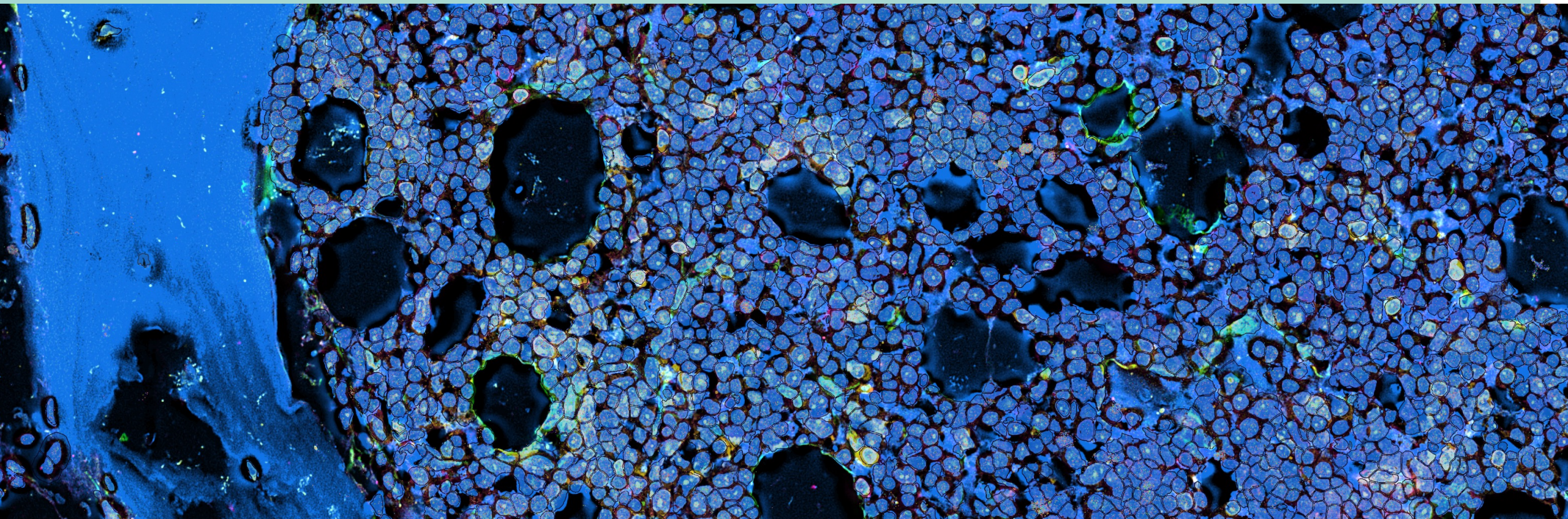
University of Basel

Cell segmentation for spatial transcriptomics (ST)

Ivan Berest, 03.06.2026



Outline

1	Intro to the cell segmentation with ST
2	Imaging-based ST segmentation methods
3	Sequencing-based ST segmentation methods
4	Spillover/bleeding challenge
5	Analysis example

Why location matters: from bulk to spatial

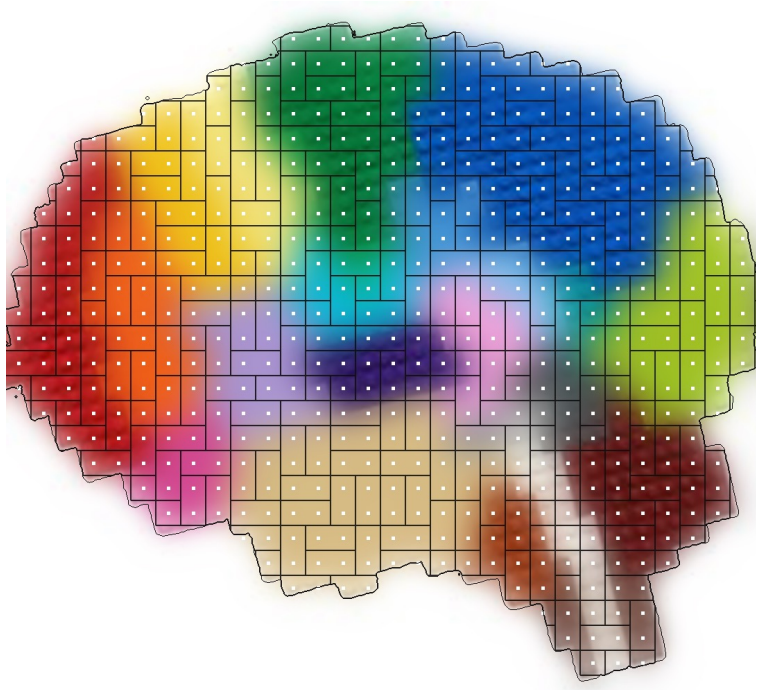
bulk omics

single-cell omics

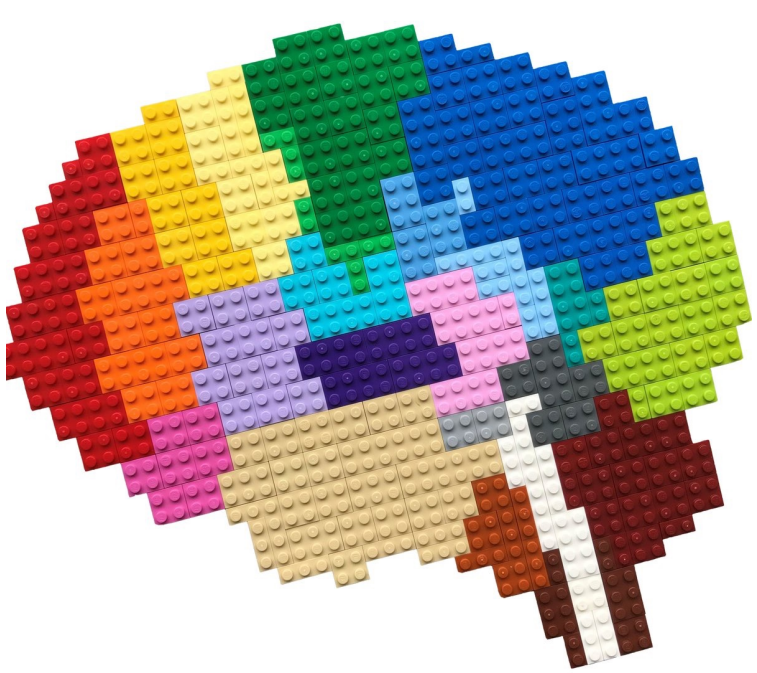
spatial omics

Segmentation is necessary — and never perfect

usual input

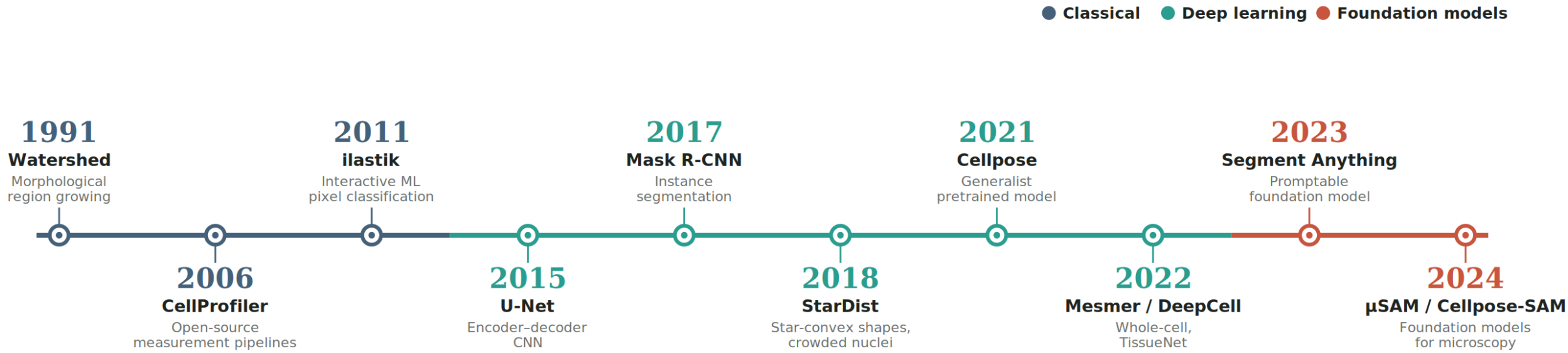


cell segmentation



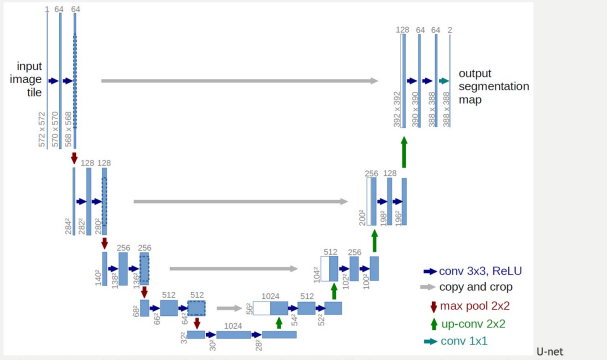
real scenario

Cell segmentation is not new concept: whole era in imaging!



U-Net: Convolutional Networks for Biomedical Image Segmentation

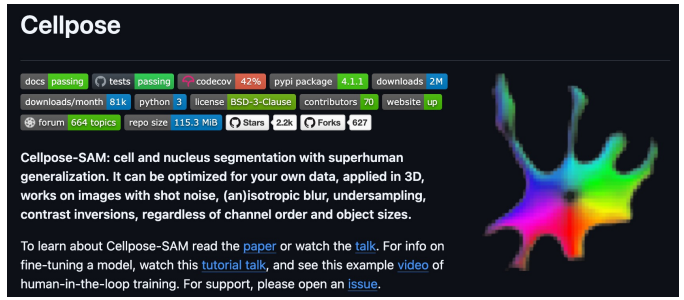
The u-net is convolutional network architecture for fast and precise segmentation of images. Up to now it has outperformed the prior best method (a sliding-window convolutional network) on the ISBI challenge for segmentation of neuronal structures in electron microscopic stacks. It has won the Grand Challenge for Computer-Automated Detection of Caries in Bitewing Radiography at ISBI 2015, and it has won the Cell Tracking Challenge at ISBI 2015 on the two most challenging transmitted light microscopy categories (Phase contrast and DIC microscopy) by a large margin (See also our annoucement).



U-net architecture (example for 32x32 pixels in the lowest resolution). Each blue box corresponds to a multi-channel feature map. The number of channels is denoted on top of the box. The x-y-size is provided at the lower left edge of the box. White boxes represent copied feature maps. The arrows denote the different operations.

