IMAGE ANALYSIS

AN OVERVIEW OF TOOLS

BY NOORSHER AHMED

The Evolution of Imaging

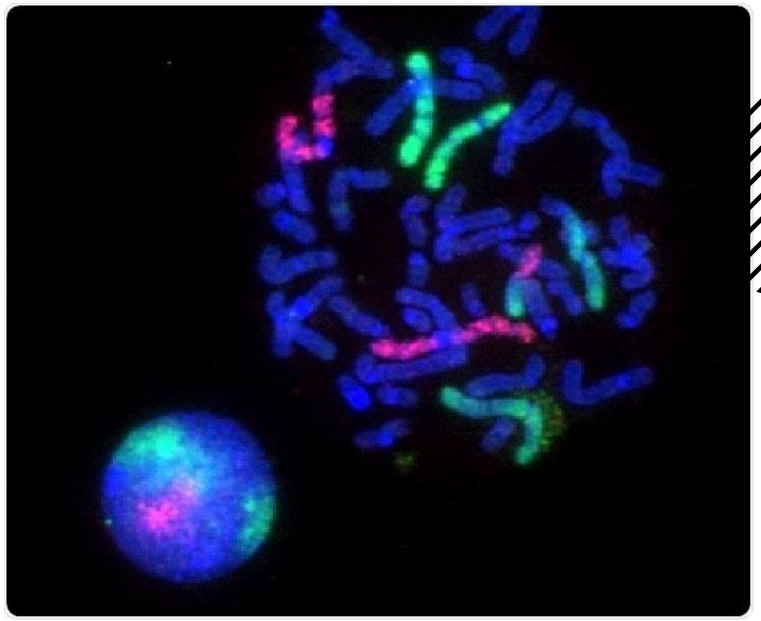
Light Microscopy

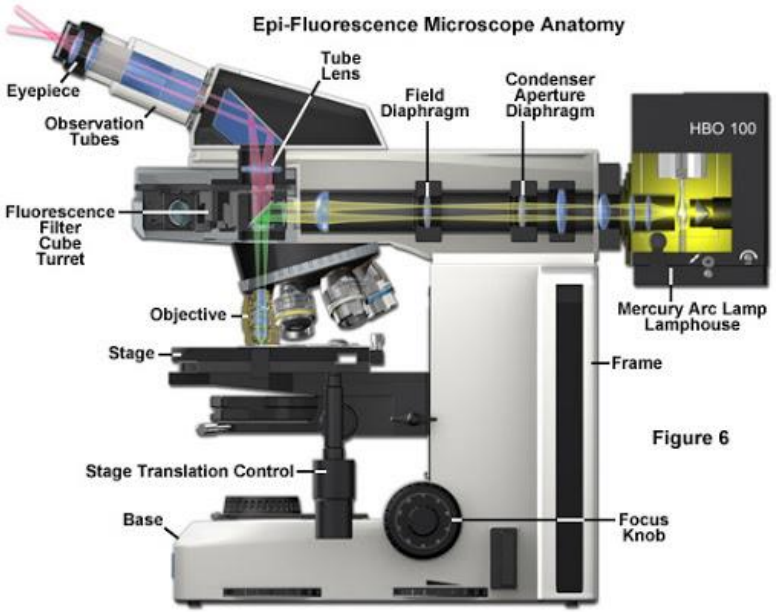
- Classic white light as source
- Low magnification
- Low resolution
- Large field of view

The Evolution of Imaging

Epi-fluorescent Imaging

- Specified wavelength excitation
- Can use fluorescently tagged proteins
- Same mag/resolution as light microscopes

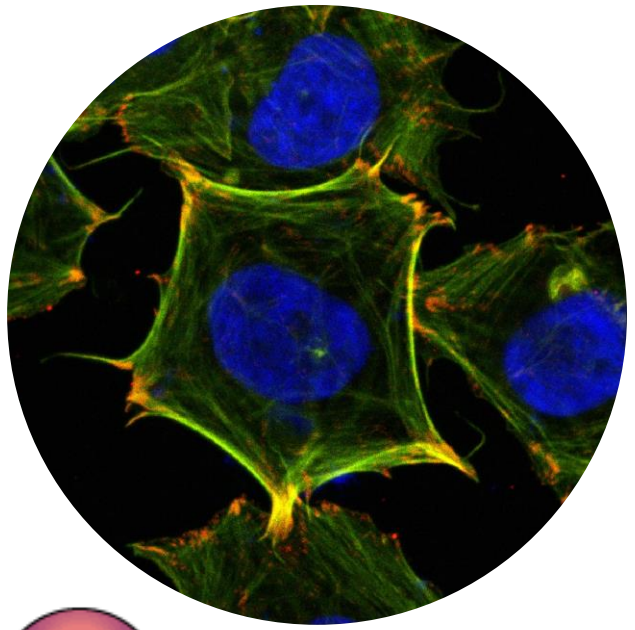


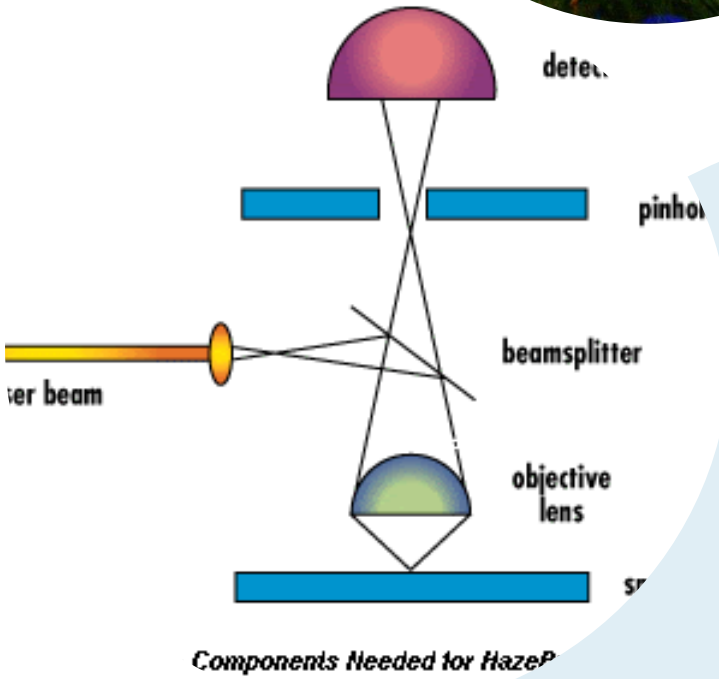


The Evolution of Imaging

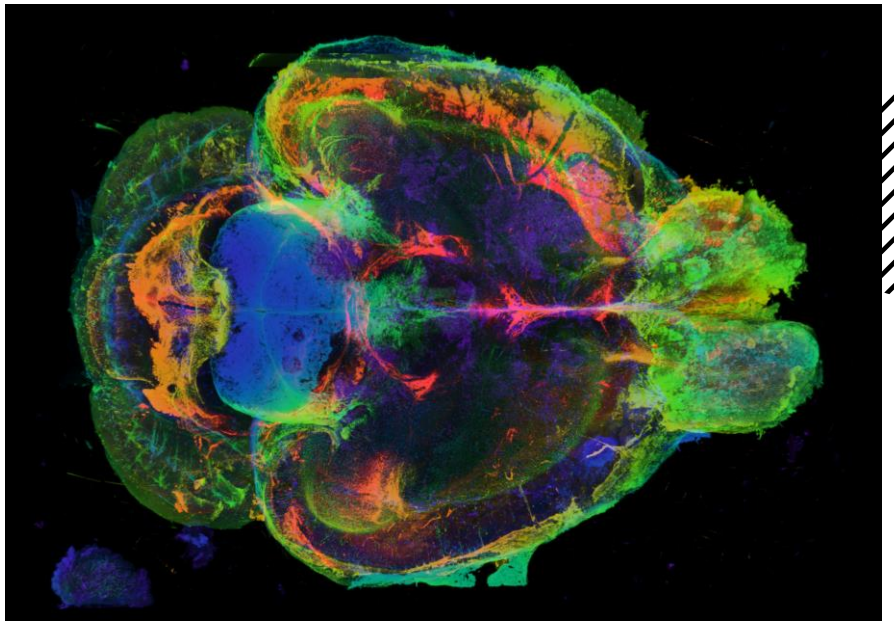
Confocal Microscopy

- Laser light source of specific wavelength
- 100x magnification possible
- Higher resolution
- “3D” imaging (z-stacks)



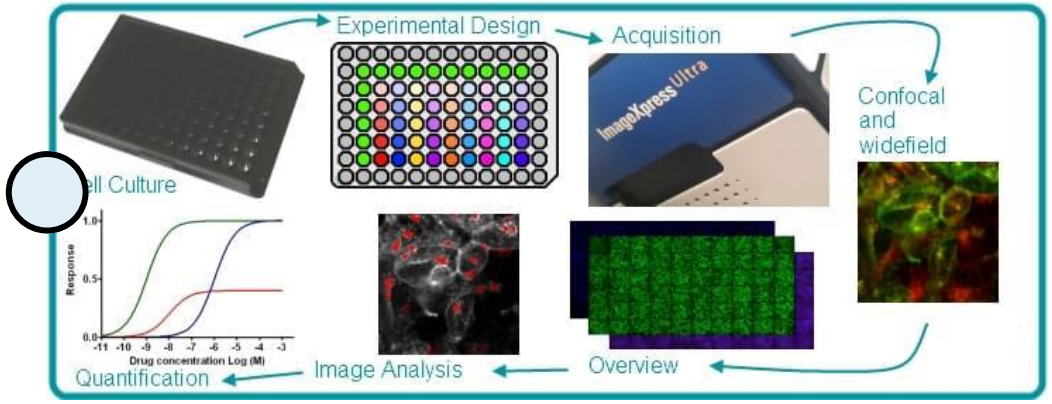


The Evolution of Imaging



High Content Imaging

- Combine Confocal Imaging with Robotics
- Thousands to millions of cells
- High mag/resolution



