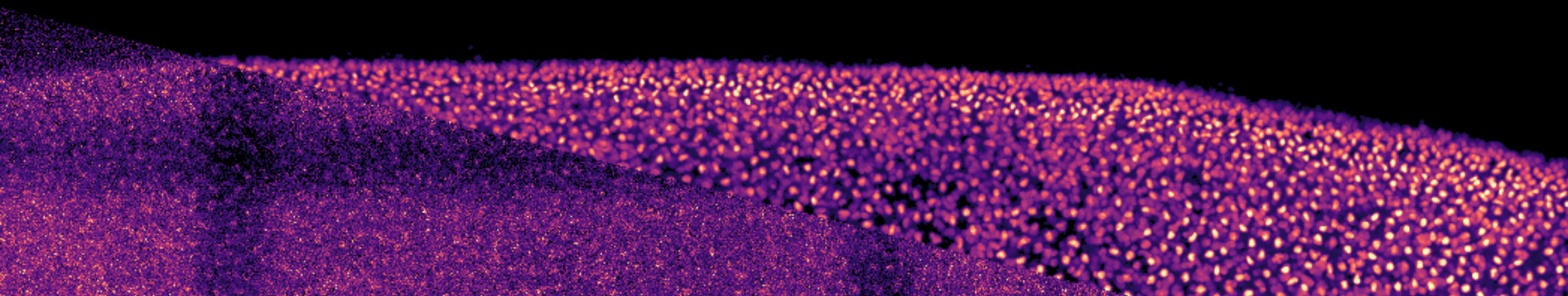
Image analysis for microscopy with machine learning

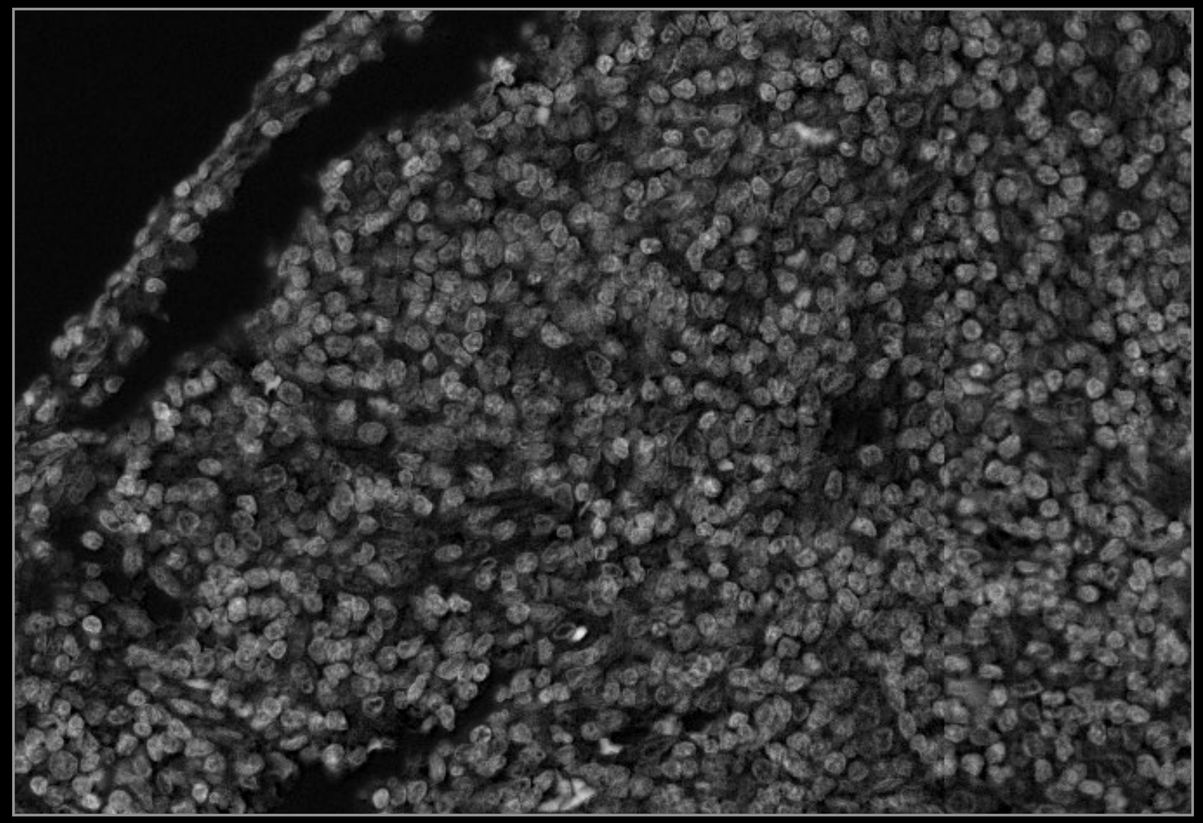
BIO-221
October 31st, 2024

Albert Dominguez Mantes
Weigert Group, EPFL 🇨🇭/TU Dresden🇩🇪
https://www.epfl.ch/labs/weigert-lab/



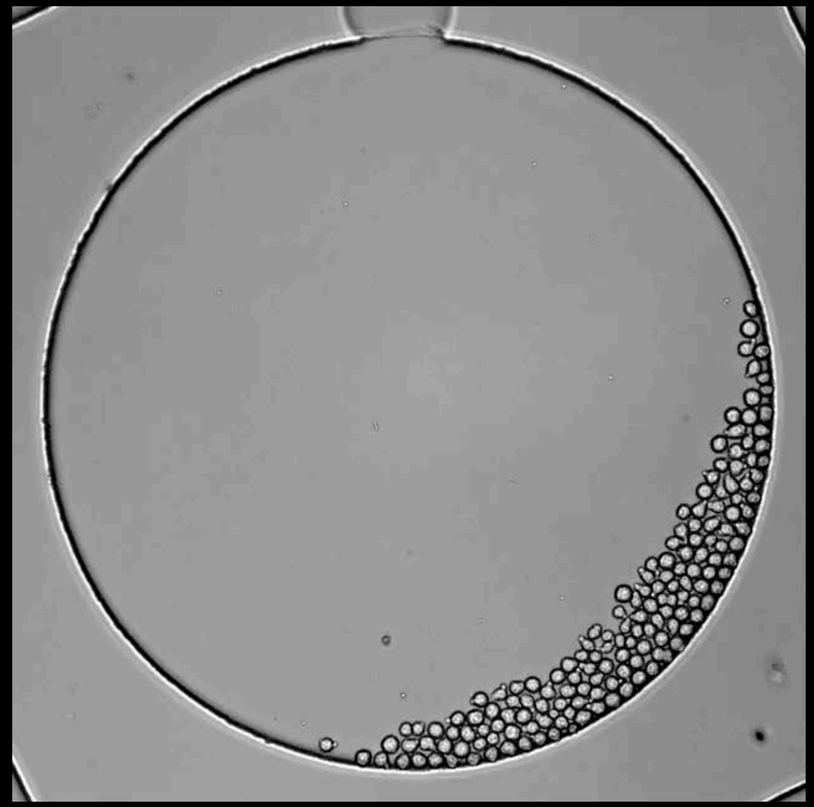
Typical Data in Microscopy

2D (fluorescence)



Data from Anna Maria Tsakiroglou (Manchester)

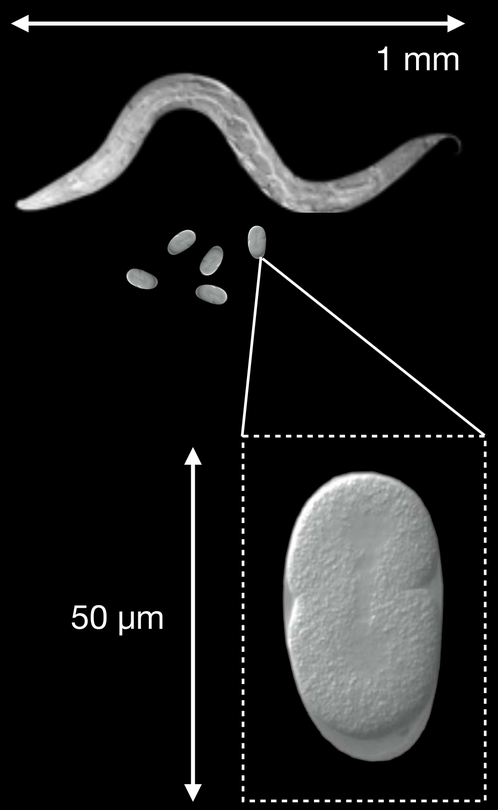
2D + time (brightfield)



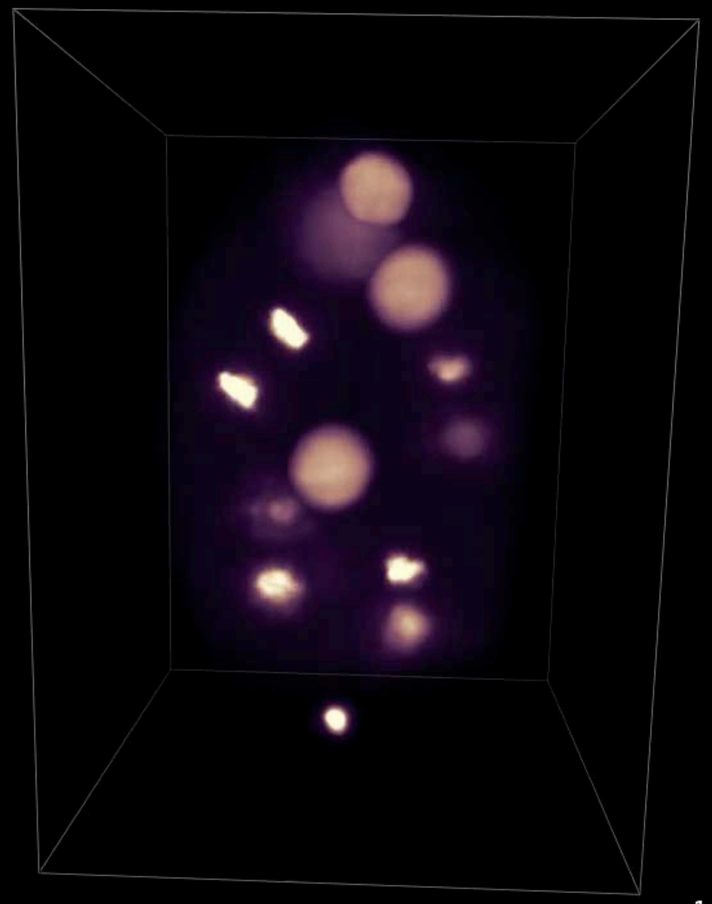
Mouse stem cells, data from cell tracking challenge

Typical Data in Microscopy

3D + time - fluorescence microscopy of developing organisms



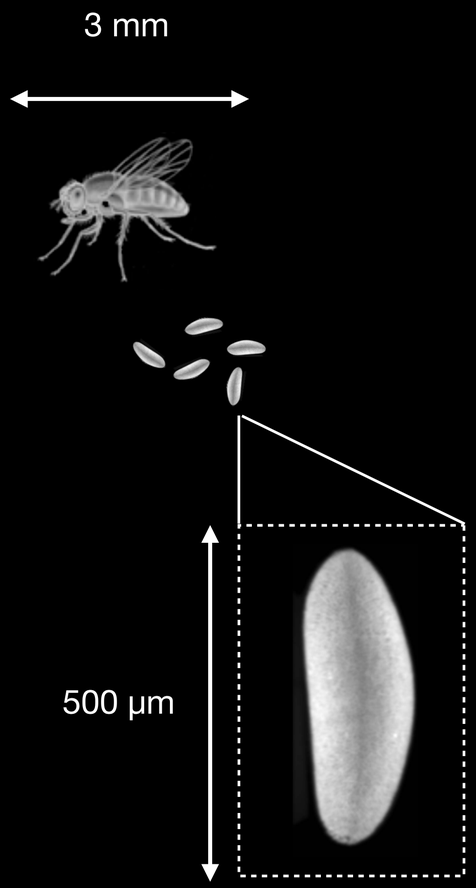
C. elegans

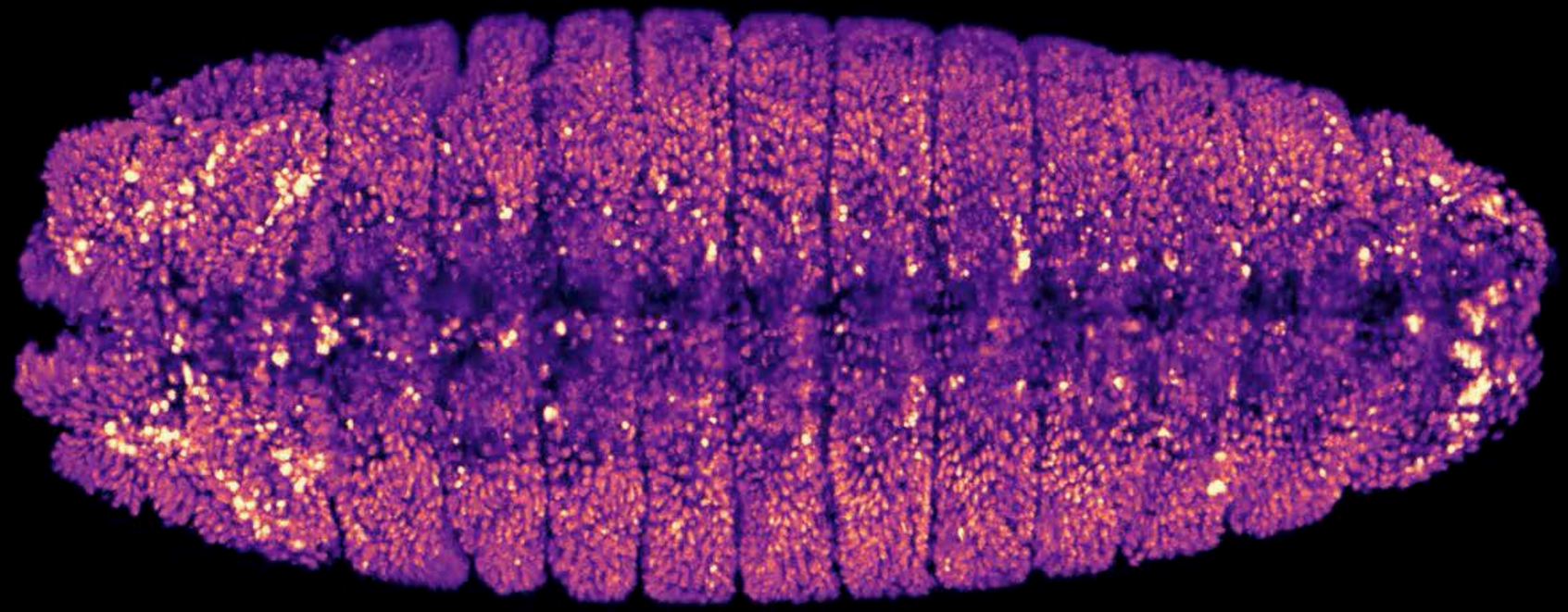


Data: Gene Myers Lab (Dresden)

Typical Data in Microscopy

3D + time - fluorescence microscopy of developing organisms





Drosophila melanogaster

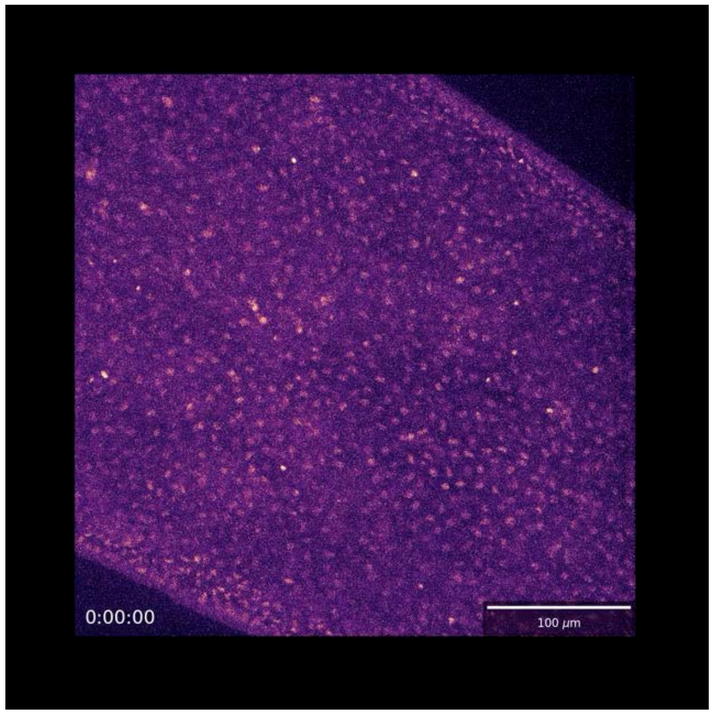
Royer et al. (2016)

