Image Processing with

CellProfiler™

cell image analysis software

CM 515 - Rosi Danzman

Getting CellProfiler

Go to: https://cellprofiler.org/

-> Click Download button

-> select OS and follow install instructions

Troubleshooting:

-> online forum: https://forum.image.sc/tag/cellprofiler

-> Github - windows issues: https://github.com/CellProfiler/CellProfiler/wiki/Windows-Installation-Troubleshooting

Images in Research

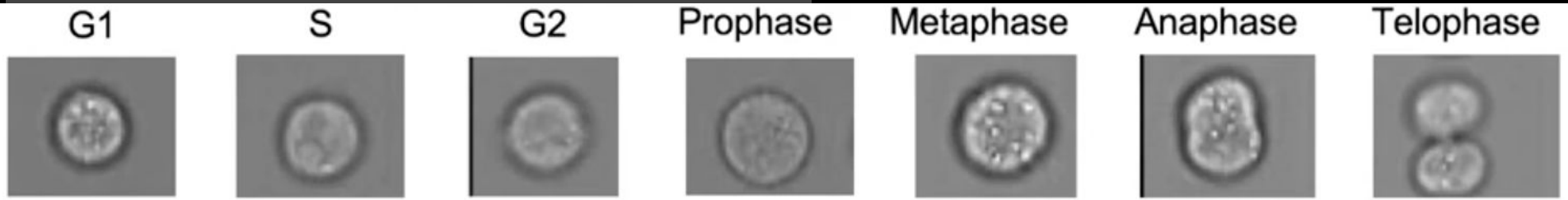
Images contain copious information, much of which is untapped

Images can be used to quantify biological phenotypes

What is the area of this nucleus?

How many seeds are present?

What stage of the cell cycle are these cells at?




Huawei P30 Pro (Auto)
Samsung Galaxy S10+ (Auto)
Google Pixel 3 (Auto)
Huawei P30 Pro (Night)
Samsung Galaxy S10+ (Night)
Google Pixel 3 (Night)

What is image processing?

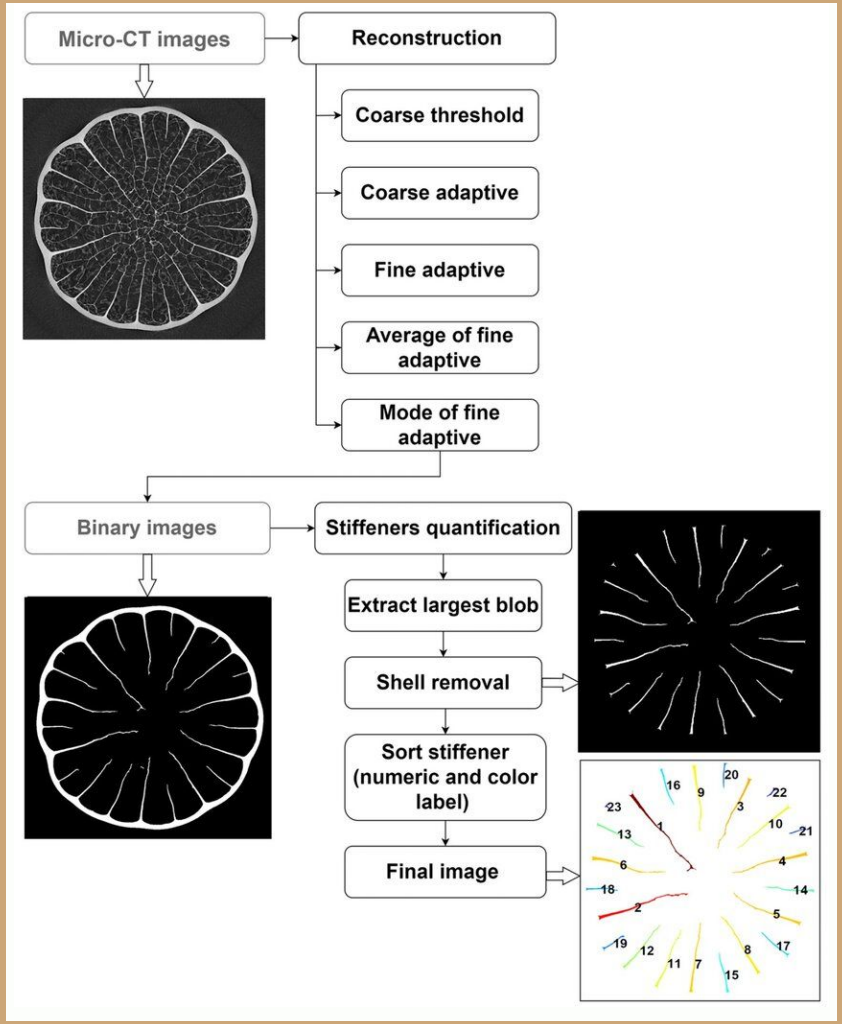
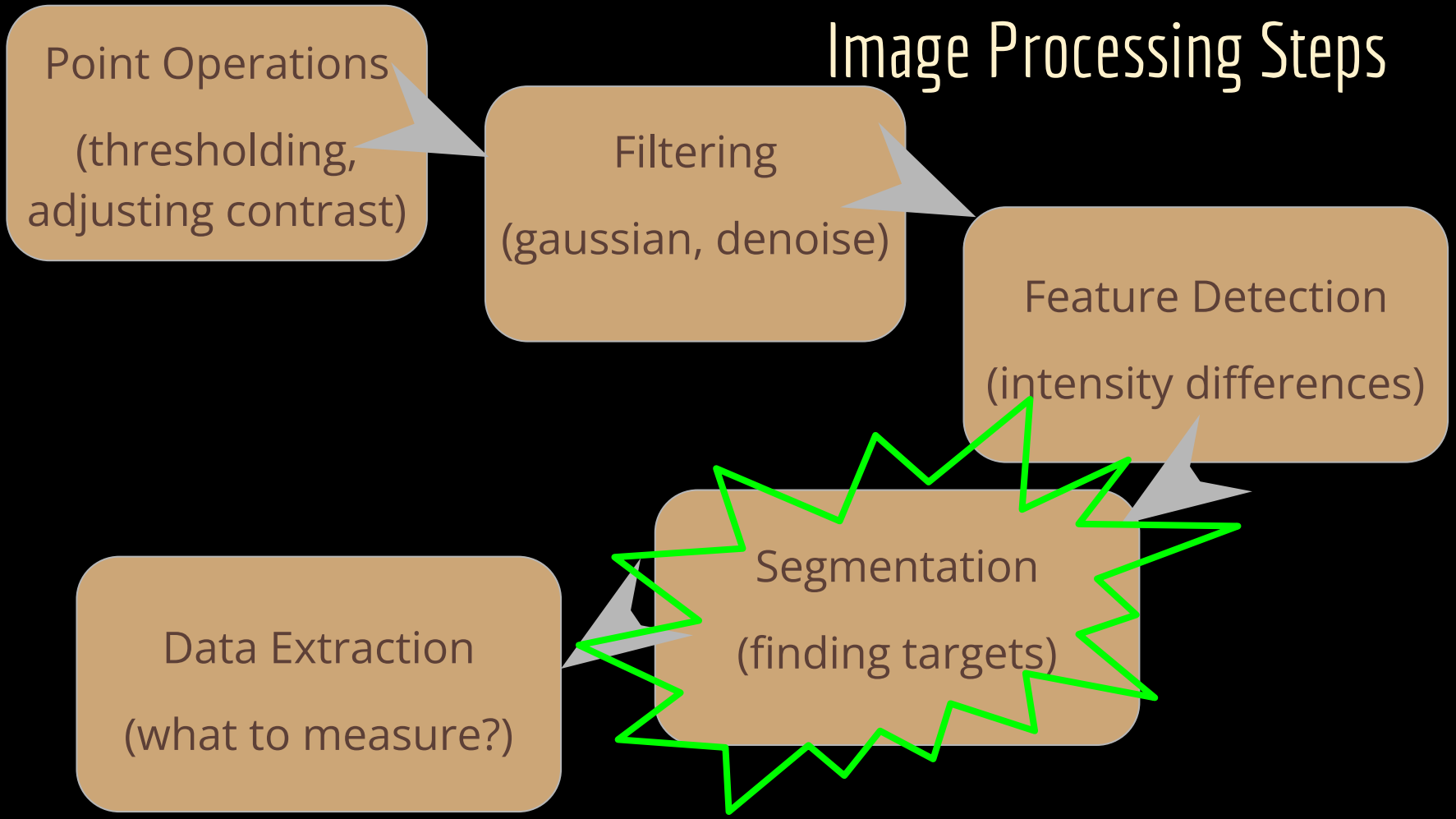
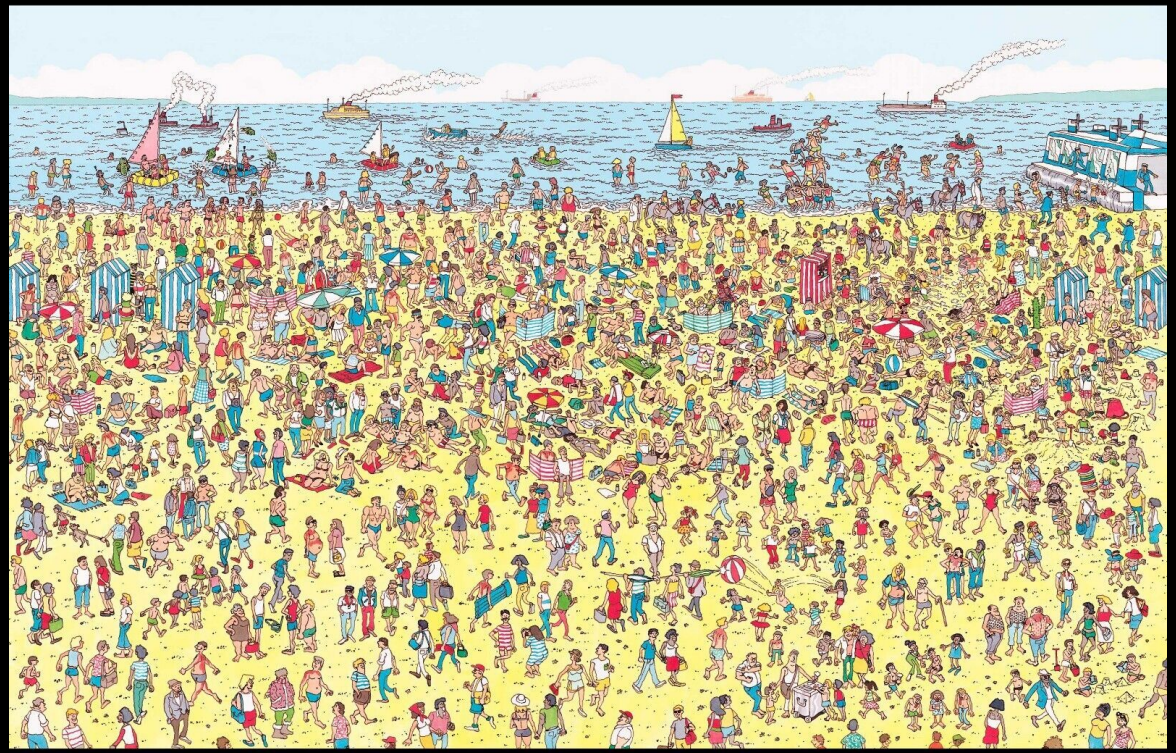


