New Image Analysis Resources at The Rockefeller University

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Date: Wednesday, April 6th, 2022

Seminar time: 10:00am – 11:00am

Workshop time: 11:00am – 12:30pm

Place: A Level Training/Classroom, Welch Hall, The Rockefeller University

Seminar slides and workshop material will be made available on our GitHub page:

https://github.com/ImageAnalysis-RockefellerUniversity

Outline

- Segmentation
- Cell/particle Counting and Tracking
- Image denoising

<u>Open-source Softwares</u>

ImageJ/Fiji, QuPath, Napari, CellProfiler, Icy

<u>Commercial Softwares</u>

Imaris, Arivis, Aivia, Huygens, MetaMorph

<u>Machine/Deep Learning frameworks</u>

Weka, ilastik, Labkit

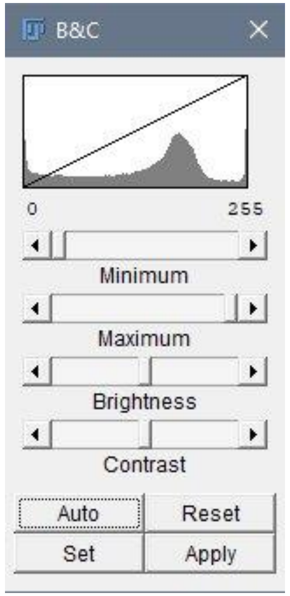
StarDist, Cellpose, DeepImageJ, ZeroCostDL4Mic

What is a Raster Image?

- Raster images are composed of a grid of pixels
- Each pixel contains intensity information
- Number of bits (N) determine the range of intensity levels

Image Type	**Range of intensity levels (0 to 2^N-1)**
8-bit	0 - 255
12-bit	0 - 4095
16-bit	0 - 65,535
RGB color (3 x 8 bits)	0 - 255 per channel

Histogram



8-bit image (256 shades)

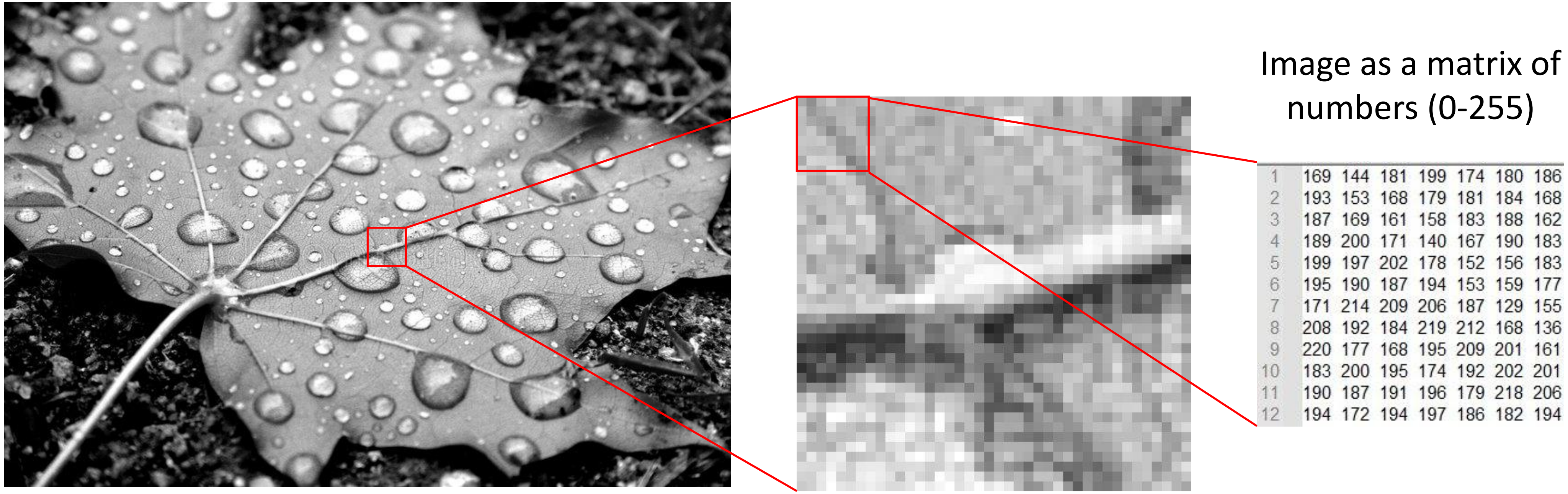


Image as a matrix of numbers (0-255)

1	169	144	181	199	174	180	186
2	193	153	168	179	181	184	168
3	187	169	161	158	183	188	162
4	189	200	171	140	167	190	183
5	199	197	202	178	152	156	183
6	195	190	187	194	153	159	177
7	171	214	209	206	187	129	155
8	208	192	184	219	212	168	136
9	220	177	168	195	209	201	161
10	183	200	195	174	192	202	201
11	190	187	191	196	179	218	206
12	194	172	194	197	186	182	194

Segmentation

Identifying object(s) of interest in an image

- cells, nuclei, membrane, transcription sites etc.

Segmentation is usually followed by quantitative analysis of object(s)

- number of cells/nuclei, mean fluorescence intensity, shape etc.

Divide image into areas representing object(s) of interest and background

Segmentation is not an easy task to solve in most practical cases

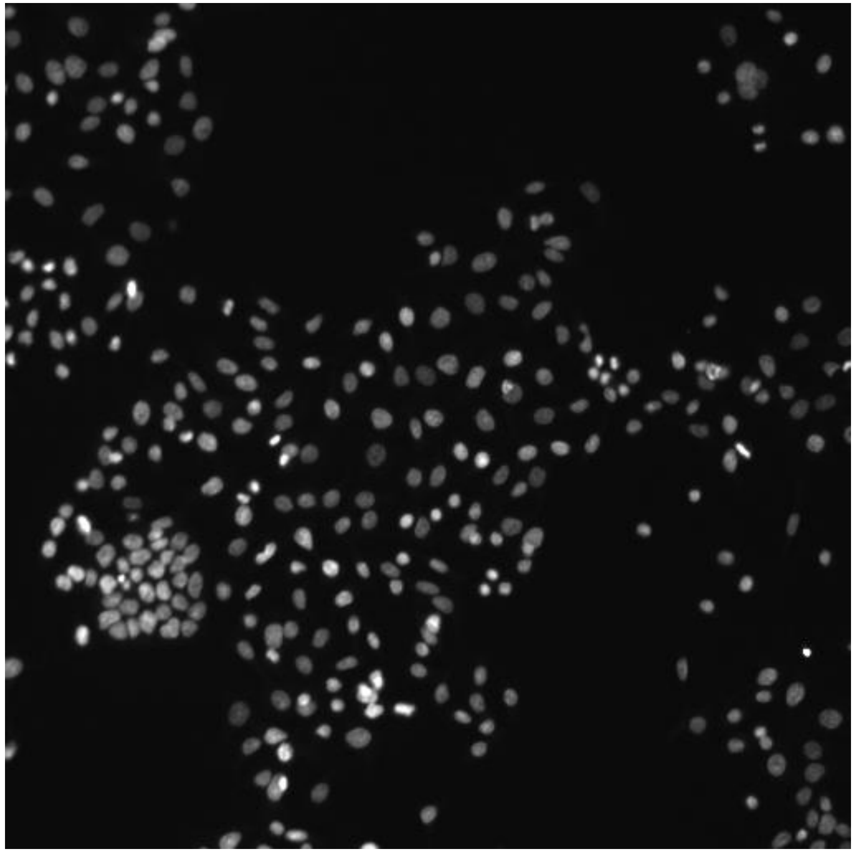
- Signal variability throughout the image
- Noise, blur and other distortions caused by the imperfect imaging conditions

Segmentation tools

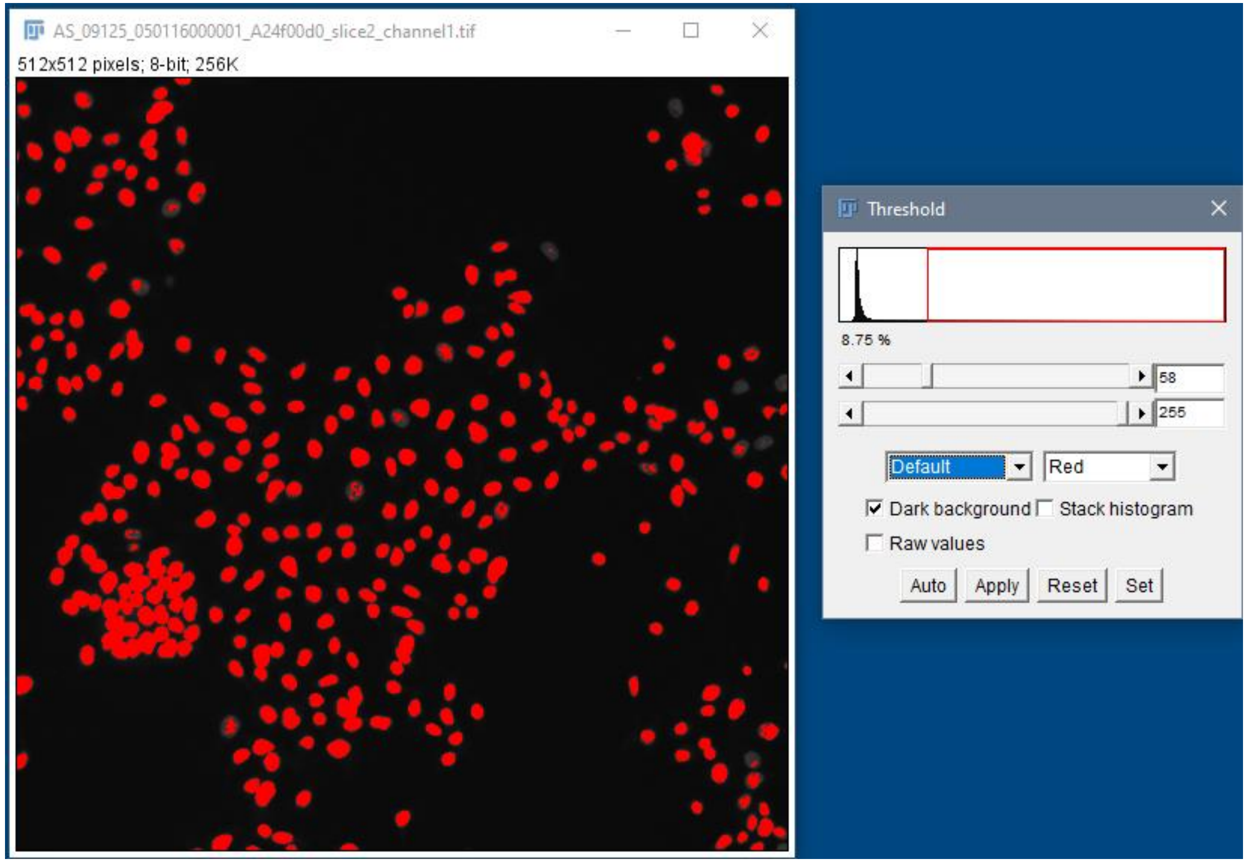
- Global thresholding, local thresholding
- Image processing filters – Gaussian, Median, Sobel etc.
- Machine learning methods (supervised learning) - Weka, Labkit, ilastik
- Deep Learning methods - StarDist, Cellpose
- 3D segmentation - Imaris, Arivis

Segmentation using global thresholding

Nuclei stained with Hoechst



Threshold 1



AS_09125_050116000001_A24f00d0_slice2_channel1.tif

512x512 pixels; 8-bit; 256K

Threshold

8.75 %

58

255

Default

Red

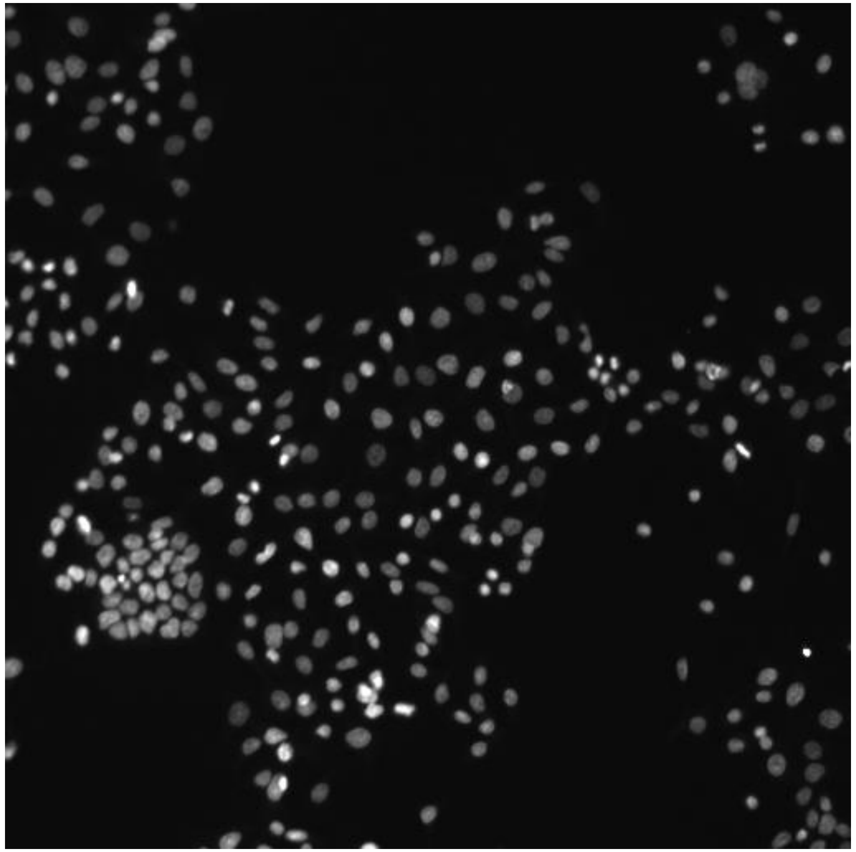
☑ Dark background ☐ Stack histogram

☐ Raw values

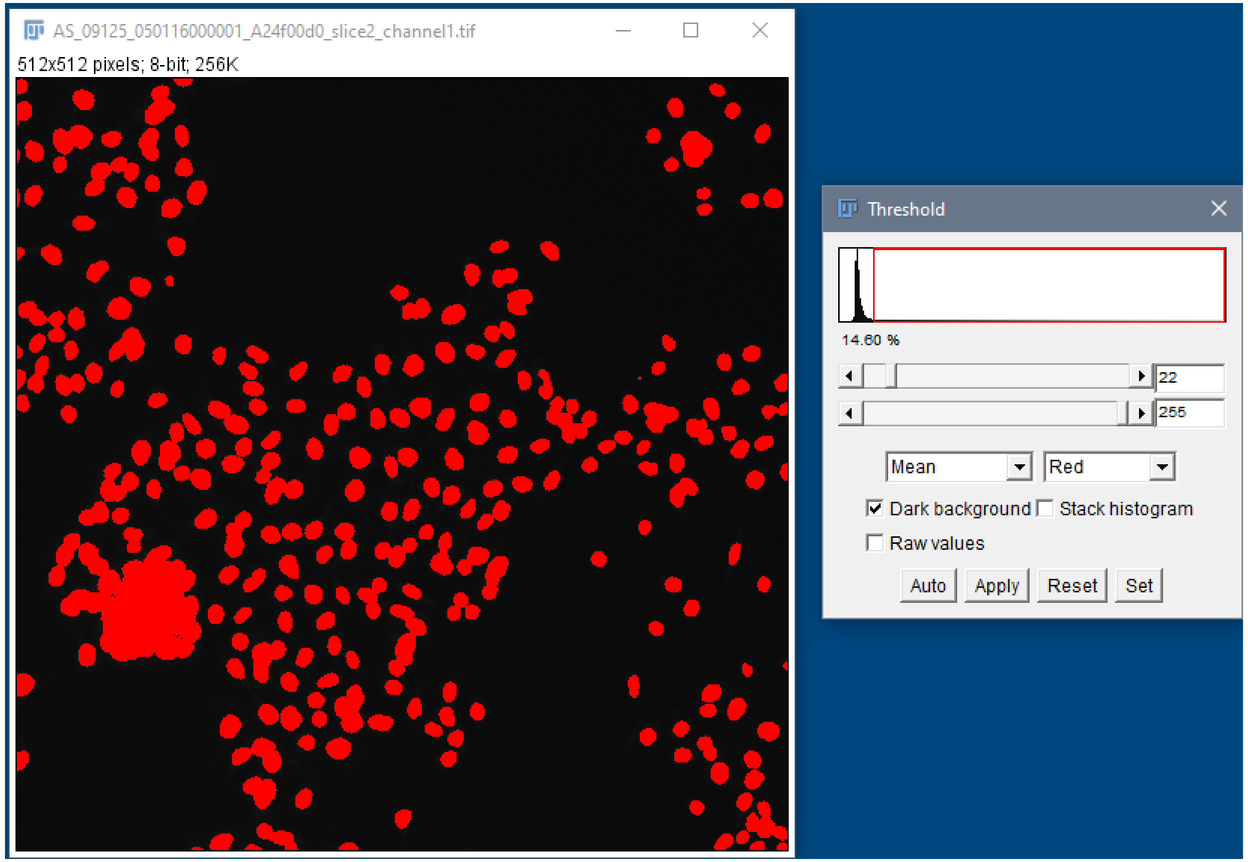
Human HT29 colon cancer cells, Image from Broad Bioimage Benchmark Collection, Ljosa et al. 2012 Nat Methods