Image Analysis for Microscopy

Extraction of quantitative biological information from digitised camera images

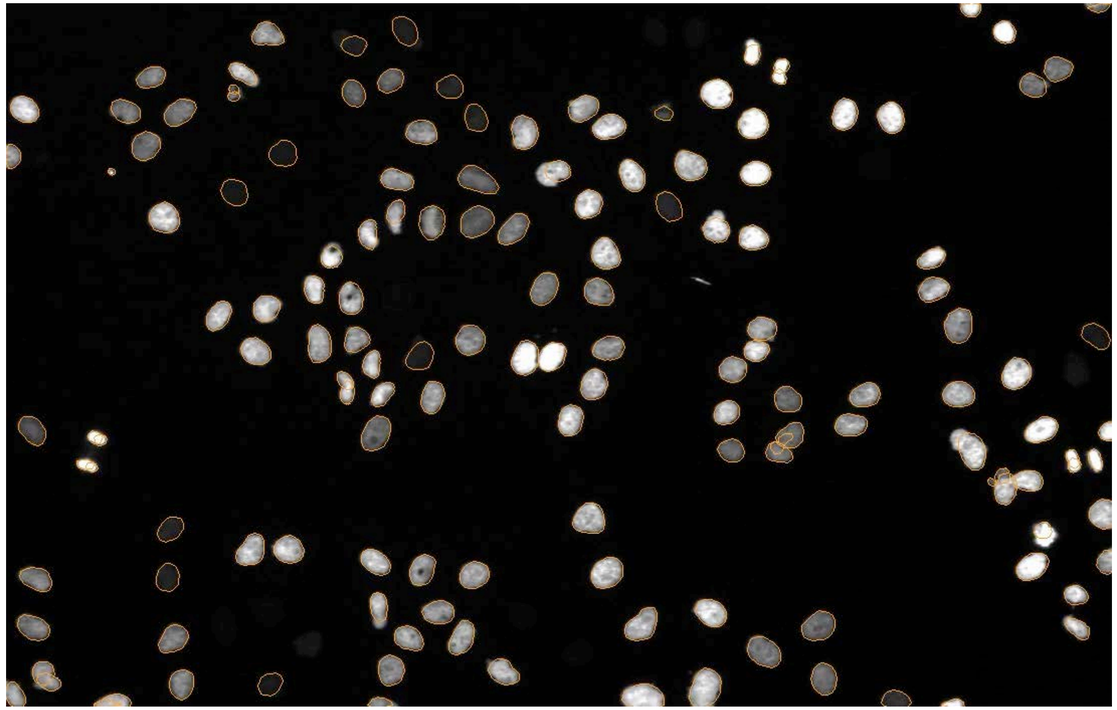
Image denoising/restoration

Remove camera noise, remove blur…



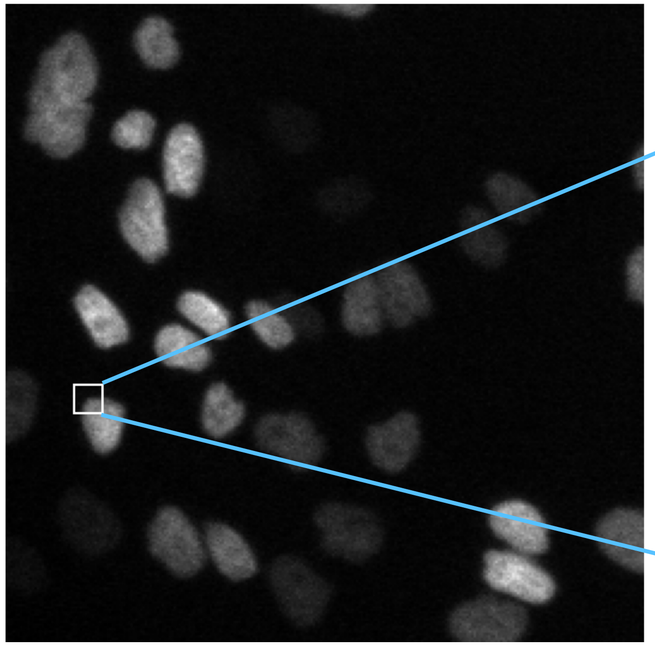
Object Detection and Segmentation

Find contours of cells, detect spots, track cells over time, …



What Is It Like to Be a Machine?

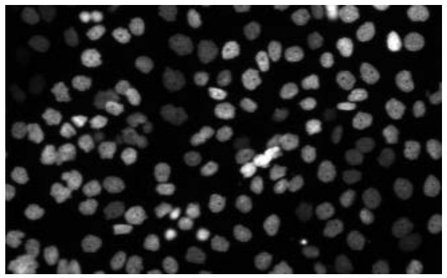
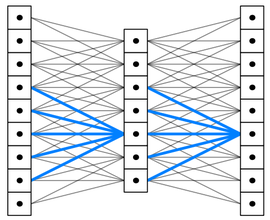
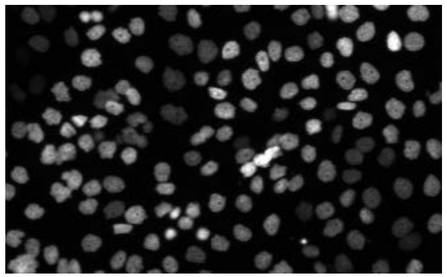
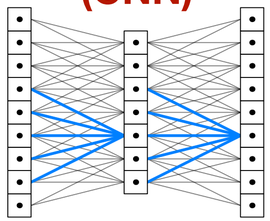
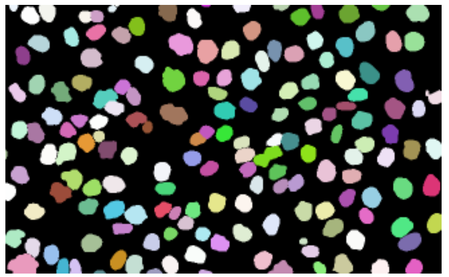
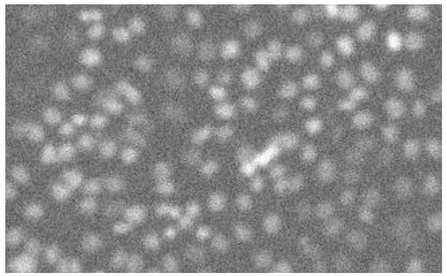
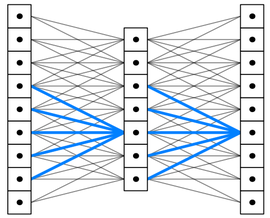
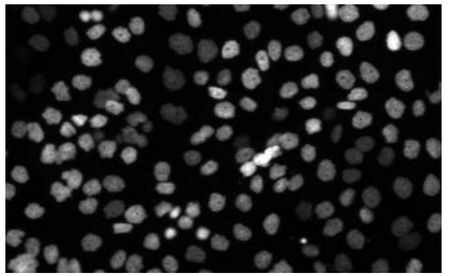
Acquired Image



Pixel level

13	7	10	11	18	17	19	6	11	5	12	13
12	11	6	7	12	13	13	8	11	7	10	13
10	9	17	11	17	17	13	19	14	14	16	31
9	6	14	21	20	16	21	17	30	24	18	14
19	14	23	28	18	23	17	29	24	16	31	26
14	19	17	15	24	21	26	41	48	48	48	56
11	11	14	23	33	46	106	111	112	116	108	103
16	16	17	26	41	80	153	188	169	199	203	182
16	12	40	35	77	123	158	223	229	207	240	209
13	25	16	50	112	175	203	227	234	252	222	224
18	18	16	54	131	198	221	233	247	199	219	232
21	18	36	73	126	187	234	256	240	225	236	258

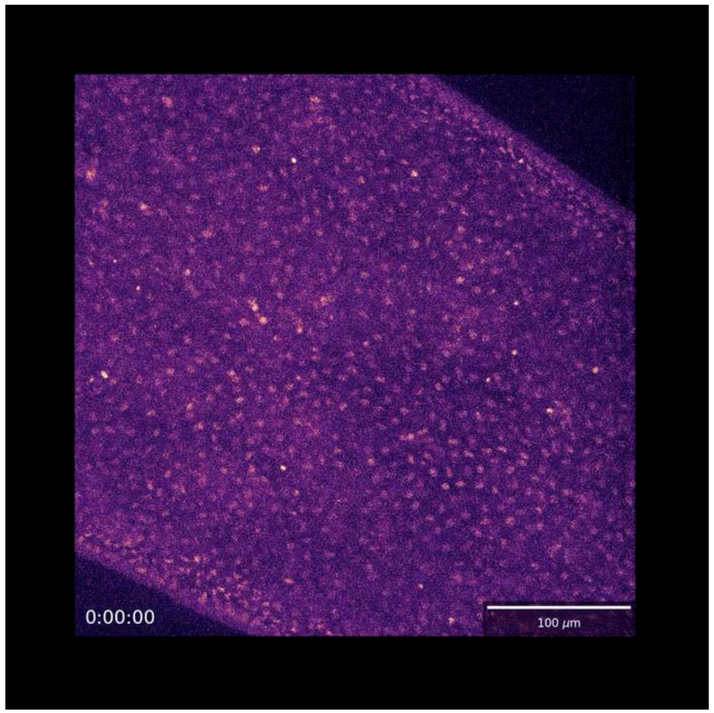
Artificial neural networks are powerful for many bioimage tasks

Input		Output	Task
Image 	 **Convolutional Neural Networks (CNN)**	*Discrete-valued class* “wildtype” or “mutant”	**Classification**
Image 		*Label Mask (class per pixel)* 	**(Dense) Segmentation** **Object Detection**
Image 		*Image* 	**(Dense) Regression** **Image2Image** **Image Restoration**

Extraction of quantitative biological information from digitised camera images
(Computer Vision)

Image Processing/Restoration

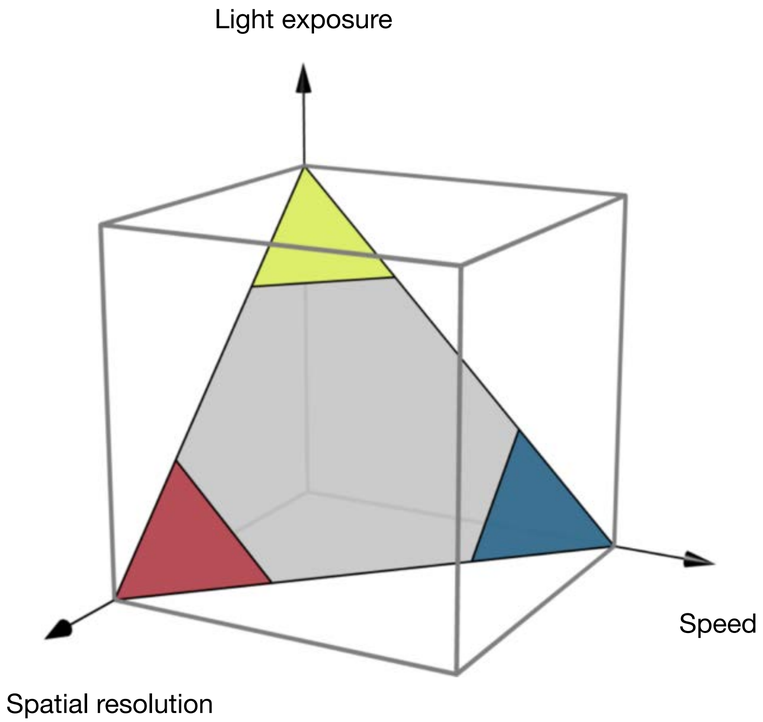
Remove camera noise, remove blur…

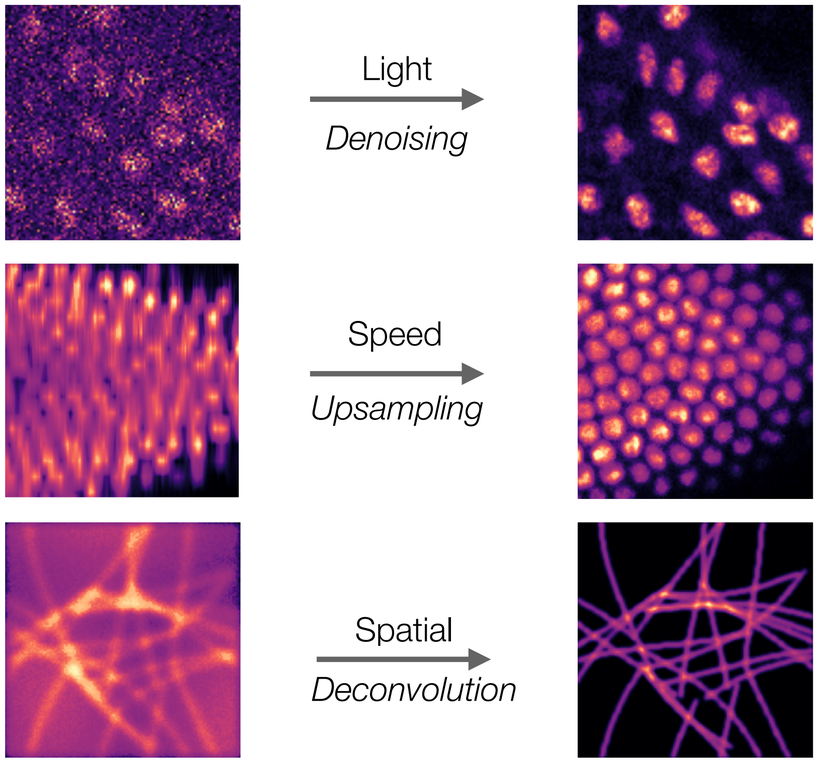


Object Detection and Segmentation

Find contours of cells, track cells over time, …

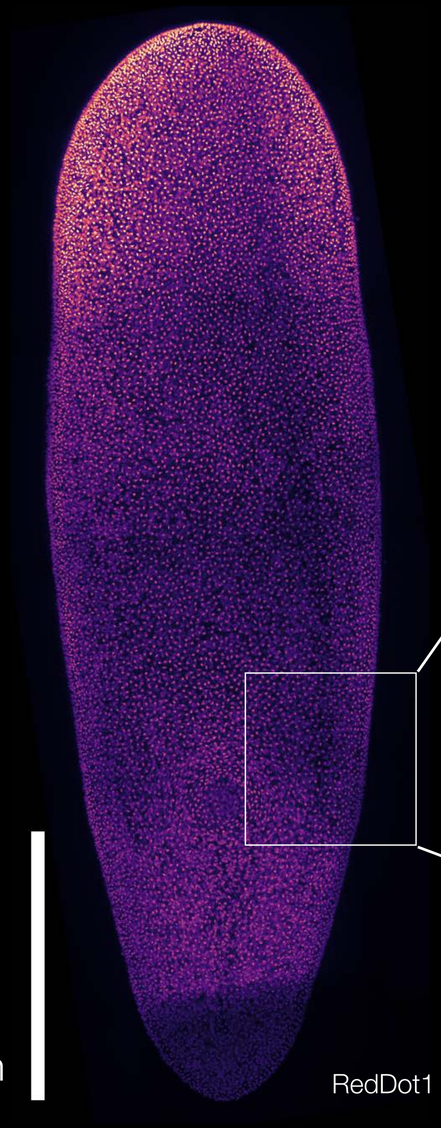
Image Restoration





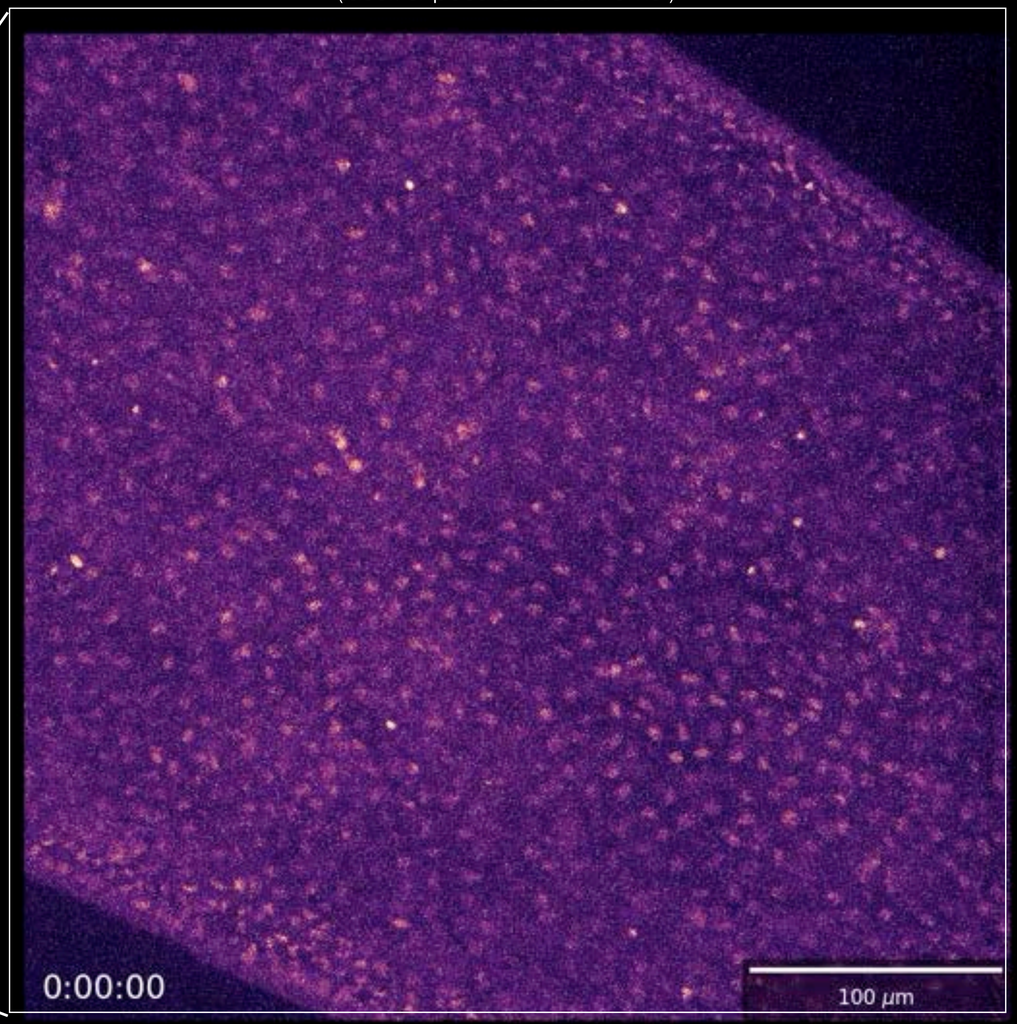
Necessity of low light imaging

Planaria (Flatworm)