2018 Data Science Bowl

Find the nuclei in divergent images to advance medical discovery



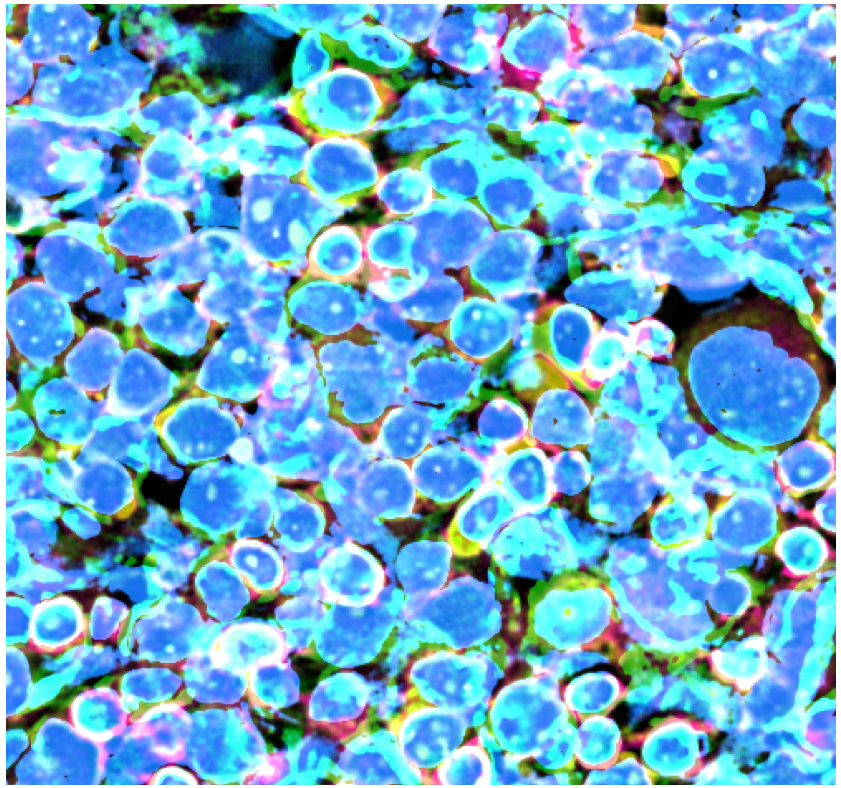
Article | Open access | Published: 08 December 2025

CellSAM: a foundation model for cell segmentation

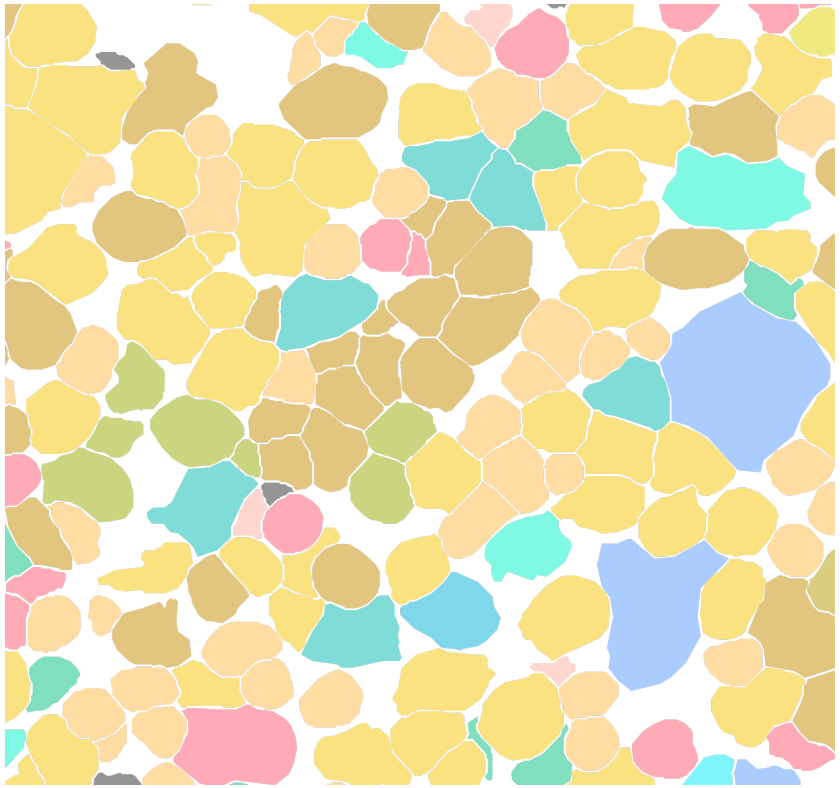
Markus Marks, Uriah Israel, Rohit Dilip, Qilin Li, Changhua Yu, Emily Laubscher, Ahamed Iqbal, Elora Pradhan, Ada Ates, Martin Abt, Caitlin Brown, Edward Pao, Shenyi Li, Alexander Pearson-Goulart, Pietro Perona, Georgia Gkioxari, Ross Barnowski, Yisong Yue & David Van Valen

Nature Methods **22**, 2585–2593 (2025) | Cite this article

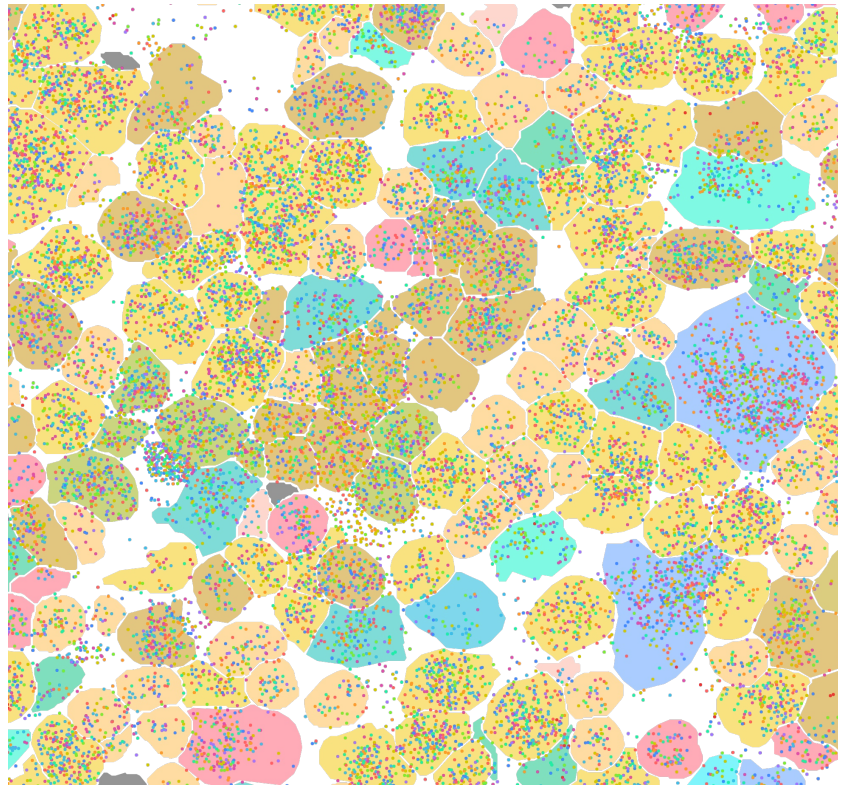
New challenges for cell segmentation with spatial transcriptomics



IF image



segmentation mask



overlaid transcripts

Transcripts for the cells can improve cell segmentation based only on the image?

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1	Intro to the cell segmentation with ST
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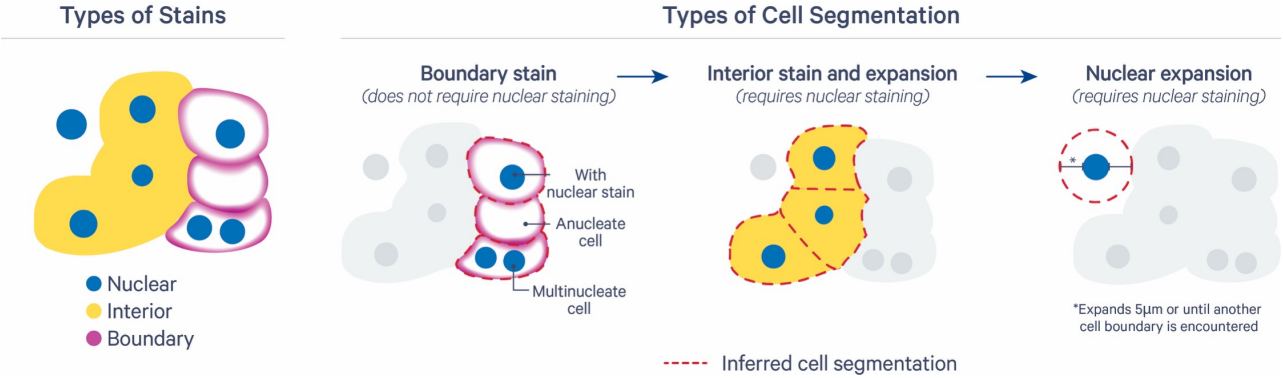
3	Sequencing-based ST segmentation methods
4	Spillover/bleeding challenge
5	Practical workflow

Xenium Ranger segmentation algorithms

Xenium onboard analysis (XOA) happening directly on the machine



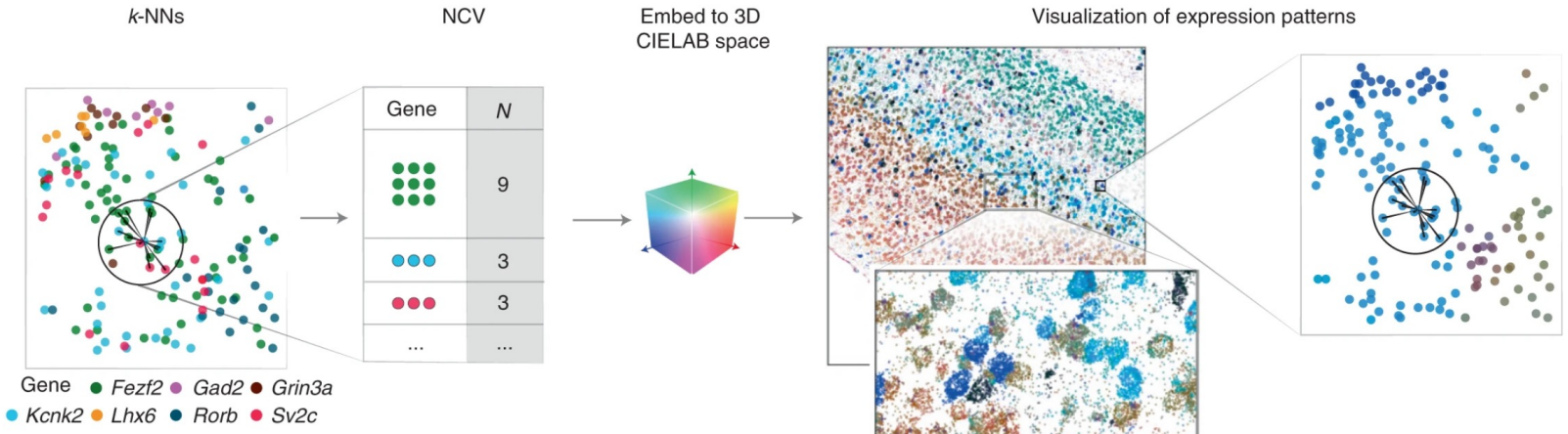
- DAPI nuclear staining
- ATP1A1/CD45/E-Cadherin boundary staining
- 18S RNA + alphaSMA/vimentin protein interior

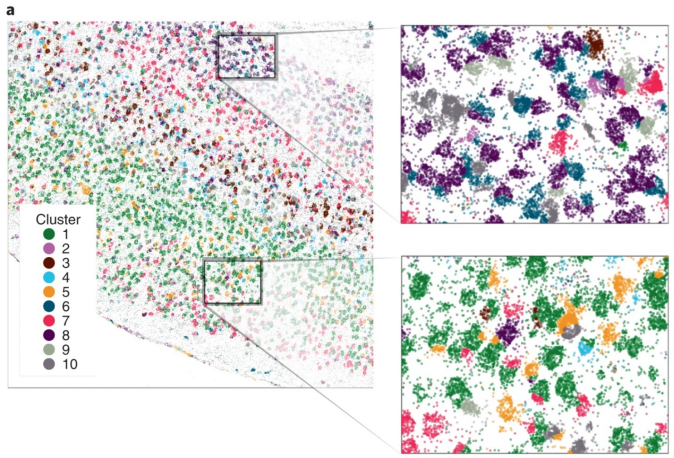
Possible to rerun segmentation later with *xeniumranger resegment* pipeline

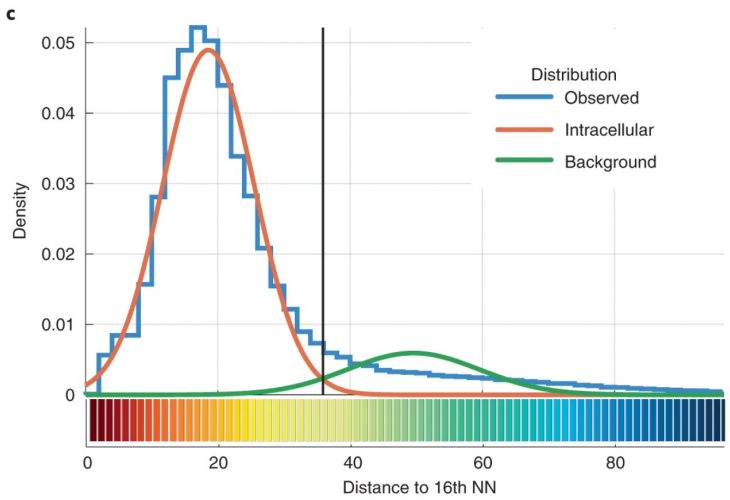
Prioritization order:

1. Segment cells based on their cell boundary stain
2. Segment cells based on expansion from the nucleus to the cell interior stain edge
3. Nuclear expansion (default 5 µm)

