A picture is worth 1000 hypotheses: using computers to analyze biological images

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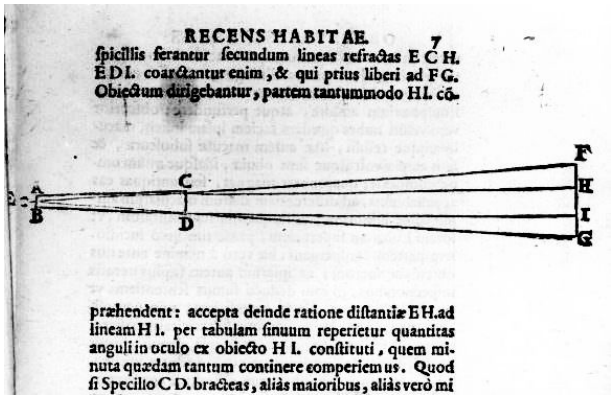
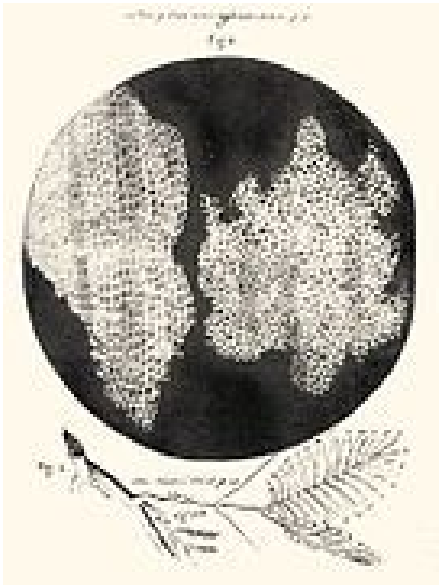
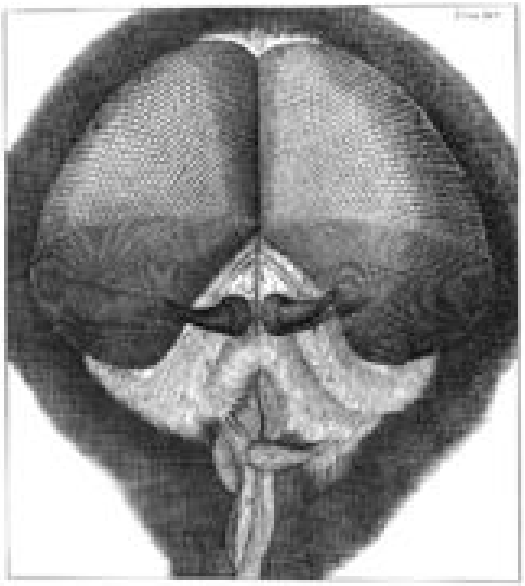
The origins of the microscope

- A microscope sees objects that are too small to see by the human eye.
- Optical microscopes were invented almost simultaneously with the telescope.
  - Galileo invented versions of both circa 1600 shortly after the original was developed in the Netherlands
  - The original compound microscopes can be described as “two lenses in a tube”



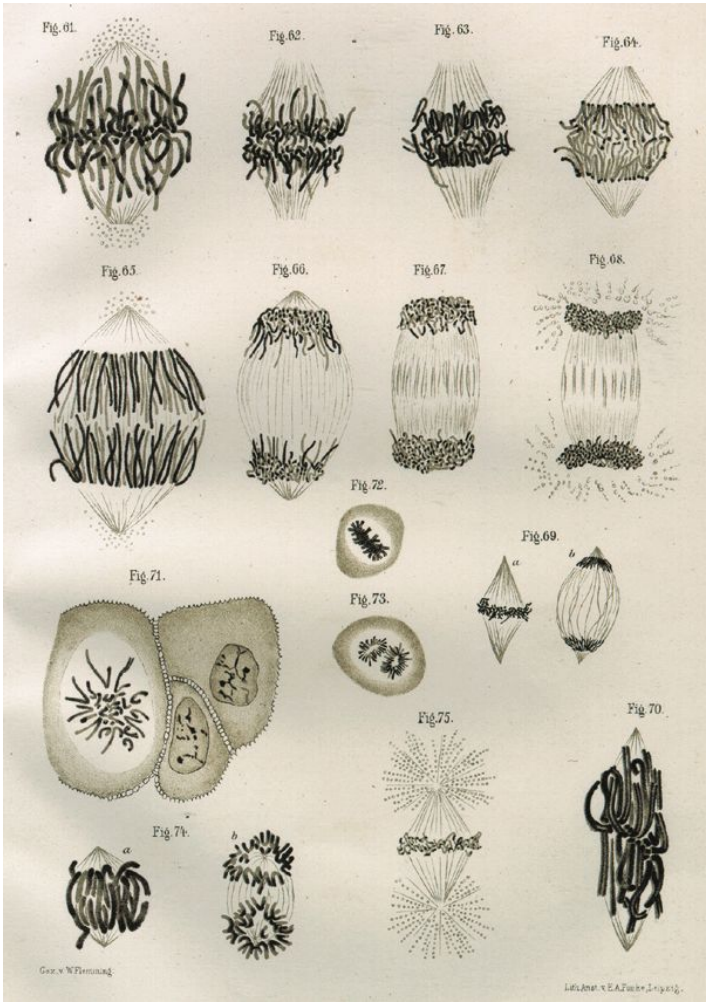
“Images” were originally hand drawings

- Robert Hooke’s Micrographia is a landmark publication documenting what microscopy reveals about the natural world (1665)
- Coined the word “cell” to describe cork

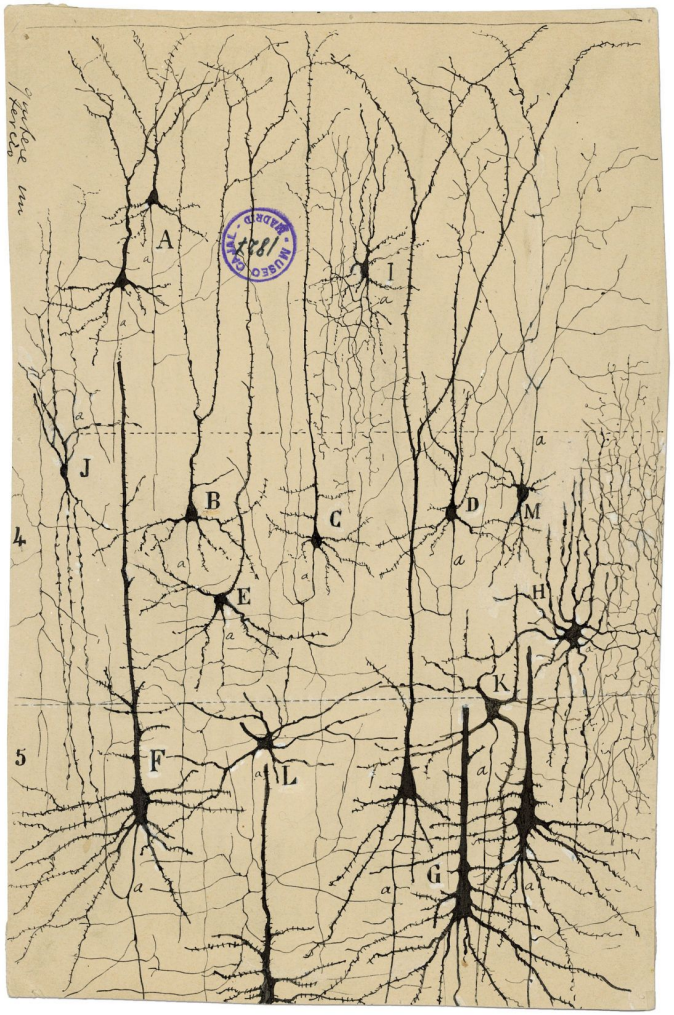

“Cell Theory” was driven by microscopy.

- Cell Theory states that all living organisms are made up of cells.
  - Introducted by Matthia Schleiden after observing hundreds of plants.
- Walther Flemming’s observations in 1882 of mitosis revealed details about the mechanics of how cells divide.
  - Key to explaining how organisms made of cells can reproduce.



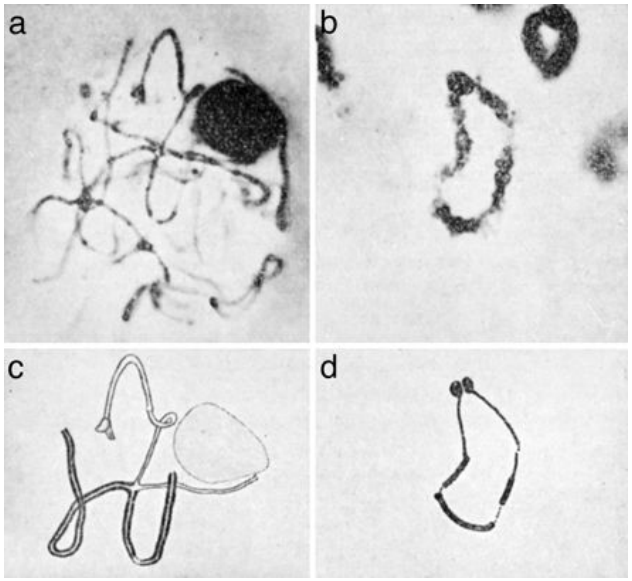
Many discoveries were made by treating biology samples with chemicals

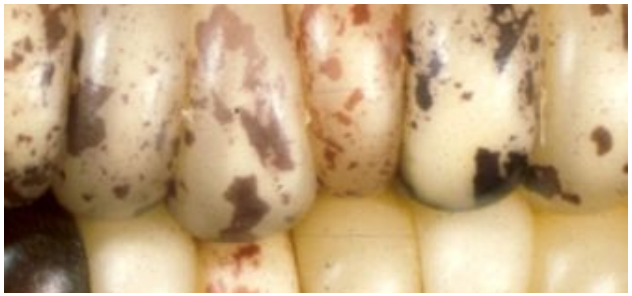
- Innovations in staining biological samples revealed previously unseen aspects of biology
- In 1906, Santiago Cajal won the Nobel Prize for his work investigating neurons using a silver based stain



Microscopy contributes to many fields of biology

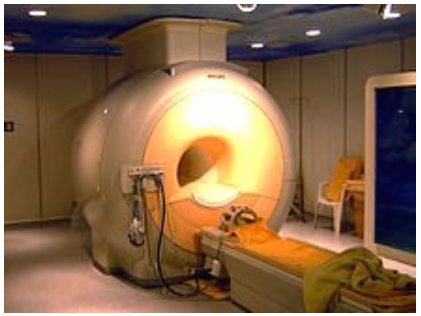
- Barbara McClintock received the Noble Prize in 1983 for her discovery of mobile genetic elements.
  - This discovery gave insight into why spots appear in certain corn species. It is a genetic mechanism also responsible for the dysregulation of genes in cancer.