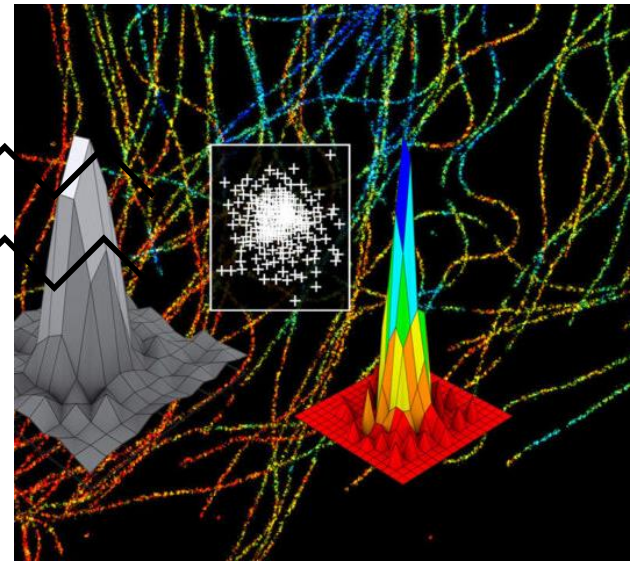
Big Data

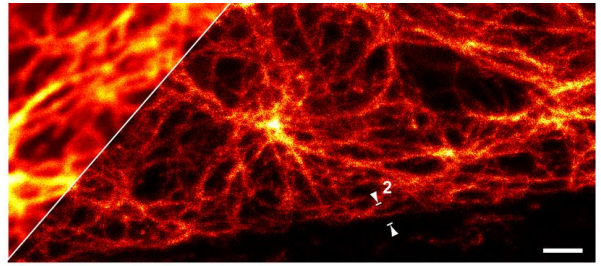
The Evolution of Imaging

Super-resolution Microscopy

- Extremely high resolution (single molecule!)
- High magnification
- Large data formats
- Thousands of particles/features per cell

Big Data





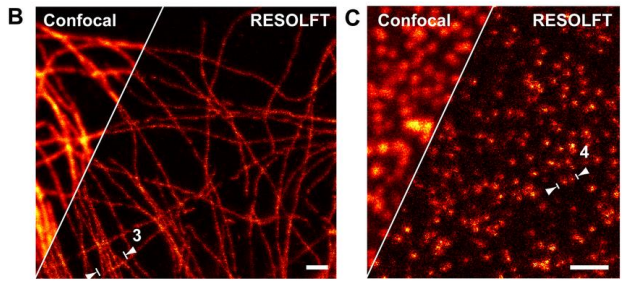
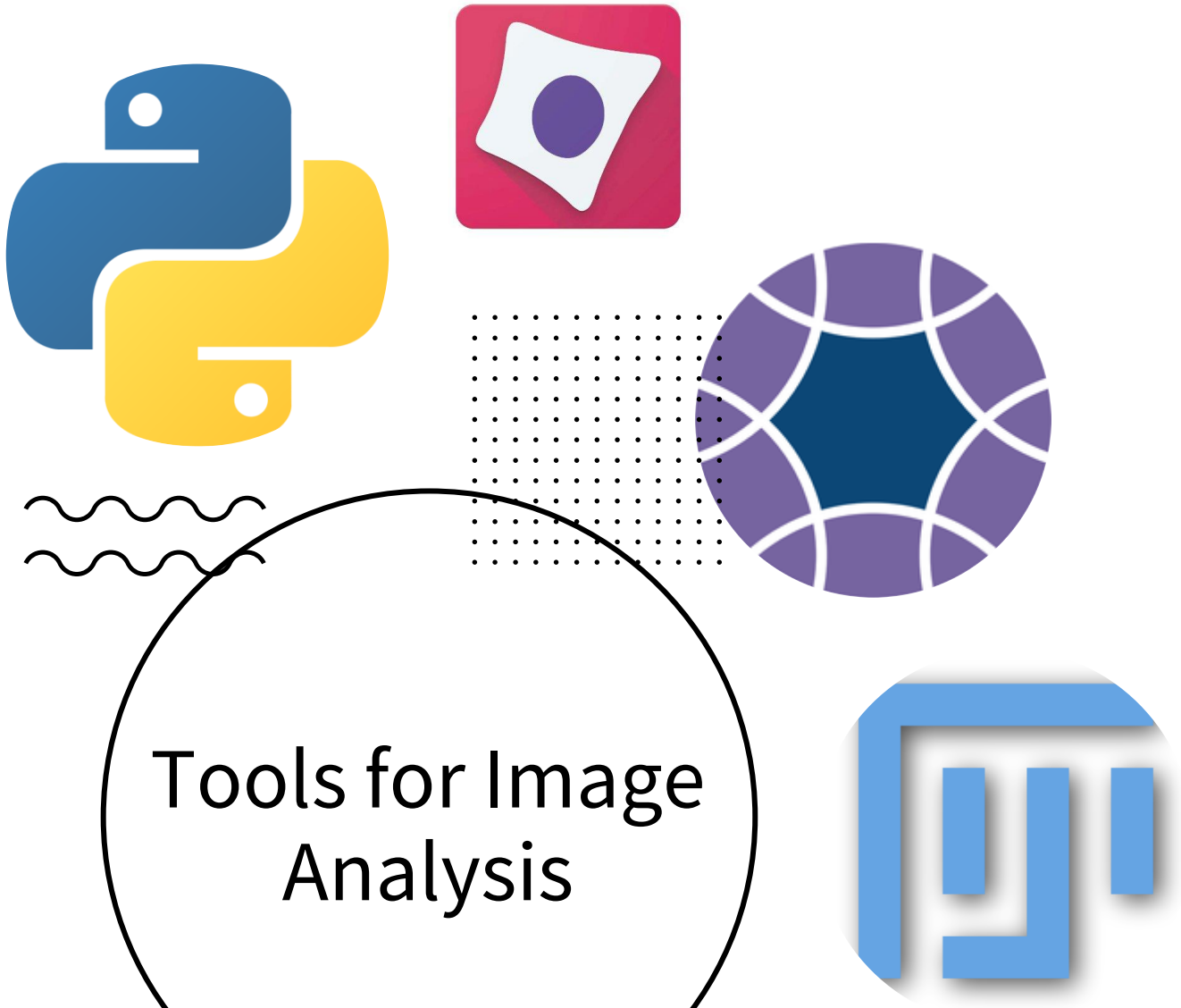


Image Analysis Tasks

- You have your images, now what?
  - Count cells/particles
  - Segment cells, tissue, etc.
  - Fluorescent area / cell area ratio
  - Comparing fluorescent signal between samples/cells
  - Track cells/organism through time series
  - Changes in morphology

…Doing this by hand is a pain…

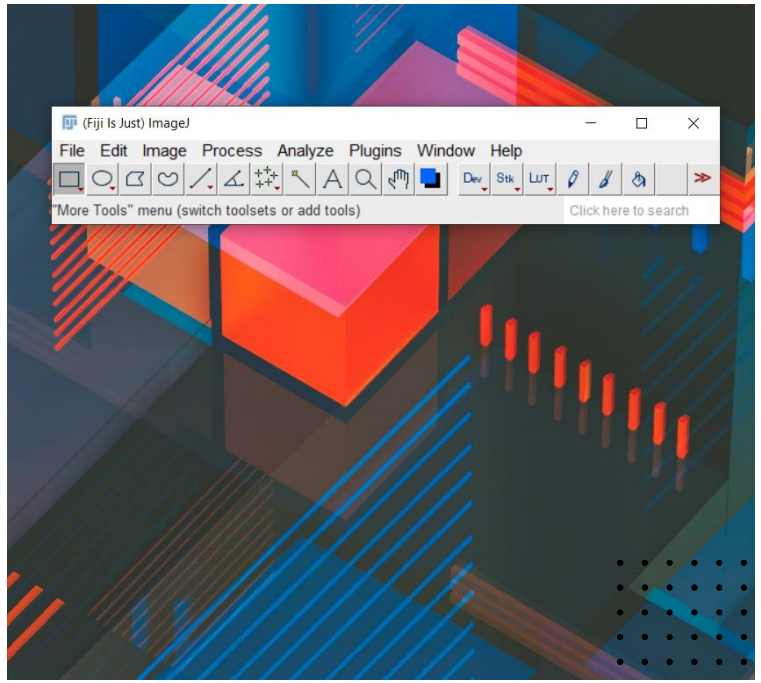


- FIJI
- CellProfiler
- AICS Segmenter
- Custom code…

FIJI (FIJI Is Just ImageJ)