Bayesian Segmentation of Spatial Transcriptomics Data (*Baysor*)







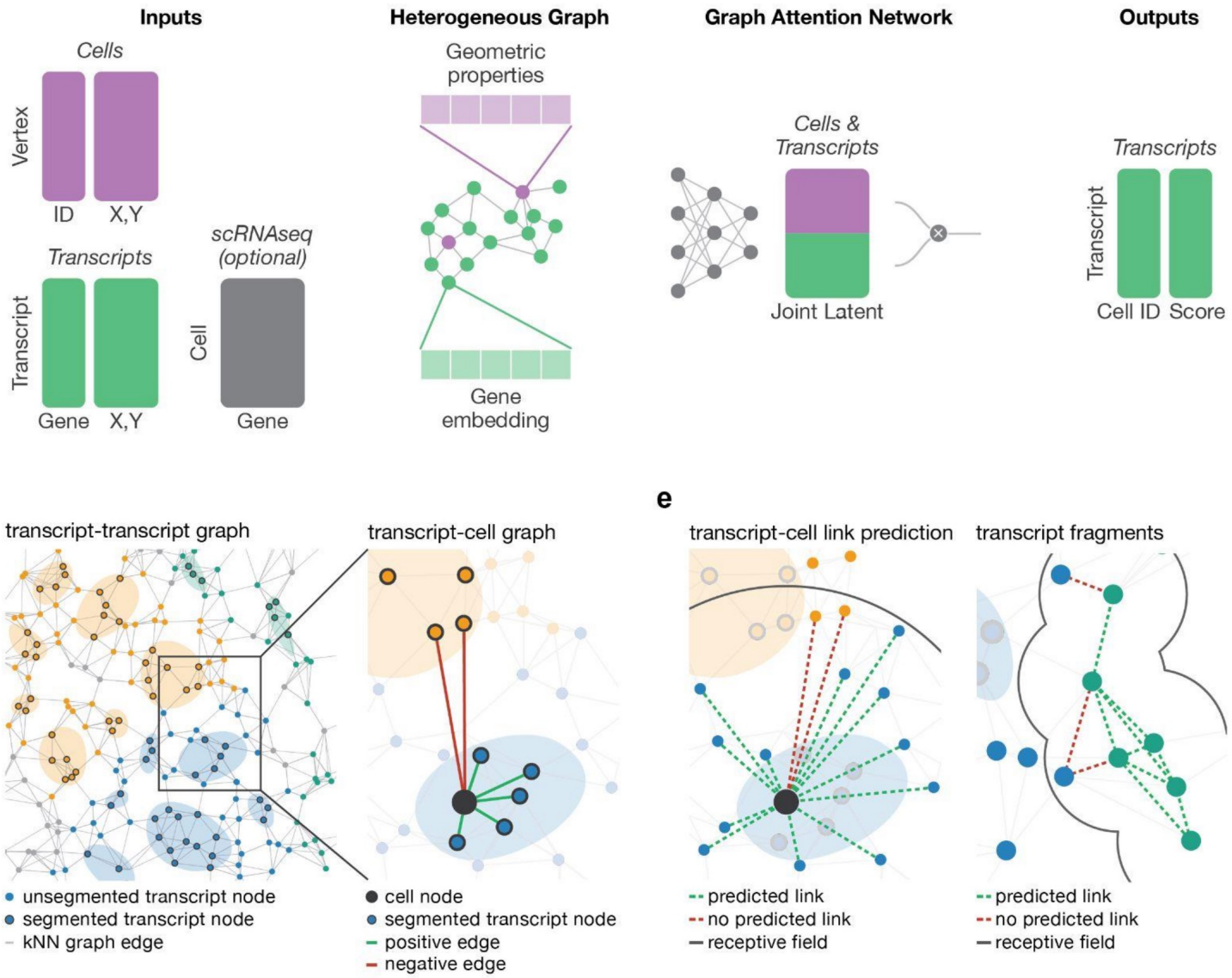
First “big” method that used segmentation-free approach based on transcriptional composition of the cells

Baysor can take advantage of auxiliary stains by incorporating a precalculated segmentation as a probabilistic prior (user can modify weight of the prior)

Really demanding for memory and running time (easy up to 1TB memory, 100s of threads and several days run for 5k Xenium panel) bejsor

https://github.com/kharchenkolab/Baysor

Using graph neural network (GNN) to solve transcripts assignment (*Segger*)



Use as input initial segmentation (i.e. from xeniumranger). Can be supervised by co-expression patterns with additional matching scRNA-seq.

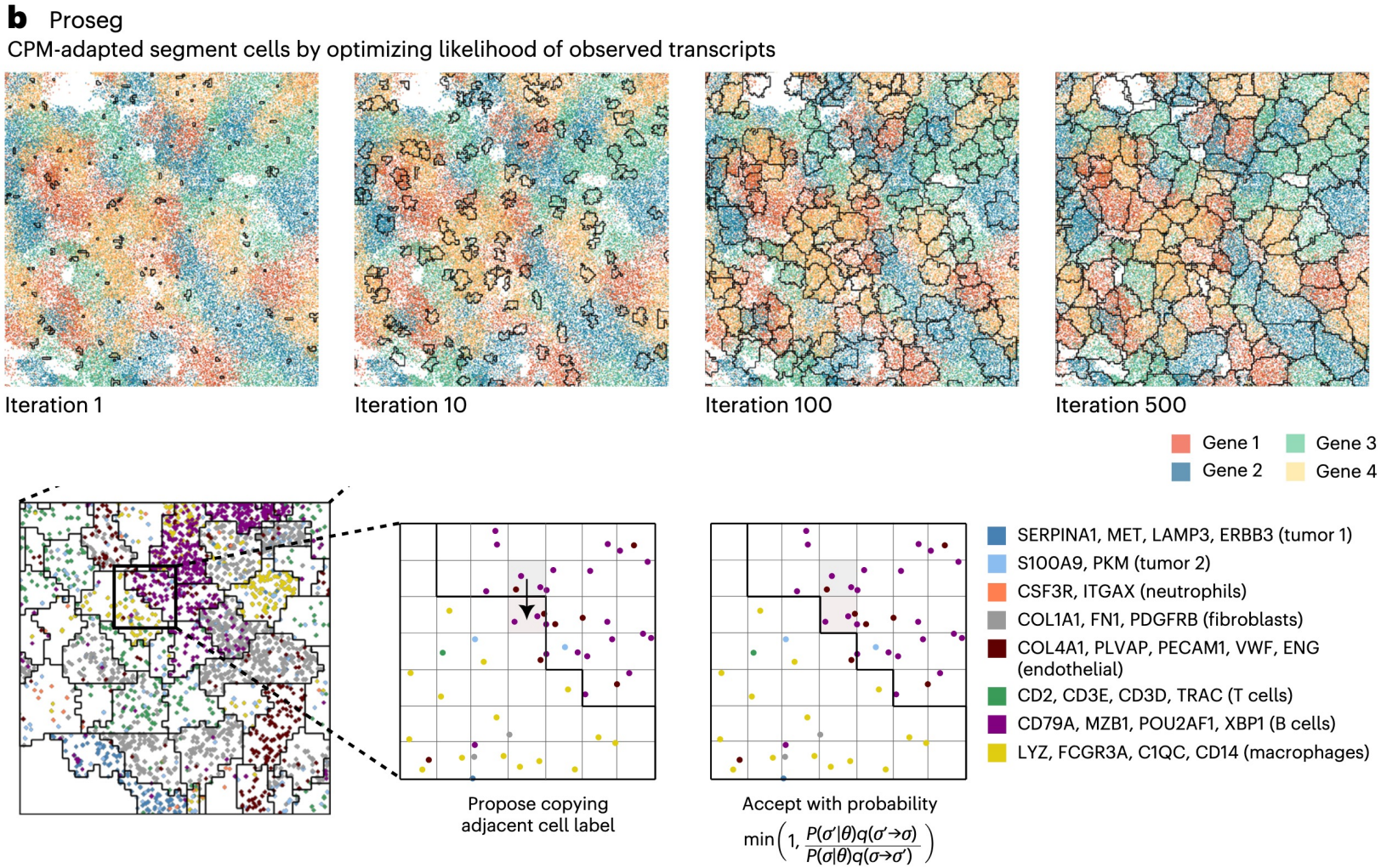
Reframes segmentation problem to the link-prediction problem on a graph (transcripts and cells are nodes). GNN learn meaningful patterns based on transcript colocalization + nuclear/membrane staining

Because of the GNN can be used for the cases with ambiguous border transcripts

Using GPU to run, faster than Baysor, but still takes some time

https://github.com/gerstung-lab/segger

Probabilistic cell segmentation for in situ spatial transcriptomics (*Proseg*)



What set of cell shapes would best explain where the observed transcripts actually landed?

Initialize cell morphologies from nuclear segmentation and iterated/expanded until best fits all transcripts

Models leaky cells (particular in Xenium data) by adding prior on the distance transcripts migrate from true position

Quite fast, current “gold standard”

https://github.com/dcjones/proseg

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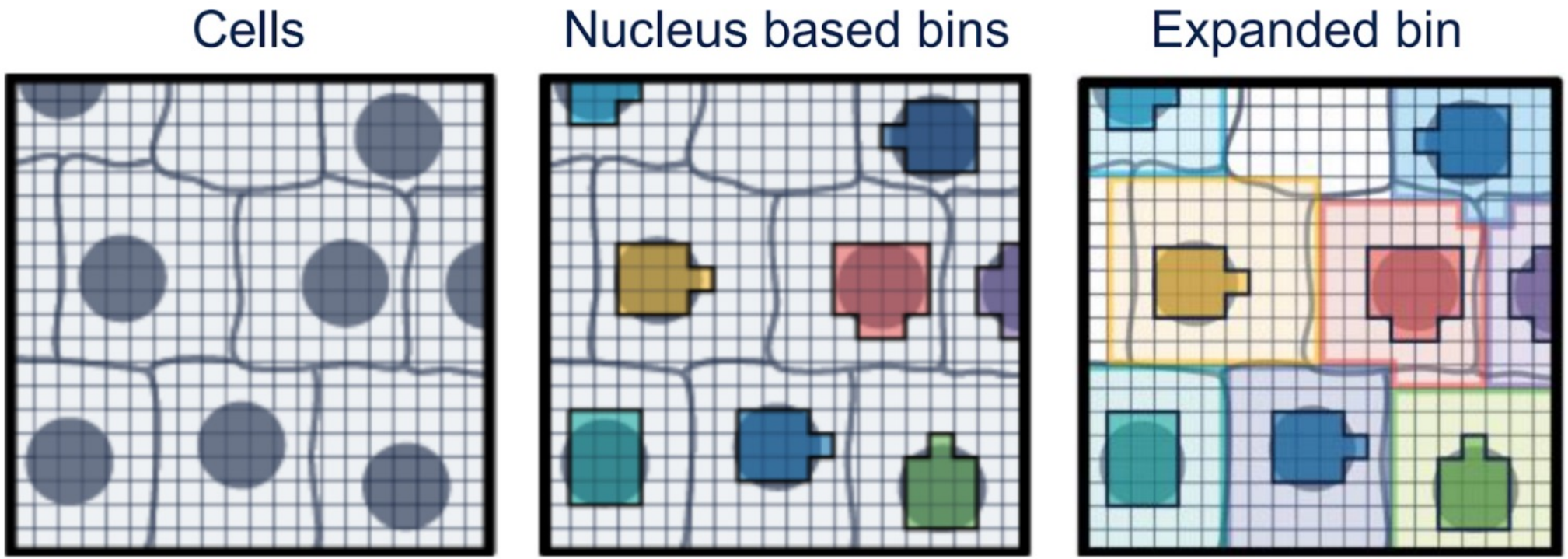
Cell segmentation from 10x *Space Ranger*