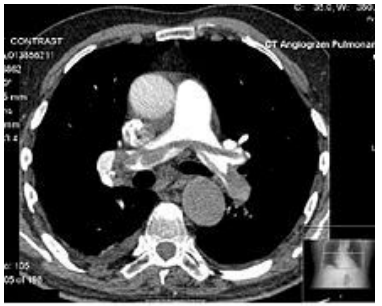


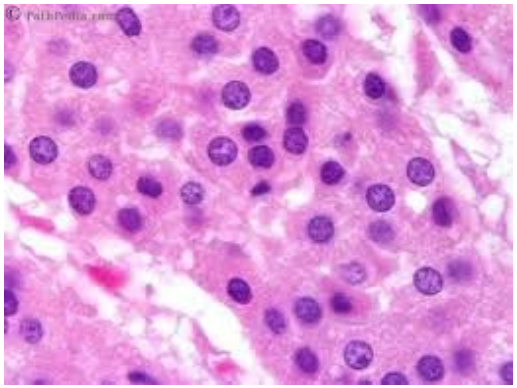


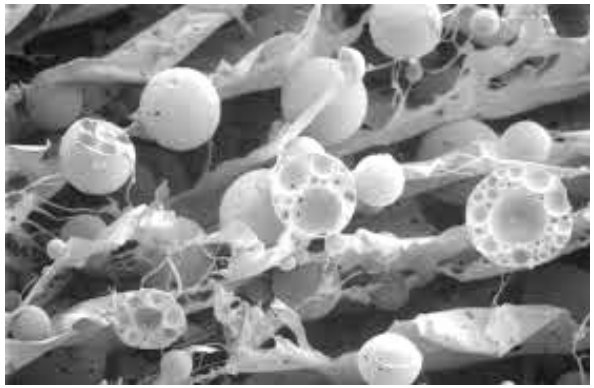
The computer revolution led to the diversification of microscopy and images

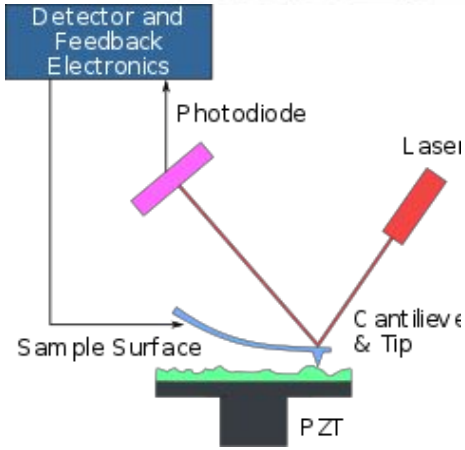
- Images come from many different sources and modalities
  - MRI and CT Scans
  - Atomic Force Microscopy
  - Electron Microscopy
  - *Epifluorescence and Light Microscopy*





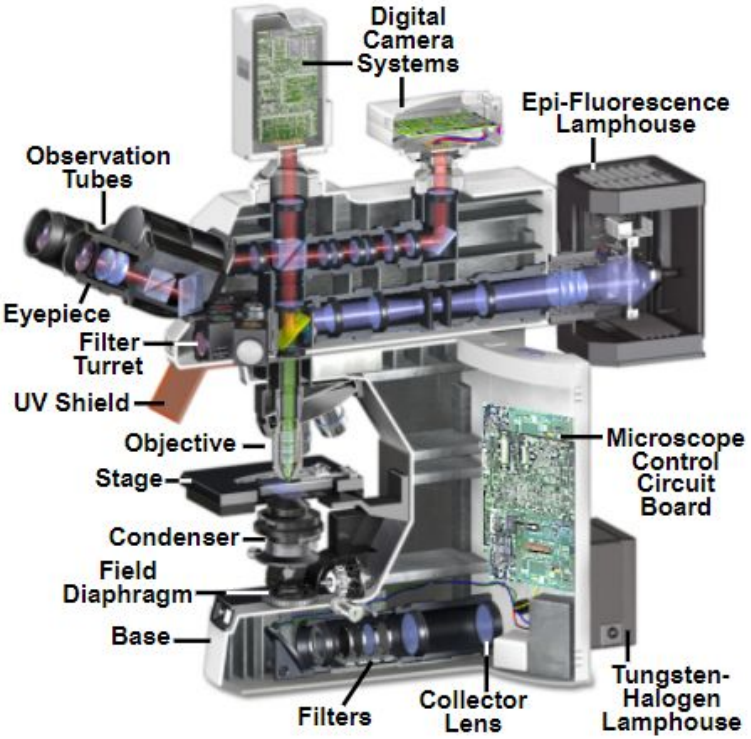


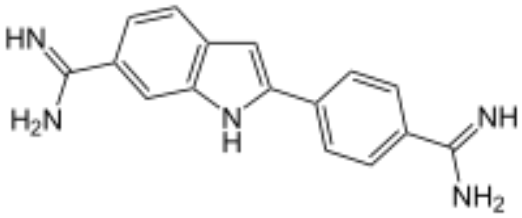


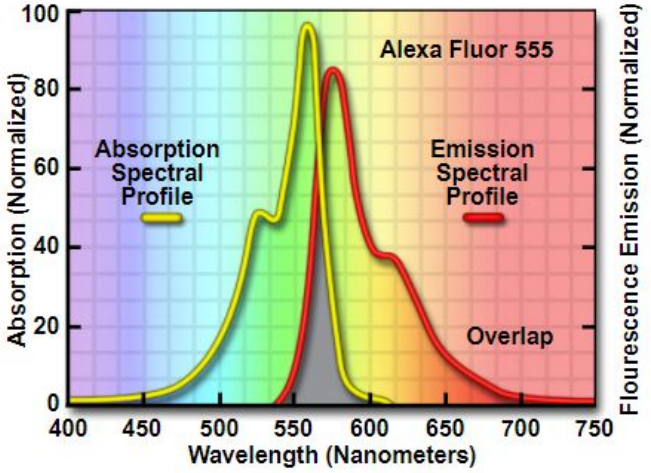


Fluorescence microscopy is essential to modern biology research

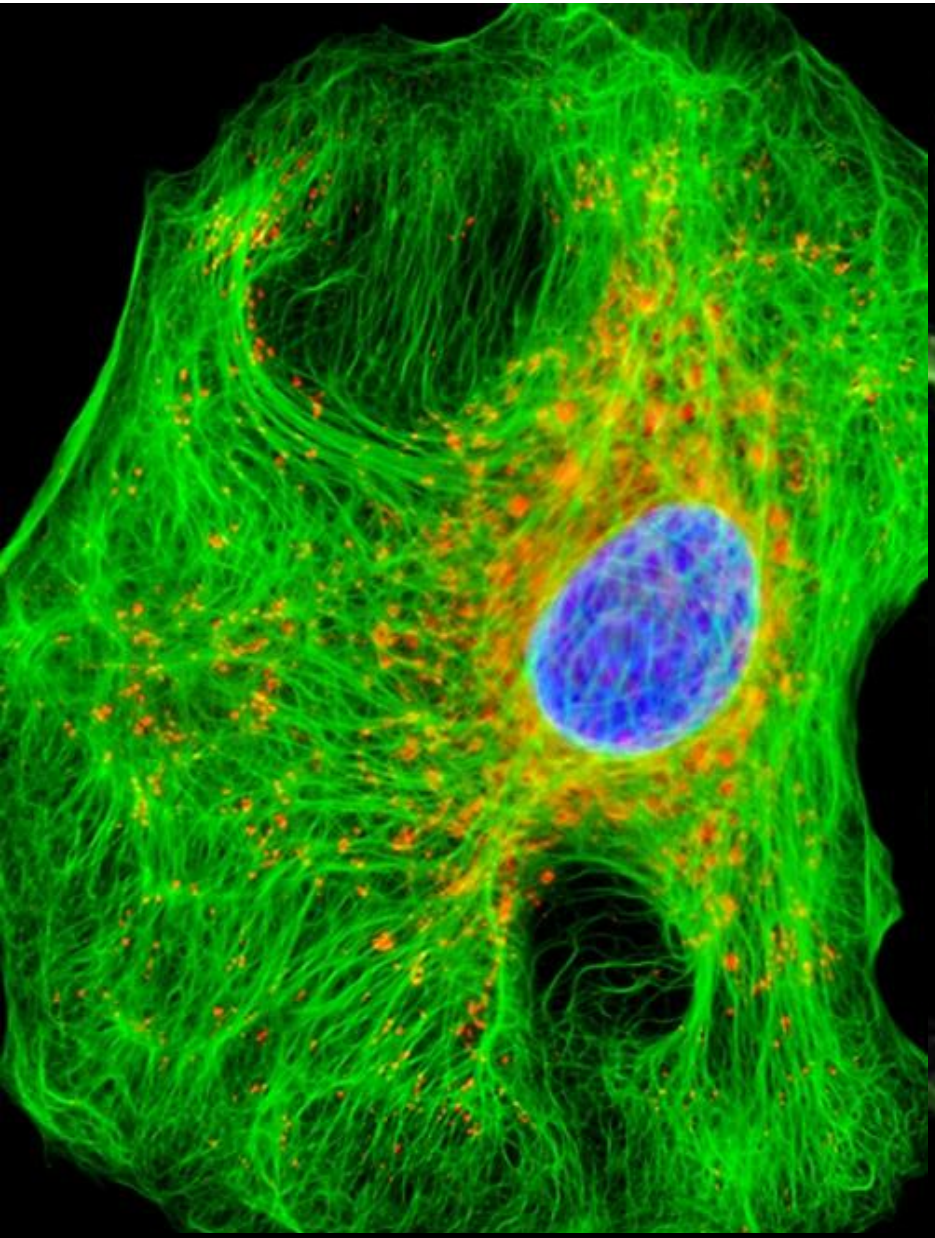
- Microscopes today are a sophisticated combination of lenses, mirrors, and electronics.
- Fluorescence: when a fluorescent compound is excited by light, it then emits light at a slightly longer wavelength after it “relaxes”.
- Fluorescent antibodies, protein-tags, and DNA oligos illuminate details within cells with great specificity.