Low light dosage / low SNR

(laser/exposure: 0.3%/10ms)

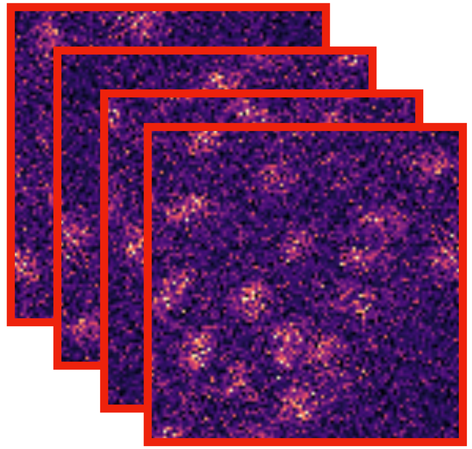


Data from Tobias Boothe

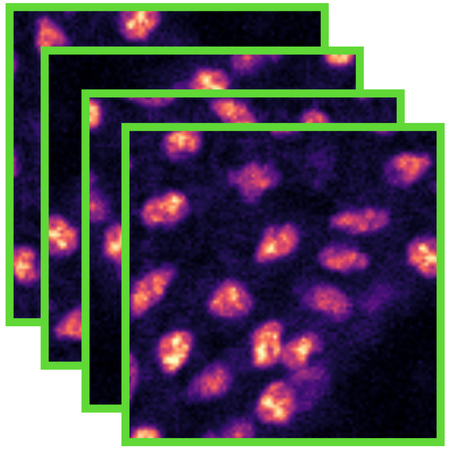
CARE: supervised deep learning for image restoration

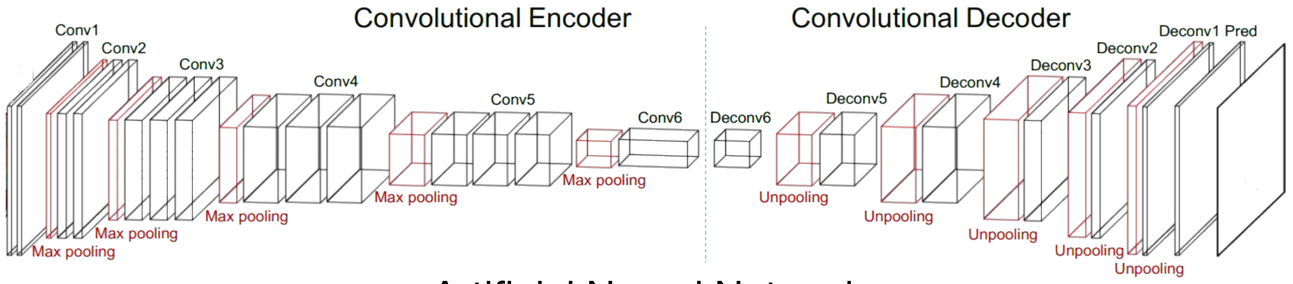
Pairwise training data of input/output pairs
Learn a function that implements the mapping noisy -> clean

Input



Output

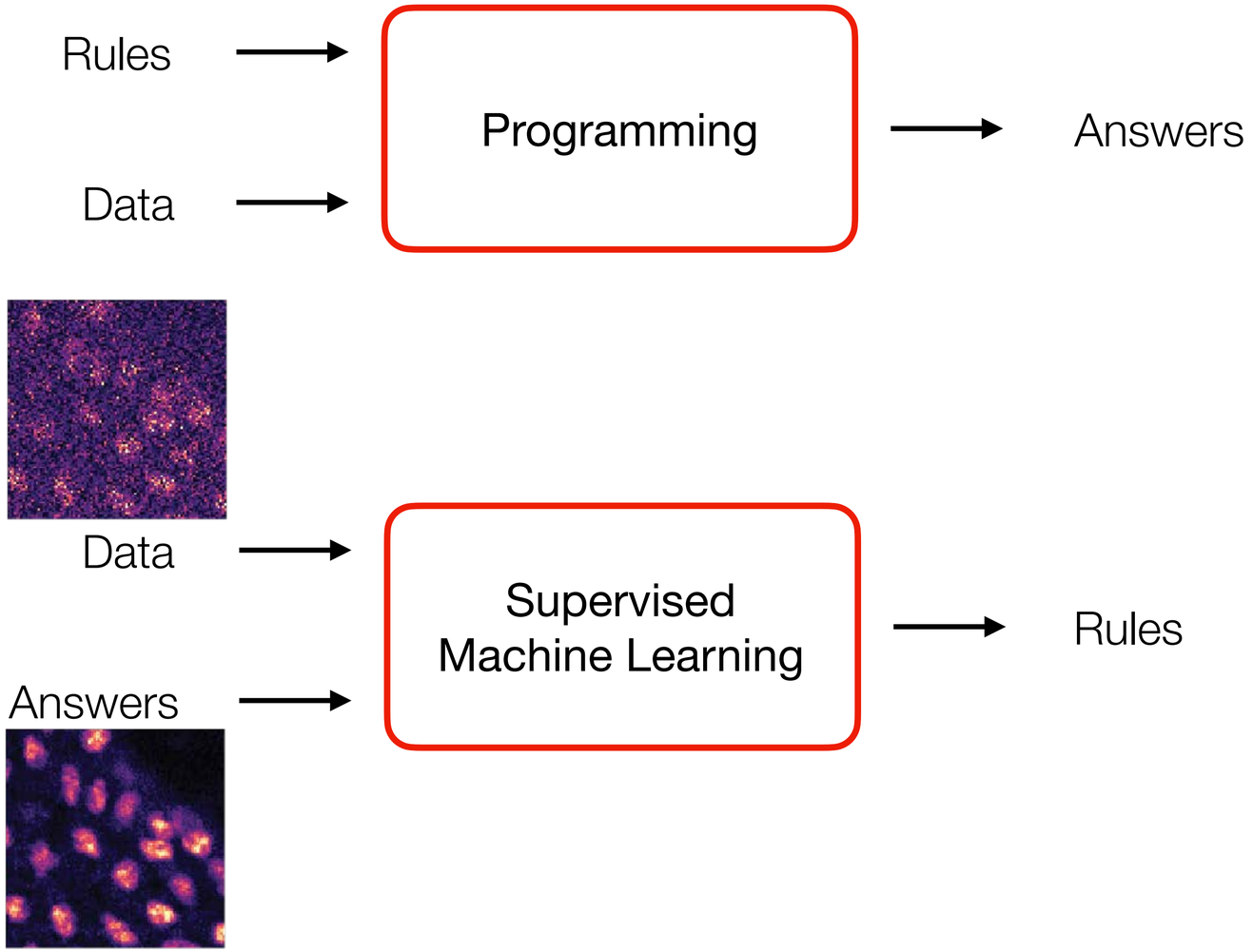




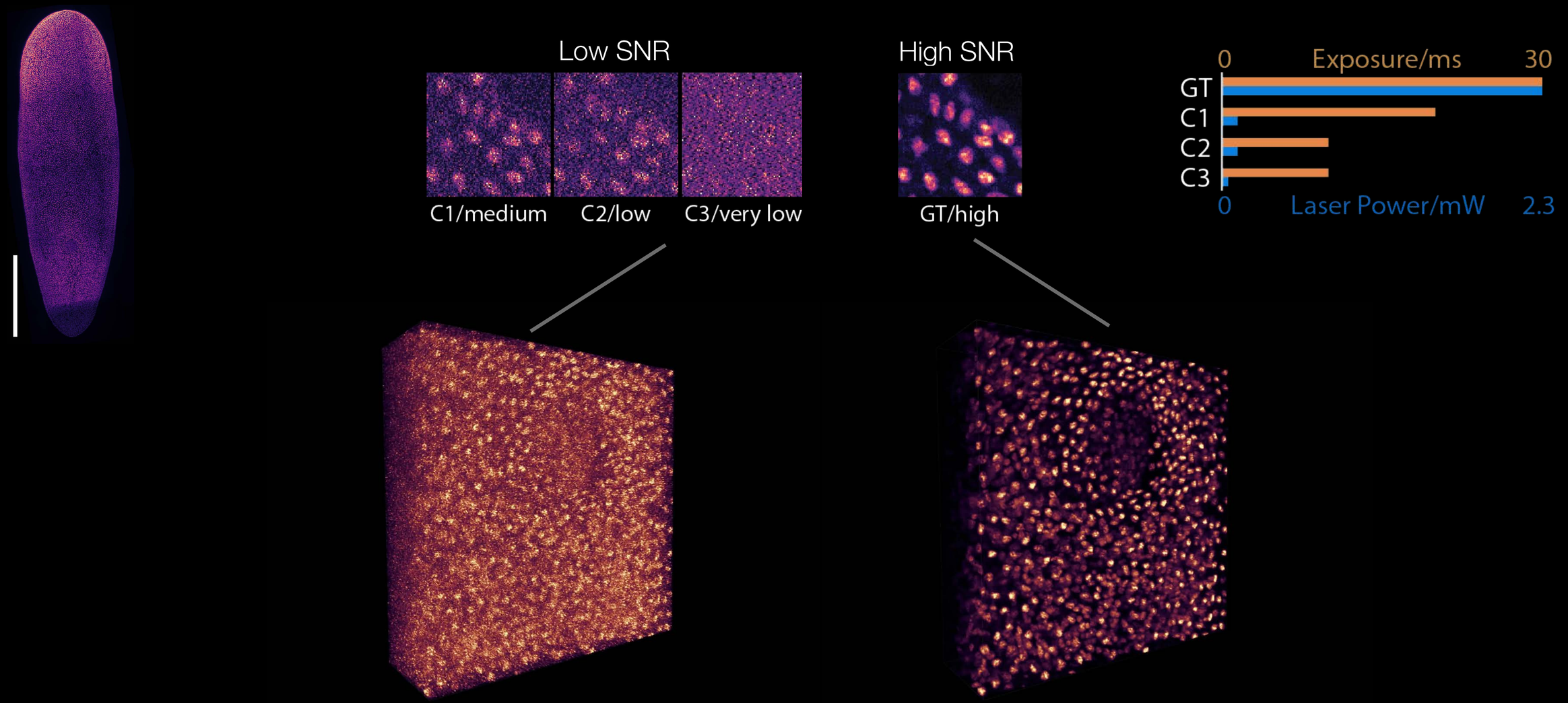
Artificial Neural Network

Weigert et al. *Content-aware image restoration: pushing the limits of fluorescence microscopy* (Nature Methods 2018)

ELI5: supervised machine learning

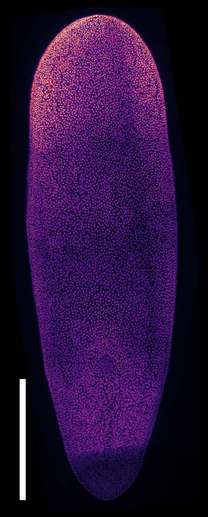


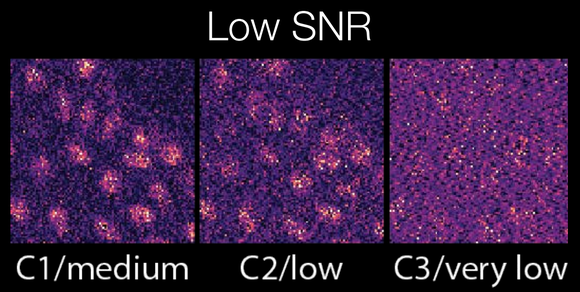
CARE: supervised deep learning for image restoration

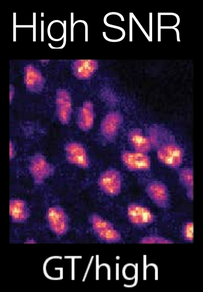


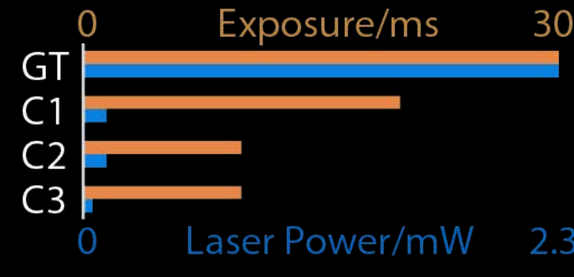
Weigert et al. *Content-aware image restoration: pushing the limits of fluorescence microscopy* (Nature Methods 2018)