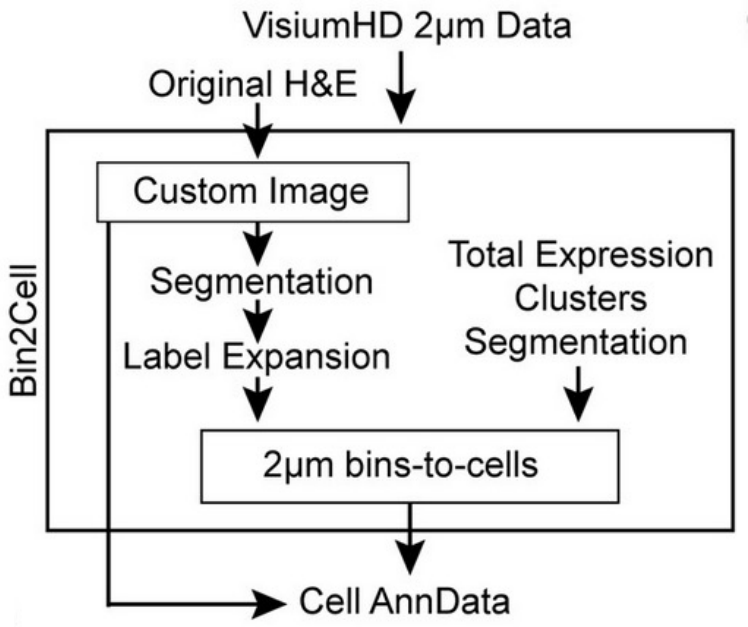


Trained StarDist v6 model on 17k image patches from 150 H&E tissue images for nuclei segmentation

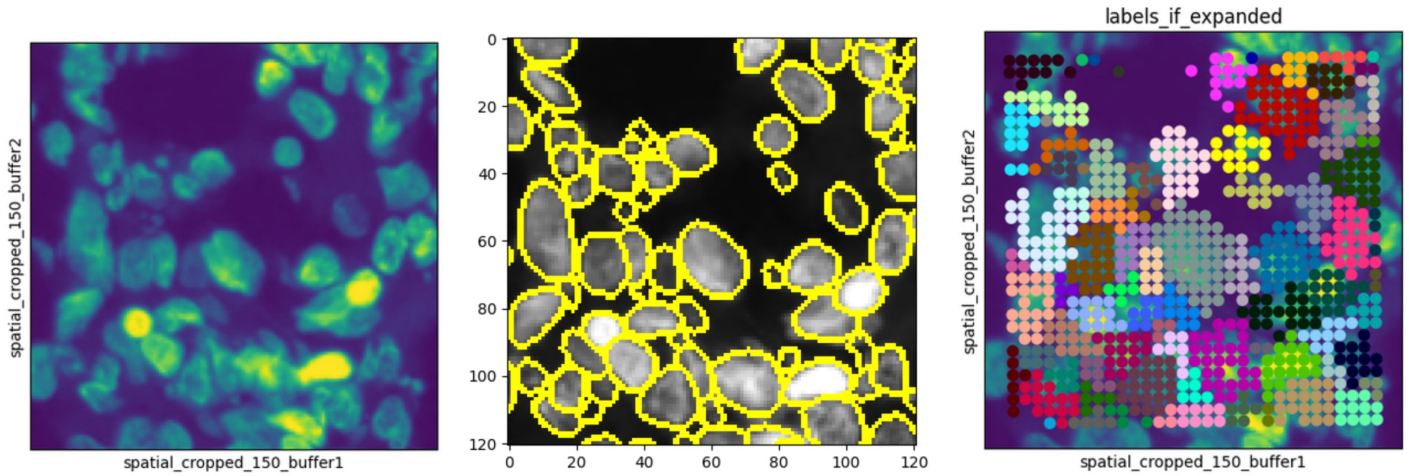
Each spot is assigned to the closest nucleus up until a maximum distance specified by *--nucleus-expansion-distance-micron*

Starting from Space Ranger v4.0

Bin2cell merge VisiumHD bins into cells



- Use cell segmentation algorithm (StarDist) on pre-trained H&E or nuclear marker model
- Label expansion of a fixed distance (default 2 bins)

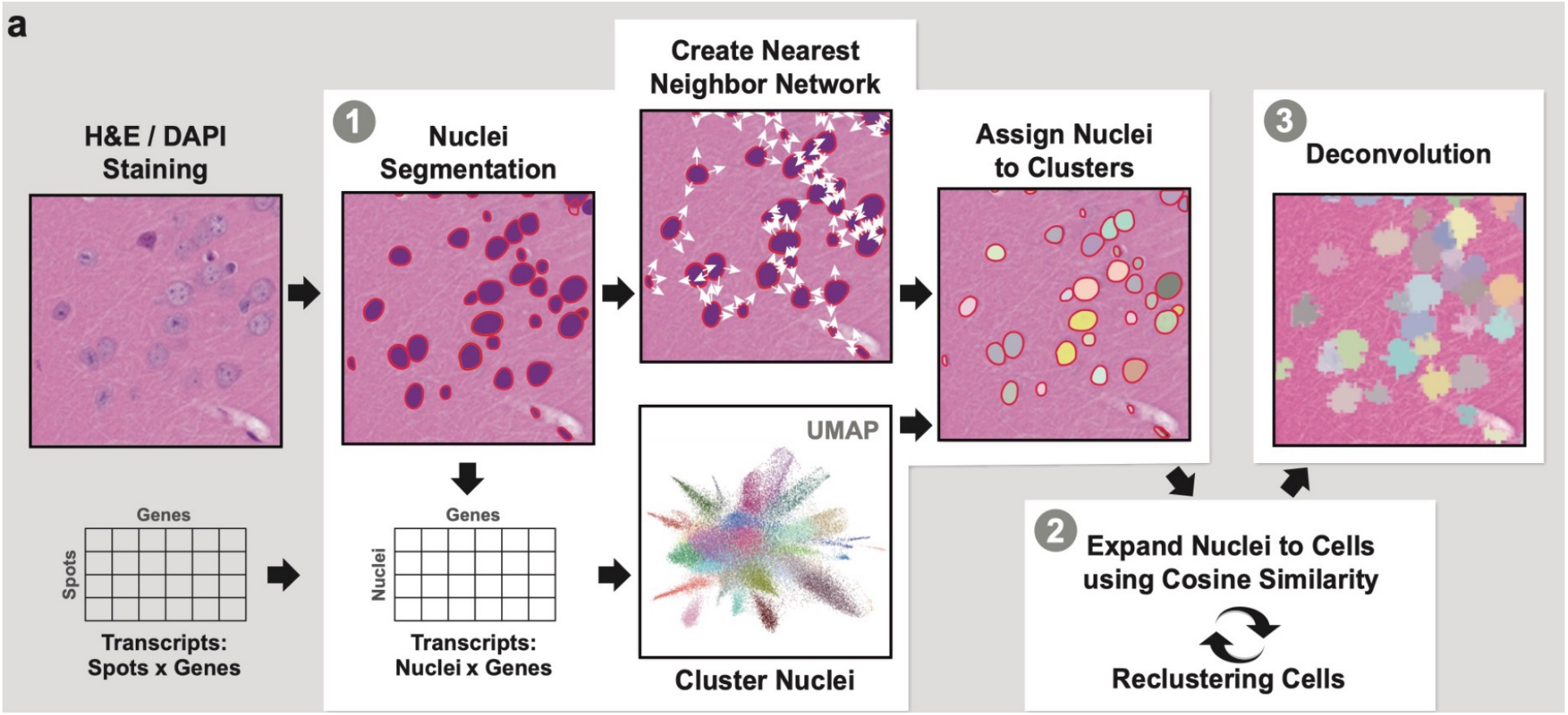


Combine label expanded cell segmentation with segmentation based on GEX counts (fill cells without nucleus)

If bin overlap segmentation masks (cell) add proportional counts of the spot to the respective cells

https://github.com/Teichlab/bin2cell

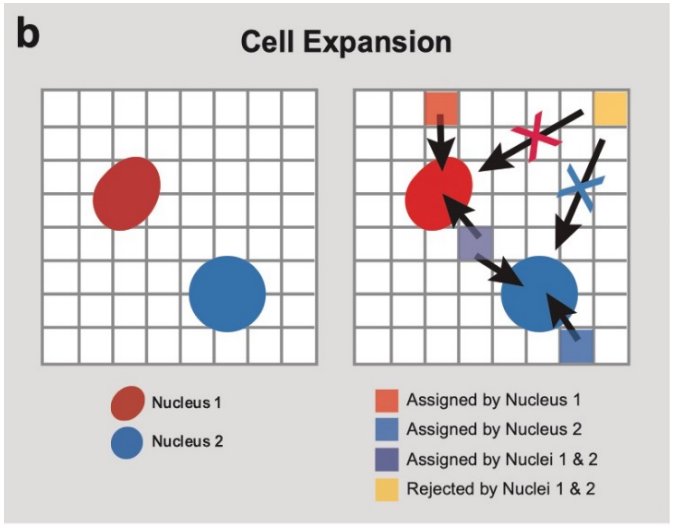
Segmentation and Manifold Unrolling Framework (*SMURF*)

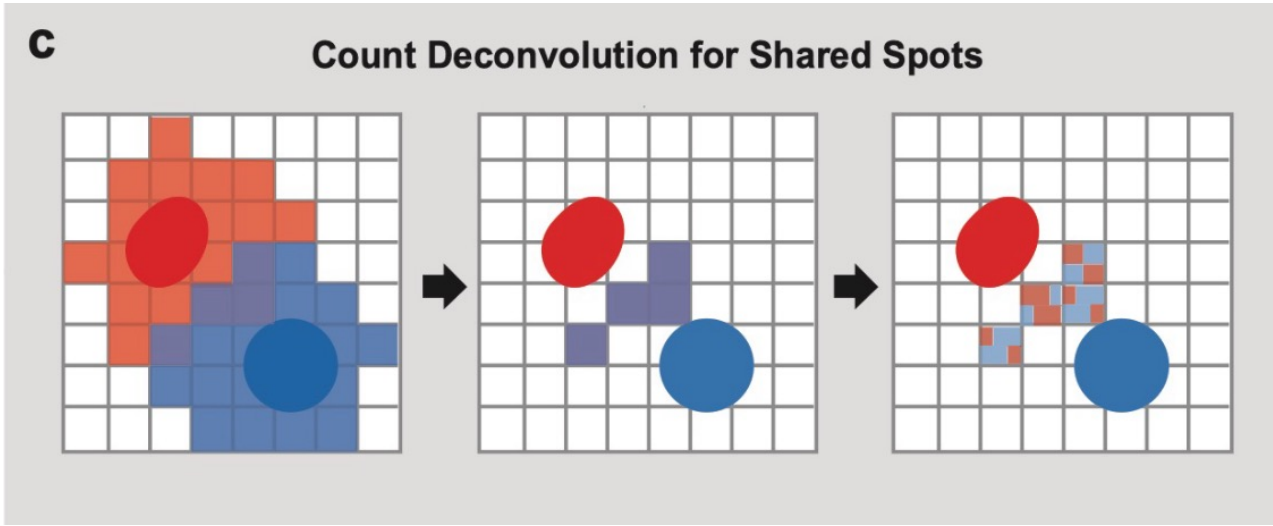


Works with associated H&E or IF stainings + uses prior nuclei segmentation (i.e. StarDist)
Calculate nearest neighbor scores for cells and cluster nuclei data with further assignment to clusters
Link imaging data to spots (50 pixels in one spot)

https://github.com/The-Mitra-Lab/SMURF

Segmentation and Manifold Unrolling Framework (*SMURF*)





- Adds nearby spots to the nucleus gene expression matrix to increase UMI counts by using concentric expansion
- In each iteration check if the resulted expanded cells increase cosine similarity between cells of the same cluster
- Repeated until clustering is stabilized based on normalized mutual information (NMI)