Microscopes produce images that contain a wealth of information



http://www.microscopyu.com

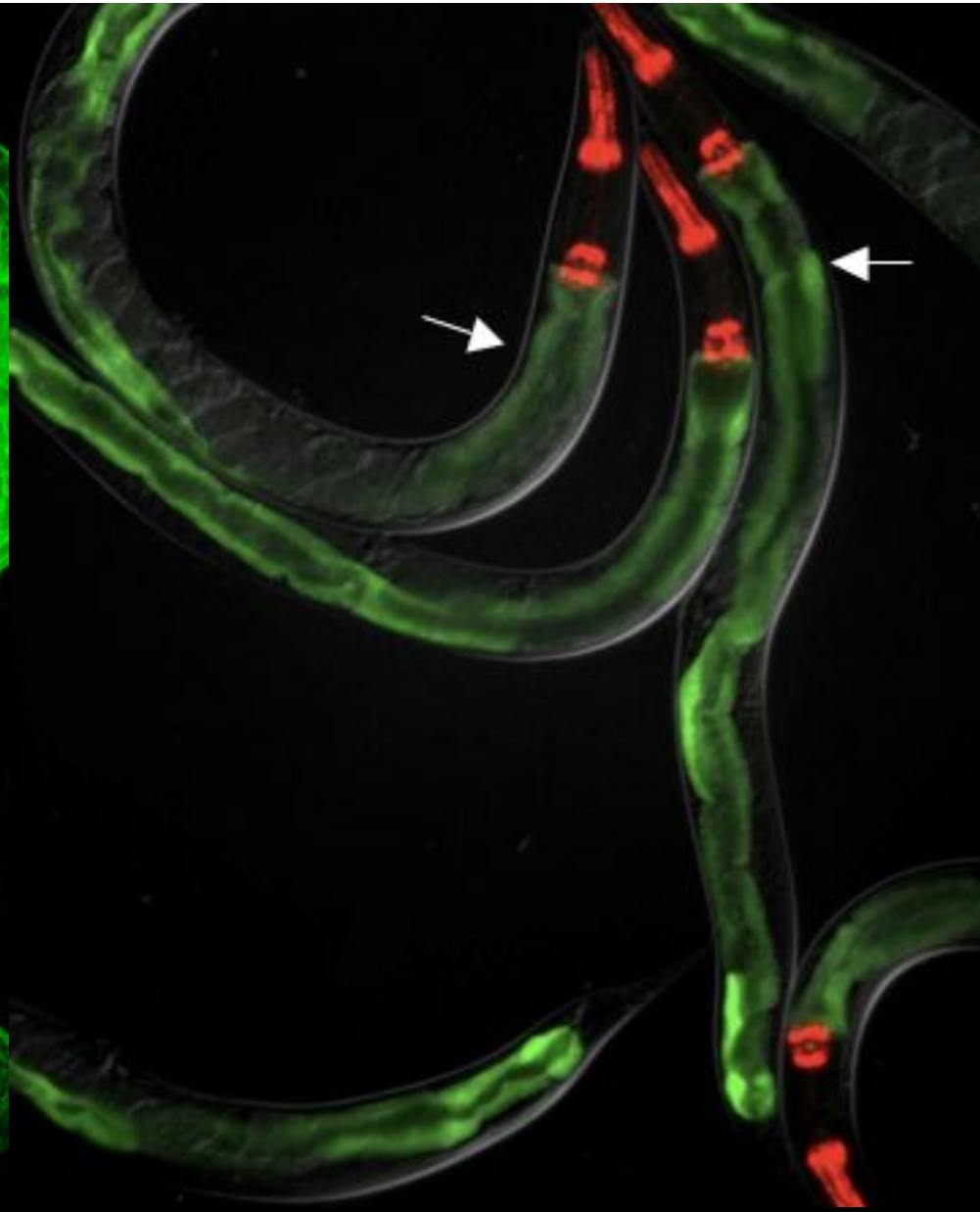
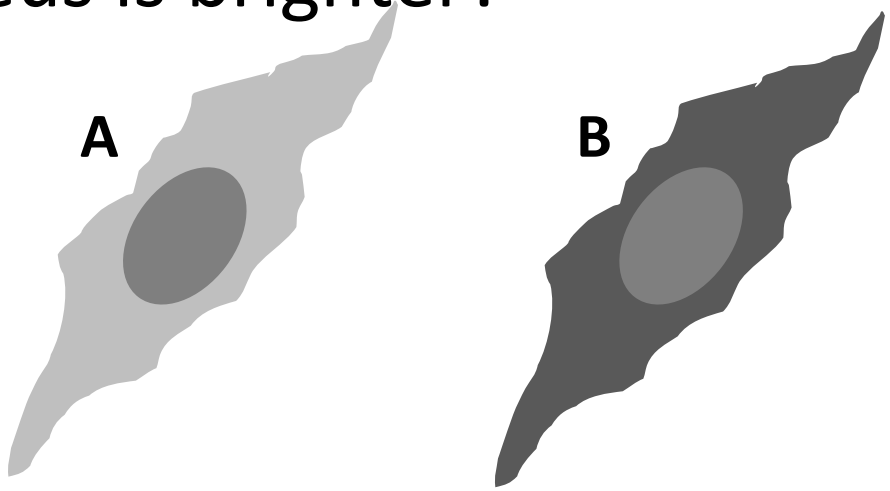


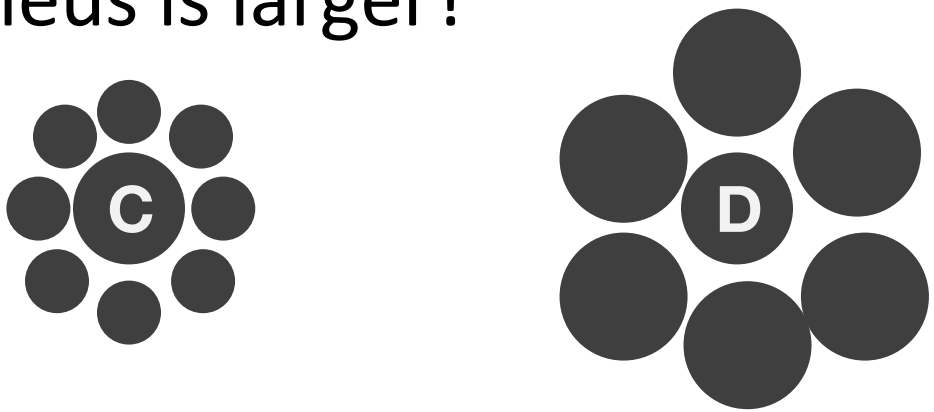
Image: Javier Irazoqui

Quantifying images makes analysis reproducible, automated, and statistical

Which nucleus is brighter?



Which nucleus is larger?



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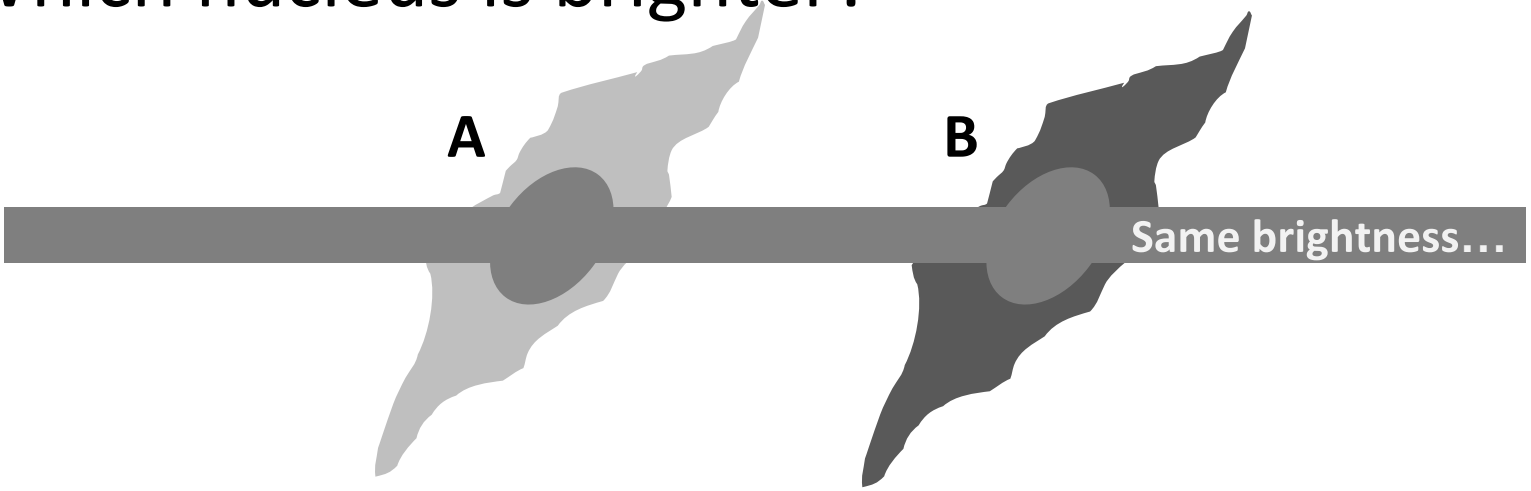
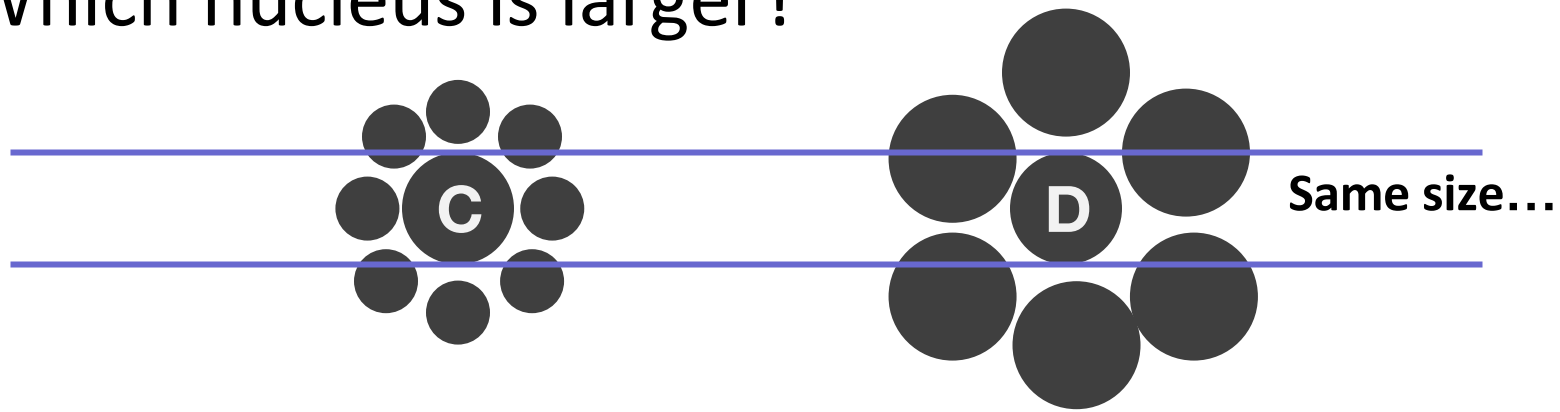
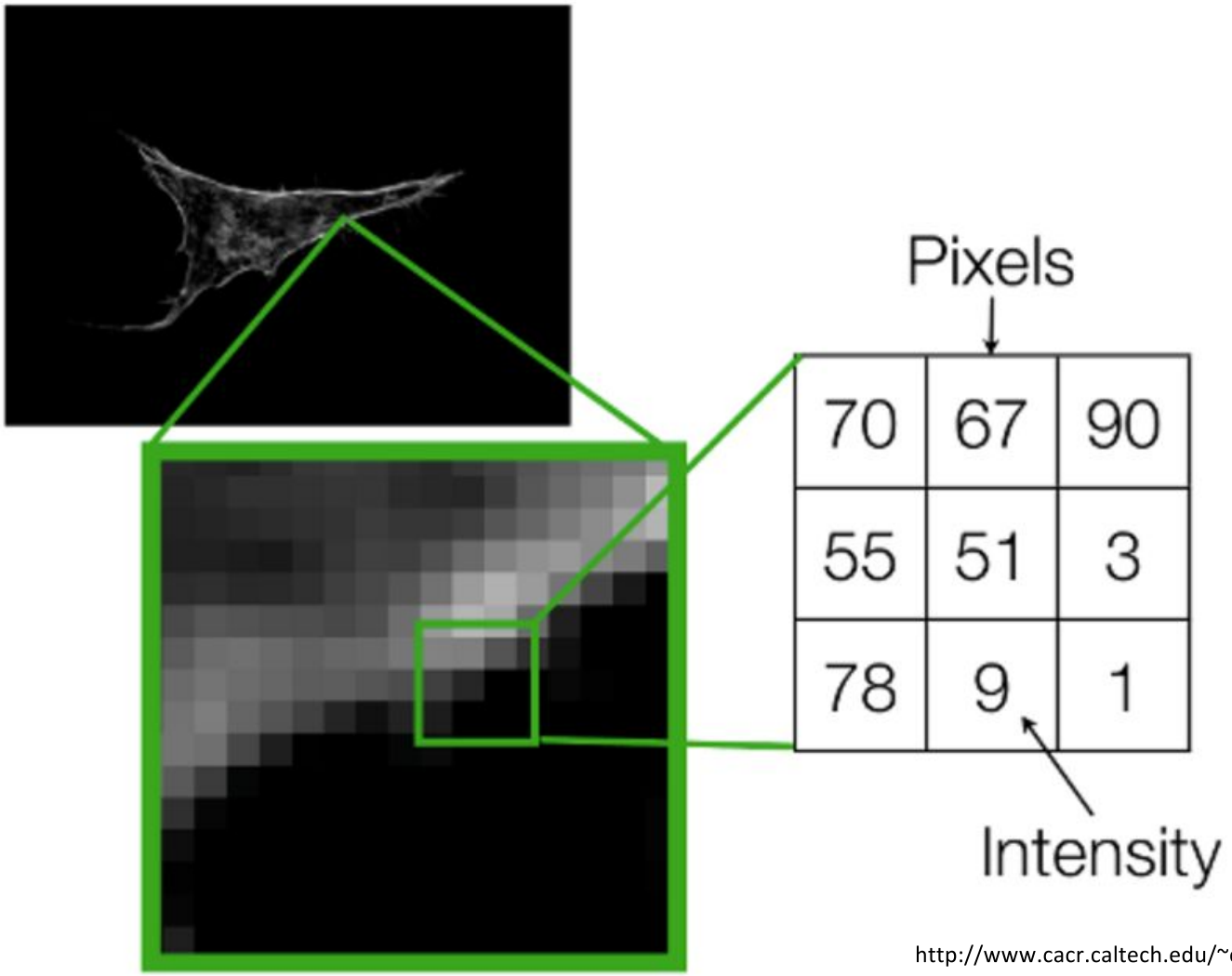


Which nucleus is larger?