CARE: supervised deep learning for image restoration

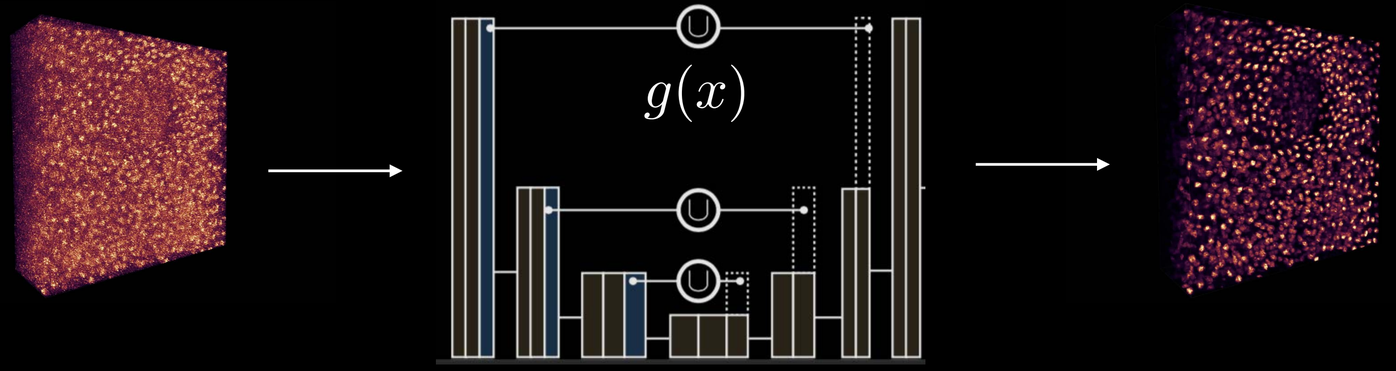








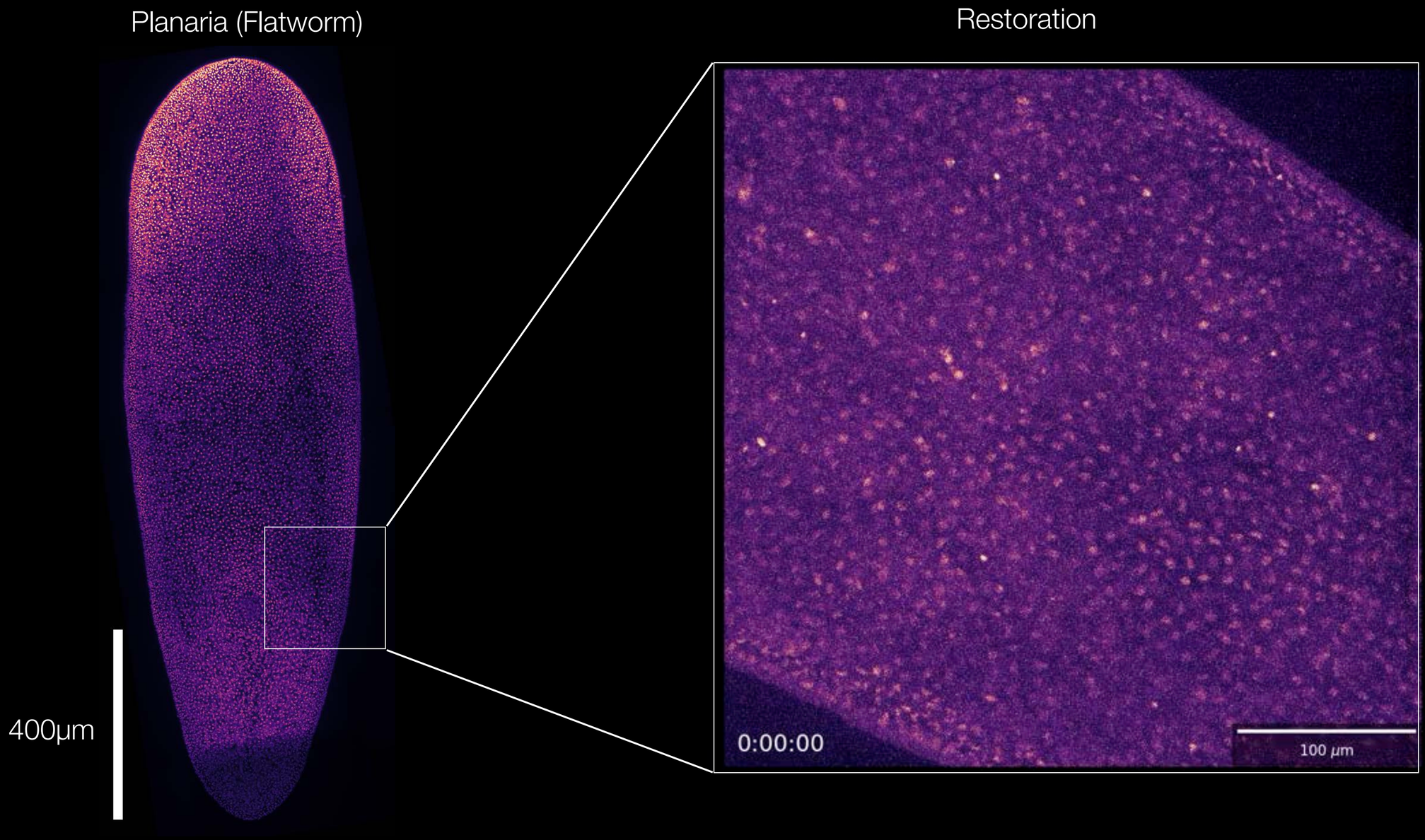
Regression model
(convolutional neural network)



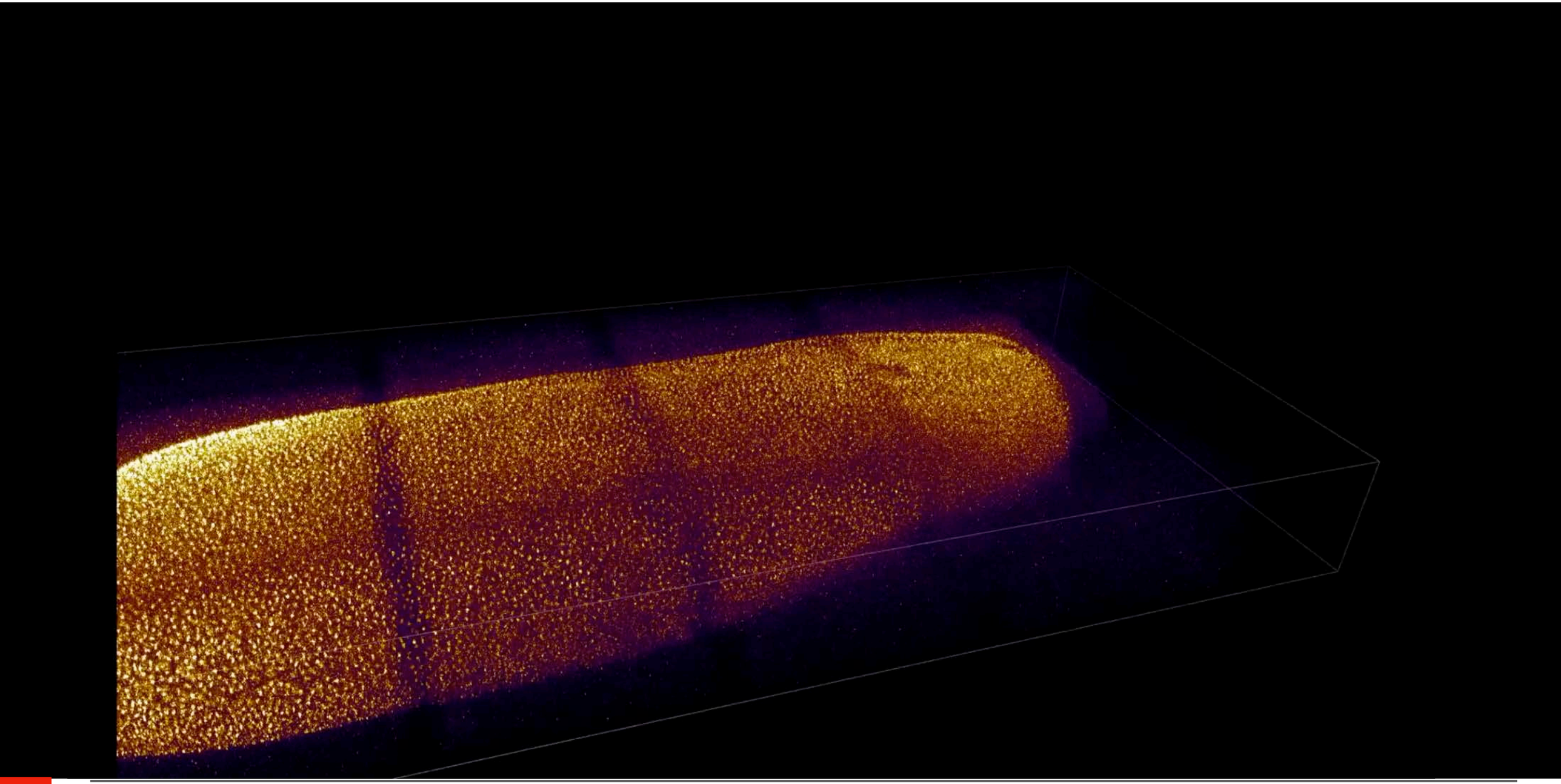
- Trained on 96 pairs (160GB)
- Training time ~ 8hrs

Weigert et al. *Content-aware image restoration: pushing the limits of fluorescence microscopy* (Nature Methods 2018)

CARE: supervised deep learning for image restoration



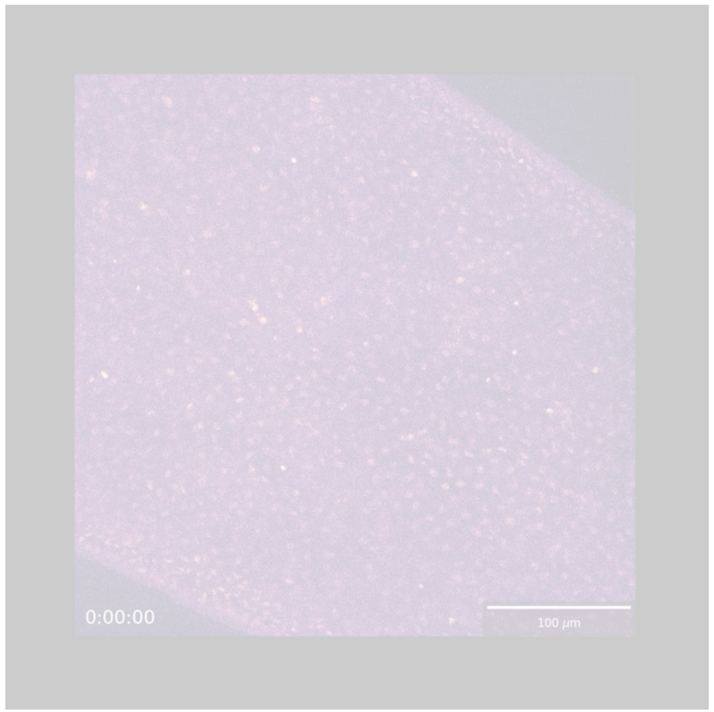
Weigert et al. *Content-aware image restoration: pushing the limits of fluorescence microscopy* (Nature Methods 2018)



Extraction of quantitative biological information from digitised camera images
(Computer Vision)

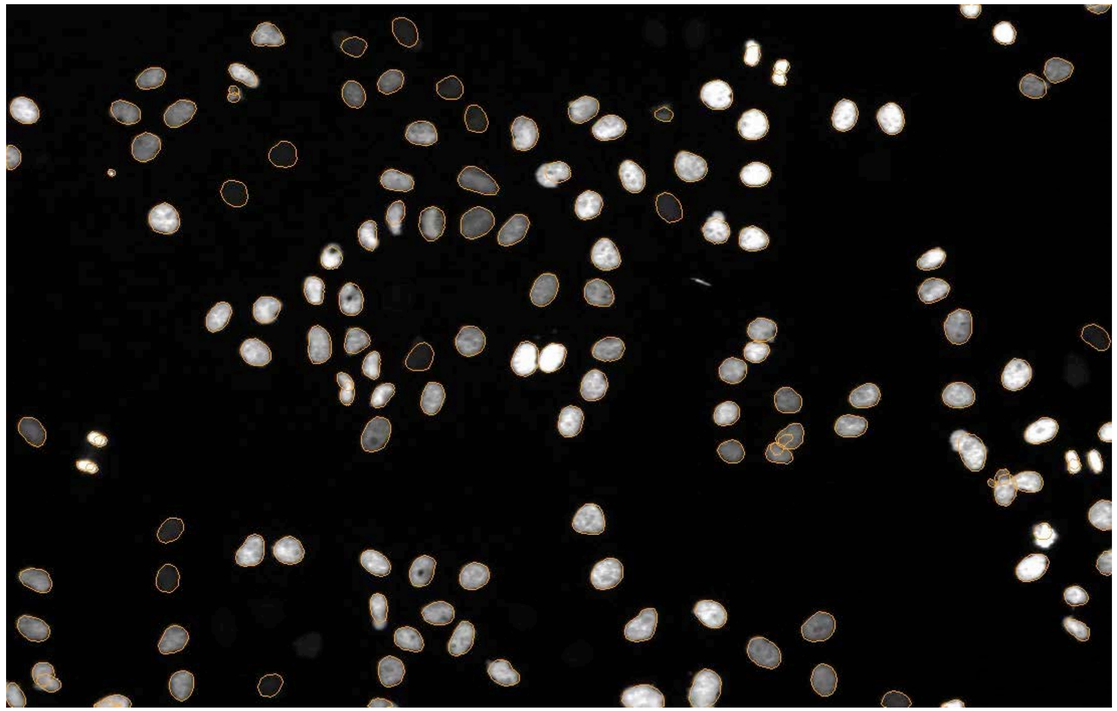
Image Denoising/Restoration

Remove camera noise, remove blur…



Object Detection and Segmentation

Find contours of cells, detect spots, track cells over time, …



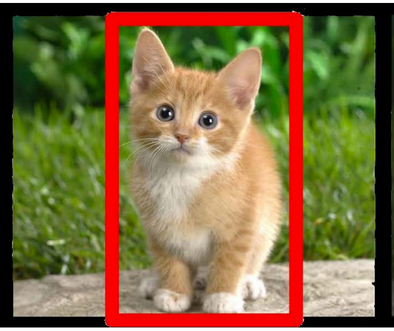
Common computer vision tasks

Single Object

Image Classification

Cat

Object Detection (Localization)



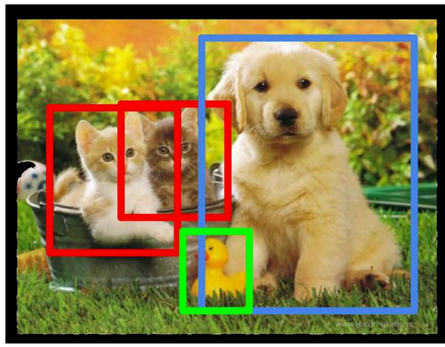
Cat

Dense/Semantic Segmentation

Cat

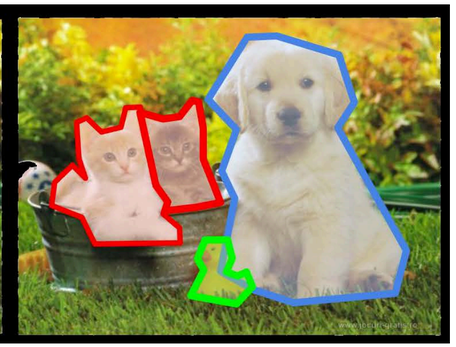
Multiple Objects

Object Detection



Cat, Dog, Duck

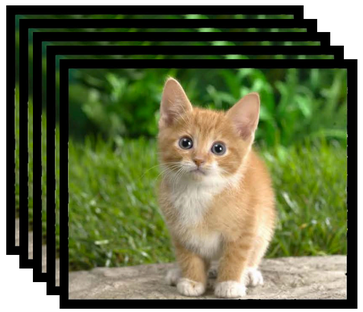
Instance Segmentation



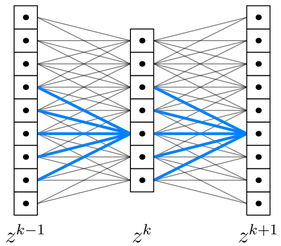
Cat, Dog, Duck

Currently the most successful paradigm: Supervised deep learning

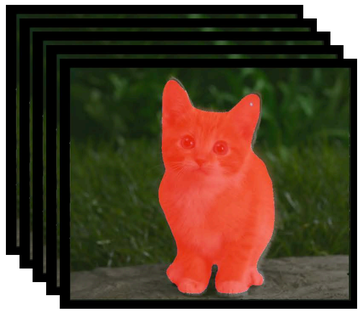
Input



Deep Neural Network



Output

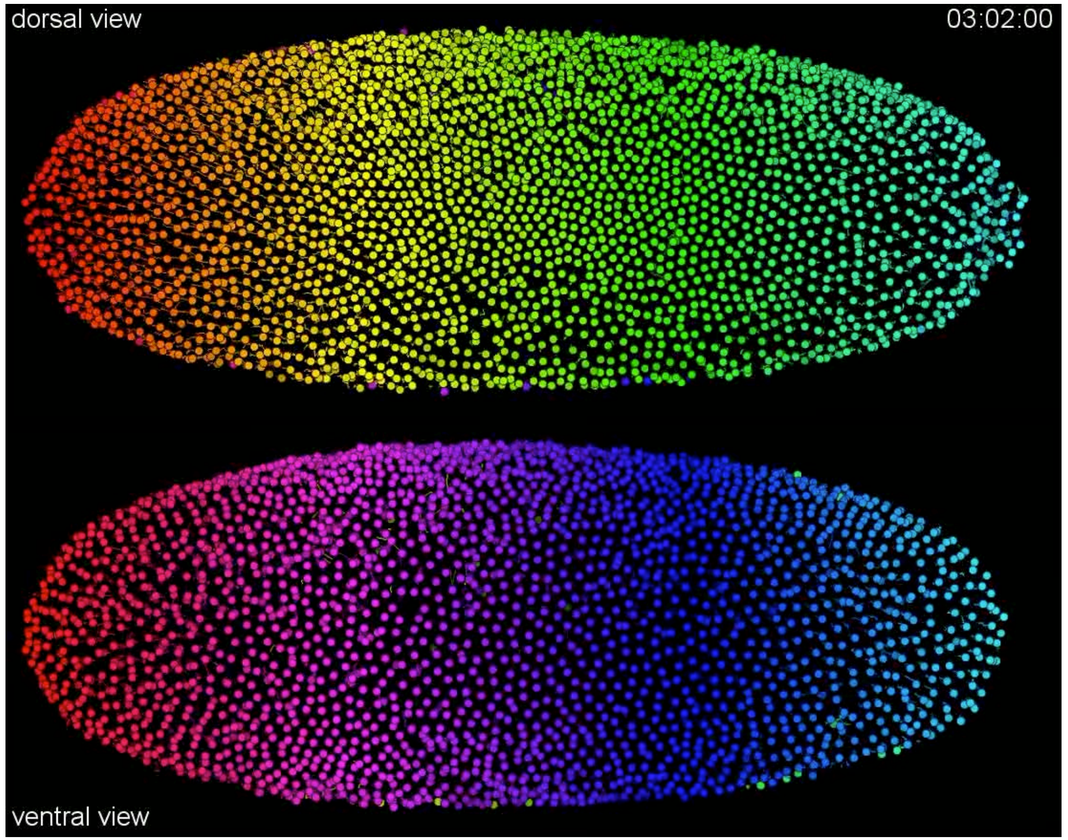


U-Net Ronneberger et al (2015)
YOLO Redmond et al (2016)
Mask-RCNN He et al (2017)