Image Processing Steps



Segmentation - finding a target

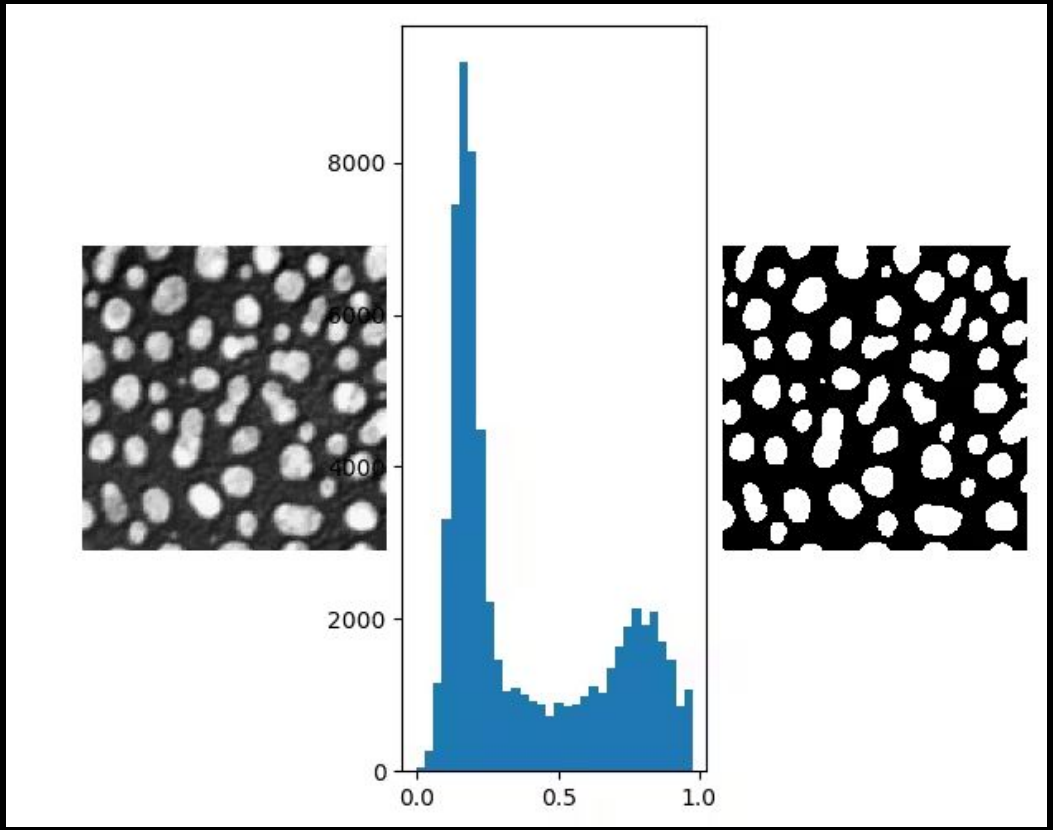
Must identify boundaries to measure something



