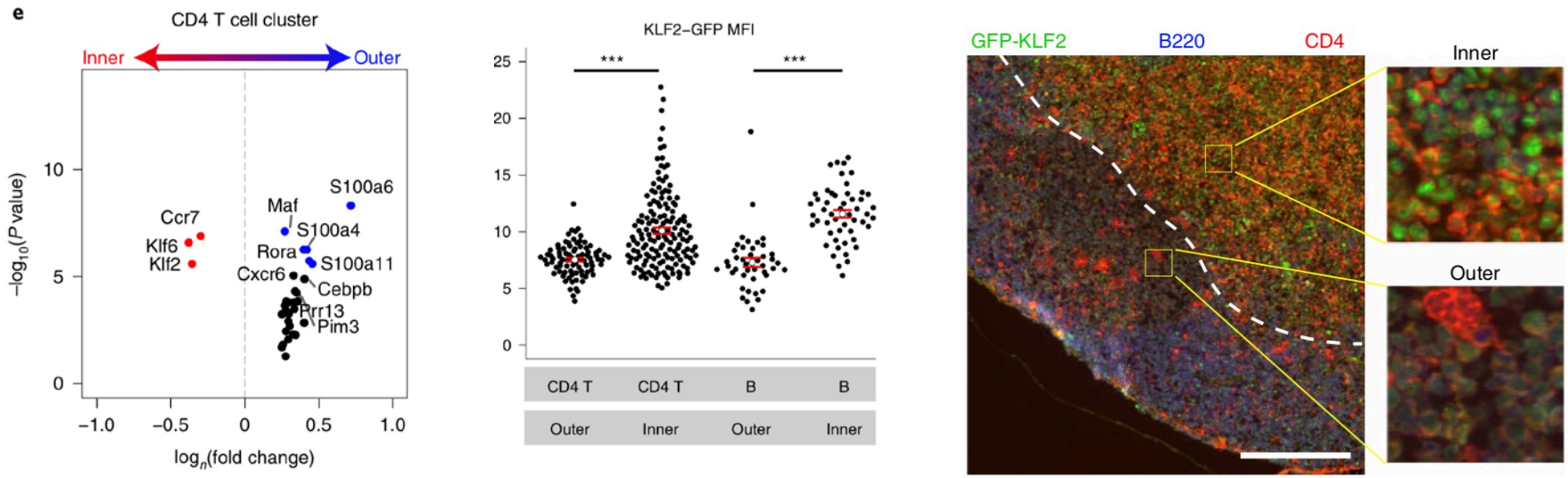
Mapping cortex vs. medulla in a lymph node:



Identification of main immune cell populations using marker genes

overlayed with zipcode entity

Enrichement of "outer" cells in B-cell cluster

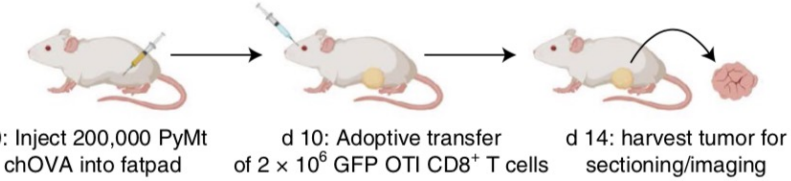


Klf2: gene and protein enrichement in „inner zone" in B- and T-cells

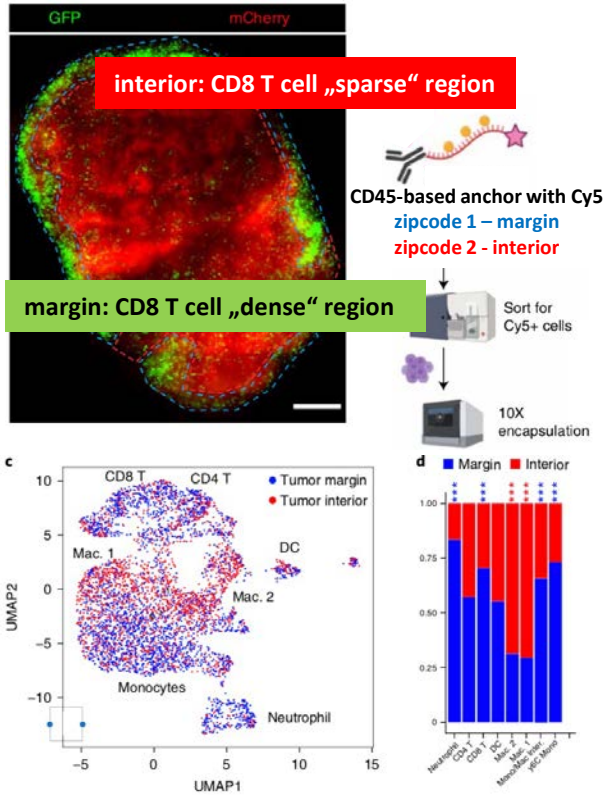
Applications of ZipSeq 3/4: tumor-infiltration

Immune cell differentiation in relation to position within tumor:

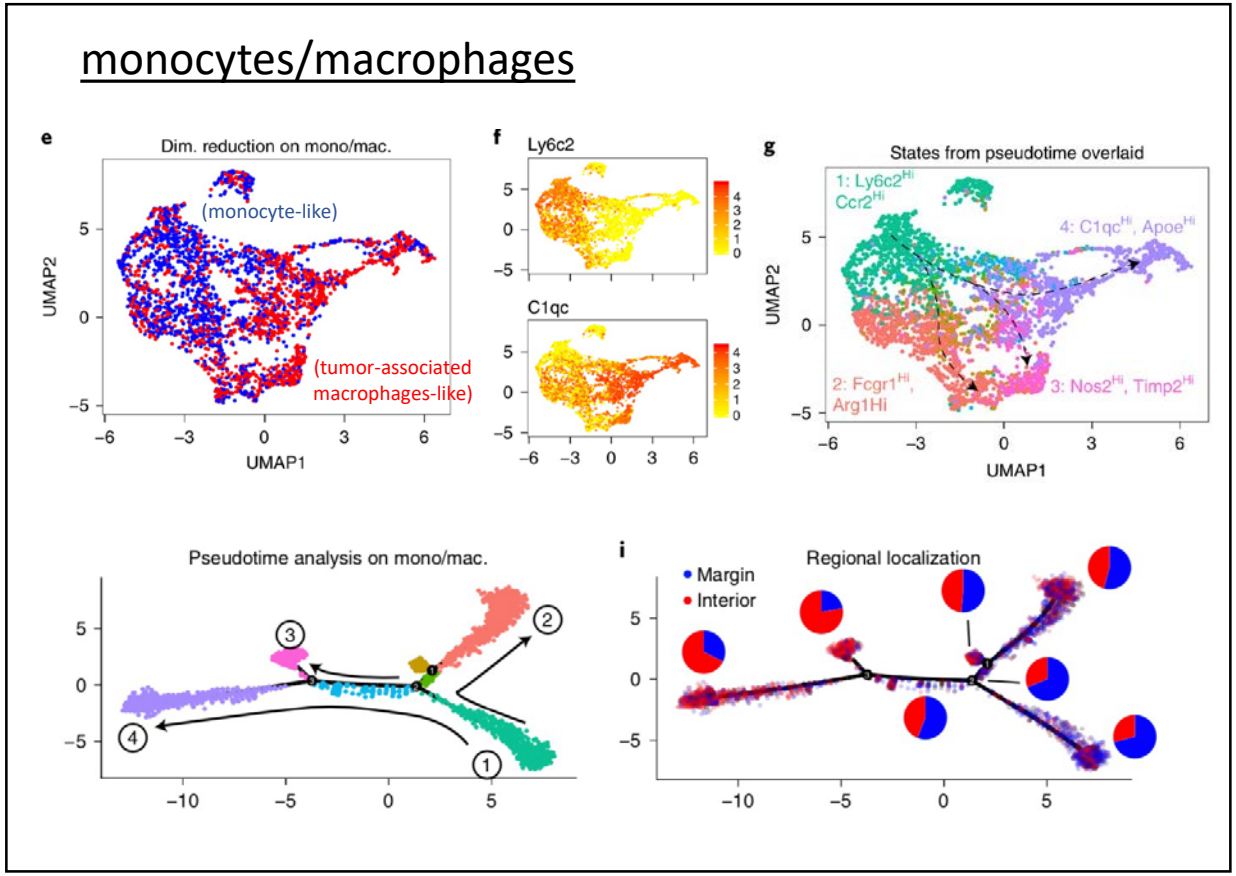
d 0 – inject PyMt-chOVA mouse breast cancer cells:
mCherry and ovalbumin coexpressed under MMTV promoter, along with Polyoma middle T antigen



d 10 – transfer of CD8+ T-cells:
Isolated from an OTI UBC-GFP mouse



Lym/neutro: skewed toward marginal region
Macrophages: skewed toward the interior



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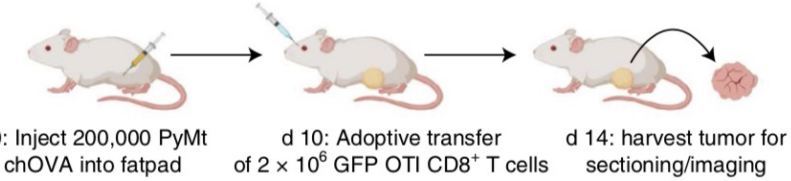