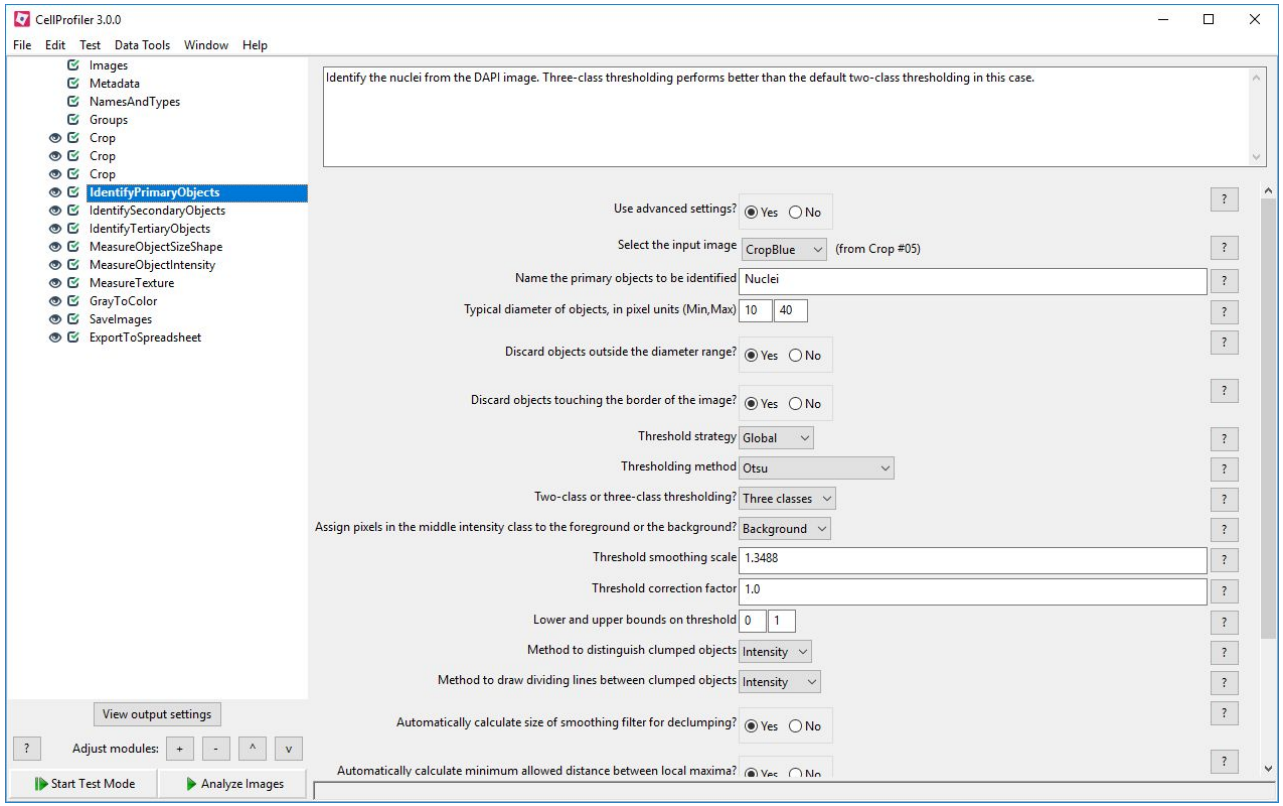
Images are numbers



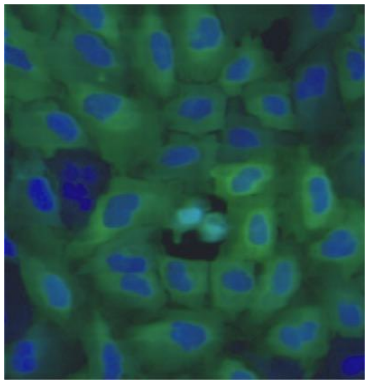
http://www.cacr.caltech.edu/~cunha/bi199/one.html

CellProfiler is a software for analyzing biological images

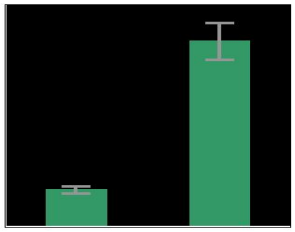
- User friendly interface provides powerful image analysis methods to non-programmers



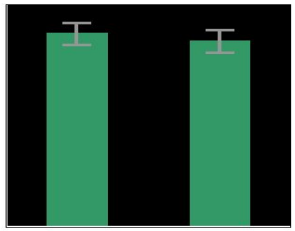
Visual appearance can be quantified



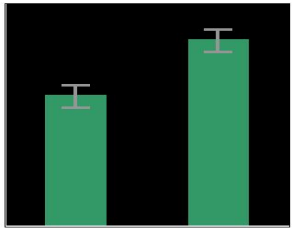
Localization



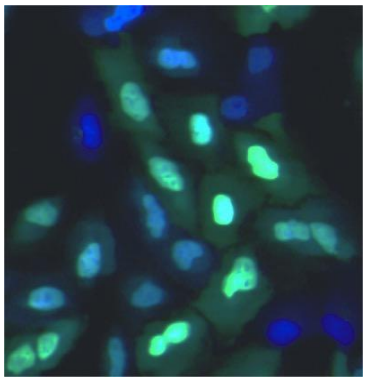
mRNA or protein levels



morphology



… + hundreds of other features



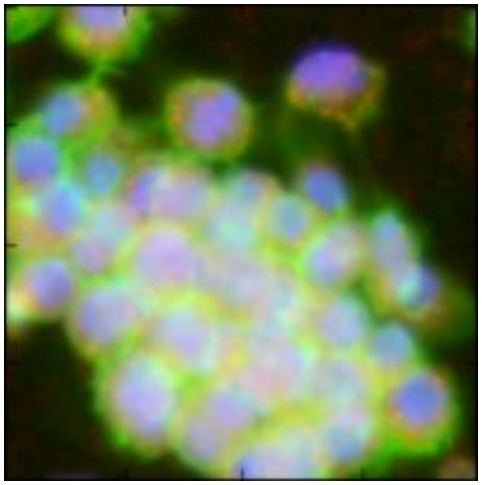
Automatic image analysis:

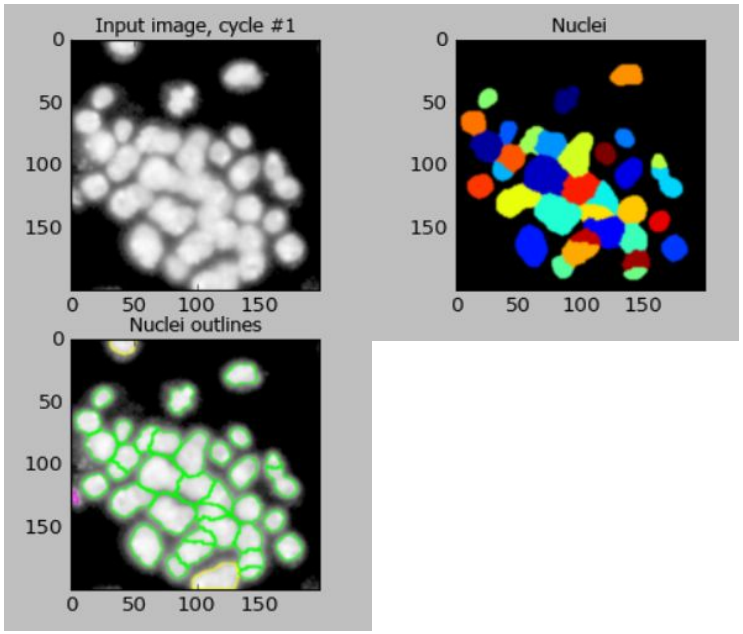
- Objective
- Quantitative, with statistics
- Can measure multiple properties at once
- Can quantify heterogeneity (single-cell measurements)
- Distinguishes subtle changes, even those undetectable by eye
- Faster, less tedious

A pipeline in CellProfiler quantifies the biology within an image

- Measure everything, ask questions later
- A typical pipeline does 3 tasks:

Process Images → **Segment Biology** → **Measurements & Features**





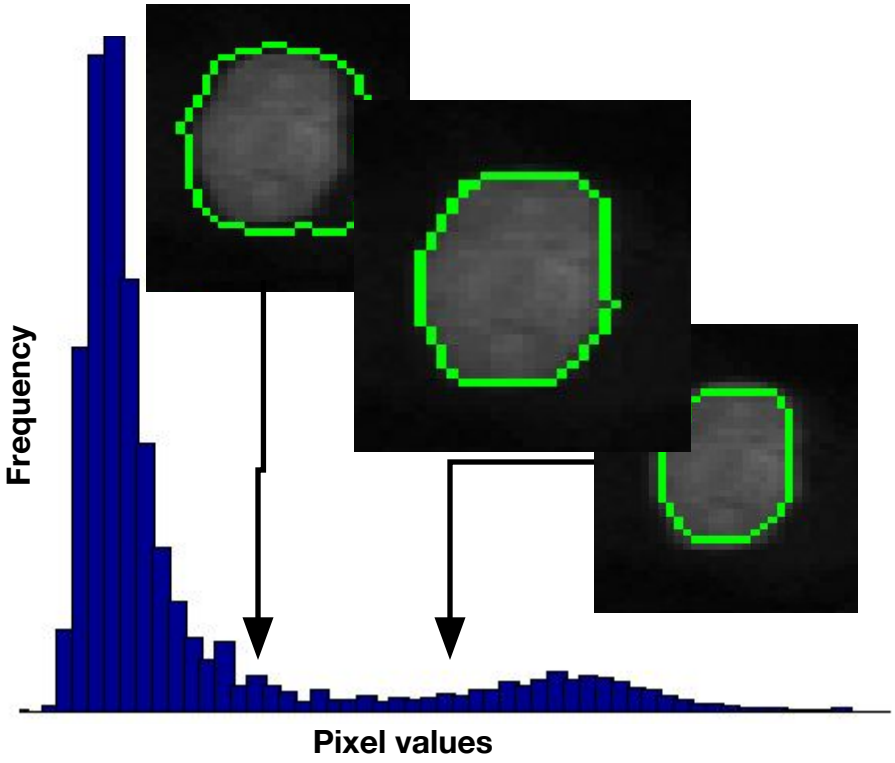
Meas. & Features

Cells

Segmentation - Image thresholding

Definition: Division of the image into background and foreground

What is the best threshold value for dividing the intensity into foreground and background pixels?