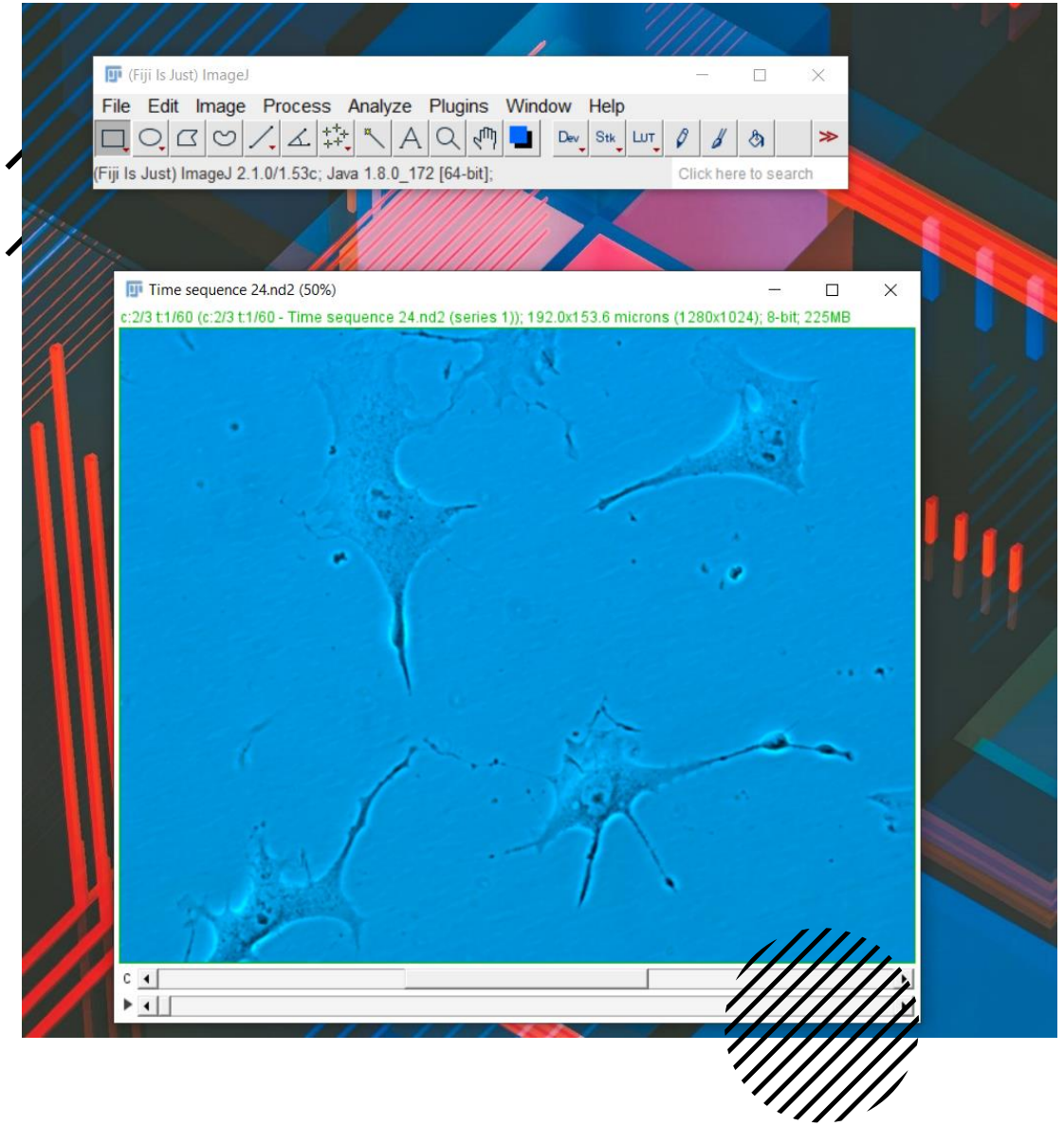
Starts with just a nifty toolbar

- Crop image
- Adjust brightness/contrast
- Annotate image
- The real power is in the menu items



FIJI

- Open any type of image or microscopy file
- Creates “Hyperstacks” for images taken at multiple positions, z-slices, channels, and time series
- Export to make cool figures!



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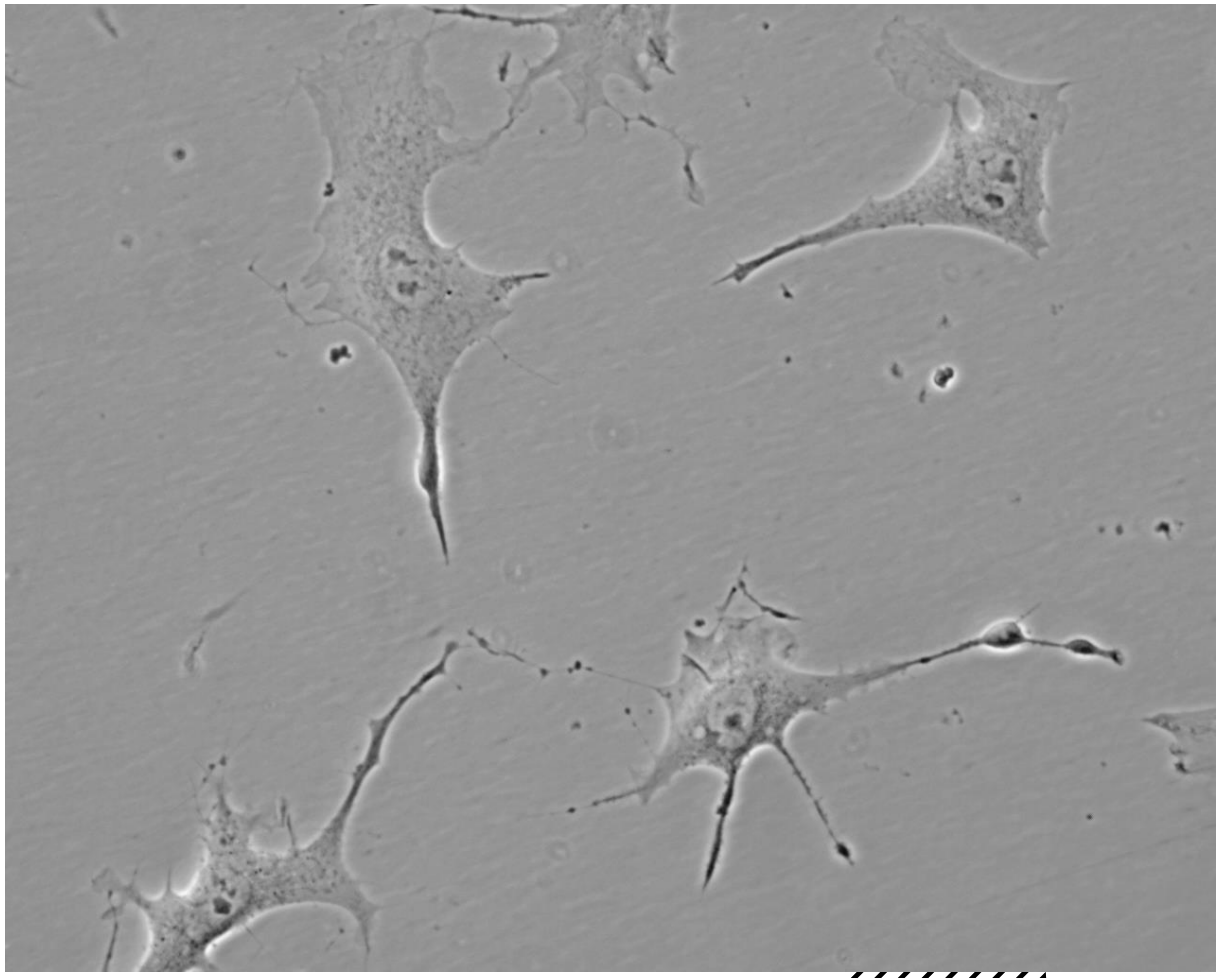
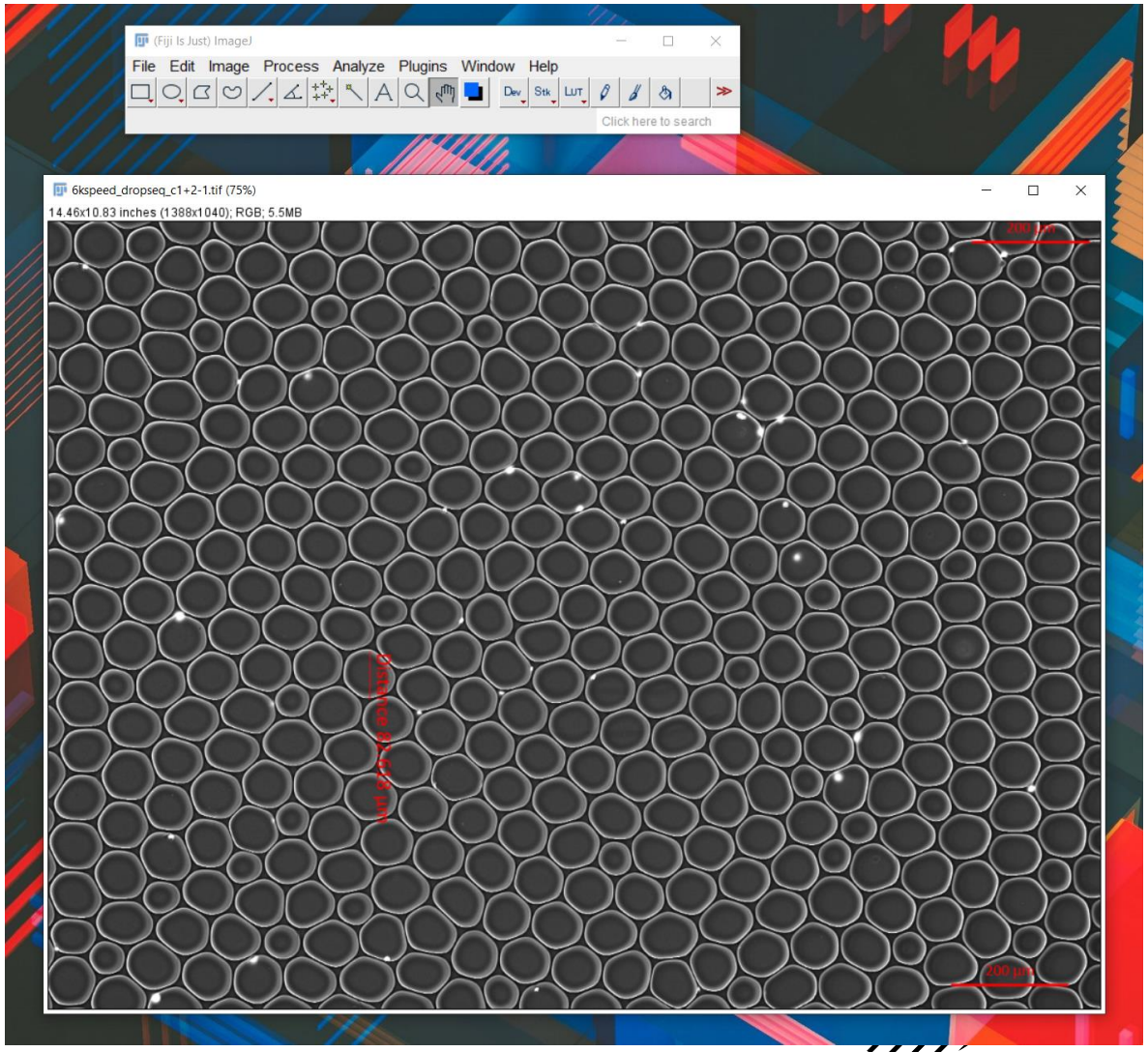