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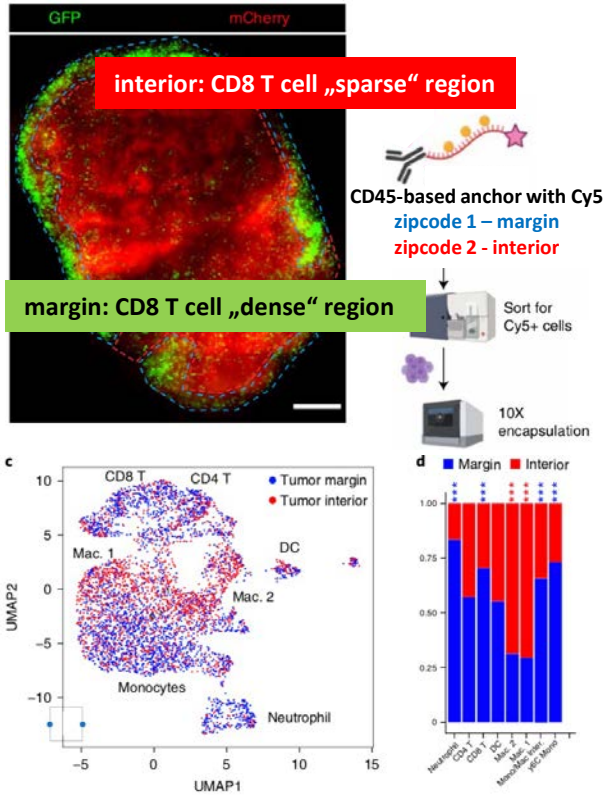
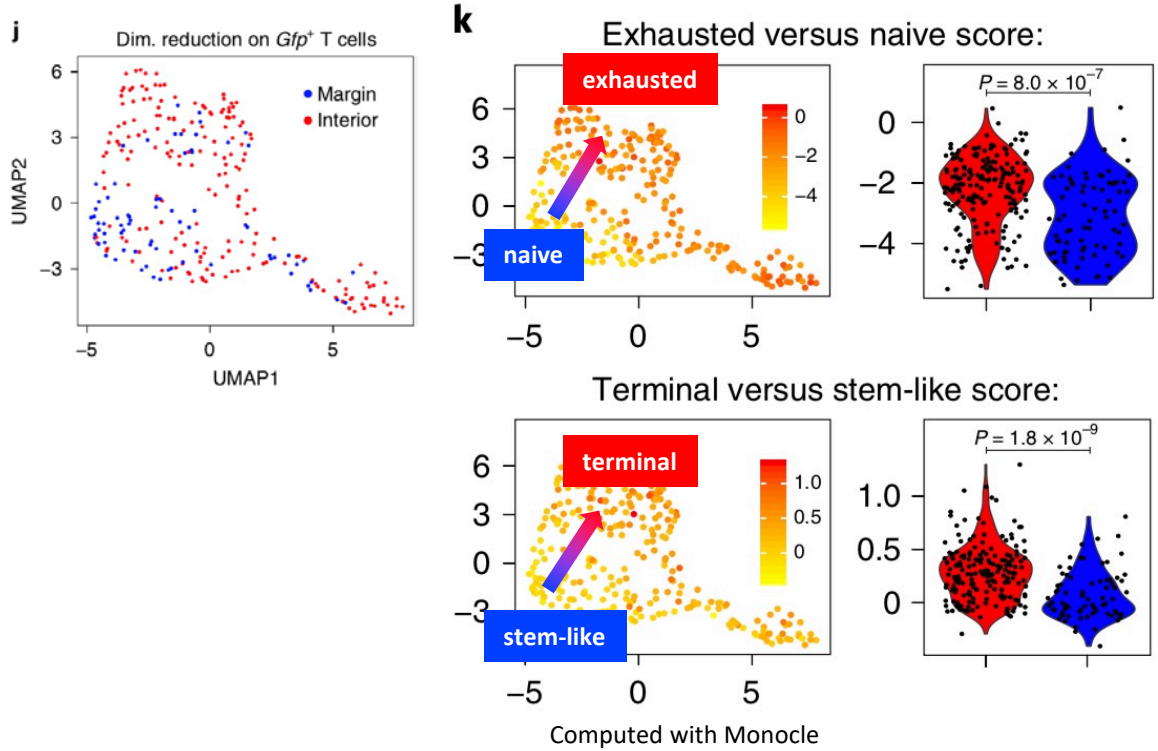


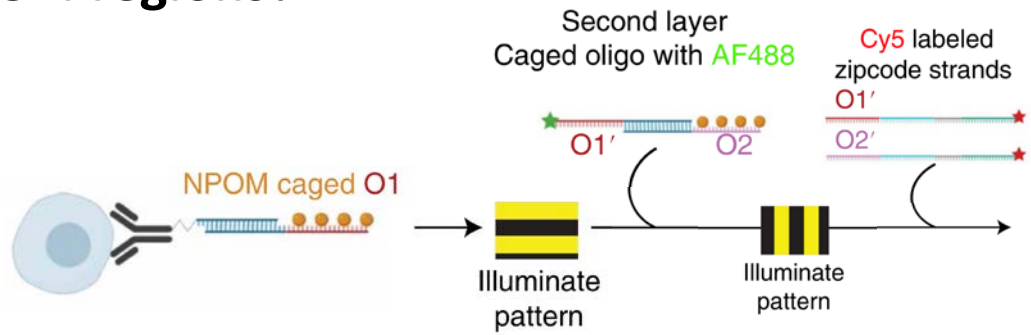
<u>Lym/neutro:</u> skewed toward marginal region
<u>Macrophages:</u> skewed toward the interior

<u>OTI T cells (Gfp-expressing):</u>

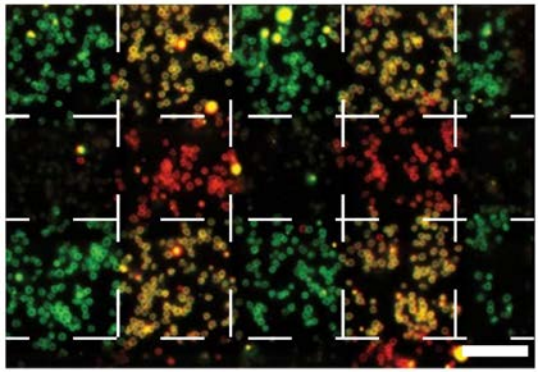


Can we define more than 2 regions?

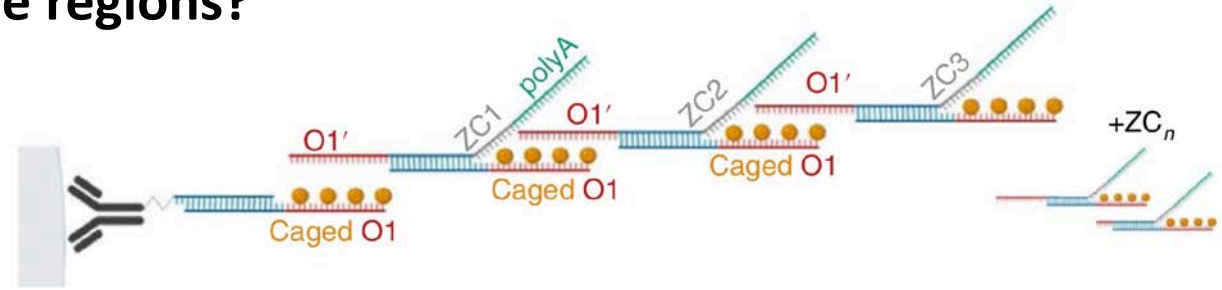
3-4 regions?

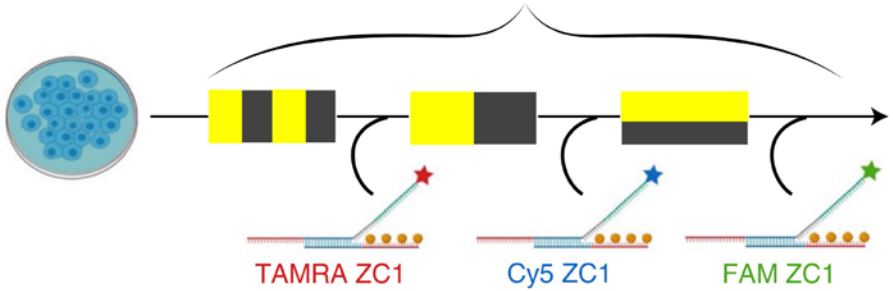


Four regions defined

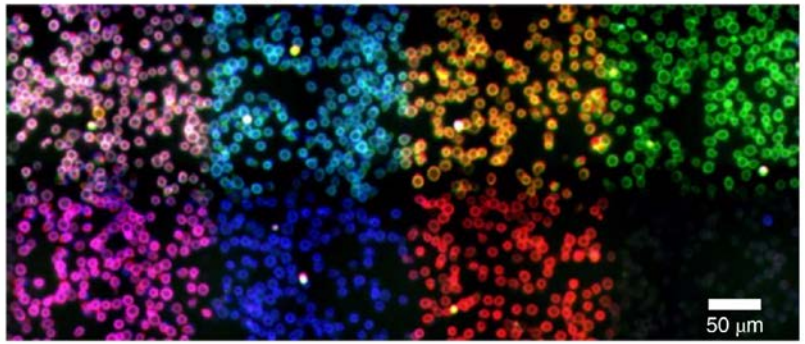


Even more regions?



