NEUBIAS: European Network of Bioimage Analysts, paving their way…

neubias
network of european bioimage analysts

BIAS
Bioimage Data Analysis Courses
(Kota Miura, EMBL)
2012-2017

Training

15 Training Schools
415 trainees
(almost 1000 applicants)

2012 2013 2014 2015 2016 2017 2018 2019 2020

EuBIAS Symposium
Barcelona Paris

1st Taggathons
(2013-2015)

EURO-BIOIMAGING

FRANCE-BIOIMAGING

Networking

Events

Career Path

Online Tools & Repositories

NEUBIAS Conference
Lisbon, Szeged, Luxembourg, Bordeaux

50 Short -term Scientific Missions, 2 books, more …

42 Countries
>250 members

BIII BioImage Informatics Index

BIAflows

NEUBIAS Academy

- Support Life Scientists, Early Careers, Bioimage Analysts, Facility Staff and Developers
- **Provide sustainable material and activities focused on:**

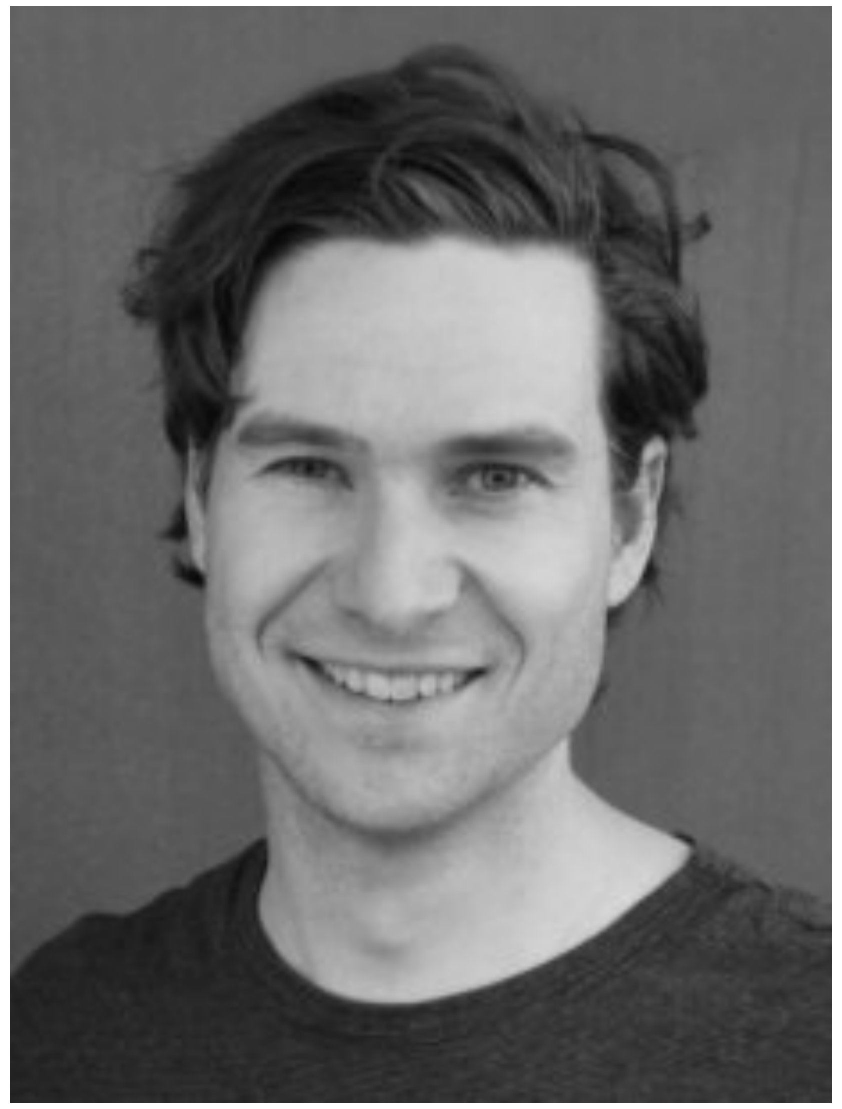
Training in Bioimage Analysis

- **Series of Webinars and online lectures**

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

http://neubias.org
https://biii.eu
https://biaflows.neubias.org
http://neubiasacademy.org

Today’s team



Martin Weigert

Group Leader at EPFL
Machine Learning and Computational Microscopy

Panelists:
We will answer your questions

Olivier Burri
EPFL,
Lausanne

Siân Culley
UCL,
London

Uwe Schmidt
MPI-CBG,
Dresden

Introduction to Nuclei Segmentation with StarDist

Martin Weigert, Olivier Burri, Siân Culley, Uwe Schmidt

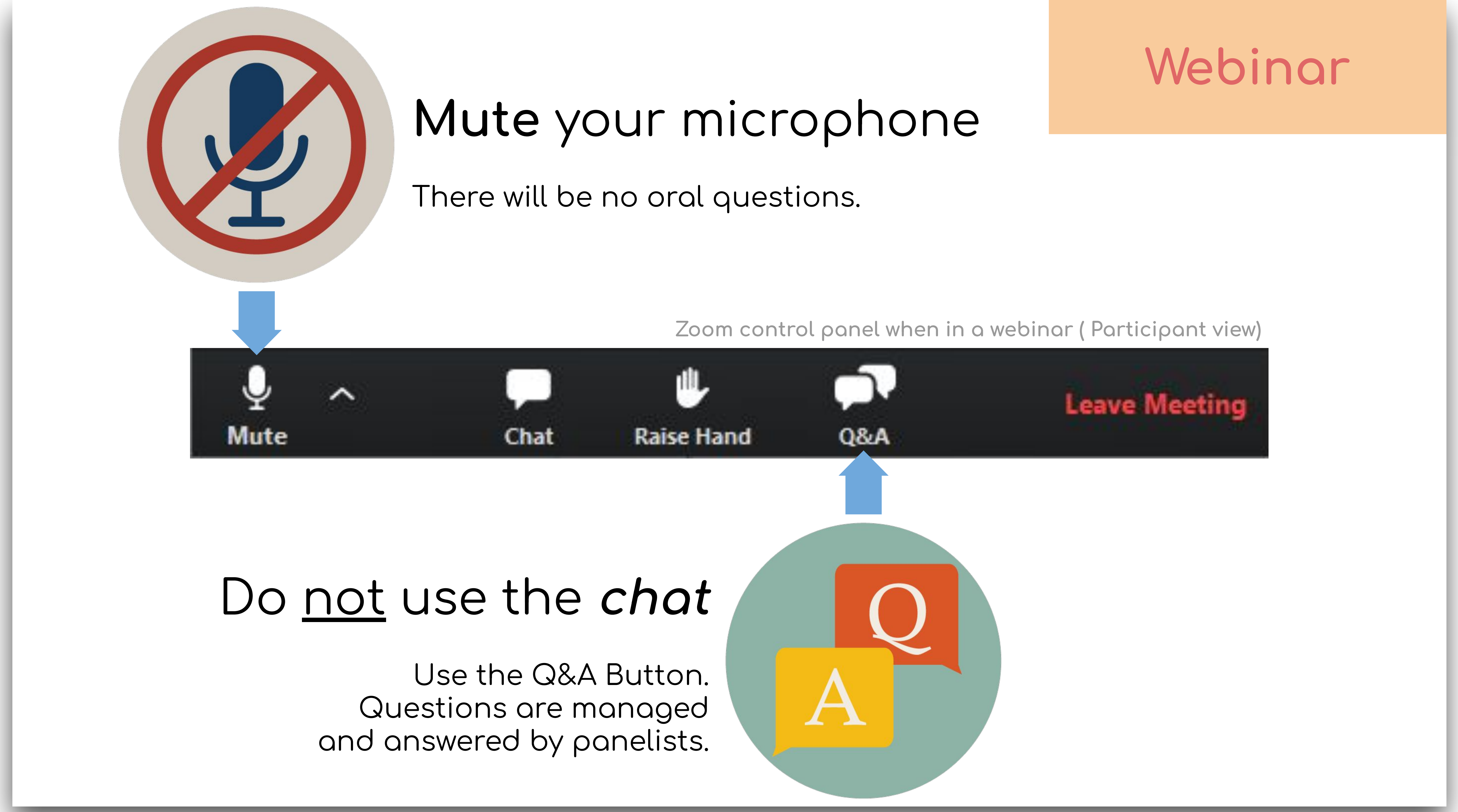
Neubias Academy @ Home, April 28th, 2020

The webinar will start 15:40 CEST

If you are already here:

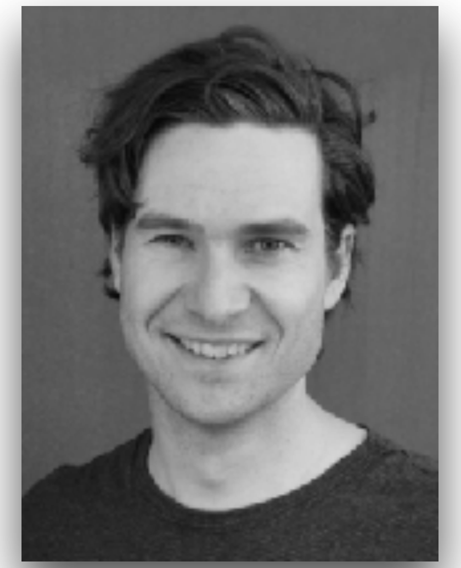
1. Please follow the instructions to the right

2. Please answer the short questions in the poll
(which should popup on your zoom screen)



Webinar Material: https://github.com/maweigert/neubias_academy_stardist

Speakers/Moderators:



Martin Weigert
EPFL, Lausanne
@martweig

Uwe Schmidt
MPI-CBG, Dresden
@uschmidt83

Olivier Burri
EPFL, Lausanne
@ChiguireKun

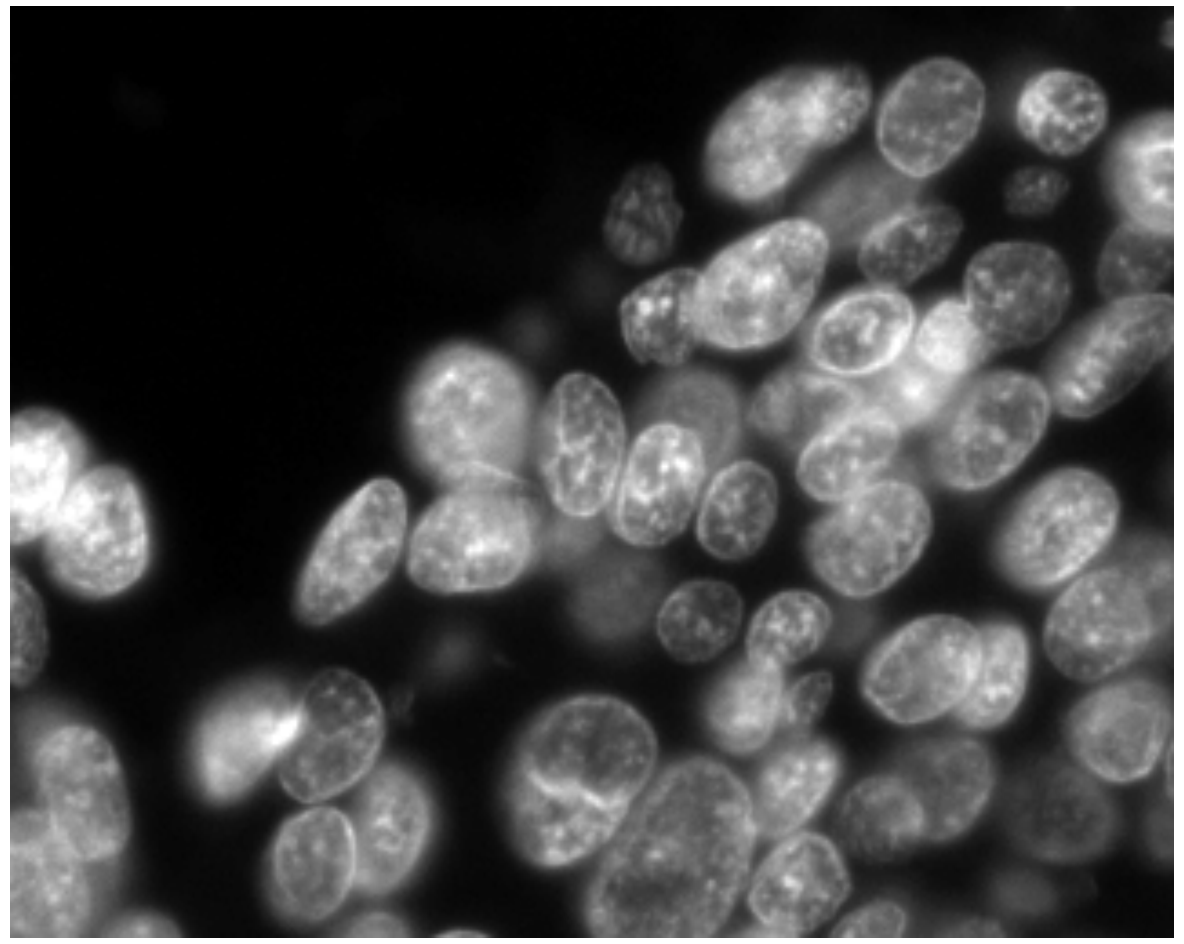
Siân Culley
UCL, London
@SuperResoluSian

1. Introduction to nuclei segmentation and StarDist
2. Questions & Answers 1
3. How to use StarDist
4. StarDist in a core facility
5. Questions & Answers 2

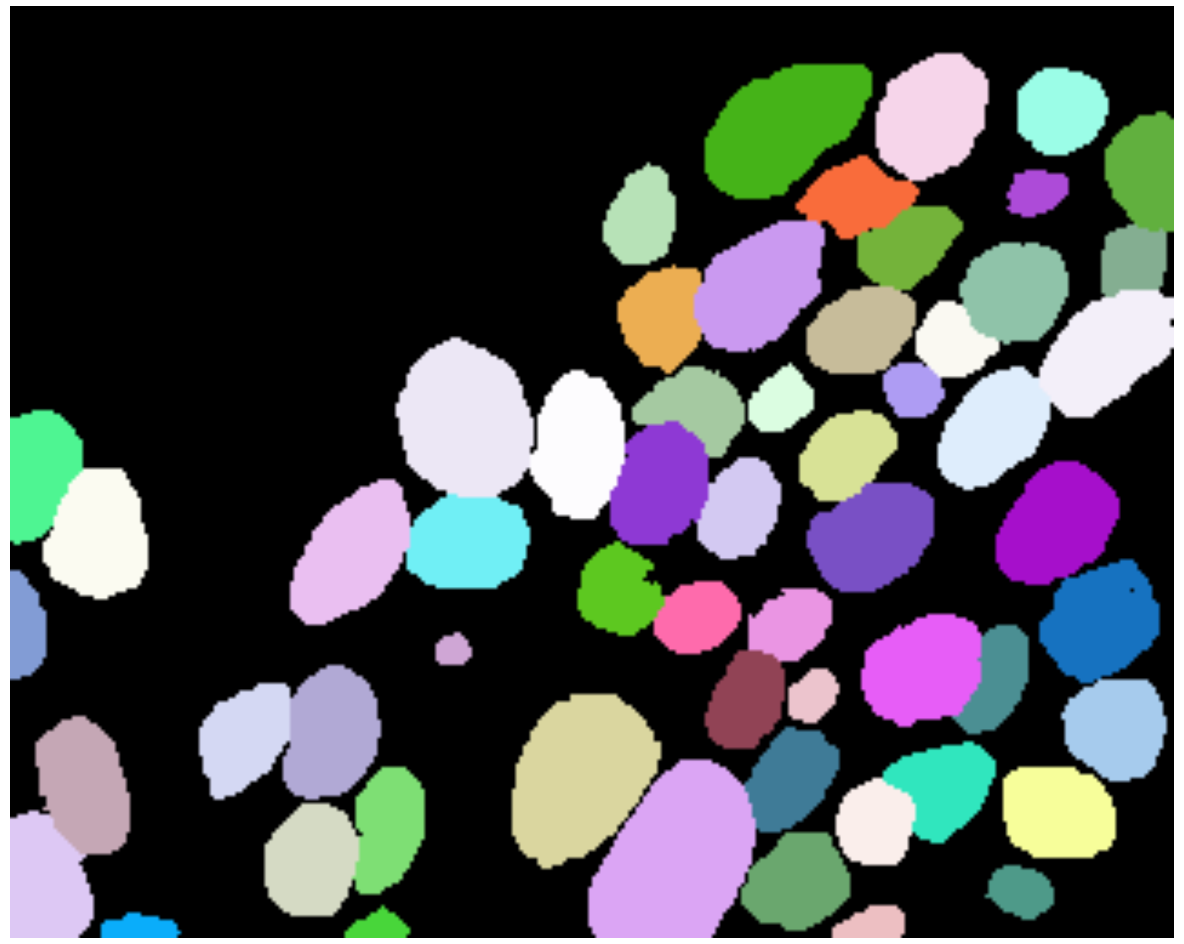
Introduction to nuclei segmentation and StarDist

Nuclei Segmentation in Microscopy

Microscopy Image
with stained nuclei



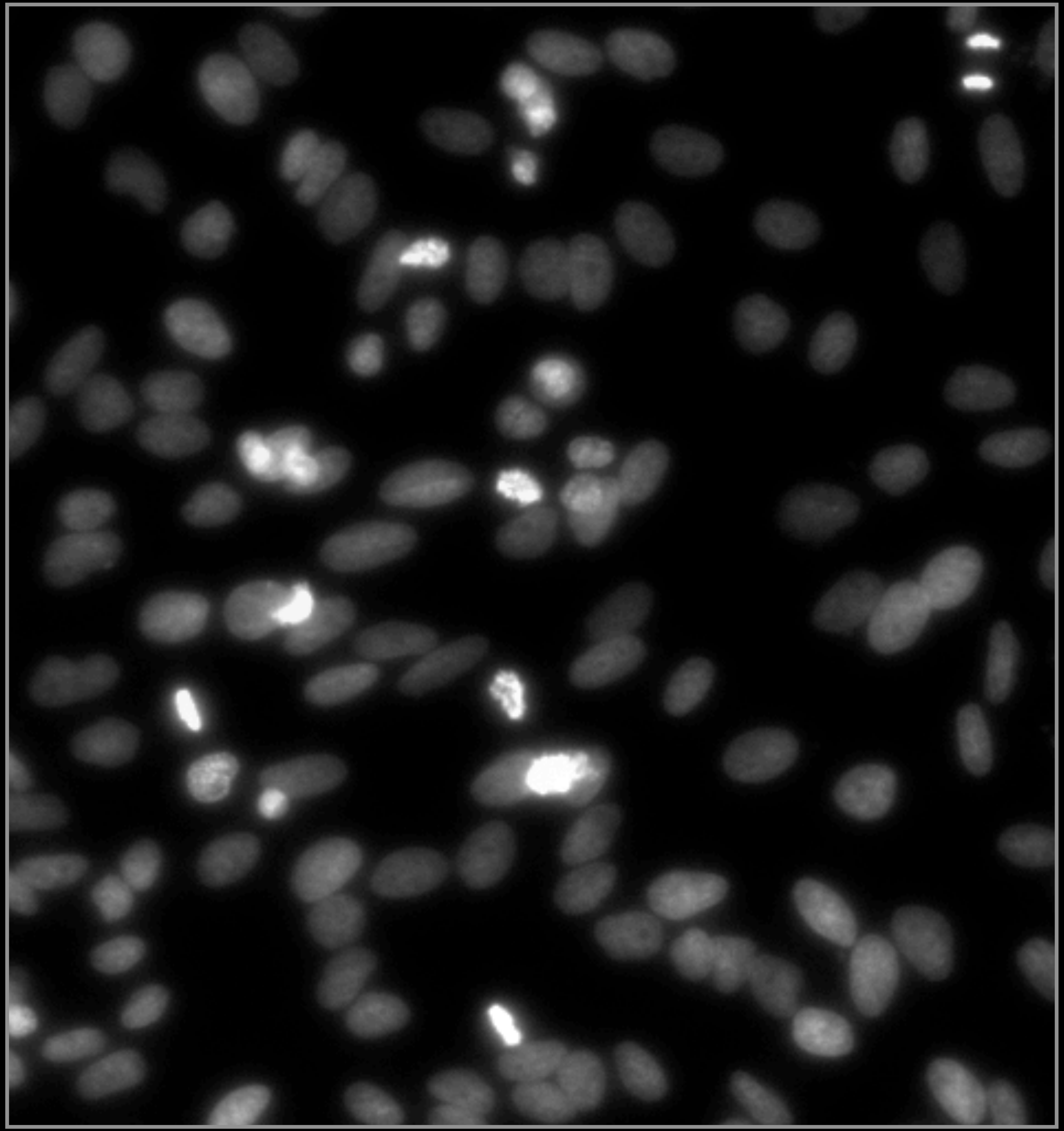
Segmentation/Detection
of each nucleus



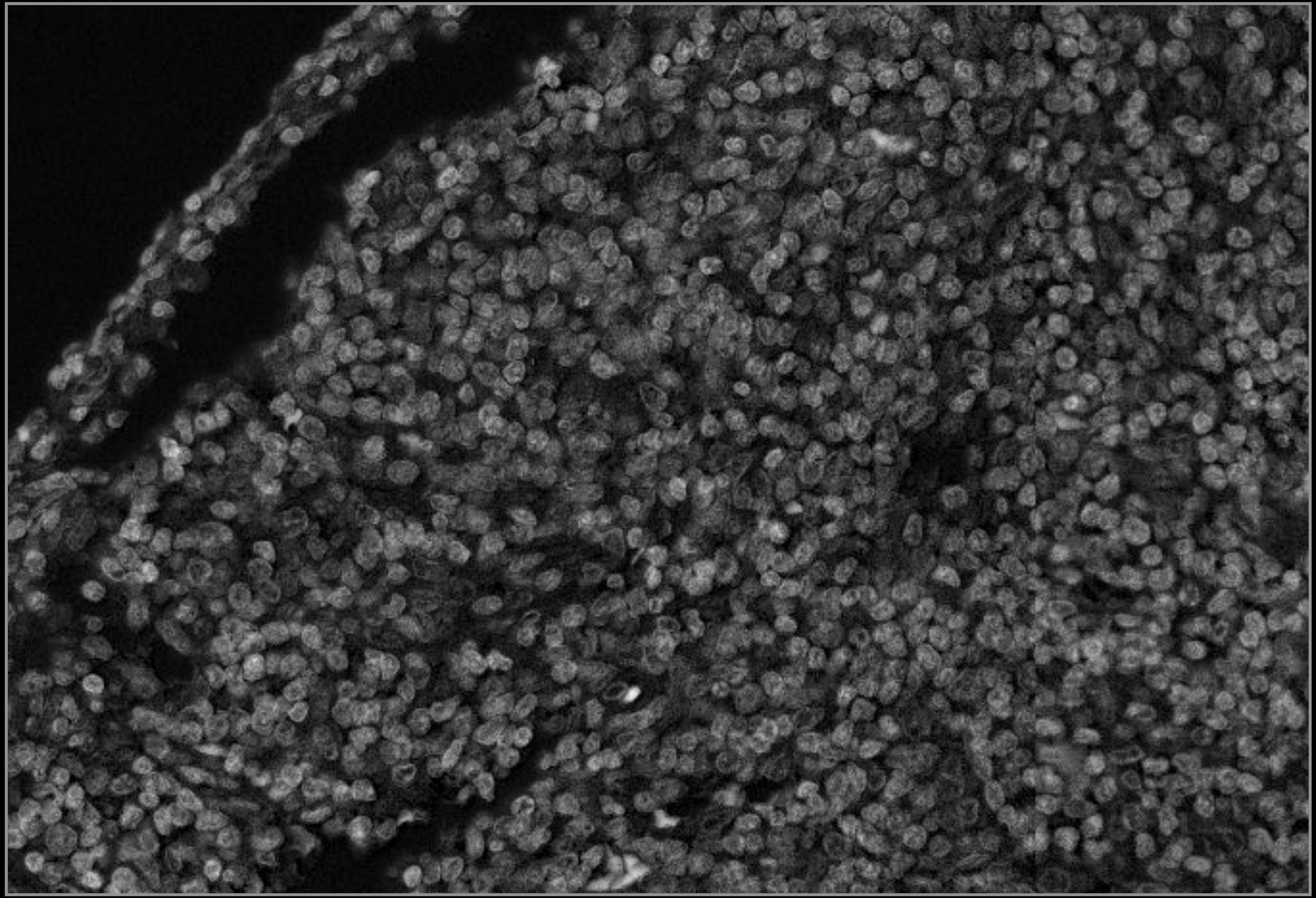
- Cell phenotypes, shapes, sizes
- Cell gene expression differences
- Lineage tracing/tracking in developing organisms

Typical Data in Microscopy

2D - Fluorescence



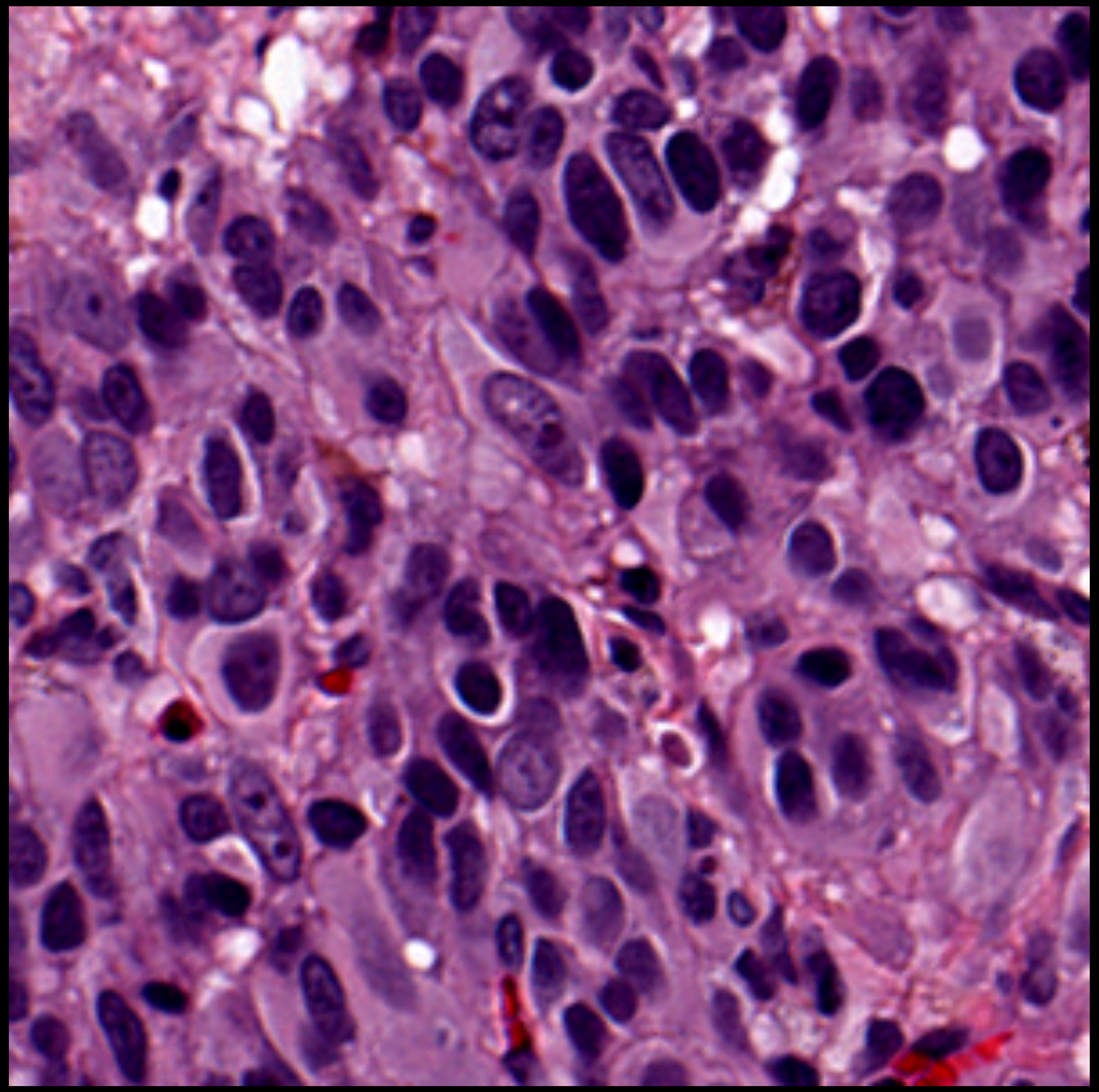
Data from Caicedo et al, 2019



Data from Anna Maria Tsakiroglou (Manchester)