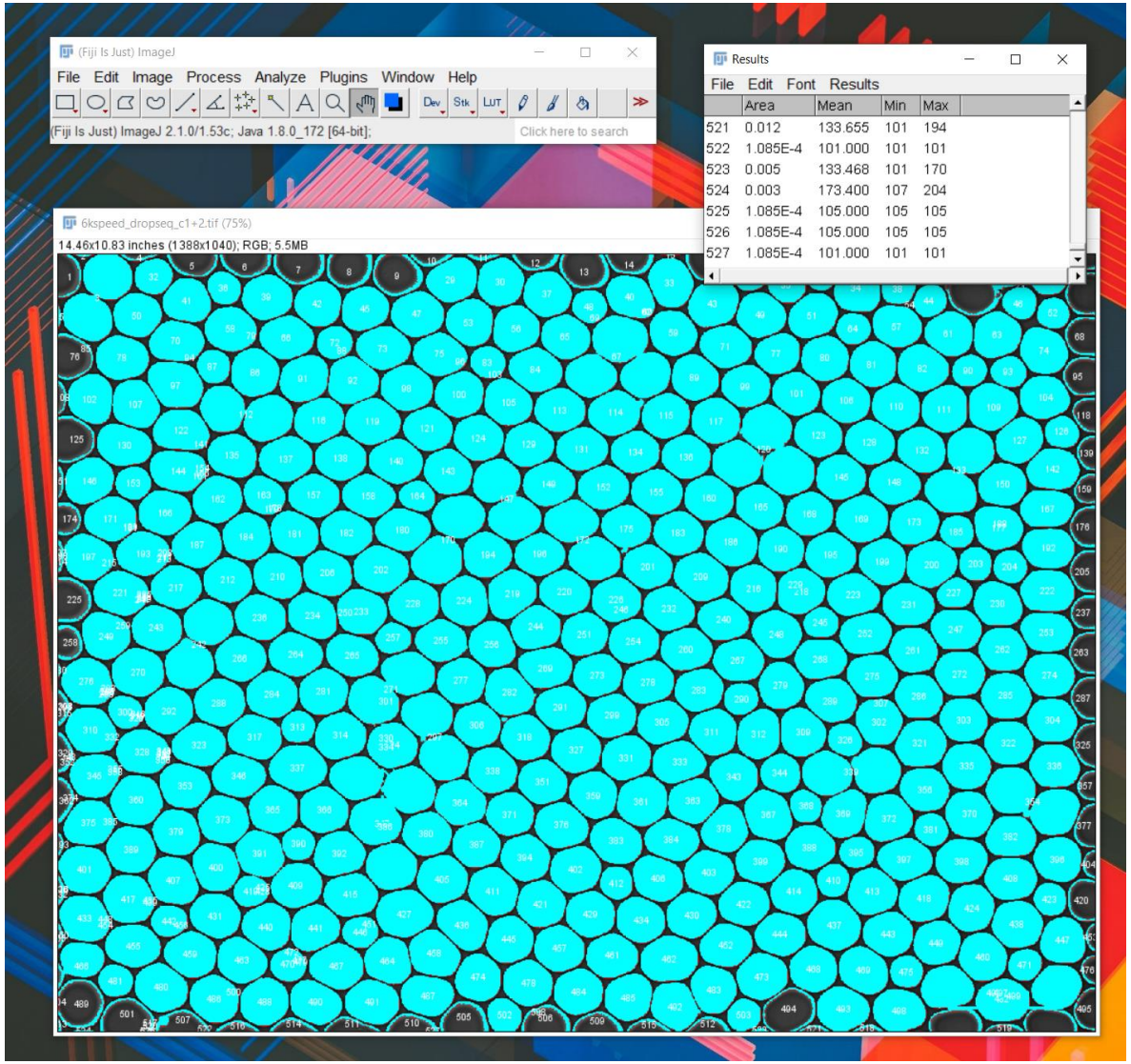
FIJI

- Automate counting features



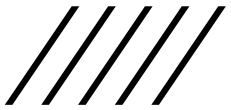
FIJI

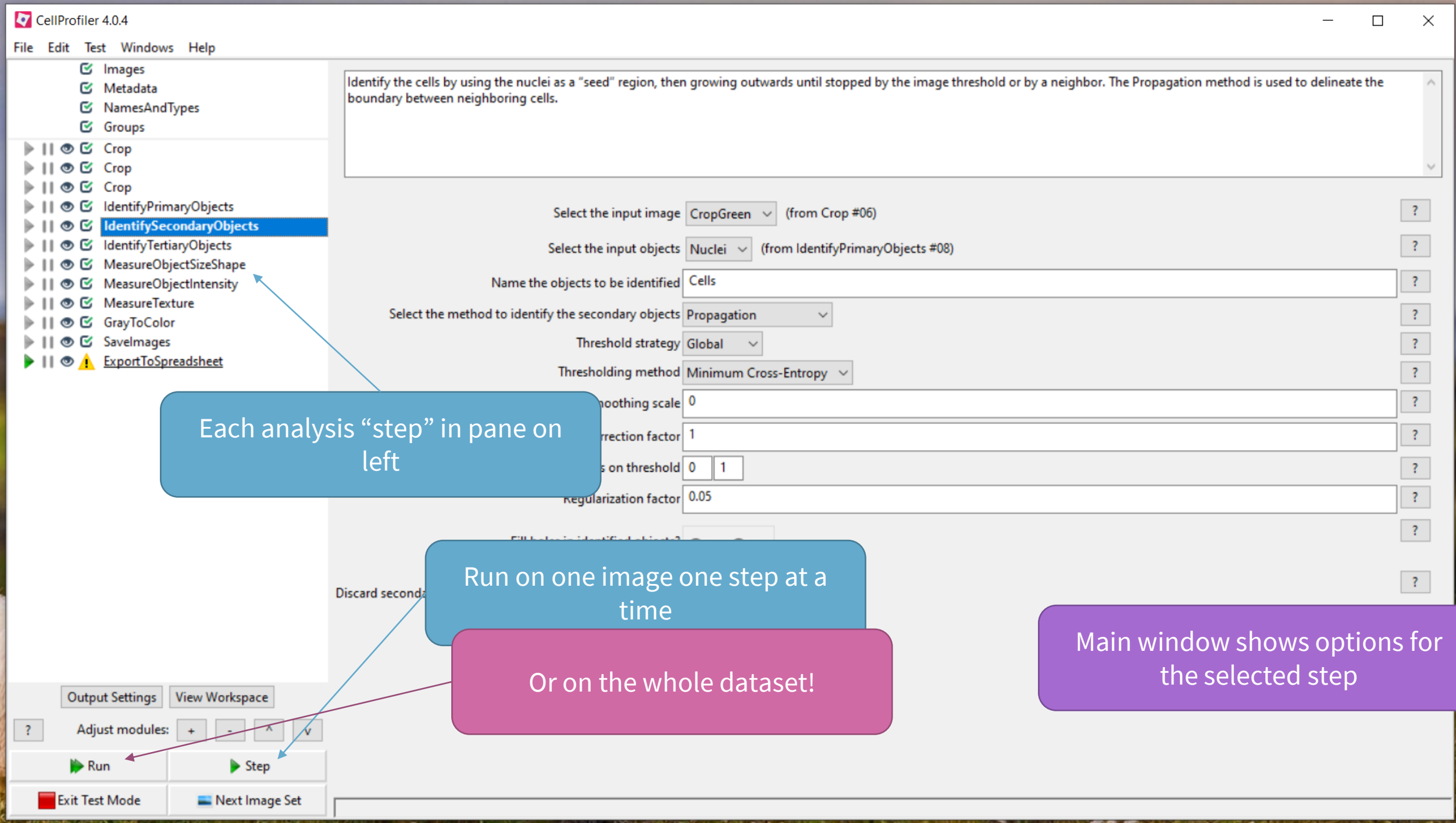
- Automate counting features
- You can also “record” yourself do an analysis and save it as a macro to apply to a whole folder of images!
- Export results into an excel file