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

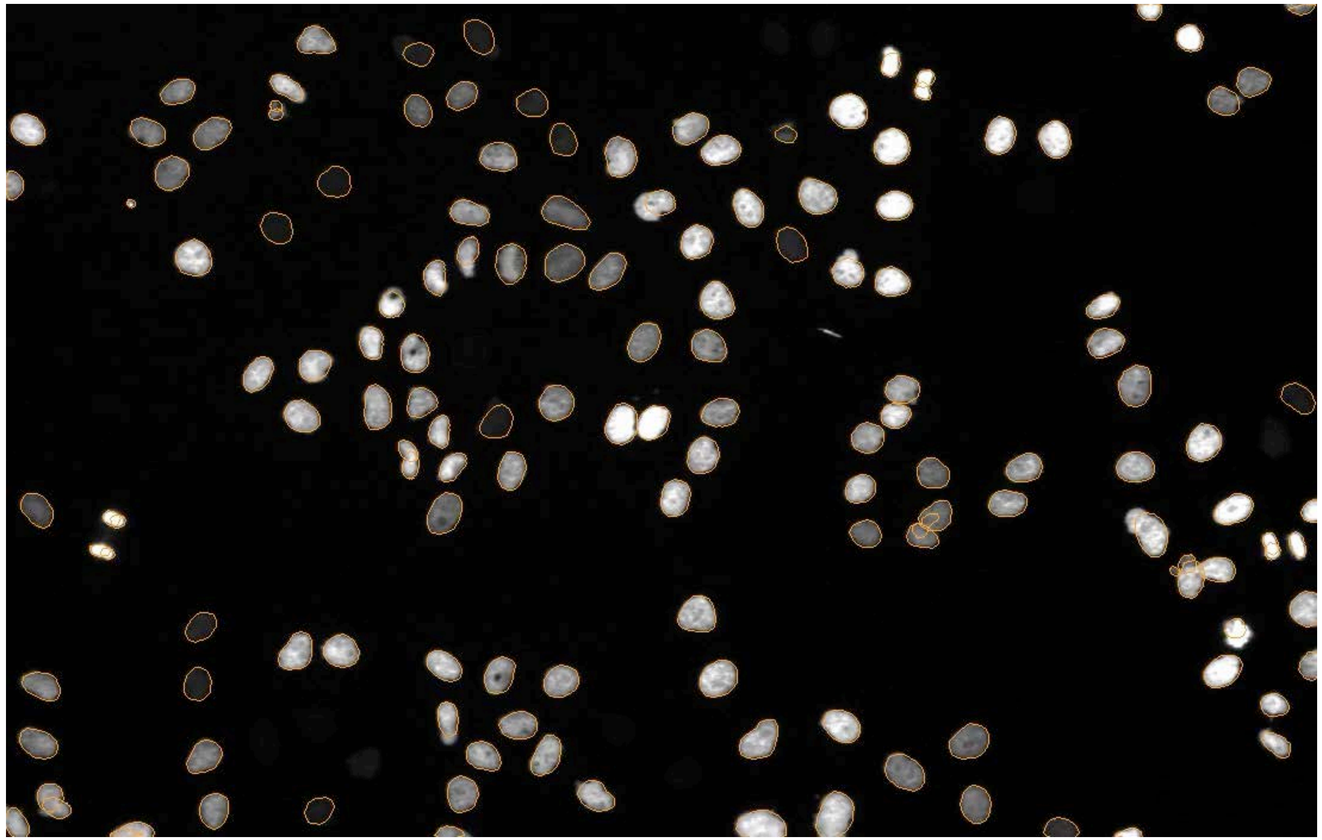
Common computer vision tasks in bioimaging

Detection + Tracking



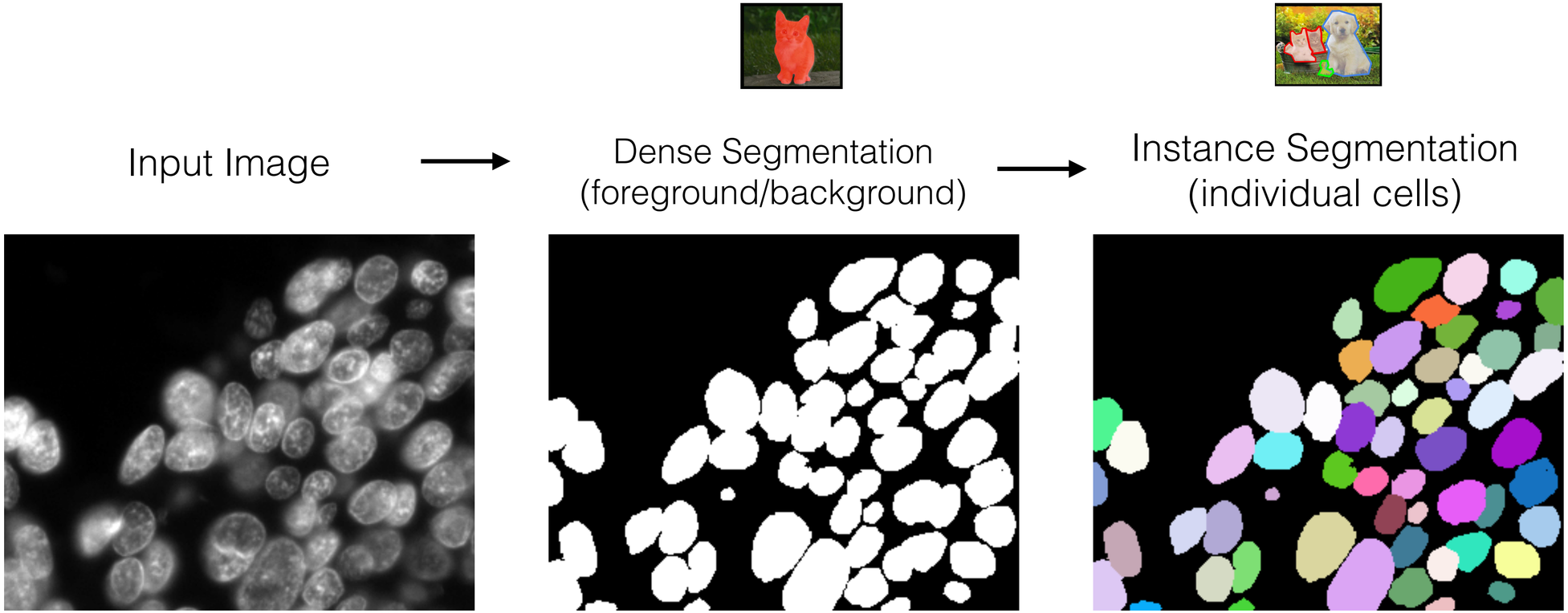
Drosophila Histone marker
Amat et al (2014)

Segmentation



HeLa cells stably expressing H2b-GFP
Data from http://celltrackingchallenge.net/

Segmentation as a computer vision problem

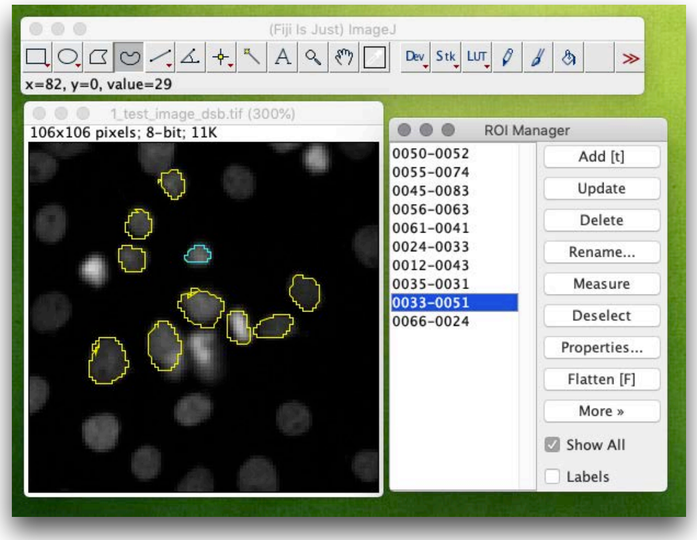


Challenges: many crowded objects, noisy images,
different from natural images (so knowledge cannot be directly transferred)

DL for segmentation: Annotations needed

Fiji/ImageJ

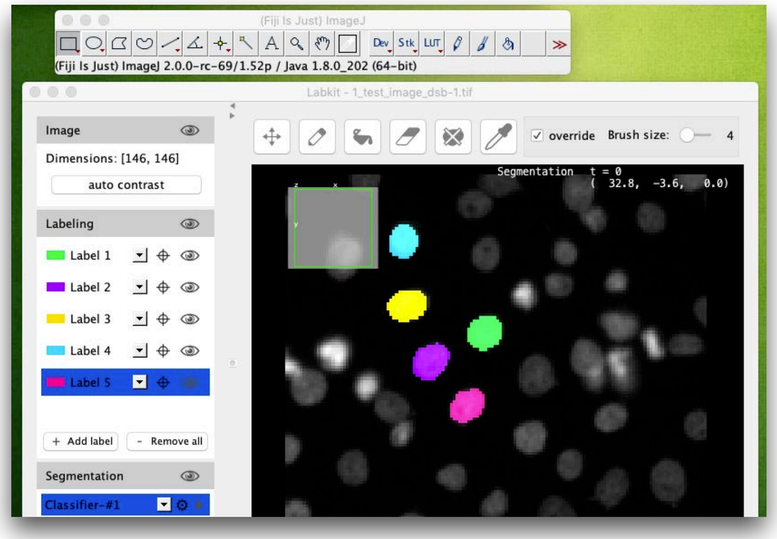
- Draw Roi per object (Roi-Manager)
- Convert to label mask (e.g. see here)



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

Fiji + LabKit

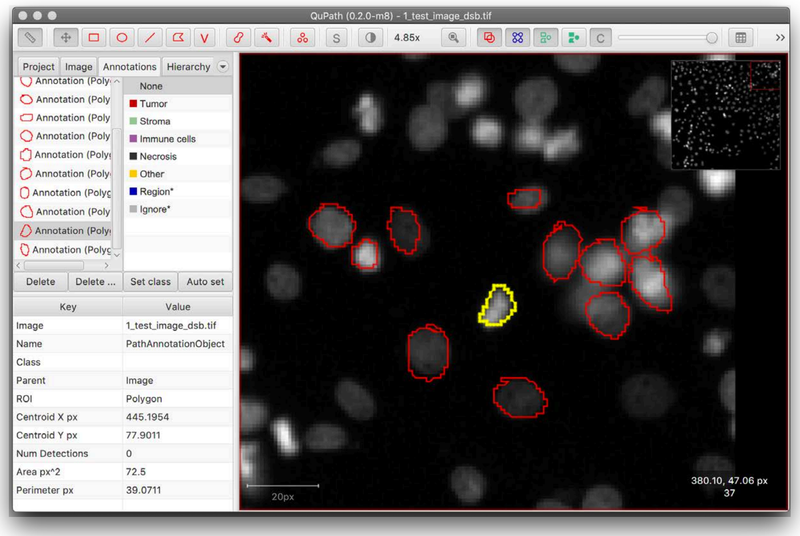
- Directly draw label mask



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

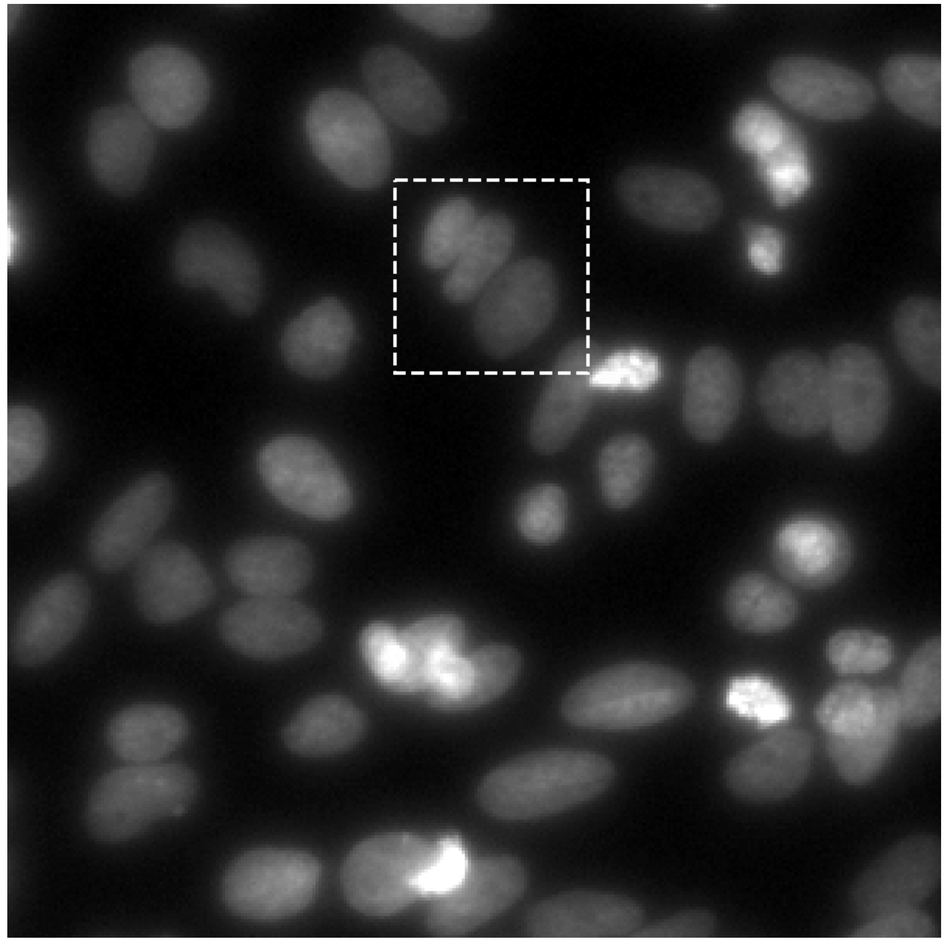
QuPath

- Draw ROI per object
- Convert to label mask

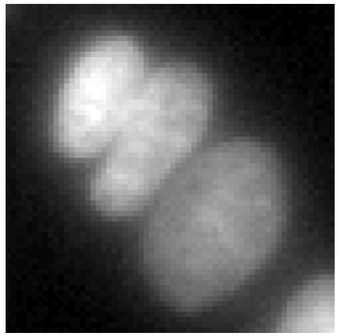


https://qupath.github.io Bankhead et al. (2017)

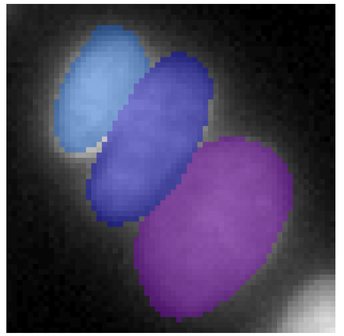
StarDist: nuclei segmentation with star-convex polygons