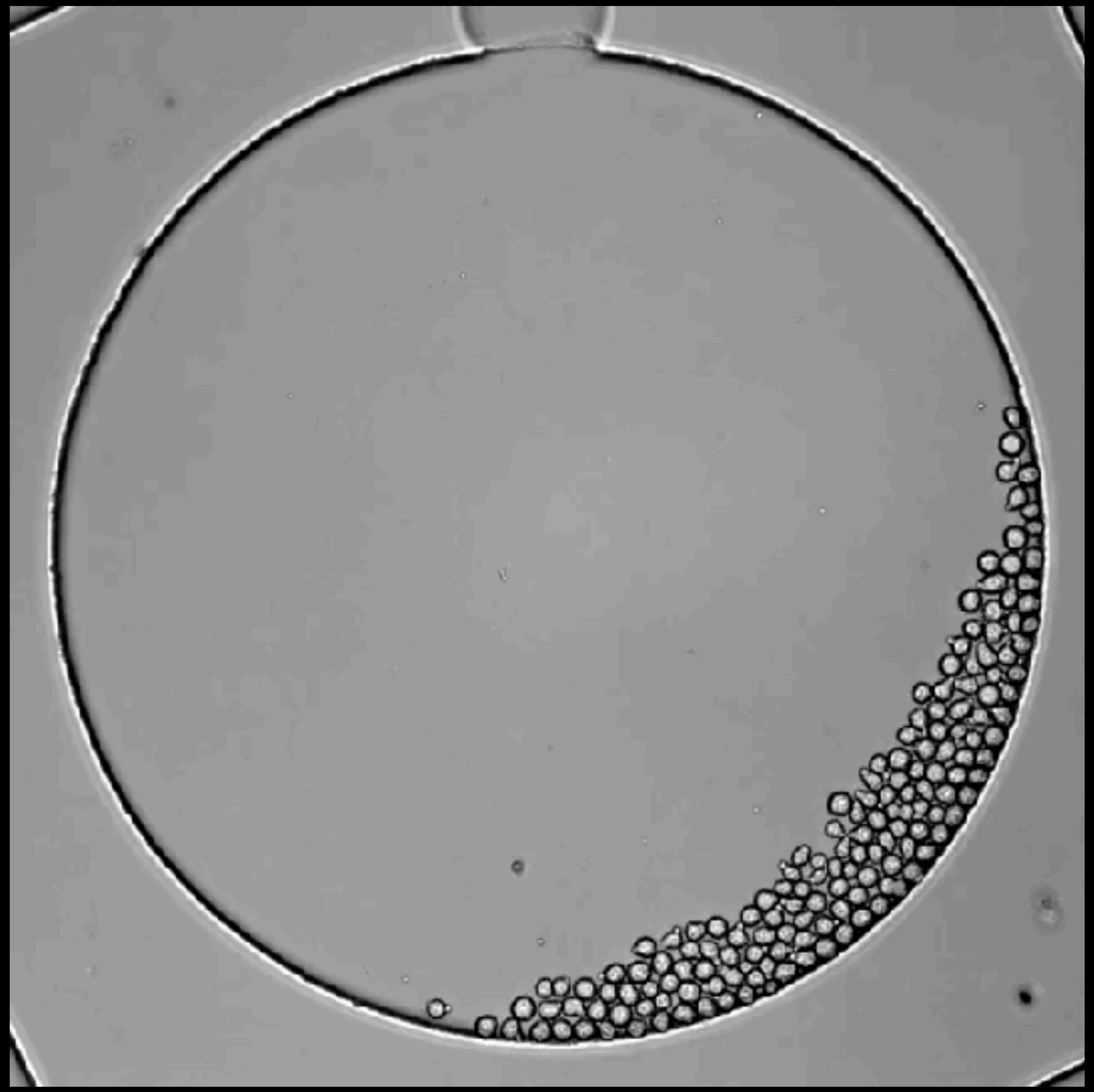
Typical Data in Microscopy

2D - RGB/Histopathology



H&E stain, data from CancerImagingArchive

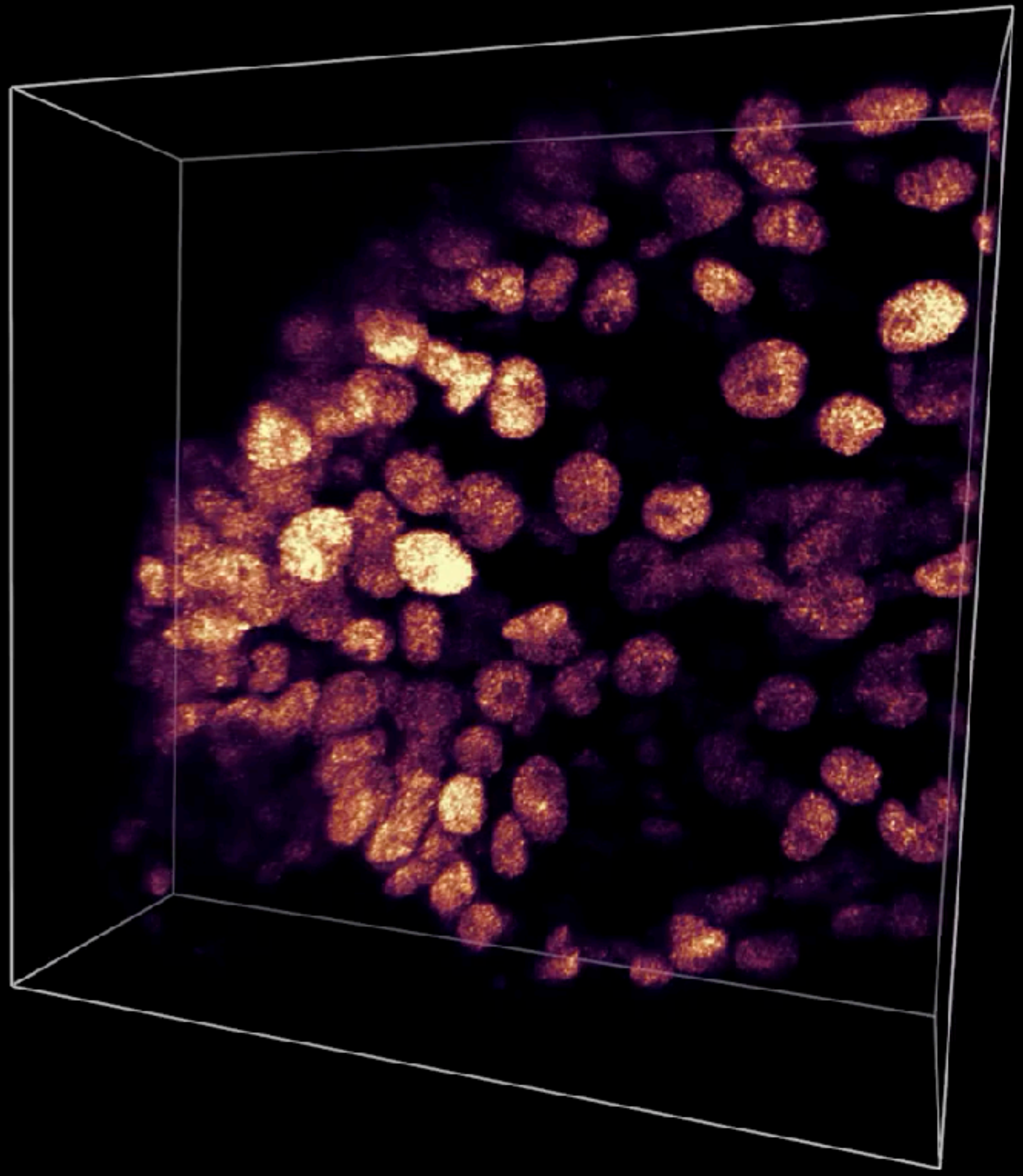
2D + time (Brightfield)



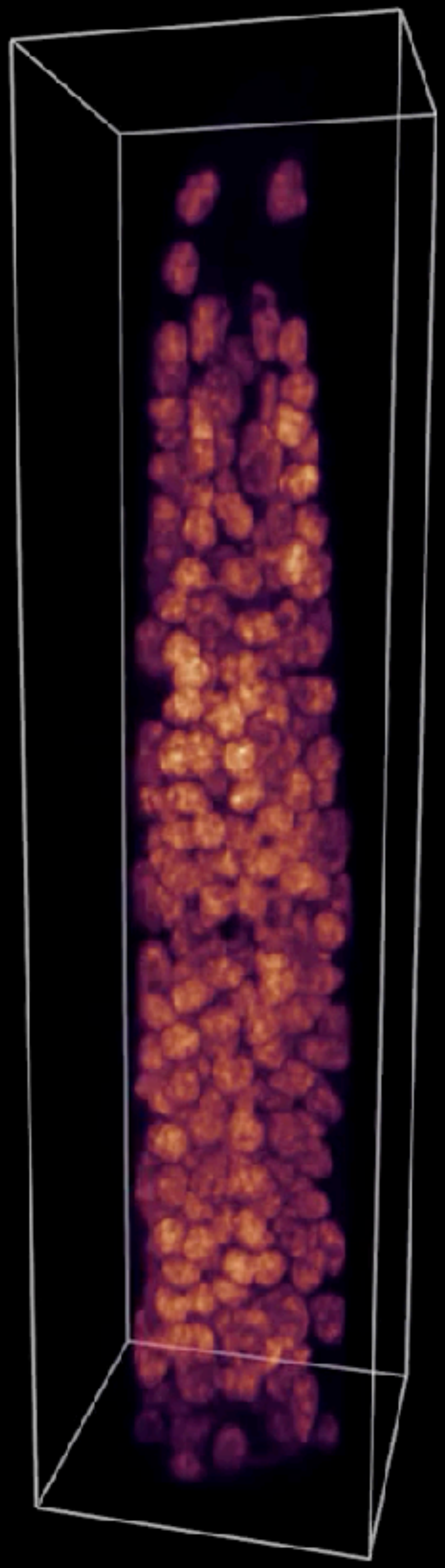
Mouse stem cells, data from cell tracking challenge

Typical Data in Microscopy

3D (+time)



Parhyale Data from Ko Sugarawa

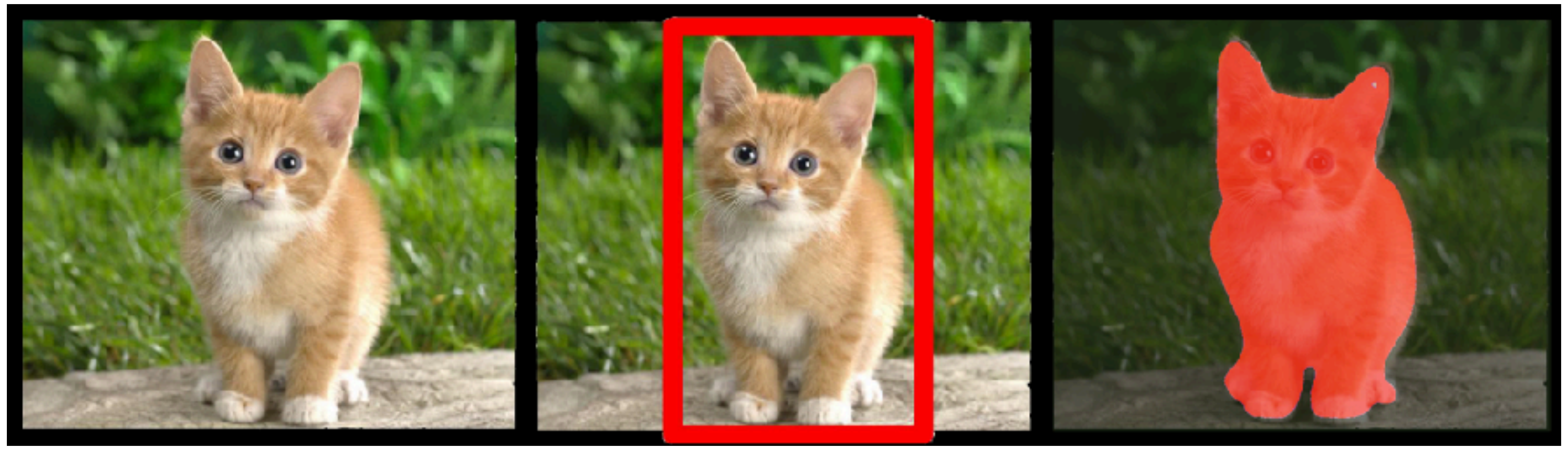


C elegans Data from Dagmar Kainmüller

Computer Vision - Common Problems

Single Object

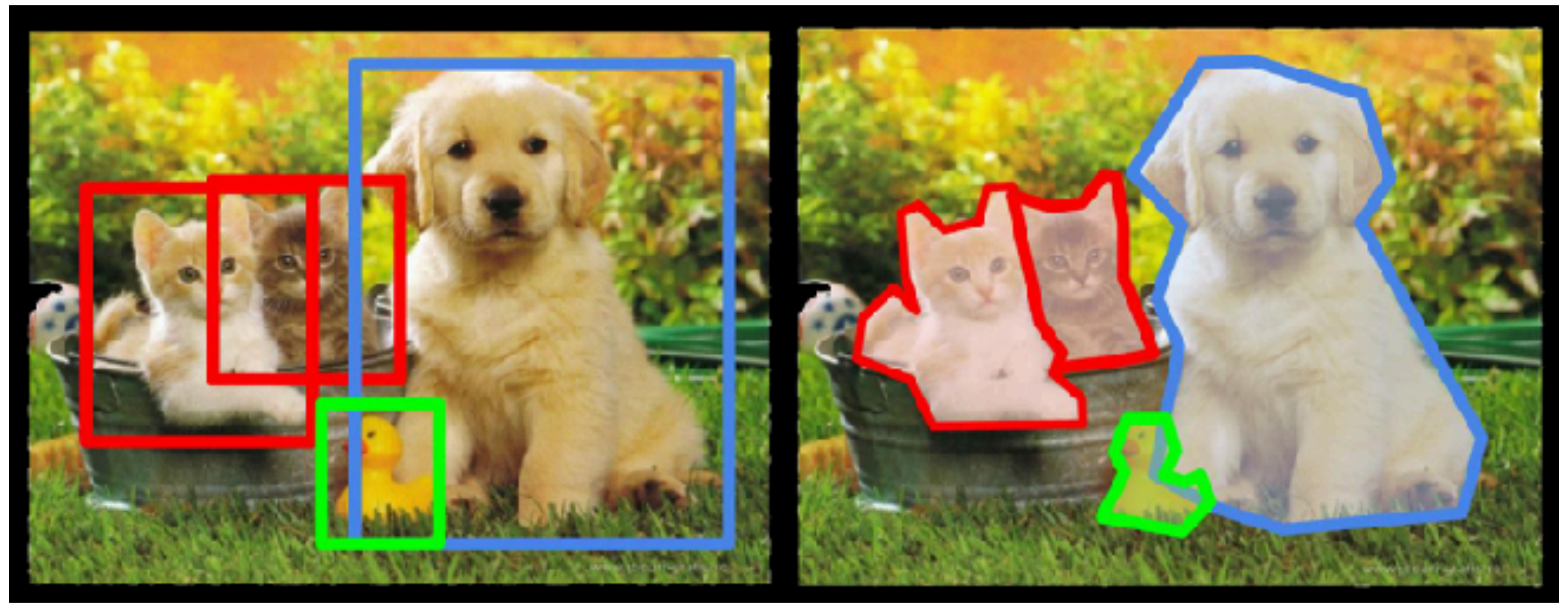
Image Classification	Object Localization	Dense/Semantic Segmentation



Cat Cat Cat

Multiple Objects

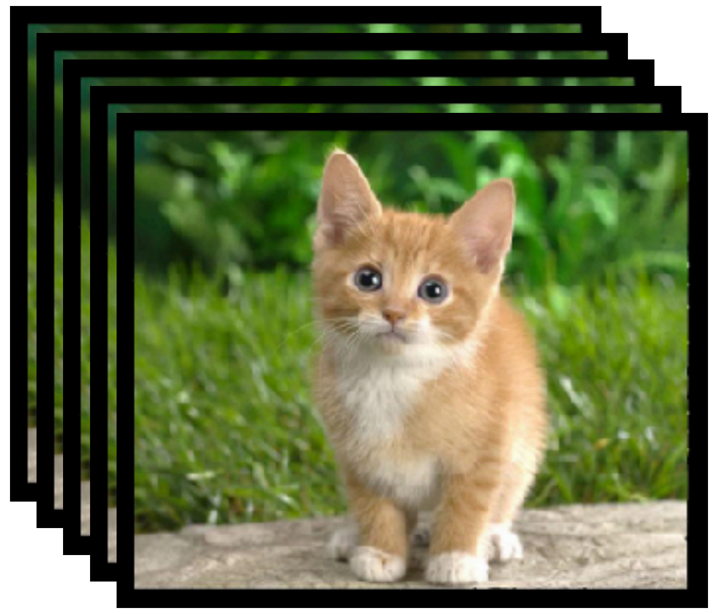
Object Detection	Instance Segmentation



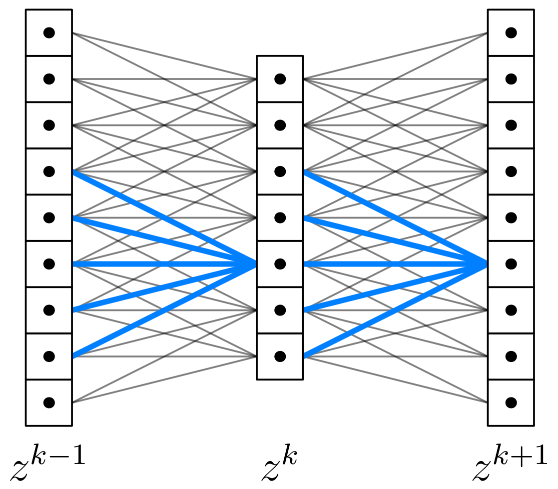
Cat, Dog, Duck Cat, Dog, Duck

Currently the most successful paradigm: Supervised deep learning

Input



Deep Neural Network

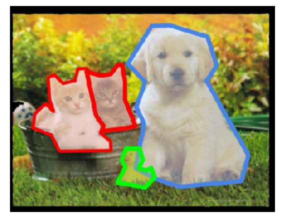


Output

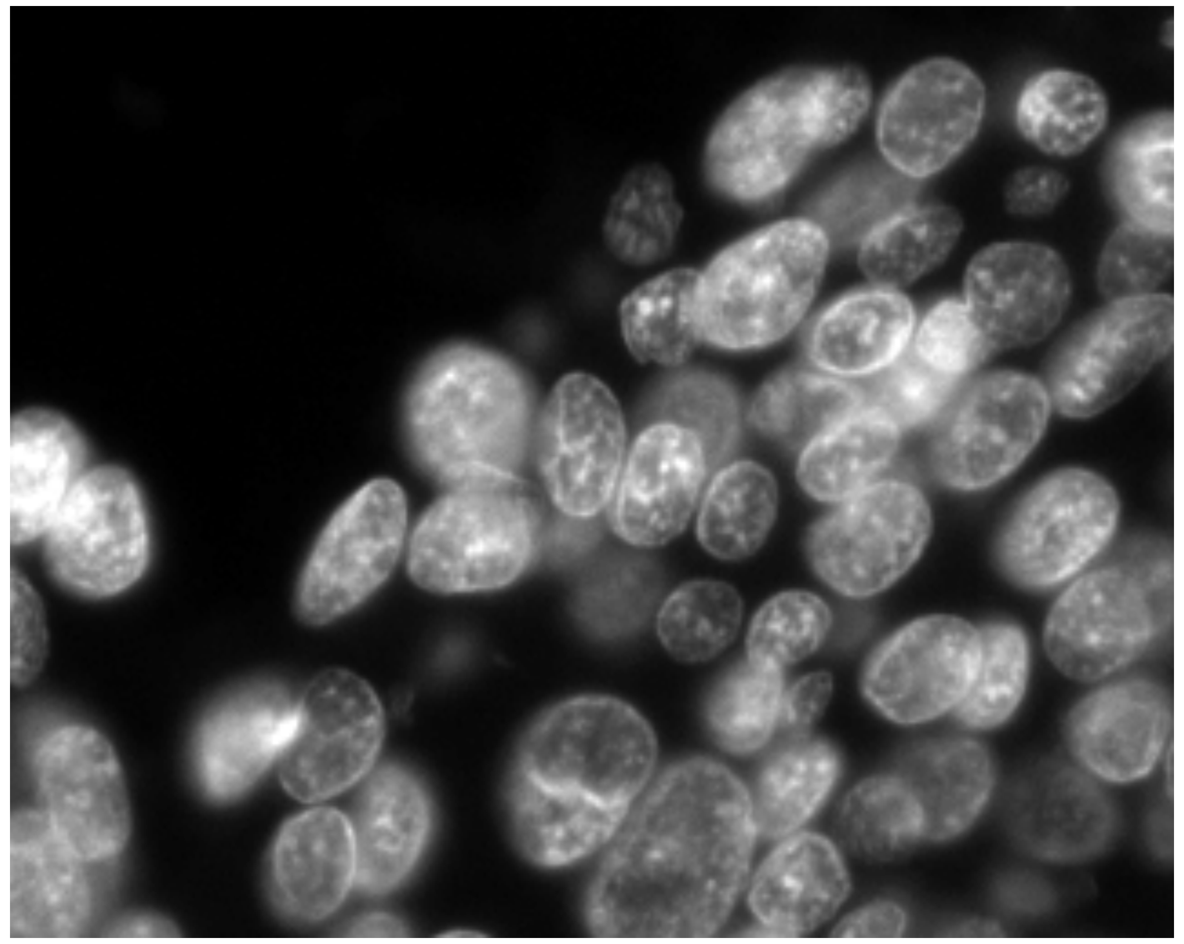
U-Net Ronneberger et al (2015)
YOLO Redmond et al (2016)
Mask-RCNN He et al (2017)

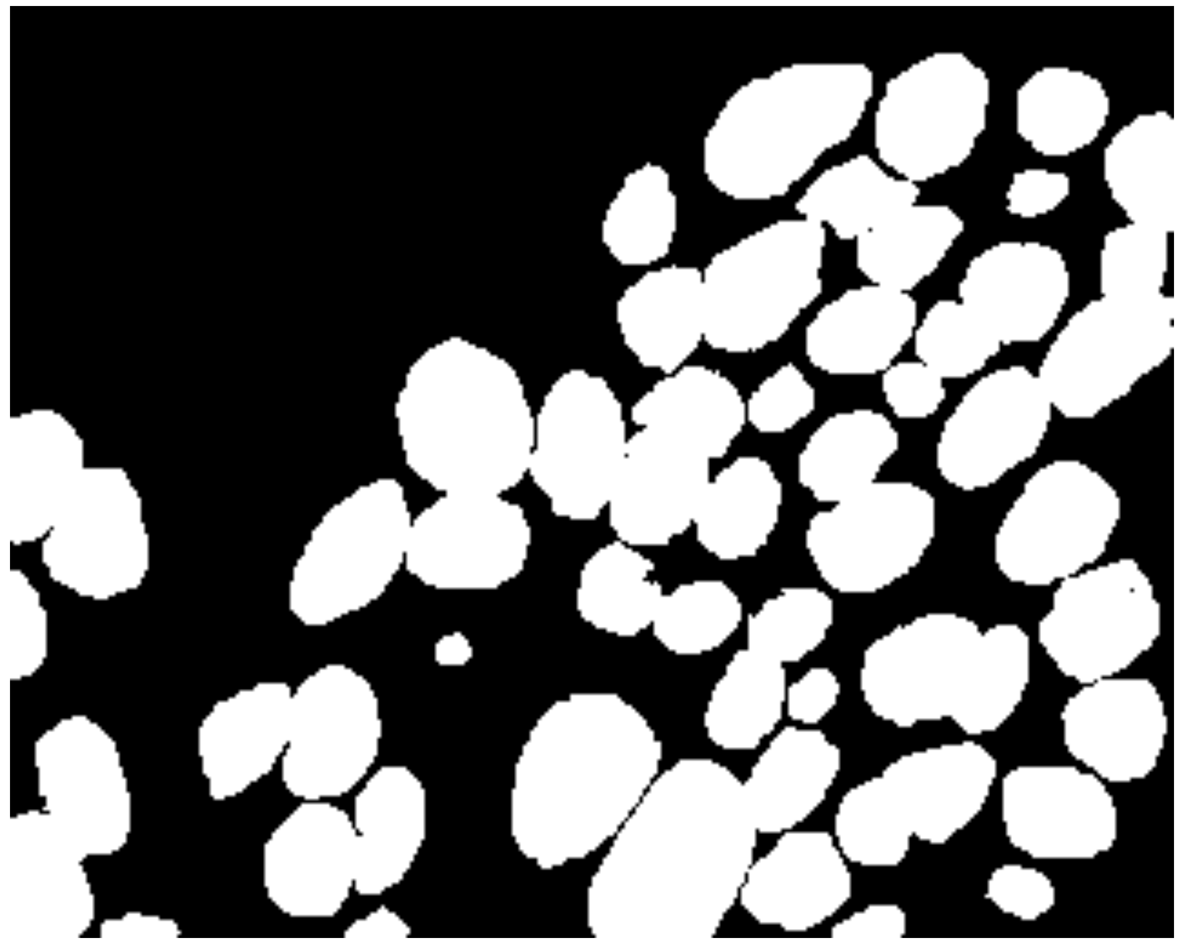
Deep (Convolutional) Neural Networks trained on annotated training data (ground truth, GT)

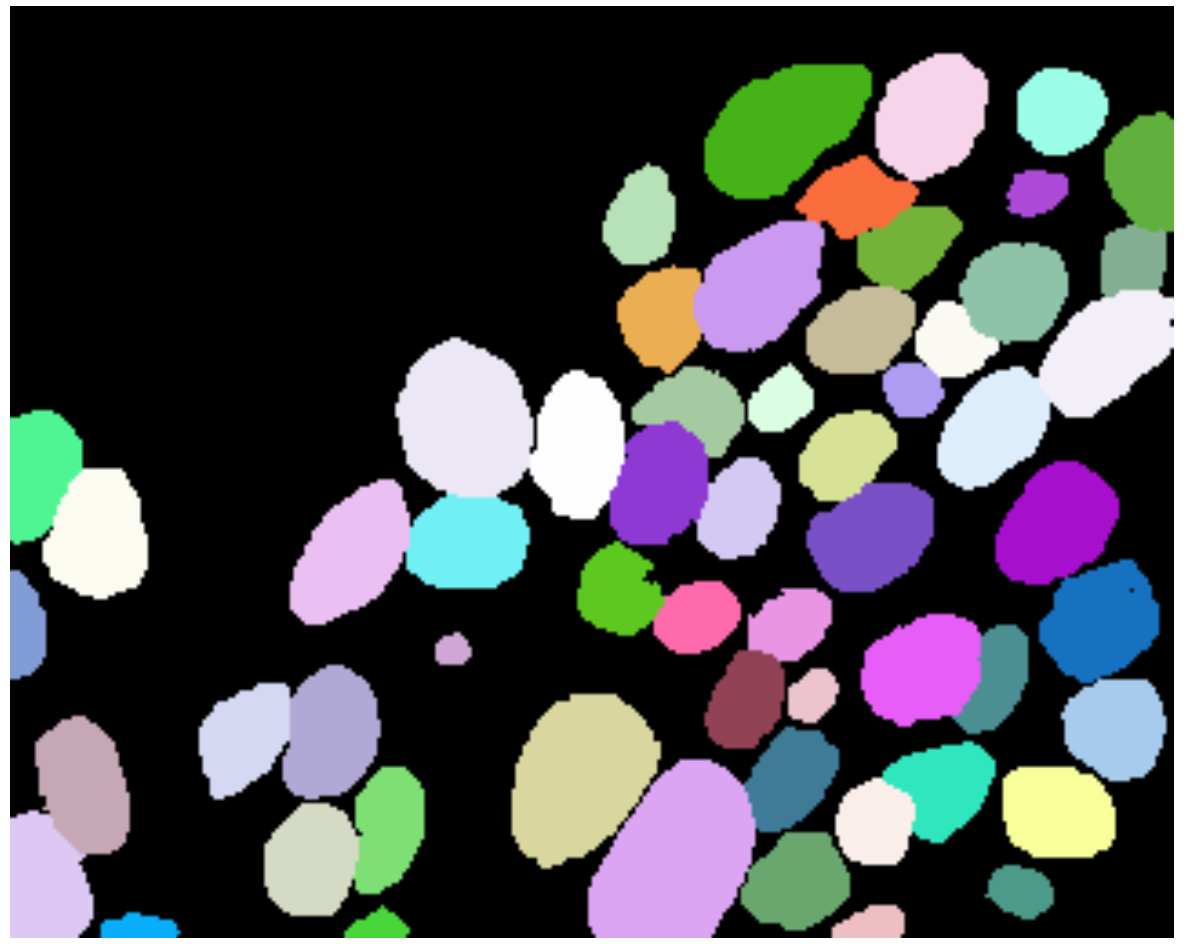
Our Problem: Nuclei Segmentation



Input Image → Dense Segmentation (foreground/background) → Instance Segmentation (individual cells)