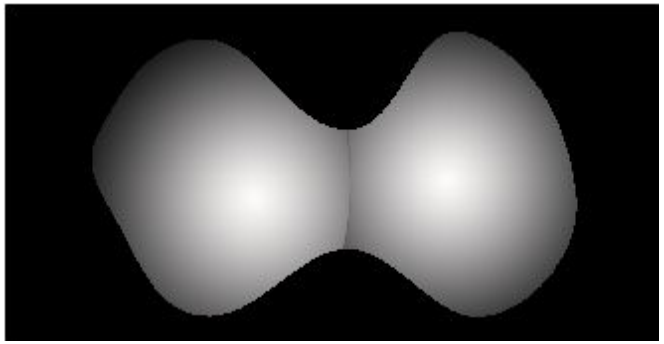


Methods:

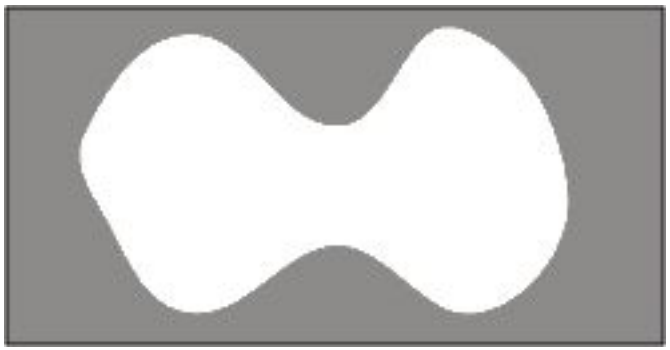
- Automatic: Good for readily identifiable foreground / background
- Otsu: Choose between 2- or 3-class if mid-level intensities present
- Background, RobustBackground: Good for images in which most of the image is comprised of background

Segmentation - Splits and Merges

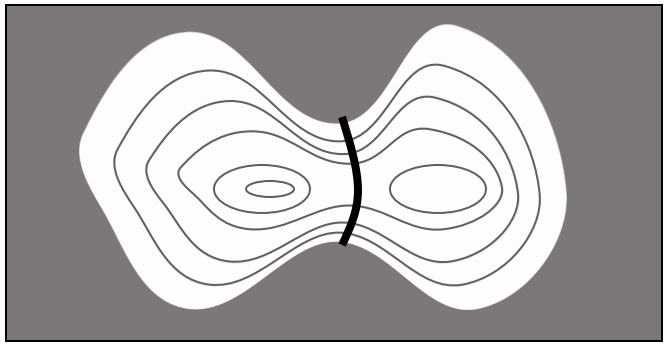
Once the images are loaded, how do you find objects of interest?



- **Step 1:** Distinguish the foreground from the background (thresholding)