Segmentation using global thresholding

Nuclei stained with Hoechst



Threshold 2



AS_09125_050116000001_A24f00d0_slice2_channel1.tif

512x512 pixels; 8-bit; 256K

Threshold

14.60 %

22

255

Mean

Red

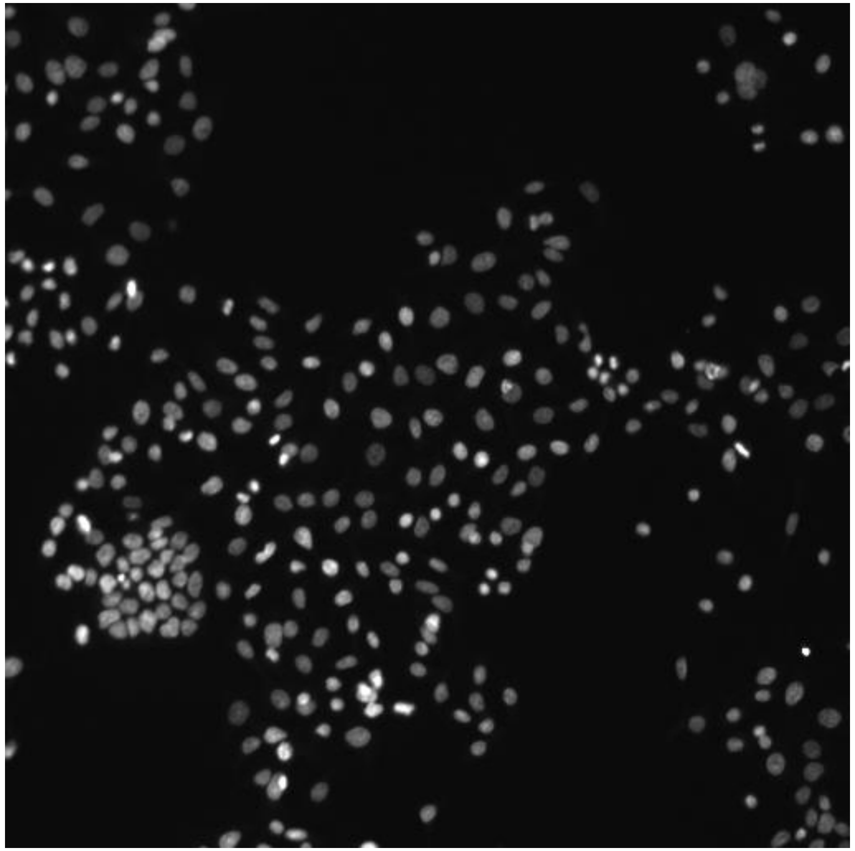
☑ Dark background ☐ Stack histogram

☐ Raw values

Human HT29 colon cancer cells, Image from Broad Bioimage Benchmark Collection, Ljosa et al. 2012 Nat Methods

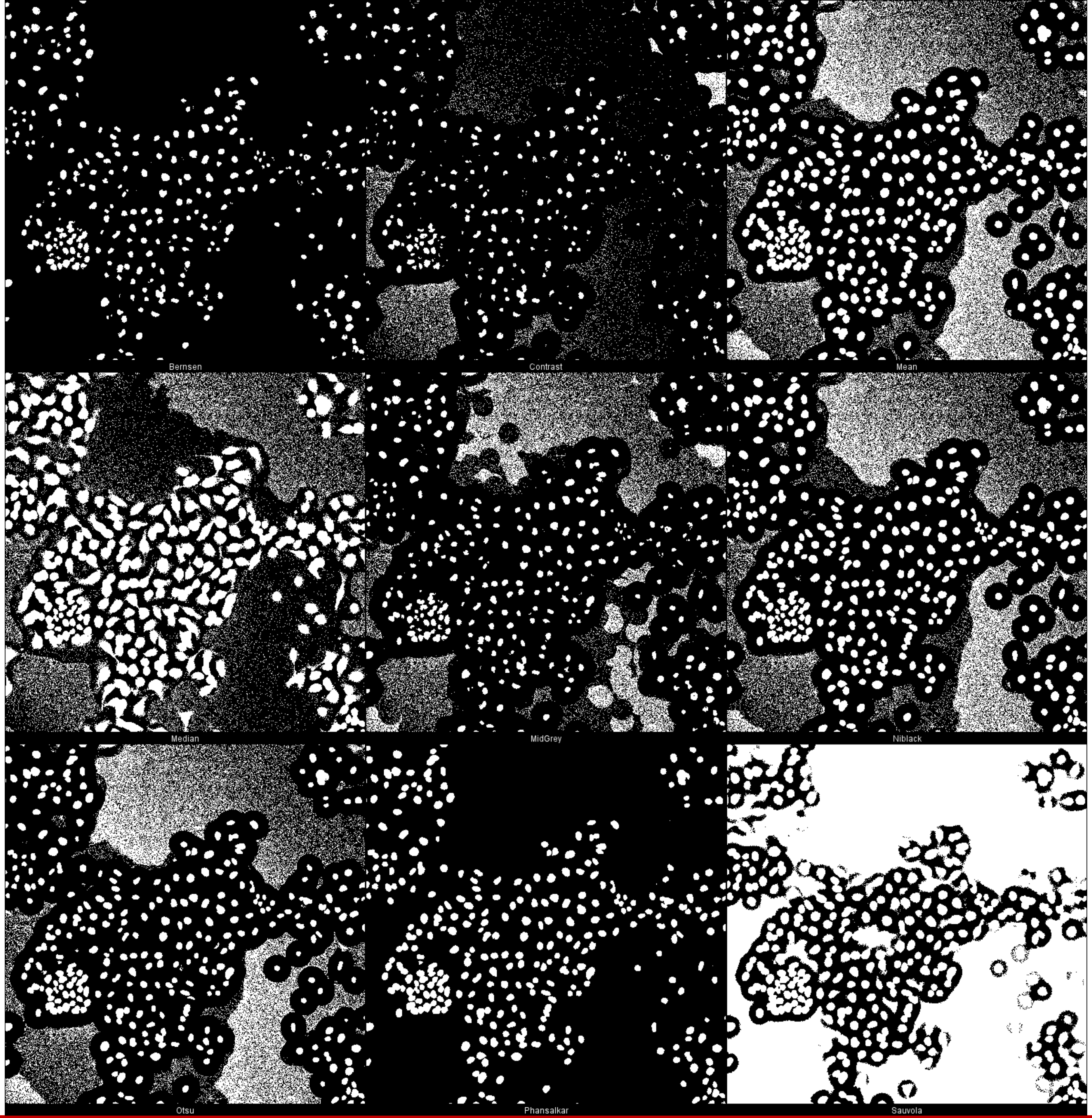
Segmentation with local thresholding

Nuclei stained with Hoechst



Human HT29 colon cancer cells, Image from Broad Bioimage Benchmark Collection, Ljosa et al. 2012 Nat Methods

Auto Local Threshold in Fiji

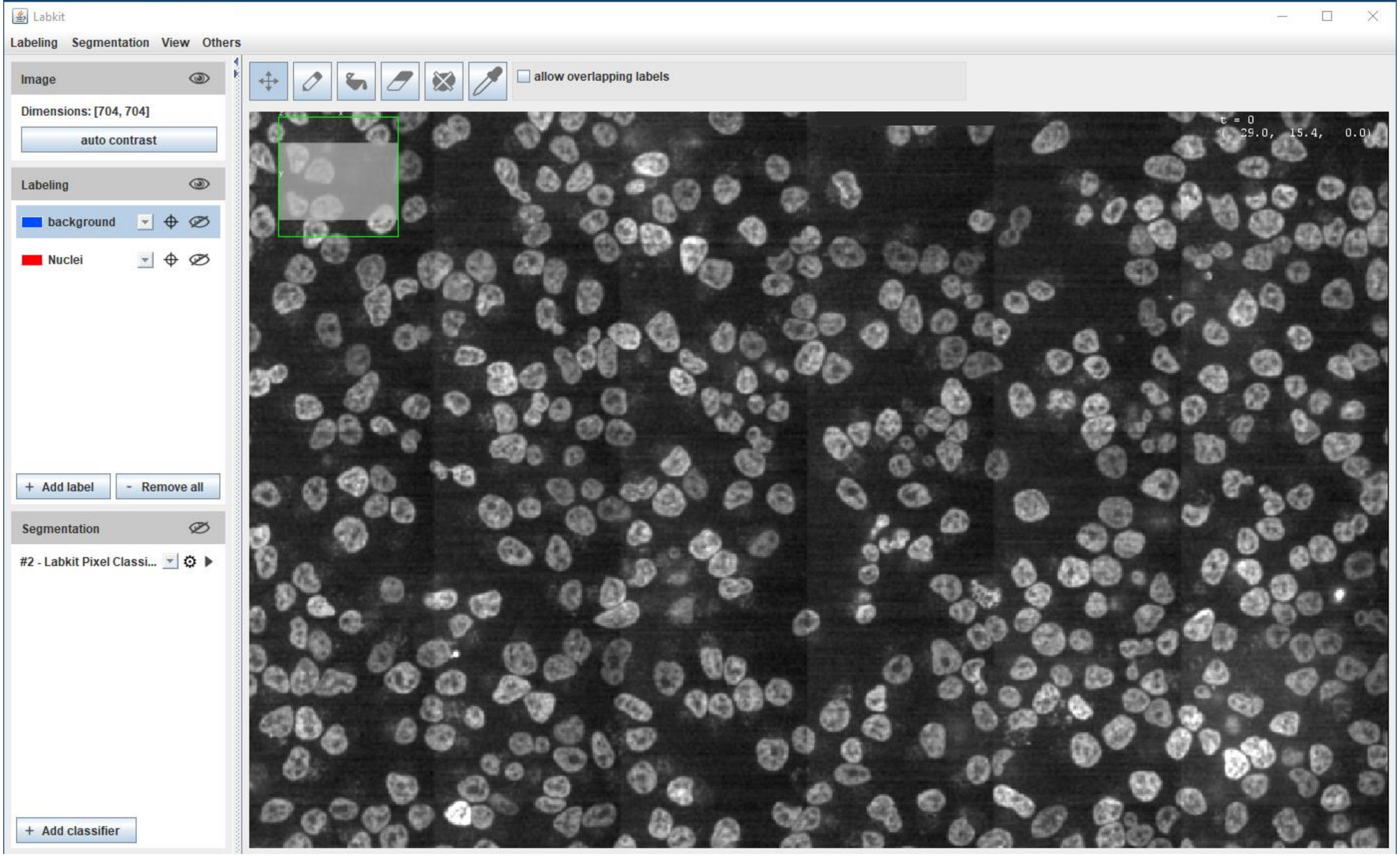


Segmentation using Machine Learning

Supervised learning - pixel classification using Random Forest classifier

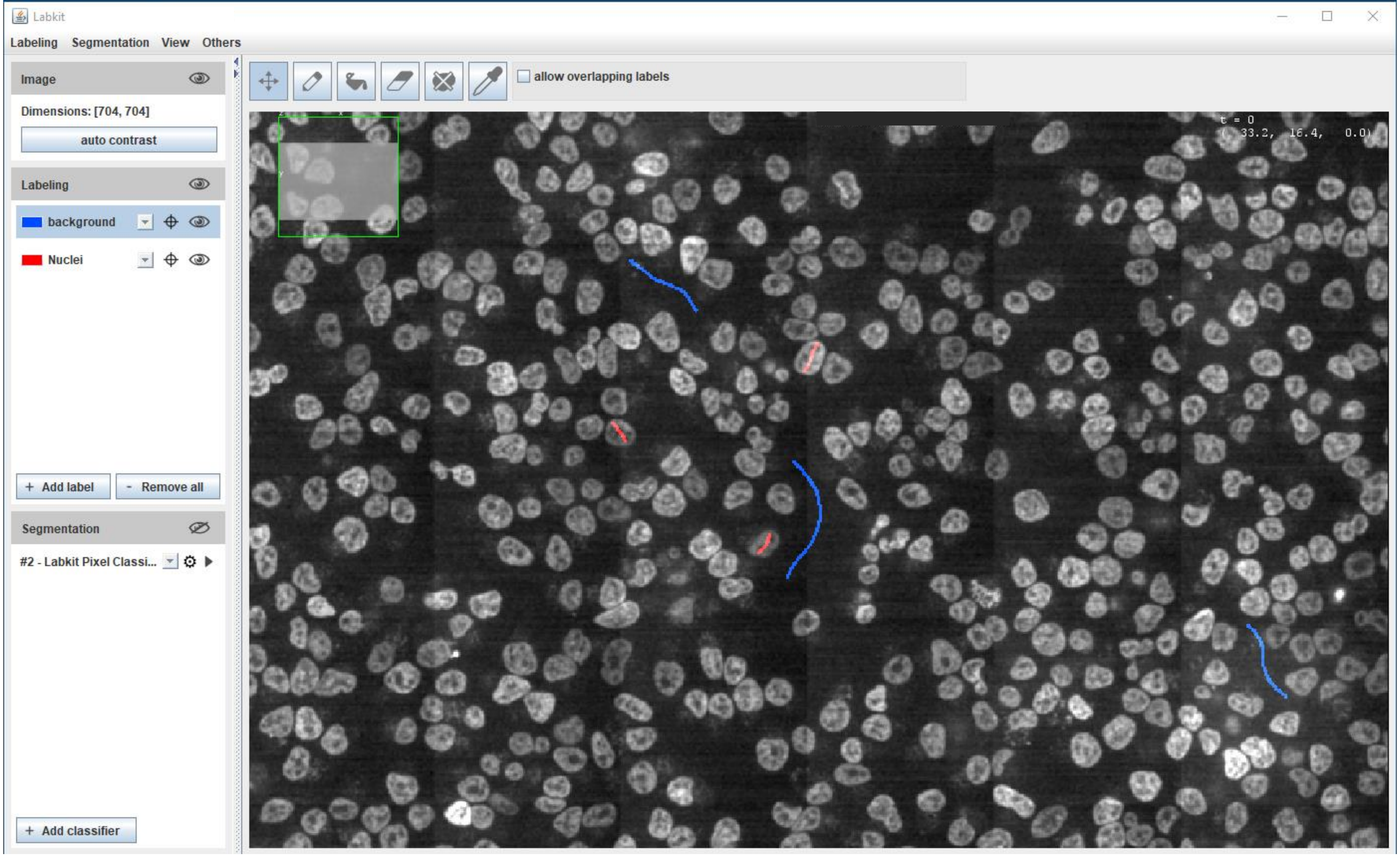
Fiji (Weka and Labkit plugins), ilastik, QuPath, Napari, CellProfiler

Requires orders of magnitude less training data/resources than Deep Learning methods