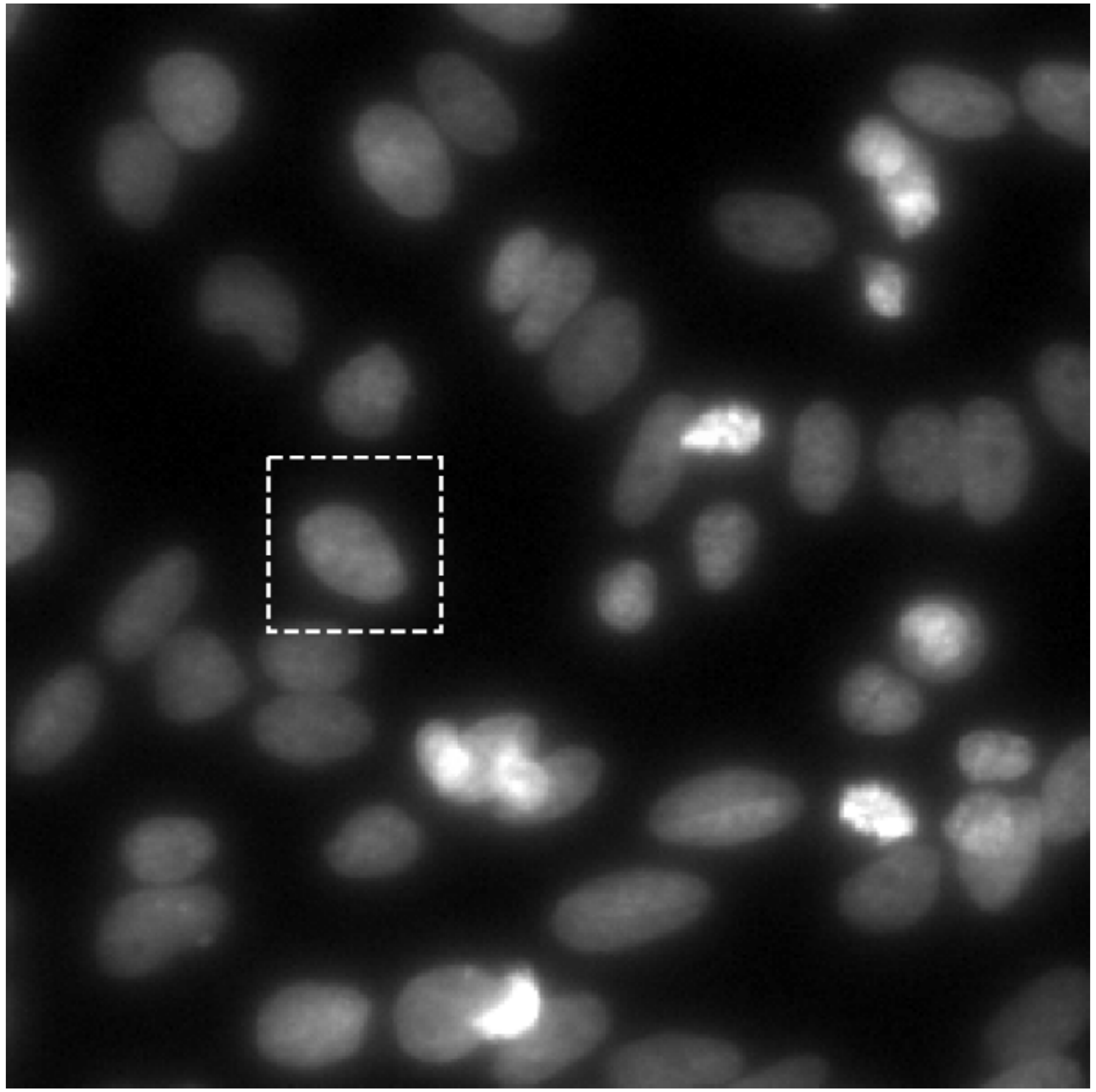






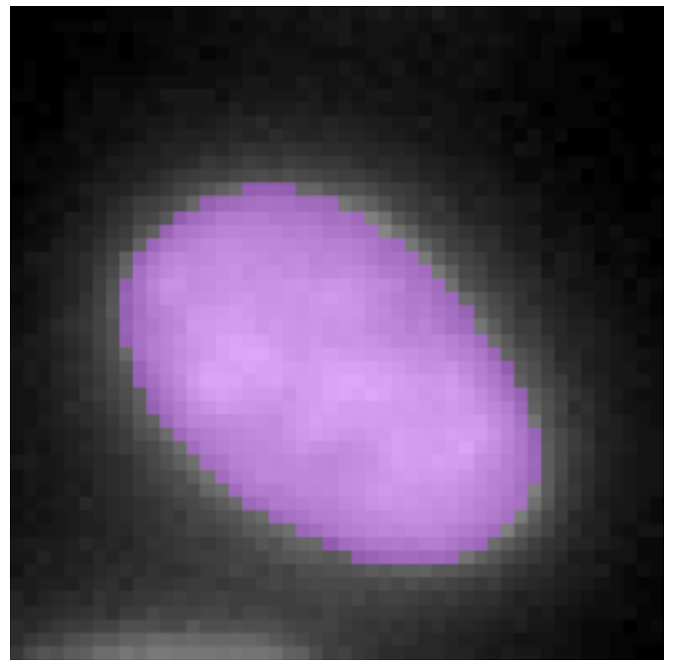
Challenges: many crowded objects, noisy images

Common (DL) approaches for nuclei segmentation



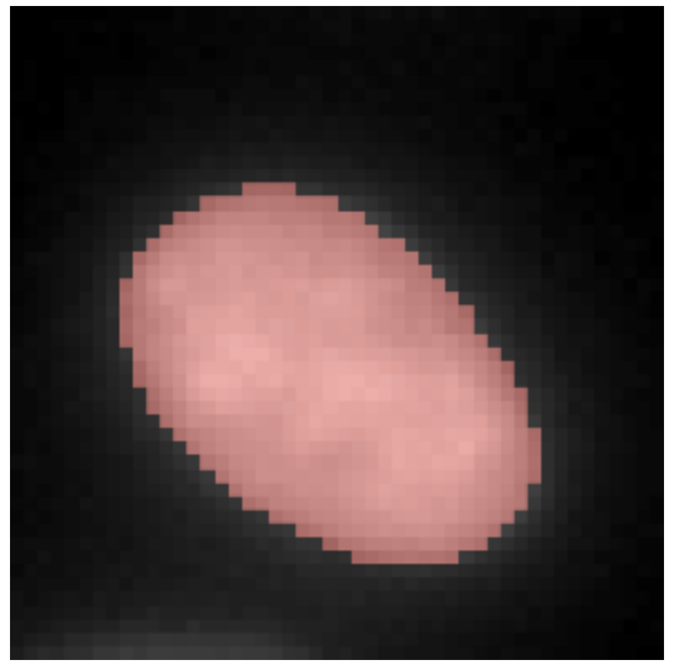
Image

GT



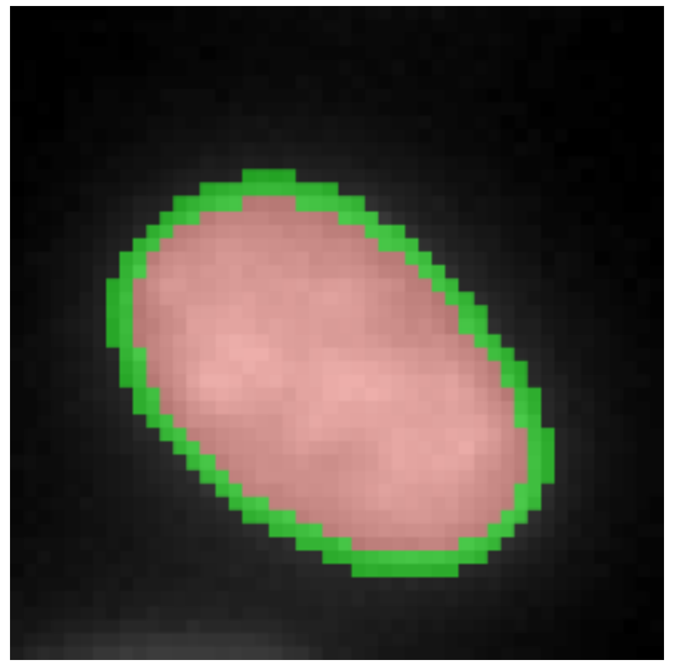
Bottom-Up

First segment, then localize



2 Class U-Net

Ronneberger et al. (2015)

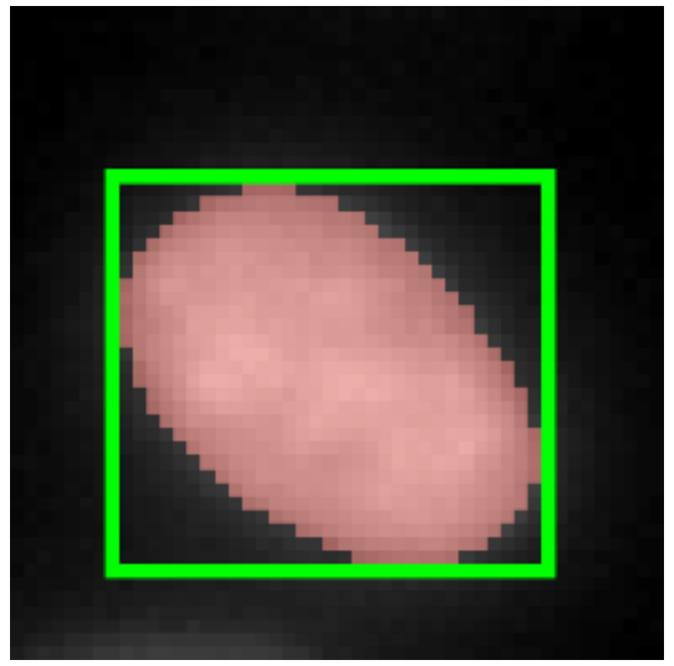


3 Class U-Net

Caicedo et al.(2019)

Top-Down

First localize, then segment



Mask-RCNN

He et al (2017)

- Affinity based methods Wolf, Pape et al. (2018), Hirsch et al. (2020)
- Embedding based methods Neven et al. (2019), Stringer et al. (2020)

Problems for crowded objects

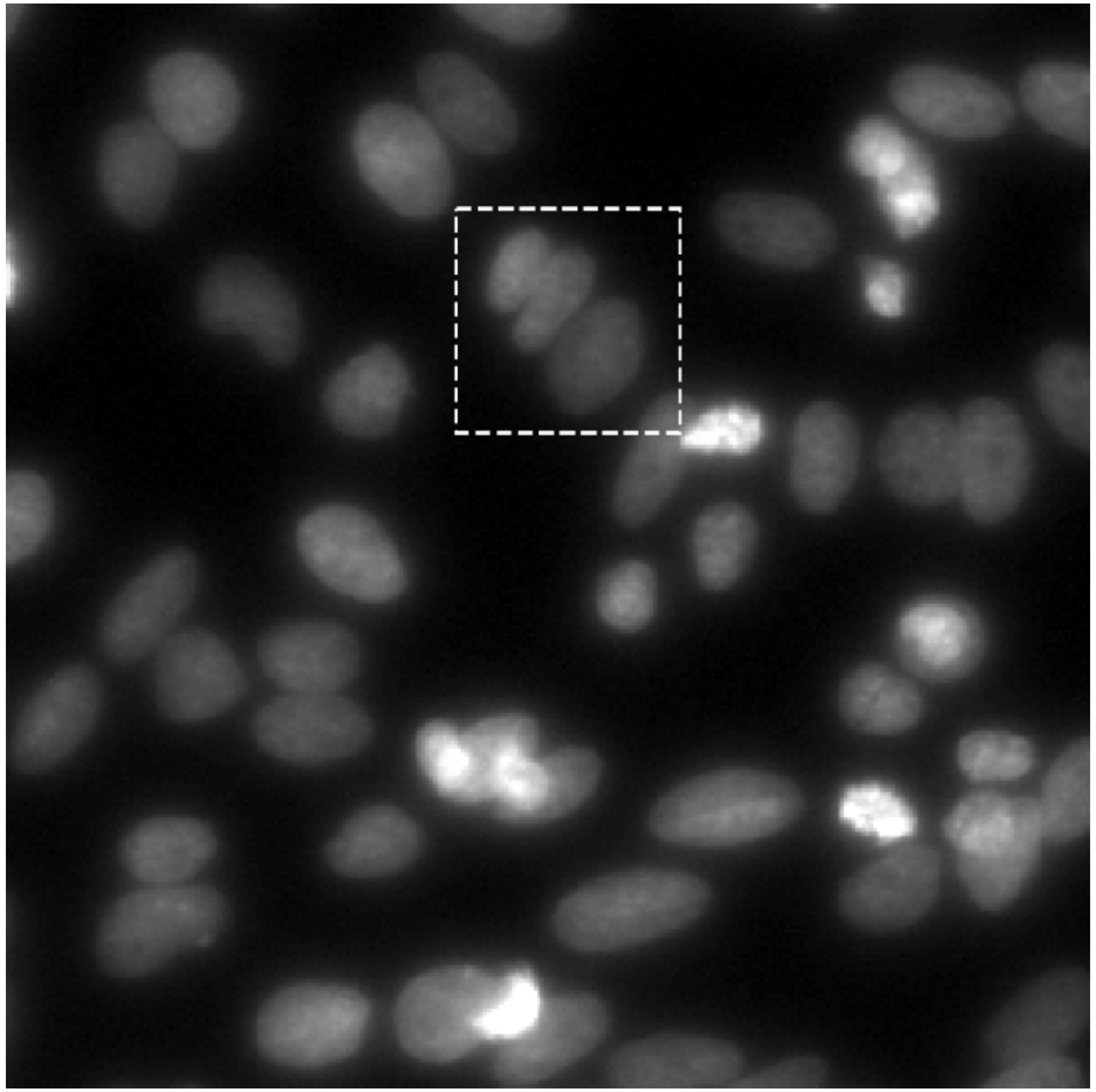
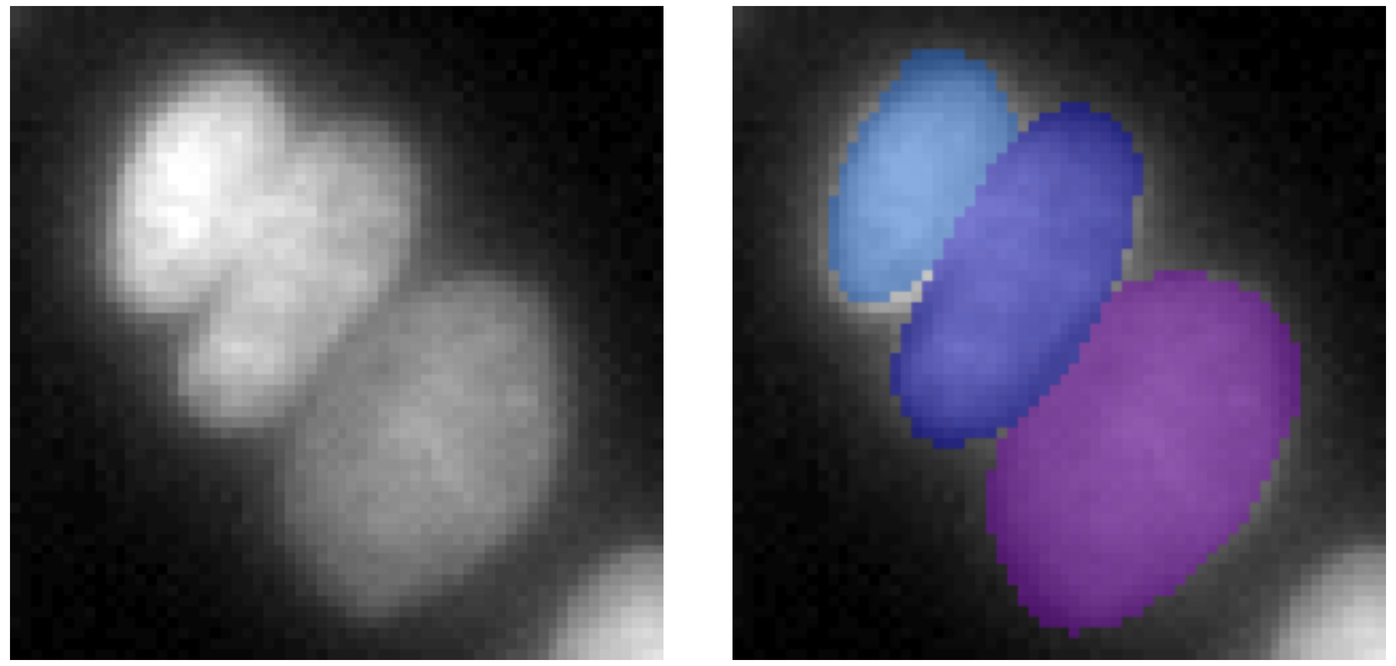