- Deconvolve counts from shared spots so that every transcript is assigned to at most one cell
- The sum of cell type proportions (weights from clustering) should match the normalized gene expression pattern for the spot

https://github.com/The-Mitra-Lab/SMURF

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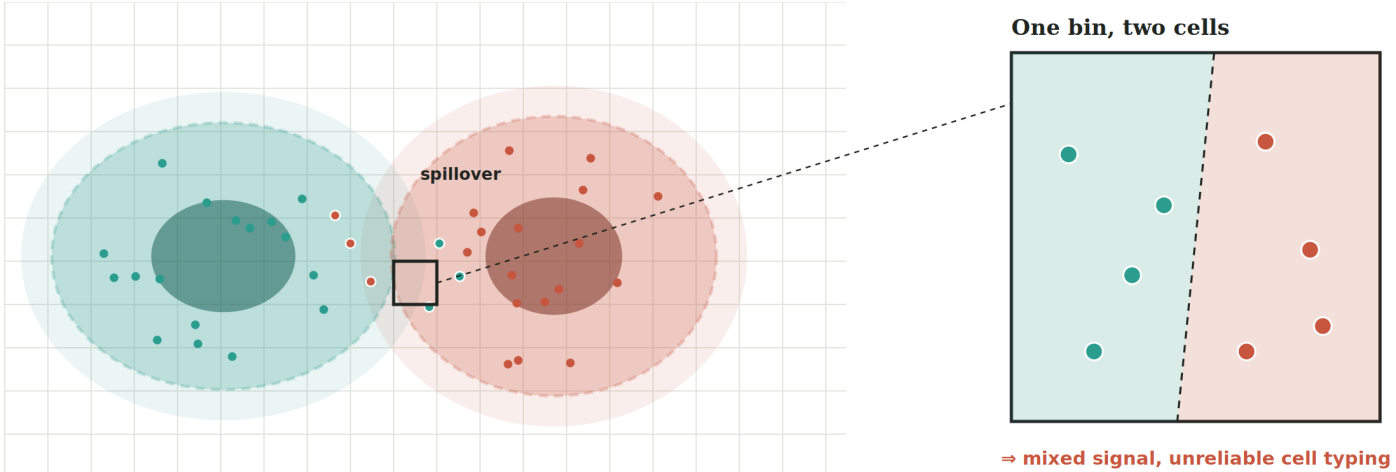
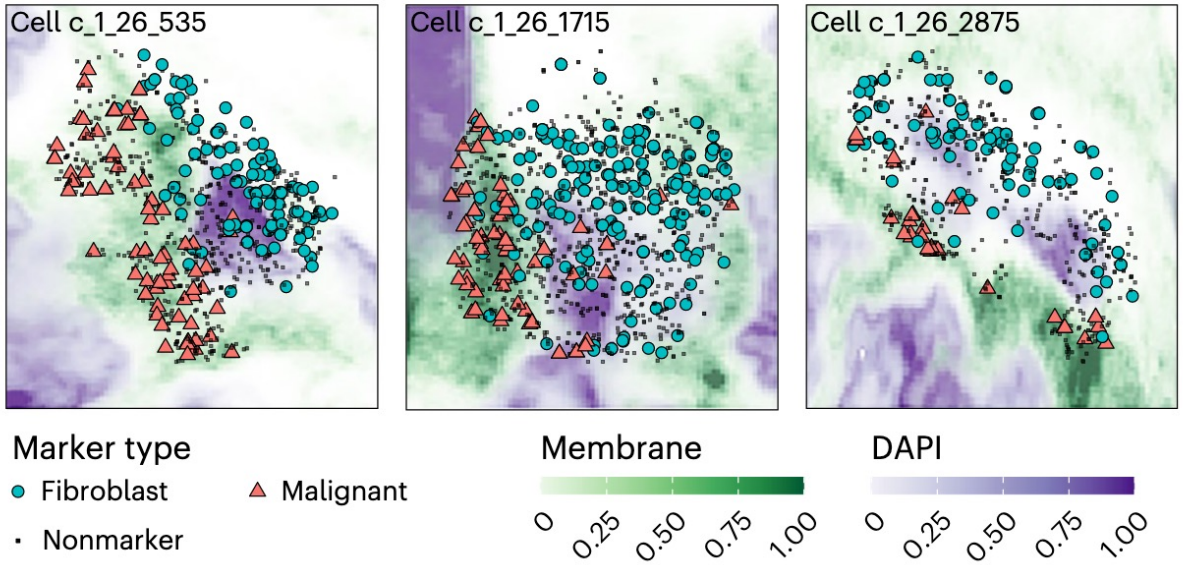
New challenges for cell segmentation with spatial transcriptomics

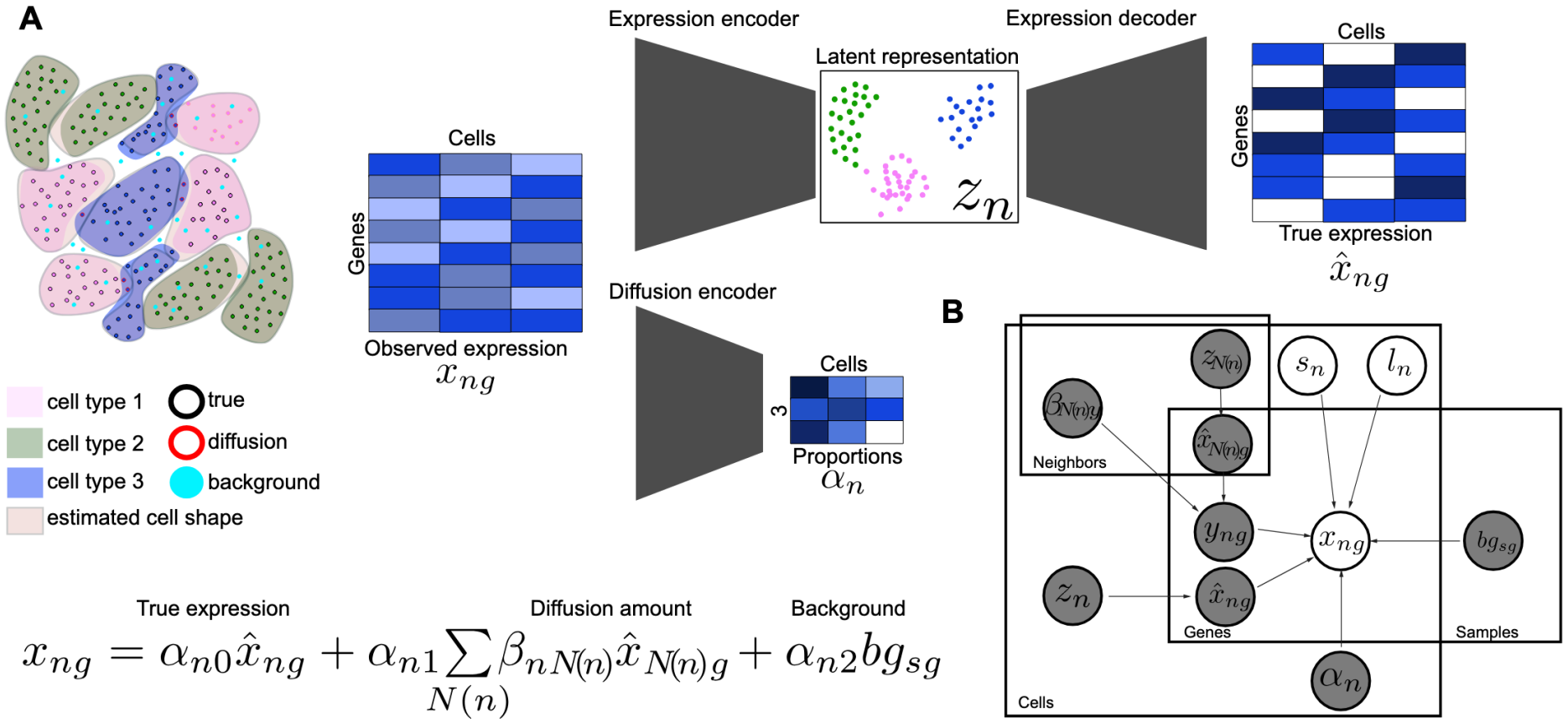


In Visium HD (sequencing-based ST) source of spillover is inherited from the technique. One bin/spot covering 2 or more cells add noise to this cells, corrupting all downstream analysis (annotation, DE expression)

Spillover effect is evident also in imaging-based ST (i.e. Xenium), where the technical source is diffusion of the RNA molecules to the neighboring cells (i.e. transcriptionally active malignant cells contaminate healthy neigboring cells)



ResolVI - addressing noise and bias in spatial transcriptomics



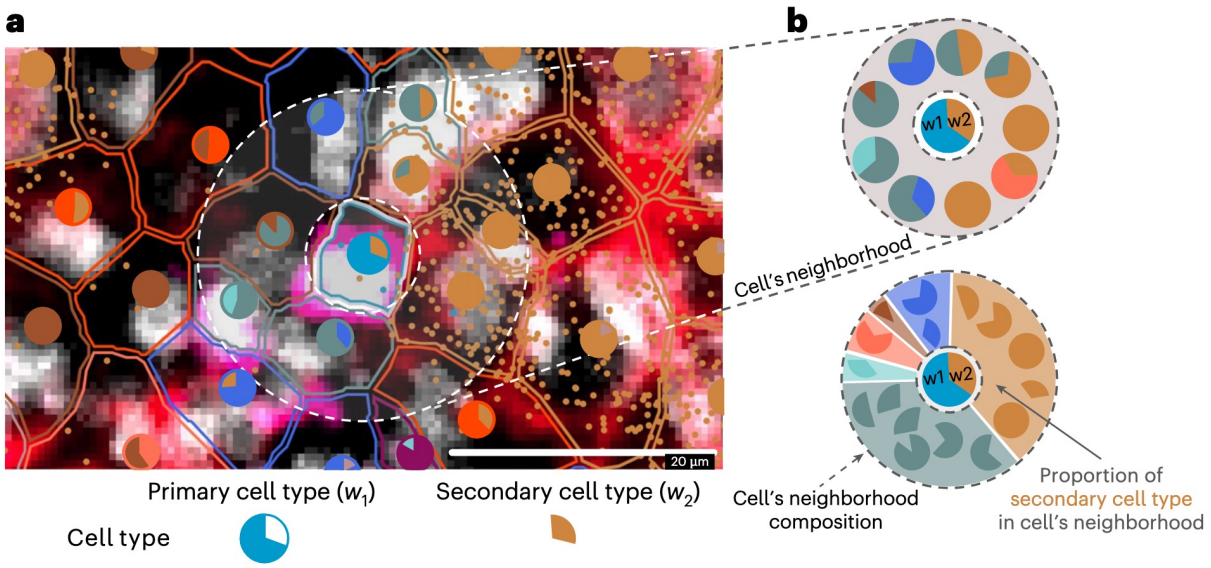
True expression — Diffusion amount — Background

$$x_{ng} = \alpha_{n0}\hat{x}_{ng} + \alpha_{n1}\sum_{N(n)}\beta_{nN(n)}\hat{x}_{N(n)g} + \alpha_{n2}bg_{sg}$$

Uses as input matrix of cell by gene expression, spatial coordinates of each cell and respective tissue slice (sample integration)

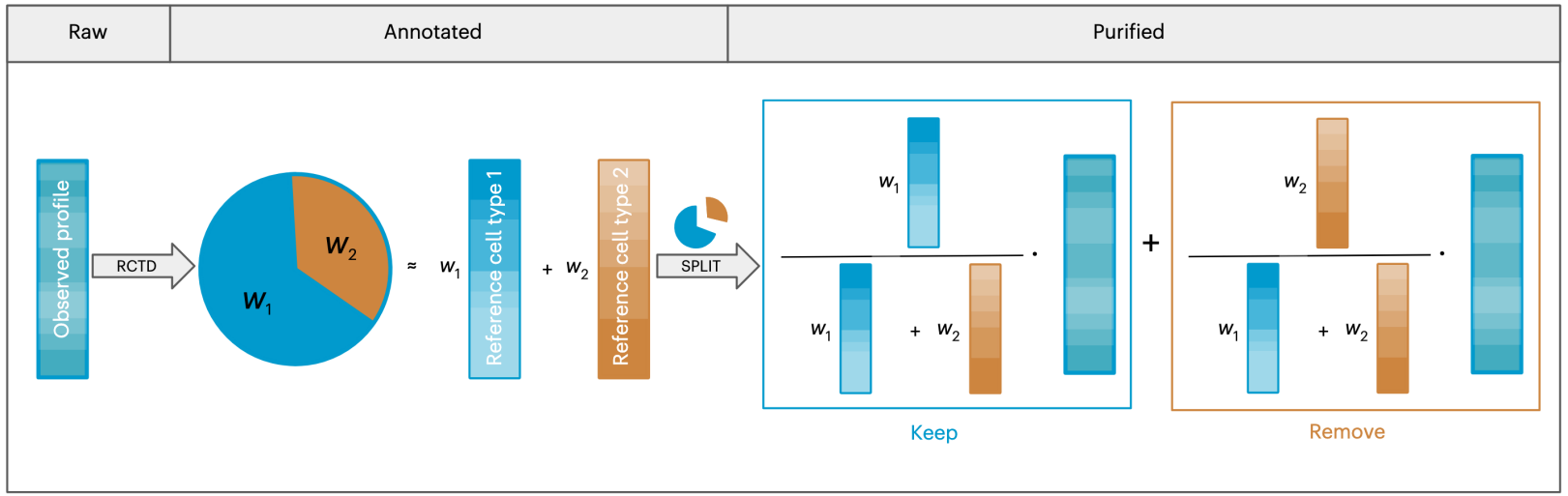
Backbone latent variable model on the variational autoencoder Estimates observed expression as a sum of true expression, expression induced by neighboring cells (diffusion) and unspecific background expression