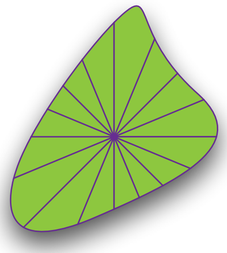
Image



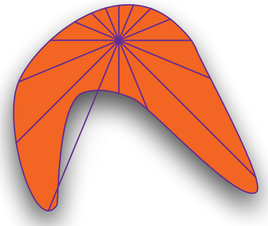
GT



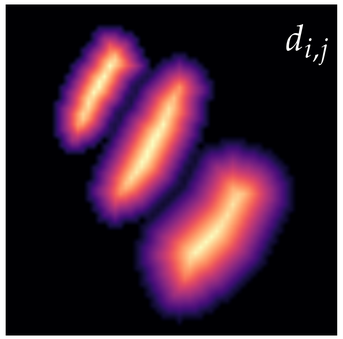
Star-Convex



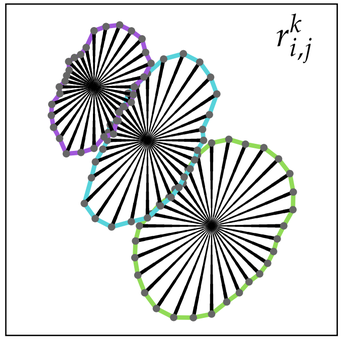
Not Star-Convex



Object Probabilities

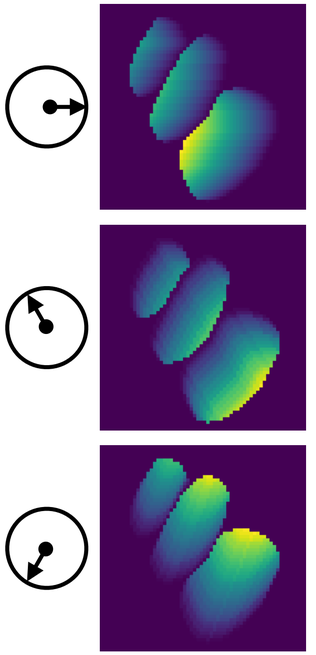


Radial Distances



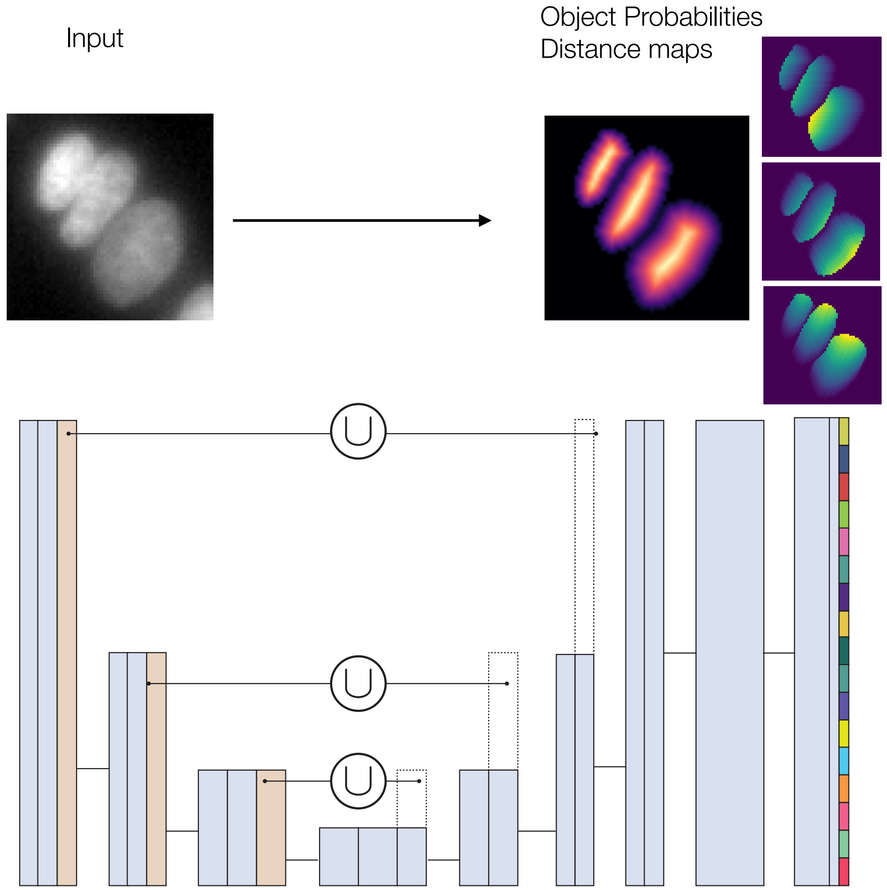
=

Distance maps (~32)



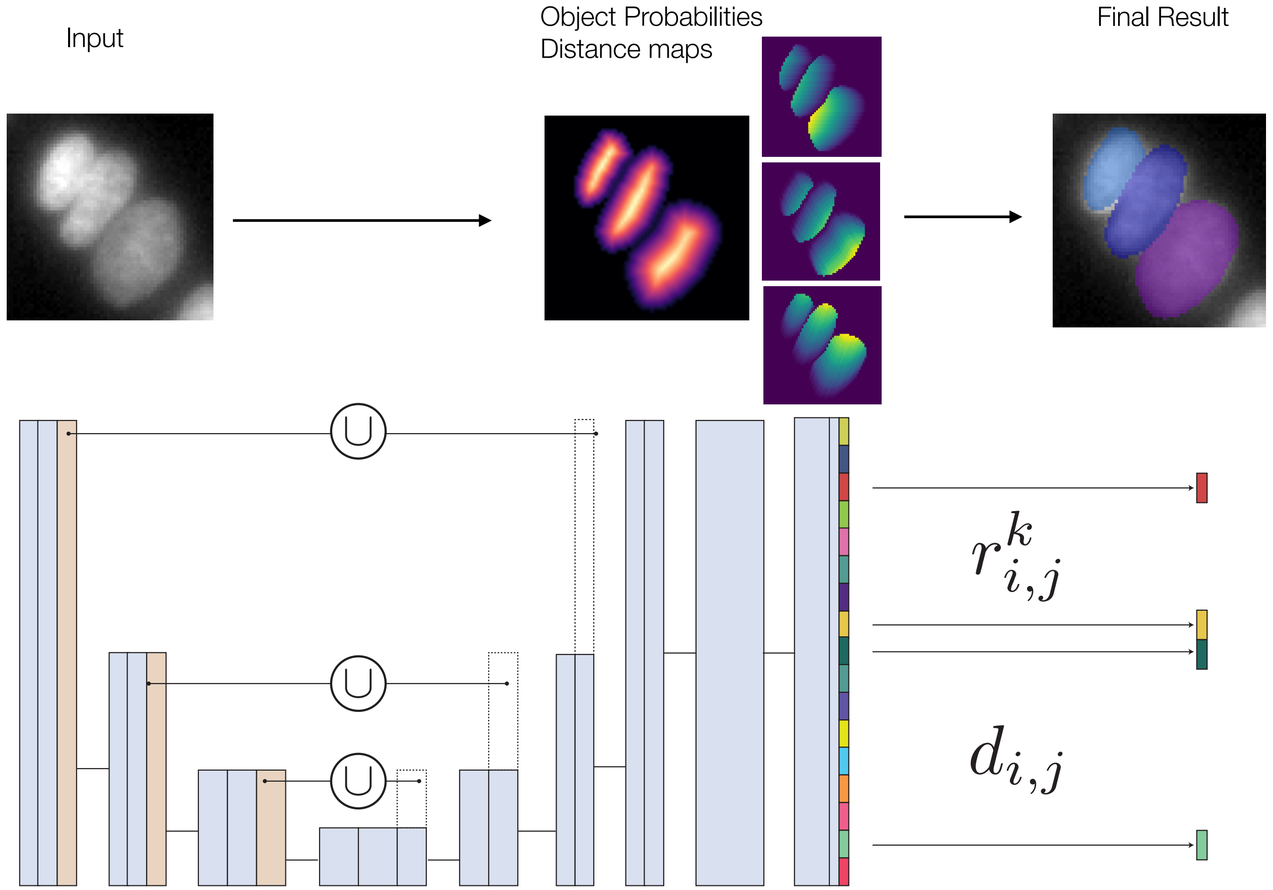
Approximate cells/nuclei with star-convex bounding polygon

StarDist: nuclei segmentation with star-convex polygons



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

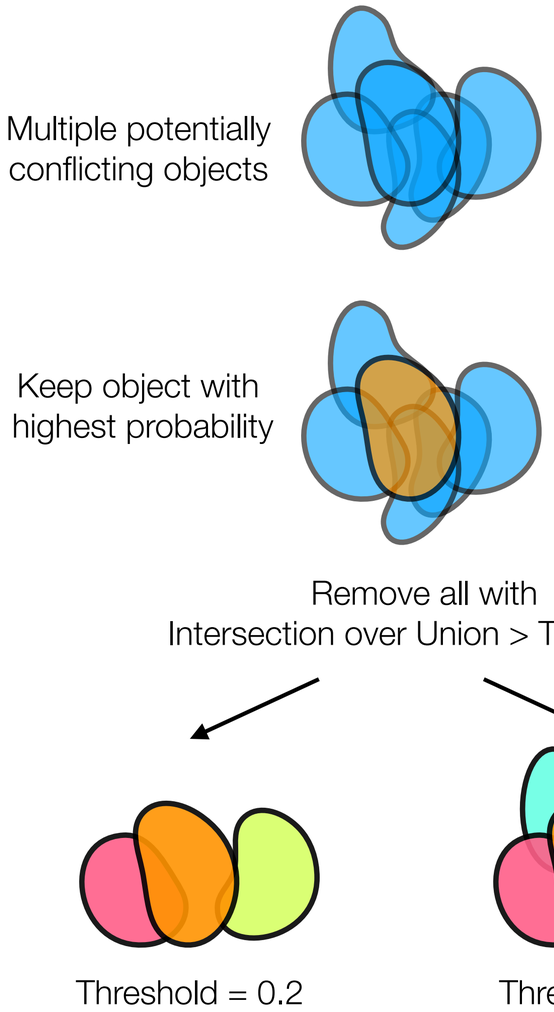
StarDist: nuclei segmentation with star-convex polygons



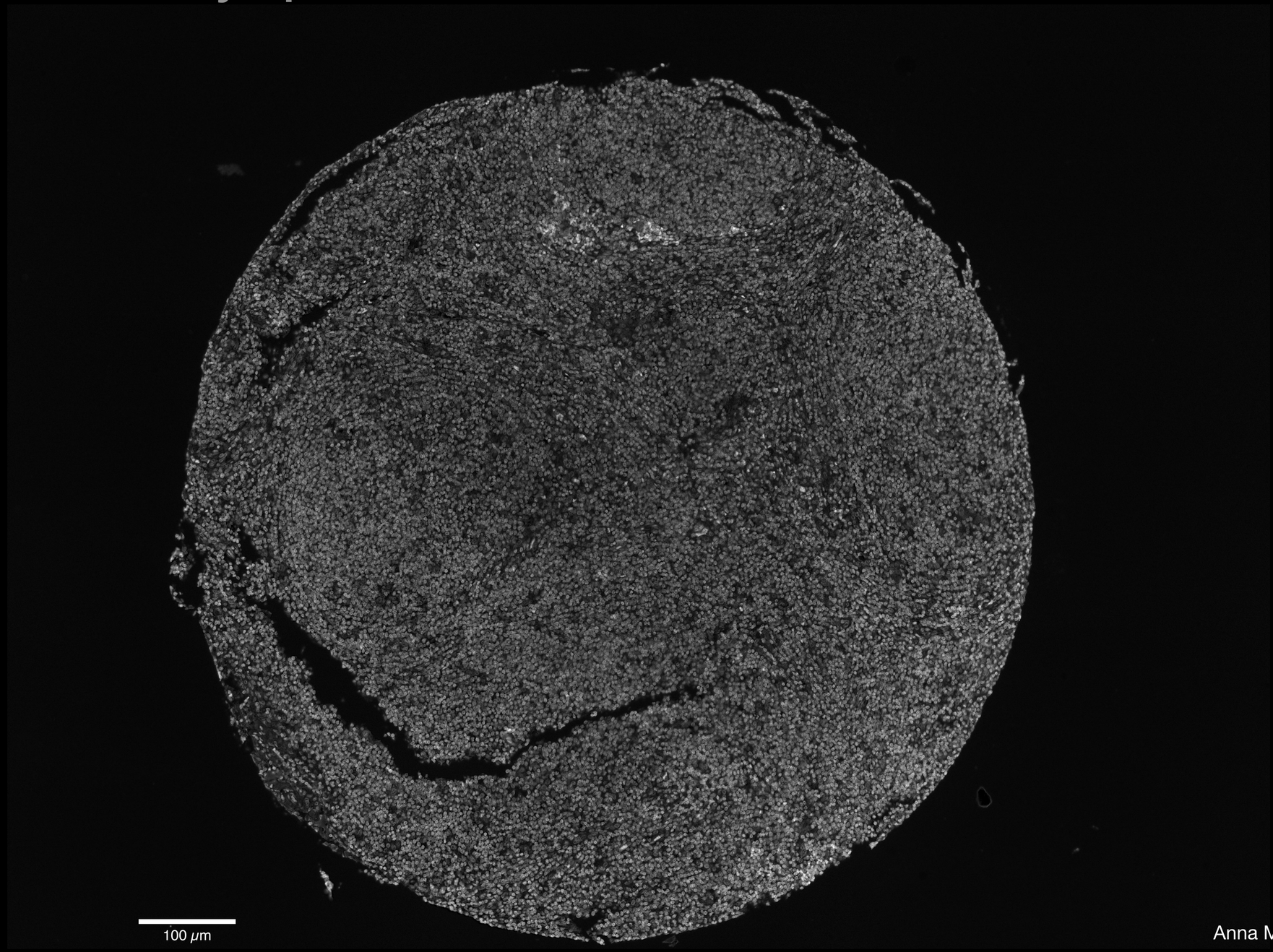
Step 1
Dense Polygon Prediction
via a CNN (e.g. U-Net, ResNet)

Step 2
Polygon Selection
(Non-Maximum Suppression NMS)

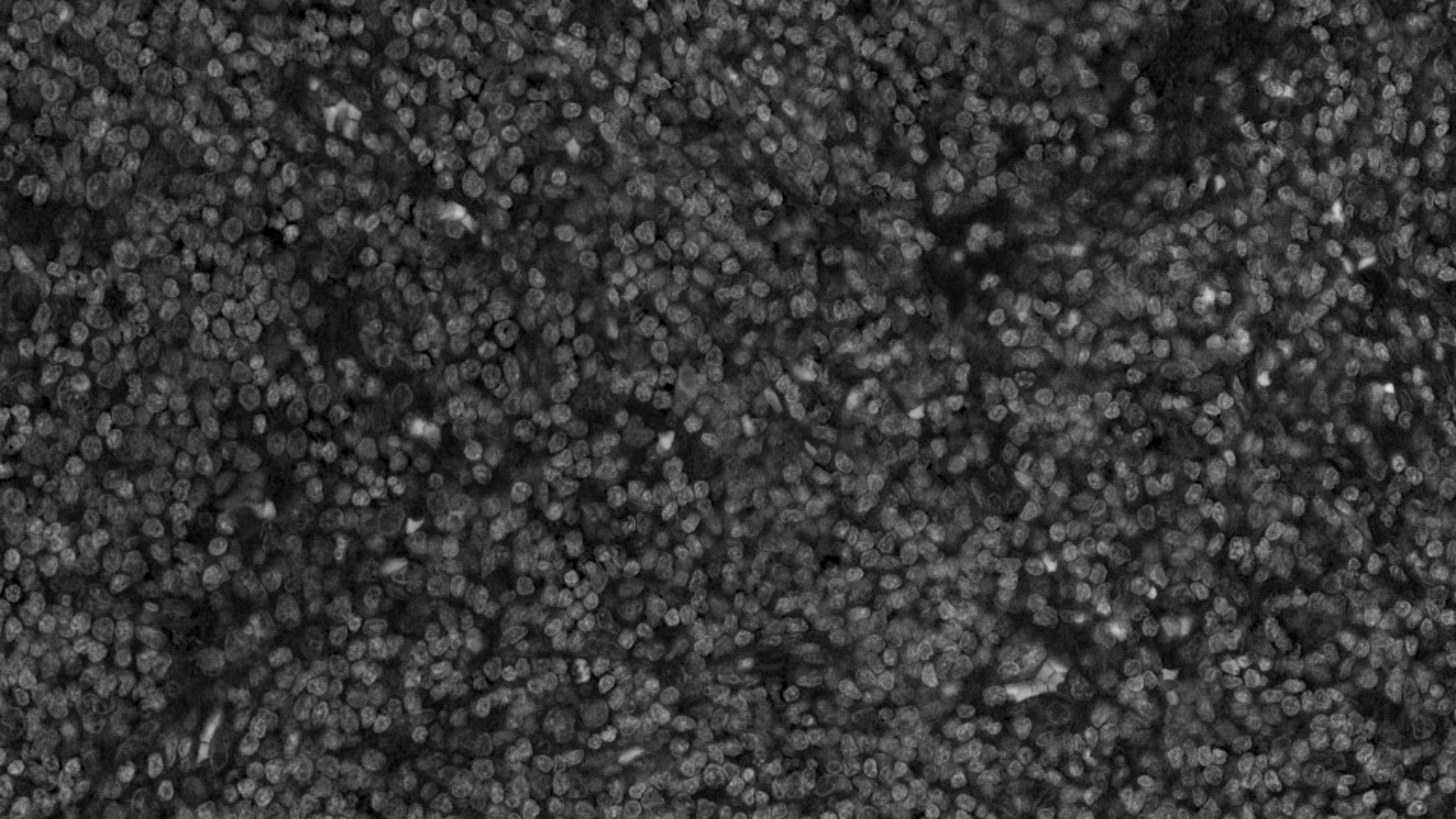
Non-Maximum-Suppression (NMS)