- **Step 2:** Split/merge “objects” properly

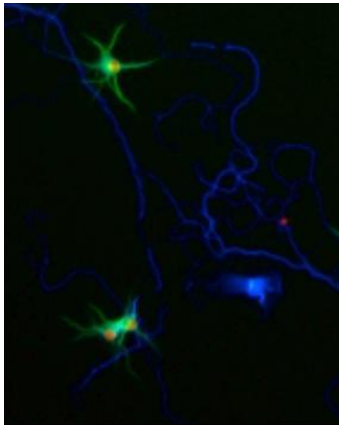


CellProfiler has quantified a wide variety of biology within images

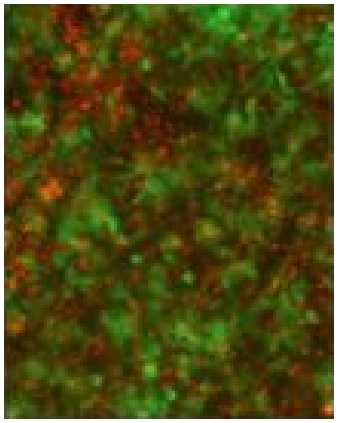
http://cellprofiler.org/examples/

Yeast

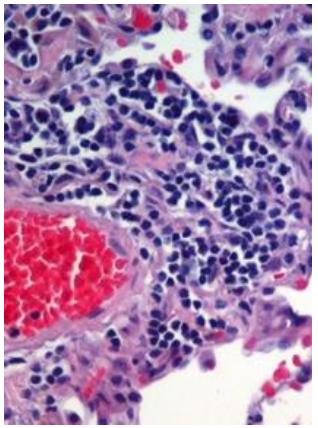
Neurons



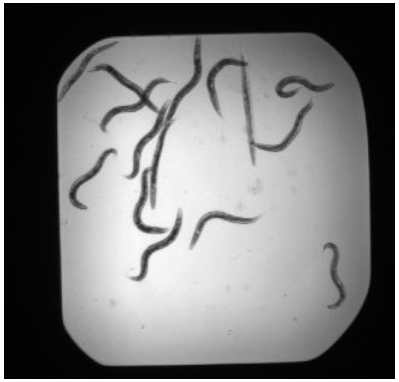
Co-cultures



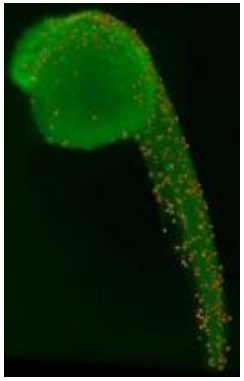
Tissue



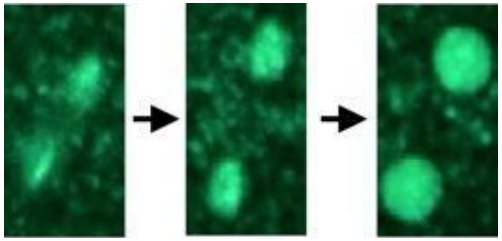
C. elegans



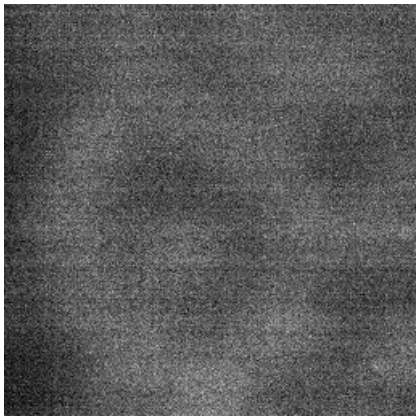
Zebrafish



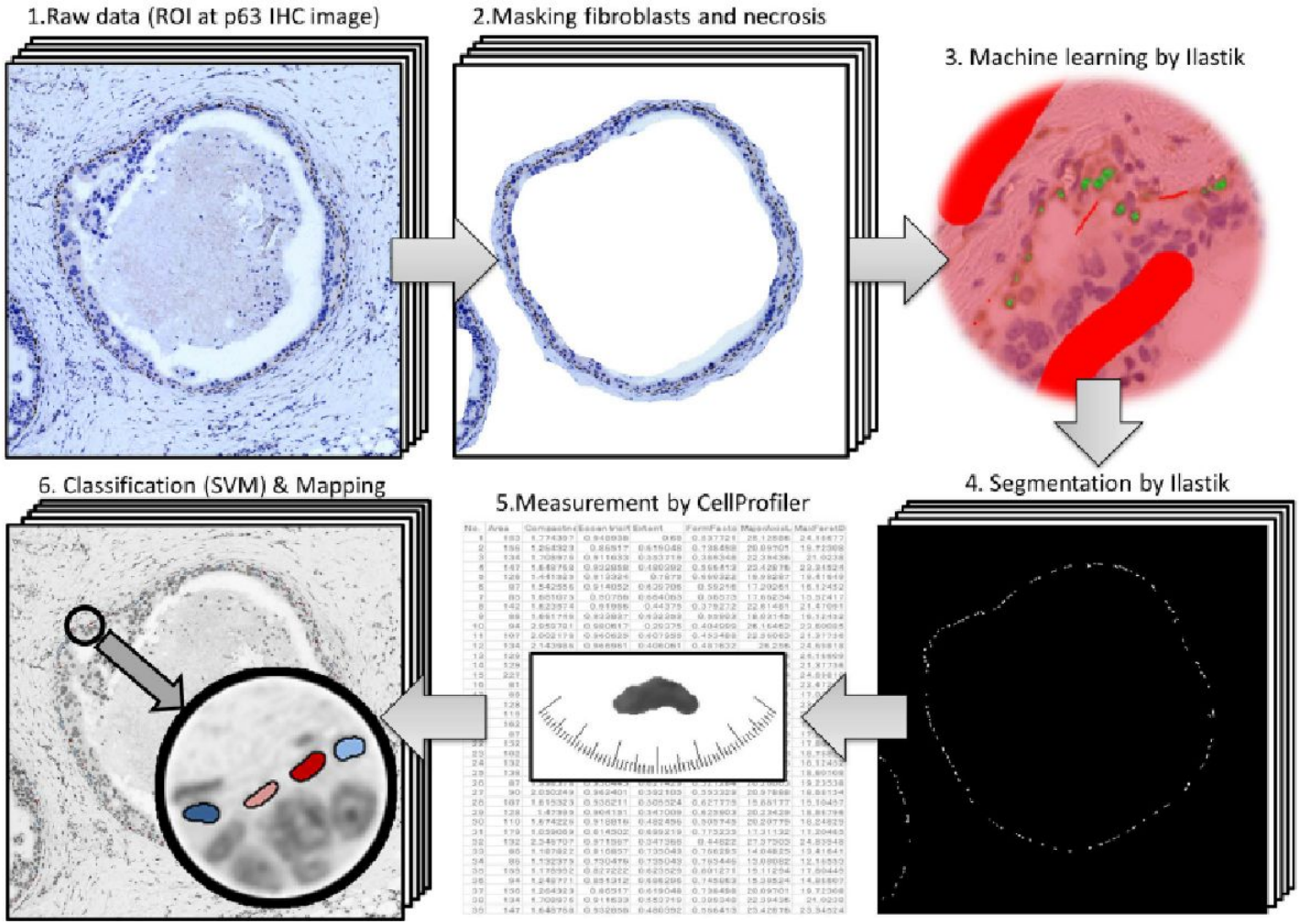
Movies



3D

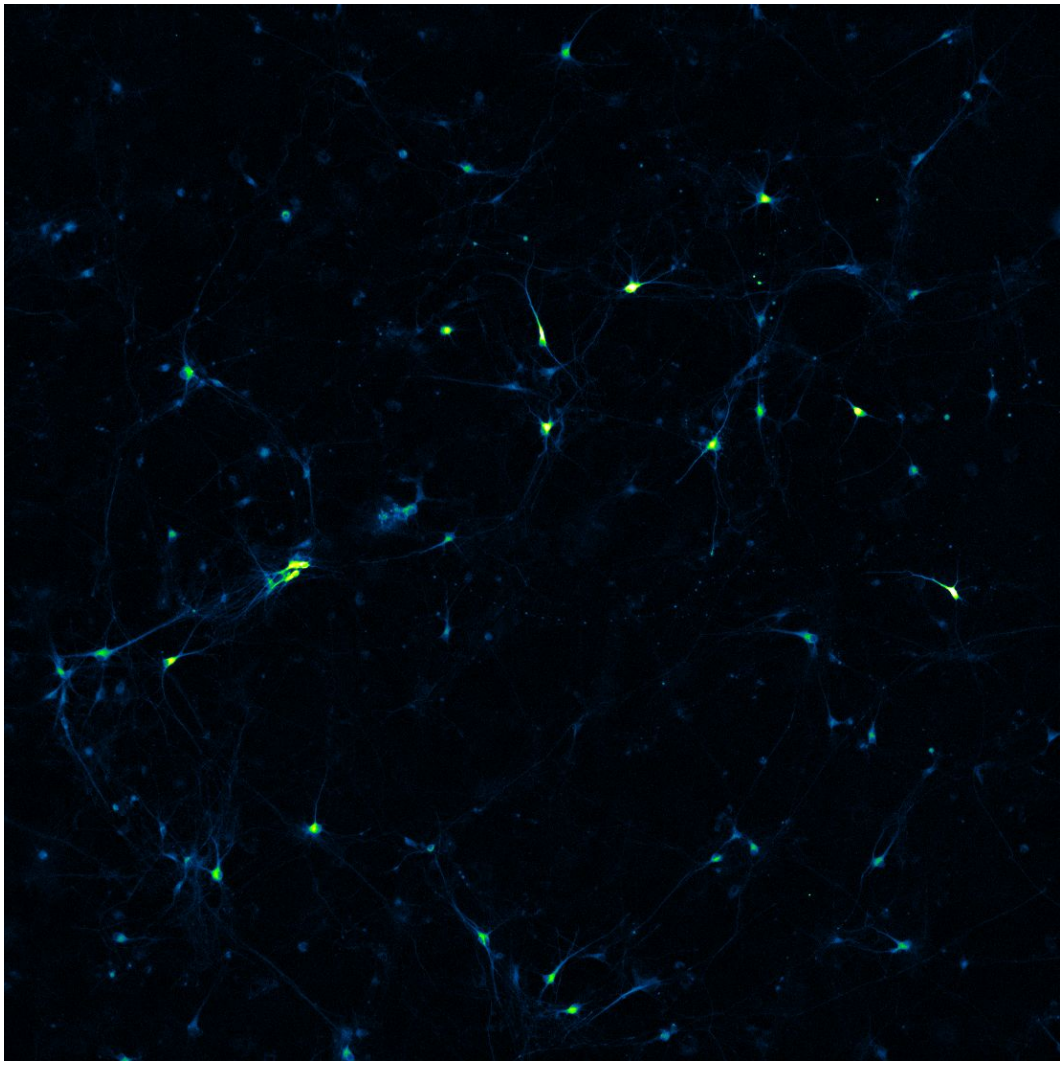


Example: Tissue analysis



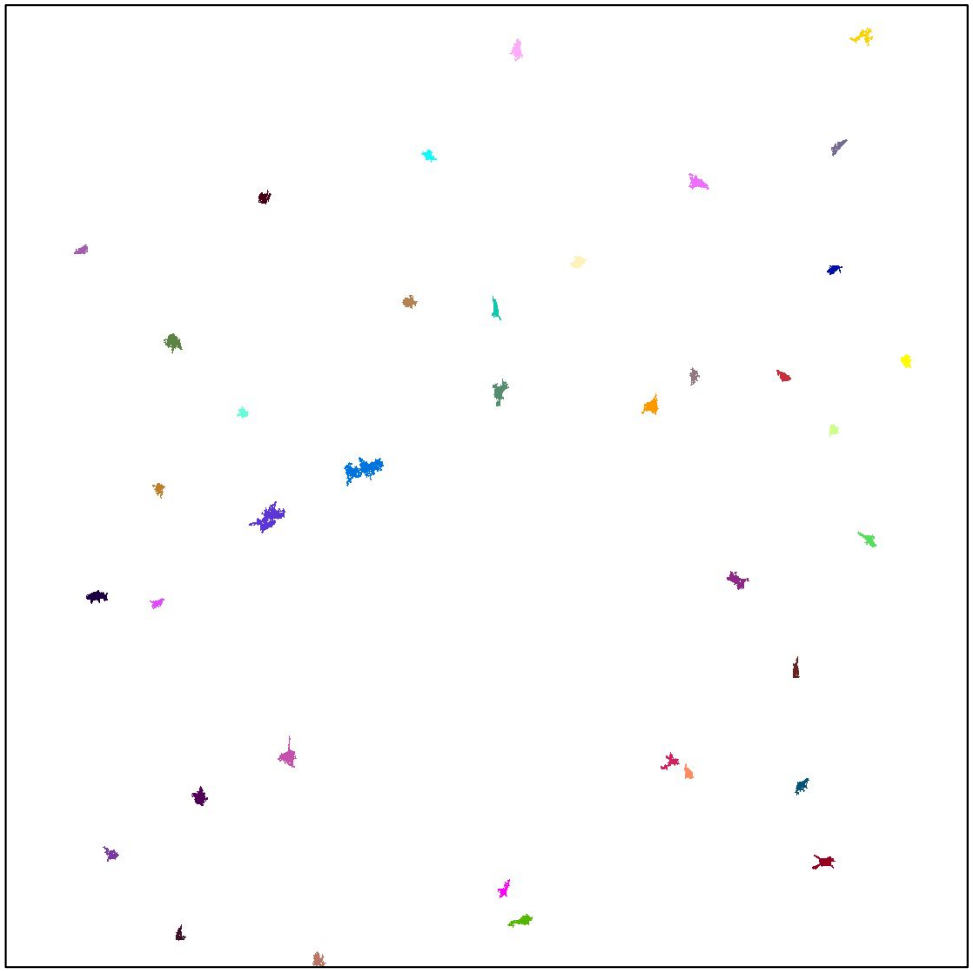
Yamamoto et al, Sci Rep 2017

Example: GCAMP fluorescence of motor neurons



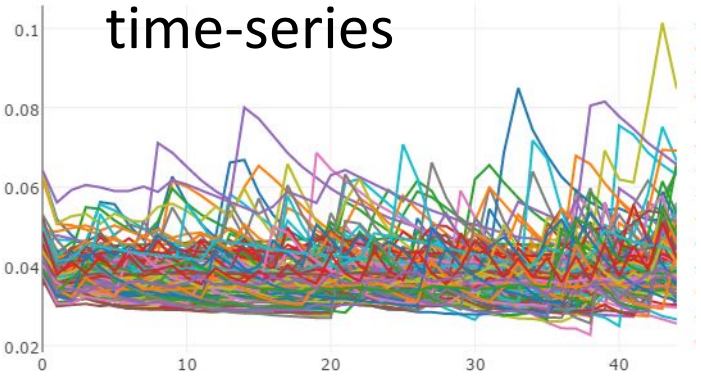
CellProfiler segments the max projection

- A CellProfiler pipeline finds regions of high intensity and segments these into