Image GT

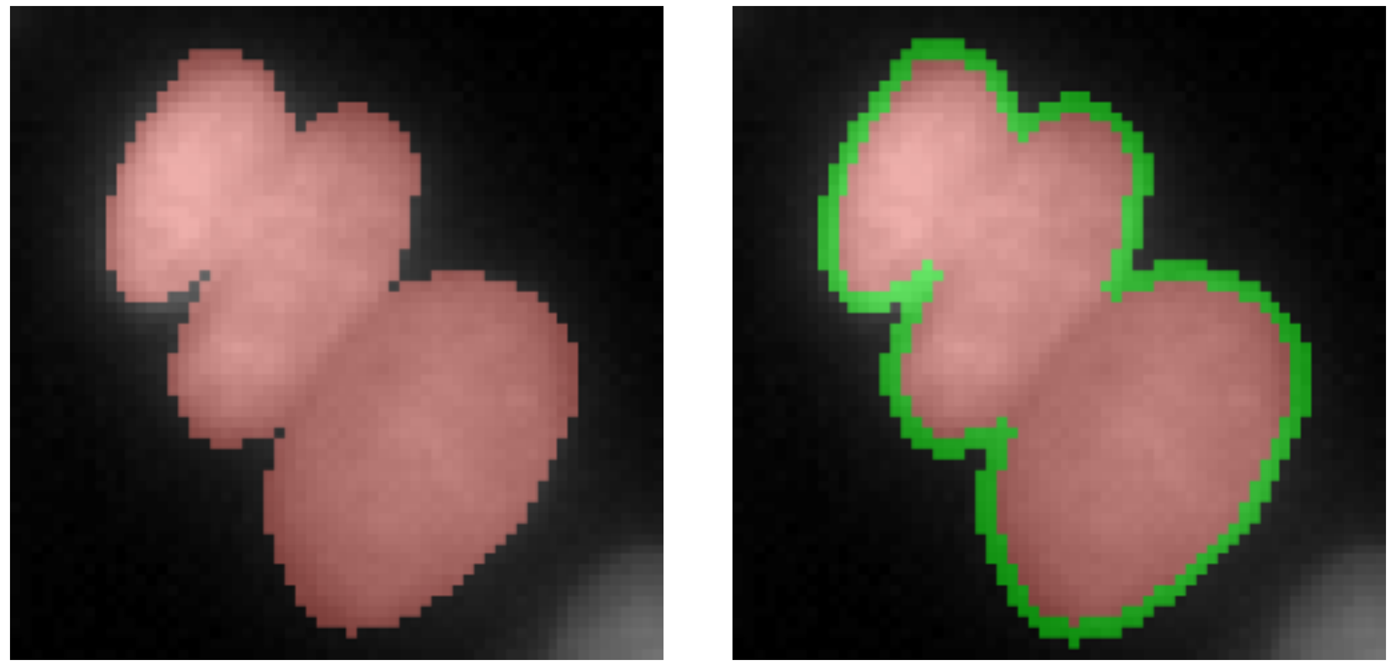


Bottom-Up

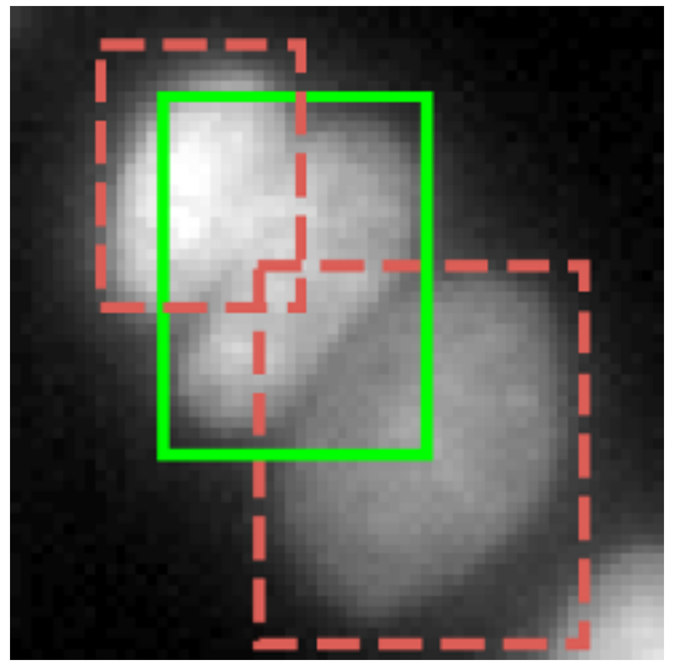
First segment, then localize

Top-Down

First localize, then segment



2 Class U-Net 3 Class U-Net

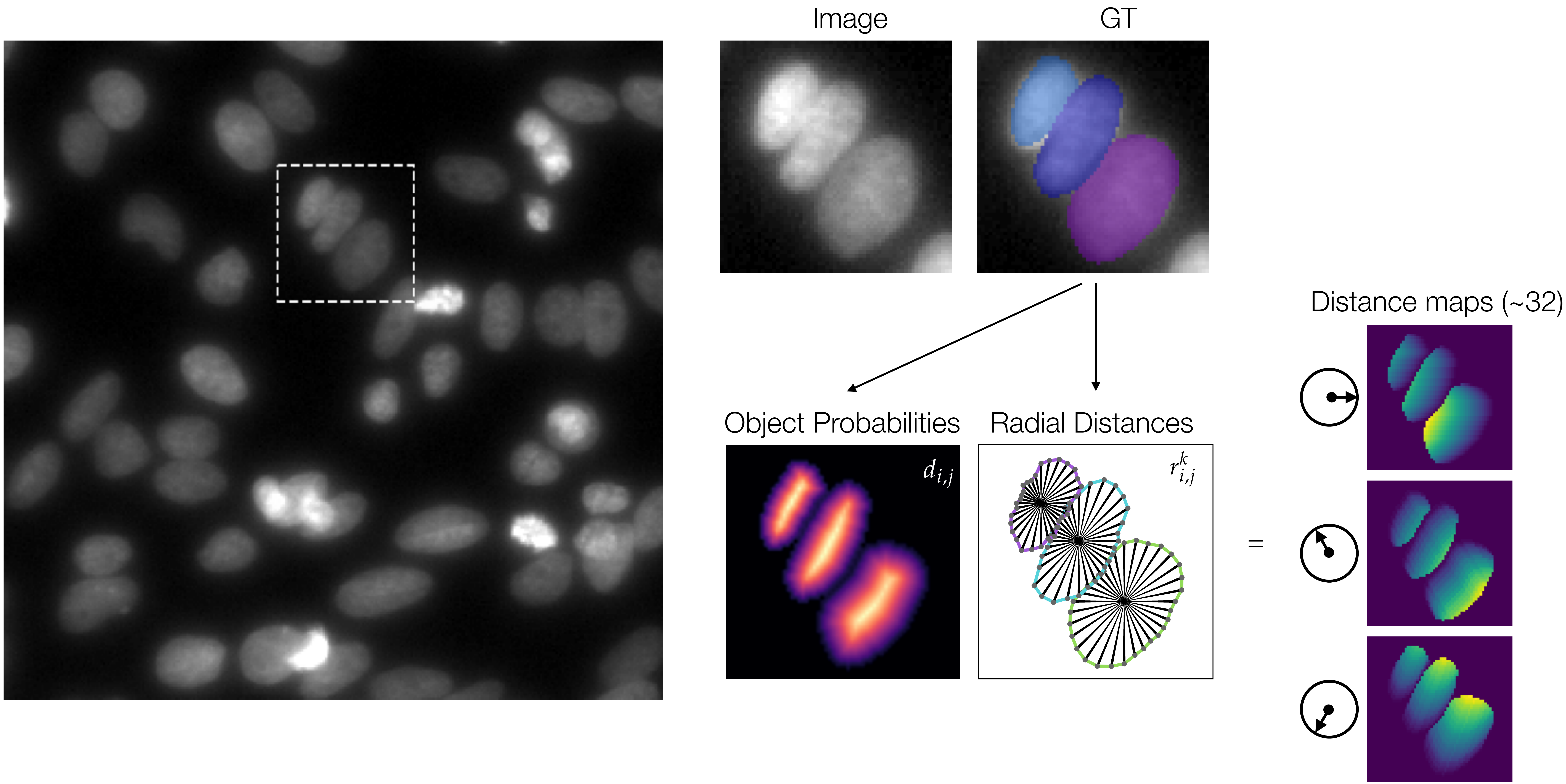


Mask-RCNN

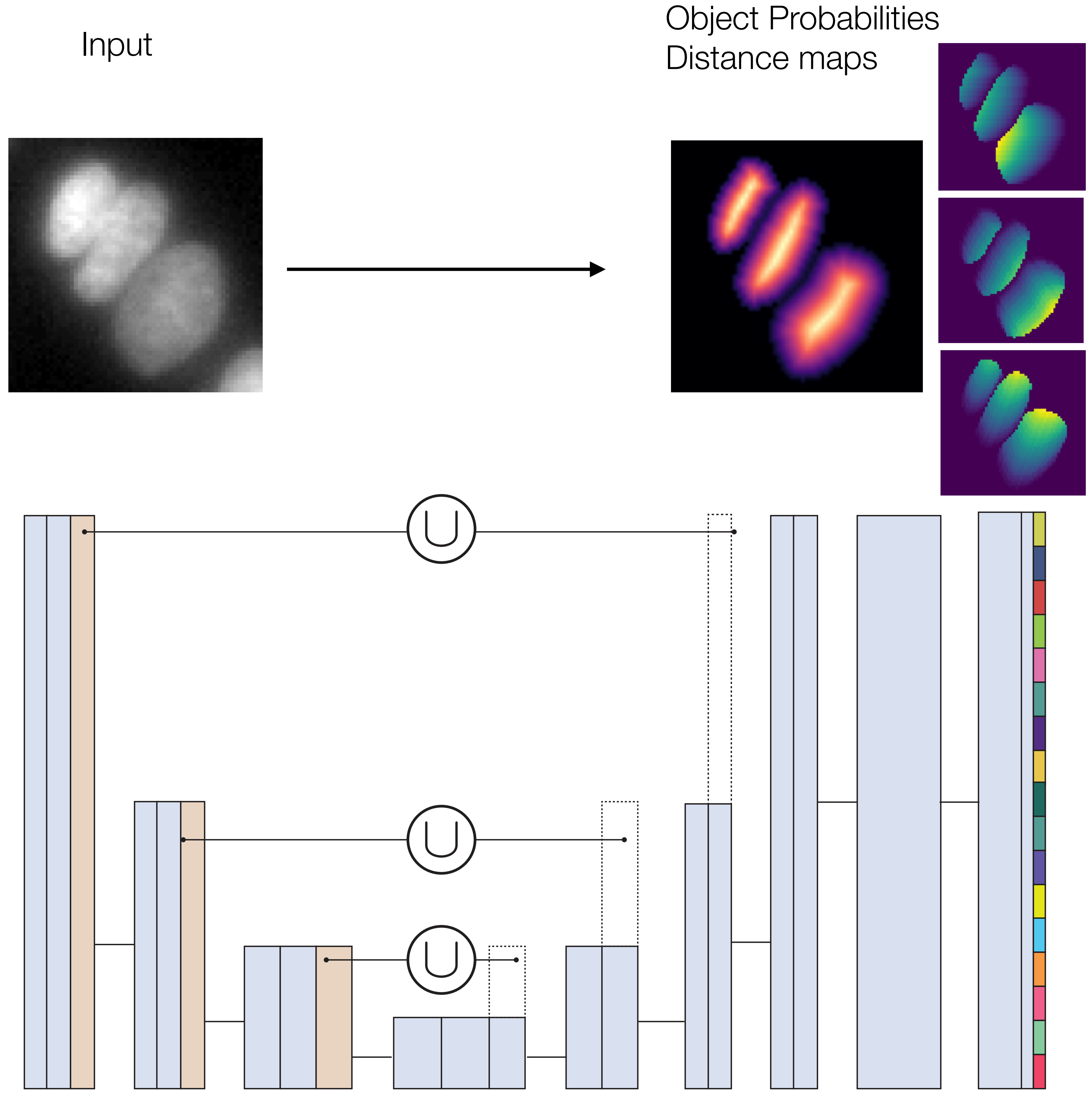
Mislocalization
Fused segmentation maps

Mislocalization
Bounding box overlap > threshold

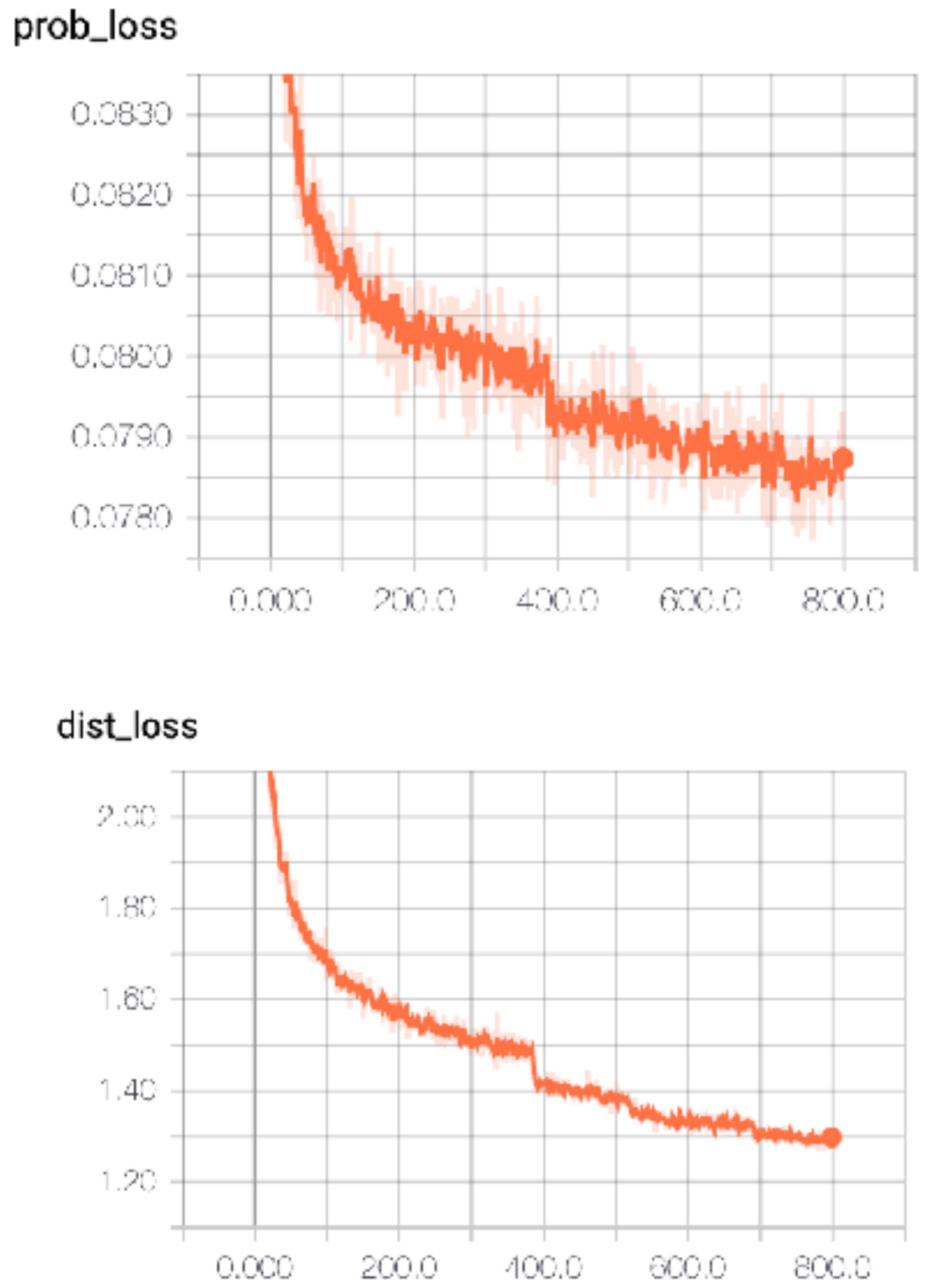
StarDist: Principle



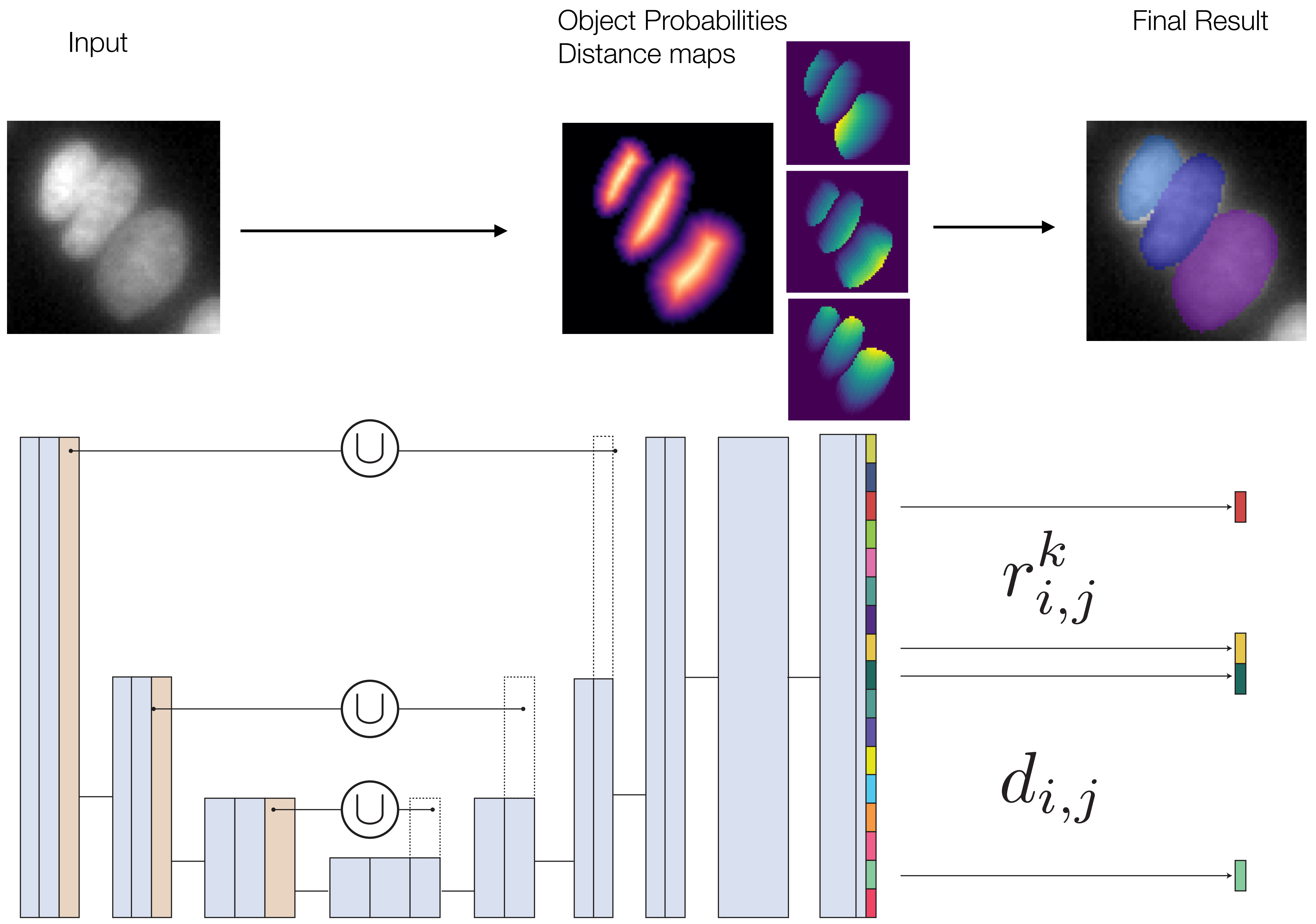
StarDist: Principle



Step 1
Dense Polygon Prediction
via a CNN (e.g. U-Net, ResNet)



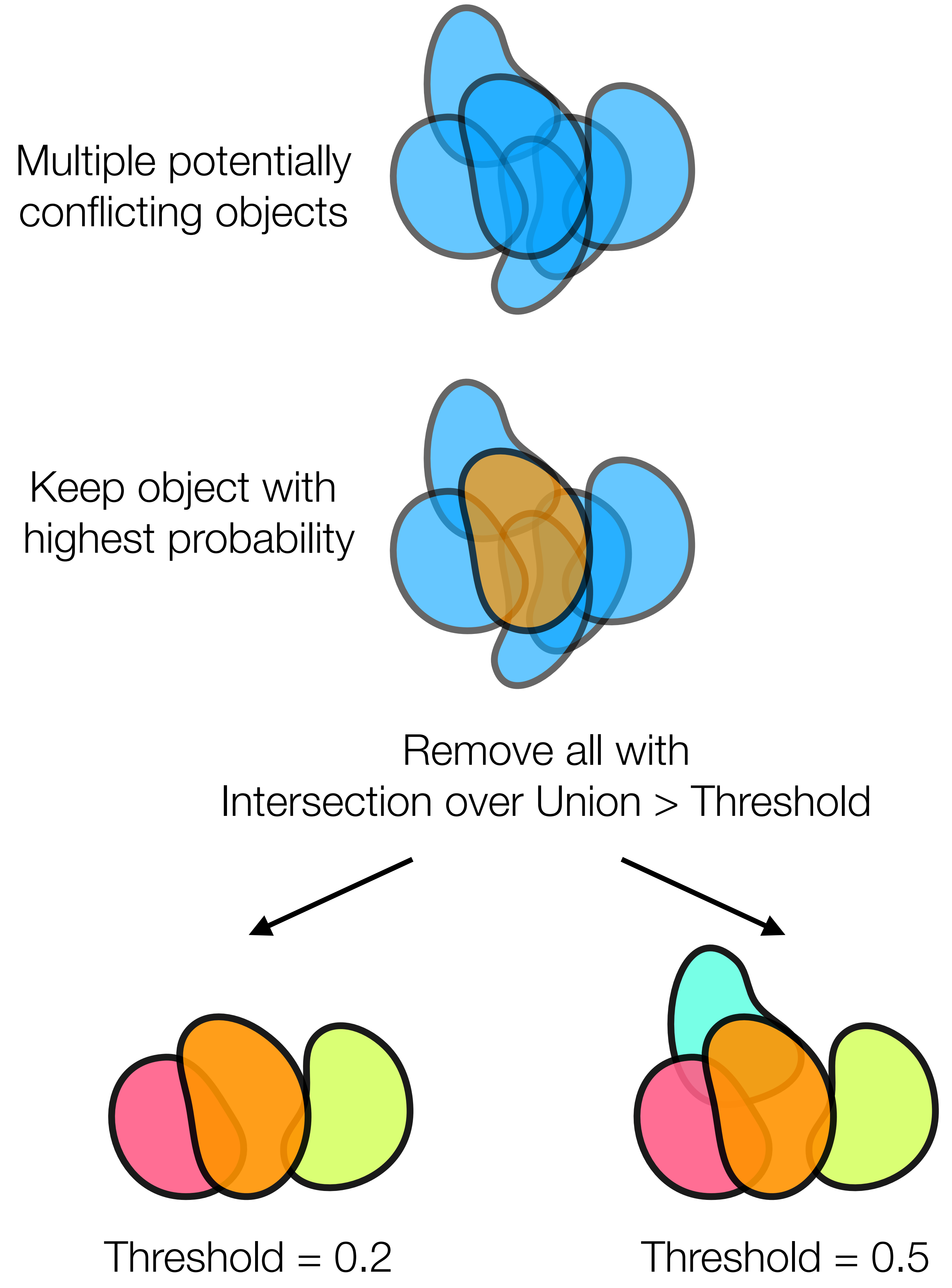
StarDist: Principle



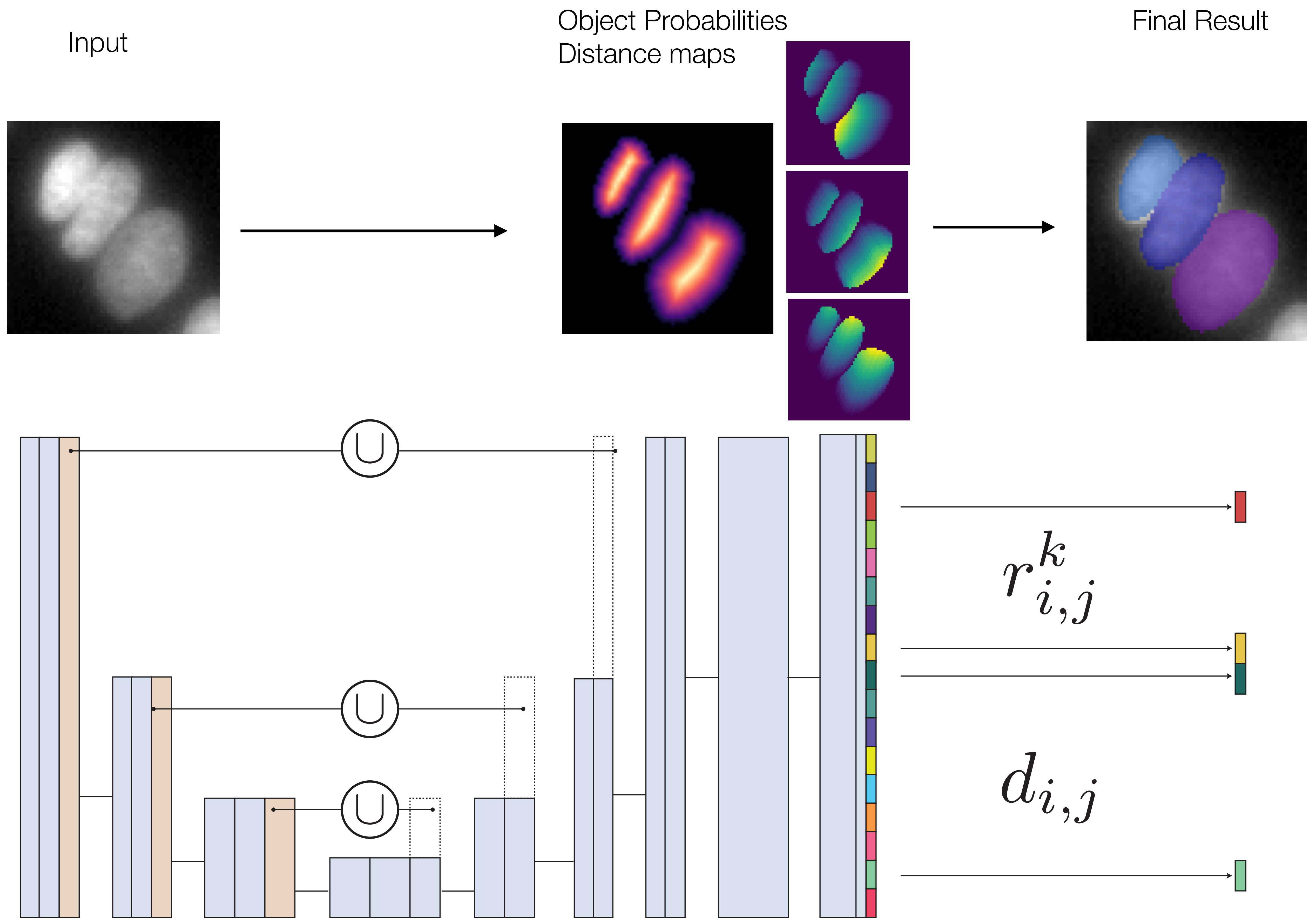
Step 1
Dense Polygon Prediction
(e.g. U-Net, ResNet)

Step 2
Polygon Selection
(Non-Maximum Suppression NMS)

Non-Maximum-Suppression (NMS)



StarDist: Principle

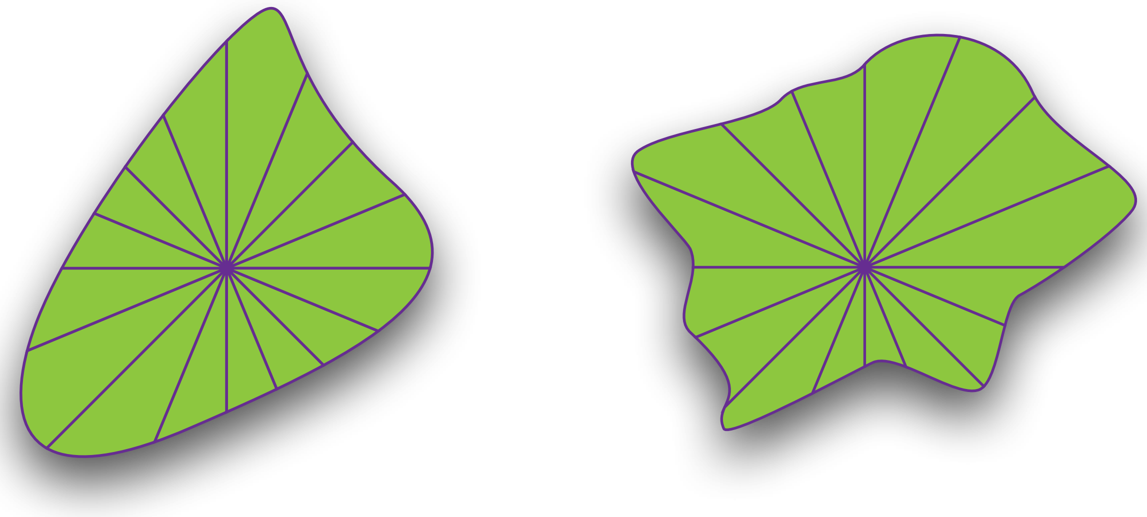


Step 1
Dense Polygon Prediction
(e.g. U-Net, ResNet)

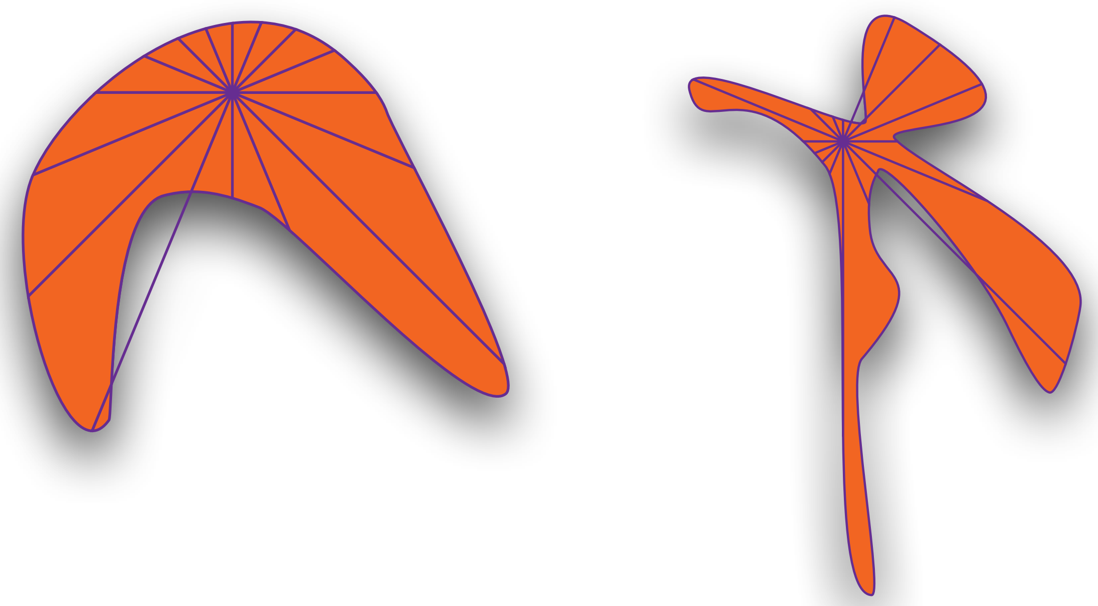
Step 2
Polygon Selection
(Non-Maximum Suppression NMS)