Spatial Purification of Layered Intracellular Transcripts (*SPLIT*)



R package that utilize first RCTD with reference/matching scRNA-seq data for initial decomposition of the cells (similarity of each cell transcriptome profile to the provided celltypes)

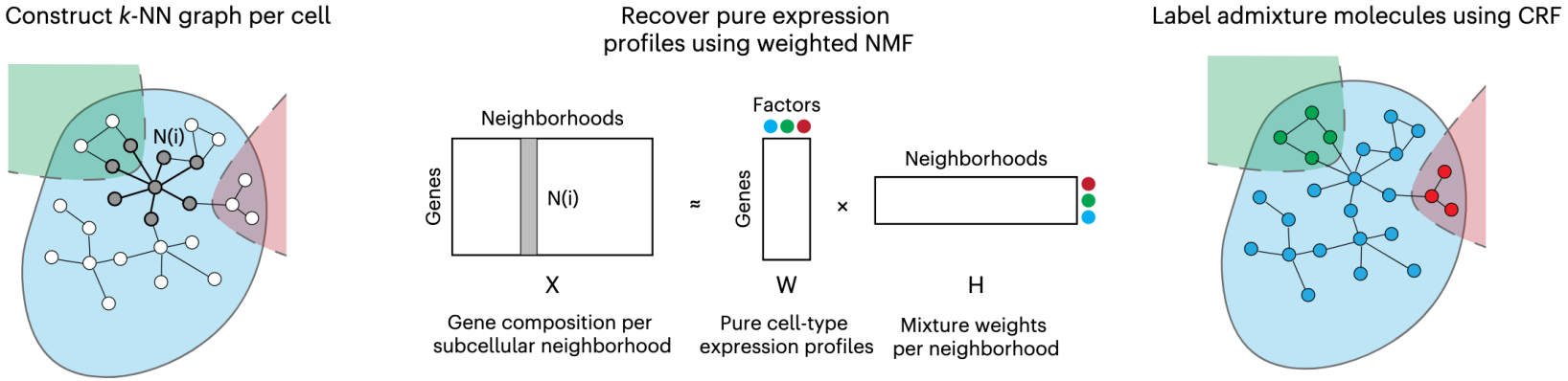
Identify doublet-cells (potential spilling)



Correct raw counts using doublet-SPLIT approach, which keep for the cells of interest (depending on the mode) only fraction of the read corresponding to the primary celltype relative to the sum of primary and secondary

https://github.com/bdsc-tds/SPLIT

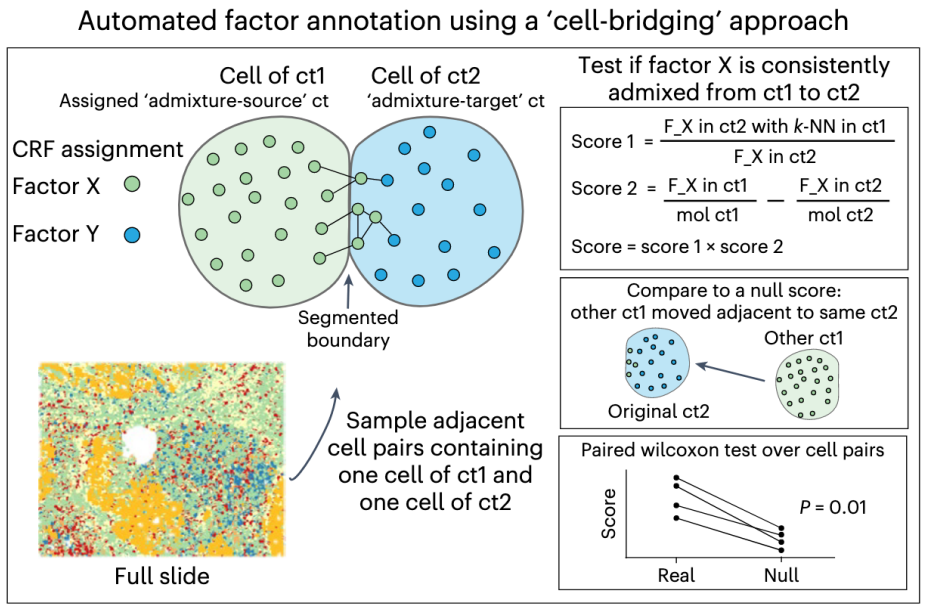
Correction with segmentation errors with *cellAdmix*

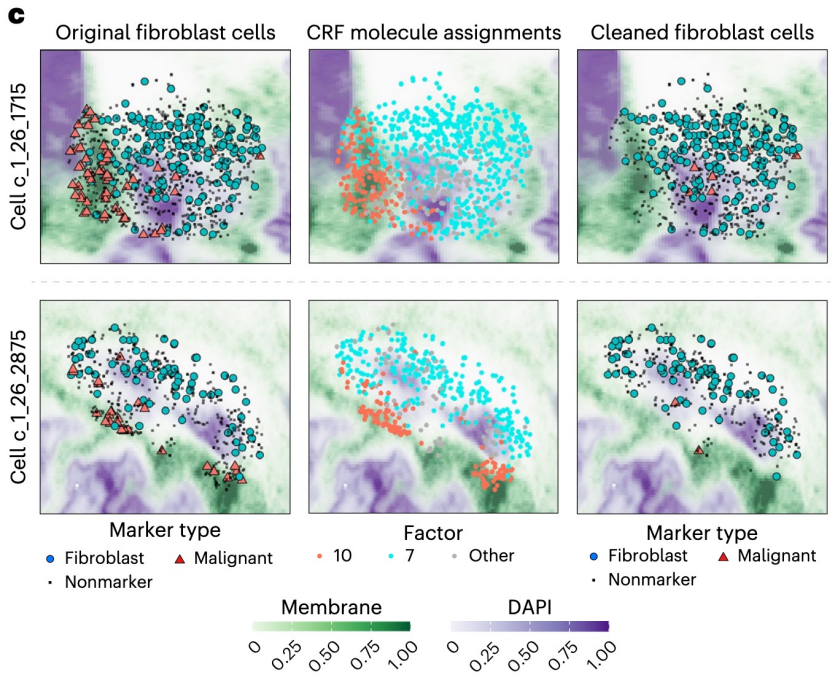


Define admixture score by using Bayesian model to estimate contamination for each gene taking to account cell type adjacency

Compute neighbourhood composition values and factorize with NMF. Conditional random field then assigns individual molecules to the recovered factors.

Via “cell-bridging” molecules whose factor assignment doesn't match their host cell's type are removed





https://github.com/kharchenkolab/cellAdmix

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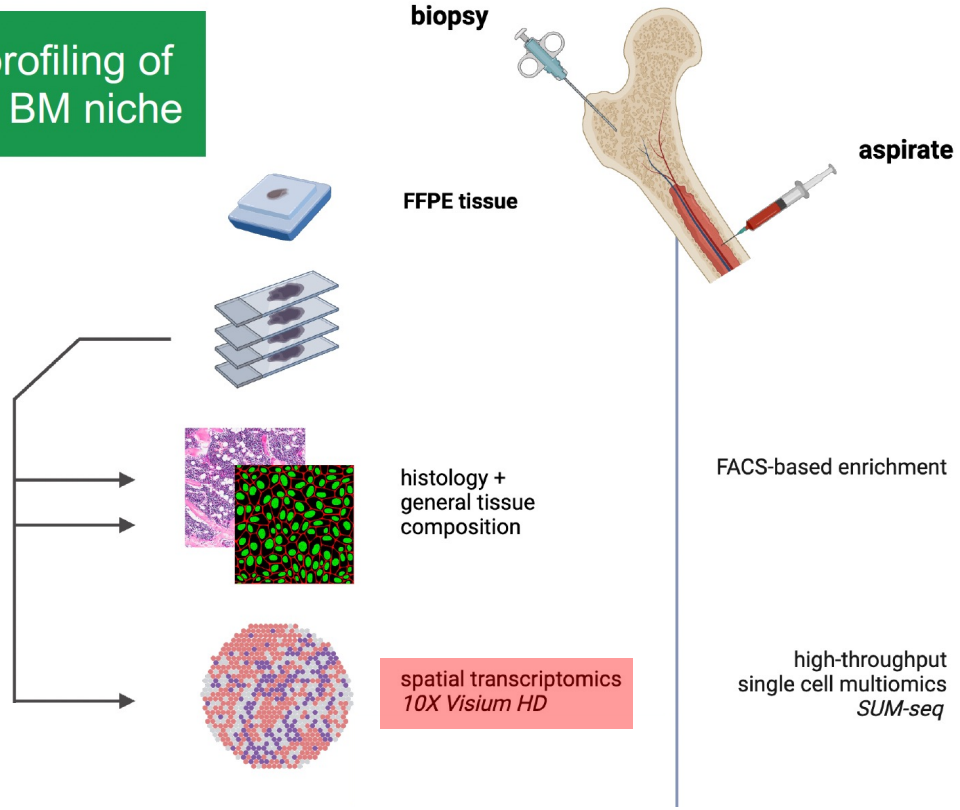
Project introduction