Computers use similarities and discontinuities

Ex: thresholding or edge detection

How segmentation is done



Thresholding - similar pixel groups ⟶ dark or bright

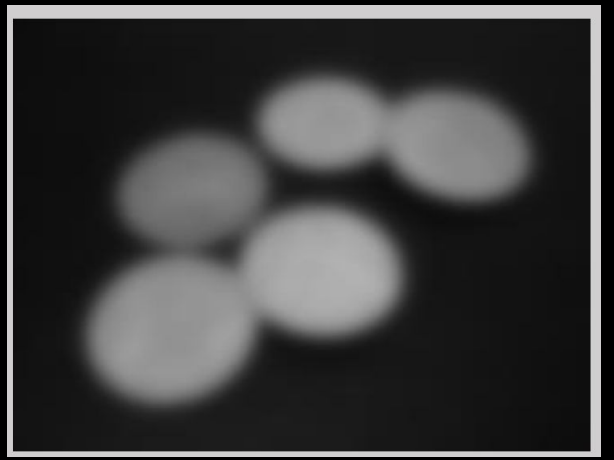
Humans can easily pick out parts of images - segment

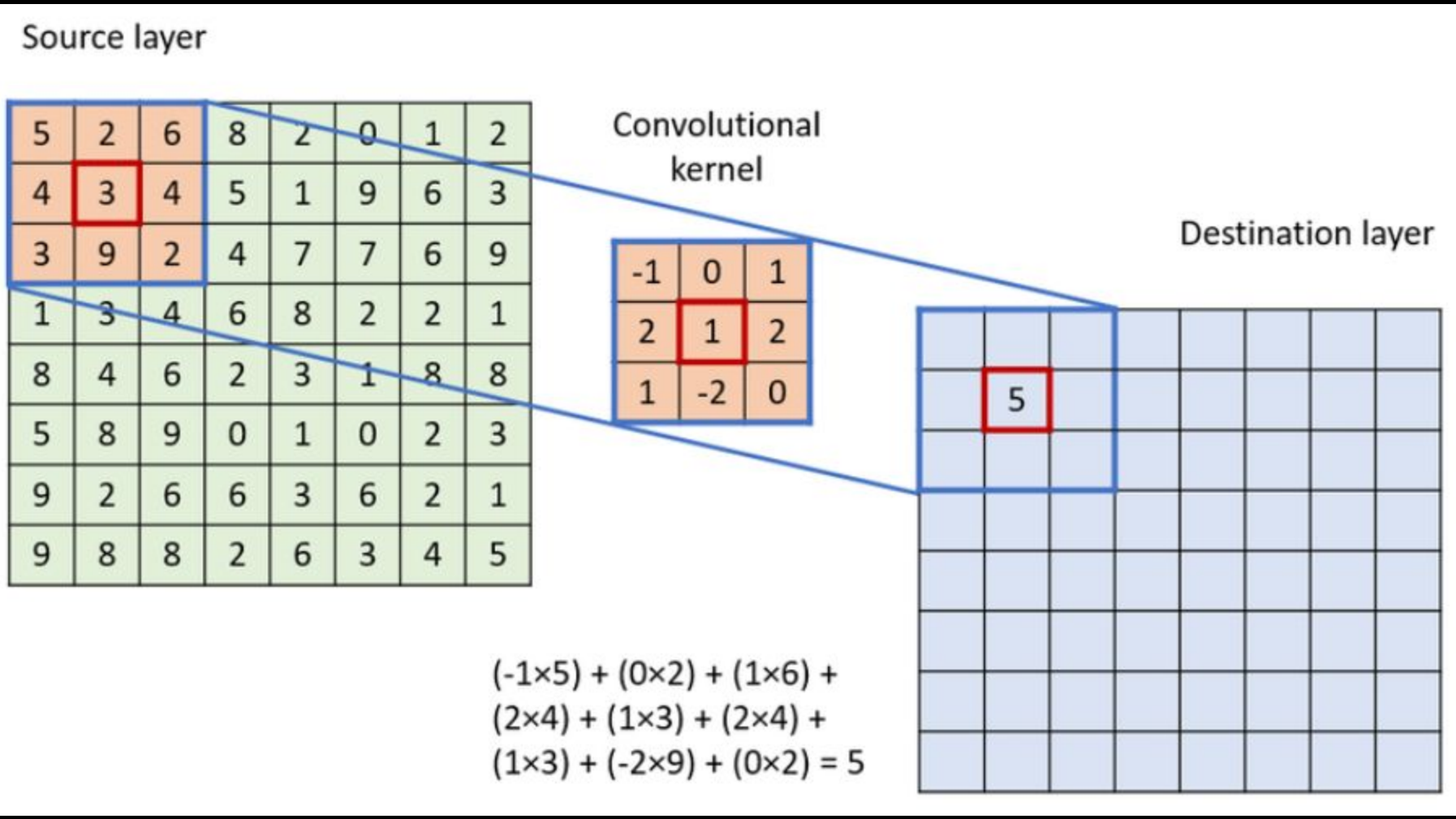
Filtering

Enhancing features before data extraction

This alters your data (pixel values)!

Filters are algorithms performed on kernels (group of pixels)




Source layer
5 2 6 8 2 0 1 2
4 3 4 5 1 9 6 3
3 9 2 4 7 7 6 9
1 3 4 6 8 2 2 1
8 4 6 2 3 1 8 8
5 8 9 0 1 0 2 3
9 2 6 6 3 6 2 1
9 8 8 2 6 3 4 5
Convolutional kernel
-1 0 1
2 1 2
1 -2 0
Destination layer
5
(-1×5) + (0×2) + (1×6) +
(2×4) + (1×3) + (2×4) +
(1×3) + (-2×9) + (0×2) = 5

Manual Segmentation

Selecting the boundaries by hand

Do we really need computers for this?

Are humans good at image analysis?

What are the challenges?