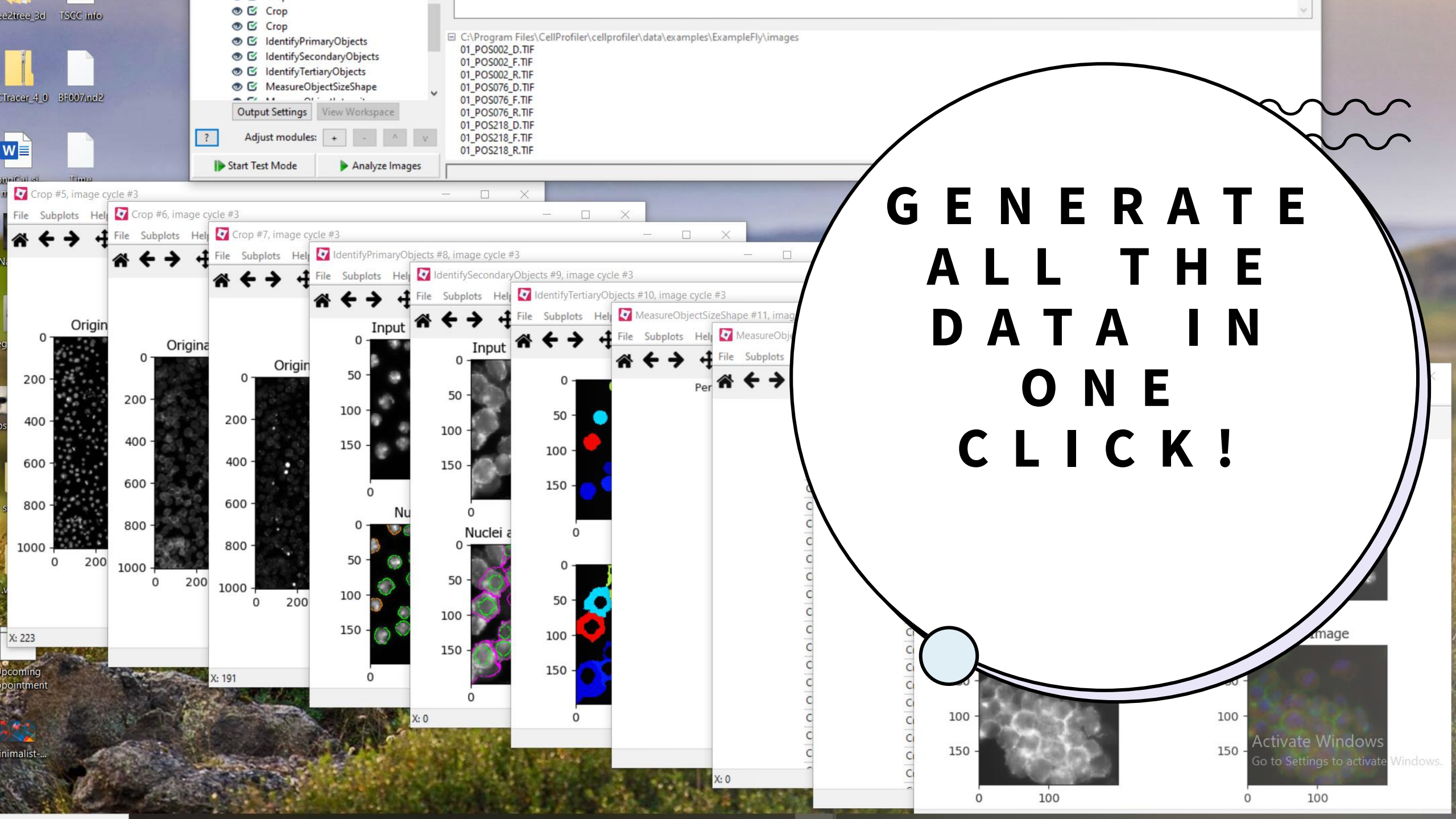


CellProfiler

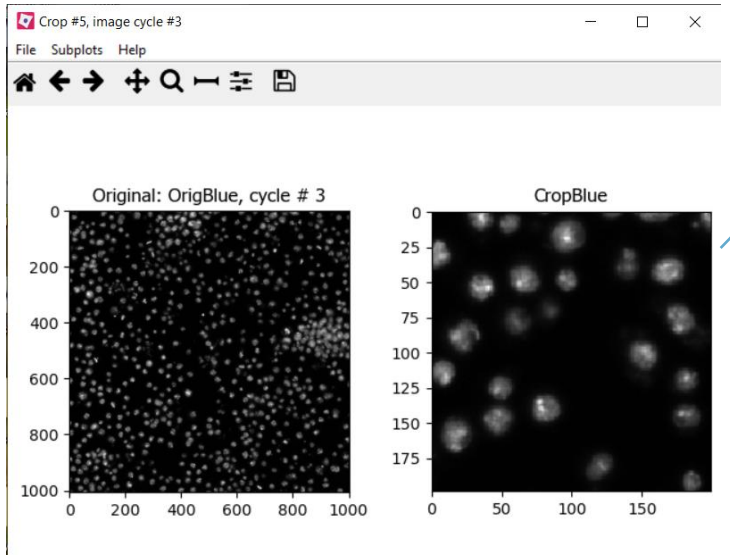
- An easy to use free software tool to build an analysis pipeline
- Apply the pipeline to thousands of images and export data in any format you want
- Hundreds of different types of analyses available from simple (co-localization) to advanced (deep learning)
- About as easy to use as FIJI but much more powerful

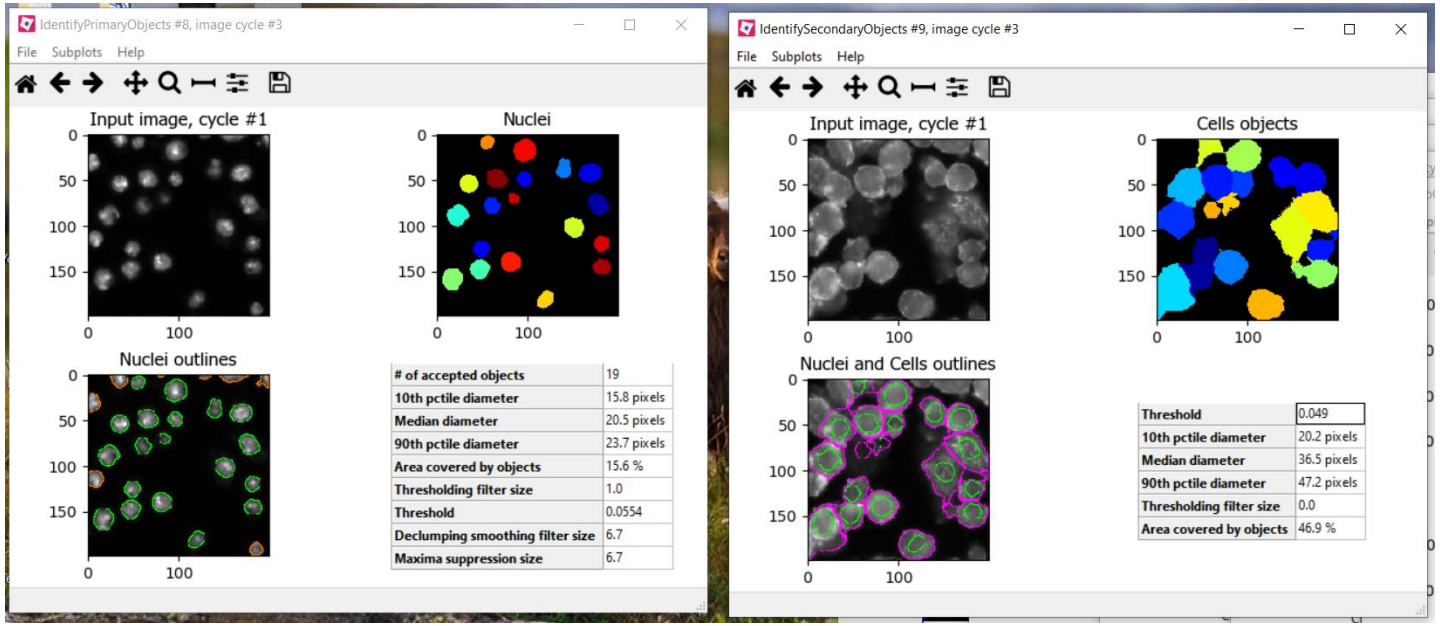



CellProfiler 4.0.4
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Crop
Crop
Crop
IdentifyPrimaryObjects
IdentifySecondaryObjects
IdentifyTertiaryObjects
MeasureObjectSizeShape
MeasureObjectIntensity
MeasureTexture
GrayToColor
SaveImages
ExportToSpreadsheet
Identify the cells by using the nuclei as a "seed" region, then growing outwards until stopped by the image threshold or by a neighbor. The Propagation method is used to delineate the boundary between neighboring cells.
Select the input image CropGreen (from Crop #06)
Select the input objects Nuclei (from IdentifyPrimaryObjects #08)
Name the objects to be identified Cells
Select the method to identify the secondary objects Propagation
Threshold strategy Global
Thresholding method Minimum Cross-Entropy
0
1
0 1
0.05
Discard seconda
Output Settings
View Workspace
Adjust modules:
Run
Step
Exit Test Mode
Next Image Set
Each analysis "step" in pane on left
Run on one image one step at a time
Or on the whole dataset!
Main window shows options for the selected step

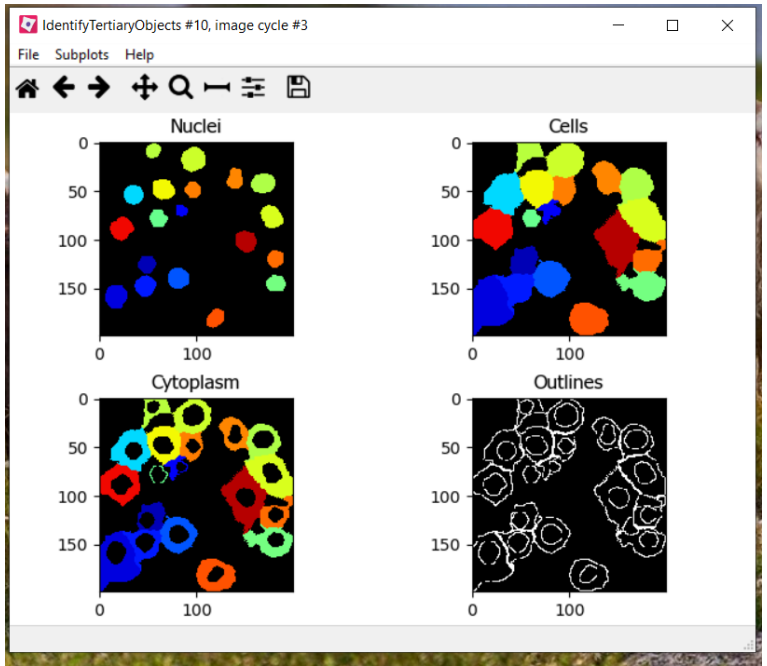

Crop
Crop
IdentifyPrimaryObjects
IdentifySecondaryObjects
IdentifyTertiaryObjects
MeasureObjectSizeShape
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
C:\Program Files\CellProfiler\cellprofiler\data\examples\ExampleFly\images
01_POS002_D.TIF
01_POS002_F.TIF
01_POS002_R.TIF
01_POS076_D.TIF
01_POS076_F.TIF
01_POS076_R.TIF
01_POS218_D.TIF
01_POS218_F.TIF
01_POS218_R.TIF
Crop #5, image cycle #3
Crop #6, image cycle #3
Crop #7, image cycle #3
IdentifyPrimaryObjects #8, image cycle #3
IdentifySecondaryObjects #9, image cycle #3
IdentifyTertiaryObjects #10, image cycle #3
MeasureObjectSizeShape #11, imag
File
Subplots
Input
Nuclei a
GENERATE ALL THE DATA IN ONE CLICK!
Activate Windows
Go to Settings to activate Windows.