Spatial profiling of diseased BM niche

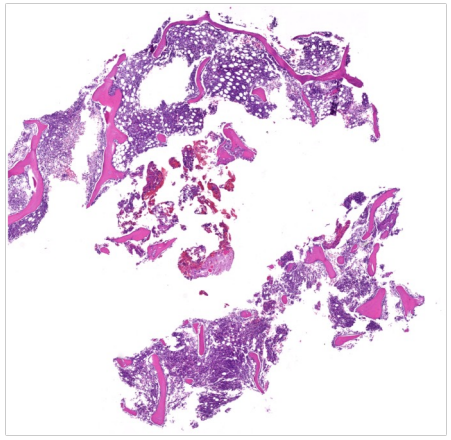
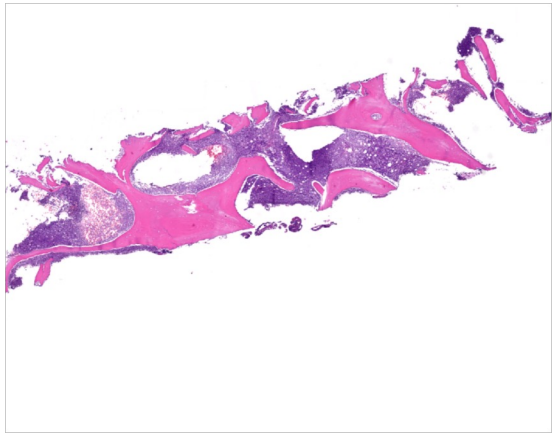
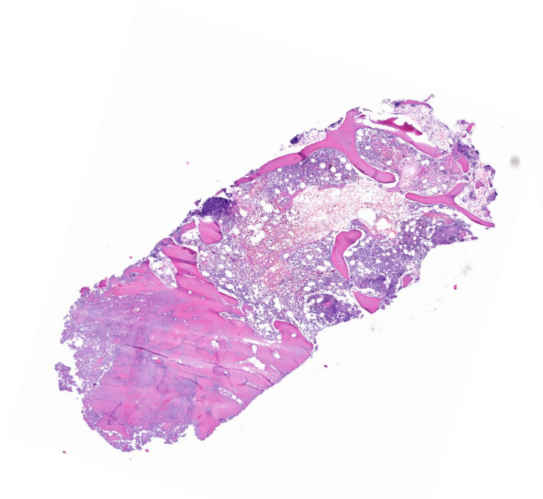
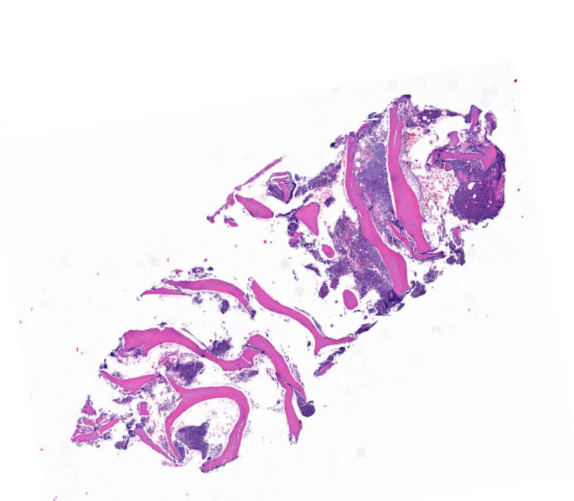
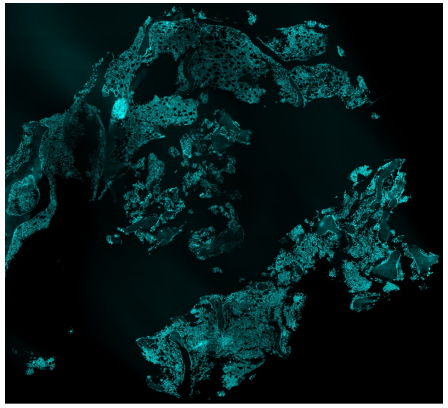
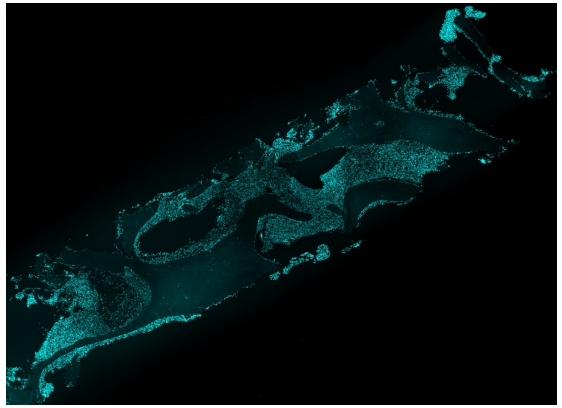
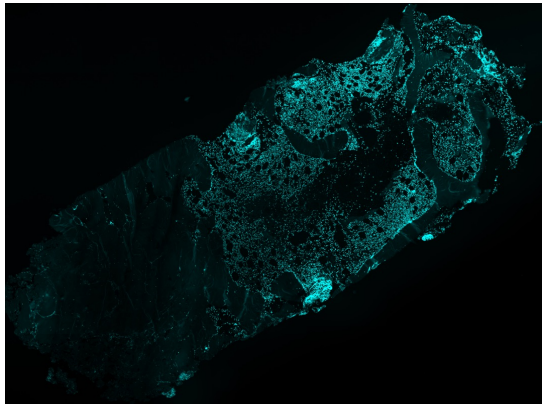
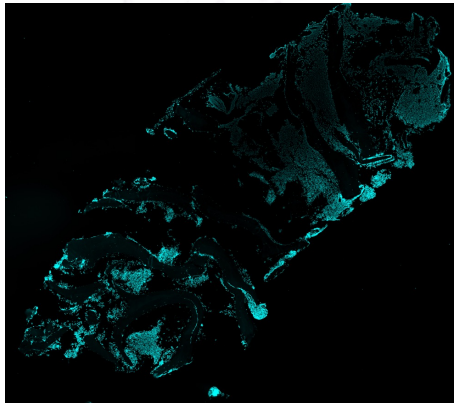


- Cohort of AML patients (various genetic background):
  - $NPM1_{mut}$ – favorable prognosis
  - $NPM1_{mut}$/$FLT3ITD_{mut}$ - intermediate
  - $NPM1_{mut}$/$FLT3ITD_{mut}$/$DNMT3a_{mut}$ – poor prognosis
  - only $FLIT3ITD_{mut}$ – poor prognosis
- Link spatial transcriptomics (FFPE) and multiomics (scATAC/scRNA) sequencing (aspirates) from the same sample
- Focus on the interactions between stromal cells and malignant cells in bone marrow

Universitätsklinikum Carl Gustav Carus

modified scheme from Karin Prummel

Patient-derived AML samples used in this analysis

$NPM1_{mut}$	$NPM1_{mut}/FLT3ITD_{mut}/DNMT3A_{mut}$	$NPM1_{mut}$	$NPM1_{mut}/FLT3ITD_{mut}$
H&E serial				
DAPI				
	56422	55572	62031	57593

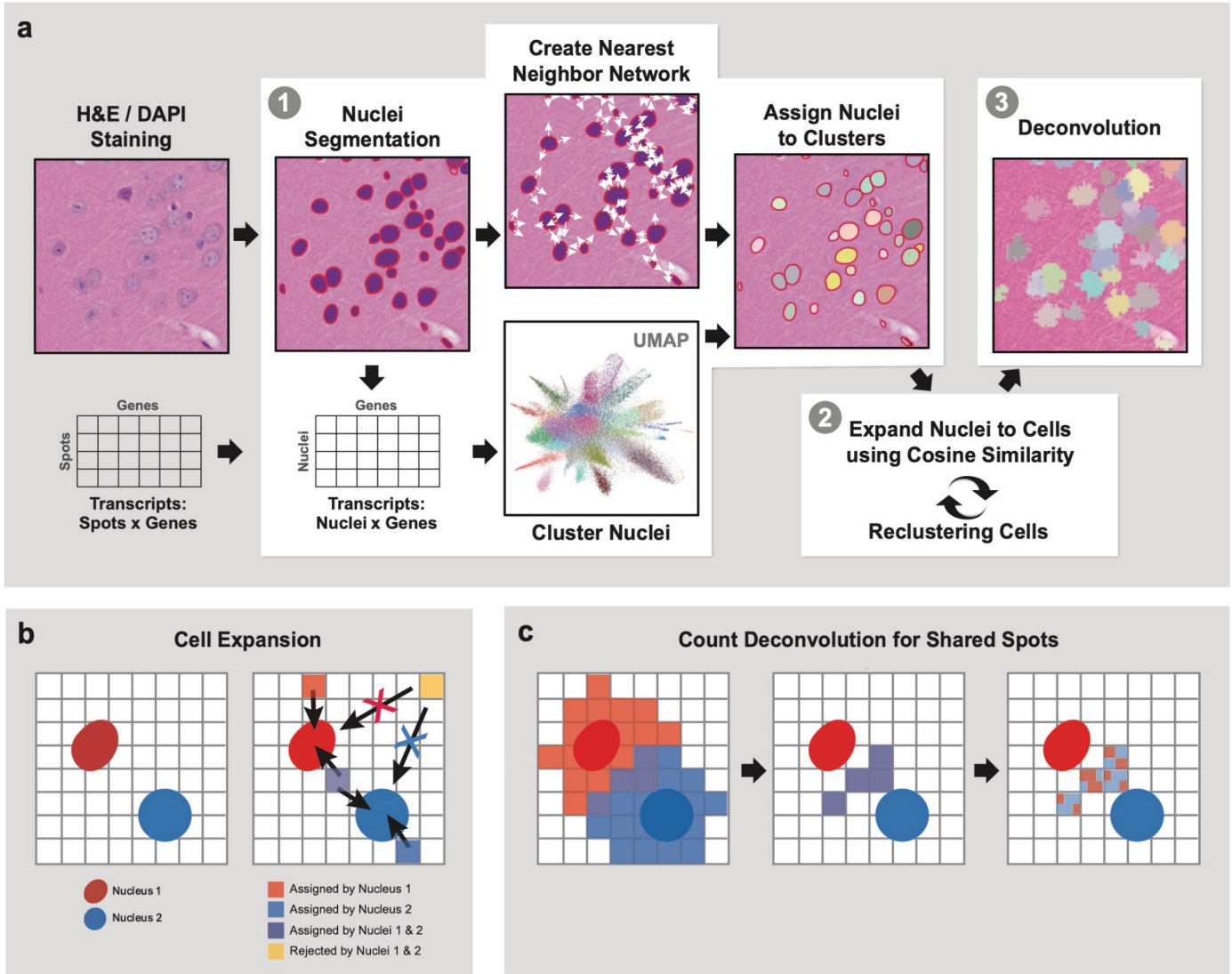
10x Spaceranger + QC

SMURF segmentation bins to cells

RCTD with BM atlas similarity to celltypes

Supervised resolVI only high confident cells

Seurat/SFE processing



Guo et al., 2025

10x Spaceranger + QC

SMURF segmentation bins to cells

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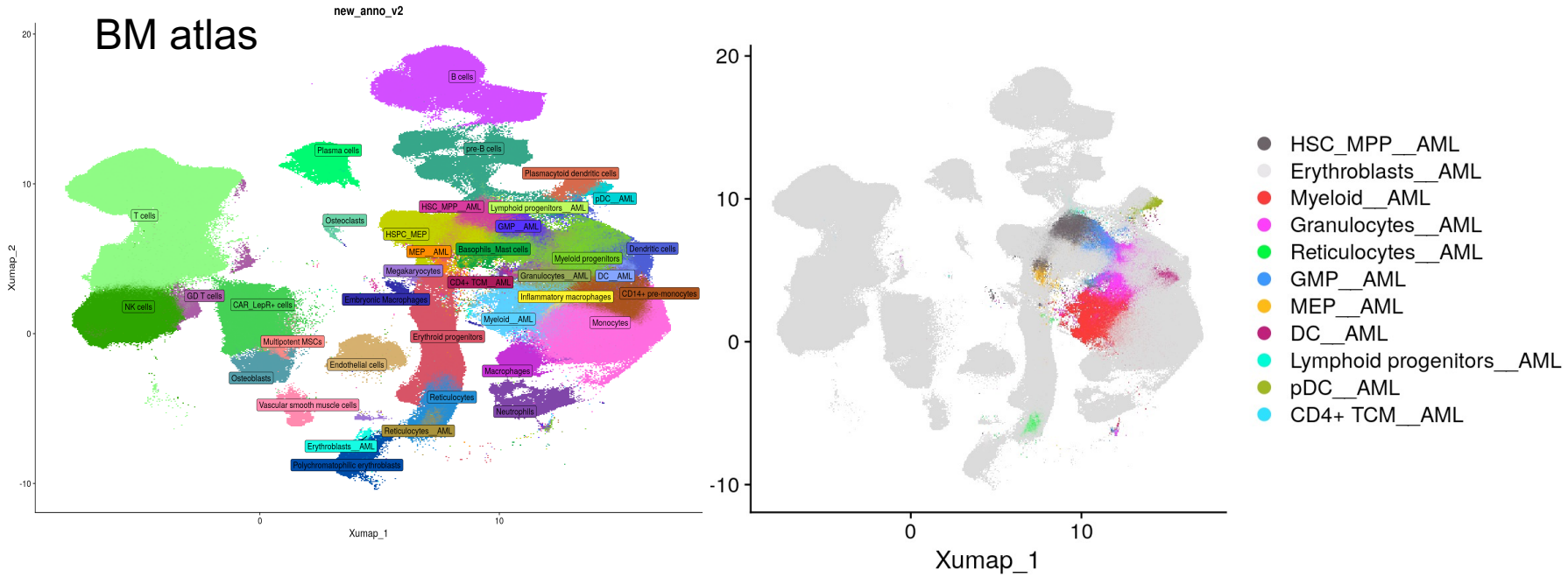
Supervised resolVI only high confident cells

Seurat/SFE processing

Analysis is based on the BM atlas with data from 46 datasets
250 donors with 1.8 million of cells