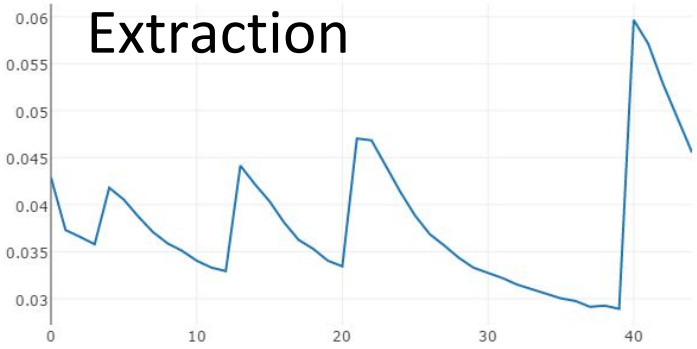


Quantify neuron spiking time-series

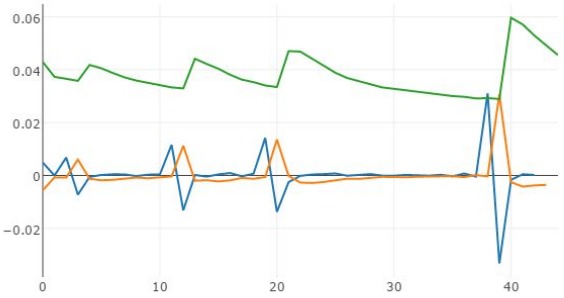
3. Correct time-series



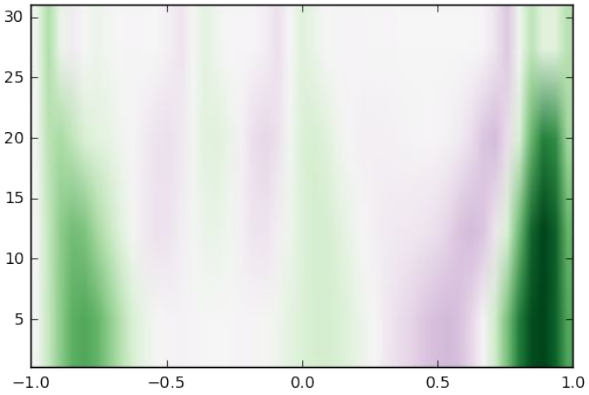
4. Feature Extraction



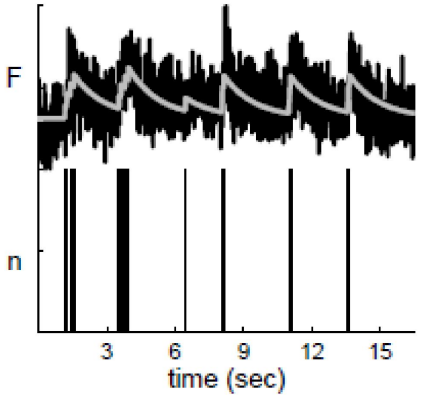
4.a Roet Peaks



4.b Wavelet Peaks

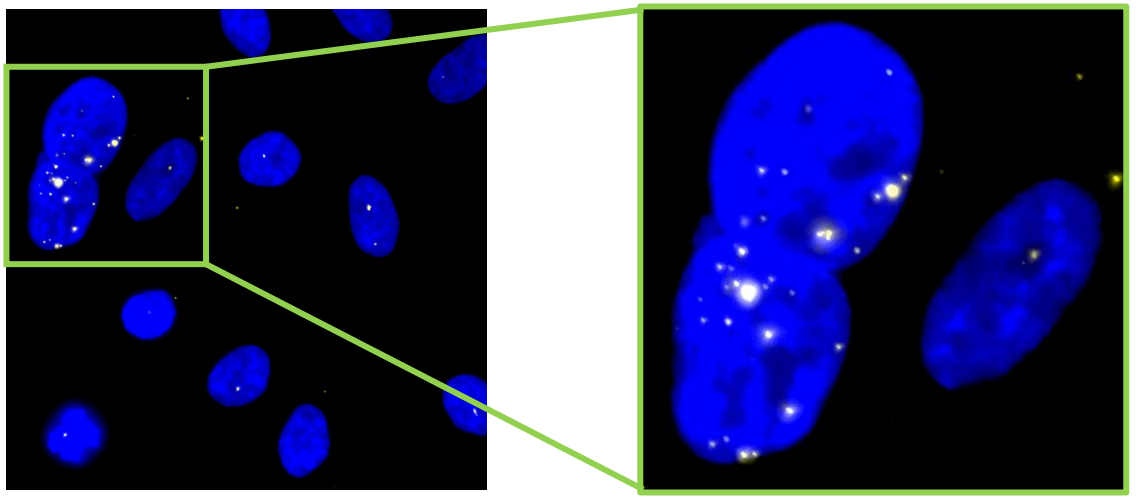


4.c oopsi spikes



mRNA ISH with cultured cells

- U2OS cells

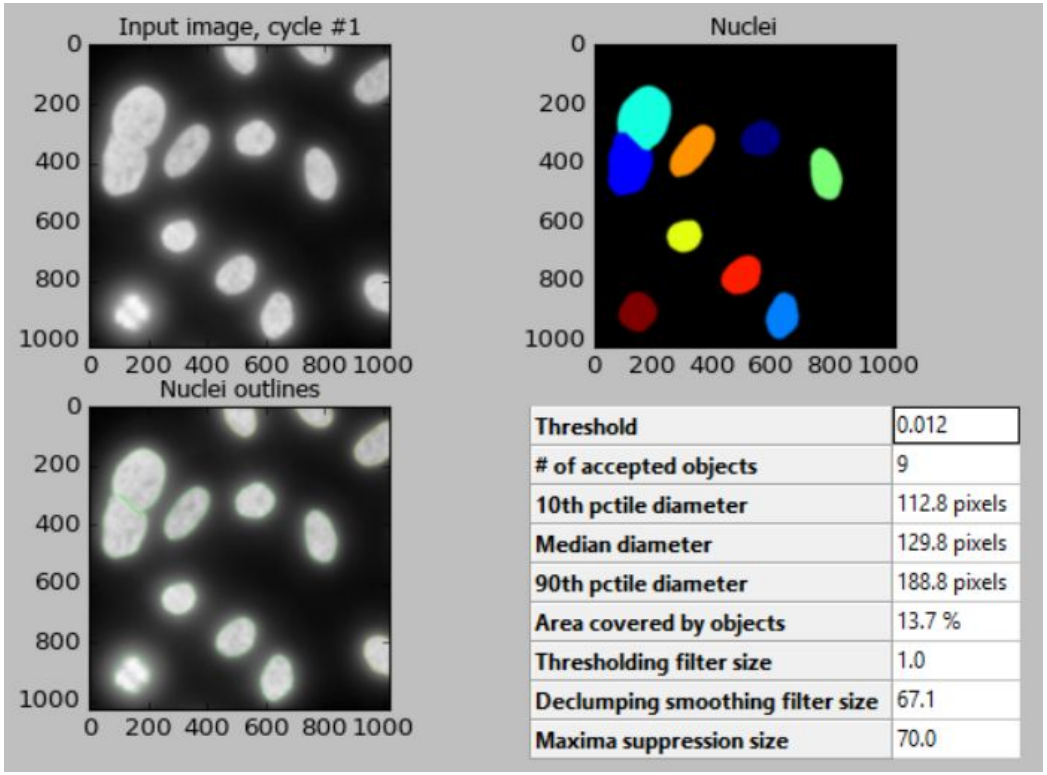


- Widefield microscope
- Z-stacks, 2-channels: nuclei and mRNA
- ISH Assay

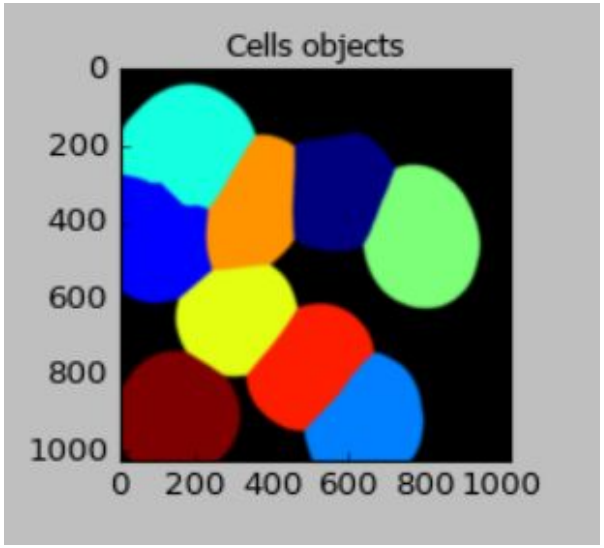
Leah Bury,
Cheeseman Lab,
Whitehead
Institute

mRNA ISH with cultured cells

- Max projections of nuclei and mRNA are inputs into CellProfiler
- Nuclei are segmented. The region around the nuclei are expanded to find mRNA near each nuclei

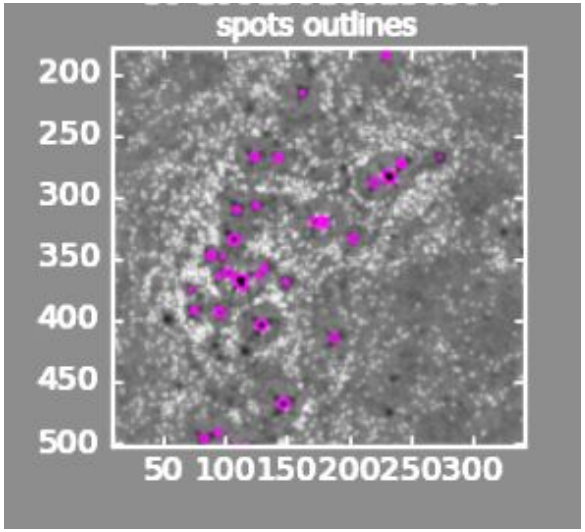


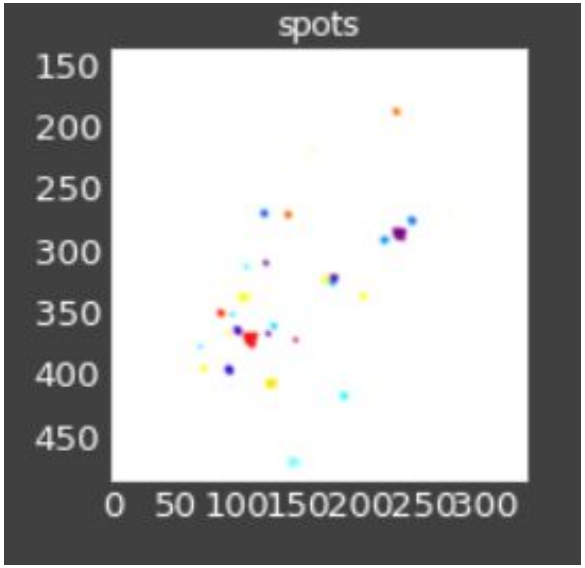
Threshold	0.012
# of accepted objects	9
10th pctile diameter	112.8 pixels
Median diameter	129.8 pixels
90th pctile diameter	188.8 pixels
Area covered by objects	13.7 %
Thresholding filter size	1.0
Declumping smoothing filter size	67.1
Maxima suppression size	70.0

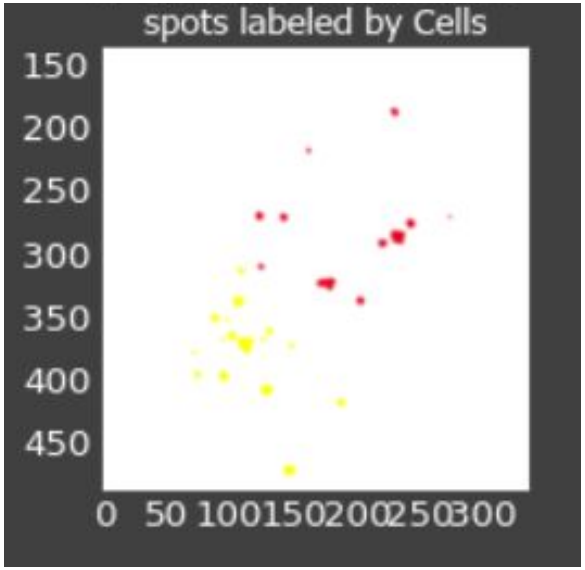


mRNA ISH with cultured cells

- Background signal is subtracted from mRNA channel and mRNA “spots” are enhanced
- Each mRNA is segmented
- mRNA is assigned to each cell by proximity to the nucleus

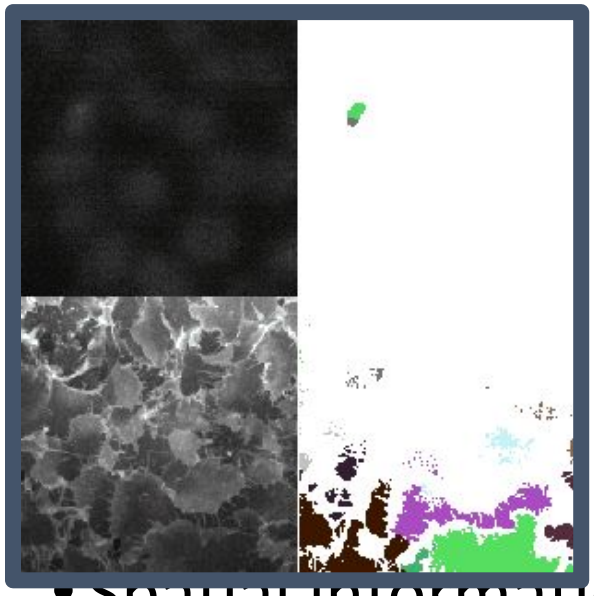


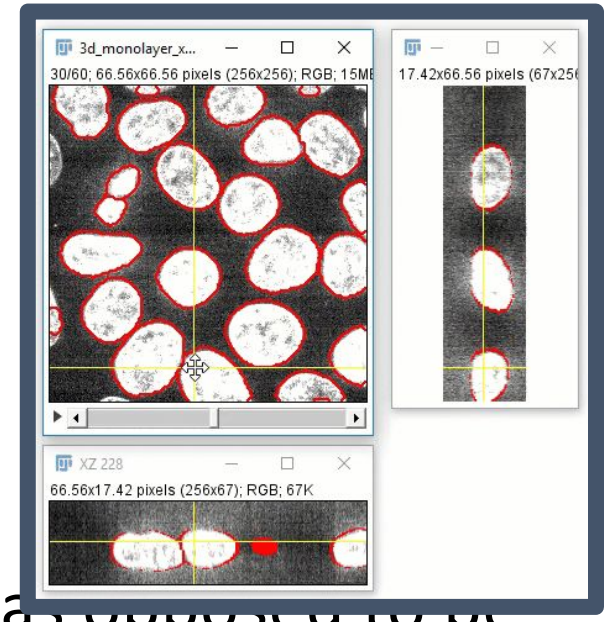




CellProfiler and 3d images

- The *Summer 2017* update of CellProfiler will include expanded 3D functionality



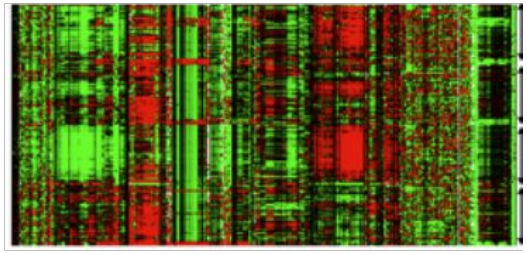


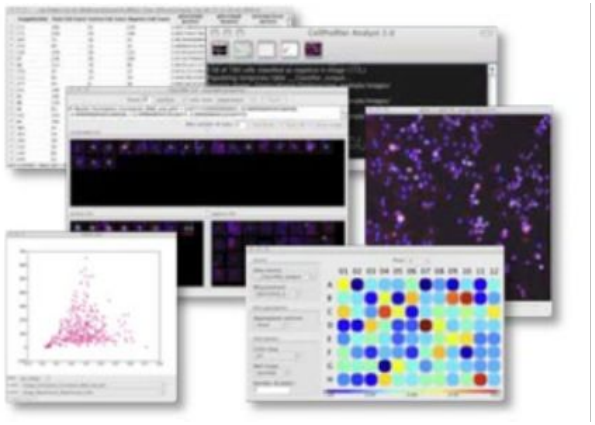
- Spatial information is preserved, as opposed to be removed through a max projection