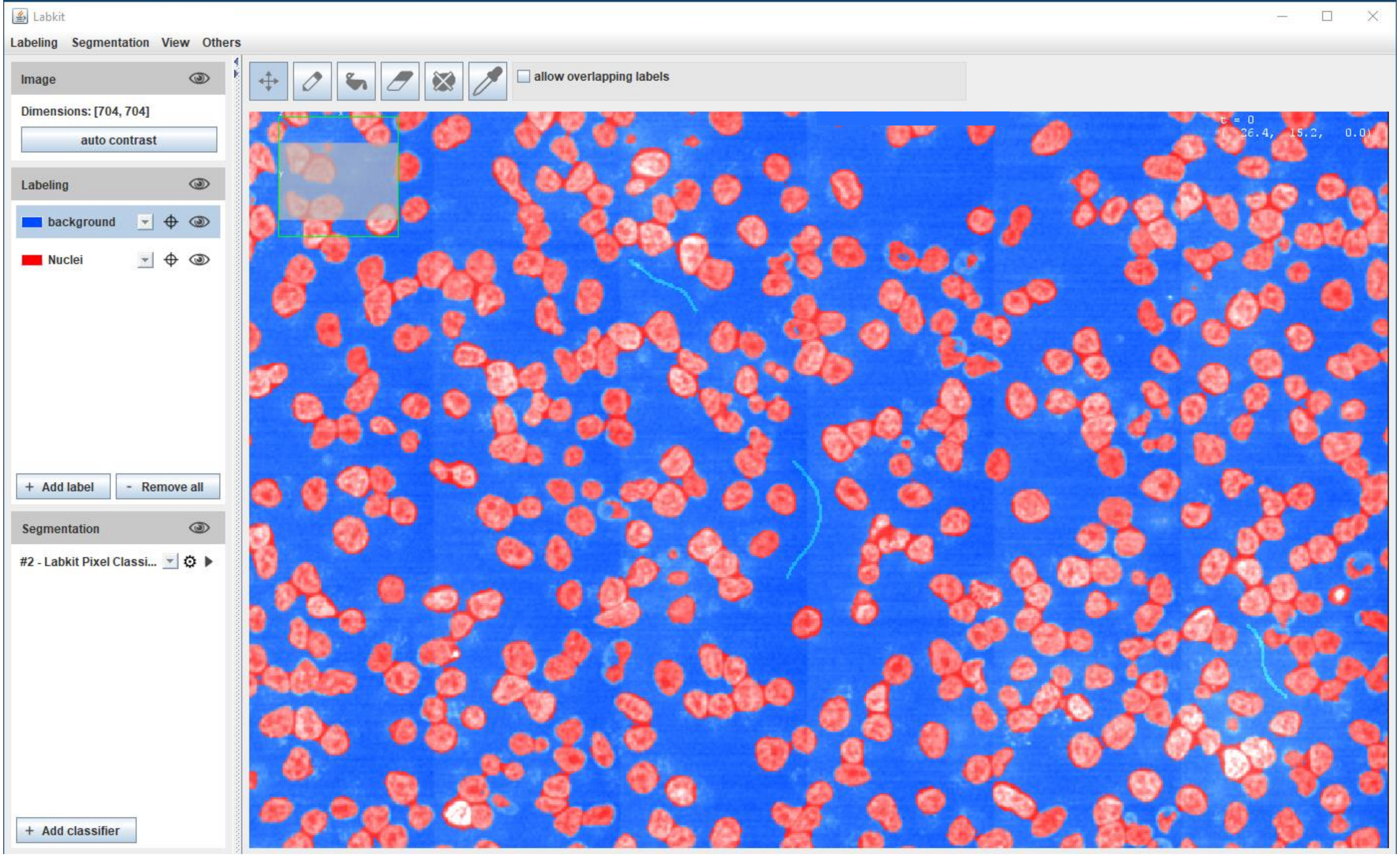
Dimensions: [704, 704]

Hoechst-stained Nuclei, image courtesy of Cherie Au, Giannakakou Lab, Weill Cornell Medicine



Dimensions: [704, 704]

Hoechst-stained Nuclei, image courtesy of Cherie Au, Giannakakou Lab, Weill Cornell Medicine



Dimensions: [704, 704]

Hoechst-stained Nuclei, image courtesy of Cherie Au, Giannakakou Lab, Weill Cornell Medicine

Segmentation using Deep Learning

Most accurate methods available for cells/nuclei segmentation

Step 1: Training

Generating a Deep Learning model is resource hungry:

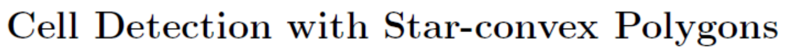
- High-end workstation
- Large amounts of training data (images and annotations)
- Training could take hours to days
- Good programming knowledge required - Python

Step 2: Prediction

Using the model from step 1 to predict the segmentation results :

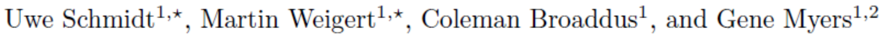
- A regular laptop is just fine
- Prediction takes seconds to mins
- Little to no programming knowledge required

StarDist (2018)



Cell Detection with Star-convex Polygons

arXiv:1806.03535



Uwe Schmidt[1,*], Martin Weigert[1,*], Coleman Broaddus[1], and Gene Myers[1,2]

Cellpose (2021)

ARTICLES

https://doi.org/10.1038/s41592-020-01018-x

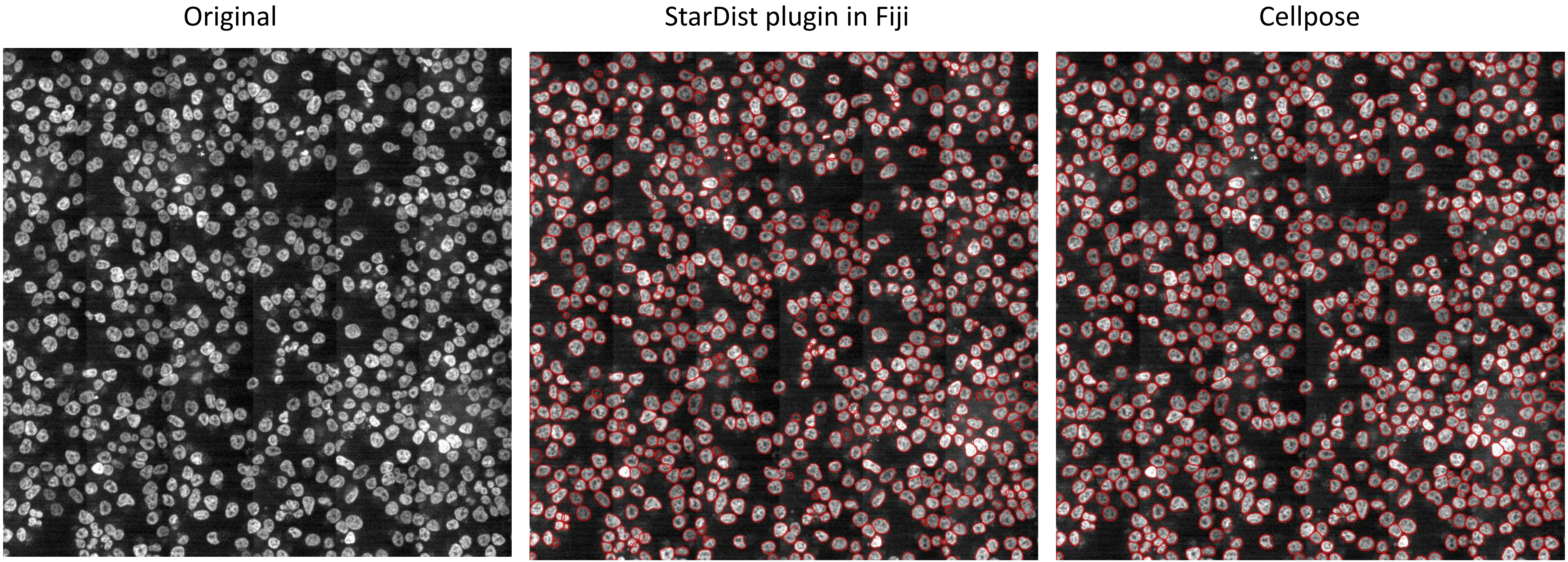
nature methods

Check for updates

Cellpose: a generalist algorithm for cellular segmentation

Carsen Stringer, Tim Wang, Michalis Michaelos and Marius Pachitariu

Segmentation using Deep Learning


Original

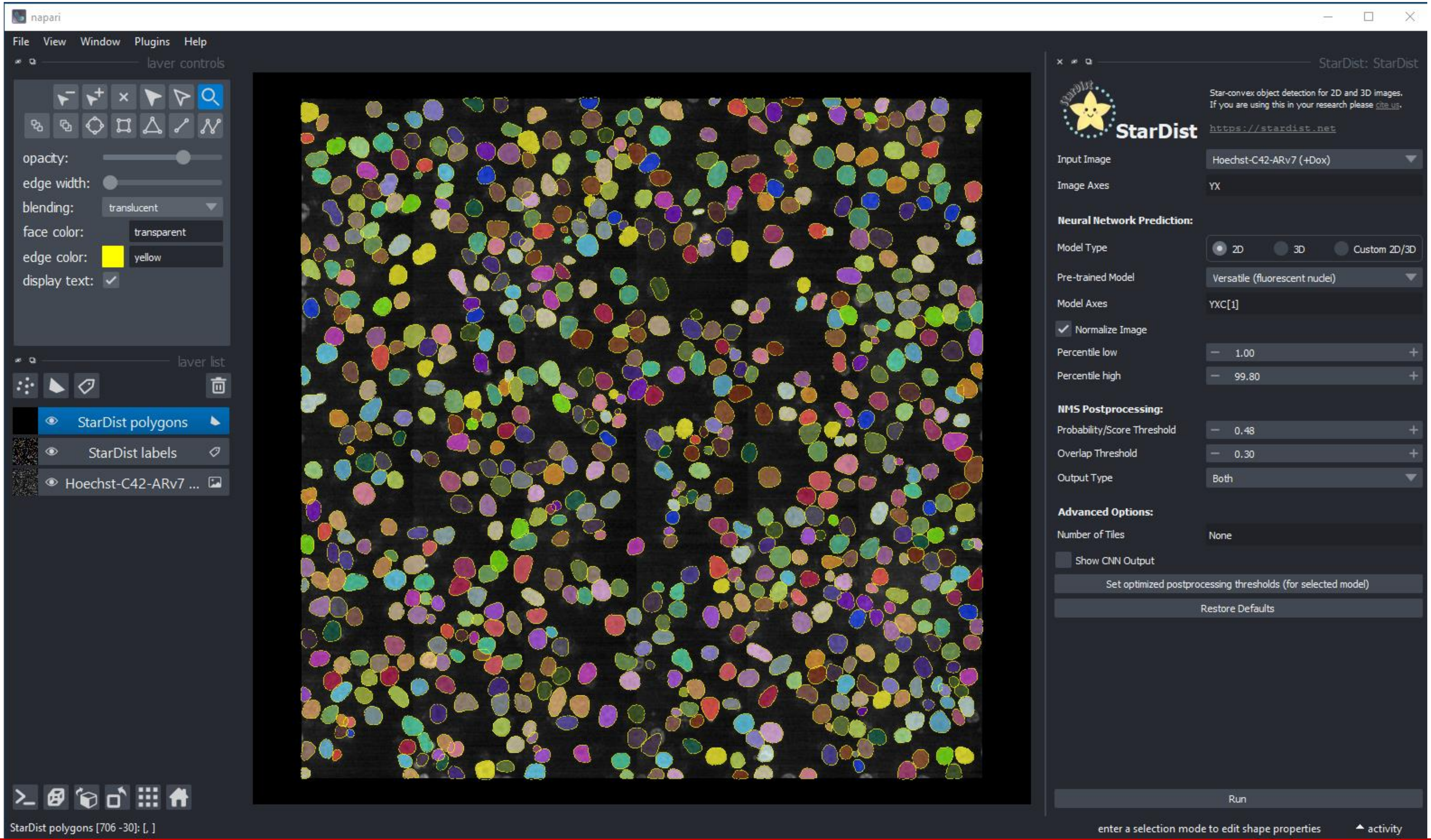
StarDist plugin in Fiji

Cellpose

Hoechst-stained Nuclei, image courtesy of Cherie Au, Giannakakou Lab, Weill Cornell Medicine

Workshop Exercise 1: StarDist based nuclear segmenation in a challenging image in Fiji

Segmentation using StarDist in Napari



opacity:

edge width:

blending: translucent

face color: transparent

edge color: yellow

display text: ☑

Star-convex object detection for 2D and 3D images.
If you are using this in your research please cite us.
https://stardist.net

Input Image: Hoechst-C42-ARv7 (+Dox)

Image Axes: YX

Neural Network Prediction:

Model Type: ● 2D ○ 3D ○ Custom 2D/3D

Pre-trained Model: Versatile (fluorescent nuclei)

Model Axes: YXC[1]

☑ Normalize Image

Percentile low: 1.00

Percentile high: 99.80

NMS Postprocessing:

Probability/Score Threshold: 0.48

Overlap Threshold: 0.30

Output Type: Both

Advanced Options:

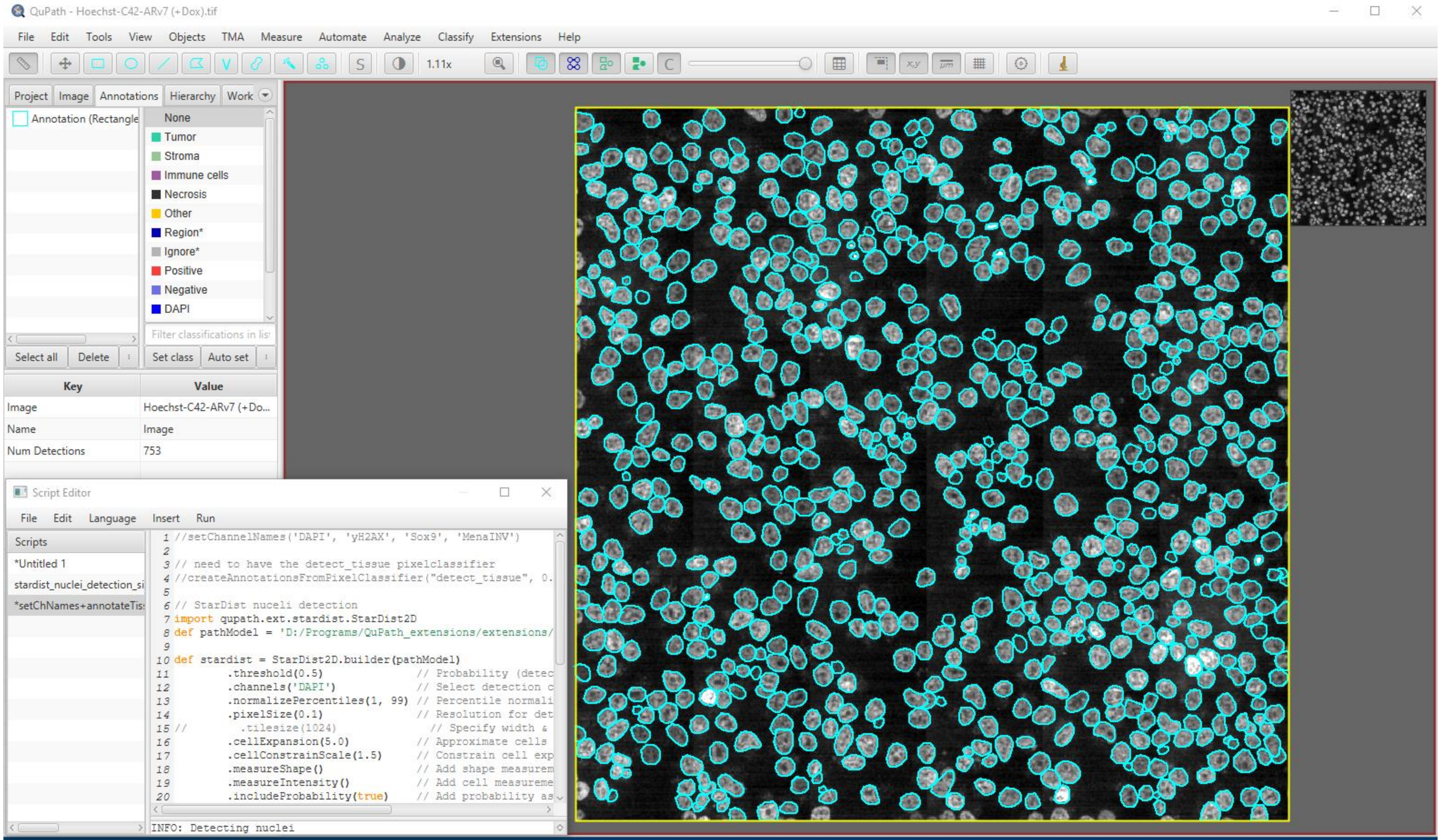
Number of Tiles: None

☐ Show CNN Output

StarDist polygons [706 -30]: [,]