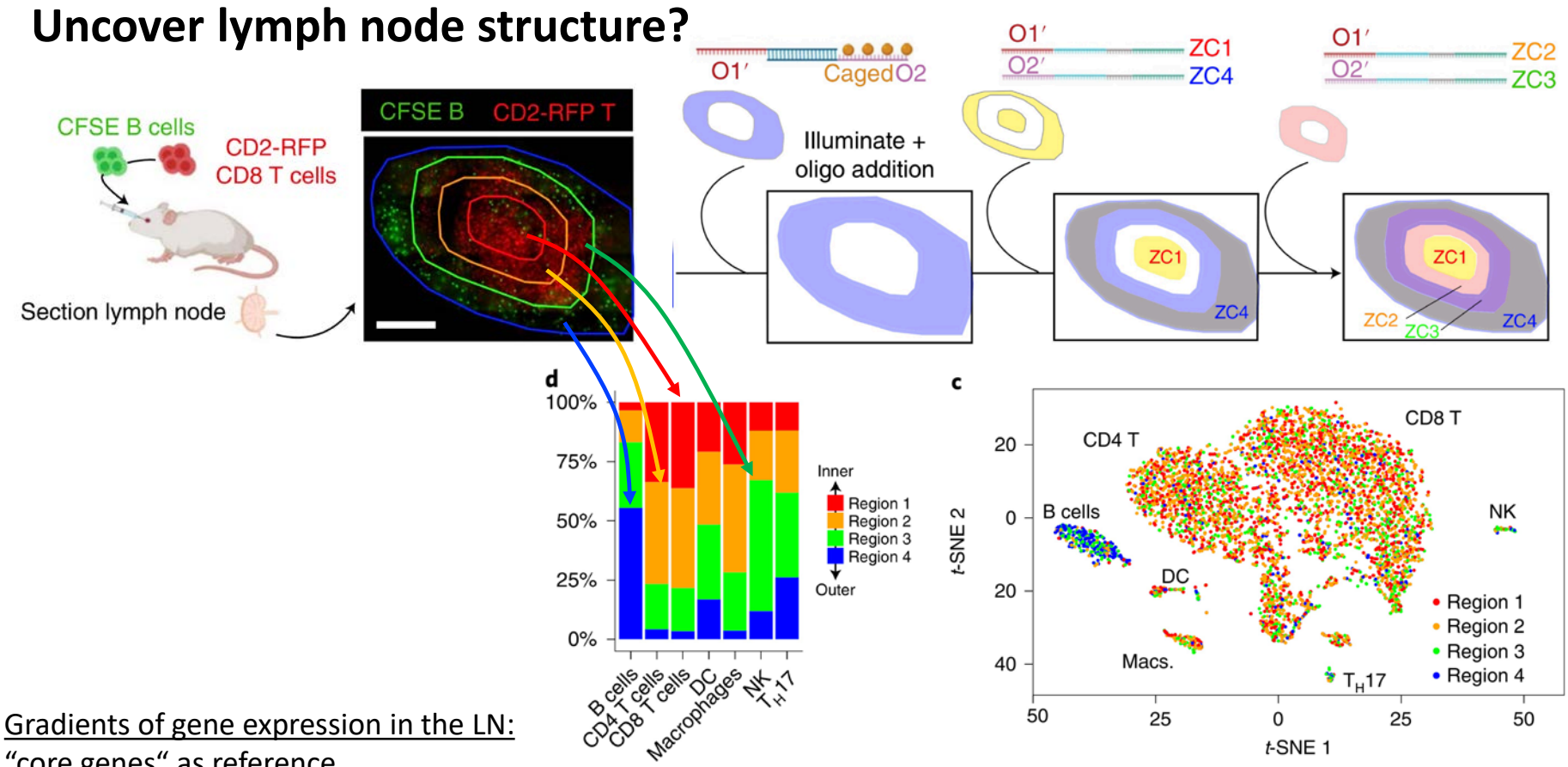


Eight regions defined

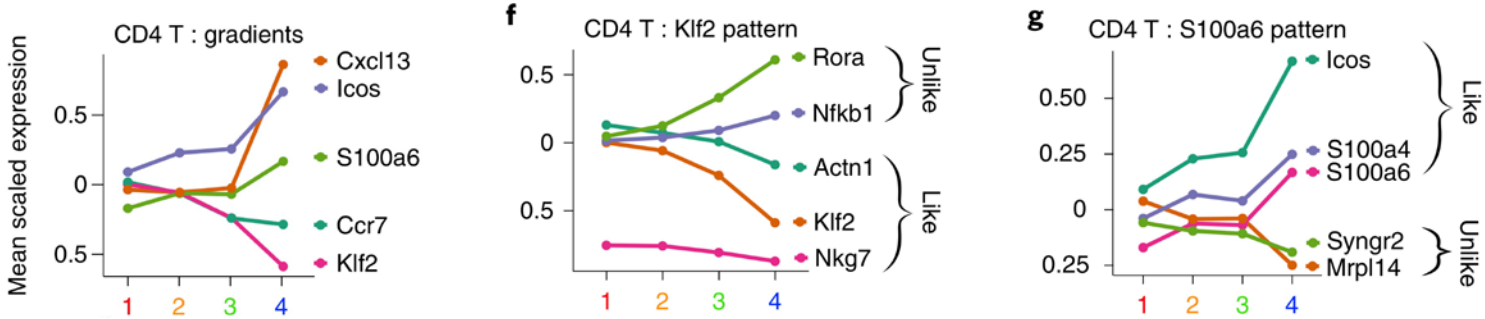


Applications of ZipSeq 4/4: spatial patterns in LN

Uncover lymph node structure?