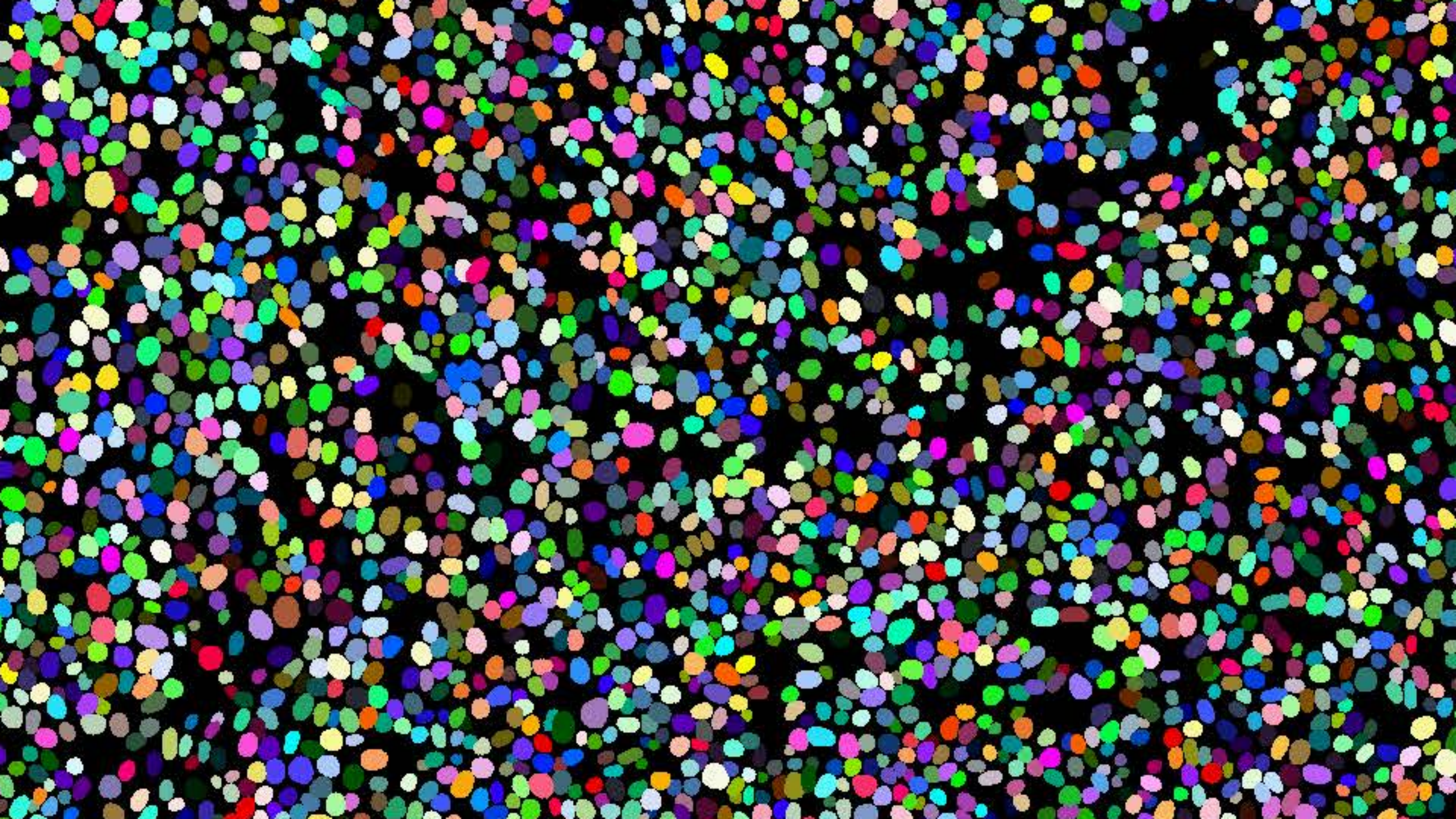


Example: crowded lymphoma cells

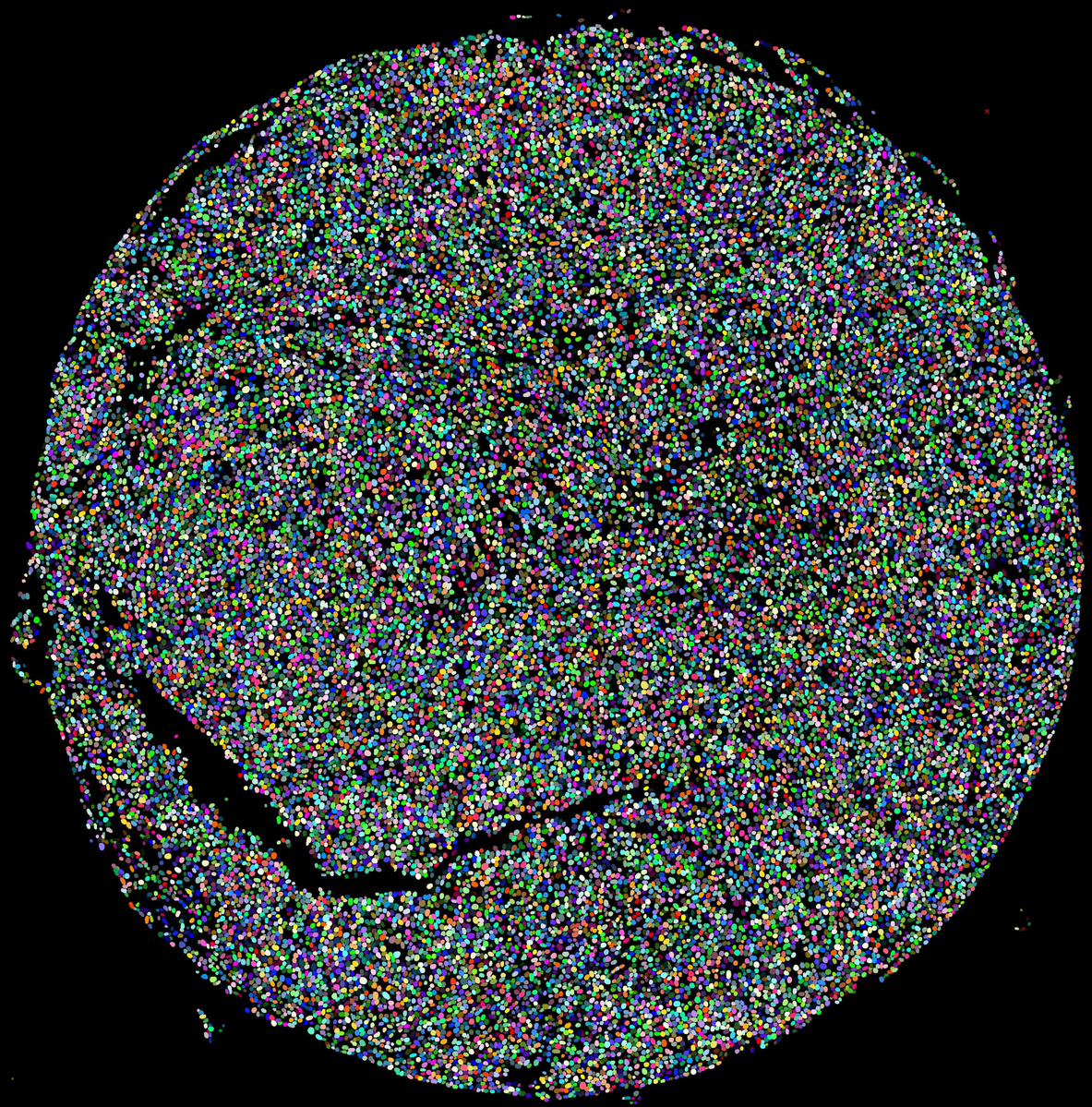


Anna Maria Tsakiroglou (Manchester)

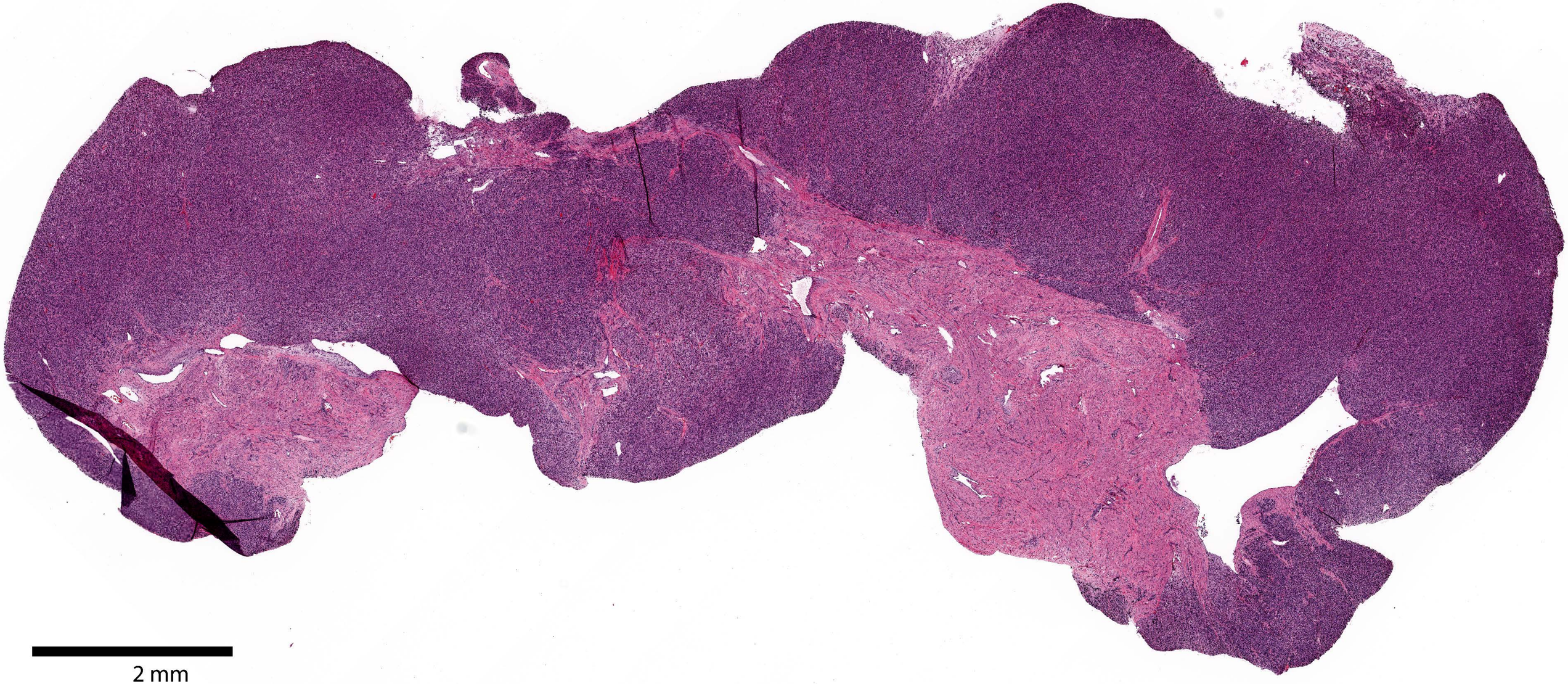




Example: crowded lymphoma cells

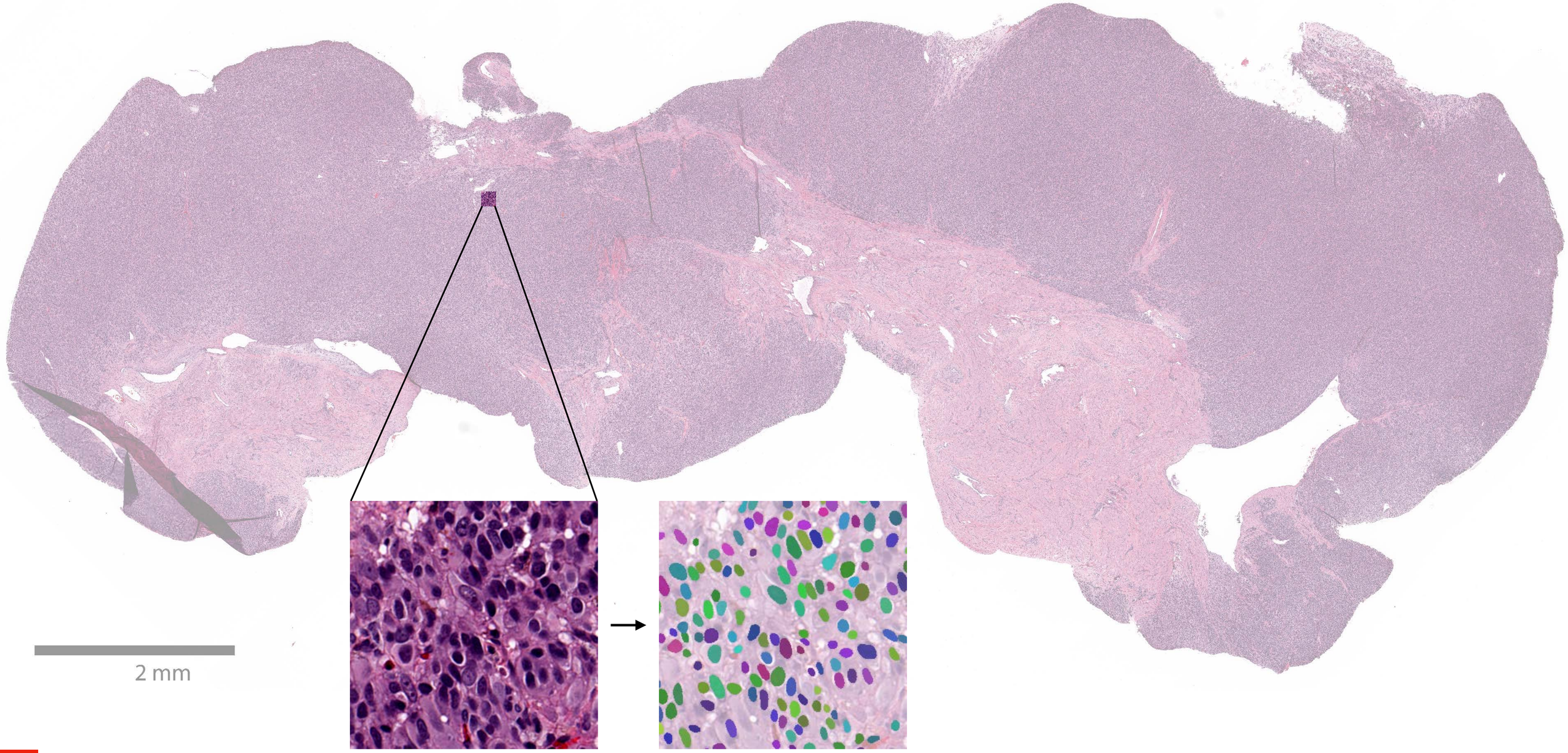


Example Pretrained: Histopathology (H&E stain)