1. Original Green and Blue channel images



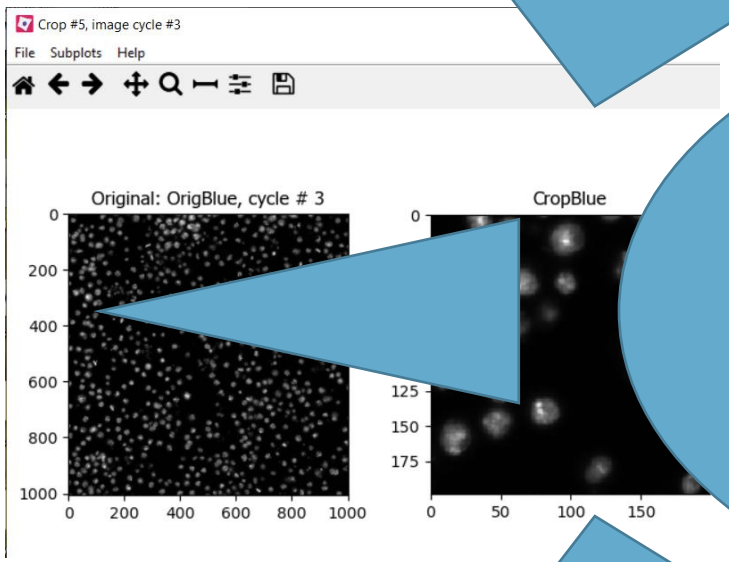


2. Segment nuclei and cells

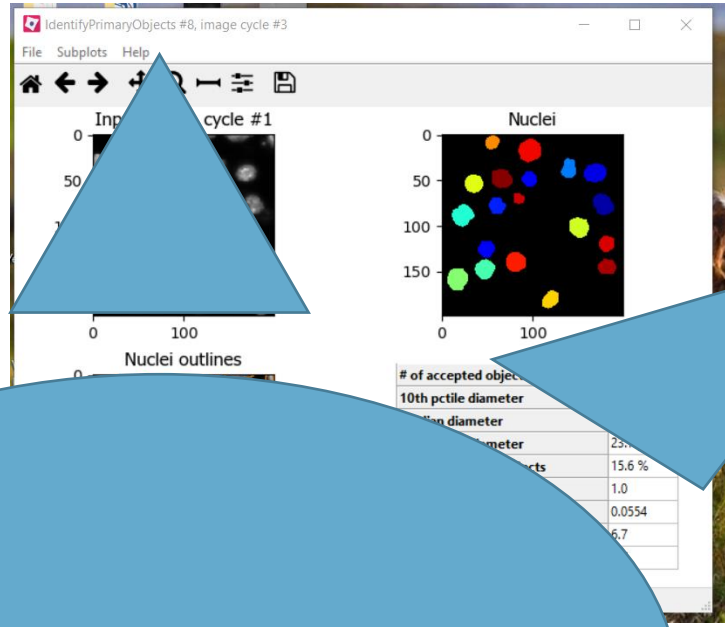


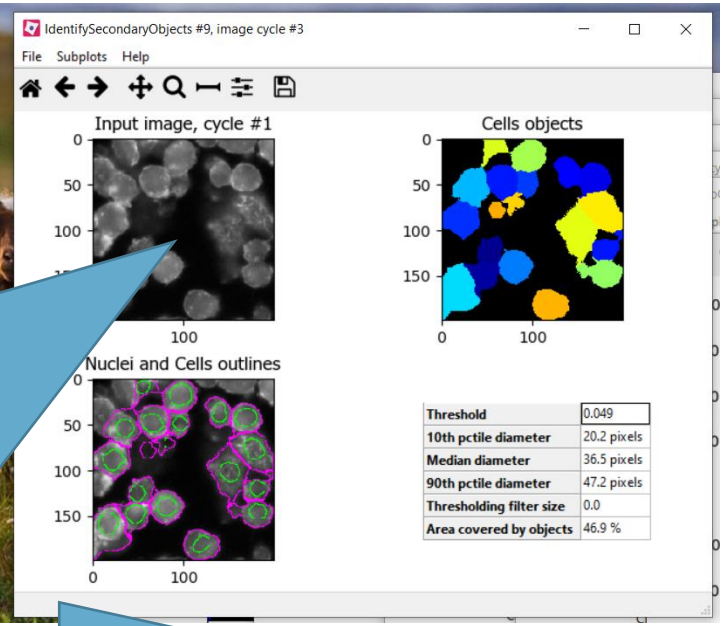
3. Subtract and get Cytoplasm area and cell outlines!

1. Original Green ar… channel images

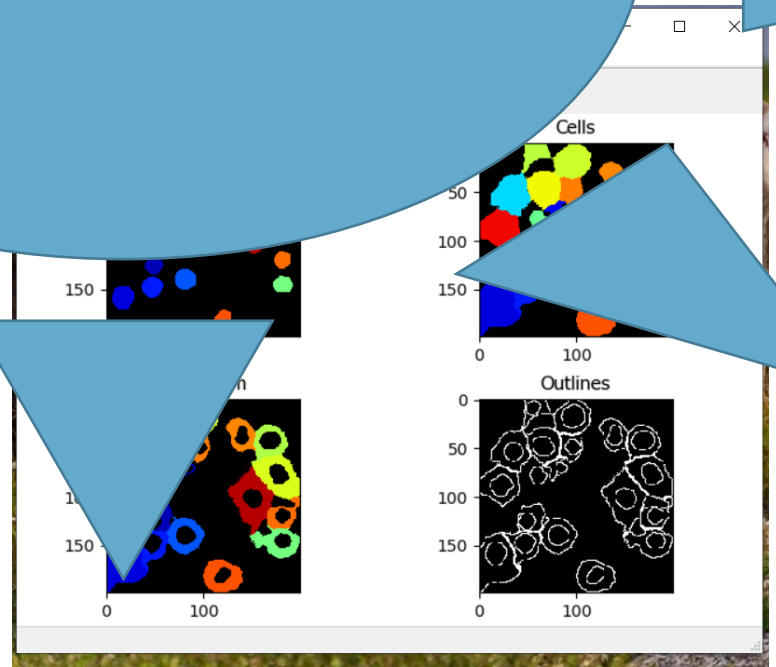


And not a line of code was needed!





…ment nuclei and …cells



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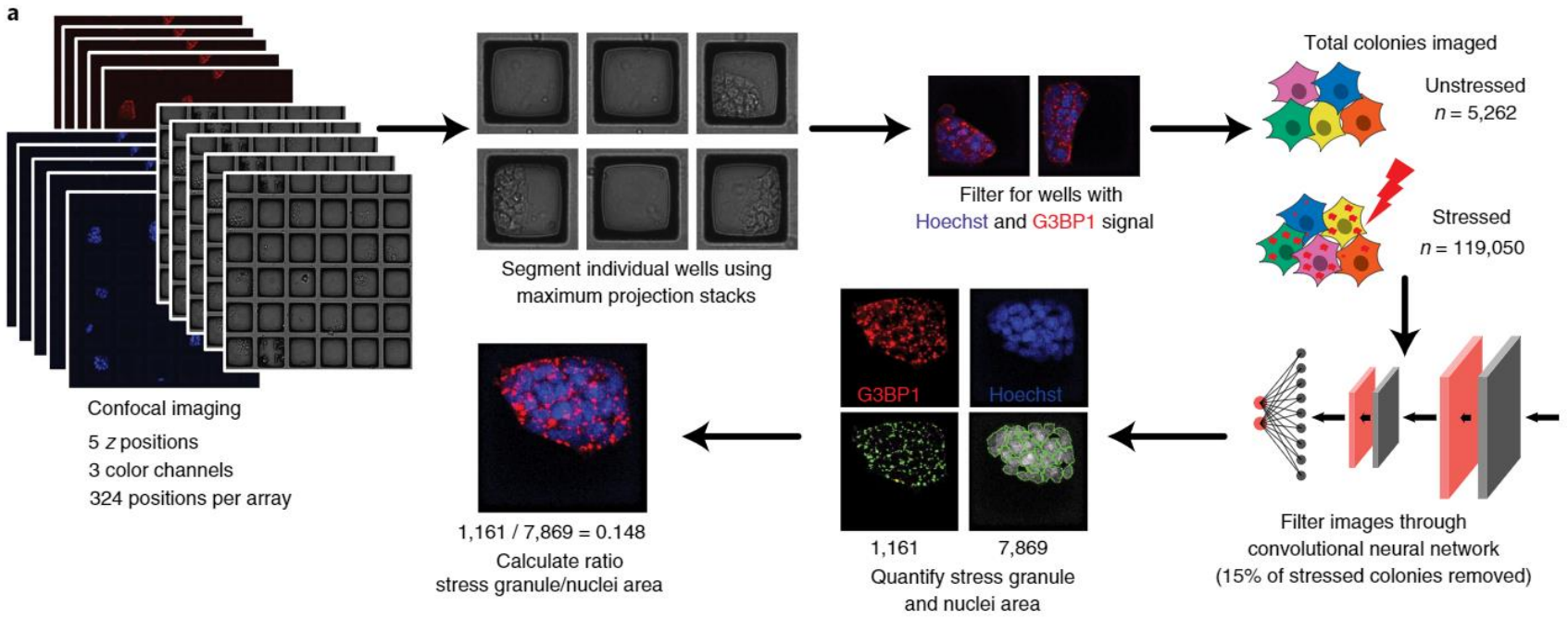
CellProfiler – A case study