Gradients of gene expression in the LN:
"core genes" as reference



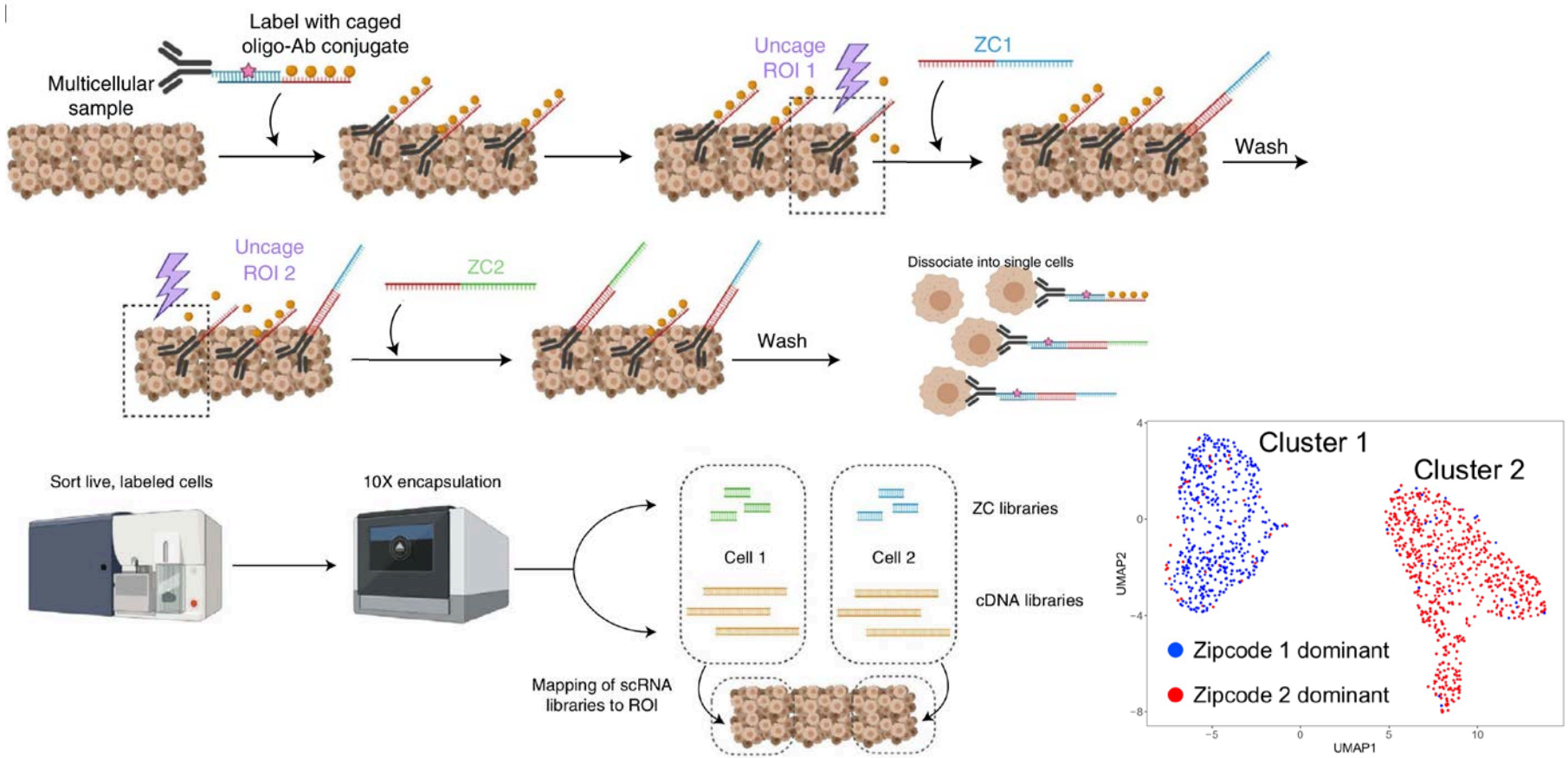
Summary ZipSeq-barcoding

“Printing“ a DNA barcode onto live cells in a spatially defined manner, which…

→ …can be read out during typical scRNA-seq workflow

→ …is compatible with many other scRNA-seq methodologies

→ …requires only caged oligonucleotides and a photo-patterning module



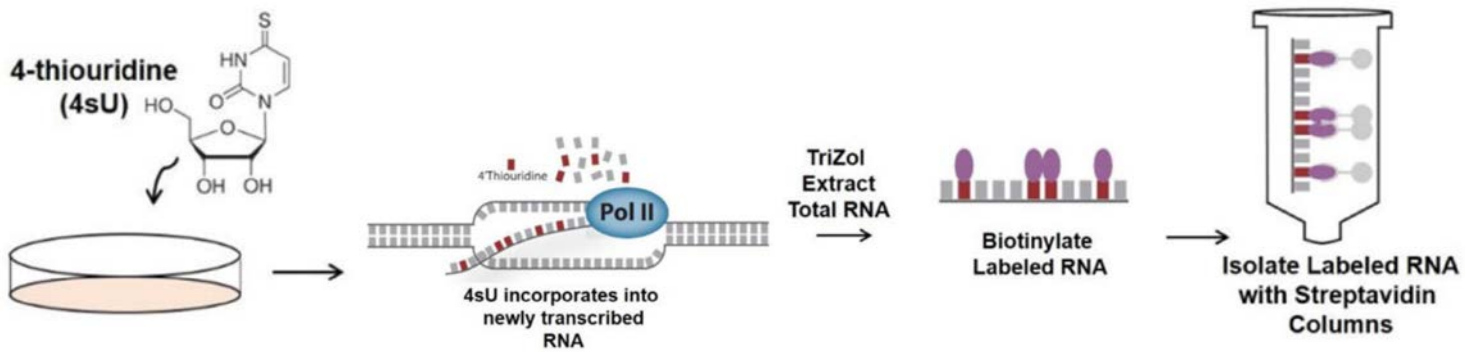
Temporally resolved scRNAseq

Background: “old way“ to detect new RNA

Most methods: RNA transcript abundance at a steady-state level, providing only a snapshot of the cellular state. Are changes in gene expression due to…:

- Rate of transcription?
- Rate of degradation?
- Both?

<u>4sU metabolic labeling and isolation of newly transcribed RNA:</u>



Rabani et al.: Metabolic labeling of RNA uncovers principles of RNA production and degradation dynamics in mammalian cells. Nature Biotechnology, 2011; 29: 436-442.
Garibaldi et al.: Isolation of Newly Transcribed RNA Using the Metabolic Label 4-thiouridine. Methods Mol Biol., 2017; 1648: 169-176.

Unresolving temporal dynamics using scRNA-seq

Problem:

Standard scRNA-seq methods capture a mixture of newly transcribed ('new') and pre-existing ('old') RNAs without being able to temporally resolve RNA dynamics.

Commonly used approaches to resolve temporal dynamics rely on RNA labelling with exogenous nucleosides (e.g. 4sU) and biochemical enrichement of labeled RNAs.

Smart-seq/plate-based methods are costly and time-consuming.

Massively parallel and time-resolved RNA sequencing in single cells with scNT-seq

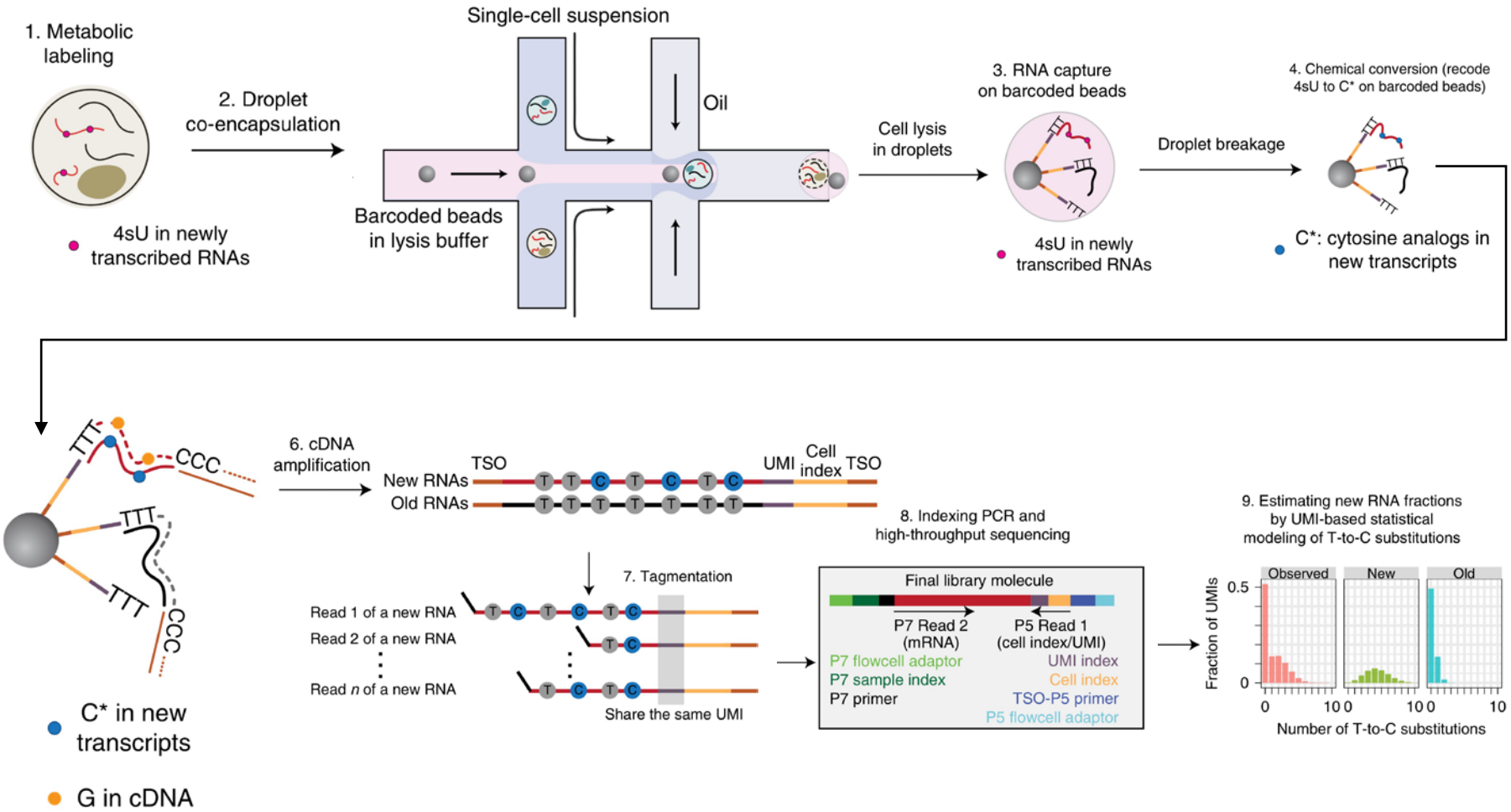
Qi Qiu[1,2,3,7], Peng Hu[1,2,3,7], Xiaojie Qiu[4,6], Kiya W. Govek[1,5], Pablo G. Cámara[1,5] and Hao Wu[1,2,3] ✉

Solution:

With single-cell metabolically labeled new RNA tagging sequencing (scNT-seq), newly transcribed and pre-existing mRNAs from the same cell can be distinguished.

Development and validation of scNT-seq

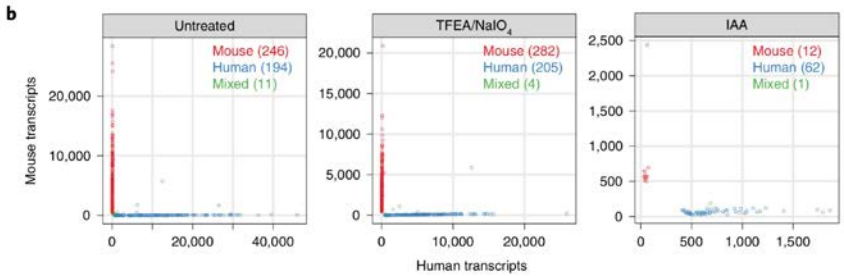
<u>Drop-seq platform:</u> unique barcoded bead design, immobilization of mRNAs → on-bead chemical conversion reactions and UMI-based sRNA-seq analysis.