Important:
Assumes objects are *star-convex*

Star-Convex

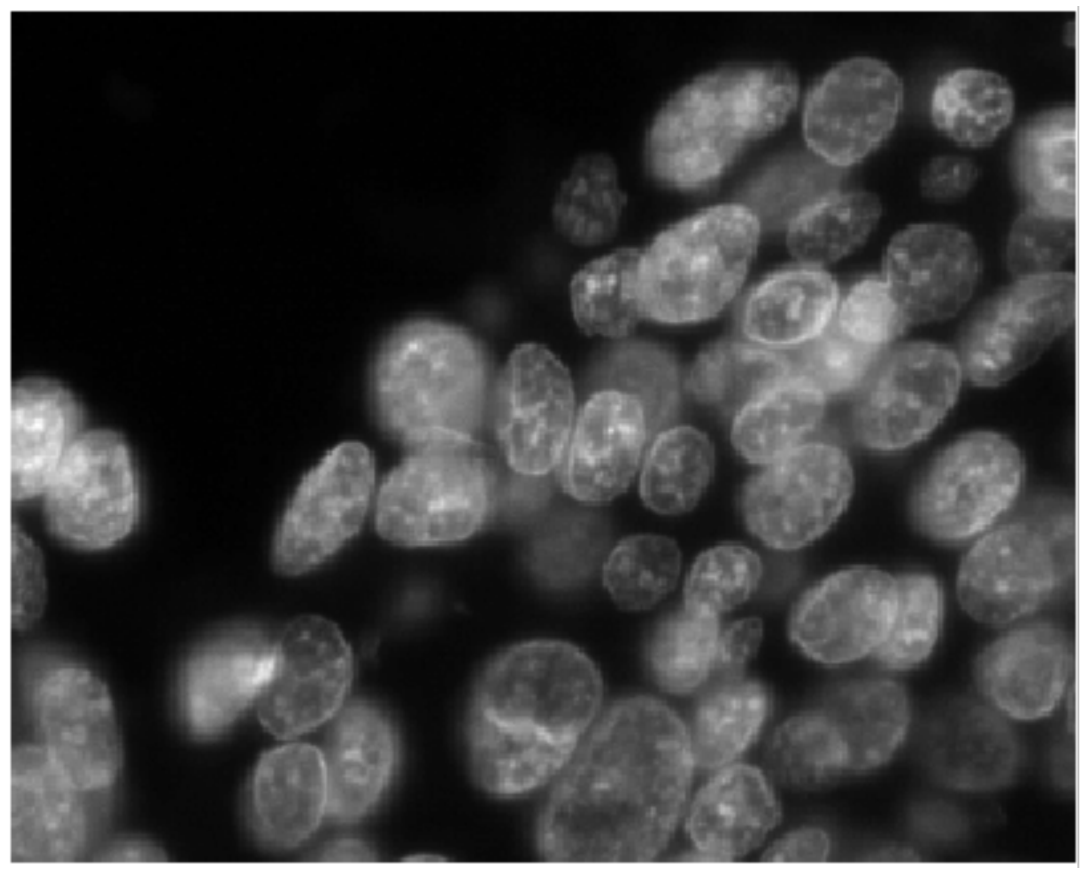


Not Star-Convex

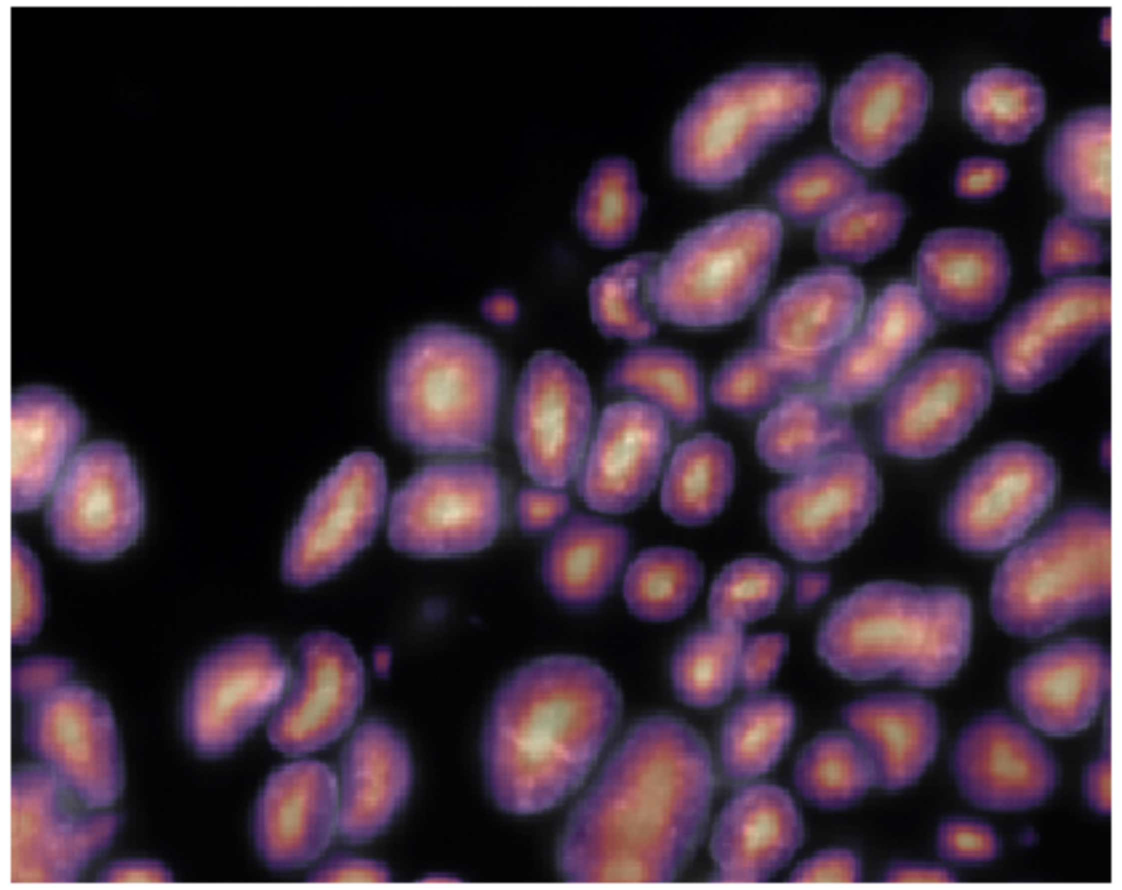


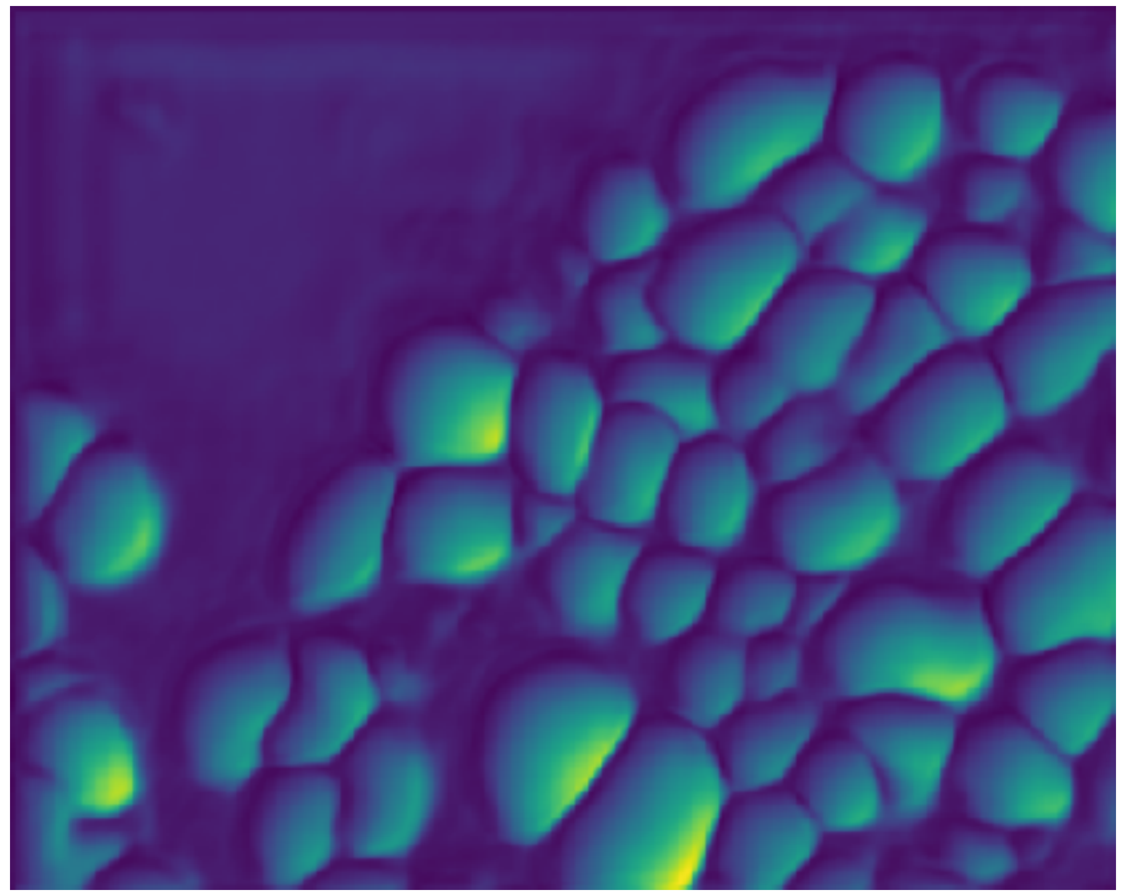
StarDist: Example

Input



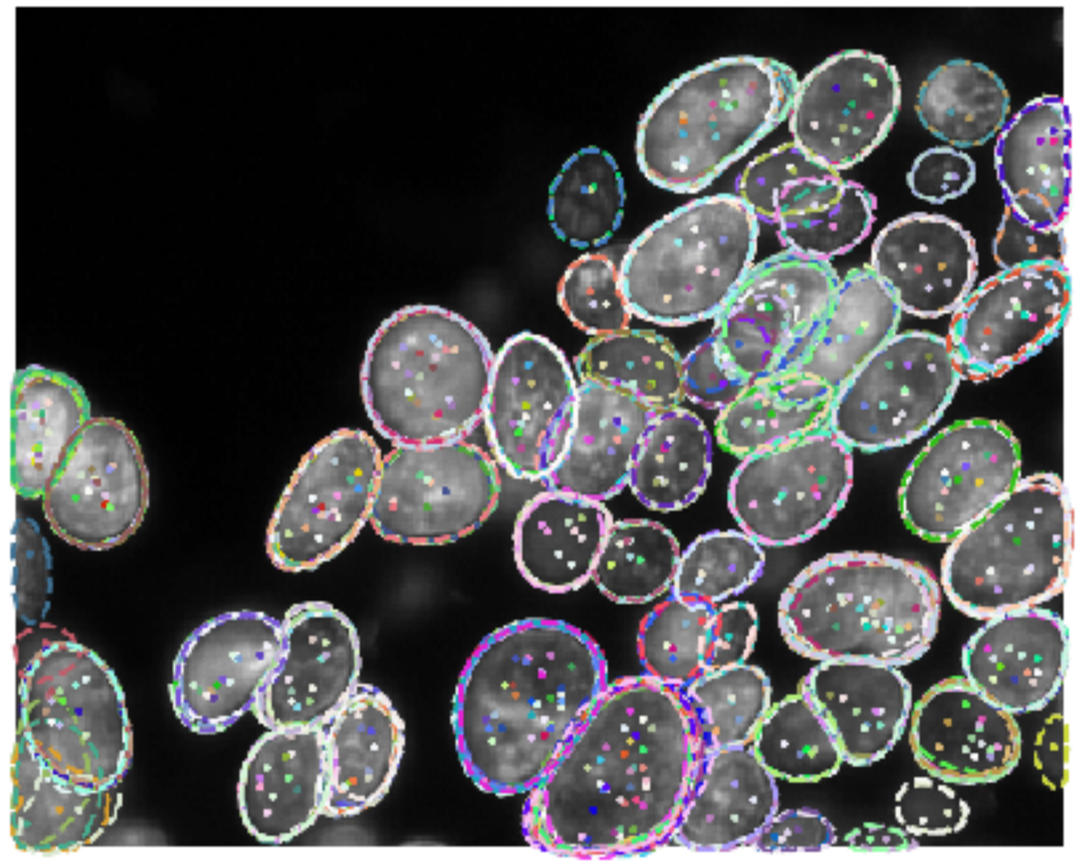
Object probabilities





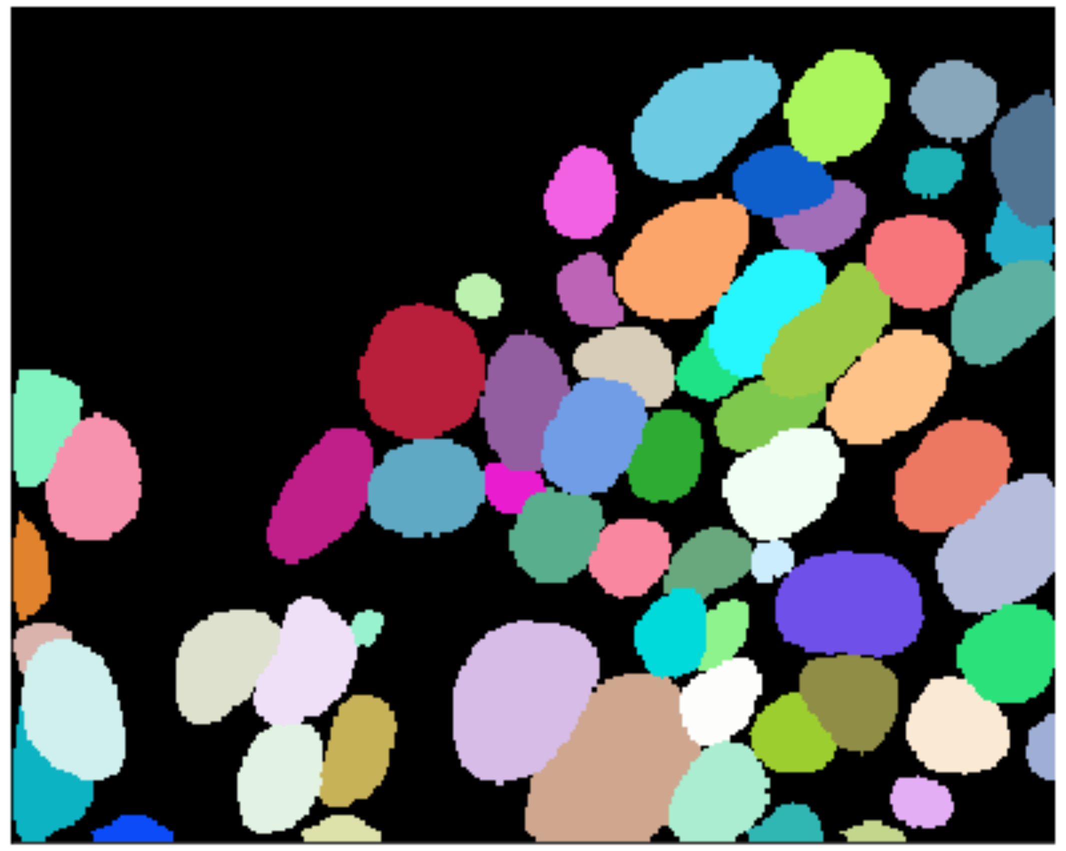
Distance maps

Star-Convex Polygon candidates



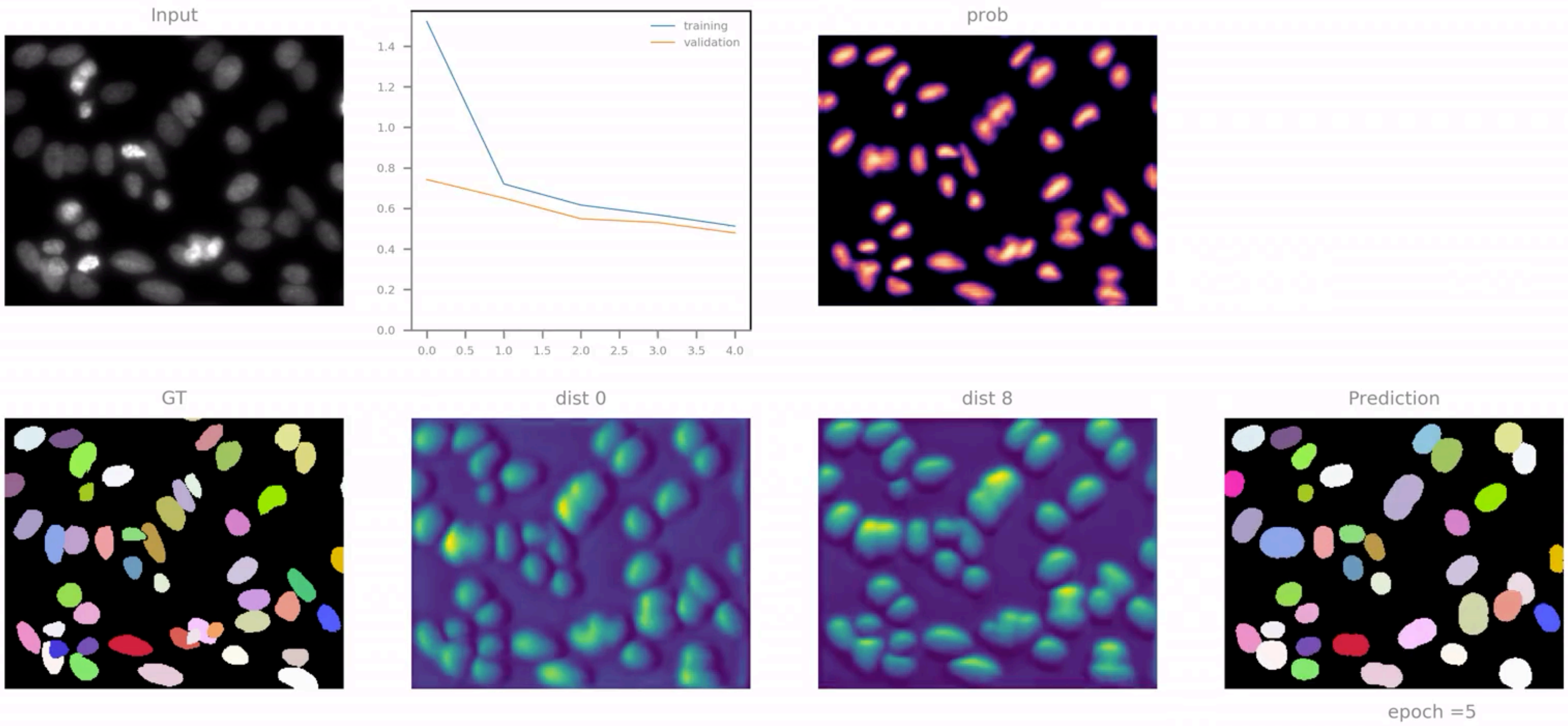
Non-Maximum-Suppression
(NMS)

IoU > 0.2 ?

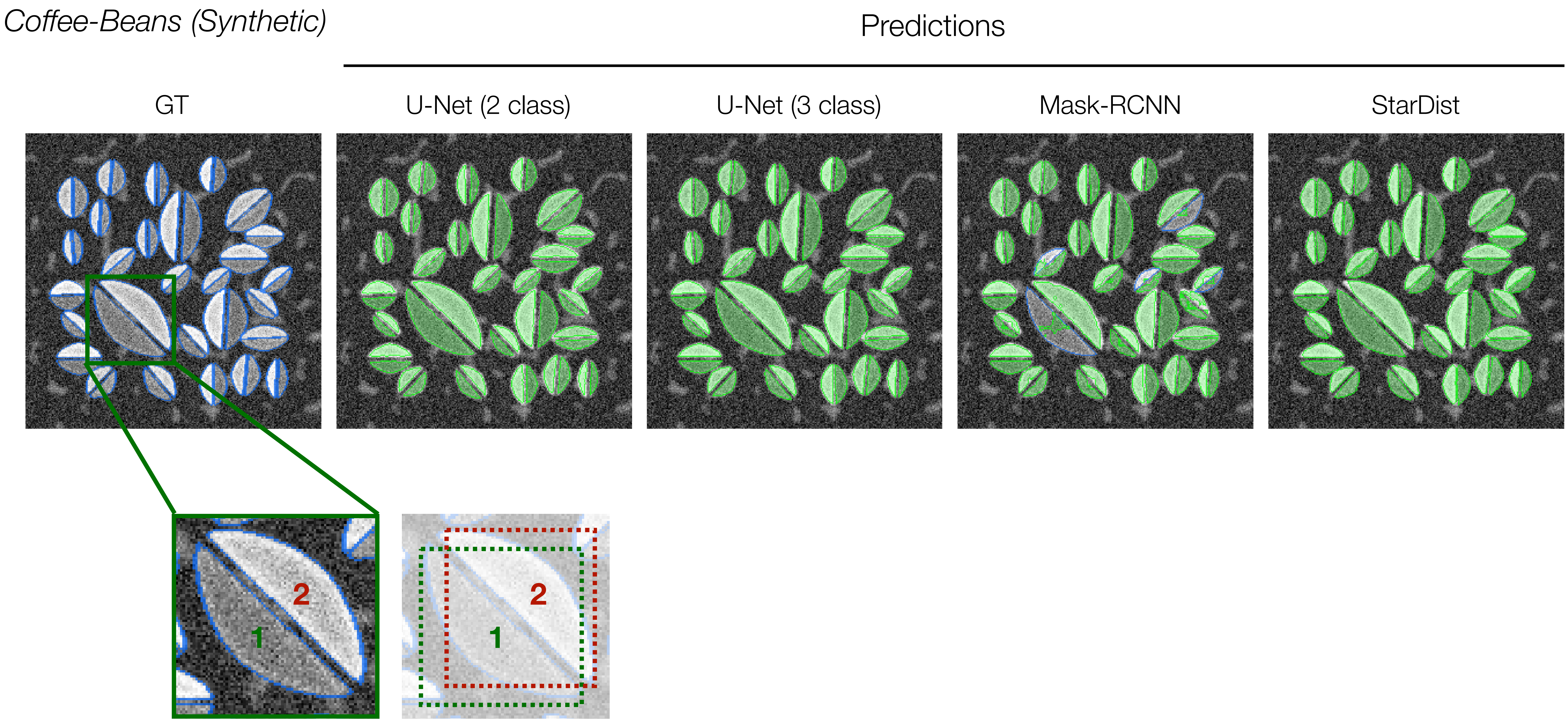


Final Result

Training process



Comparison with common methods

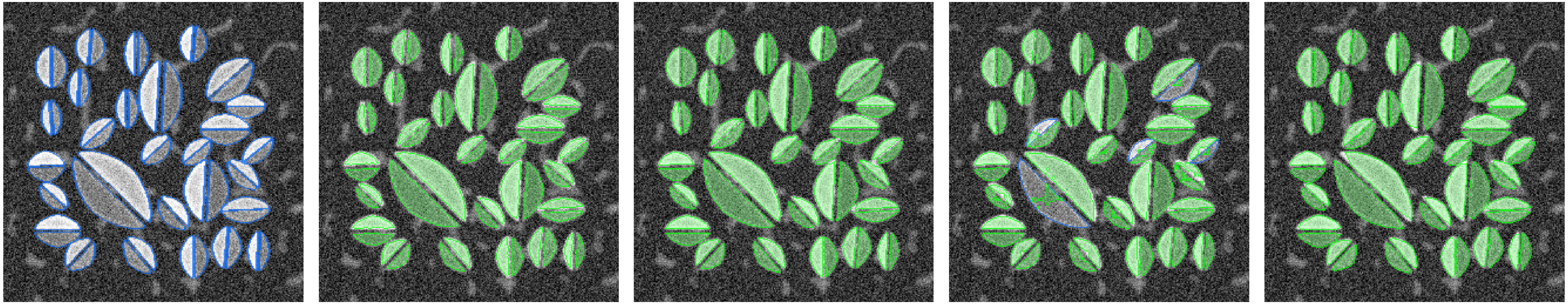


Comparison with common methods

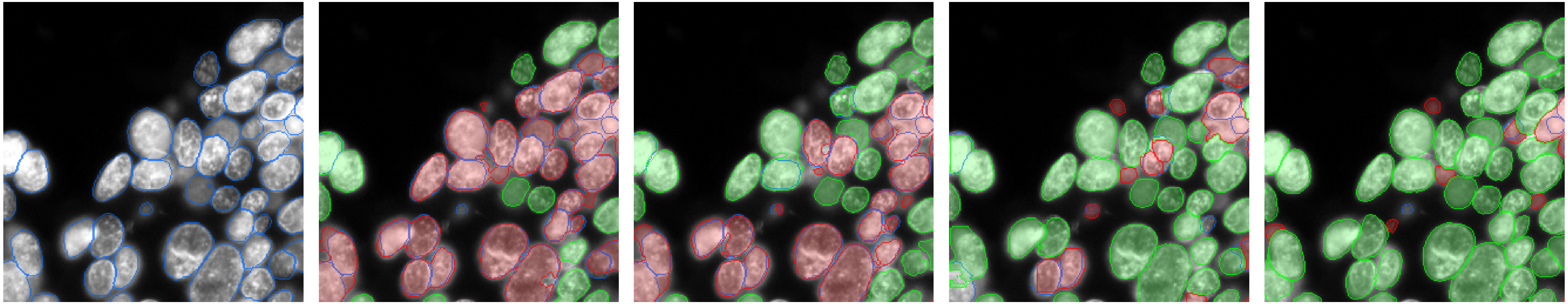
Coffee-Beans (Synthetic)

Predictions

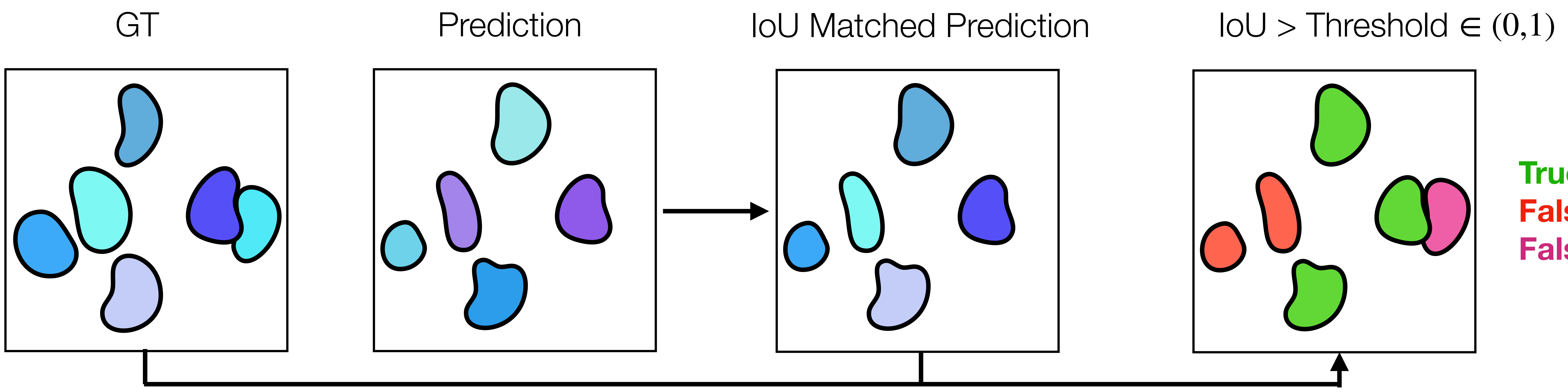
GT	U-Net (2 class)	U-Net (3 class)	Mask-RCNN	StarDist



DSB 2018 (Kaggle Challenge) Caicedo et al (2019)

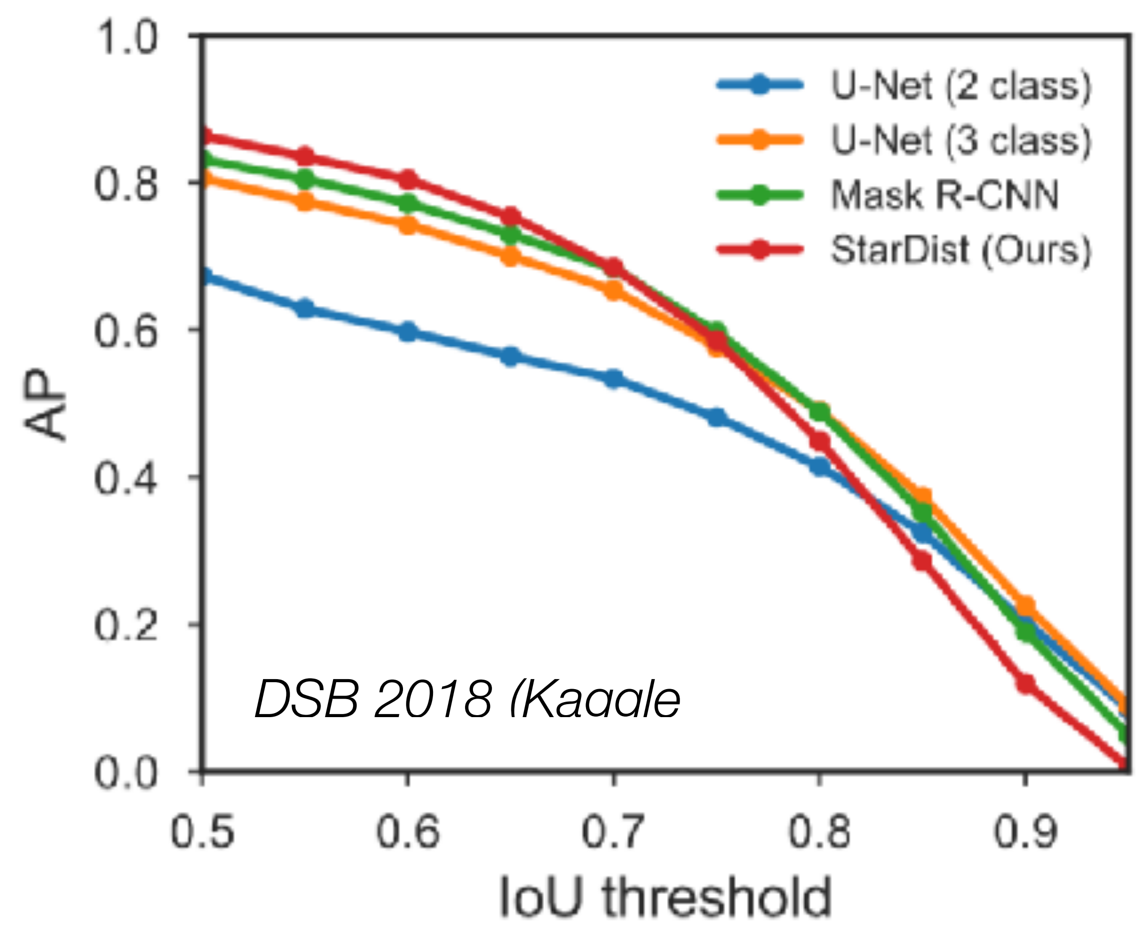


Accuracy Measures



True Positive TP
False Positive FP
False Negative FN

low threshold: already slightly overlaps are counted as TP (high AP)
high threshold: only almost complete overlaps are counted as TP (low AP)

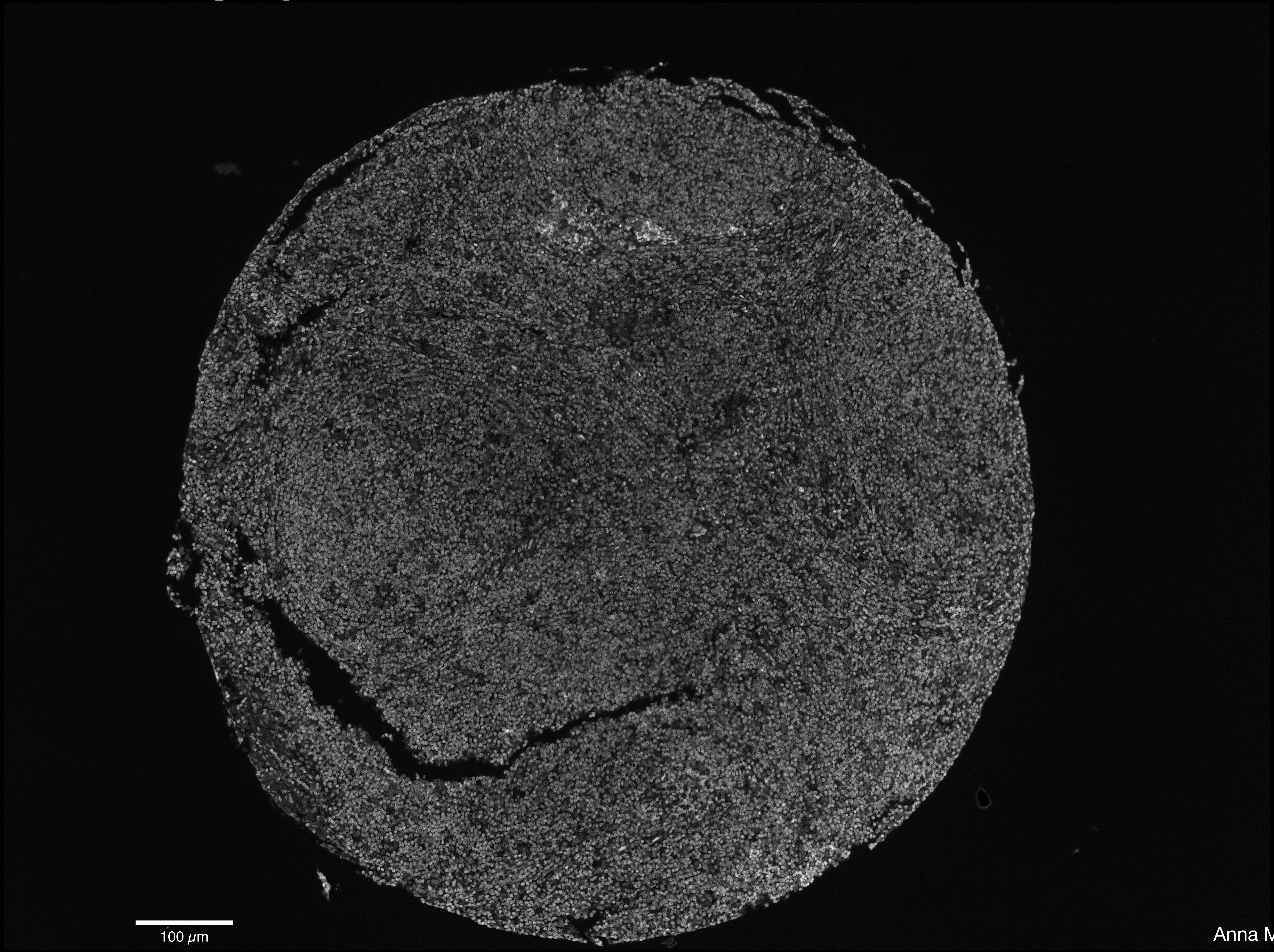


Precision $\dfrac{TP}{TP + FP}$

Recall $\dfrac{TP}{TP + FN}$

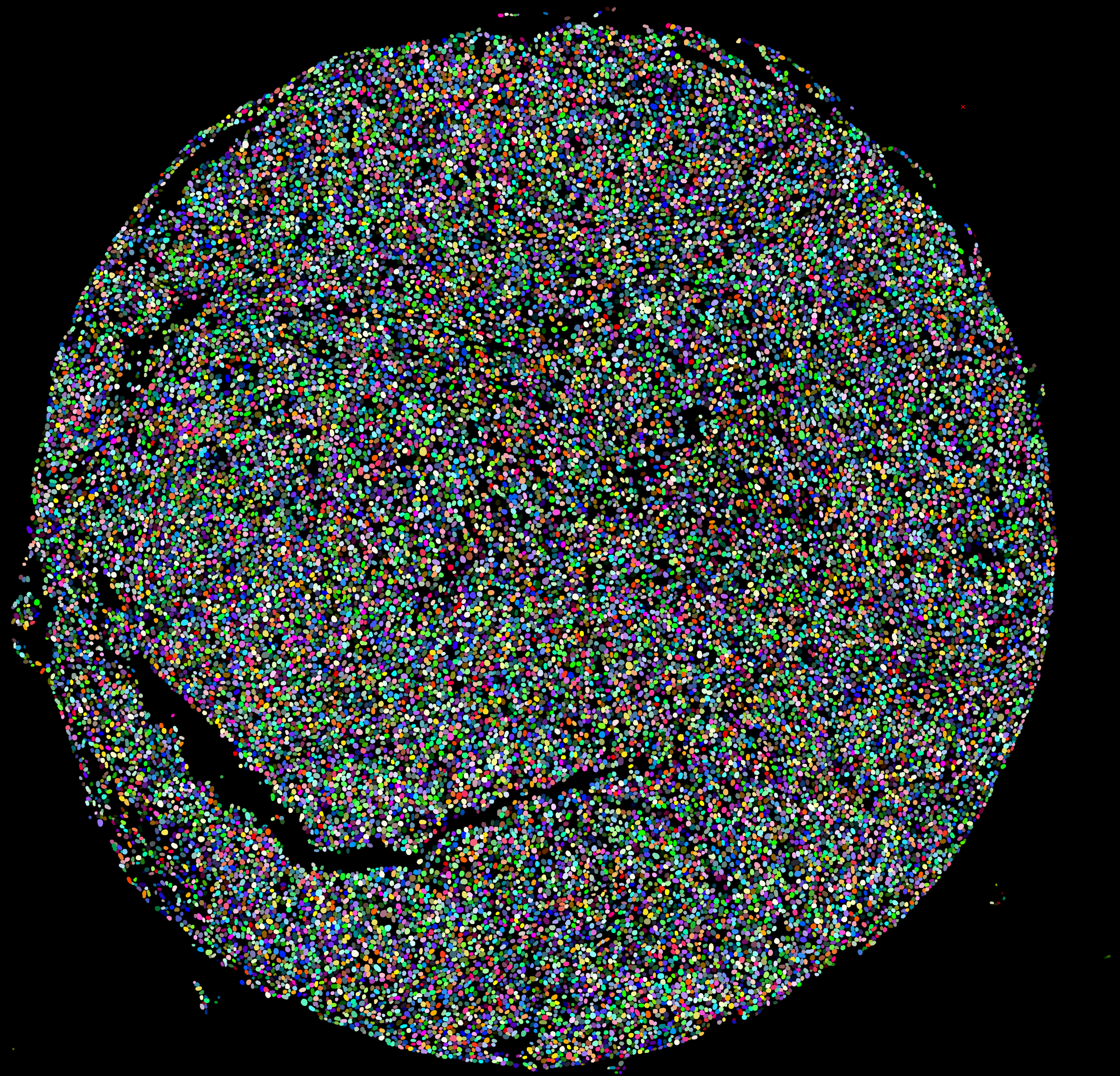
Average Precision (AP)
Accuracy $\dfrac{TP}{TP + FP + FN}$

Example: crowded lymphoma cells



Anna Maria Tsakiroglou (Manchester)

Example: crowded lymphoma cells



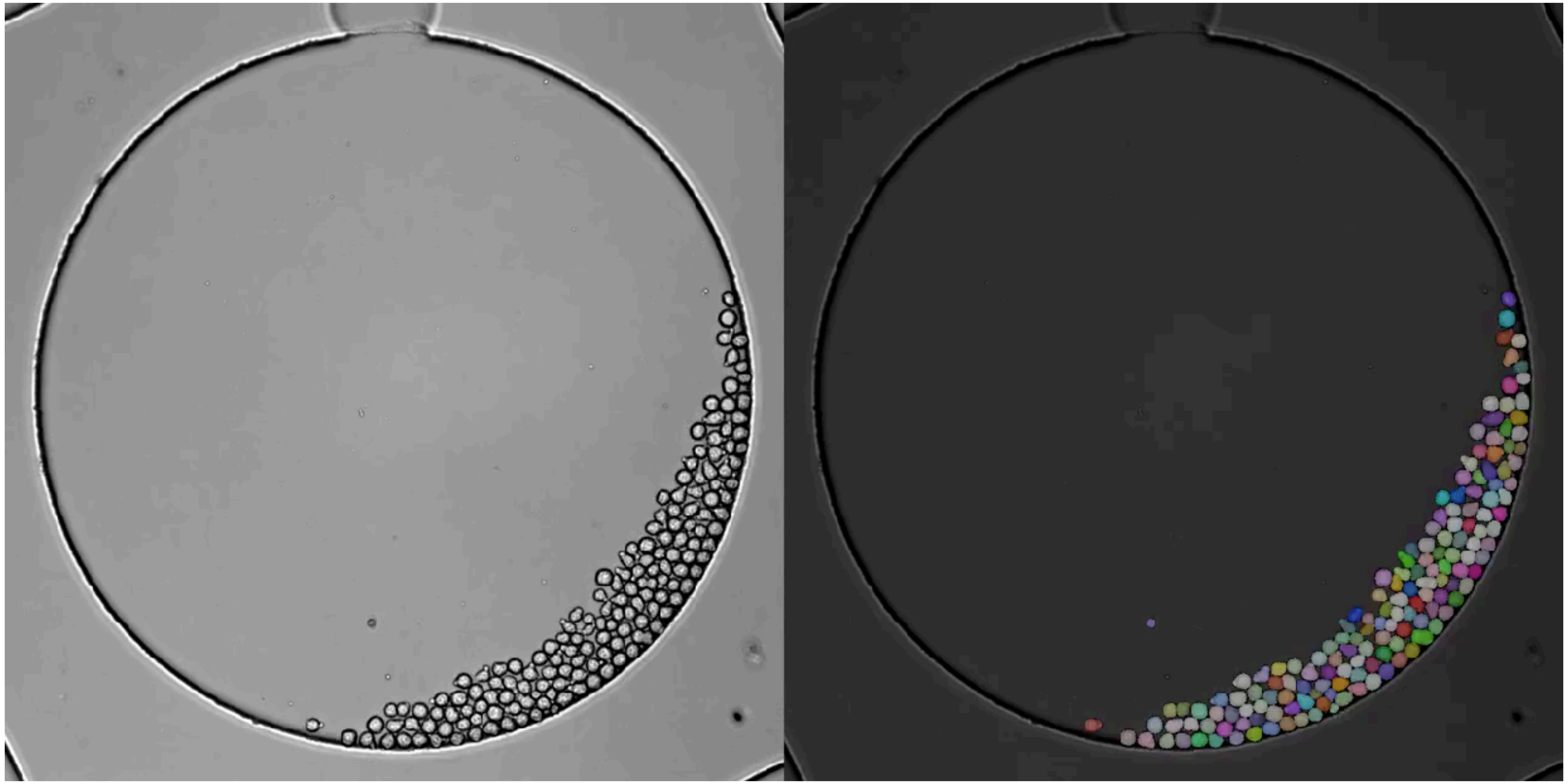
Example: Histopathology (H&E stain)