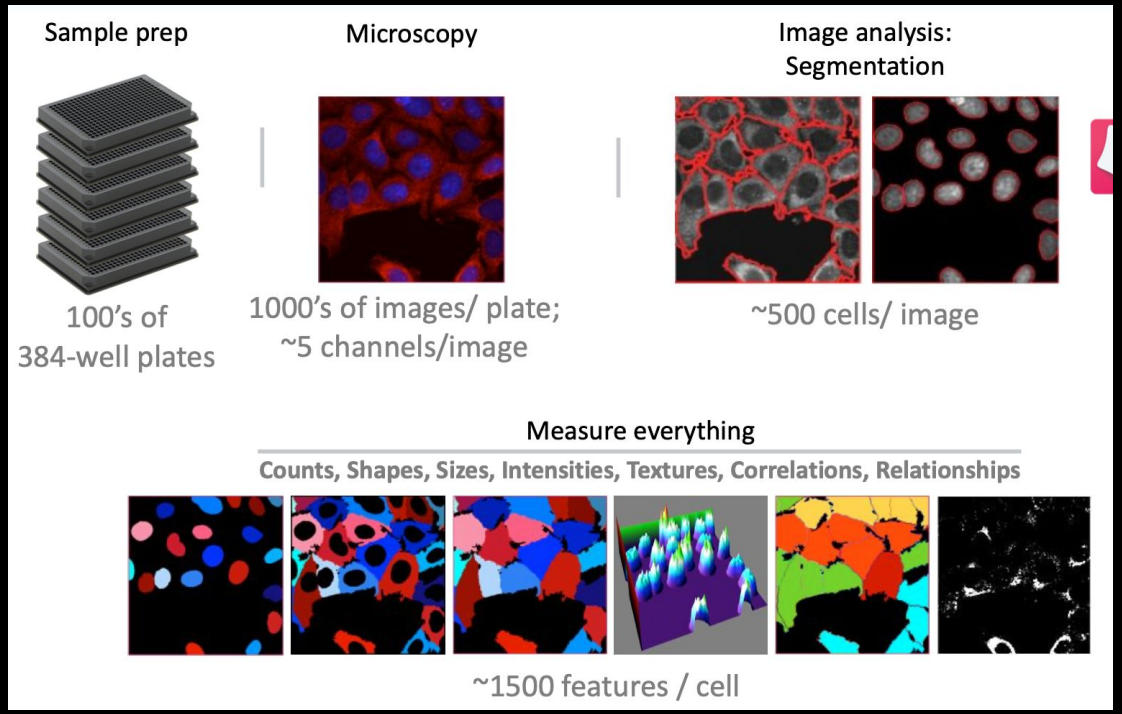
Automation/efficiency

Accuracy - human error at all stages

Consistency - manual image analysis is common, but computers are consistent

Let Computers Do the work

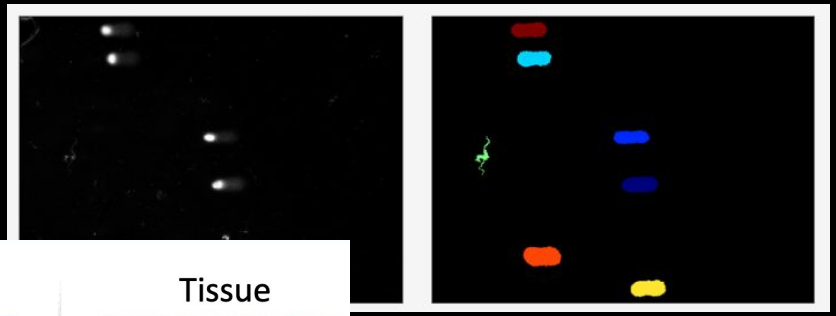
Why CellProfiler



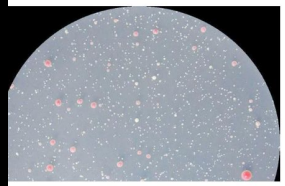
Details on how CP addresses the challenges

- Friendly user interface
- Input nearly any kind of image
- Machine learning algorithms under the hood
- Automated - process thousands of images in minutes
- Exceptional support network for troubleshooting