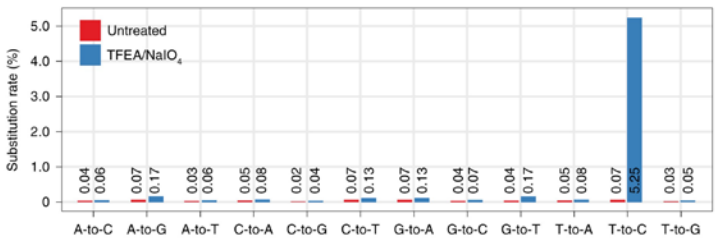
Development and validation of scNT-seq

Chemical converting of 4sU to a cytidine analog:

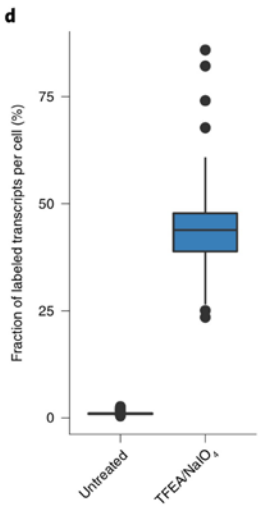
SLAM-seq: iodoacetamide (IAA)-based vs. TimeLapse: 2,2,2-trifluoroethylamine (TFEA)/sodium periodate ($NaIO_4$)-based reaction



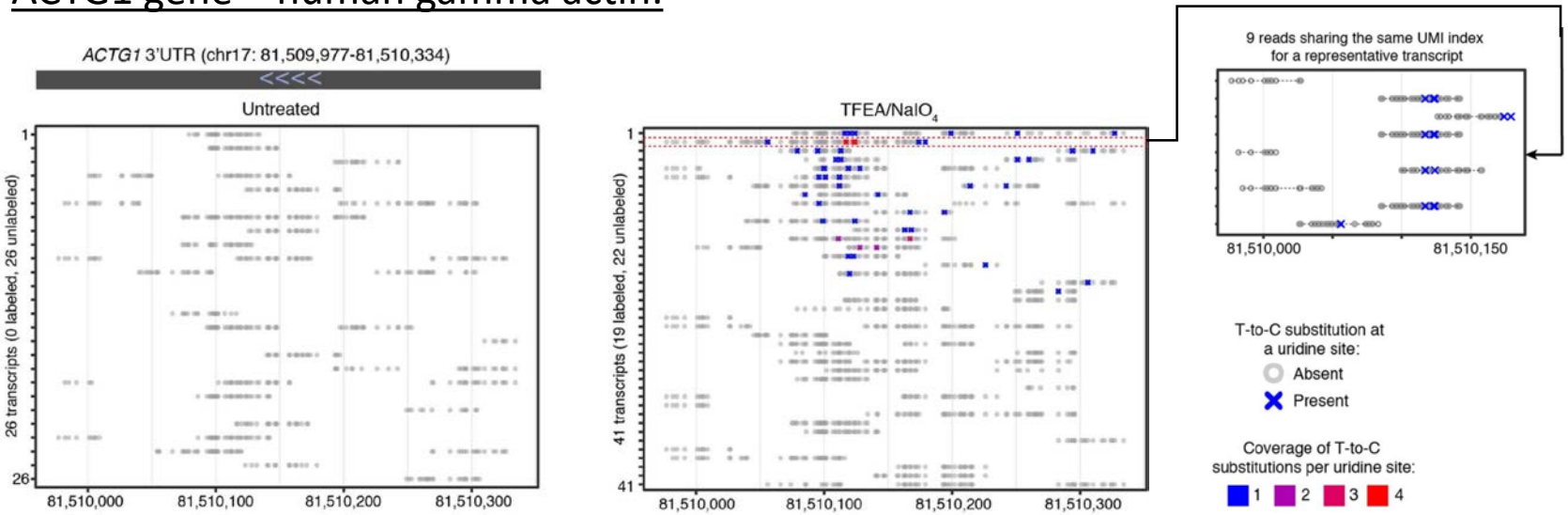
TFEA/NaIO4-based reaction outperforms IAA-based reactions



Nucleotide substitutions rates of 4sU-labeled cells (works on freshly isolated and cryo-preserved cells)



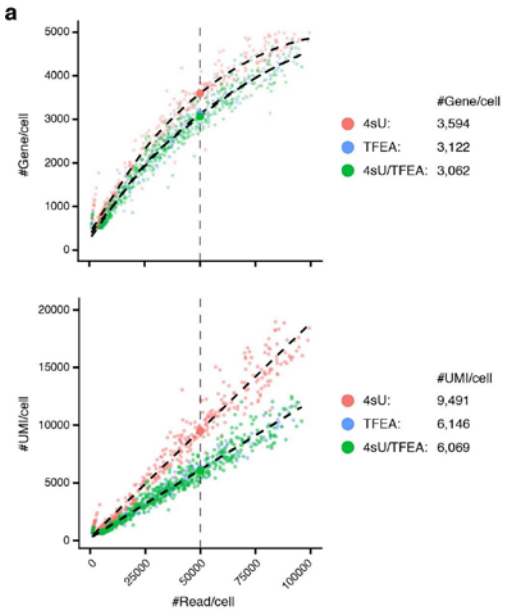
ACTG1 gene – human gamma actin:



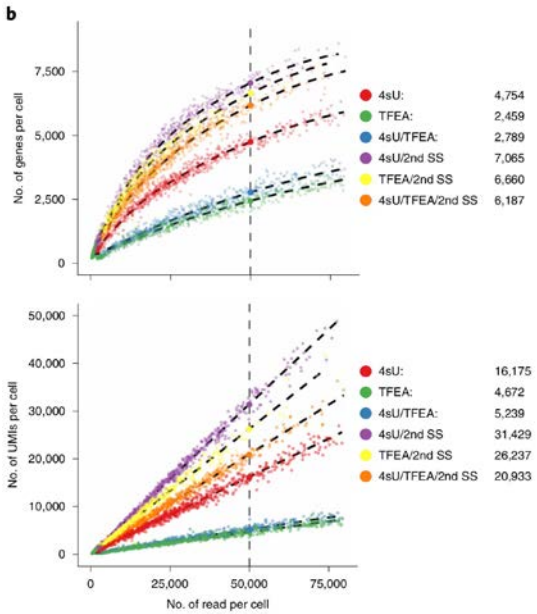
→ **Feasibility of detecting metabolically labeled new transcripts at single-cell level**

SS-synthesis reaction enhances the efficiency

Problem

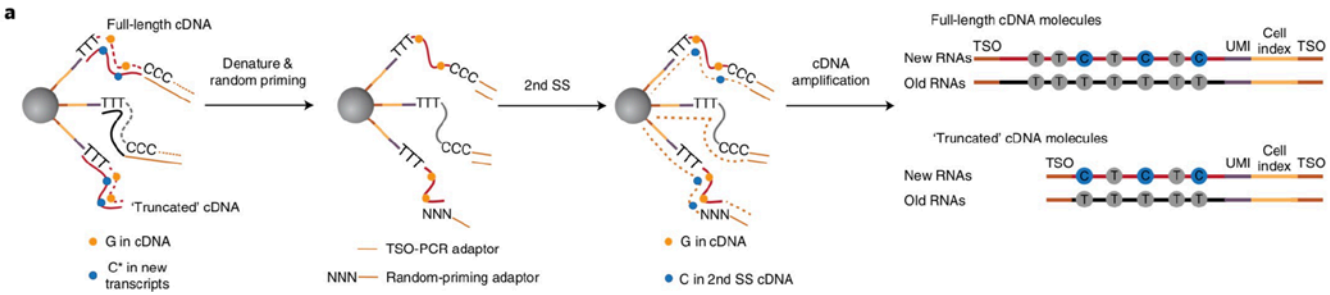


TFEA/$NaIO_4$ treatment may increase the failure rate of generating full-length cDNAs („truncated“) cDNAS during reverse transcription, which is required for the „template-switching“ reaction to add the second PCR handle for cDNA amplification.