Whole Slide H&E (Sarcoma), (15mm x 6mm, 32k x 14k pixels, 1.35 GB)

Example: Histopathology (H&E stain)

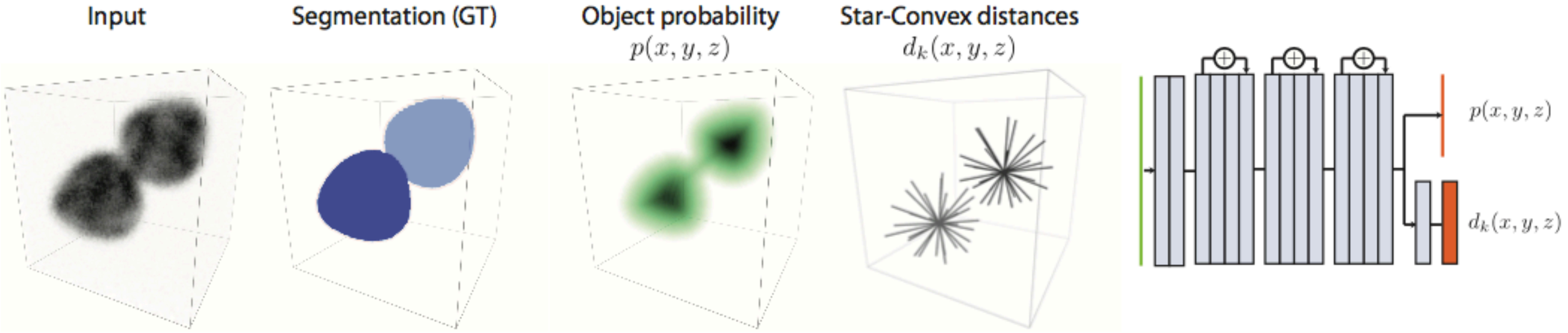

2 mm

Example: Brightfield



StarDist for 3D images

Similar approach:



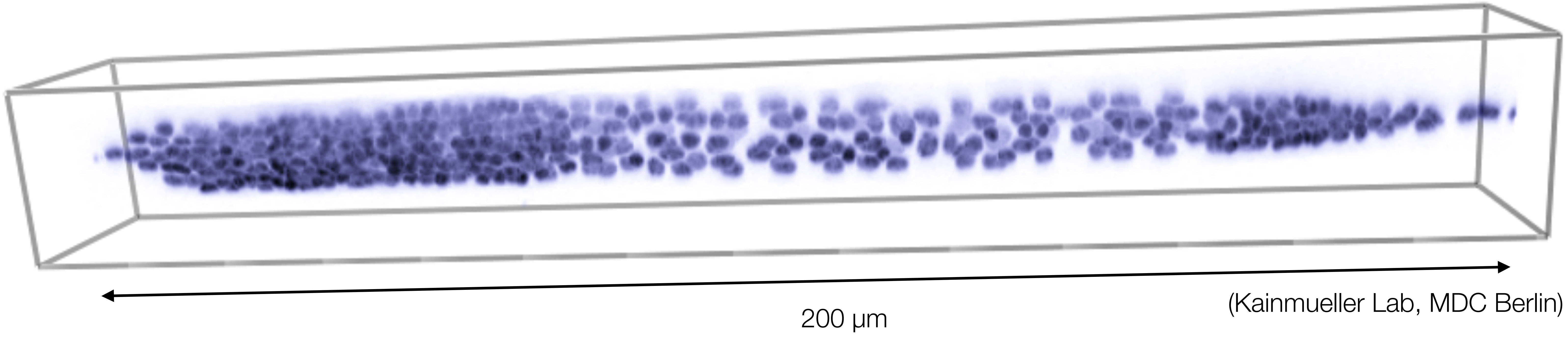
Additional Considerations:

- Ray choice: Fibonacci Lattice on sphere ~ 96 points
- Data anisotropy: Adjust rays according to GT anisotropy
- Non-Maximum Suppression of large sets of polyhedra (> 2 Mio candidates)

Examples 3D

C. elegans (L1)

- 28 Stacks
- ~15k annotated cells
- almost isotropic resolution

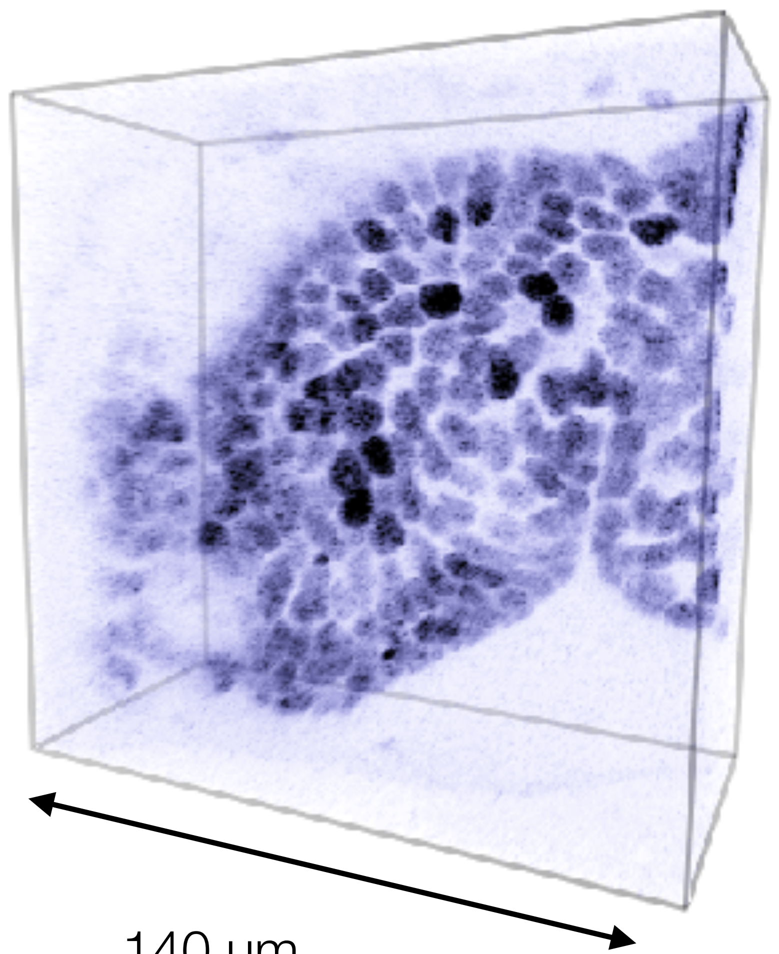


(Kainmueller Lab, MDC Berlin)

Examples 3D

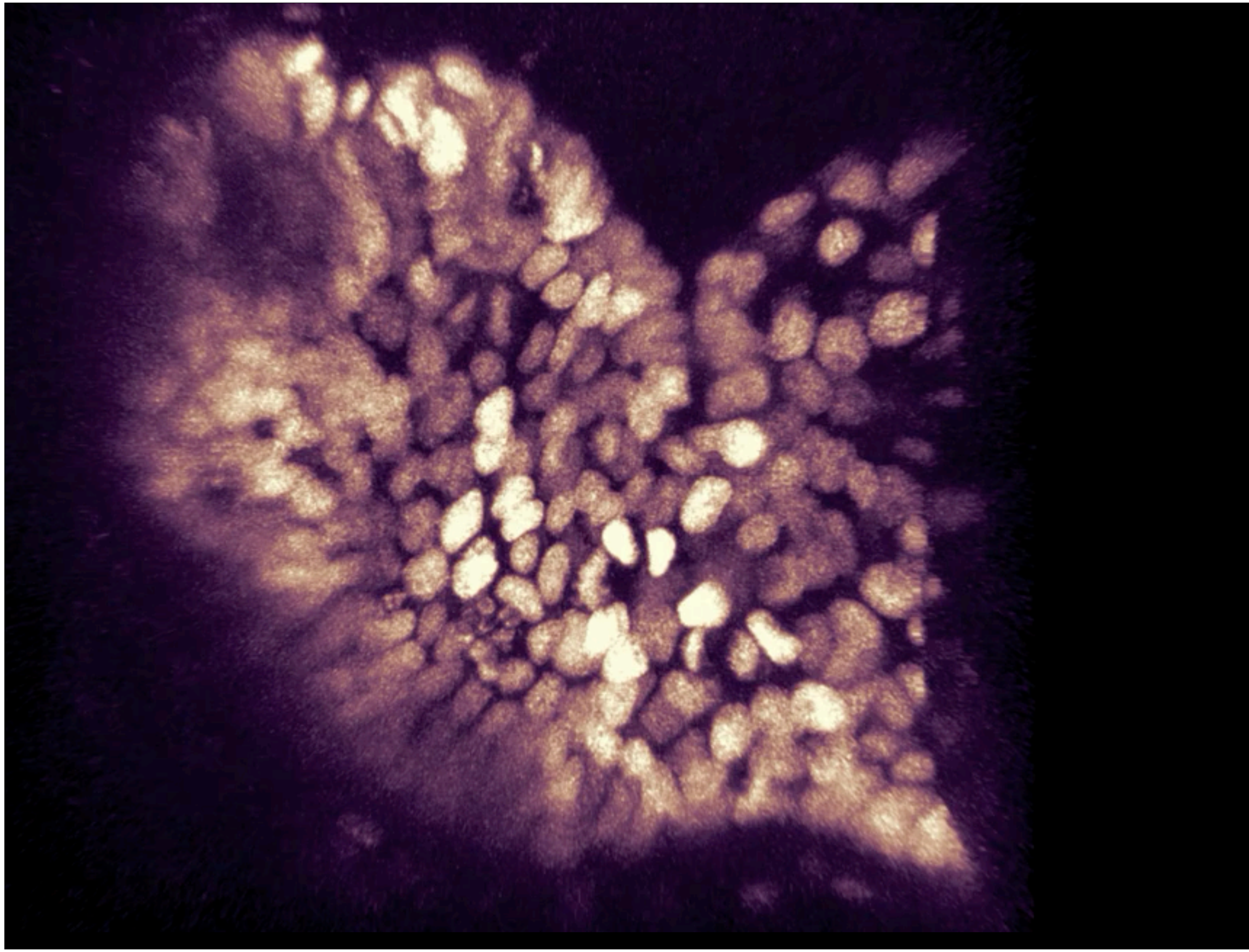
Parhyale hawaiensis

- 6 stacks
- ~1500 annotated cells
- highly anisotropic resolution



140 µm

(Ko Sugarawa, IGF Lyon)



Questions & Answers 1

How to use StarDist

How to use StarDist

Main python library

```
pip install stardist
```

https://github.com/mpicbg-csbd/stardist

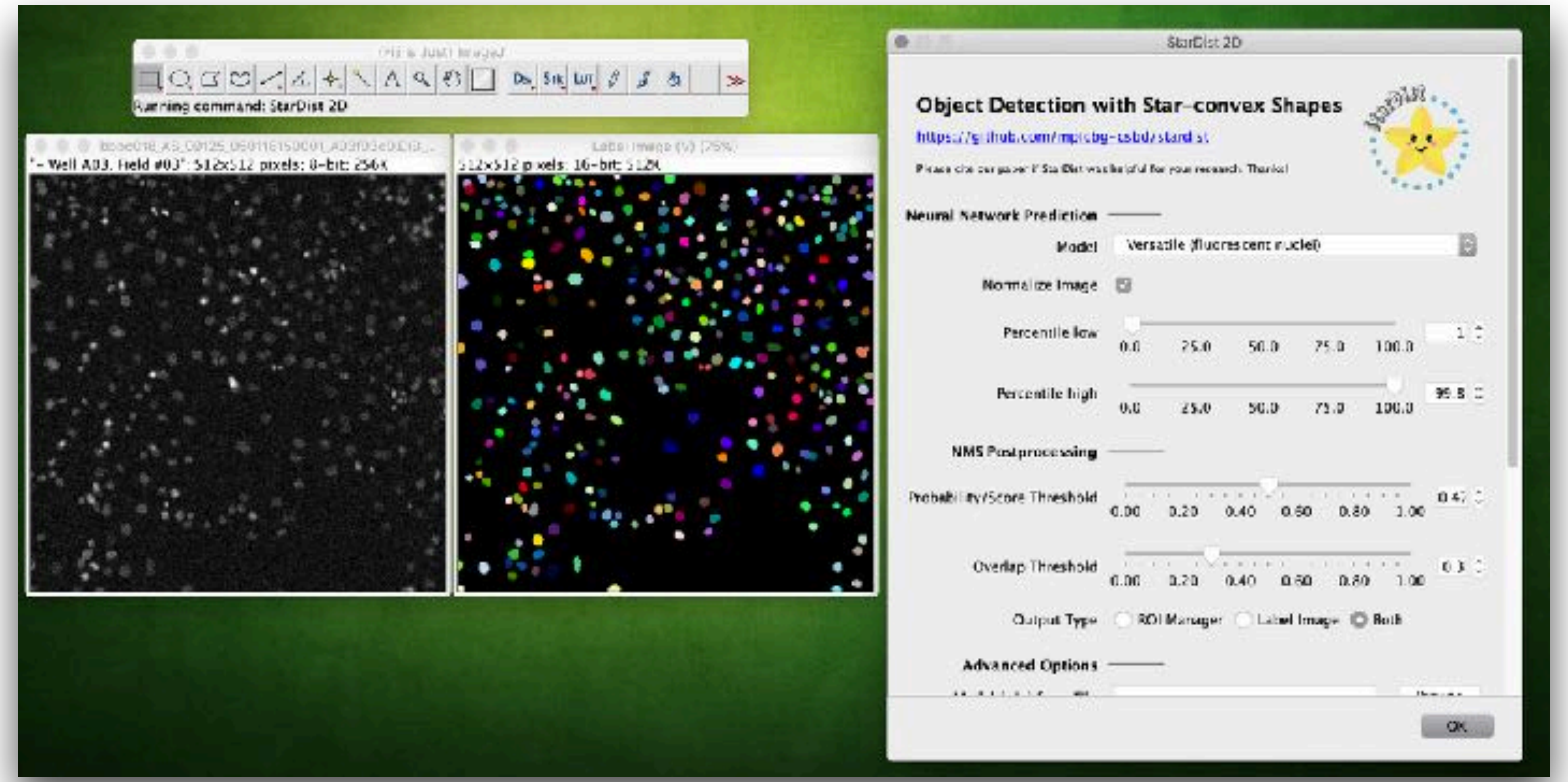
- Training and prediction for 2D/3D images
- Neural network backend keras/tensorflow via csbdeep
- Sensible training defaults
- Multi-Core NMS, tiled prediction
- Image normalization
- Segmentation/Detection measures
- Model export to Fiji

```
from stardist.models import StarDist2D, StarDist3D

model = StarDist2D(config, name = "mymodel")

Using default values: prob_thresh=0.5, nms_thresh=0.4.

model.train(X,Y,validation_data=(Xv,Yv))

Epoch 1/400
 53/100 [==============>...............] - ETA: 26s - lc
t_loss: 8.6035 - prob_kld: 0.3578 - dist_relevant_mae: 8

labels, _ = model.predict_instances(img)
```

Fiji Plugin

https://imagej.net/StarDist

- Prediction for 2D images by already trained models
- Scriptable
- CPU and GPU support (via CSBDeep-Fiji by Deborah Schmidt, MPI-CBG)



How to use StarDist on your own data

- 2D data
- similar to pretrained images (H&E, fluorescent nuclei)

→ **Try pretrained models** — python, Fiji — CPU (or GPU)

→ **Predict on your data** — python, Fiji — CPU (or GPU)

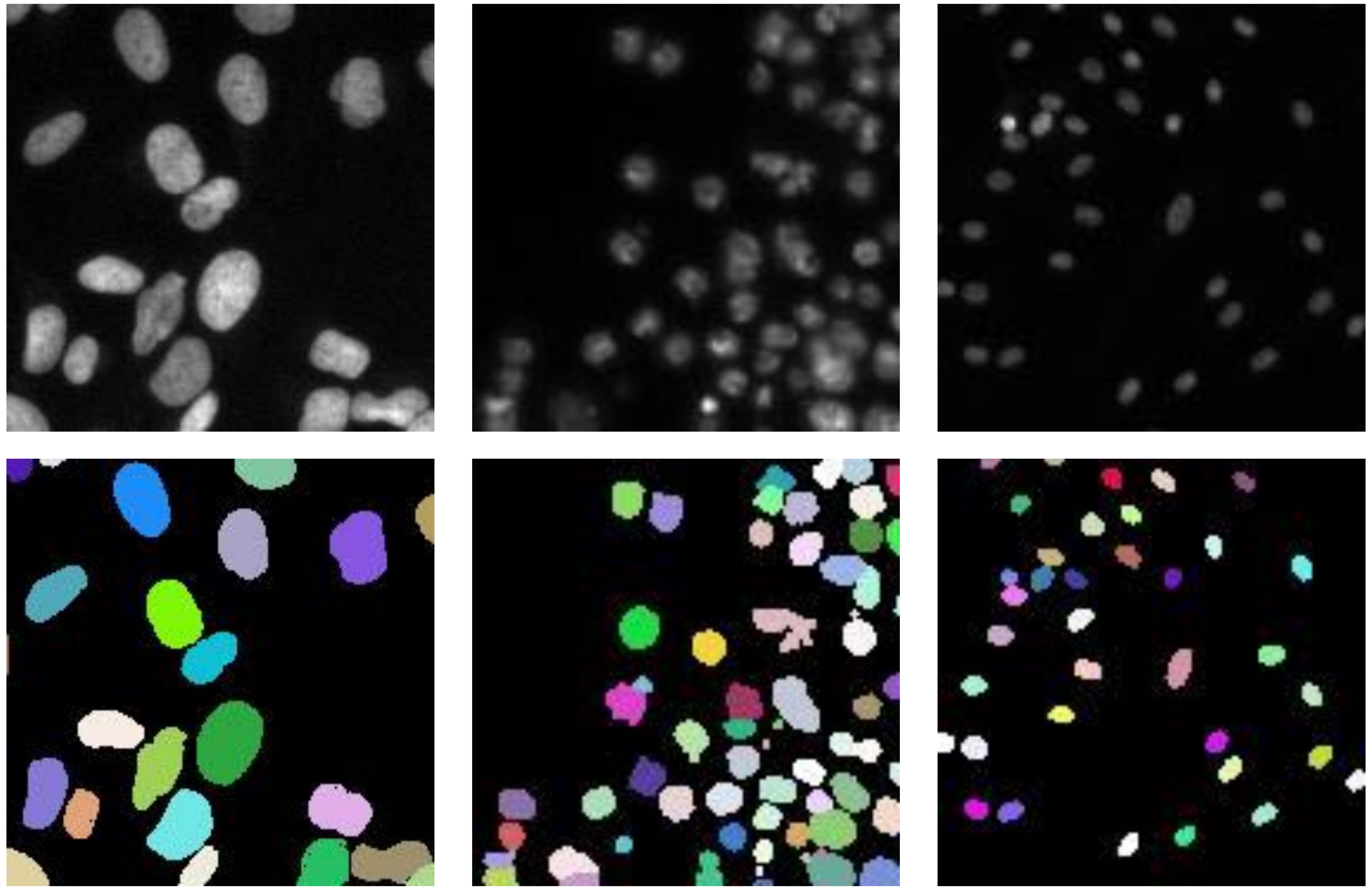
- 2D data dissimilar to pretrained
- 3D data

→ **Generate Annotations & train own model** — python — GPU

→ **Predict on your data** — python, Fiji — CPU (or GPU)

Pretrained Models (2D)

Fluorescence Microscopy
Single Channel

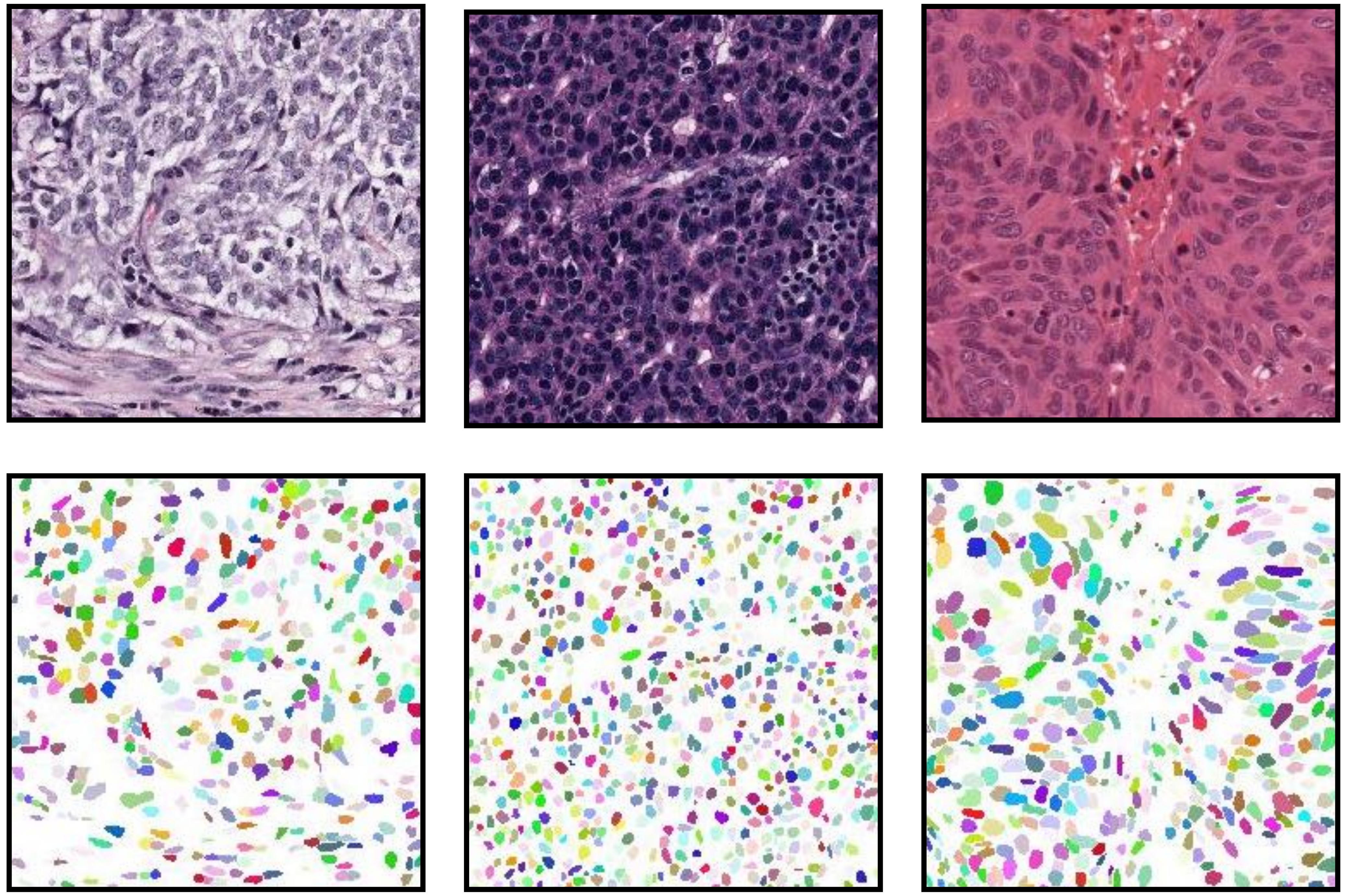


Data Science Bowl 2018

Caicedo et al. (2018)

~ 600 images (2D)
~ 20k annotations

Histopathology
RGB H&E



MoNuSeg

Kumar et al (2017)

~ 30 Images (2D)
~ 22k annotations

Pretrained Models (2D)

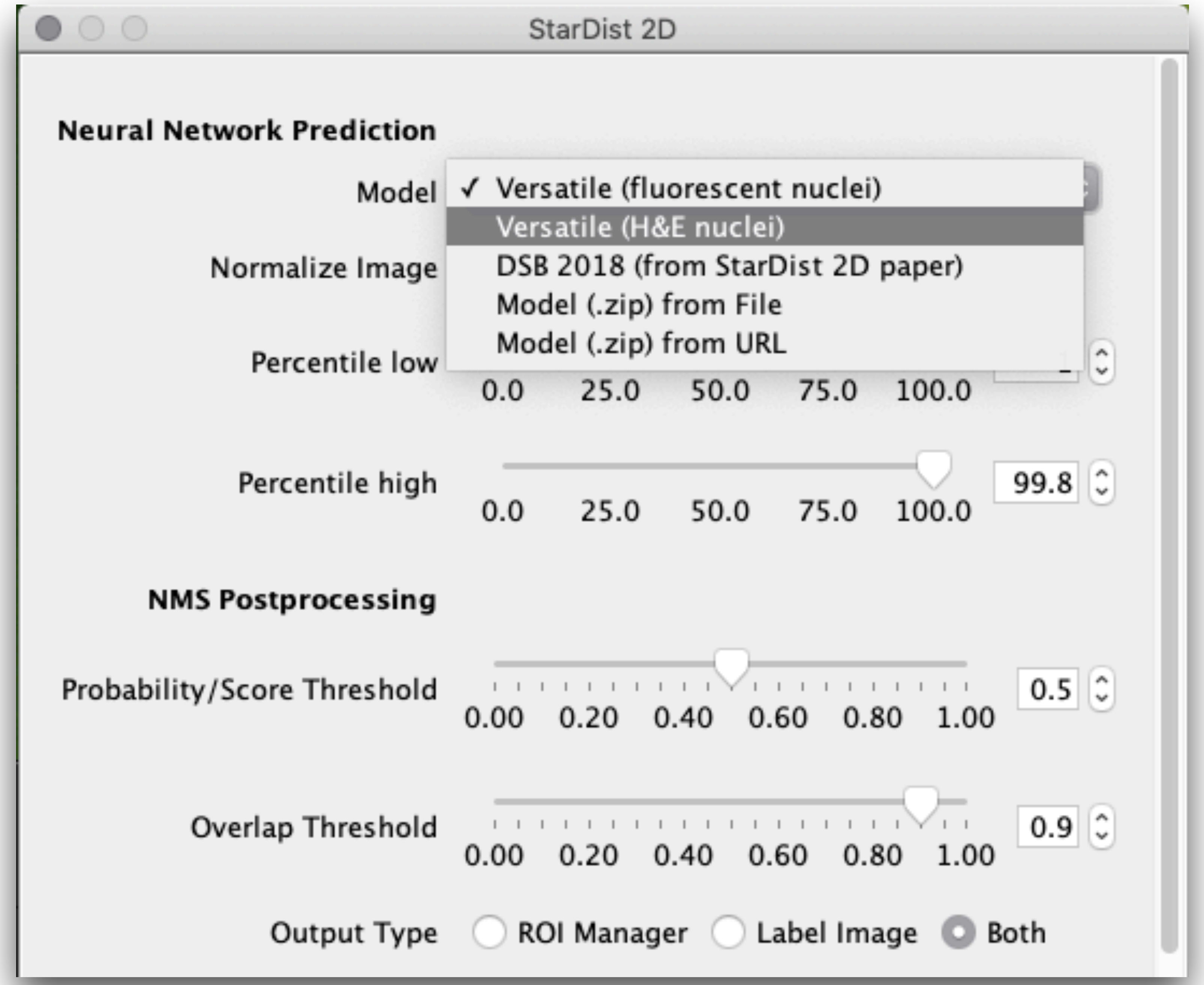
python

```python
from stardist.models import StarDist2D

StarDist2D.from_pretrained()
```

```
There are 4 registered models for 'StarDist2D':

Name                  Alias(es)
────                  ─────────
'2D_versatile_fluo'   'Versatile (fluorescent nuclei)'
'2D_versatile_he'     'Versatile (H&E nuclei)'
'2D_paper_dsb2018'    'DSB 2018 (from StarDist 2D paper)'
'2D_demo'             None
```

```python
model = StarDist2D.from_pretrained('2D_versatile_fluo')

labels, _ = model.predict_instances(img)
```

```
Found model '2D_versatile_fluo' for 'StarDist2D'.
Loading network weights from 'weights_best.h5'.
Loading thresholds from 'thresholds.json'.
Using default values: prob_thresh=0.479071, nms_thresh=0.3.
```