enter a selection mode to edit shape properties

Segmentation using StarDist in QuPath



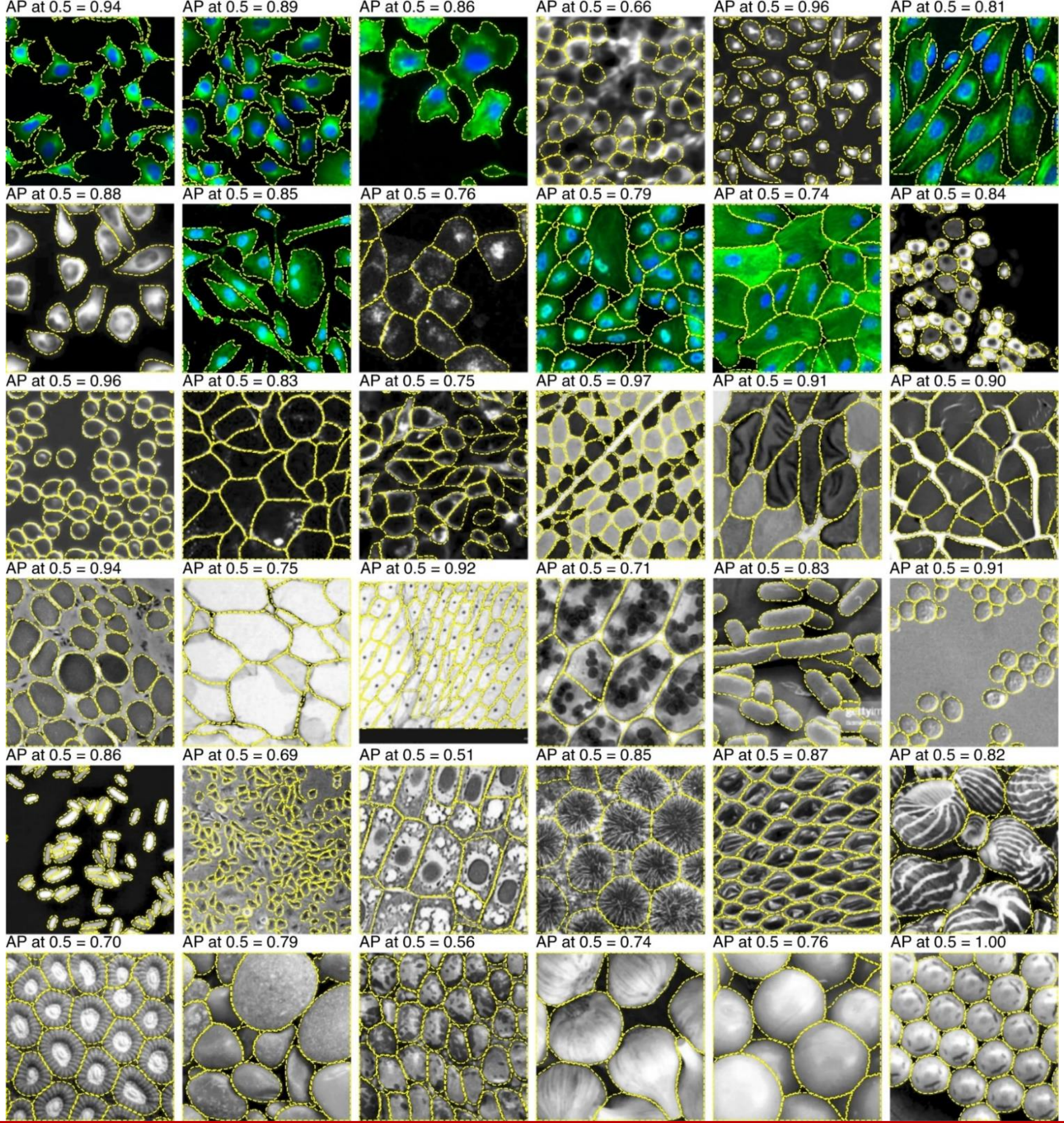
Key	Value
Image	Hoechst-C42-ARv7 (+Do...
Name	Image
Num Detections	753

```
//setChannelNames('DAPI', 'yH2AX', 'Sox9', 'MenaINV')

// need to have the detect_tissue pixelclassifier
//createAnnotationsFromPixelClassifier("detect_tissue", 0.

// StarDist nuceli detection
import qupath.ext.stardist.StarDist2D
def pathModel = 'D:/Programs/QuPath_extensions/extensions/

def stardist = StarDist2D.builder(pathModel)
        .threshold(0.5)              // Probability (detec
        .channels('DAPI')            // Select detection c
        .normalizePercentiles(1, 99) // Percentile normali
        .pixelSize(0.1)              // Resolution for det
//        .tilesize(1024)              // Specify width &
        .cellExpansion(5.0)          // Approximate cells
        .cellConstrainScale(1.5)     // Constrain cell exp
        .measureShape()              // Add shape measurem
        .measureIntensity()          // Add cell measureme
        .includeProbability(true)    // Add probability as
```

```
INFO: Detecting nuclei
```

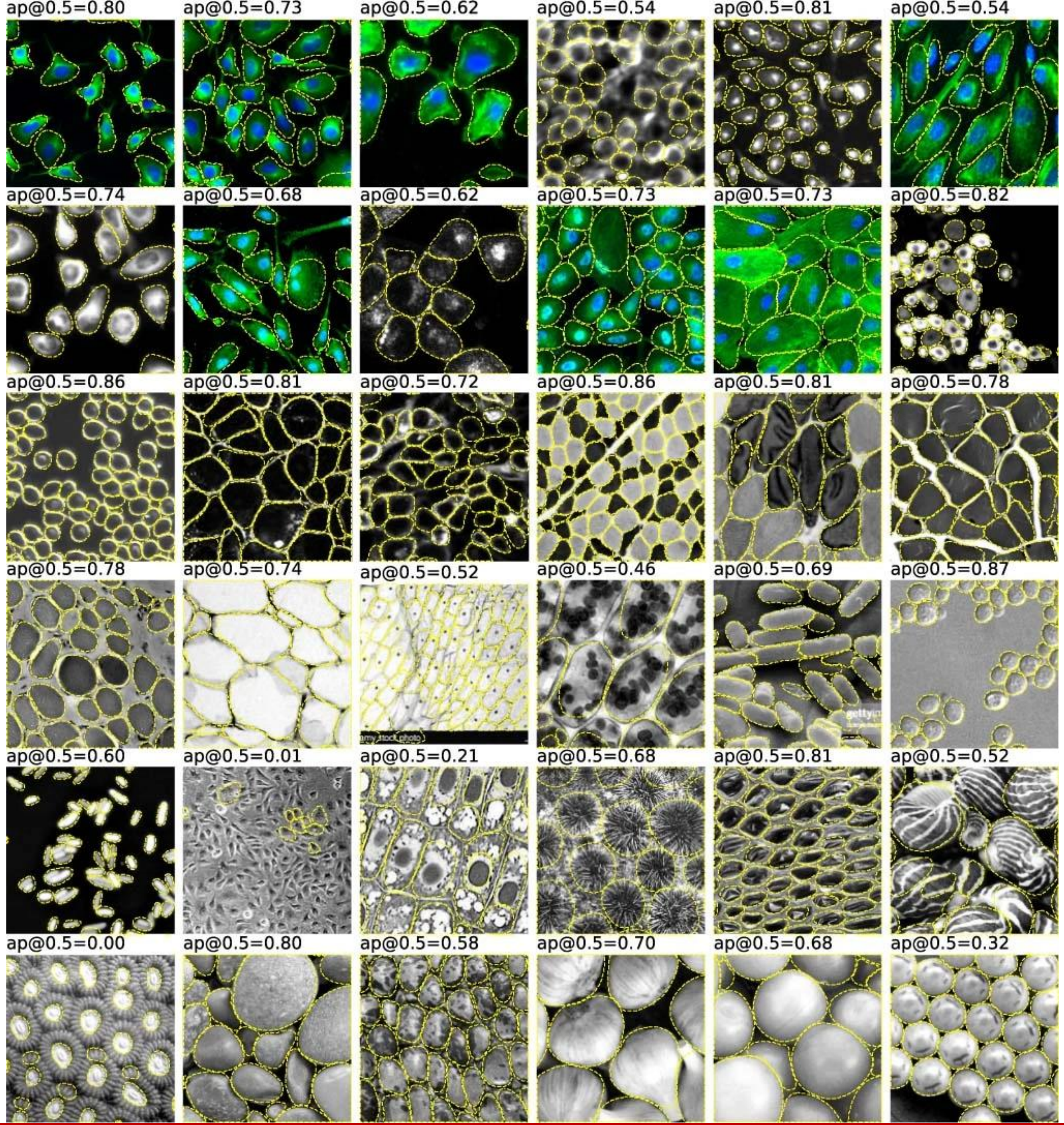
Segmentation – comparison of Deep Learning models

Cellpose



Stringer et al 2021,
Nat Methods

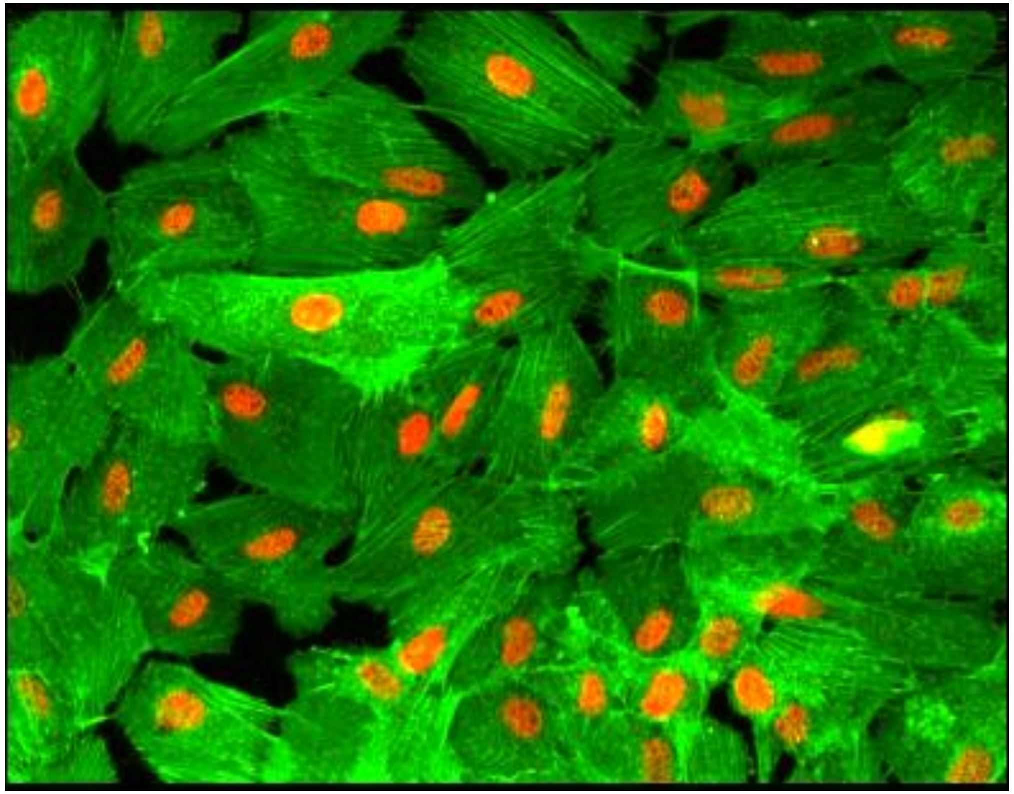
StarDist



Stringer et al 2021,
Nat Methods

Segmentation – challenging cases

Crowded fluorescent cells



Cellpose segmentation

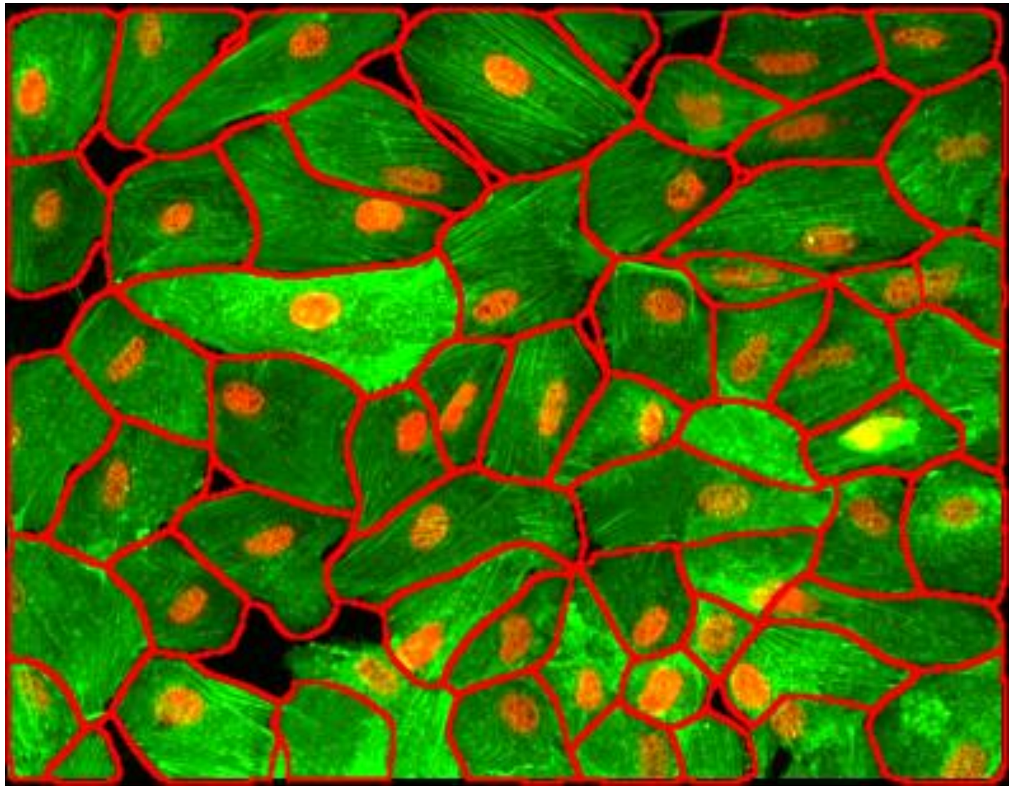
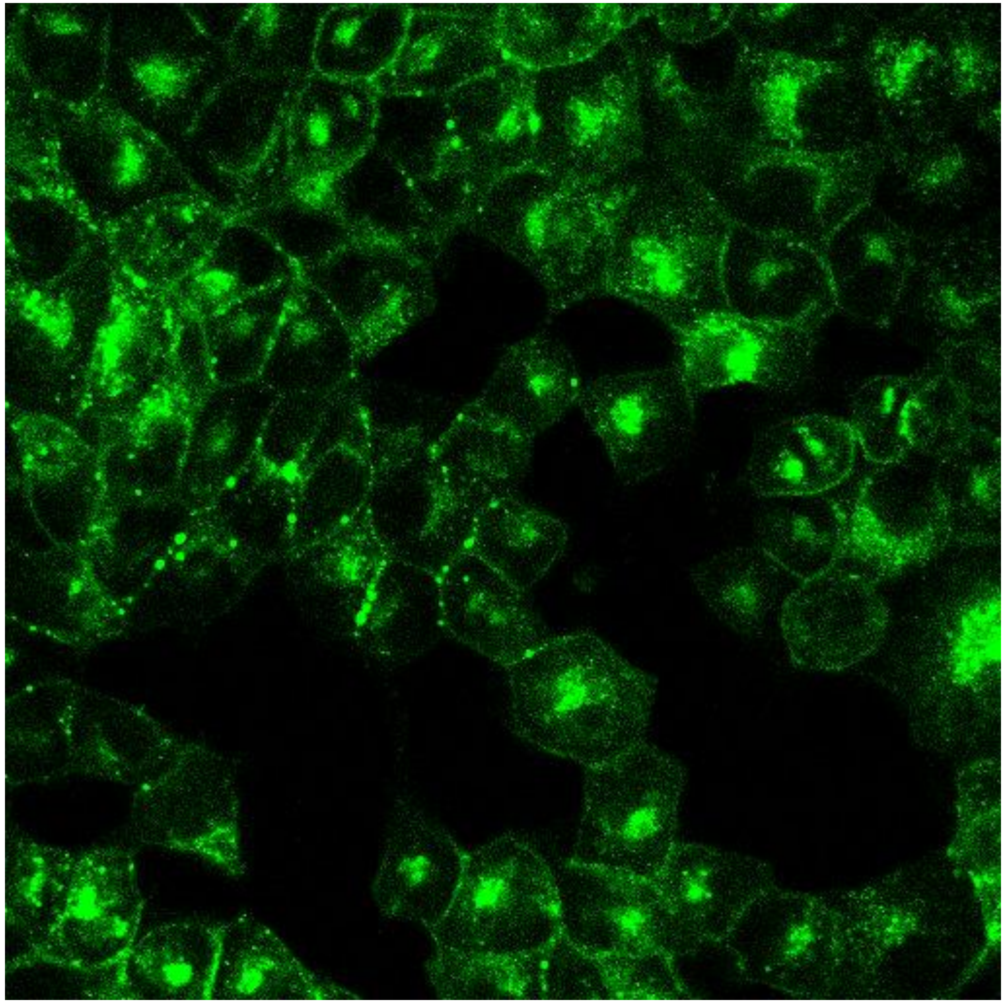