Solution: Second-strand synthesis reaction

Development of a random- primed second-strand synthesis (2nd SS) reaction to recover truncated cDNA and

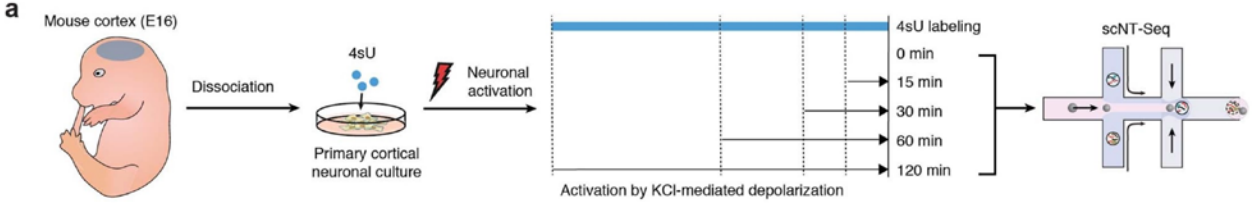


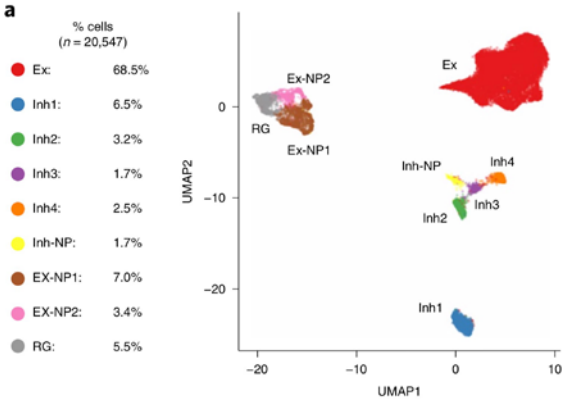
Applications of scNT-seq 1/3: activity-induced RNA

scNT-seq performance for detecting activity-induced new RNAs:

Defining patterns of activity-regulated genes (ARGs)

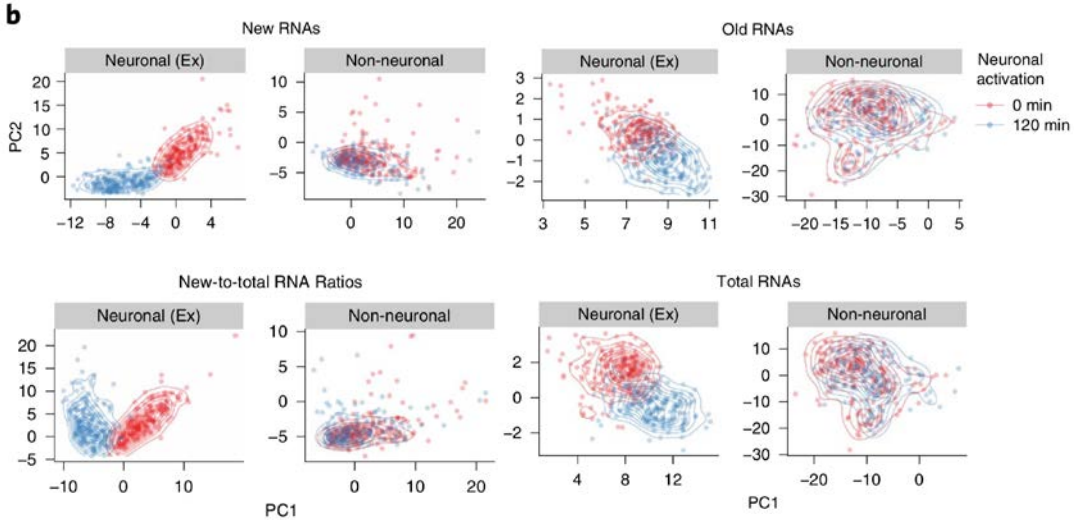
1. Experimental setup

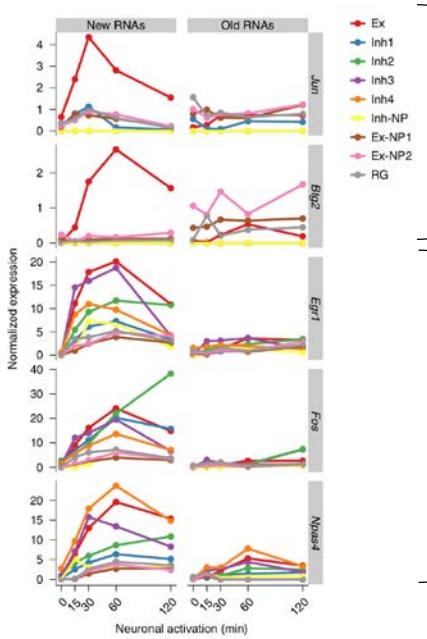




2. Identification of all major celltypes expected in embryonic mouse cortex

3. PCA of highly variable genes





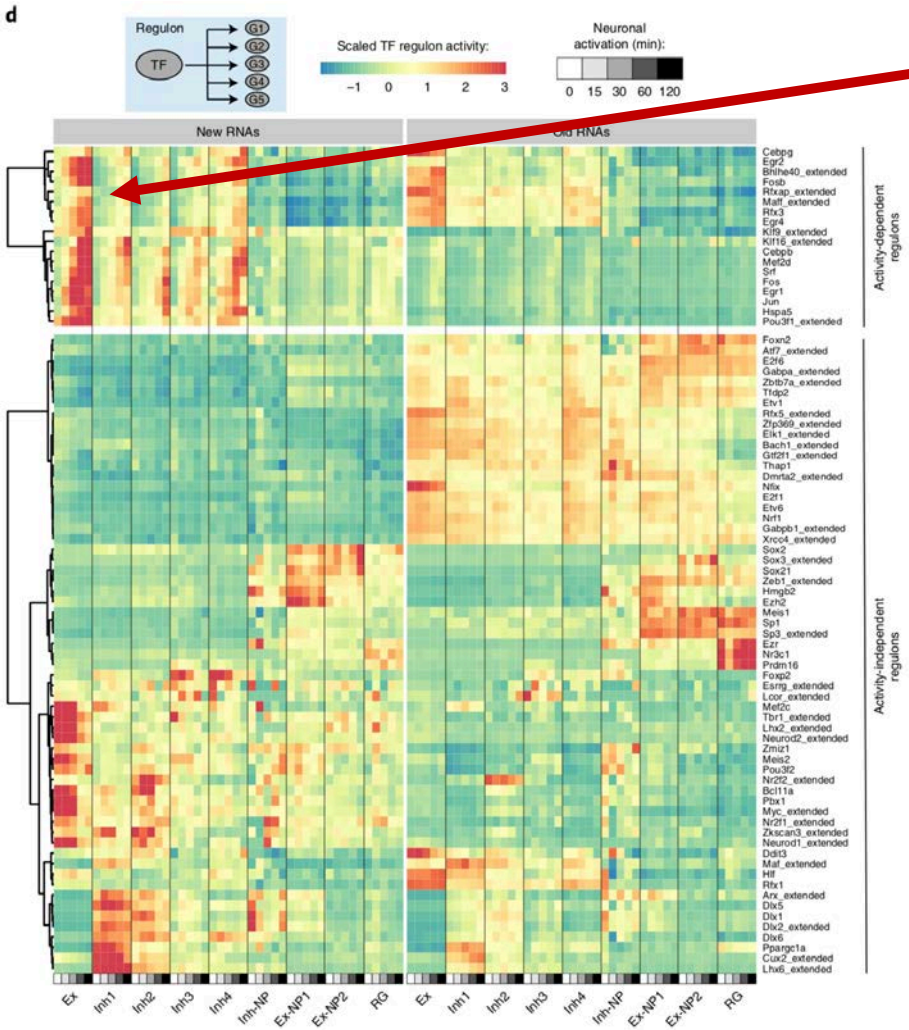
Specific induction of excitatory neurons

Induction in many cell types

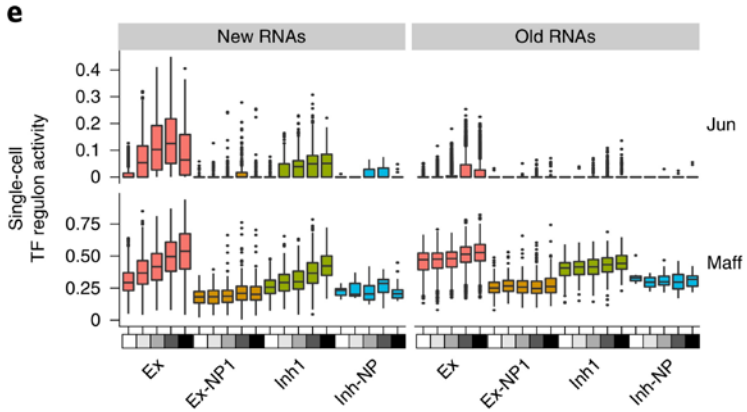
Separation of **activity-induced** from **resting state excitatory neurons**

Applications of scNT-seq 1/3: activity-induced RNA

Regulon-activity of transcription factors (TF):
Parallel analysis of dynamic regulons (external stimuli) and stable regulons (related to cellular identities)

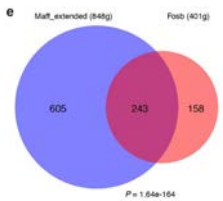


18 regulons showed significant changes in response to neuronal activity patterns



Jun: activity dependant increase in Jun-activity

Maff: not previously implicated in neuronal activation, associated with activity-dependant but also –independant regulon activities.