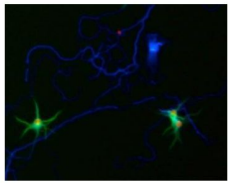
Use any images



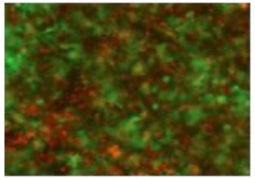
Yeast



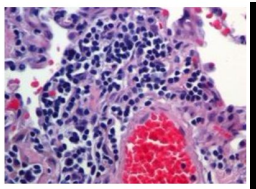
Neurons



Co-cultures

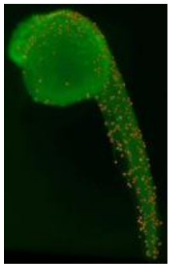


Tissue

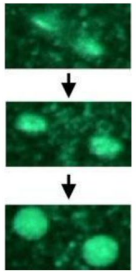


C. elegans

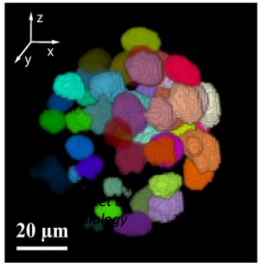
Zebrafish



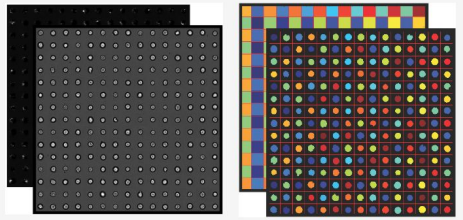
Time-lapse



3D



Imaging Flow Cytometry



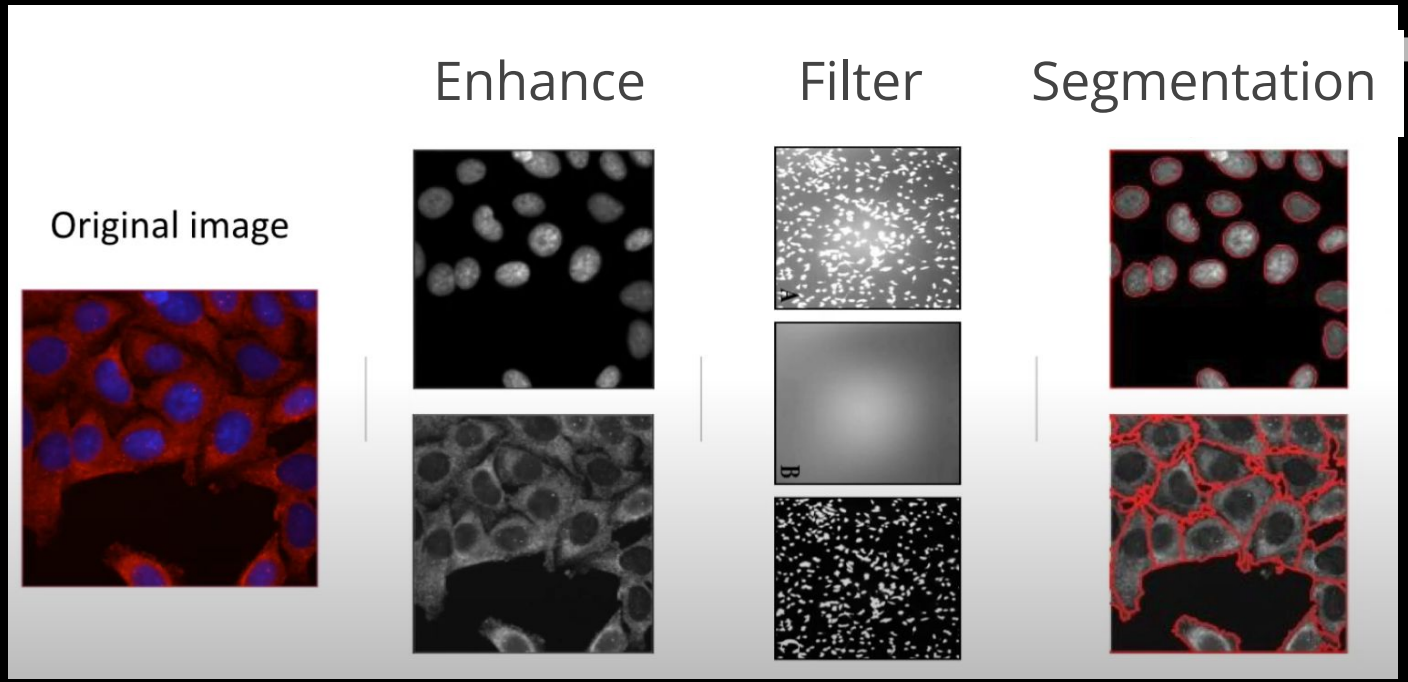
More reasons to use CellProfiler

Free and open-source (and always will be)

Cited in thousands of papers per year

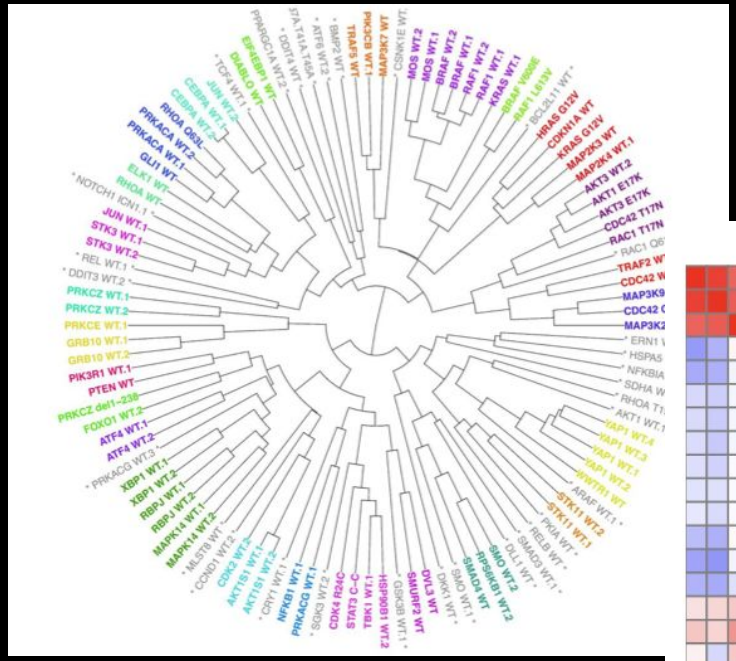
Used by the majority of top pharma companies

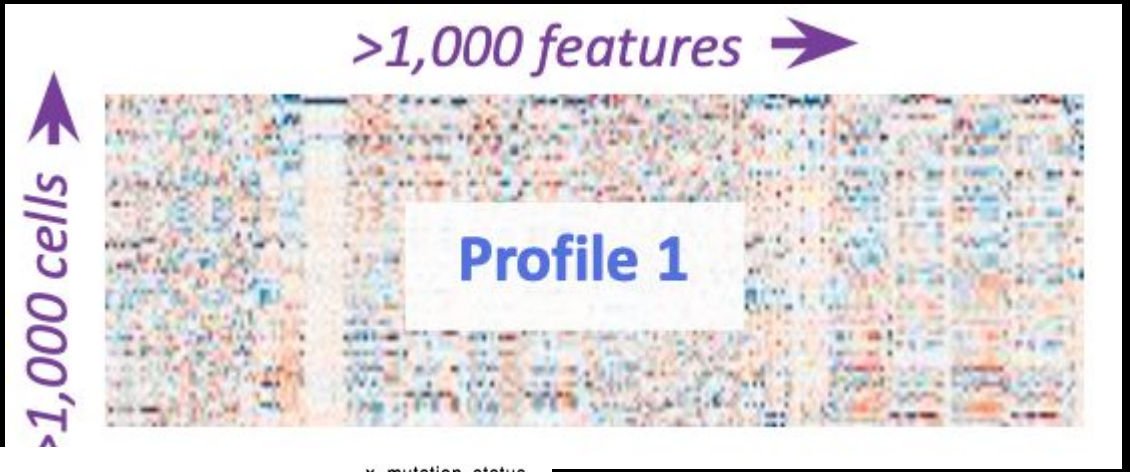
Flexible and usable by biologists (no coding necessary)