- Try different scalings
- Invert intensity, RGB -> grayscale, ….
- Play around with the prob and overlap (NMS) threshold

Demo: Pretrained models in Python and Fiji (2D)

Try pretrained models

python Fiji — **CPU (or GPU)**

→

Predict on your data

python Fiji — **CPU (or GPU)**

Training of custom models

If the pretrained models do not work on your 2D images (or your data is 3D) you need to training your own model

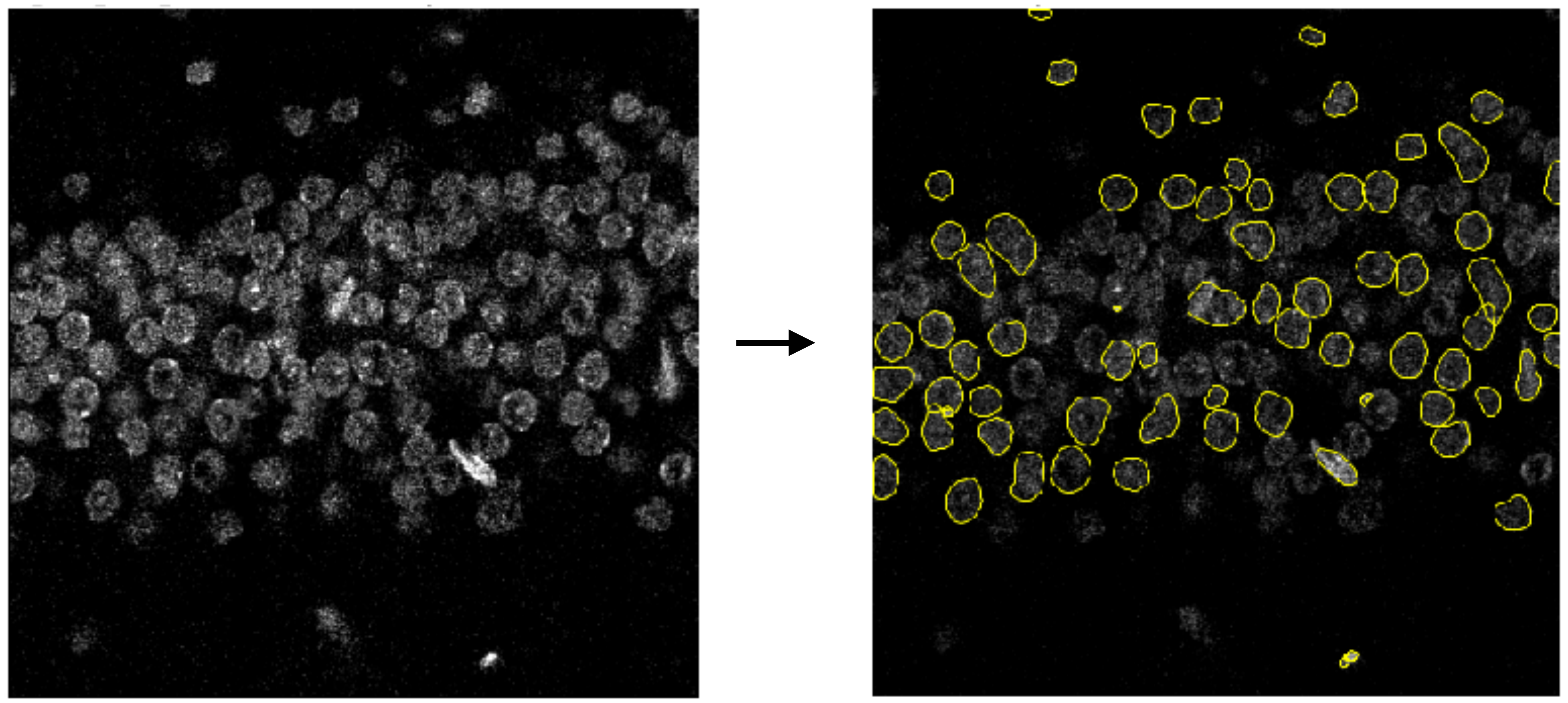
Generate Annotations & train own model

python **GPU**

→

Predict on your data

python **CPU (or GPU)**



Data from Silvia Monari, EPFL

- Needs user annotated Image/label pairs
- GPU workstation
- Python
- Training time from scratch:
  - 2D: 30min-2h
  - 3D: 4h-12h

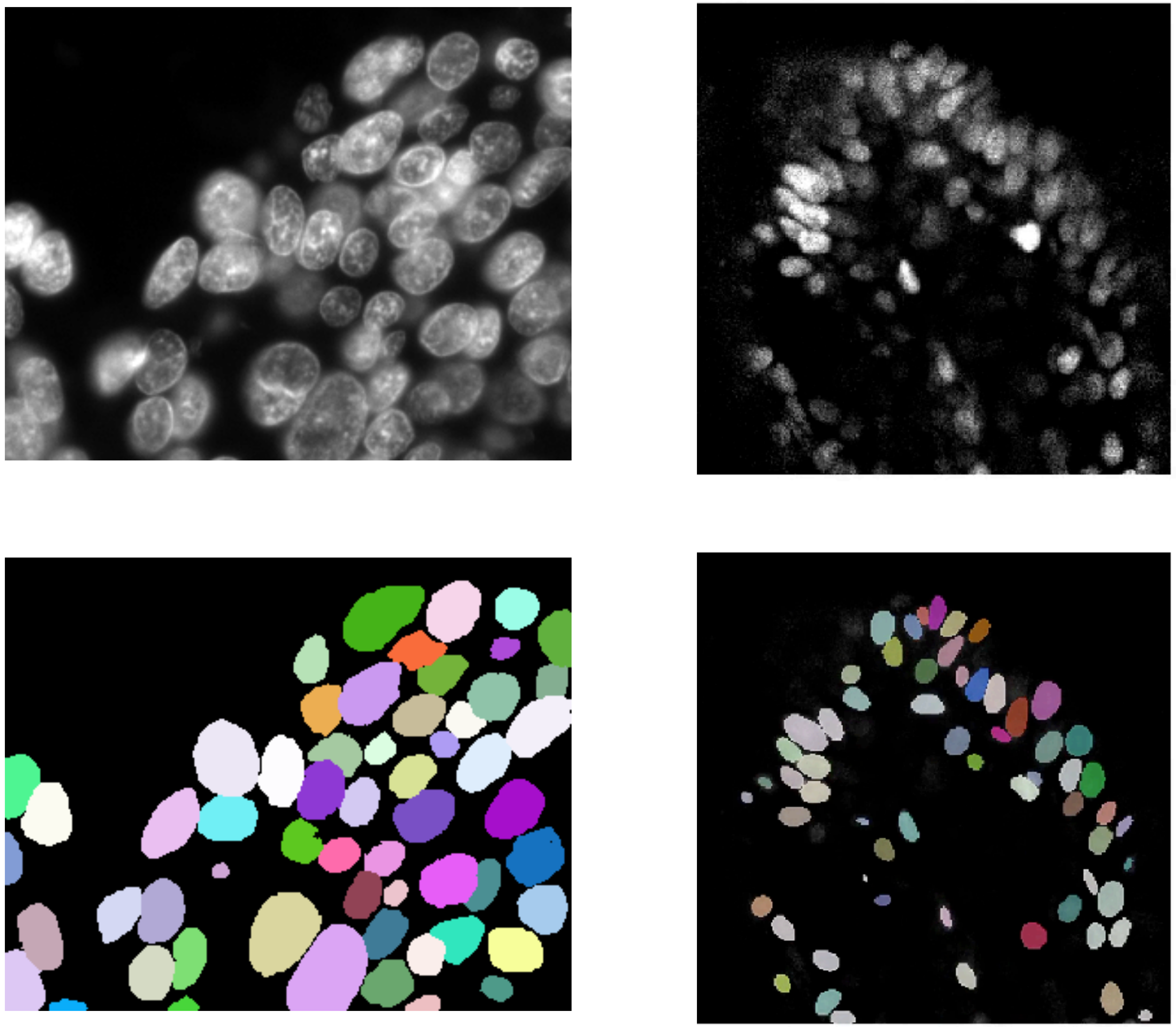
Training data generation

Generate corresponding Image/Mask pairs:

2D **3D**

Images

Masks



- Image/Mask: tif format
- Mask: Every cell pixel has to have a different label (dense labeling)
- Use crops from different regions/timepoints
- Size: at least 128^2 (2D) or 96^3 (3D)
- Number: N > 10 (2D) and N > 4 (3D)

Training data generation

```
data/
├── train
|   ├── images
|   |   ├── img_1.tif
|   |   ├── img_2.tif
|   |   ├── img_3.tif
|   |   ├── img_4.tif
|   |   ├── img_5.tif
|   |   └── img_6.tif
|   └── masks
|       ├── mask_1.tif
|       ├── mask_2.tif
|       ├── mask_3.tif
|       ├── mask_4.tif
|       ├── mask_5.tif
|       └── mask_6.tif
└── test
    ├── images
    |   ├── img_7.tif
    |   └── img_8.tif
    └── masks
        ├── mask_7.tif
        └── mask_8.tif
```

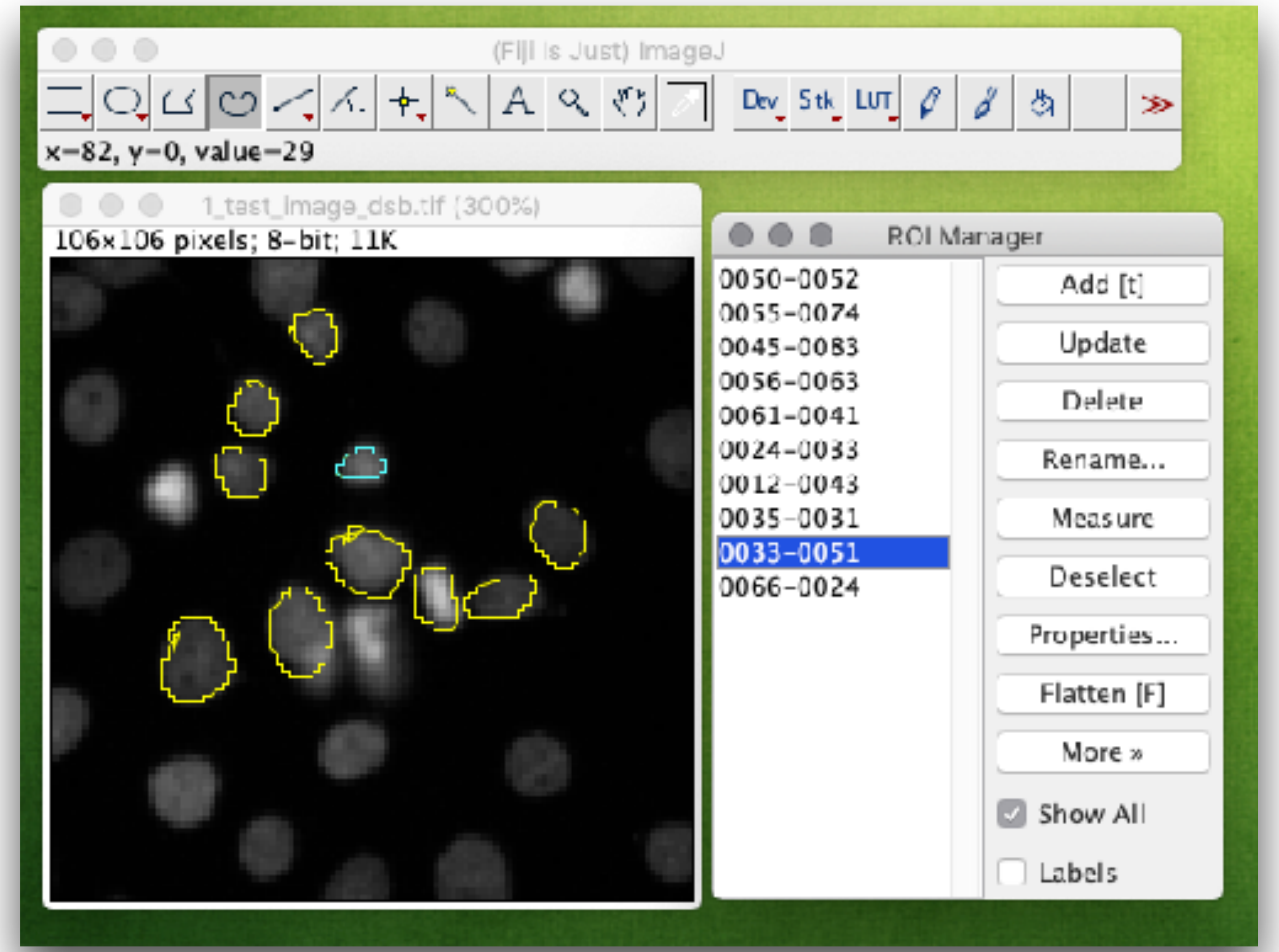
Generate corresponding Image/Mask pairs:

- Image/Mask: tif format
- Mask: Every cell pixel has to have a different label (dense labeling)
- Use crops from different regions/timepoints, e.g.
  2D: > 10 of size 128x128
  3D: > 4 of size 32x96x96
- Split into (non-overlapping) train and test for validation

Annotation Software (2D)

Fiji/ImageJ

- Draw Roi per object (Roi-Manager)
- Convert to label mask (e.g. see here)

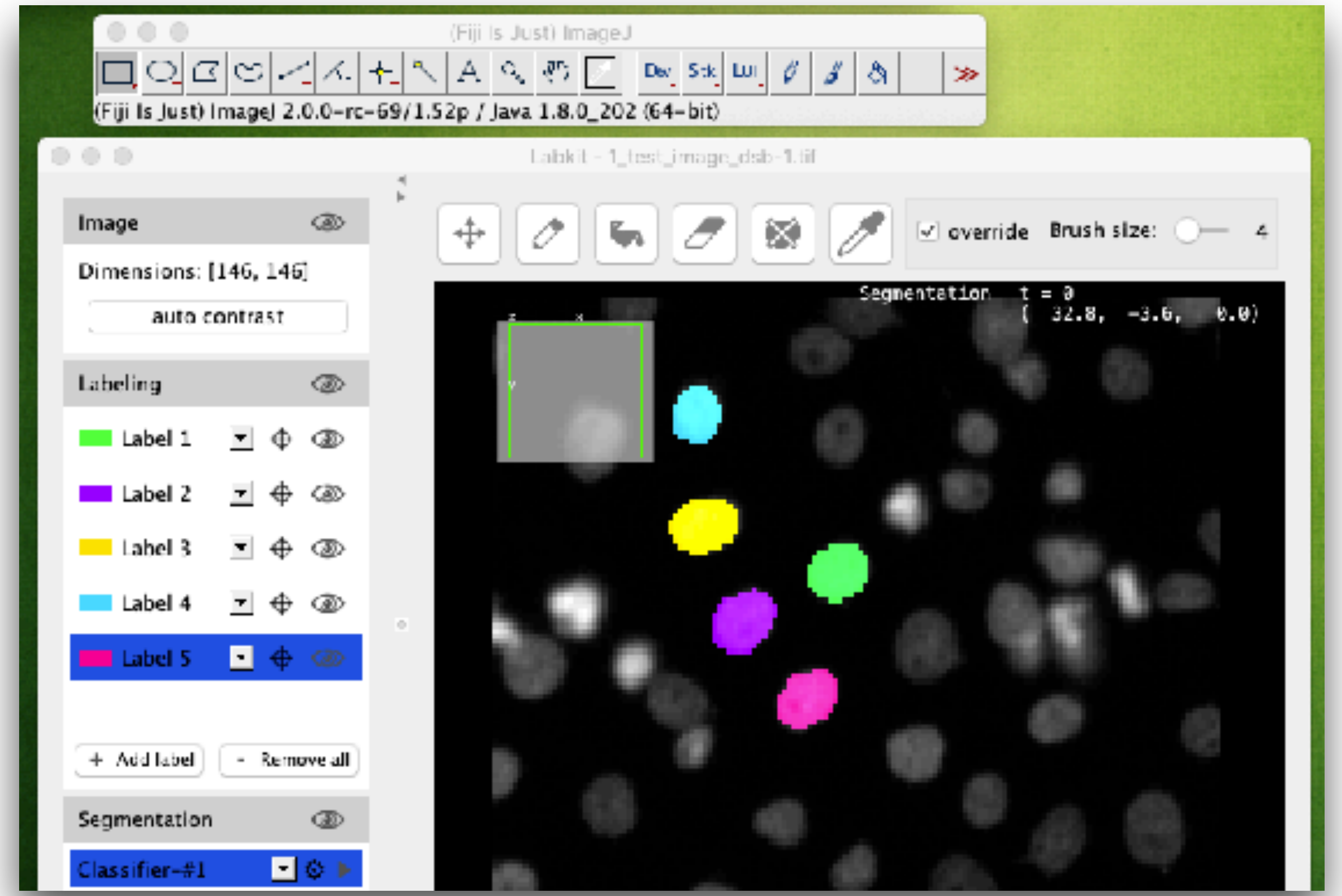


http://fiji.sc/ Schindelin et al. (2012)

Schneider, Rasband et al (2012)

Fiji + LabKit

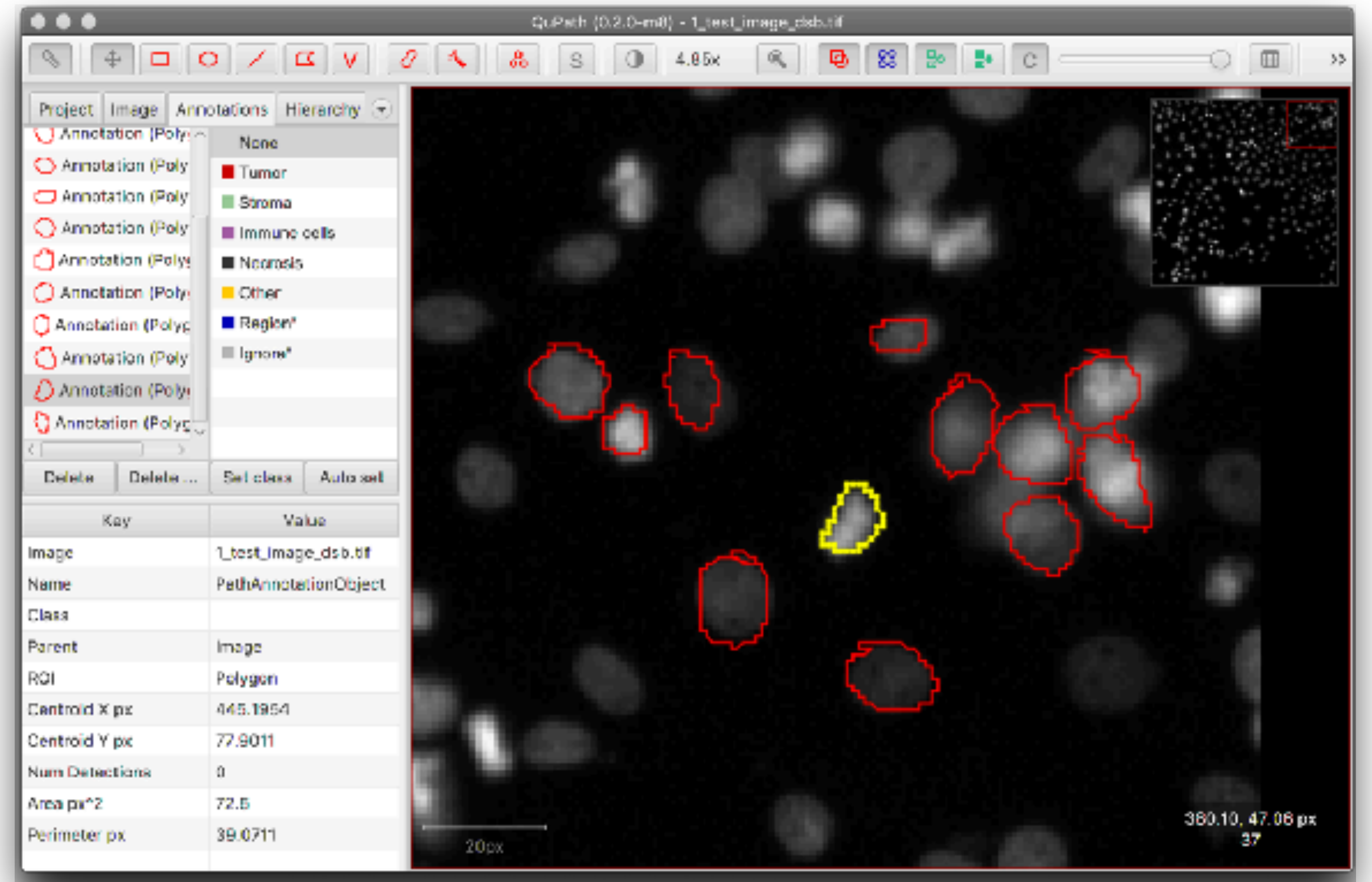
- Directly draw label mask



https://imagej.net/Labkit M. Arzt, MPI-CBG

QuPath

- Draw ROI per object
- Convert to label mask

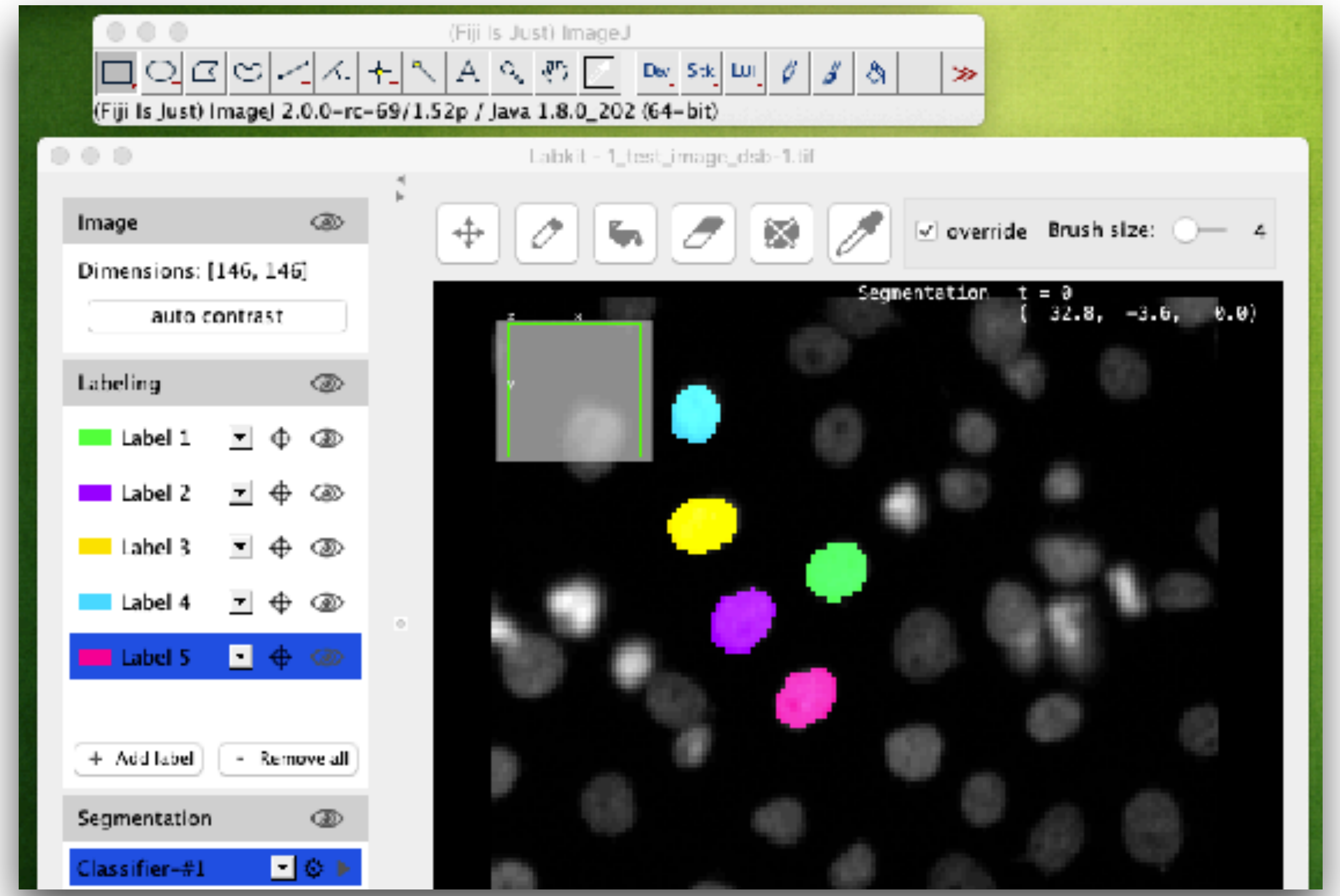


https://qupath.github.io Bankhead et al. (2017)

Annotation Software (3D)

Fiji + LabKit

- Directly draw label mask
- Reorder z -> t (to not miss a plane)

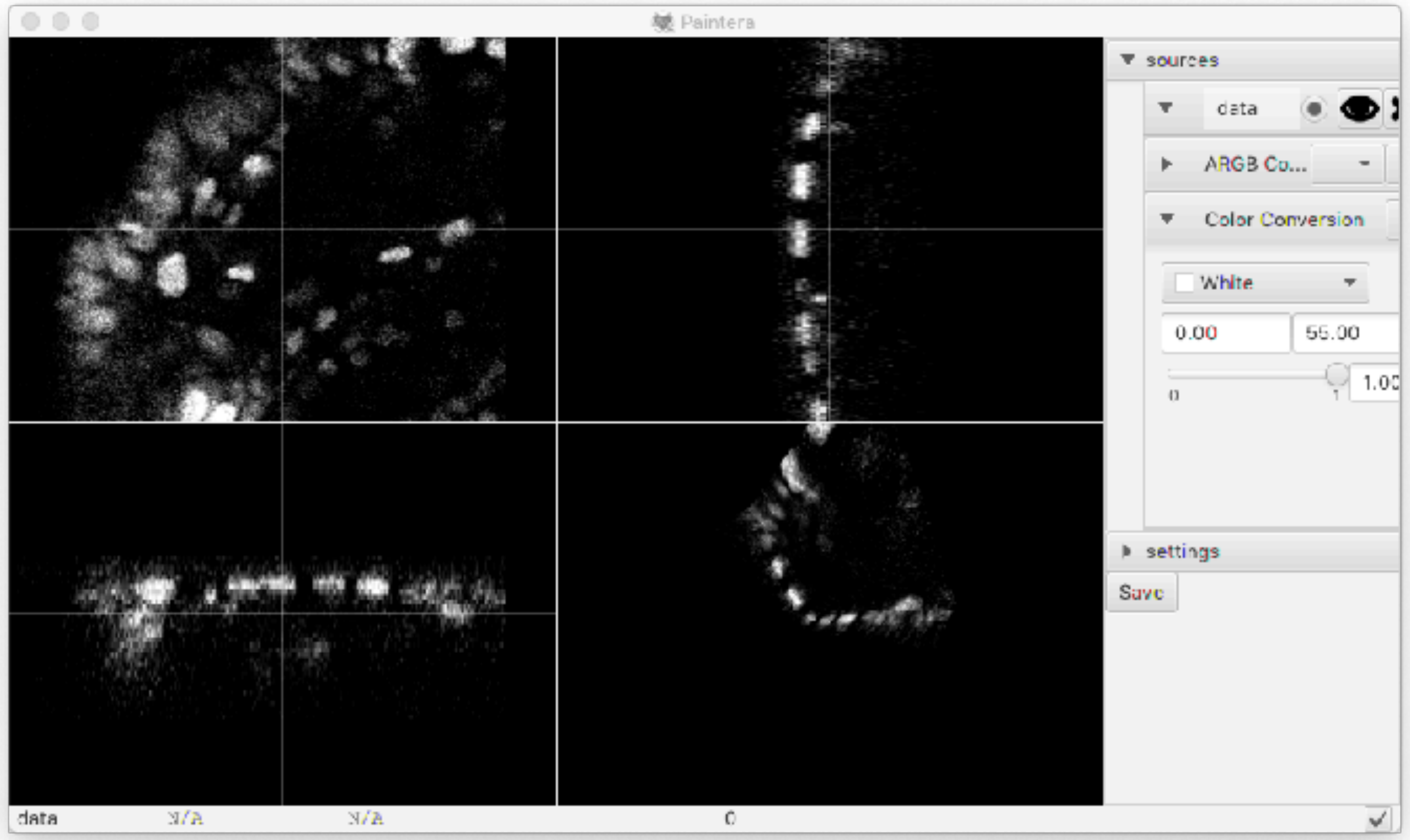


https://imagej.net/Labkit

M. Arzt, MPI-CBG

Paintera

- For very large volumes (> GBs)
- Powerful but slightly steeper learning curve



https://github.com/saalfeldlab/paintera

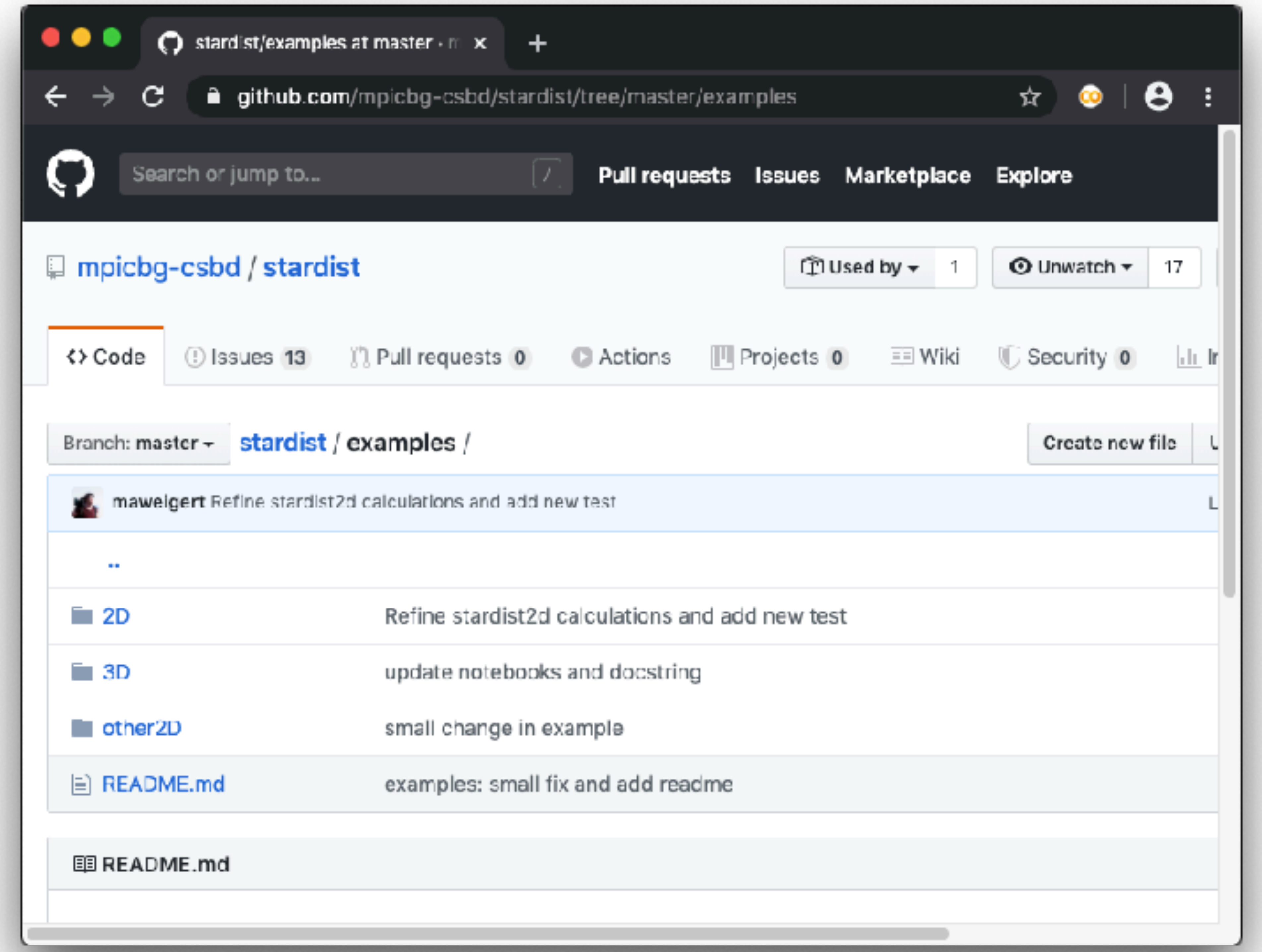
P. Hanslovsky, S. Saalfeld et al Janelia