Image from https://github.com/MouseLand/cellpose

Segmentation – challenging cases

Crowded cells, noisy signal



Cellpose segmentation

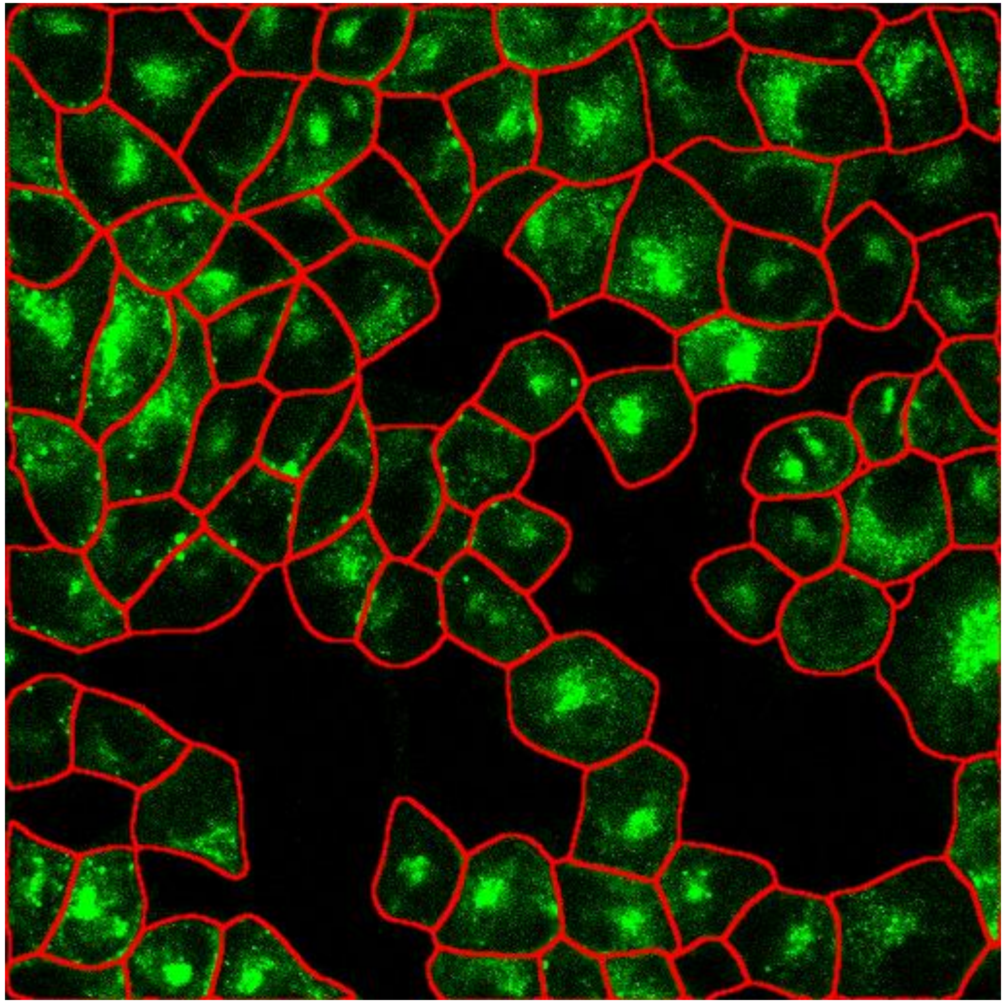
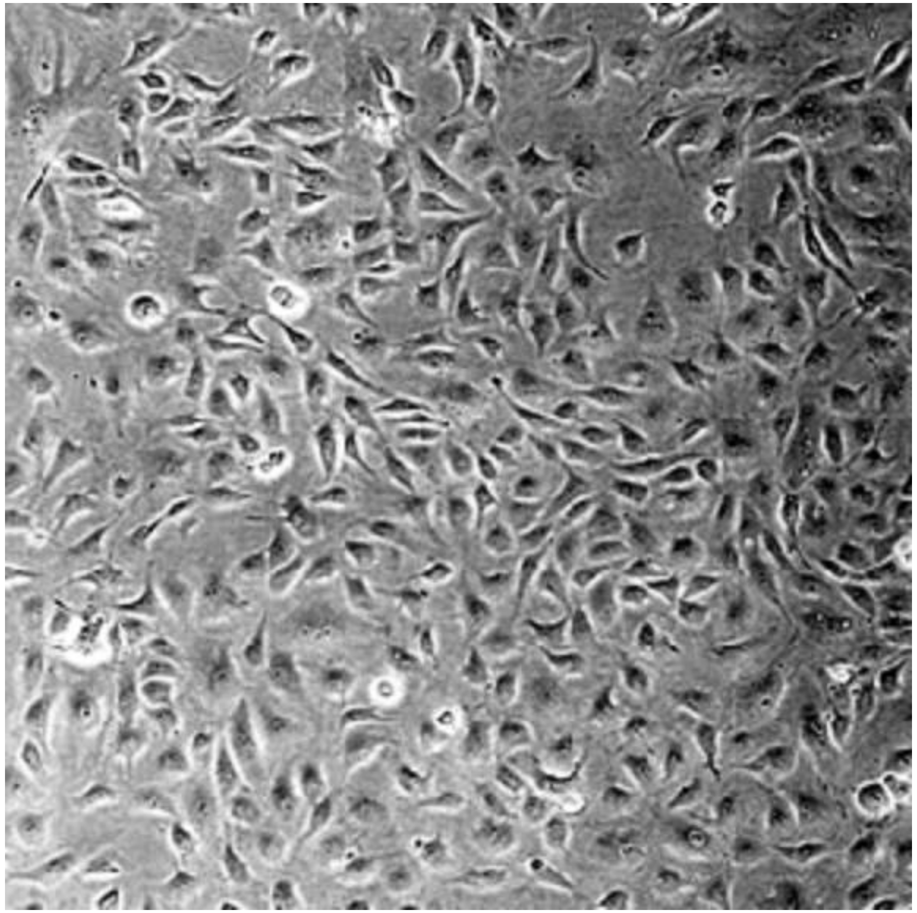


Image from https://github.com/MouseLand/cellpose

Segmentation – challenging cases

Crowded cells, uneven illumination



Cellpose segmentation

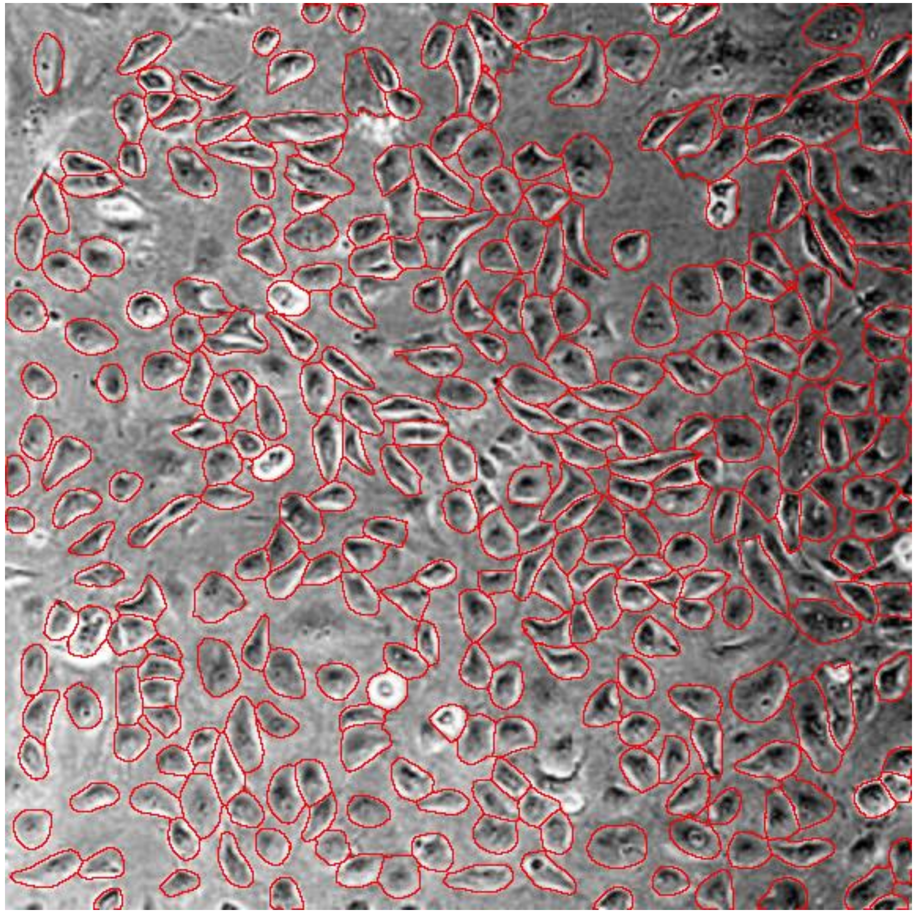
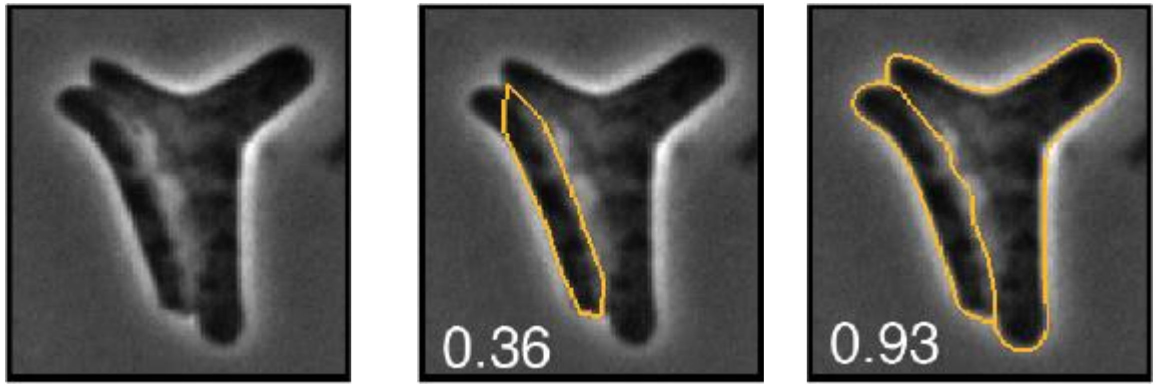


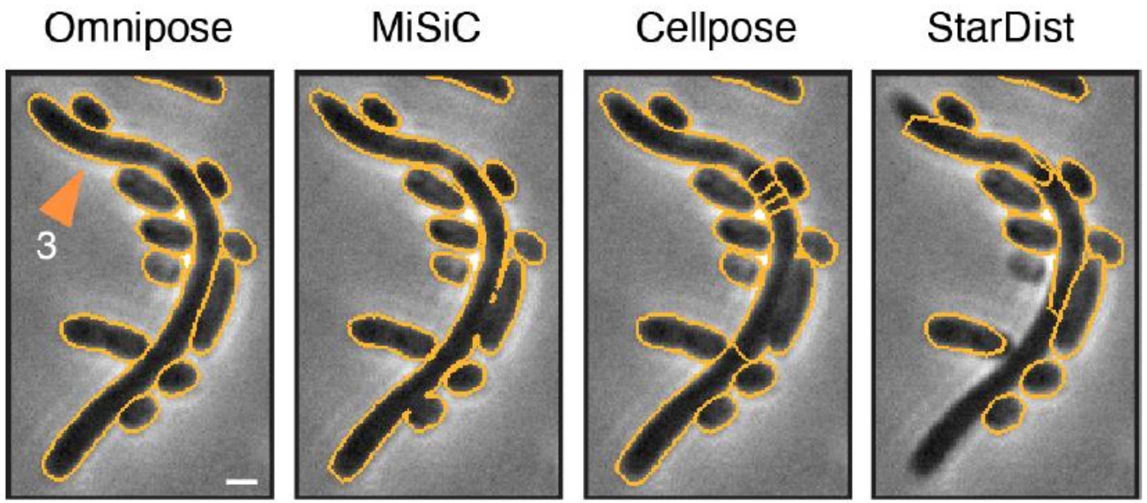
Image from https://github.com/MouseLand/cellpose

Segmentation – comparison of Deep Learning models

Phase contrast image StarDist Cellpose



Bacteria - *Escherichia coli*



Cutler et al. bioRxiv 2021

Segmentation – take home message

- There is no single “BEST” segmentation method
- Try different approaches to see which one works “BEST” for your “SPECIFIC” images

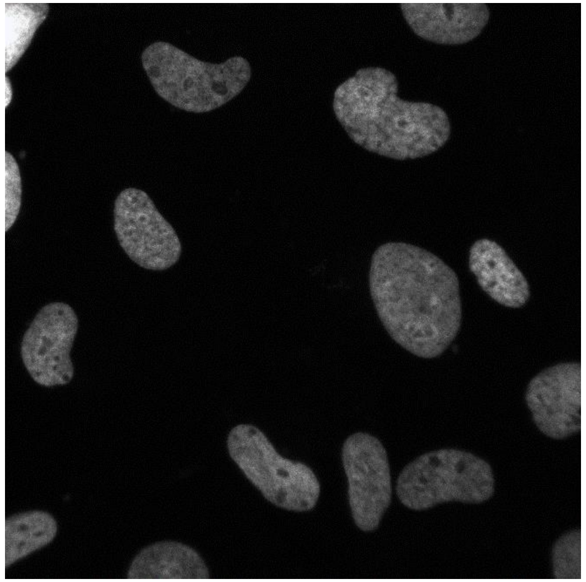
Denoising ⟺ Filtering ⟺ Masking ⟺ Segmentation

Original image

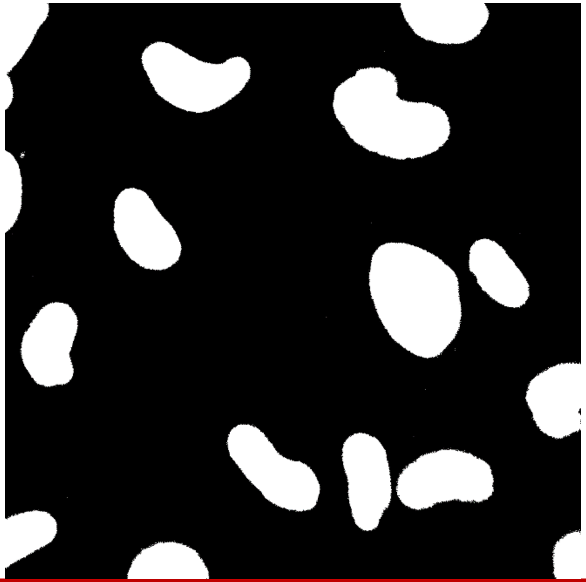
Cellpose segmentation

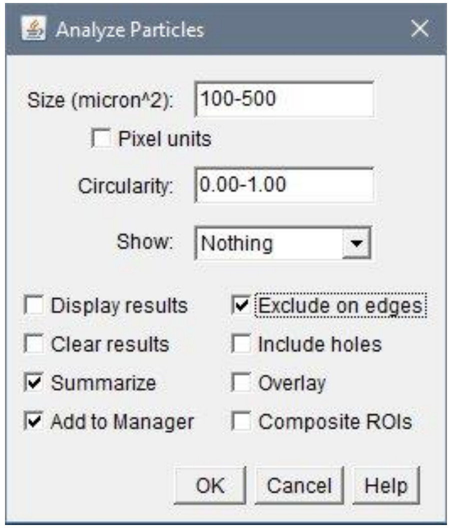
Cell/particle counting in Fiji

DAPI



segmented binary image





Analyze Particles

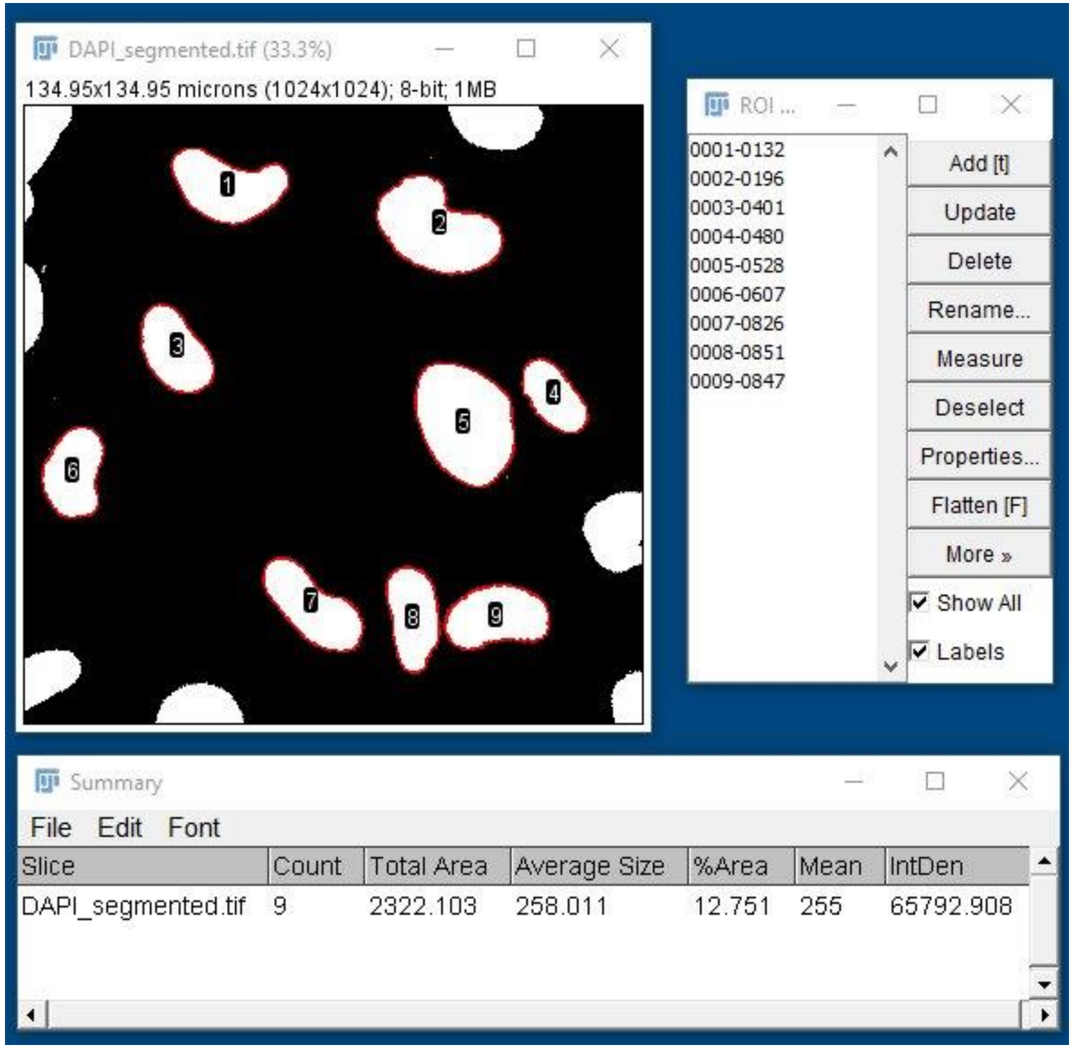
Size (micron^2): 100-500

☐ Pixel units

Circularity: 0.00-1.00

Show: Nothing

☐ Display results
☑ Exclude on edges
☐ Clear results
☐ Include holes
☑ Summarize
☐ Overlay
☑ Add to Manager
☐ Composite ROIs



DAPI_segmented.tif (33.3%)

134.95x134.95 microns (1024x1024); 8-bit; 1MB

ROI ...