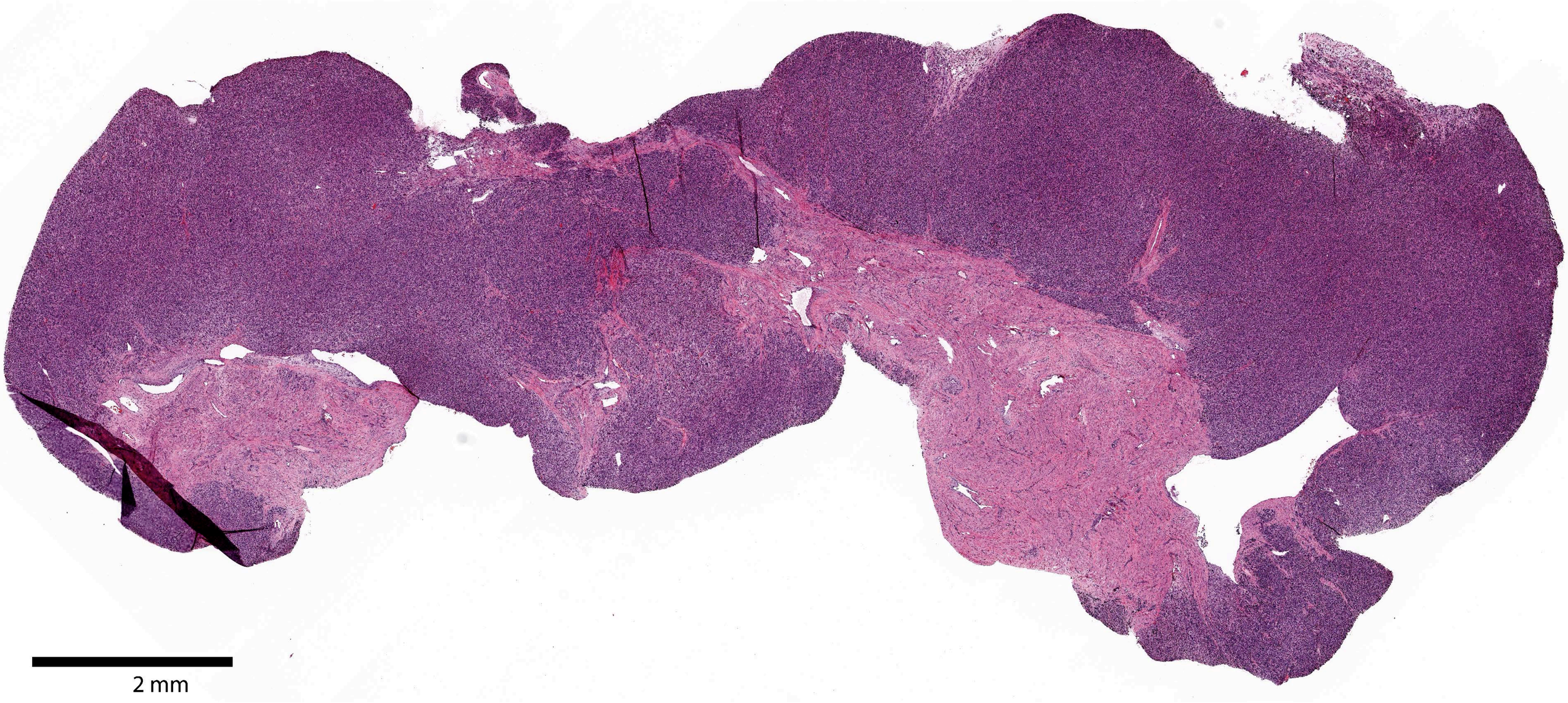


Whole Slide H&E (Sarcoma), (15mm x 6mm, 32k x 14k pixels)

Example Pretrained: Histopathology (H&E stain)

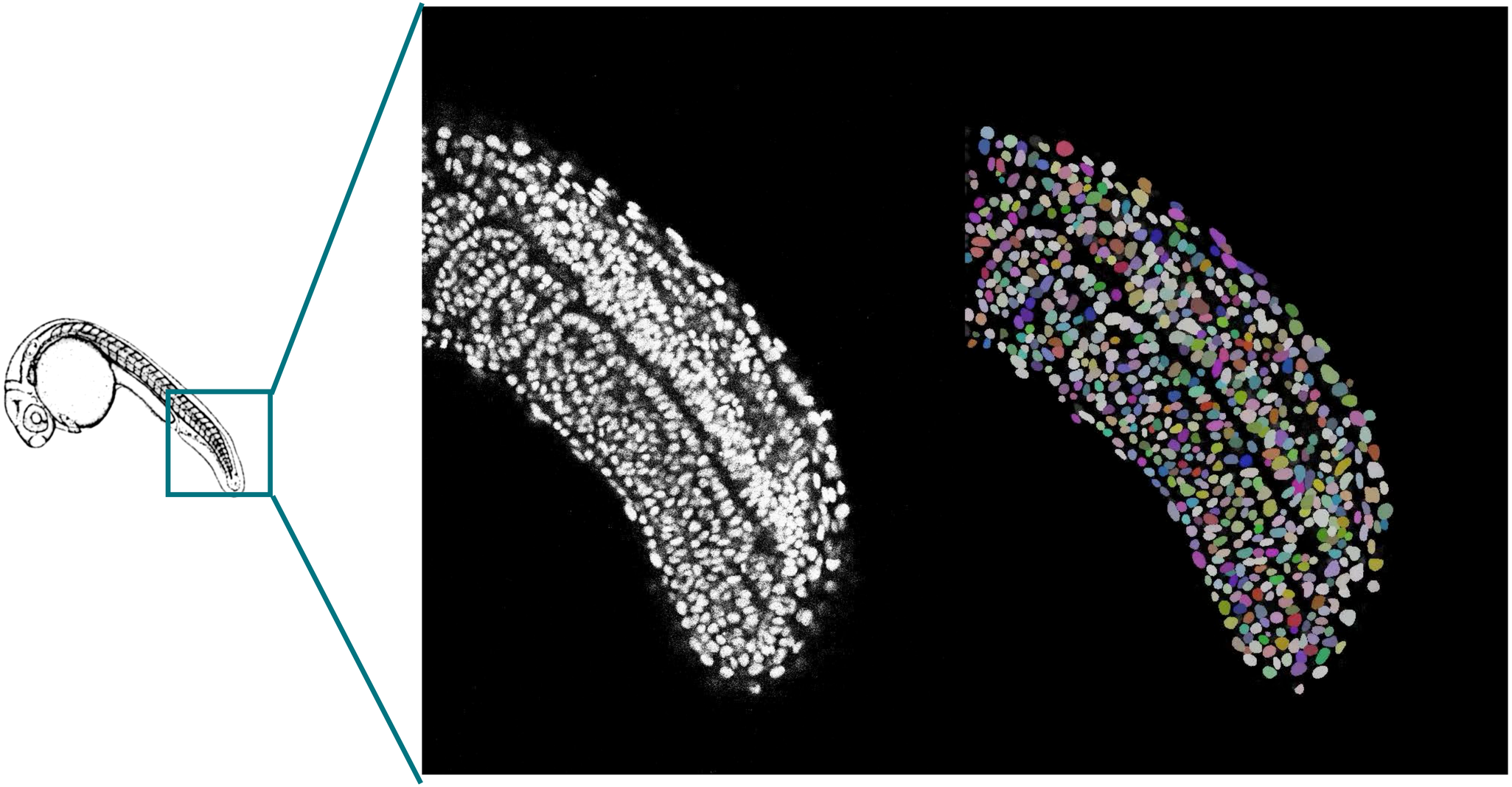


Example Pretrained: Histopathology (H&E stain)



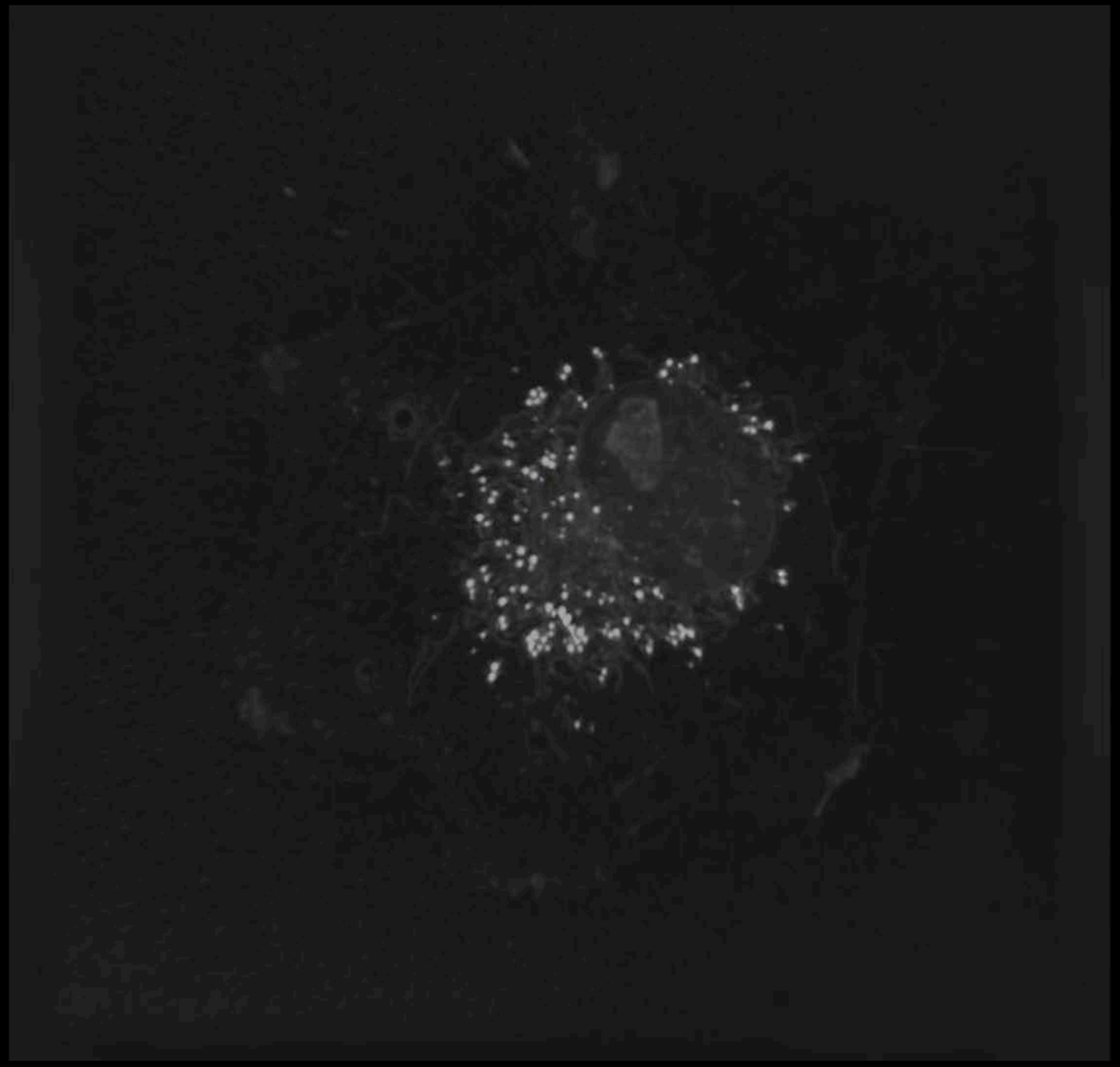
Whole Slide H&E (Sarcoma), (15mm x 6mm, 32k x 14k pixels)

Example application of pretrained models



Zebrafish tail development, Data from Oates lab, EPFL

Other bioimaging tasks: detection and localization

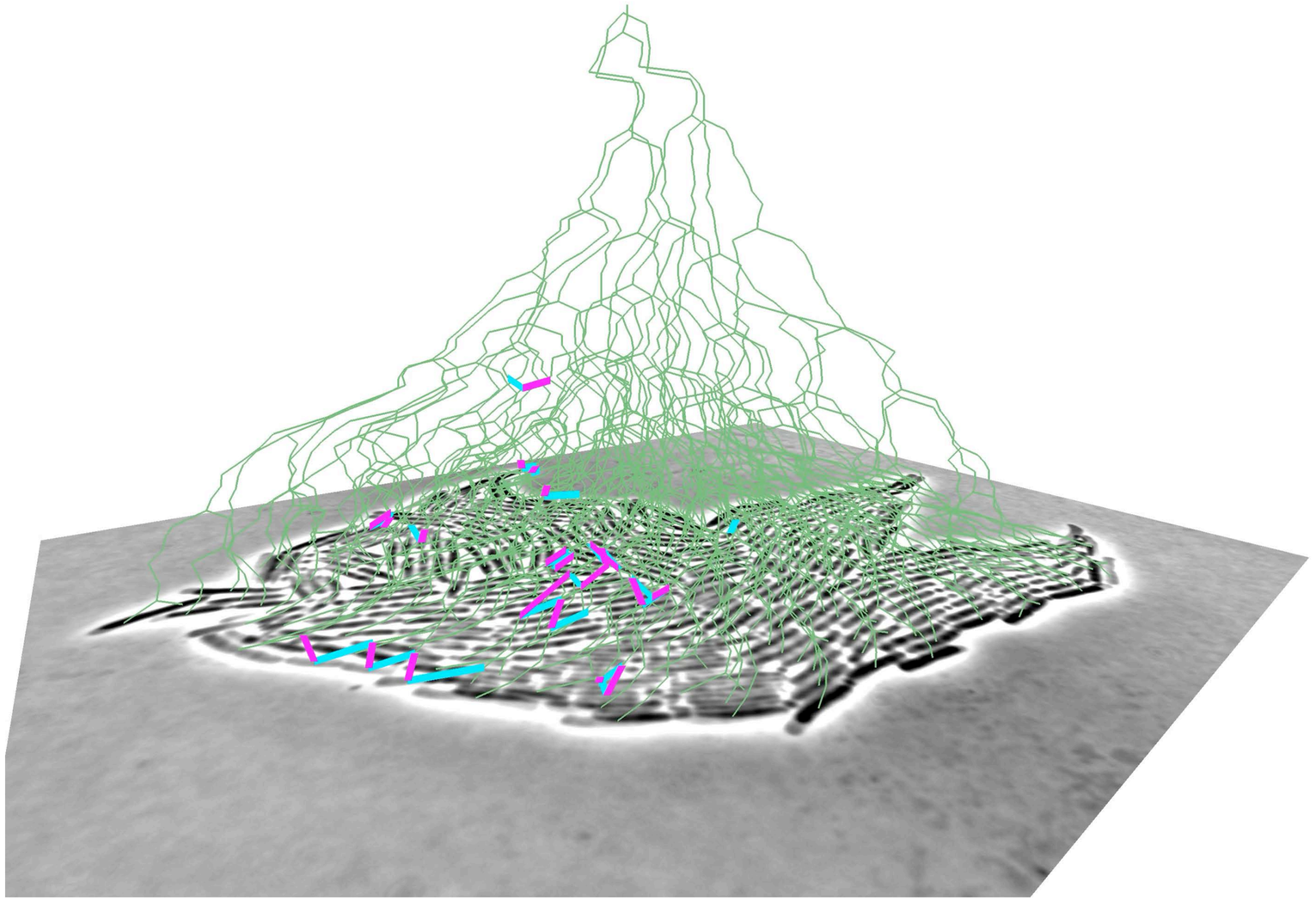


Dominguez Mantes et al. *Spotiflow: accurate and robust spot detection for fluorescence microscopy* (bioRxiv 2024, under review)

Data from Manley lab, EPFL

Other bioimaging tasks: tracking

Method	Linking	TRA ↑	AOGM ↓
Distance	greedy	0.944	1511
Distance	ILP	0.962	1065
TrackMate (overlap)	LAP	0.957	872
Delta 2.0	greedy	0.996	118
Trackastra (points only)	ILP	0.995	136
Trackastra	greedy	**0.999**	36
Trackastra	ILP	**0.999**	23

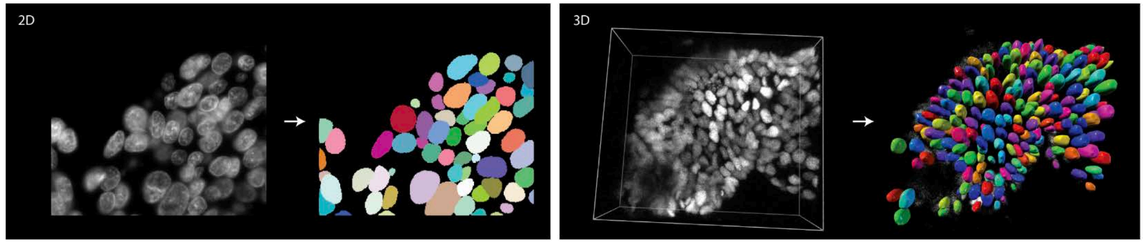


Gallusser and Weigert. *Trackastra: Transformer-based cell tracking for live-cell microscopy* (ECCV 2024)

Usable open source software is important!

pypi package 0.9.1 | Anaconda.org 0.9.1 | Test passing | Test (PyPI) failing | forum 459 topics | downloads 22k/month

StarDist - Object Detection with Star-convex Shapes



Spotiflow | Spotiflow 0.5.0 documentation | Search | Spotiflow 0.5.0 ☆57 7

Spotiflow 0.5.0 documentation

- Predicting spots using the napari plugin
- Inference via CLI
- Available pre-trained models
- Training a Spotiflow model on a custom dataset
- Fine-tuning a Spotiflow model on a custom dataset
- API Reference

Spotiflow

Spotiflow is a learning-based subpixel-accurate spot detection method for 2D and 3D fluorescence microscopy. It is primarily developed for spatial transcriptomics workflows that require transcript detection in large, multiplexed FISH-images, although it can also be used to detect spot-like structures in general fluorescence microscopy images and volumes. For more information, please refer to our paper.

Spotiflow

- Getting Started
  - Installation
  - Predicting spots in an image
    - Python API
    - Command Line Interface (CLI)
    - Napari plugin
- Contents