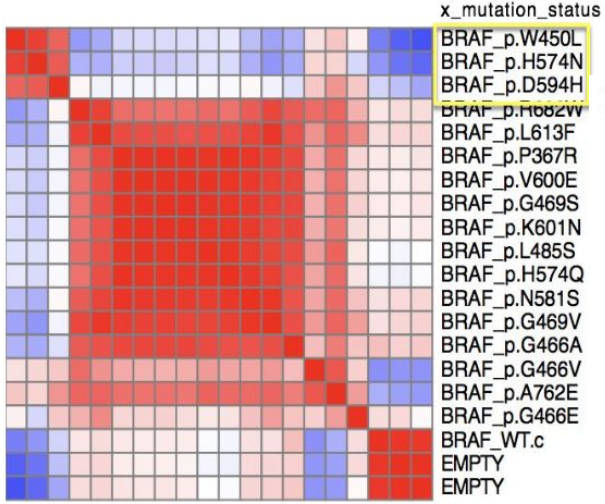

Enhance
Filter
Segmentation
Original image
Measure everything
Save data

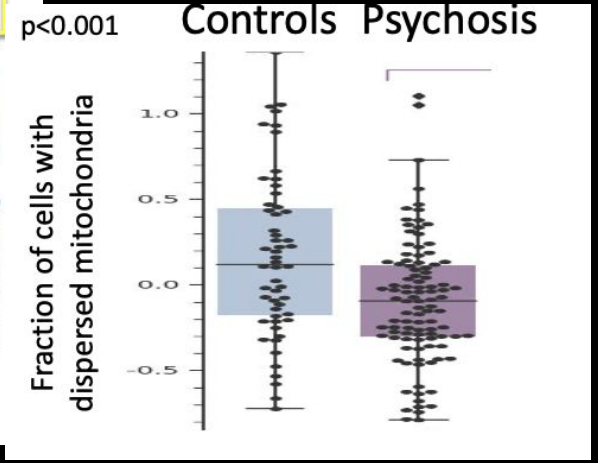
Workflow

The Data



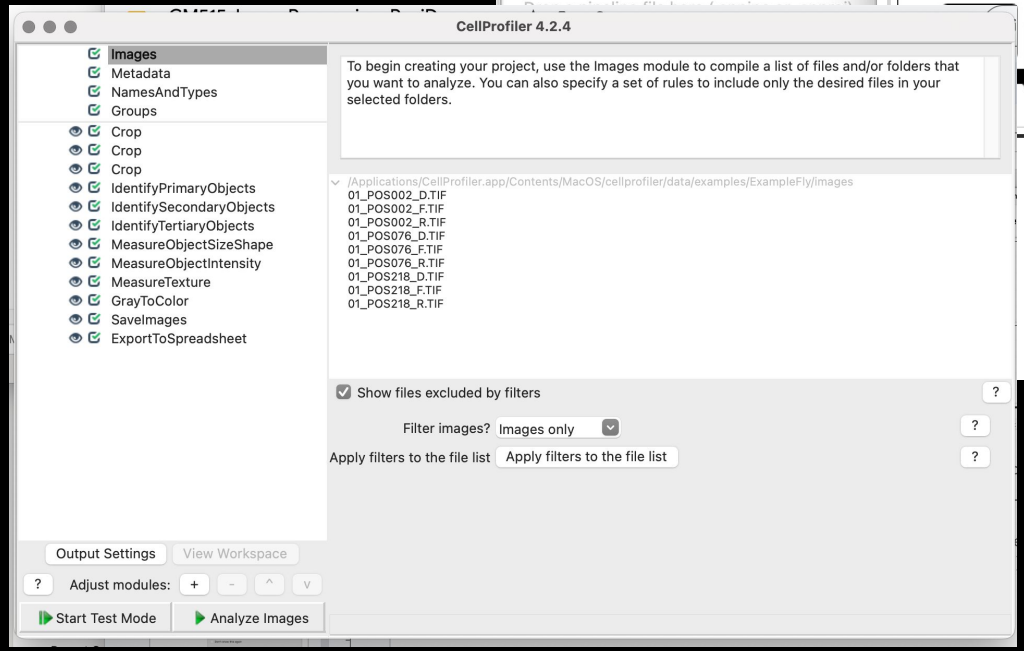






BROAD INSTITUTE IMAGING PLATFORM

Opening CP



Open program

Click on Try Example

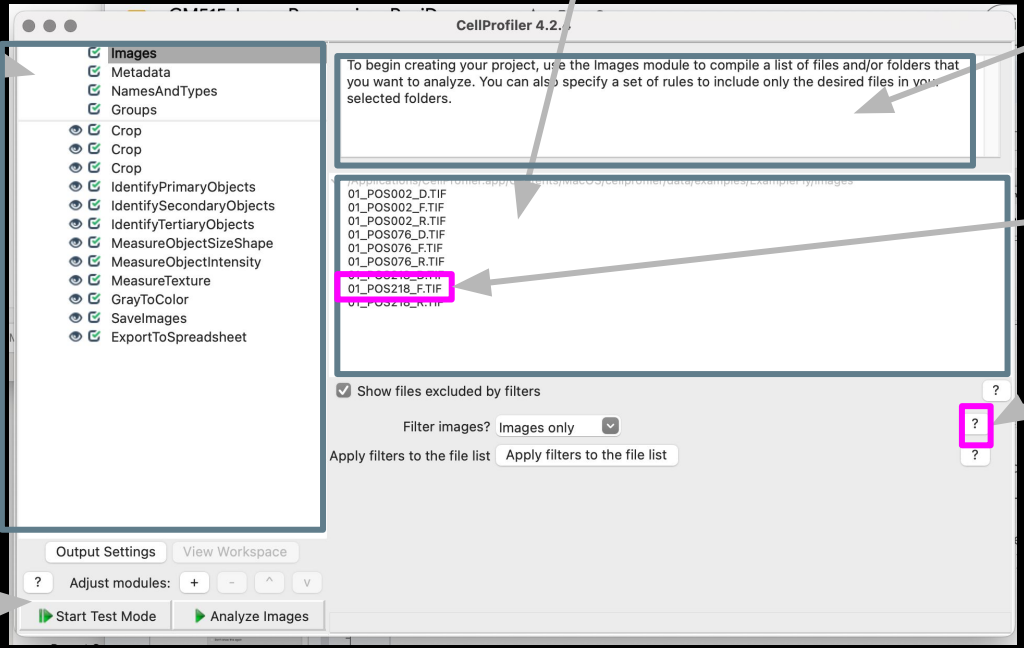
Close Welcome window (lots of helpful info here)

User Interface

Module window: add or delete modules as needed to build your pipeline

Images are loaded here, drag and drop or right click and select an option from the drop down

Information about each module: edit this so users know what to do



Examine images double click

Use the ?

They are very helpful for learning what each function does

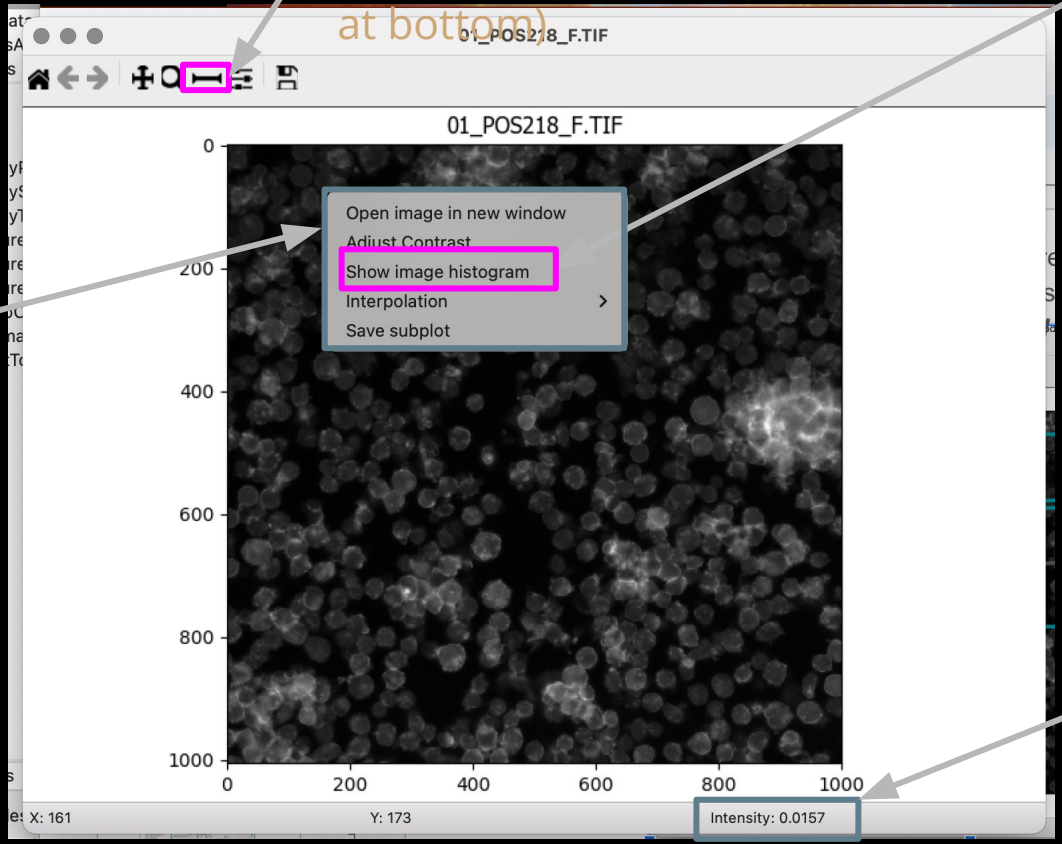
Tools for building and running the pipeline