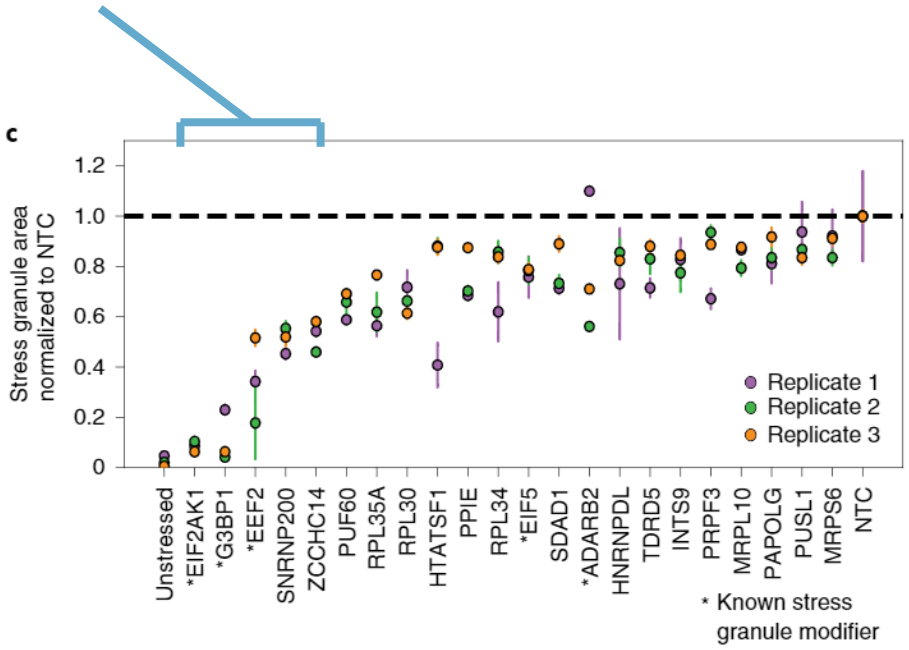


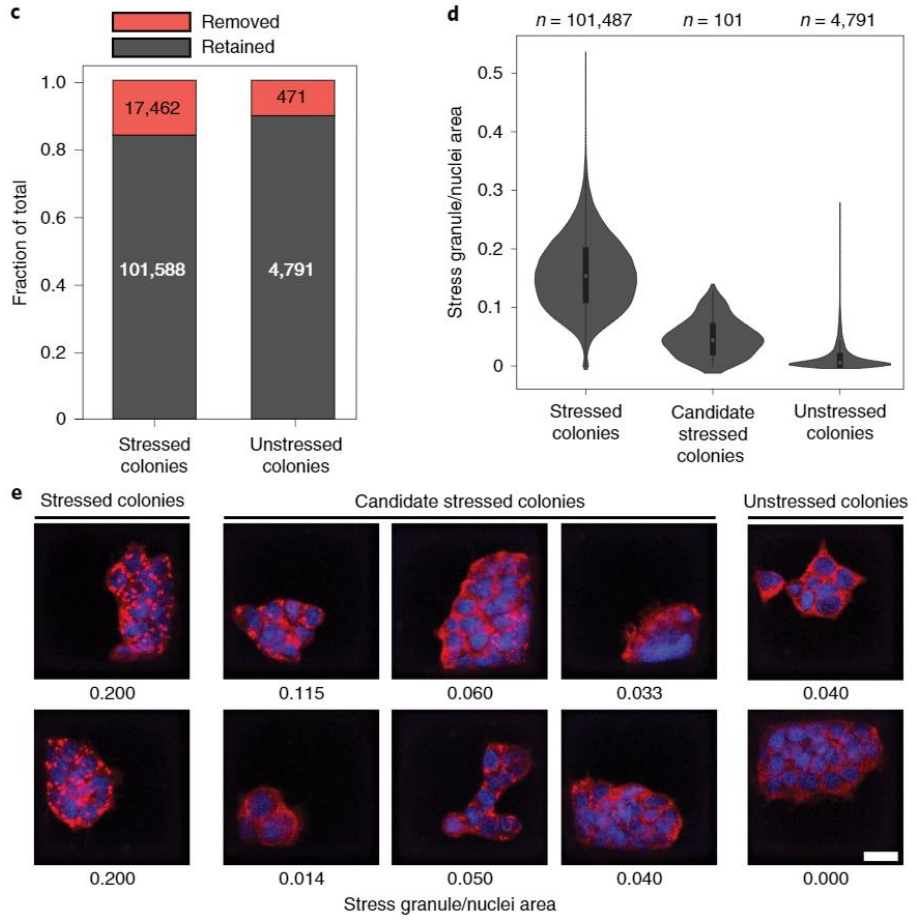
- 100k+ images
- Segment each well
- Quality control the image
- Stress granule area (G3BP1) / DAPI area ratio
- Look for wells with cells with very low ratio
- Sequence cells to find gene knockdown

Wheeler, E. C. *et al*. *Nat Methods* 1–7 (2020)

CellProfiler – A case study

CellProfiler can help find the needle in the haystack!



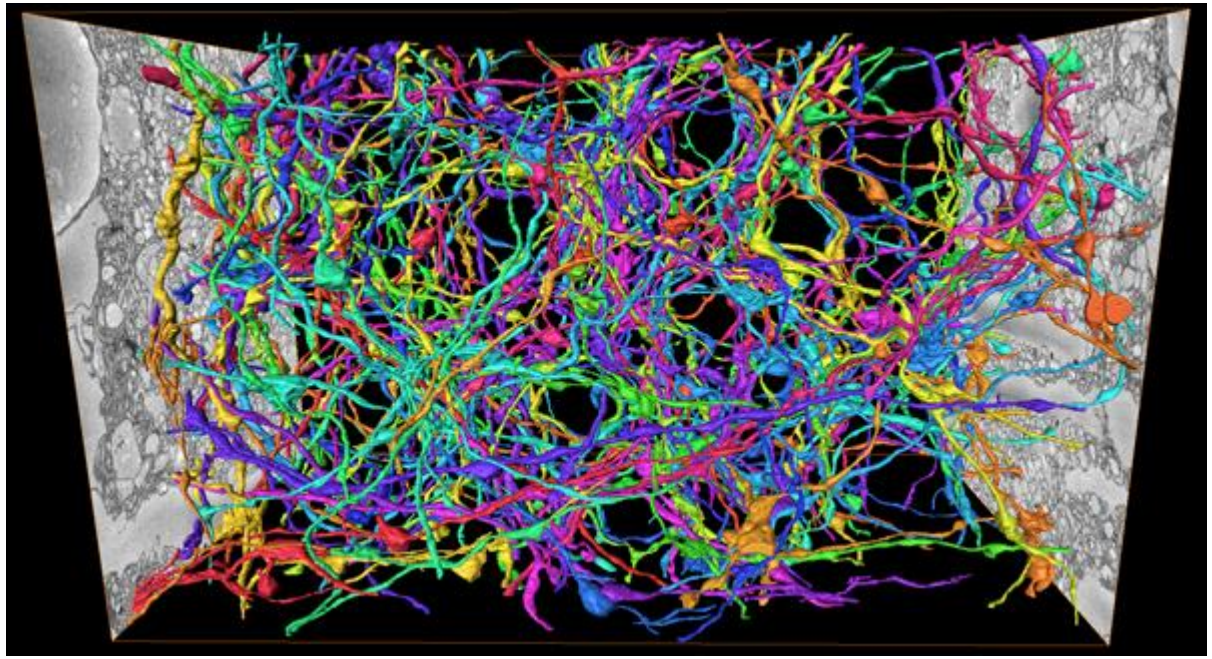


Wheeler, E. C. *et al*. *Nat Methods* 1–7 (2020)

Image Analysis wizardry in Python

- Highly custom pipelines using libraries used across disciplines for image analysis
  - Scikit-image
  - OpenCV
  - Numpy (because images are just matrices after all…)
  - PyTorch/Tensorflow (for machine learning)
- Apply analysis on terabytes of data on supercomputing cluster (TSCC, AWS, etc.)

What is image analysis with code used for?



- Connectomics reconstruction (neurons)

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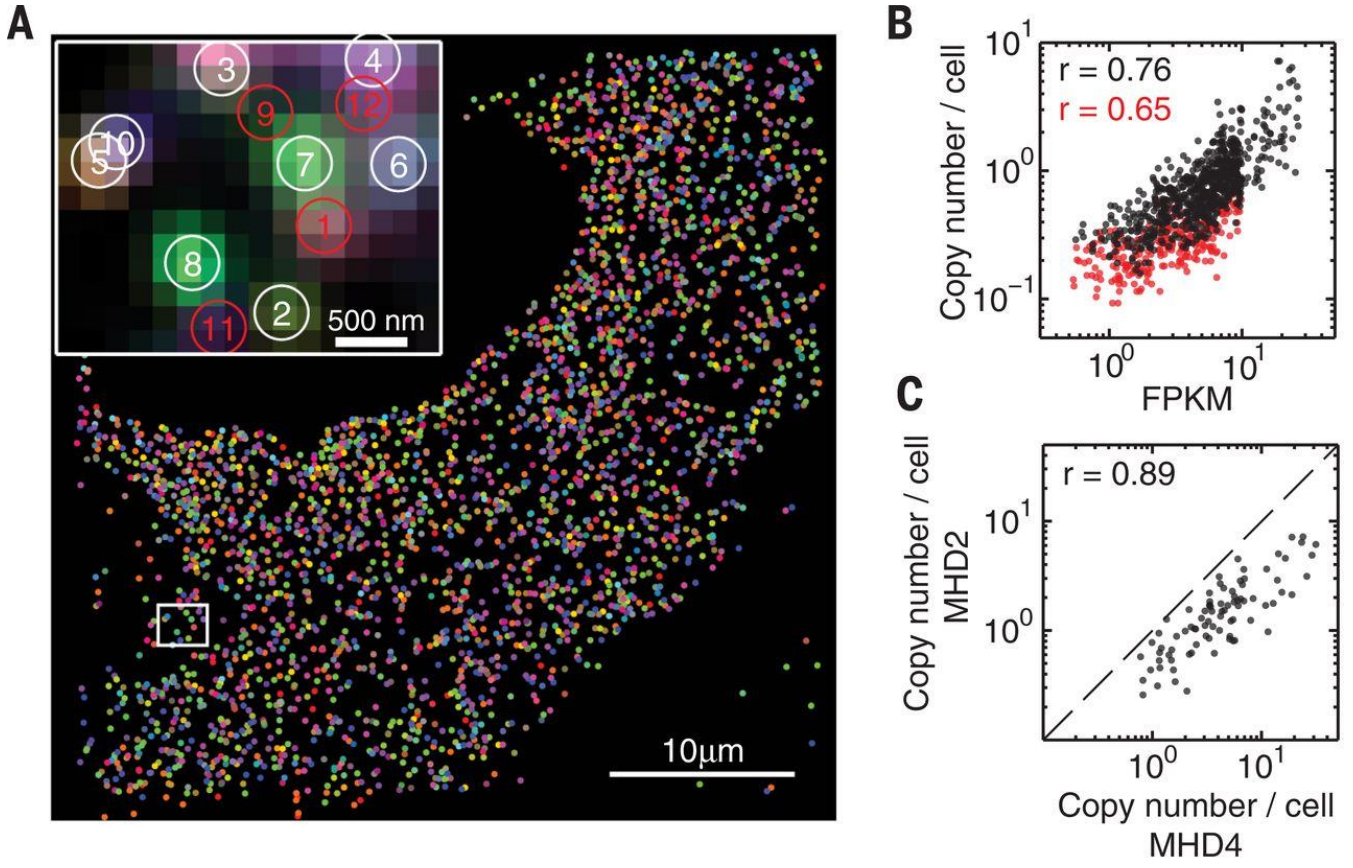