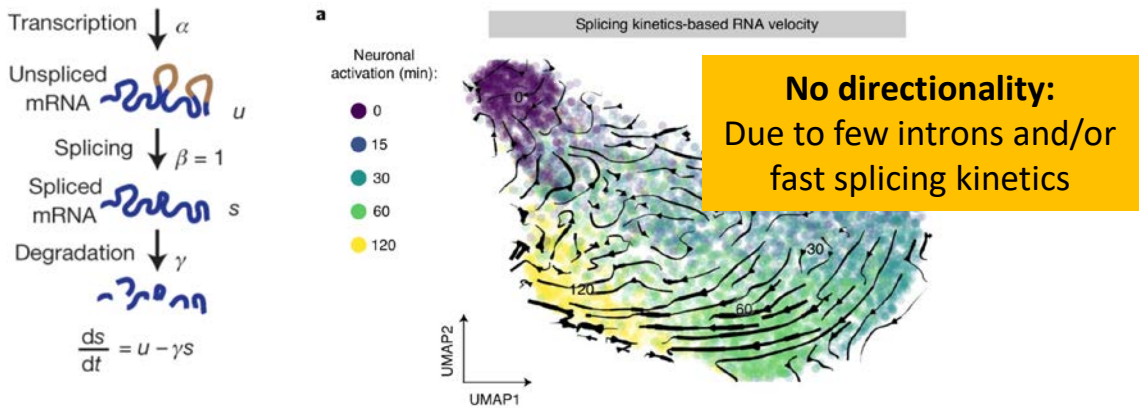


Maff targets overlap with those of Fosb. Maff is functionally related to neuron projection and synapse

Applications of scNT-seq 2/3: velocity analysis

Metabolic labeling-based time-resolved RNA velocity analysis:

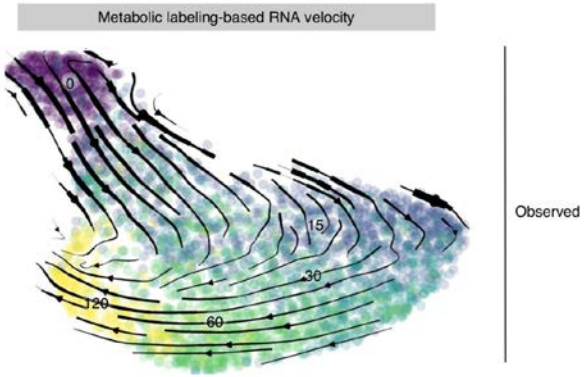
Estimating RNA velocity by spliced vs. unspliced mRNA



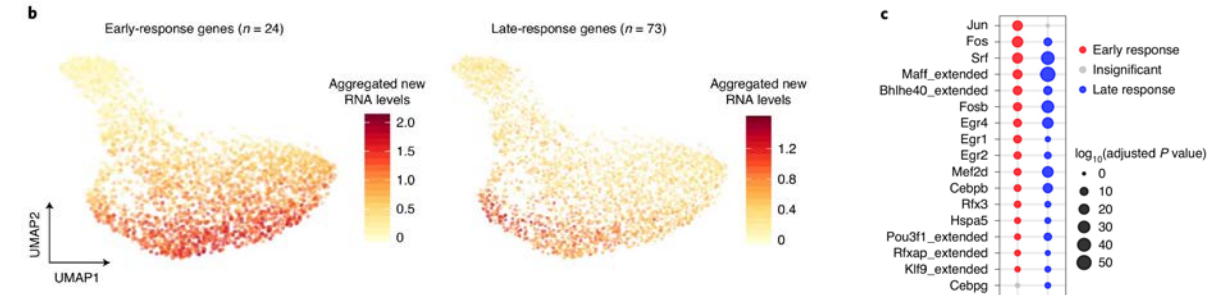
No directionality:
Due to few introns and/or fast splicing kinetics

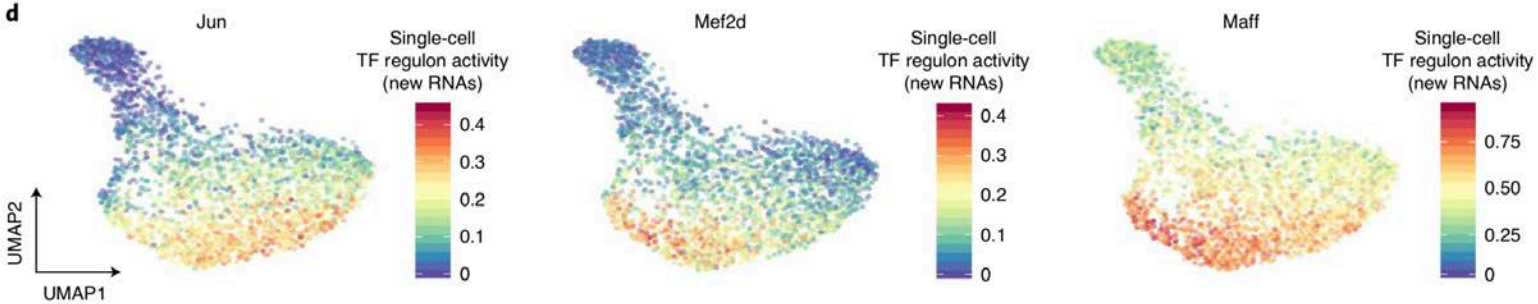
La Manno et al.: RNA velocity of single cells. Nature, 2018. 560. 494-498.

Metabolic labeling is independent (!) of gene structure



Directionality:
"Dynamo", models metabolic labeling-based scRNA-seq. Calculates time-resolved RNA velocity

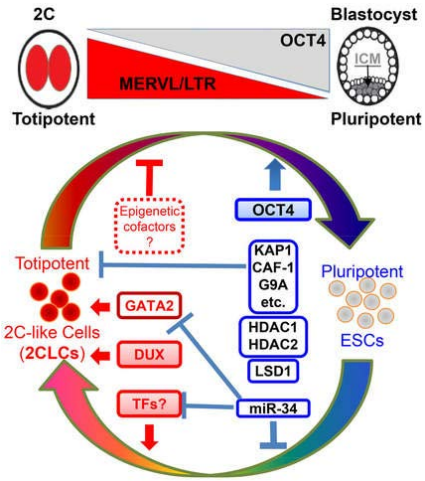




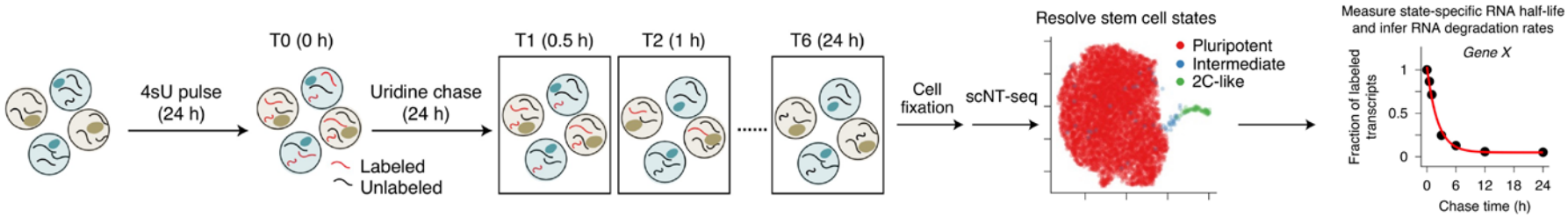
Applications of scNT-seq 3/3: RNA regulation

DNA regulatory strategies during stem cell-state transition:

All ES cells cycle in and out of a priviliged, totipotent state, resembling 2C-like ES cells. Those totipotent cells arise spontaneously in pluripotent mESC cultures. But 2C-like cells are rare (<1%) and it remains unclear, how regulation of RNA synthesis and degradation contributes to conversion of these cell states.

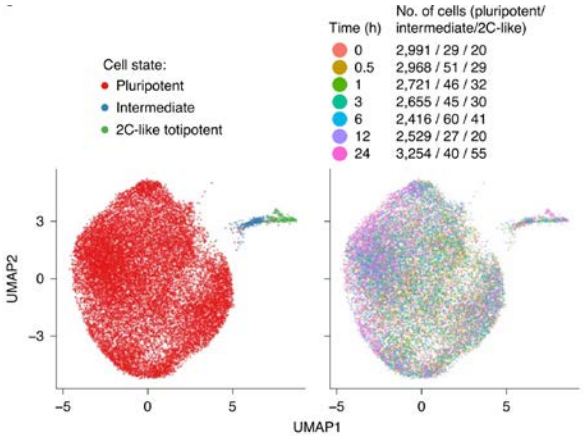


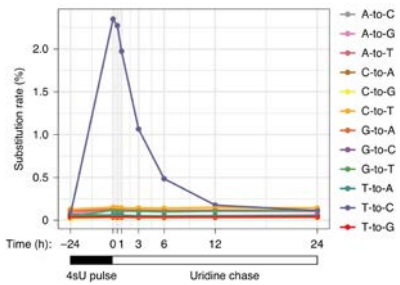
1. WT mESCs were labeled with 4sU **2. Collecting seven time points**



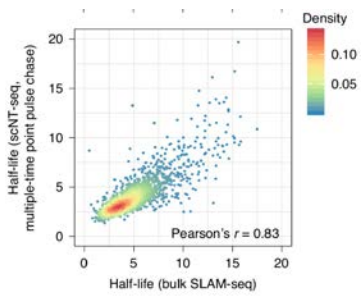
1. Determine state-specific mRNA degradation rates