Image Window

Click on line tool to measure (pixel length appears instead of intensity at bottom)

Select Show Image Histogram to see where pixel intensities fall

Right click on the image to open tool window



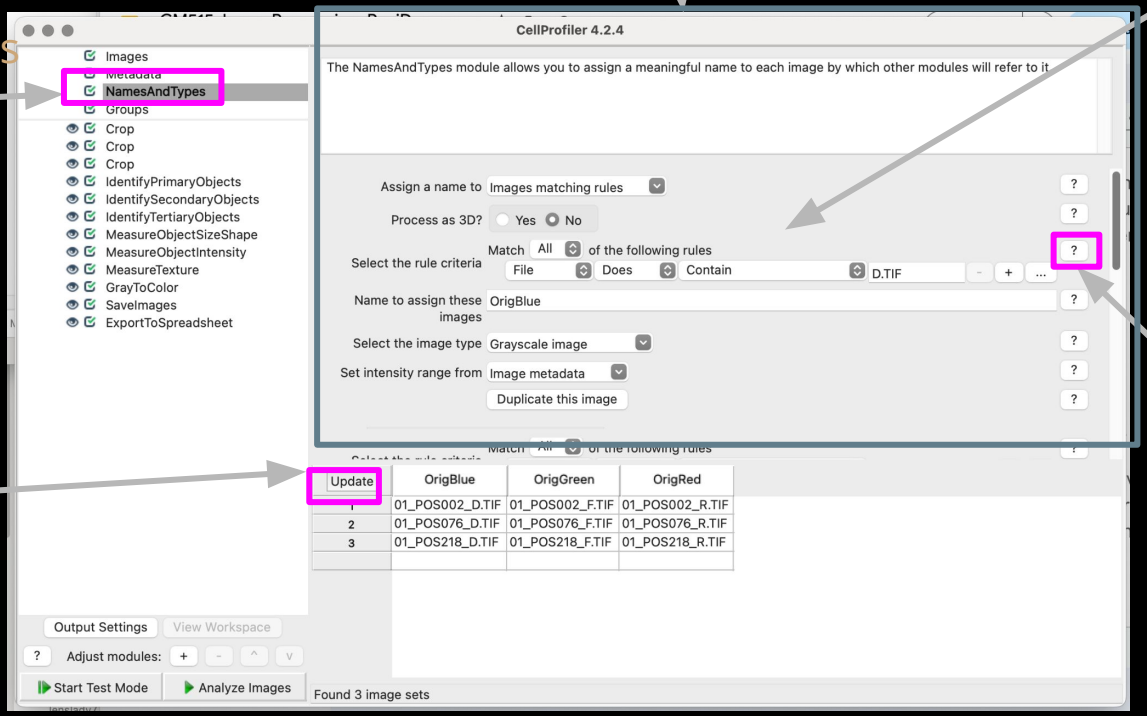
Pixel intensity is shown as you mouse over image

Names and Types

Click on the Names and Types module

The panes on the right change with each module.

Scroll down in this area to see more parameters

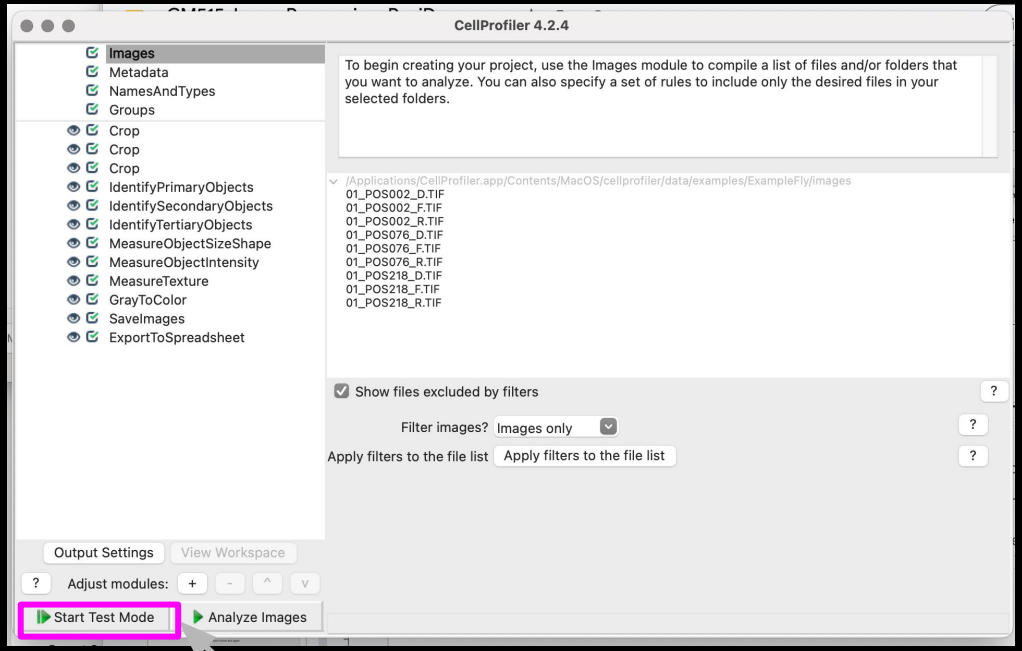


Use the ?

This tells you how to use this function

Click update button to populate image list

Run the Pipeline - Test Mode



Click the Start Test Mode button to test the pipeline

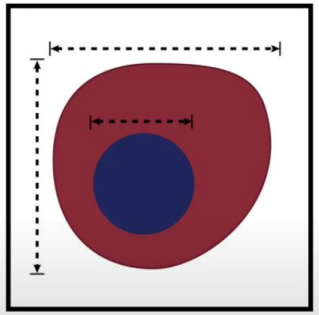
Segmentation

What can we measure?