- Connectomics reconstruction (neurons)
- Single molecule detection

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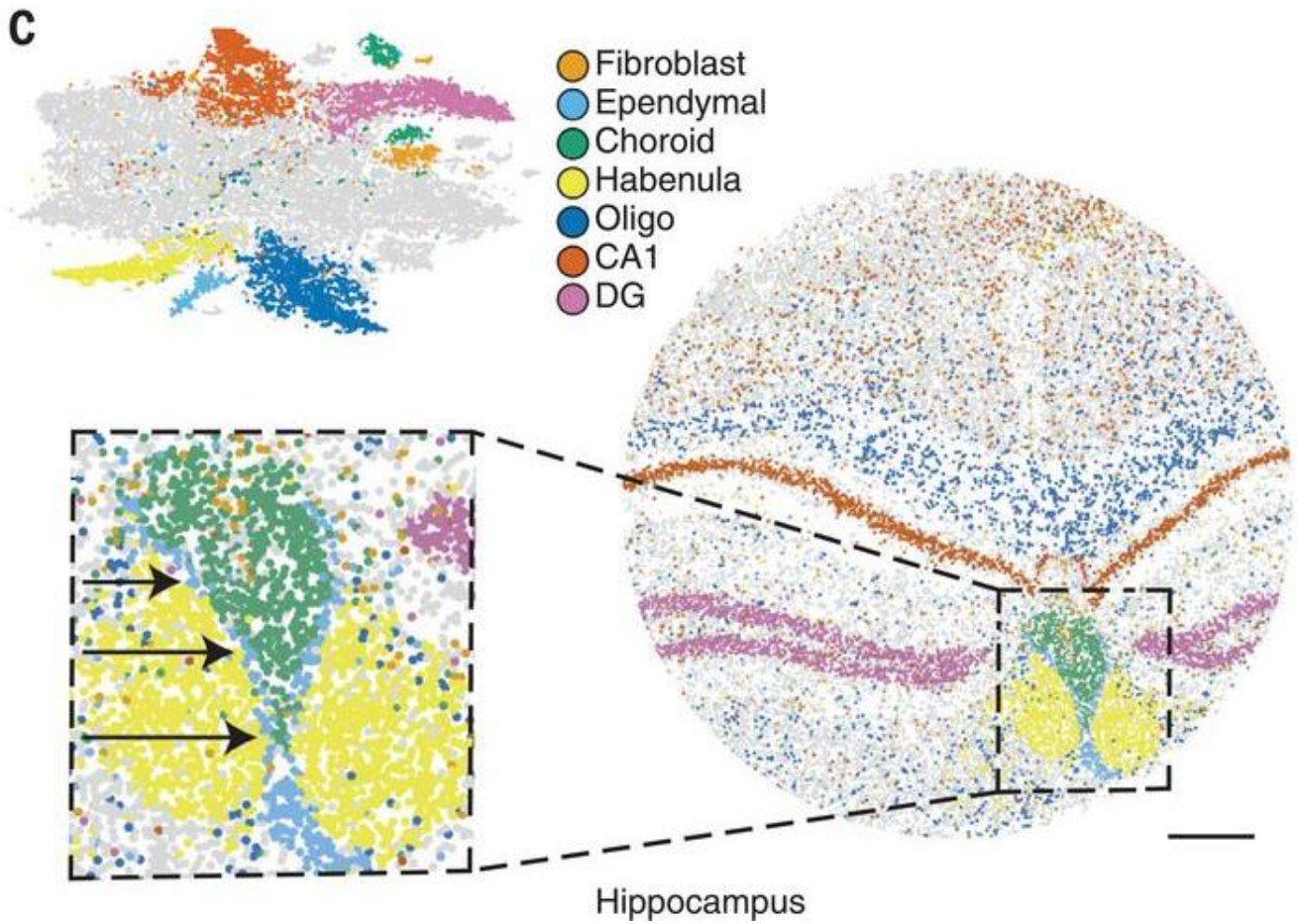
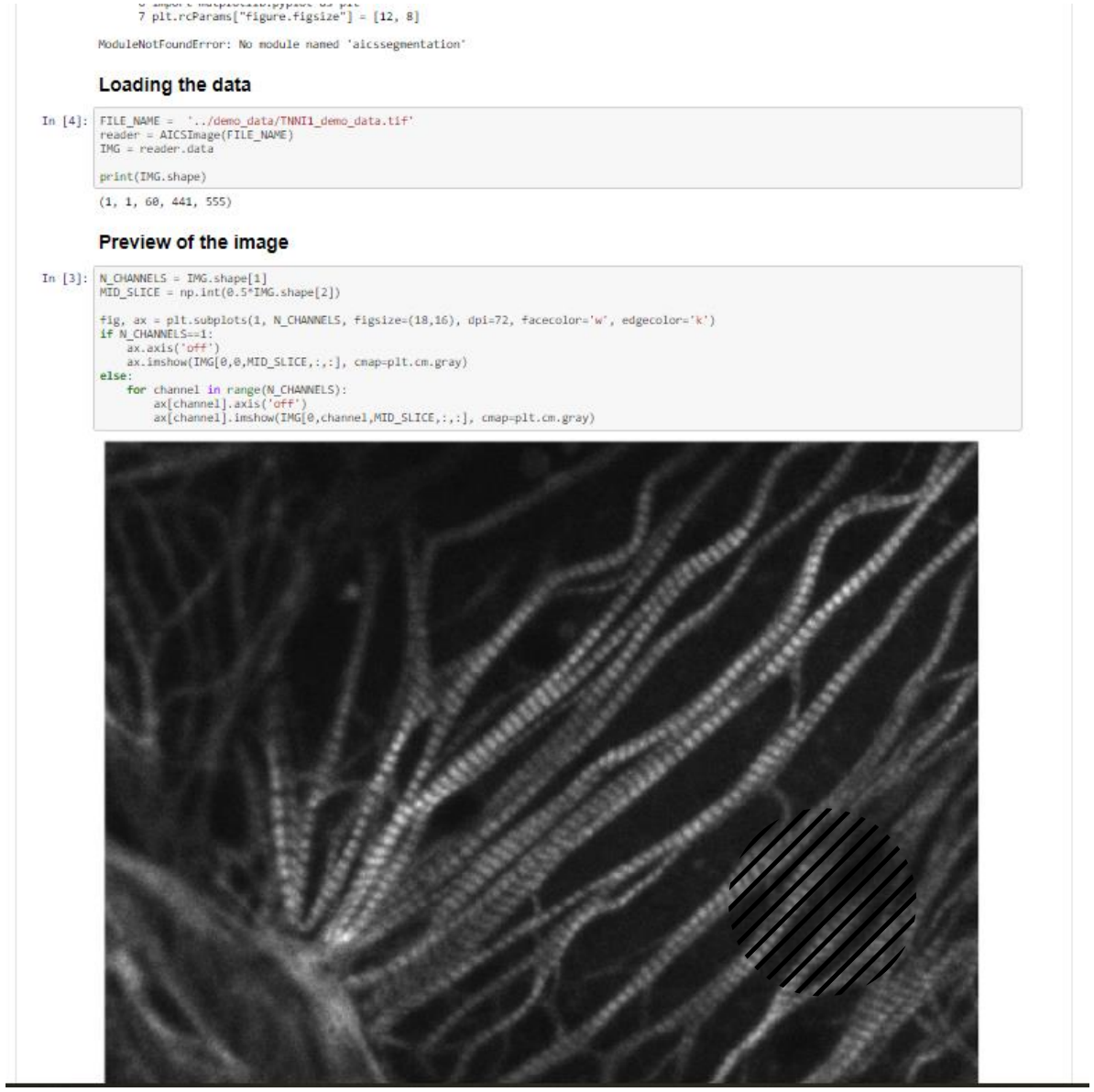


- Connectomics reconstruction (neurons)
- Single molecule detection
- Combine single-cell sequencing with imaging

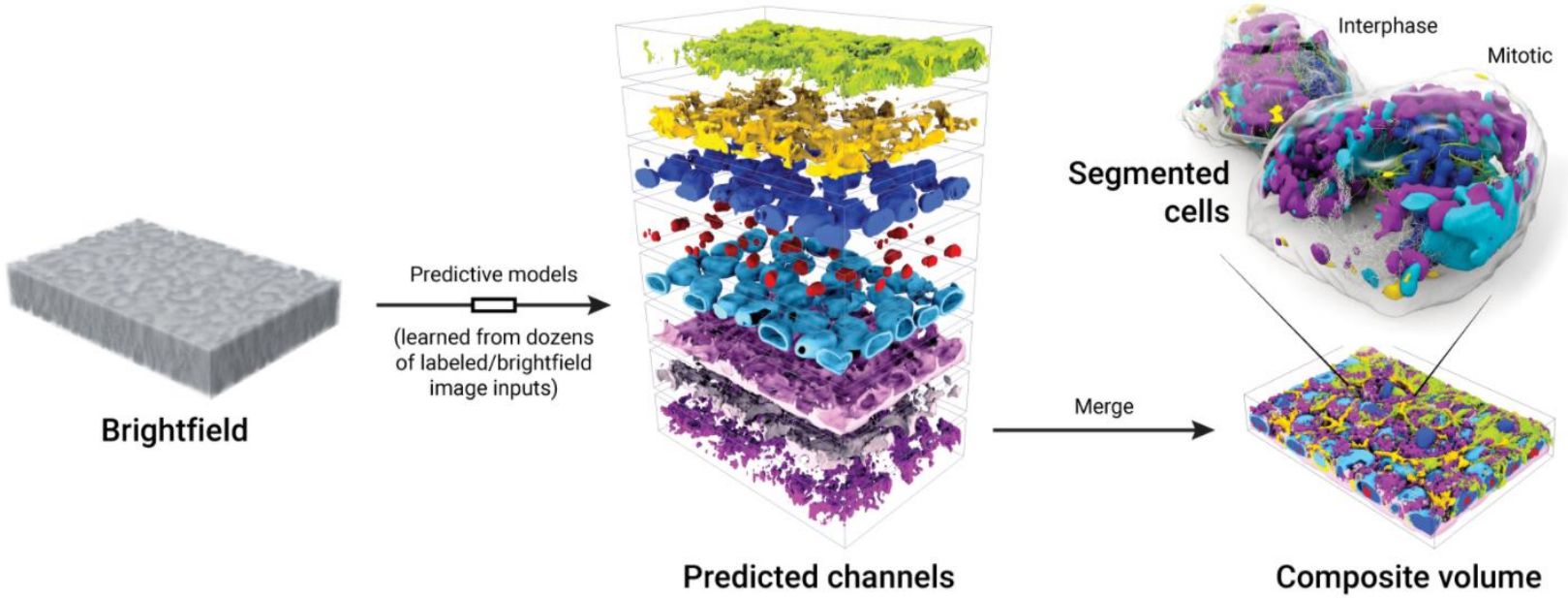
AICS Segmenter

- Has very easy to use functions to segment features in confocal images
- Run inside of a Jupyter Notebook
- Nifty functions to visualize in 3D
- https://www.allencell.org/3d-cell-viewer.html



Label-free “staining”

- Classically: limited in number of stains you can image
- Now: Image brightfield → predict all the other stains
- https://www.allencell.org/label-free-determination.html



Summary

- For your own sake, please don’t do image analysis by hand
- You can do really simple analyses with almost no learning curve (FIJI)
- You can do really advanced high-throughput analysis with no coding! (CellProfiler)
- Using python for image analysis, you can defy the limitations imposed by the laws of physics!!