Training of a custom model

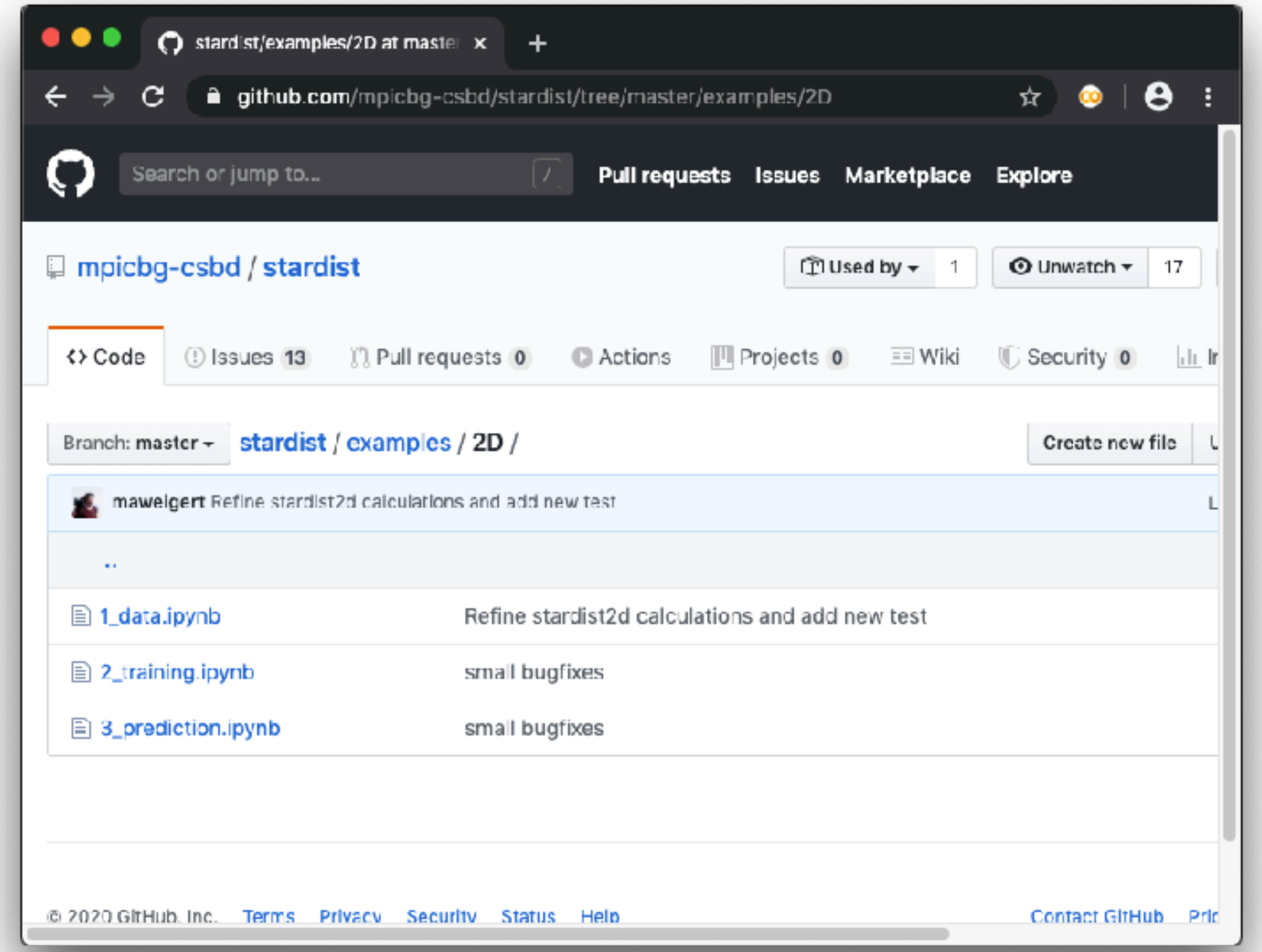
Demo Jupyter Notebooks for 2D and 3D data that you can adapt and run on your (or your facilities) GPU-workstation

https://github.com/mpicbg-csbd/stardist/tree/master/examples

Jupyter Notebooks examples in 2D and 3D

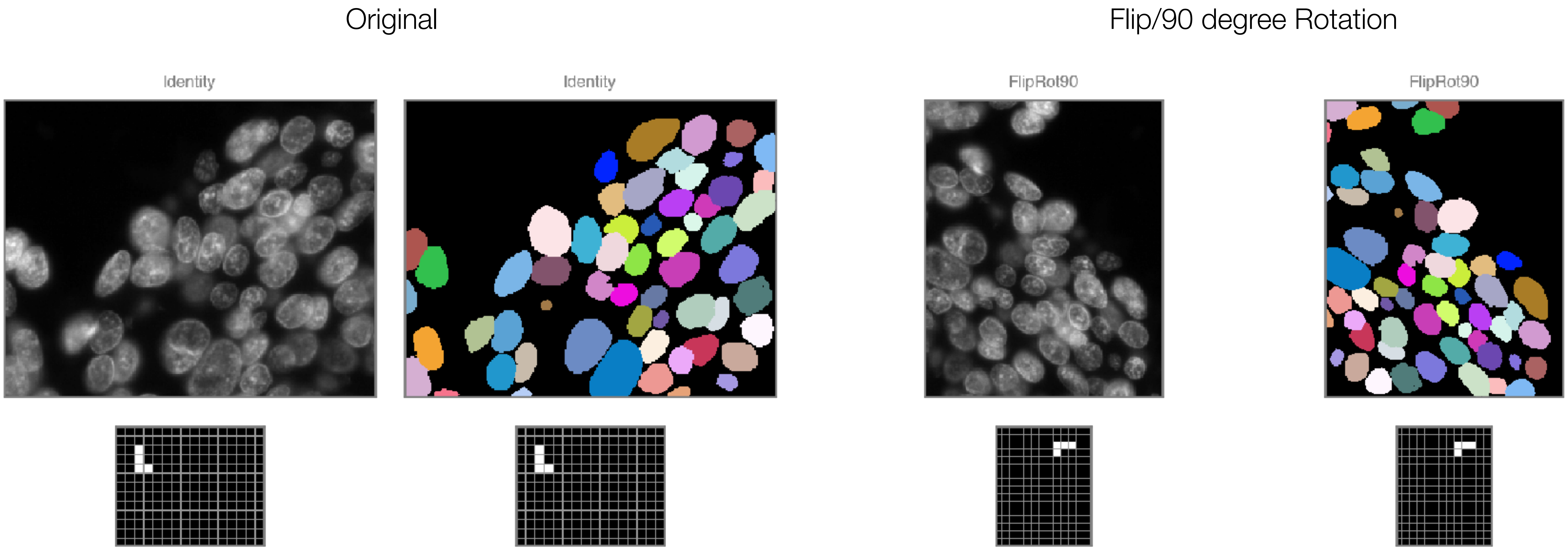


1. Data preparation/inspection
2. Training of the StarDist model
3. Prediction on new images



Data Augmentation

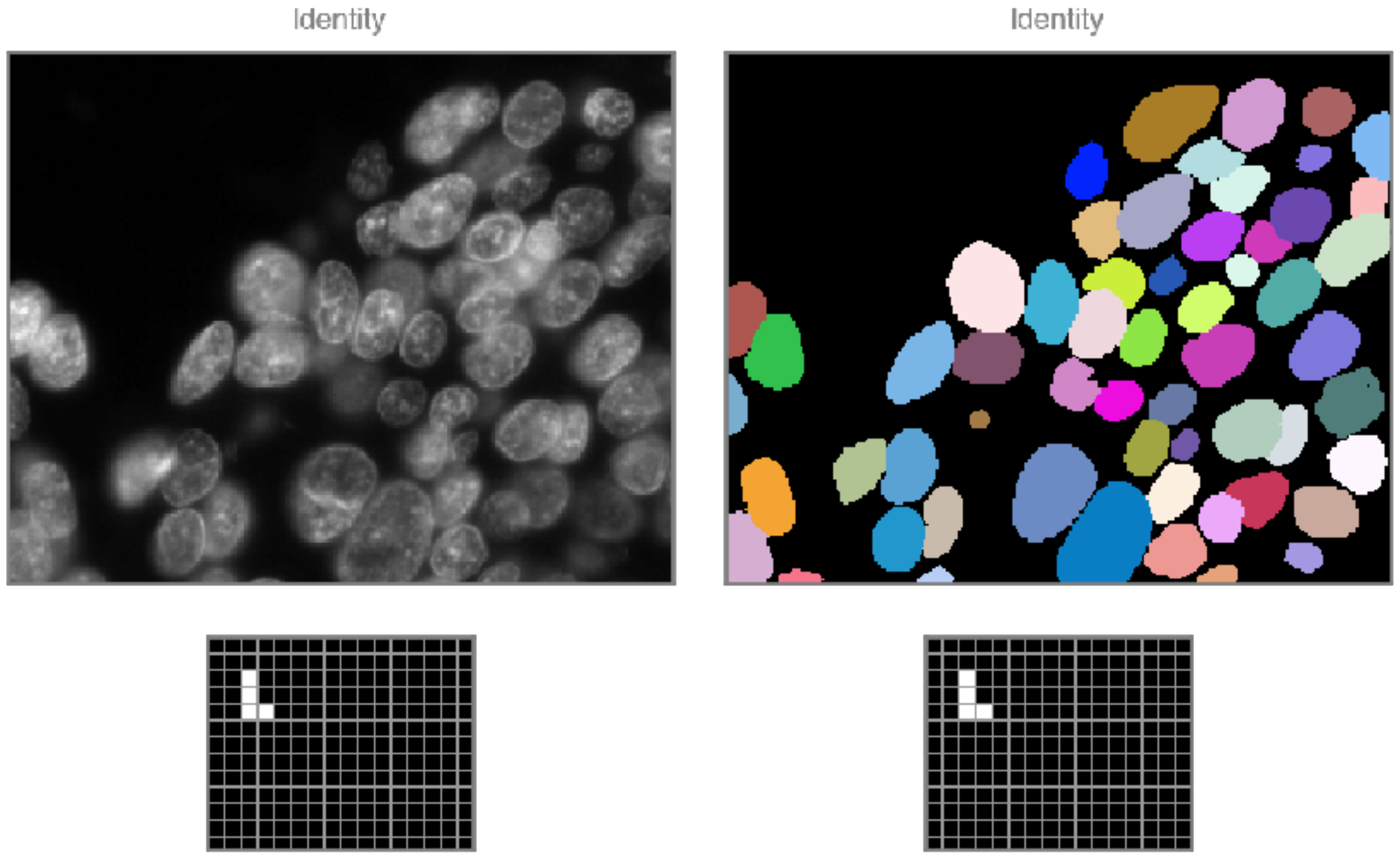
Artificially create more training data by transforming existing images/masks into different, yet plausible versions



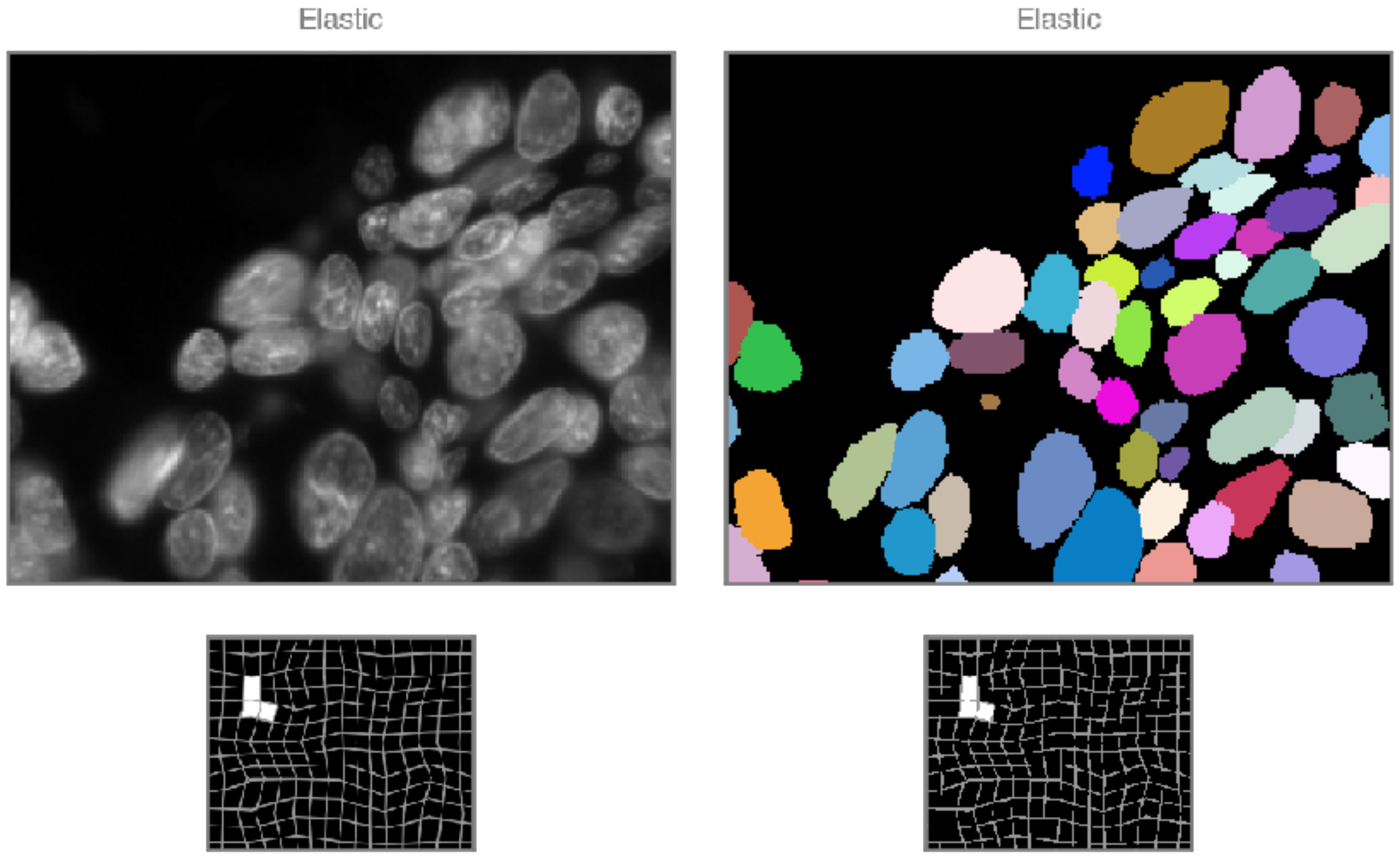
Data Augmentation

Artificially create more training data by transforming existing images/masks into different, yet plausible versions

Original

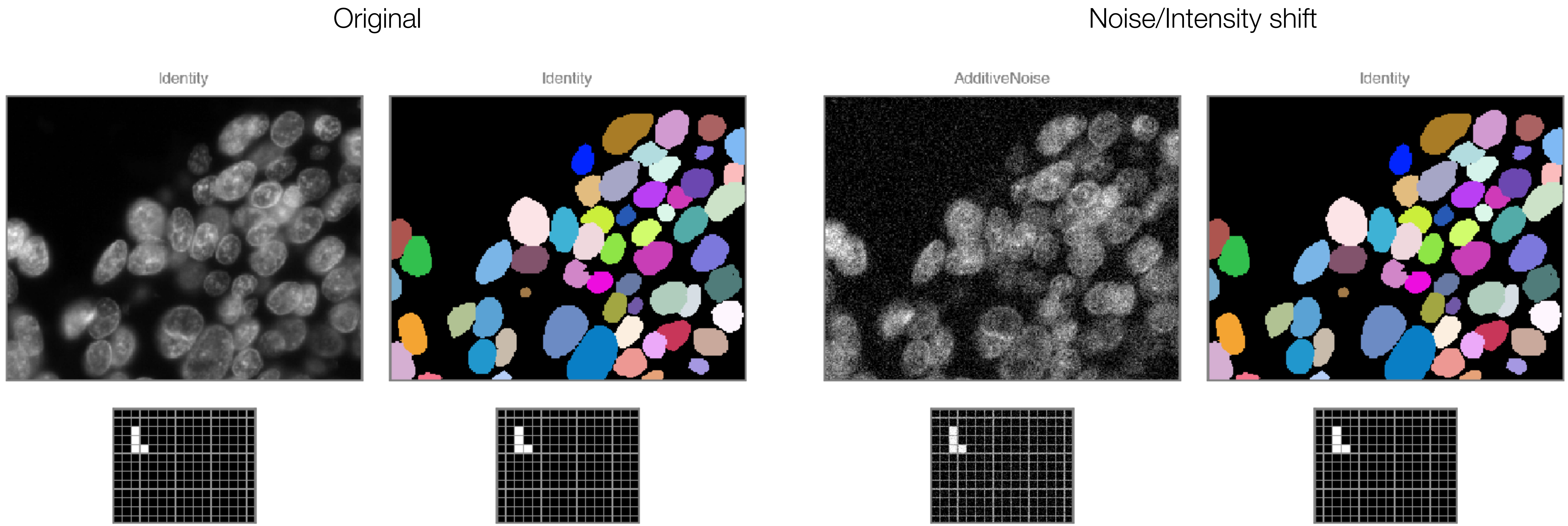


Elastic deformation



Data Augmentation

Artificially create more training data by transforming existing images/masks into different, yet plausible versions



Demo: Training of custom models (python)

Generate Annotations & train own model

python **GPU**

→

Predict on your data

python **CPU (or GPU)**

Google colab

Webinar Demo on Google Colab
(Click to try it out!)

Where to ask questions / get help?

Github project issues page

- Technical questions
- Bugs, unexpected behavior
- Missing functionality

https://github.com/mpicbg-csbd/stardist/issues

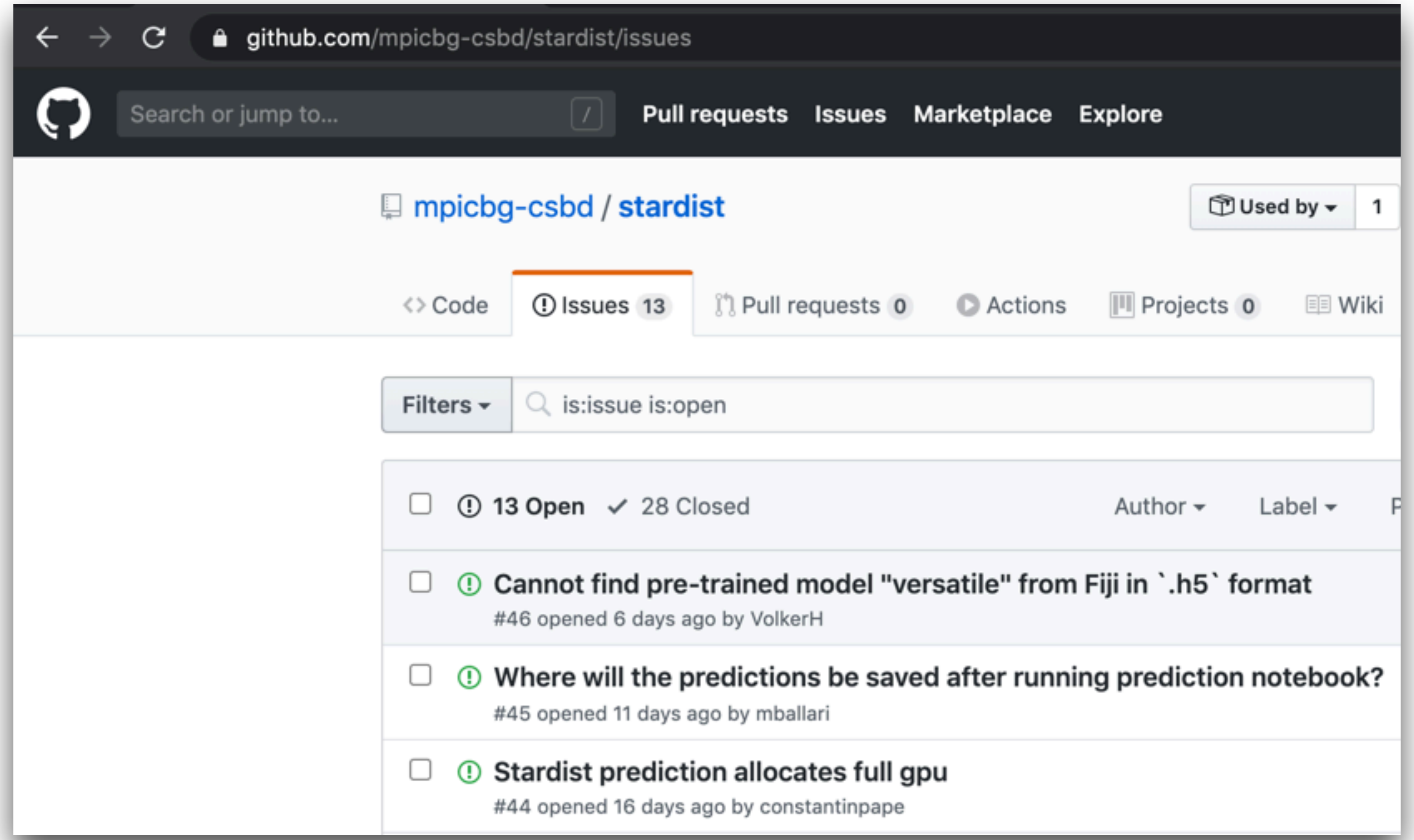
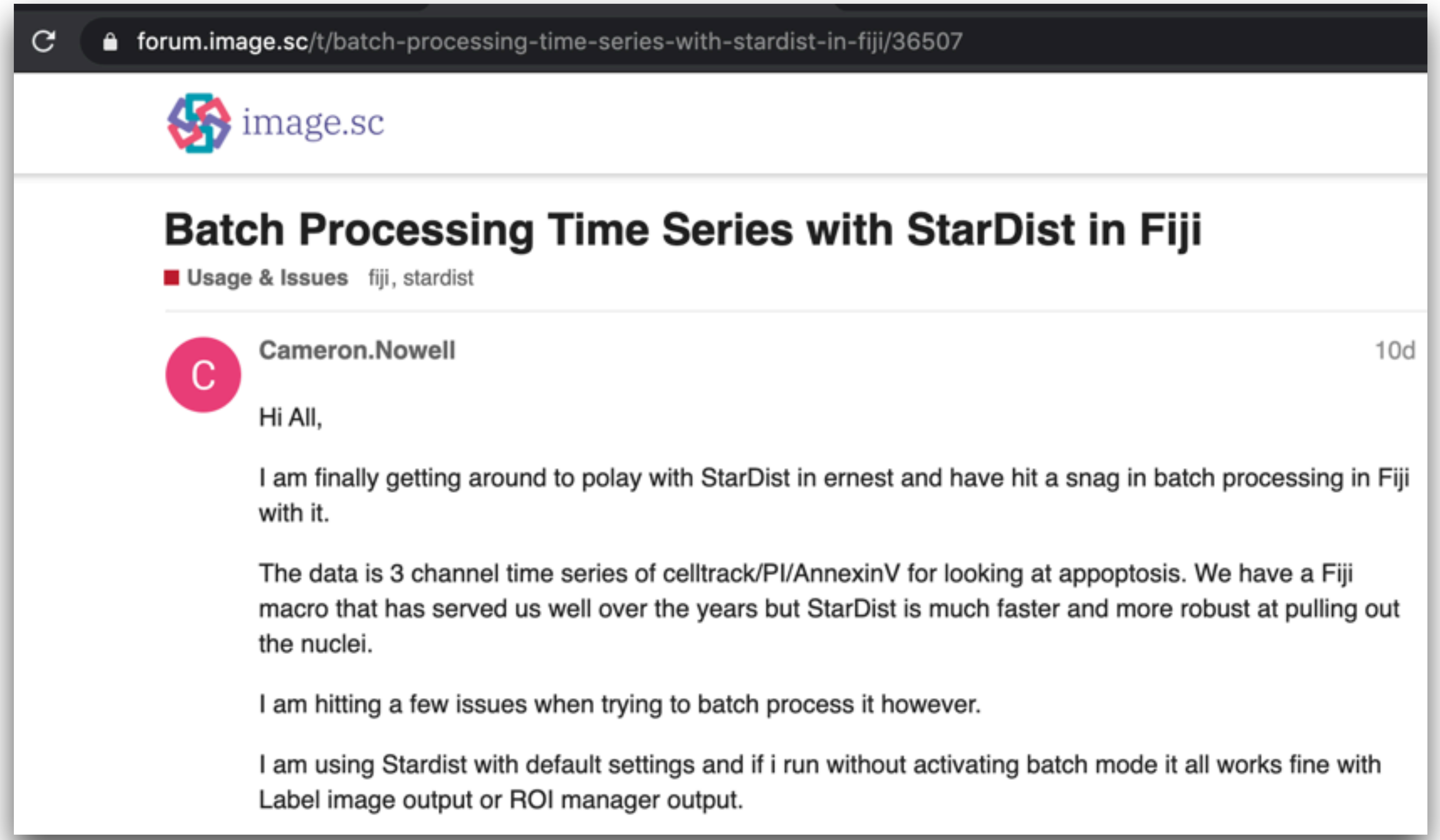


Image.sc forum

- Usage questions
- Best practices
- Problems with training/data

https://forum.image.sc (use tag "stardist"!)



StarDist in a core facility

Questions & Answers 2

Acknowledgments

Neubias Team: Julien Colombelli, Romain Guiet …

MPI-CBG/CSBD Dresden

- Gene Myers
- **Uwe Schmidt**
- Robert Haase
- Coleman Broaddus

Institut de Génomique Fonctionnelle de Lyon
École Normale Supérieure de Lyon

- Ko Sugawara
- Michalis Averof
- Frederike Alwes

UCL/LMCB London

- Sian Culley

University of Manchester

- Anna Maria Tsakiroglou

EPFL Lausanne

- Olivier Burri
- Romain Guiet
- Daniel Sage
- Silvia Monari
-

Janelia Research Campus, Virginia

- Stephan Saalfeld
- Phillipp Hanslovsky

Max-Delbrück Center (MDC) Berlin

- Dagmar Kainmüller