0001-0132
0002-0196
0003-0401
0004-0480
0005-0528
0006-0607
0007-0826
0008-0851
0009-0847

☑ Show All
☑ Labels

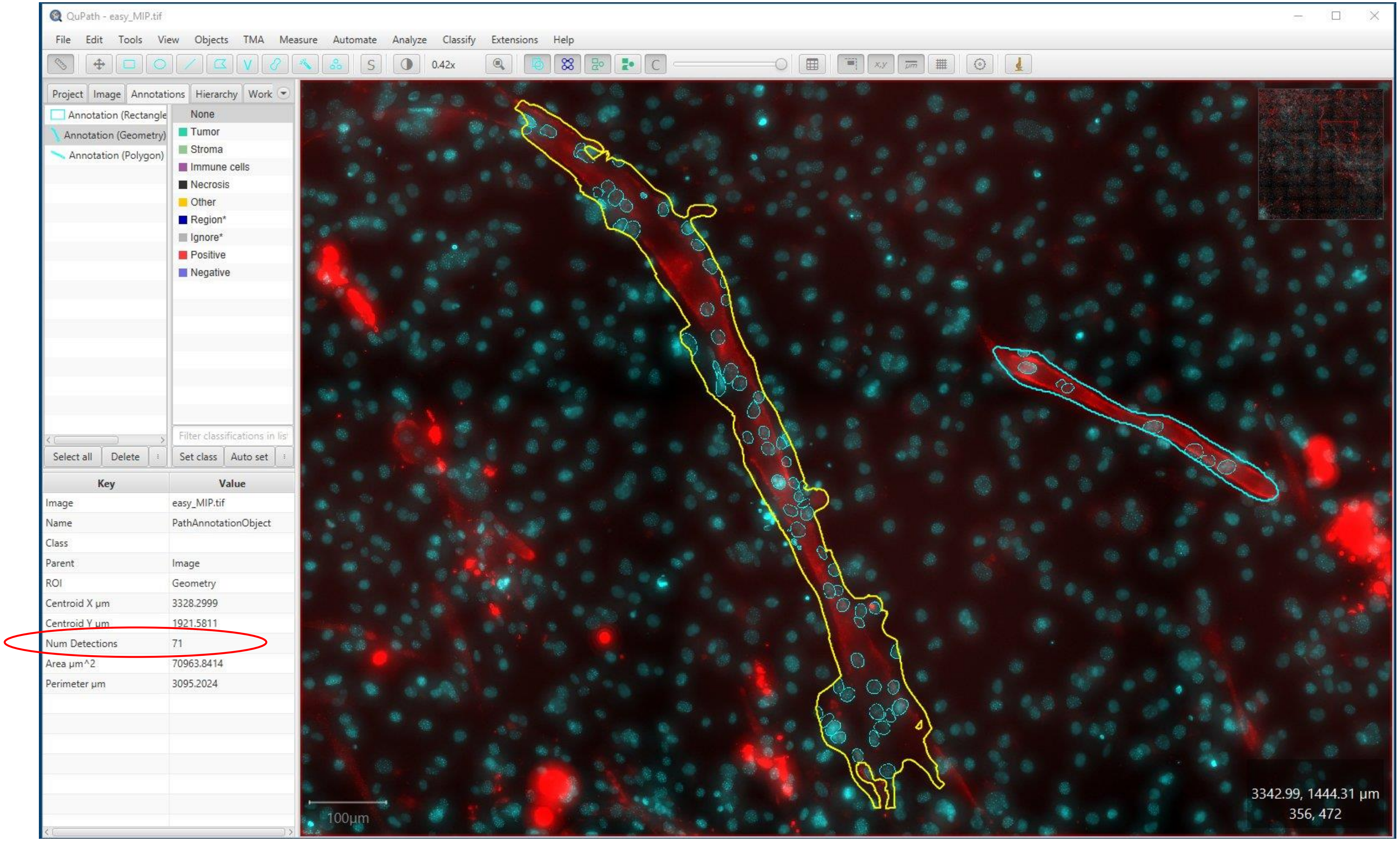
Summary

Slice	Count	Total Area	Average Size	%Area	Mean	IntDen
DAPI_segmented.tif	9	2322.103	258.011	12.751	255	65792.908

Image courtesy of Uwe Schaefer, Tarakhovsky Lab

Cell/particle counting in QuPath

Nuclei segmented with StarDist



Key	Value
Image	easy_MIP.tif
Name	PathAnnotationObject
Class	
Parent	Image
ROI	Geometry
Centroid X µm	3328.2999
Centroid Y µm	1921.5811
Num Detections	71
Area µm^2	70963.8414
Perimeter µm	3095.2024

Image courtesy of Yakshi Dabas, David Allis Lab

Cell/particle counting in 3D using Imaris

3D volume rendering showing green and magenta particles

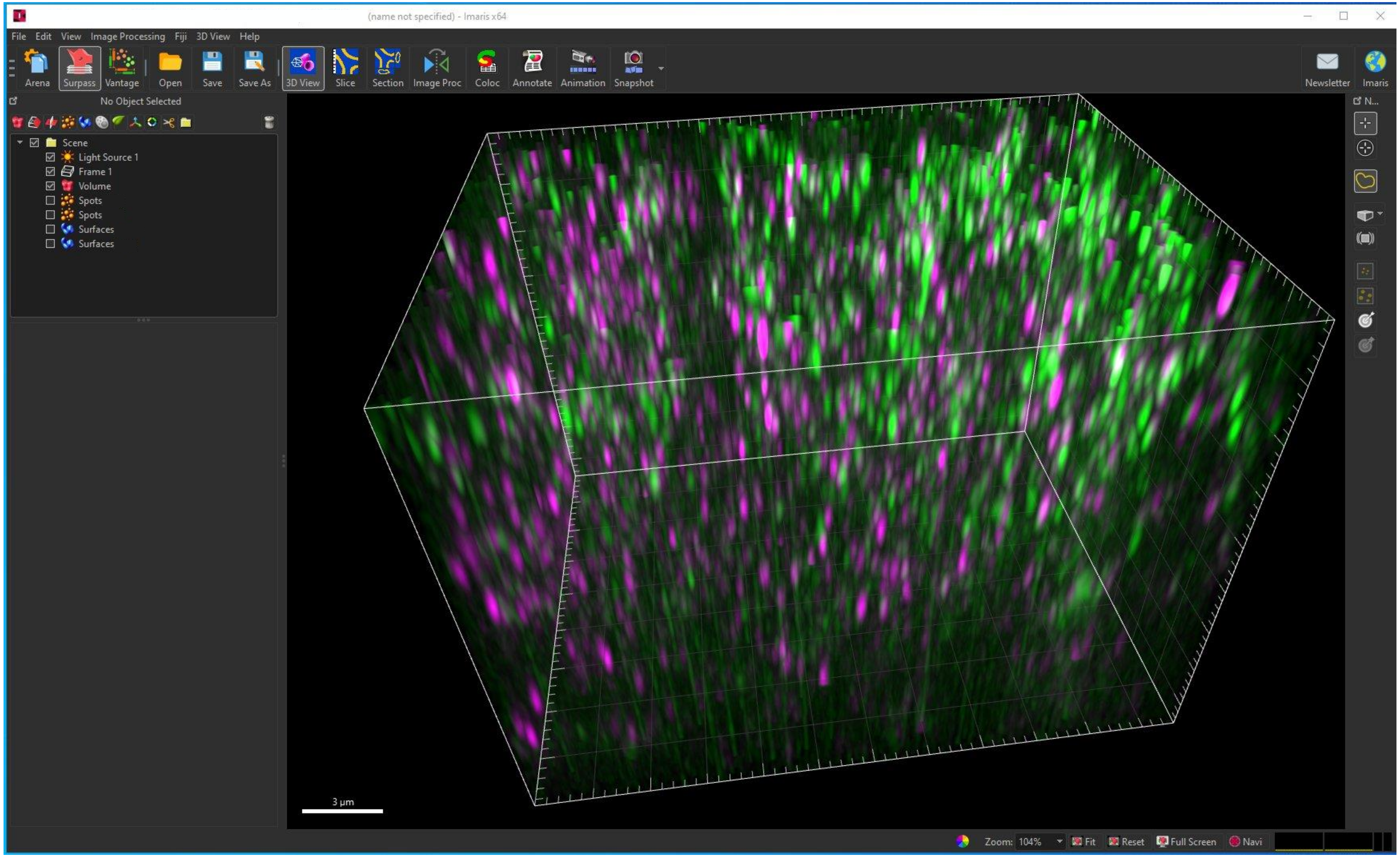
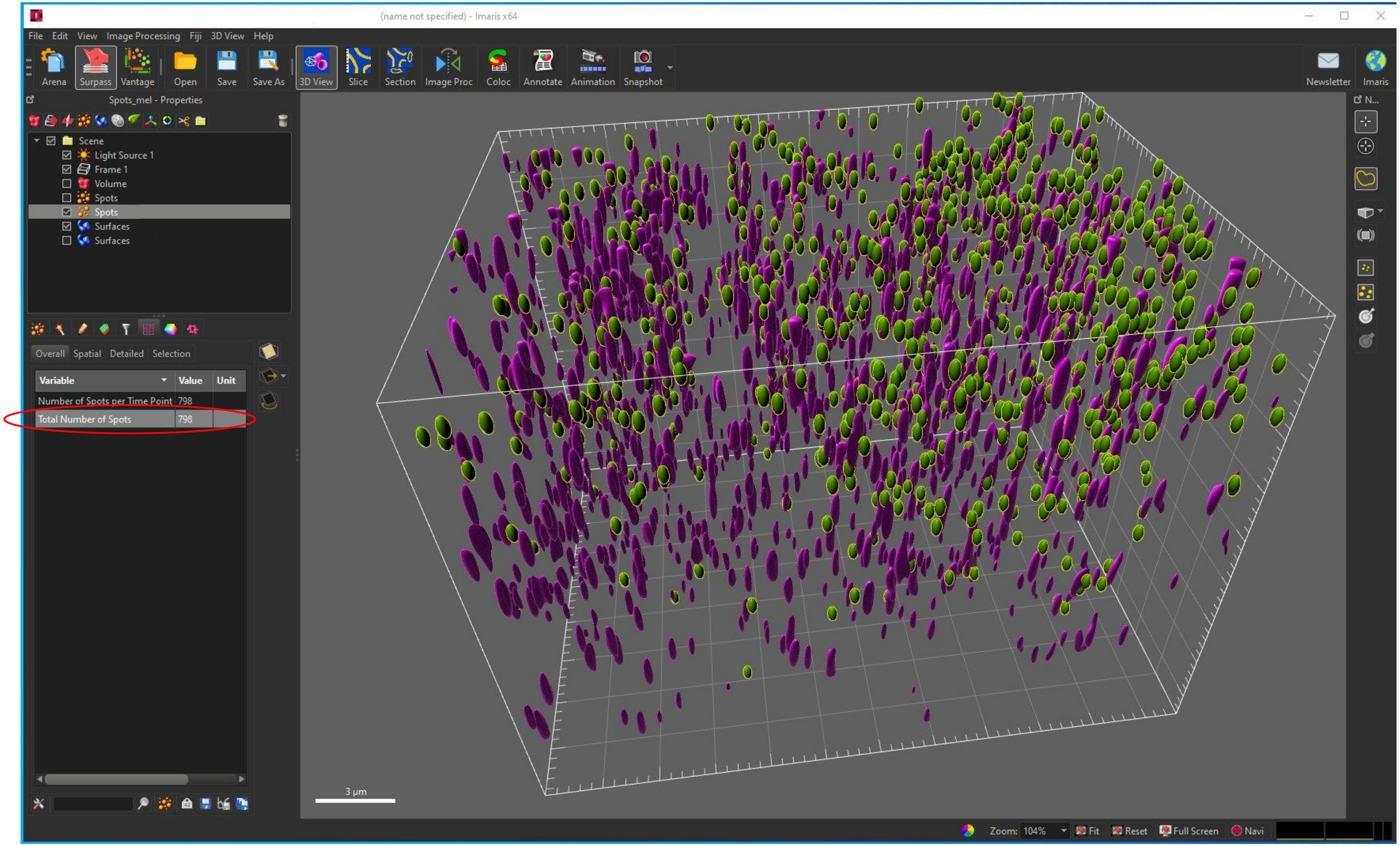


Image courtesy of Toby Lieber, Agnel Sfeir Lab, MSKCC

Cell/particle counting in 3D using Imaris

3D volume rendering showing green and magenta particles segmented and quantified

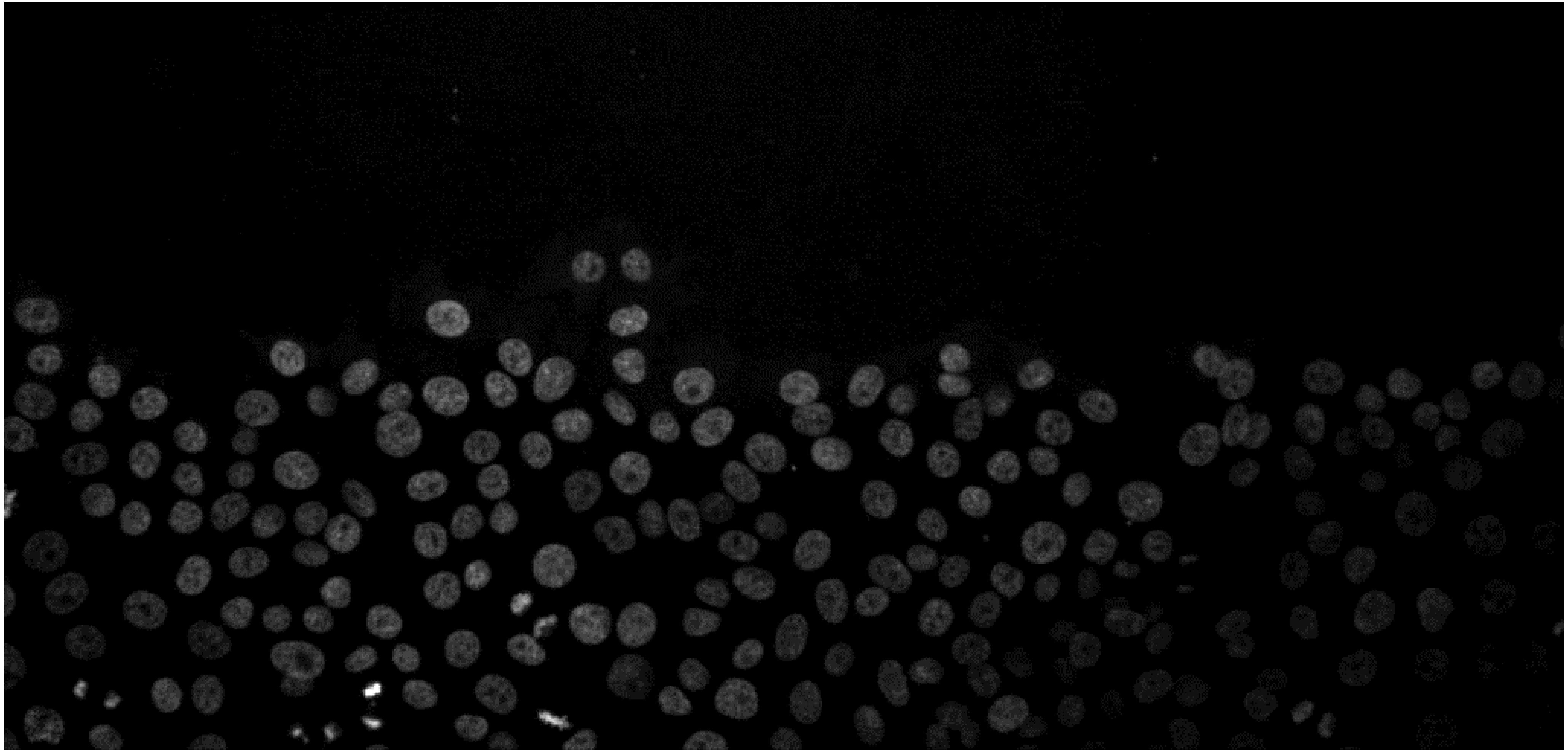


Variable	Value	Unit
Number of Spots per Time Point	798	
Total Number of Spots	798	