Using reference atlas transcriptional profiles per celltype to unmix ST data

Cable et al., 2022

10x Spaceranger + QC

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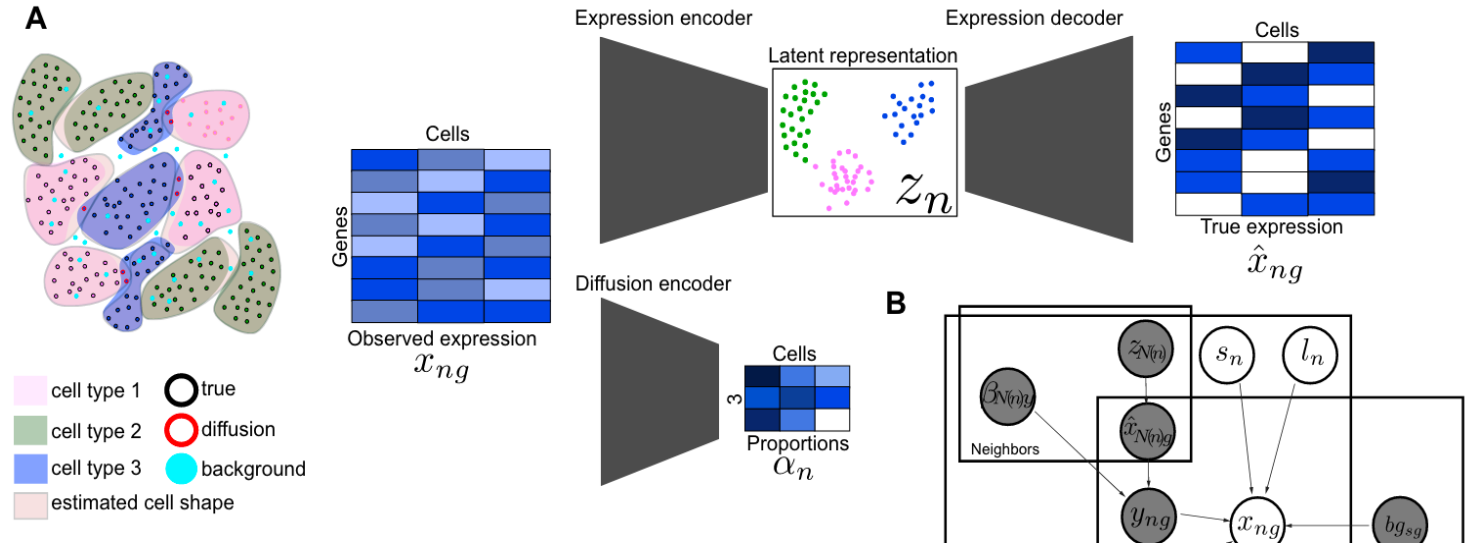
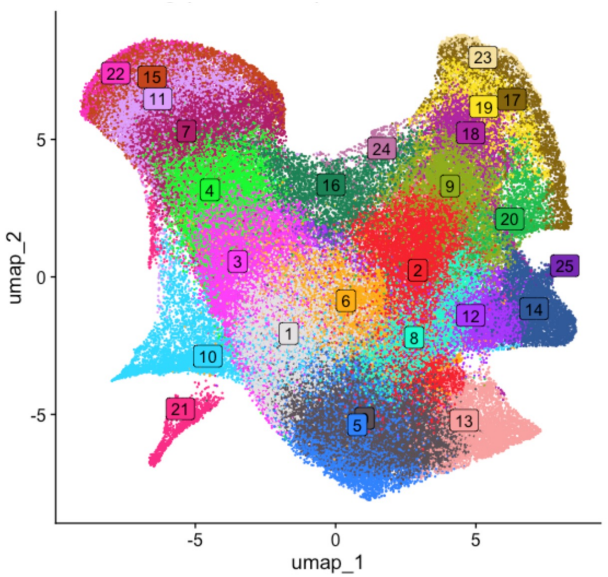
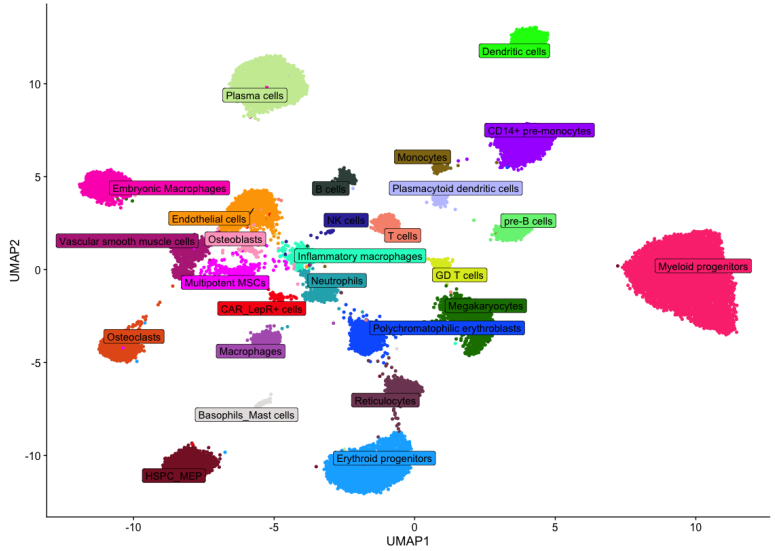


Use supervised resolVI with confident set of cells (primary – secondary > 0.5) ~10-15% of cells





Ergen et al., 2025

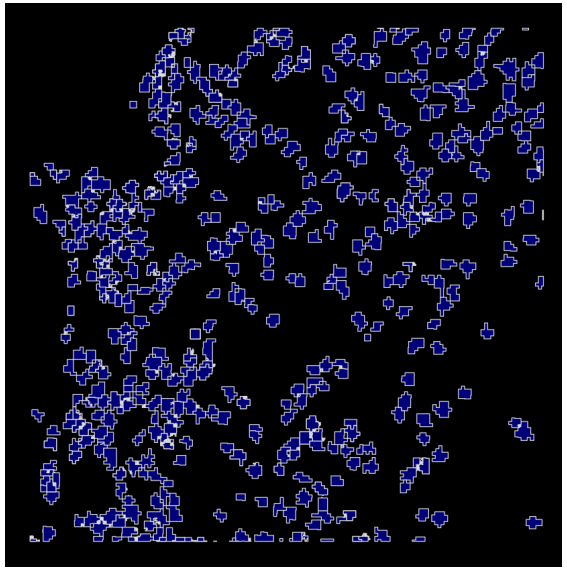
10x Spaceranger + QC

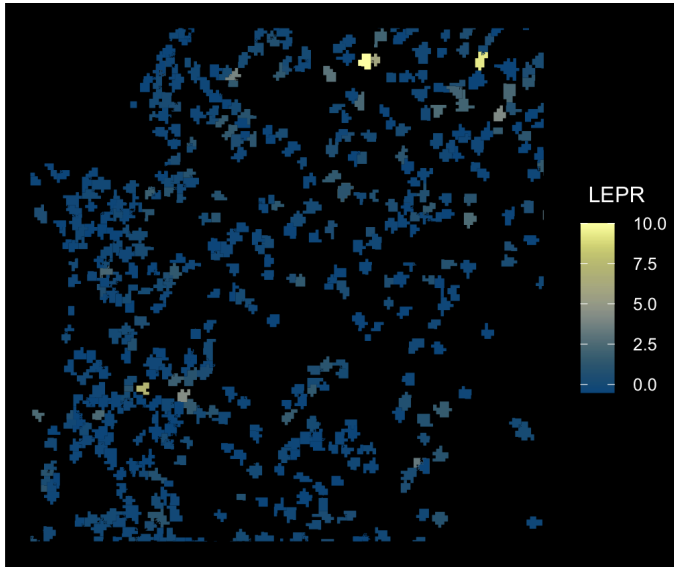
SMURF segmentation bins to cells

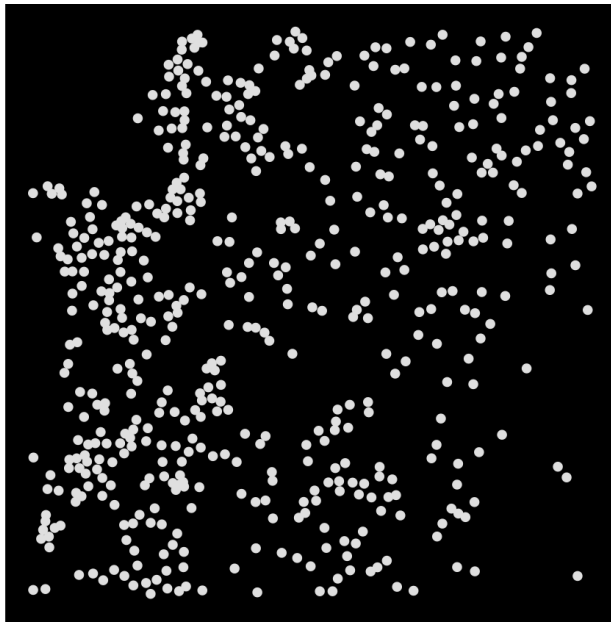
RCTD with BM atlas similarity to celltypes

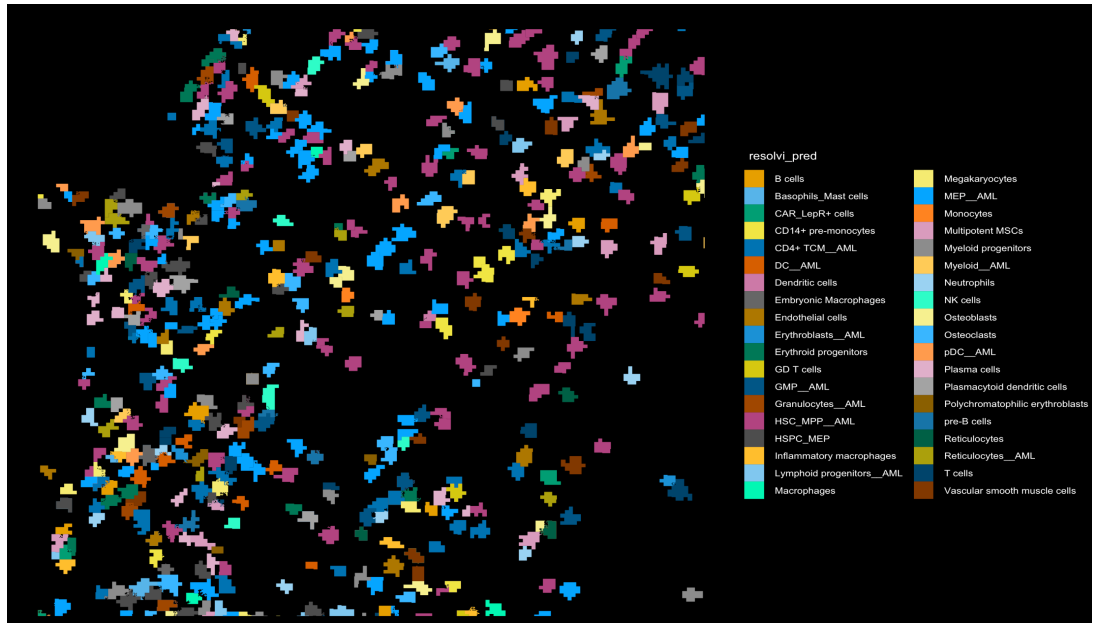
Supervised resolVI only high confident cells

Seurat/SFE processing









Make Seurat/SpatialFeatureExperiment objects for downstream processing

Moses et al., 2023

Snakemake Xenium pipeline

10x Xenium in Basel
DBM/D-BSSE

Using initial output from Xenium machine followed by resegmentation, additional segmentation with *Proseg* and output in the *SpatialFeatureExperiment* or *spatialdata* format.

Runtime: ~1day for the whole Xenium slide

Downstream steps:

- RCTD + resolVI annotation based on the reference/matching atlas
- Cropping samples (if not cropped by Xenium, too tight on the slide) based on polygons from Xenium Explorer
- Integrating samples with scvi?
- SPLIT correction and annotation?

Conclusions

Cell segmentation is **very important** (however almost **never perfect**). Should be done at the very beginning (i.e. part of the Xenium onboarding analysis) and influence all downstream steps.

Comprehensive up-to-now list of sequencing-based and imaging-based spatial transcriptomics cell segmentation tools.

Spatial transcriptomics can enhance just image-based cell segmentation, however introduce certain technology-specific biases (spillover, diffusion).

For the best result you almost always need to combine tools (i.e. SMURF + RCTD + resolVI). Still computational tools lacking interoperability between R and Python ecosystems.

University of Basel EMBL

Thank you
for your attention.

Zaugg lab

Gwen Bauersachs
Mikael Marttinen
Kristy Ou
Charles Dussiau
Karin Prummel
Jupa Jauregui

Sandra Kessler
Pavel Kos
Aryan Kamal
Maksim Kholmatov
Jonas Rauchhaus
Sara Lobato

Evi Vlachou
Hanna Norbäck
Andrei Stapran
Efi Kormari
Elena Stefanovska
Amandine Kremer

Laura Bakker
Yulia Ermakova
Andrea Polazzi
Luke Mittelberger
Karin Fava
Maria Tapken