More than 150 parameters

For each identified structure of interest, measure:

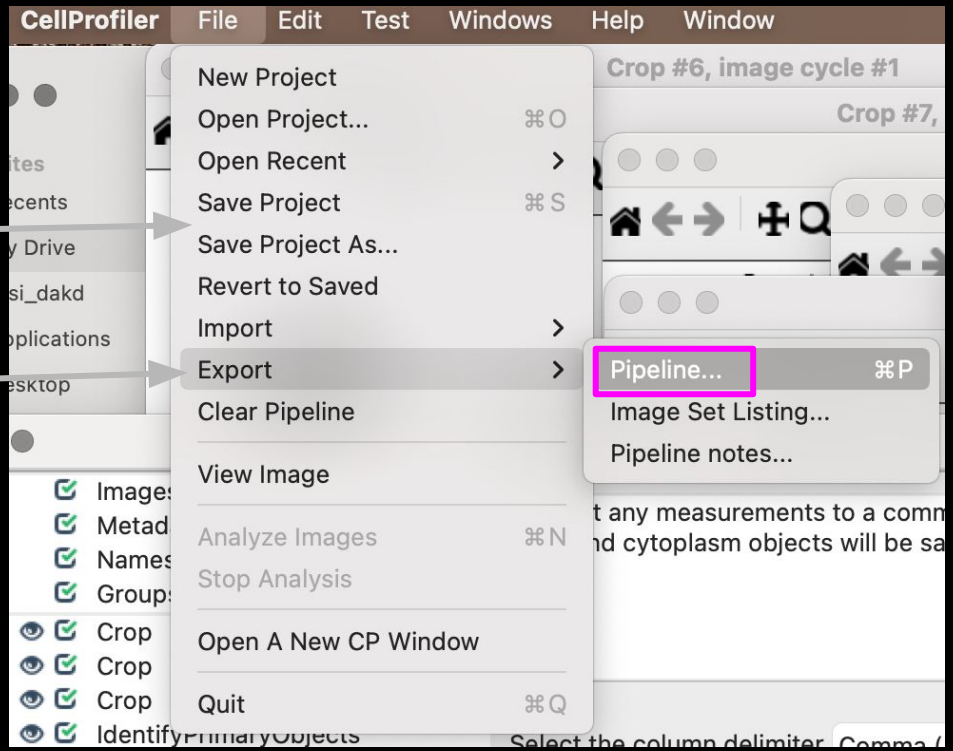
- Counts
- Size
- Shape
- Texture (smoothness)
- Intensity
- Intensity patterns
- Spatial relationships



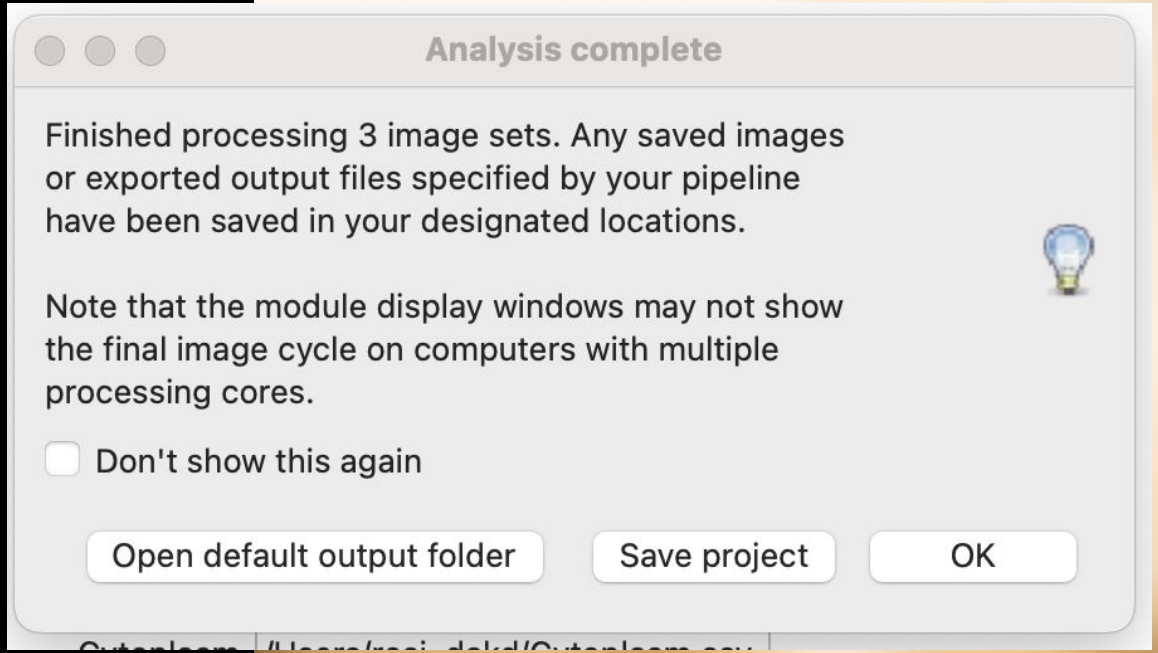
Complete list here: https://cellprofiler-manual.s3.amazonaws.com/CellProfiler-3.0.0/modules/measurement.html

Saving the Project and/or Pipeline

Save project vs export pipeline



Winning!!



Expect many hours of failure to reach a working pipeline

Next time:

More details on image processing
CellProfiler input modules
a few other modules
How to save the output
What to do with the output
In class practice
GET MAGE FROM GITHUB- Brain_input.jpg

Homework: Two parts (outlined in following two slides)

1) Image Processing: Use your own images (or borrowed) to construct a new pipeline (5 points)
2) Questions: Answer the 3 questions in 600 words or less. (20 points)

Turn in: 1) a screenshot of your pipeline

2) Homework Questions (word or pdf)

Due date: Sunday May 4th, 2025 by midnight (almost two weeks from today)

Grades: based on answers to the questions and attempt at pipeline (this does not have to be fully functional)

Homework: Image processing

Process your own images (5 points):

- Use 2-3 of your or a colleagues images (or our images uploaded to this module on canvas)
- Build a pipeline to:
  - Save a modified image for each input image
  - Extract data: at least 3 measurements of your choosing to save to .csv file

Include: Meaningful notes, attempted multiple modules

Suggestions:

Use .tif files

Be careful with metadata and Names and Types (this is usually where problems happen)

Don't be ambitious - only use a few images at most and limit the number of modules used.

Post on the forum for help

Use ImageJ to pre-processes images if needed

Work together

*Utilize all resources at your disposal

Homework: Questions

Answer the following questions in less than 600 words. Include references. (20 points)

1. Where do you see image analysis fitting into your future project? What data you hope to collect from your images (if you use images)?
2. What was the most challenging part of working through the example in CellProfiler? Why was it challenging?
3. Find an image from a recent paper you read. What type of data did they collect from the image and what technique/software did they use to analyze it? Provide the reference.