Image courtesy of Toby Lieber, Agnel Sfeir Lab, MSKCC

Cell/particle tracking in Fiji

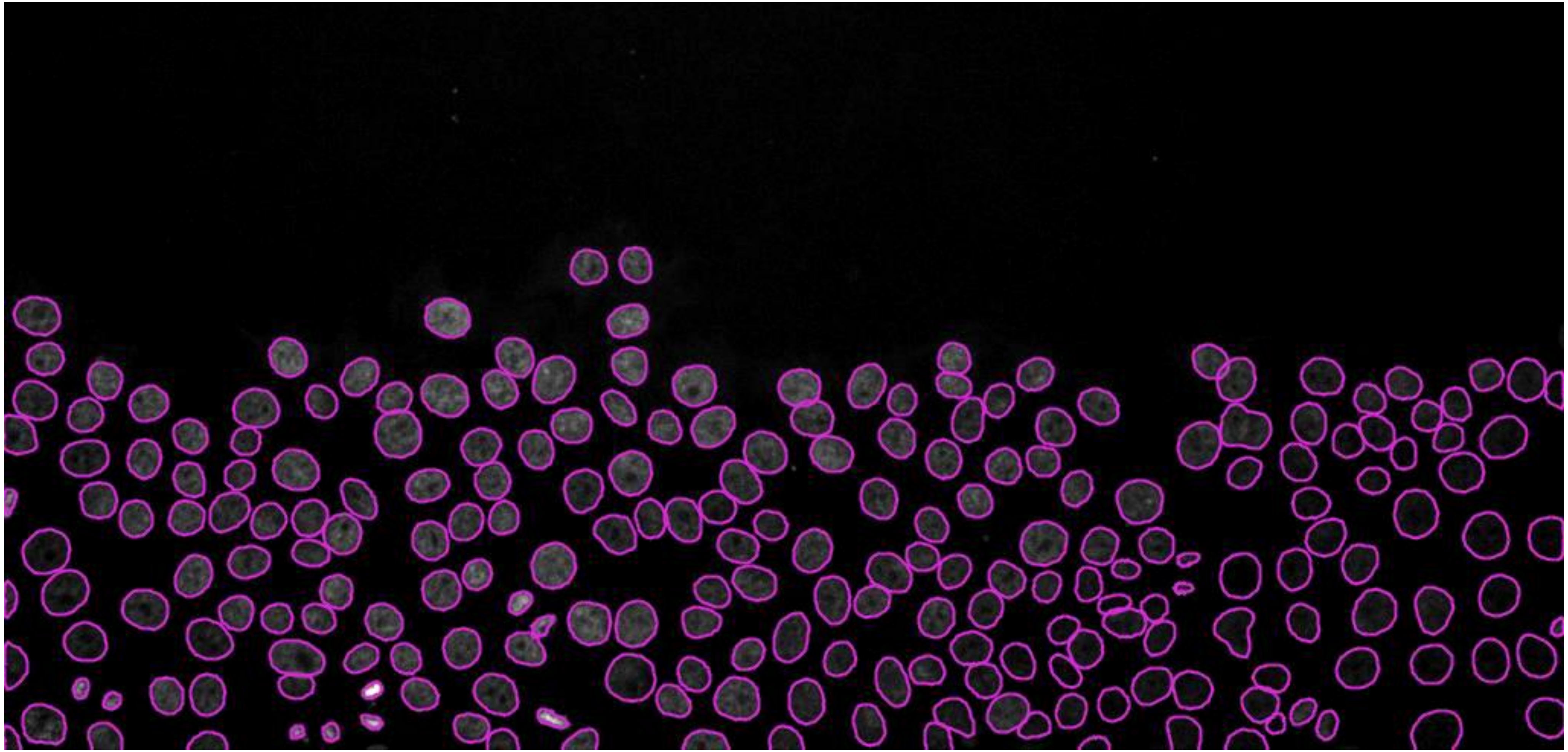
Cancer cell migration



https://doi.org/10.5281/zenodo.5206107

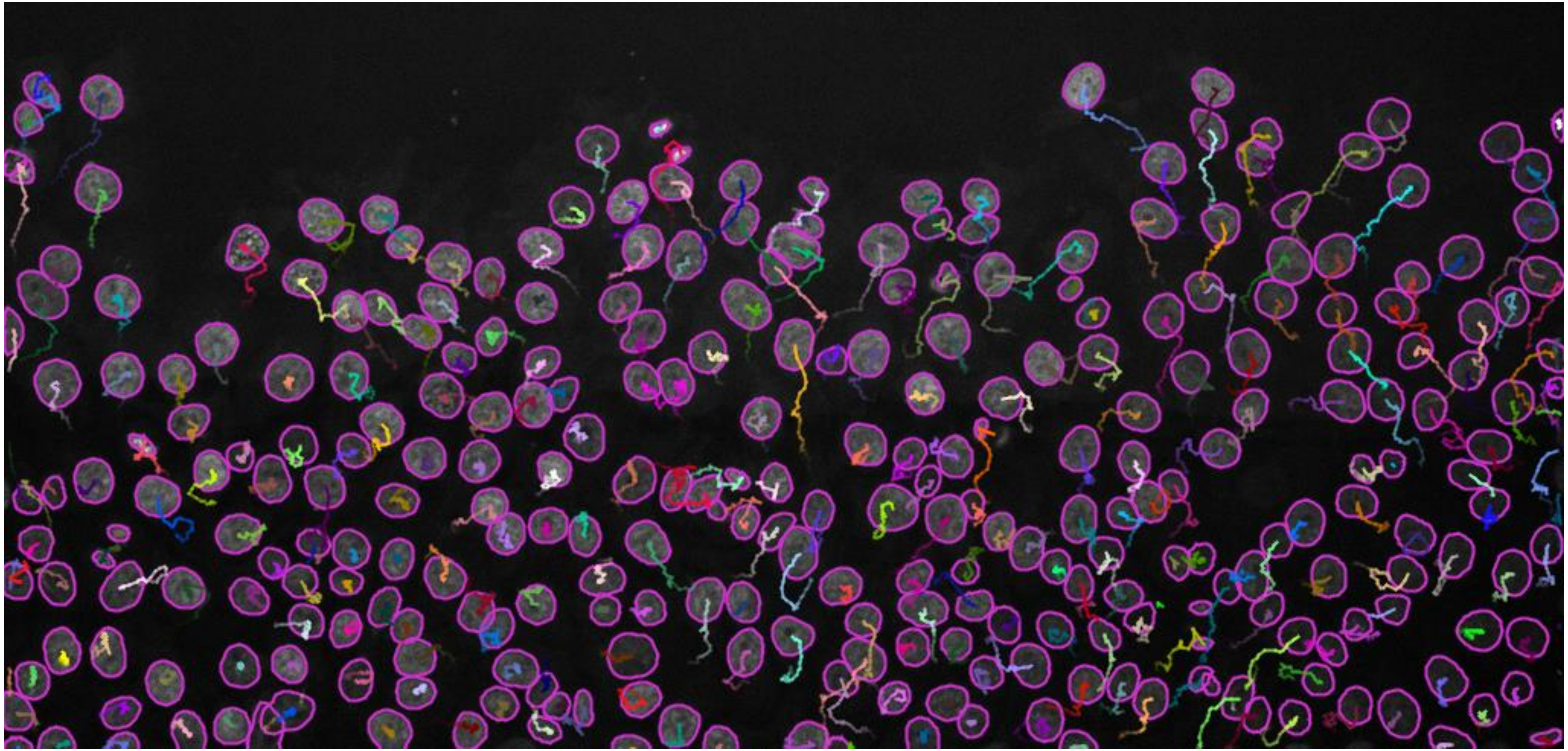
Cell/particle tracking in Fiji

StarDist based cell segmentation in TrackMate plugin



Cell/particle tracking - Fiji

Tracking results with the TrackMate plugin



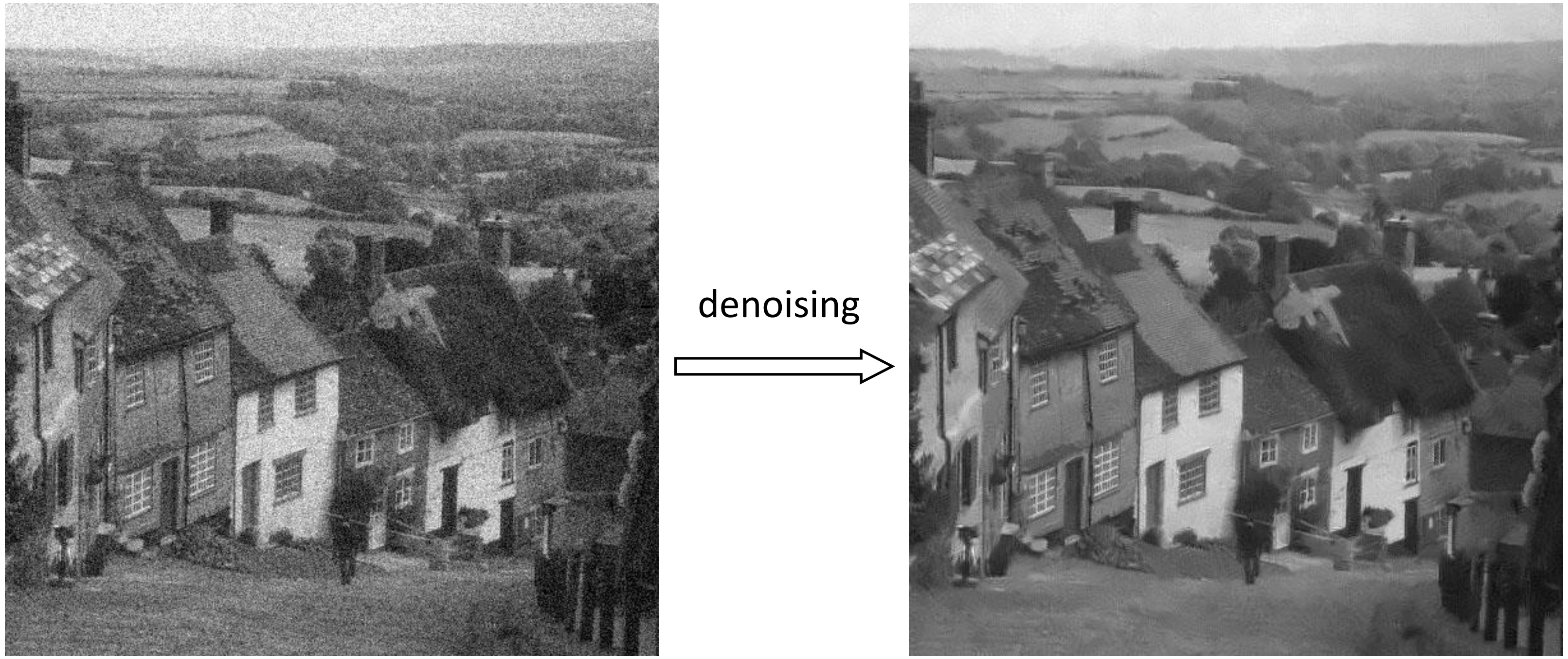
Workshop Exercise 2

3D tracking with TrackMate

3D tracking in Imaris

Denoising

- The task of removing noise from an image
- Not used solely for making an image look “good”, but when done properly, it can greatly improve the segmentation accuracy
- Beware - denoising changes pixel values in the image!



Gold Hill, Shaftesbury, UK

Why acquire noisy images in the first place?

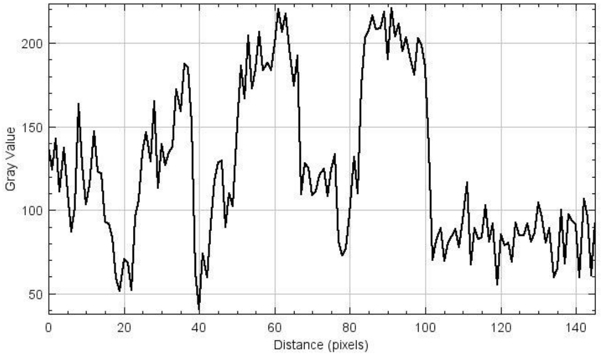
- Low protein expression
- High protein expression has unwanted phenotype
- Photobleaching
- Detection noise due to inherent uncertainly of photon detection

Denoising - tools

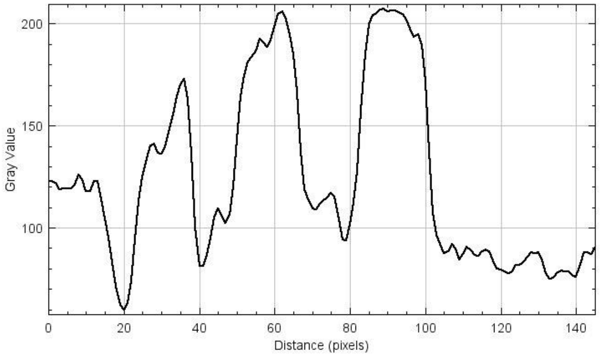
- Classical Filters – Gaussian, Median, Non-local means
- Deep Learning methods (open-source): CARE, Noise2Noise, Noise2Void
- Deep Learning methods (commercial): RCAN in Aivia

Denoising using image processing filters

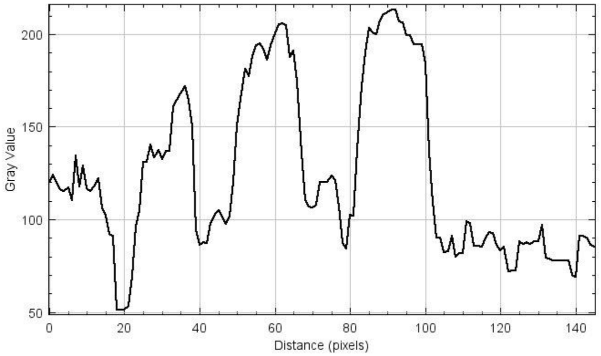
Noisy image



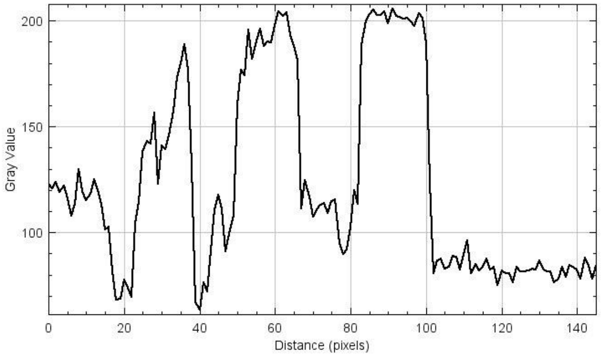
Gaussian filter



Median filter



Non-local means filter



Denoising using Deep Learning methods (open-source)

CARE (Content Aware Restoration):

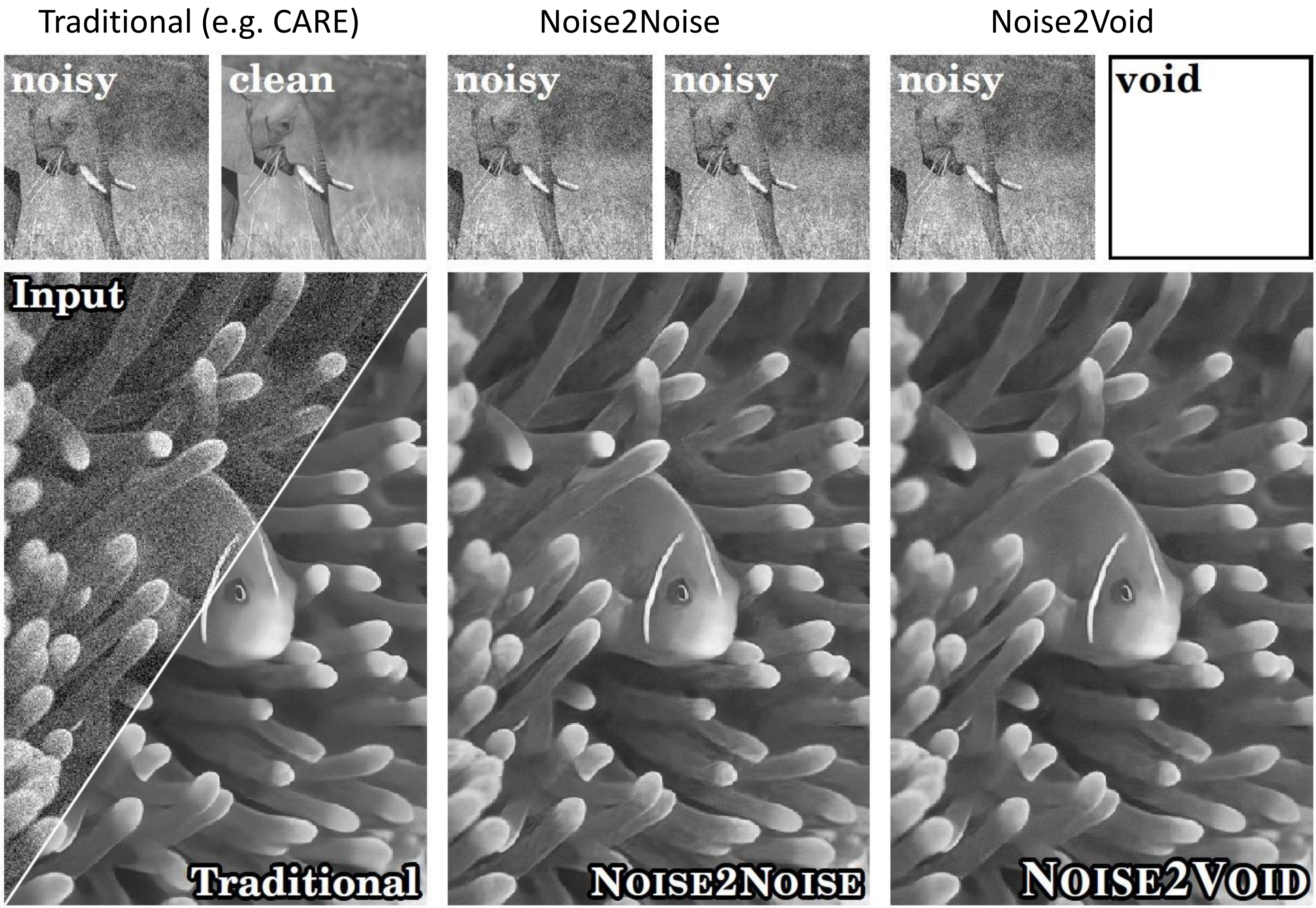
A pair of high and low SNR images required for training the model