Applications of scNT-seq 3/3: RNA regulation

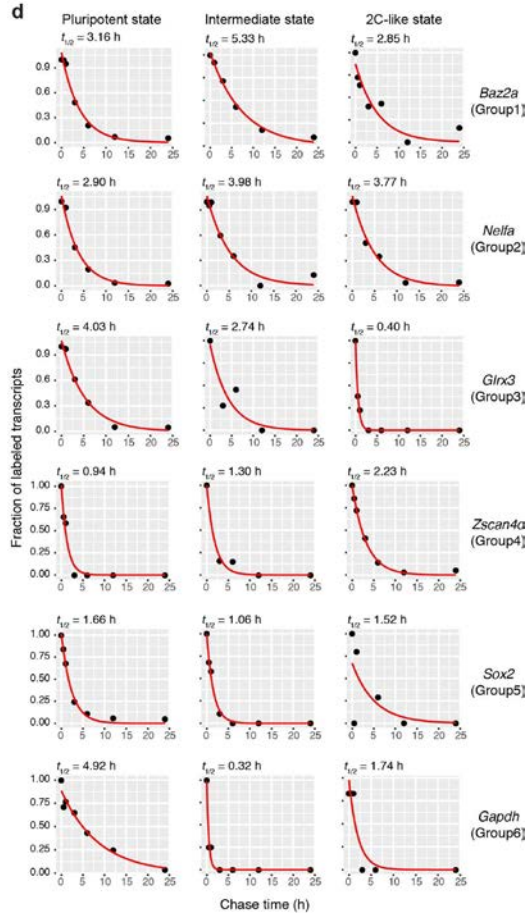


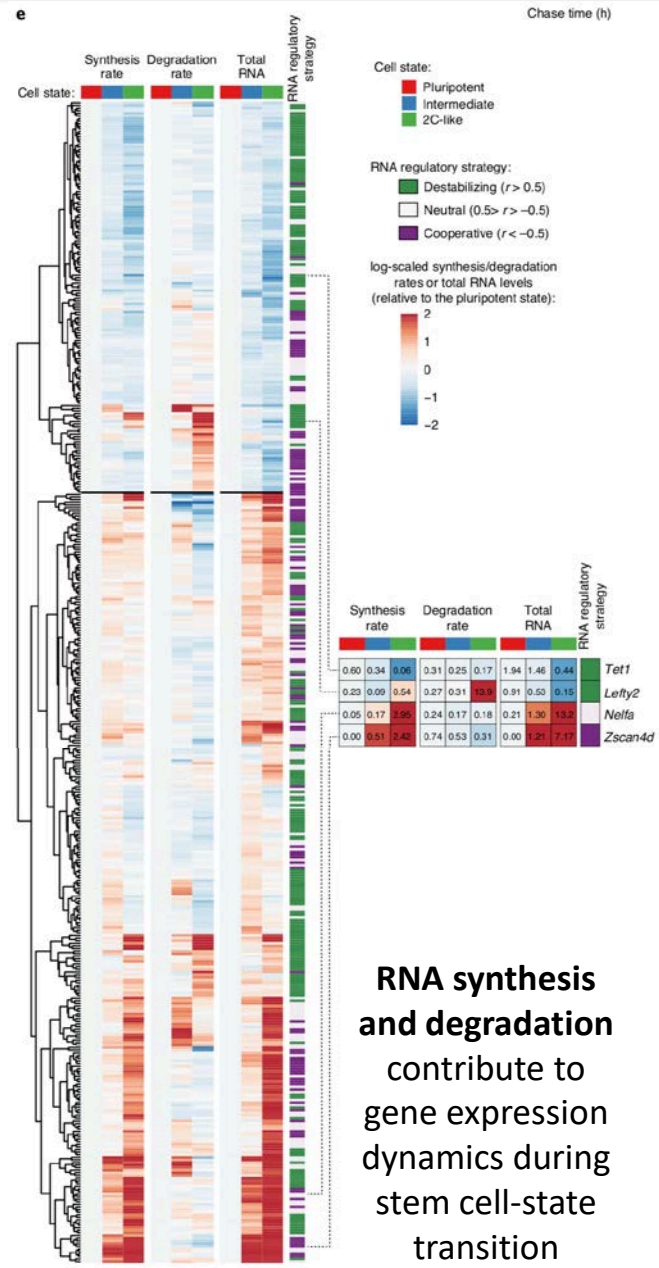


accumulation of T-to-C substitutions after 24hs and decrease to baseline after chase



RNA half-life determined by scNT-seq is concordant with bulk SLAM-seq assays





RNA synthesis and degradation contribute to gene expression dynamics during stem cell-state transition

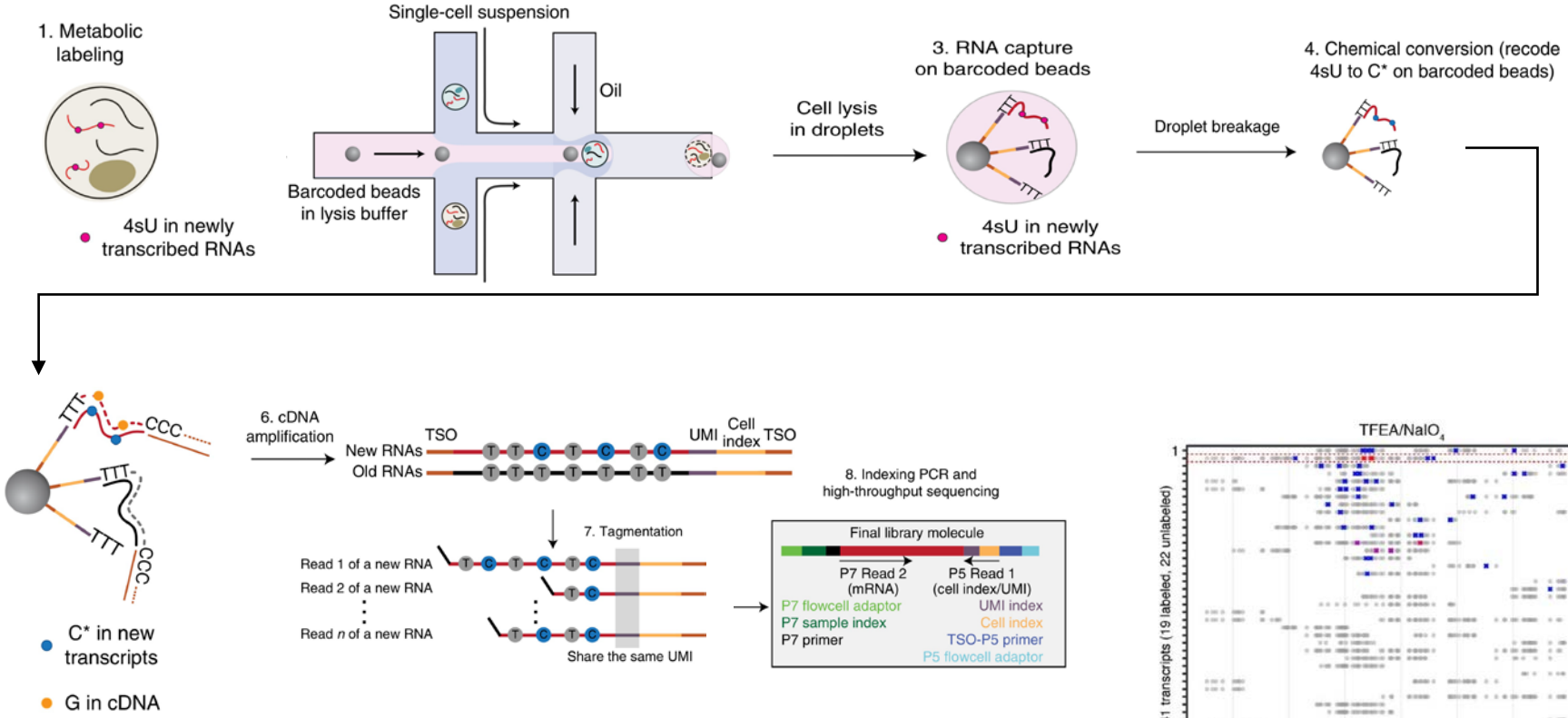
Summary scNT-seq

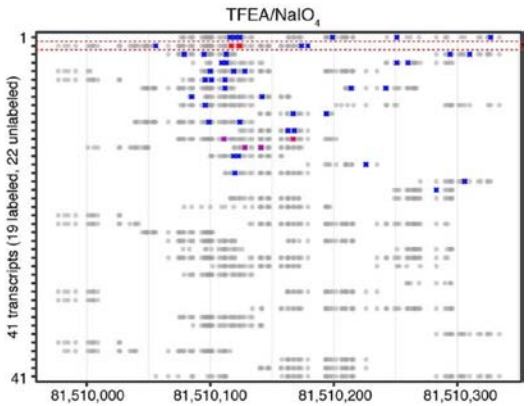
Detects „new“ (metabolically 4sU-labeled) RNA at single-cell level, what…:

→ …can be performed using the Drop-seq platform and TimeLapse chemistry

→ …is faster/cheaper compared to Smart-seq/plate-based methods

→ …allows accurate quantification of new transcript levels due to use of UMIs





Thank you for your attention!