Liya Ding,
Allen Institute

CellProfiler™ Analyst
data exploration software

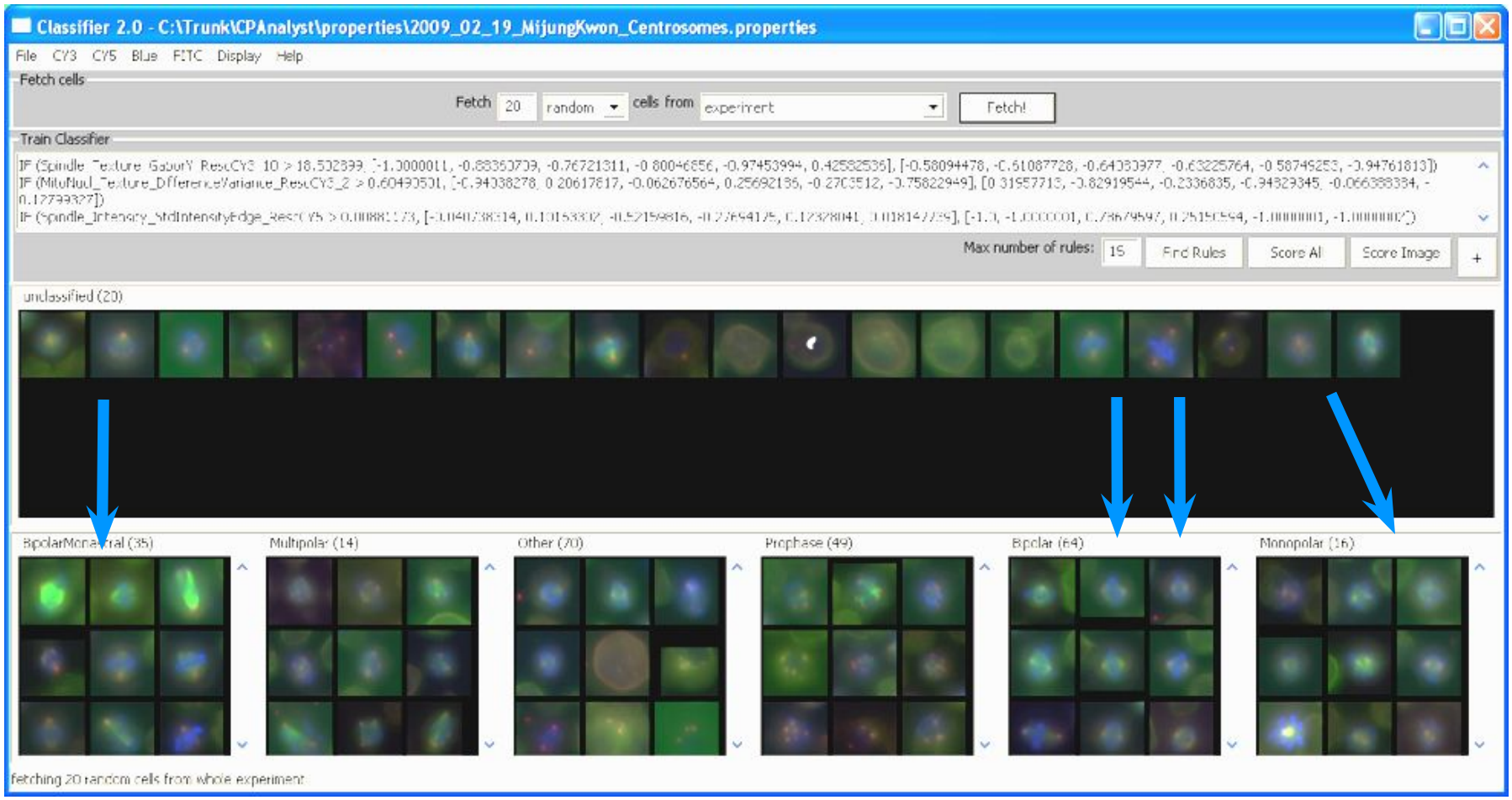
- **Explore** large, image-derived data sets
- **Score phenotypes** with machine learning





- **Goal:** Support data analysis and exploration based on individual cells

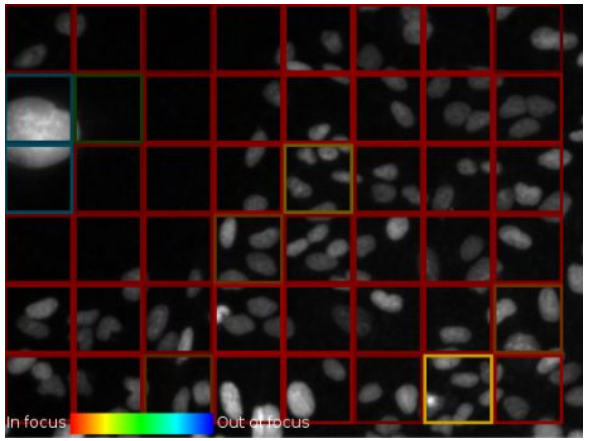
Phenotyping / Classification / Machine learning

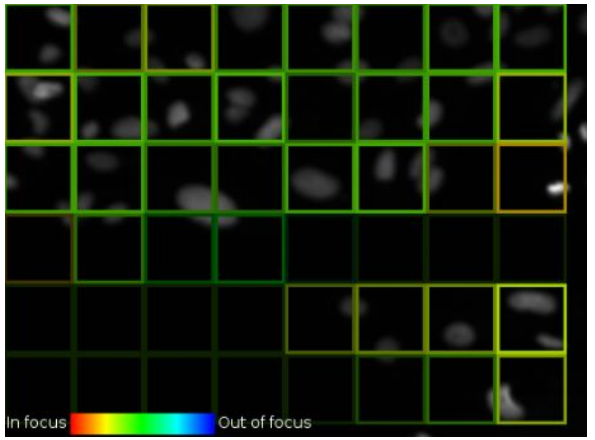


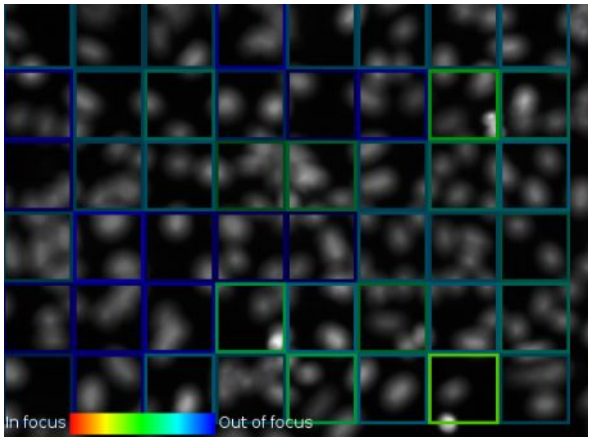
CellProfiler™ Analyst
data exploration software

Future Directions

- Distributed CellProfiler: a project for processing images with CellProfiler on cloud services such as AWS or Google Cloud.
- Deep Learning Modules: pre-trained DNN models can be added to CellProfiler pipelines
  - **MeasureImageQuailty** will determine what parts of the image are in-focus.





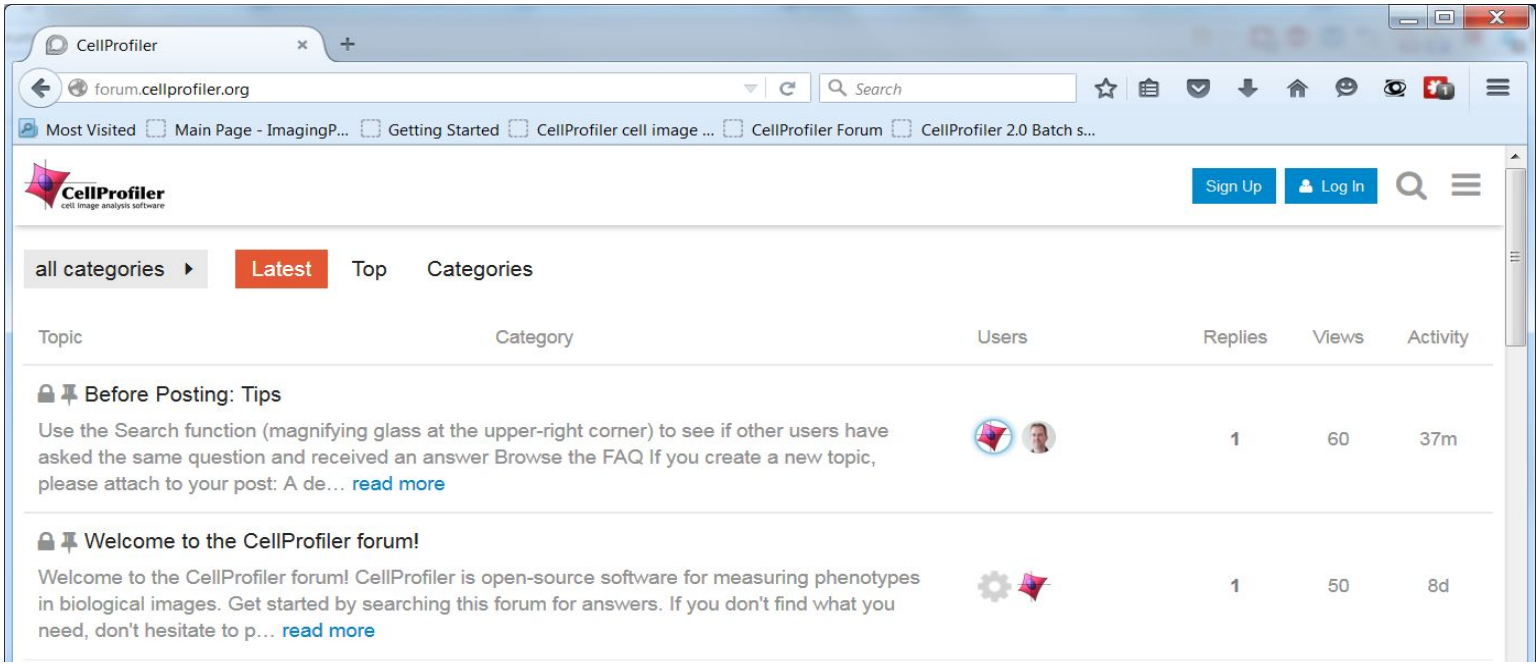


Conclusion

1. CellProfiler can quantify images from **diverse** biological contexts in an automated workflow
2. CellProfiler can analyze complex image sets including **3D**, **time-series**, **tissue** and **high-content screening**.
3. CellProfiler is **open-source** and actively developed, so it will grow with the data as image sets become larger and more complex and incorporate the latest methods

CellProfiler help is found online

- Documentation and examples are at cellprofiler.org
- Direct help from the Broad Institute and other users can be found at the forum.cellprofiler.org



Thank you!

The Anne Carpenter Lab and Imaging Platform