Noise2Noise:

A pair of noisy images required for training the model

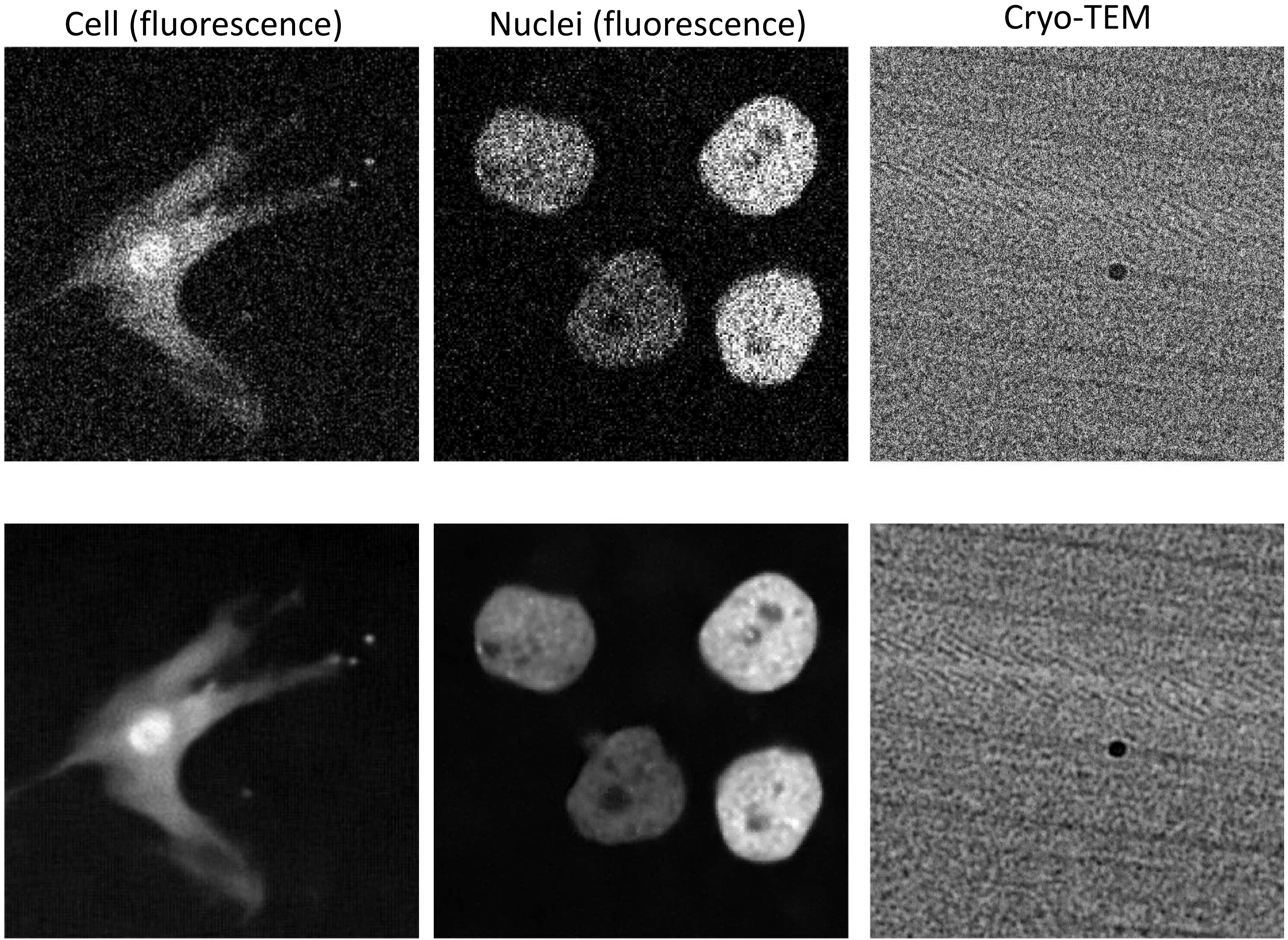
Noise2Void:

Single noisy images are enough for training the model



Krull et al.
https://doi.org/10.48550/arXiv.1811.10980

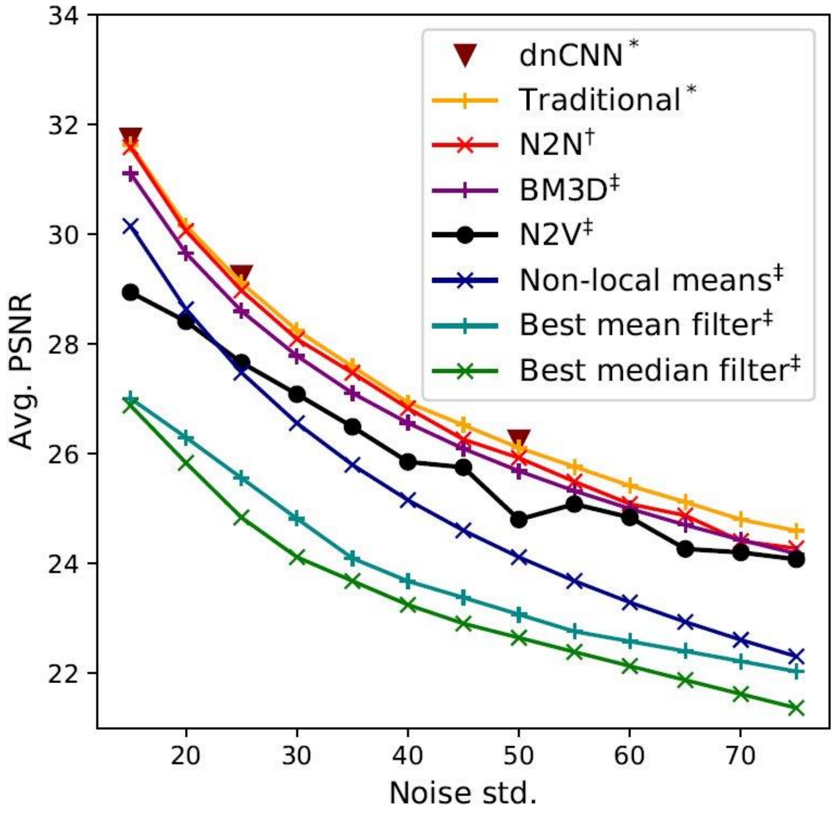
Denoising using Noise2Void



Workshop Exercise 3

Krull et al.
https://doi.org/10.48550/arXiv.1811.10980

Denoising – limits of Noise2Void




Input image

Traditional (e.g. CARE)

Noise2Void

N2V cannot distinguish between the signal and structured noise that violates the <u>assumption of pixel-wise independence of noise</u>

N2V can not be expected to outperform methods that have additional information during training (CARE, N2N)

Krull et al. https://doi.org/10.48550/arXiv.1811.10980

Denoising using Deep Learning methods (commercial: Aivia)

Aivia Deep Learning Models

Denoising_Actin_iSIM-60x

Denoising_ER_iSIM-60x

Denoising_Generic(mito+MT)_iSIM-60x

Denoising_Golgi_iSIM-60x

Denoising_Lysosome_iSIM-60x

Denoising_Microtubule_iSIM-60x

Denoising_Mitochondria_iSIM-60x

Expansion_Microtubule+decon

Expansion_Mitochondria+decon

Restoration

Segmentation

Segmentation_3D_EM_ISBI_2013

Segmentation_3D_EM_UW_WongLab

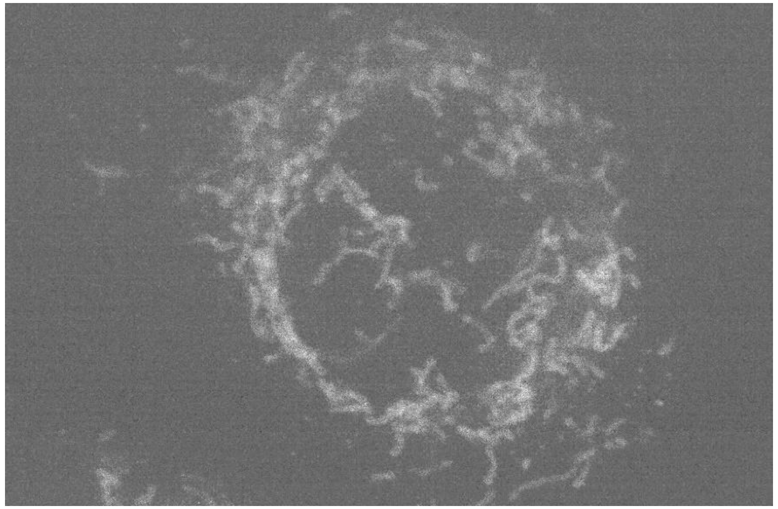
Virtual_Staining_Actin_20x

Virtual_Staining_Microtubule_20x

Virtual_Staining_Nucleus_20x

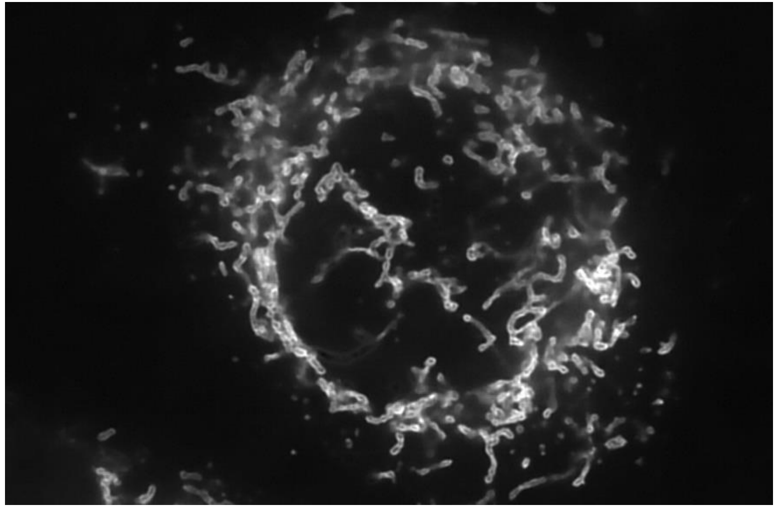
Original image

Mitochondria membrane
captured on an iSIM



Model trained on RCAN architecture from Hari Shroff lab (NIBIB, NIH)
Chen et al. Nat. Methods 2021

Denoised image



Images from https://www.aivia-software.com

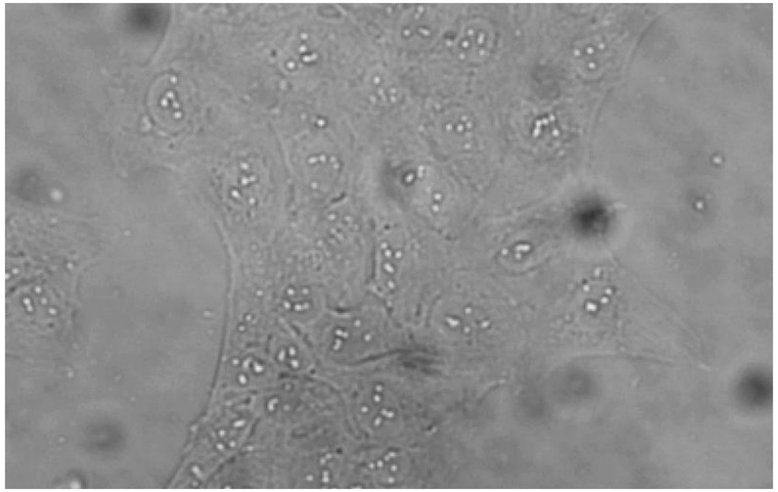
Virtual staining using Aivia

Aivia Deep Learning Models

Denoising_Actin_iSIM-60x
Denoising_ER_iSIM-60x
Denoising_Generic(mito+MT)_iSIM-60x
Denoising_Golgi_iSIM-60x
Denoising_Lysosome_iSIM-60x
Denoising_Microtubule_iSIM-60x
Denoising_Mitochondria_iSIM-60x
Expansion_Microtubule+decon
Expansion_Mitochondria+decon
Restoration
Segmentation
Segmentation_3D_EM_ISBI_2013
Segmentation_3D_EM_UW_WongLab
Virtual_Staining_Actin_20x
Virtual_Staining_Microtubule_20x
Virtual_Staining_Nucleus_20x

Original image

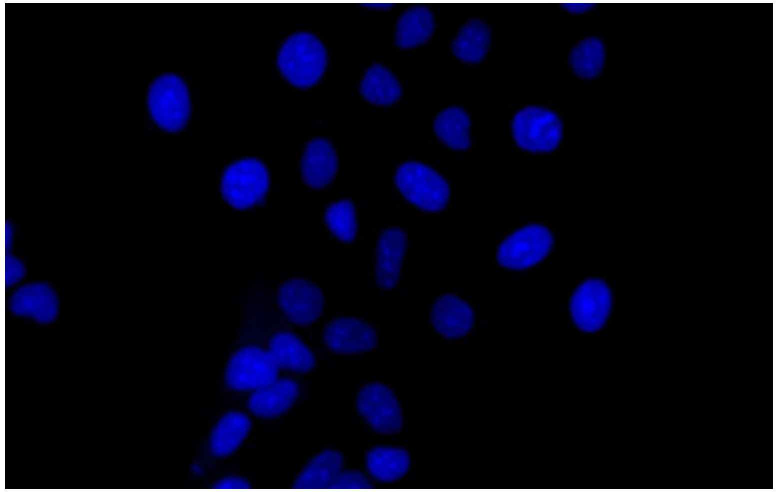
Brightfield image (DIC, 20x)



Model trained on RCA U-Net architecture ⇩

Predicted image

Fluorescence image (simulates DAPI)



Images from https://www.aivia-software.com

Image analysis help at BIRC

- Help users analyze their data
  Suggestion on the best method/software/pipeline for user data analysis

  - Open-source: Fiji, QuPath, ilastik, DeepImageJ, ZeroCostDL4Mic, Napari, CellProfiler
  - Imaris – 2D/3D large data, segmentation, counting, tracking; currently lacks deep learning methods
  - Arivis – Light-sheet data (multi-GB to TB)
  - Aivia – AI-powered 3D image analysis (e.g. segmentation, denoising)
  - Huygens – deconvolution, 2D/3D chromatic aberration correction

- Writing customized image analysis scripts, pipelines, batch processing

- Experimental design discussion with a team of microscopy and image analysis staff, to ensure that the imaging data acquired will be appropriate for the planned analysis and research questions

Training

One-on-one Image analysis training

Regular workshops on image analysis

- basic ImageJ/Fiji workshop
- advanced ImageJ/Fiji workshop
- commercial analysis softwares – Imaris, Arivis, Huygens, Aivia
- Colocalization and distance analysis
- New tools for Methods reporting in manuscripts – MethodsJ2
- Handling big data – BigDataViewer in Fiji, Imaris, Arivis
- Data management and figure generation in OMERO
  May 4th – Jason Swedlow, OME

"Image Analysis User Group" – NEW!!!

- anybody who is interested in image analysis discussion
- Regular meetings in an informal setting where members will take turn presenting on a specific topic
- new method/pipeline/software/paper
- First meeting in May 2022
- Contact vsharma01@rockefeller.edu to join the group

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our users!