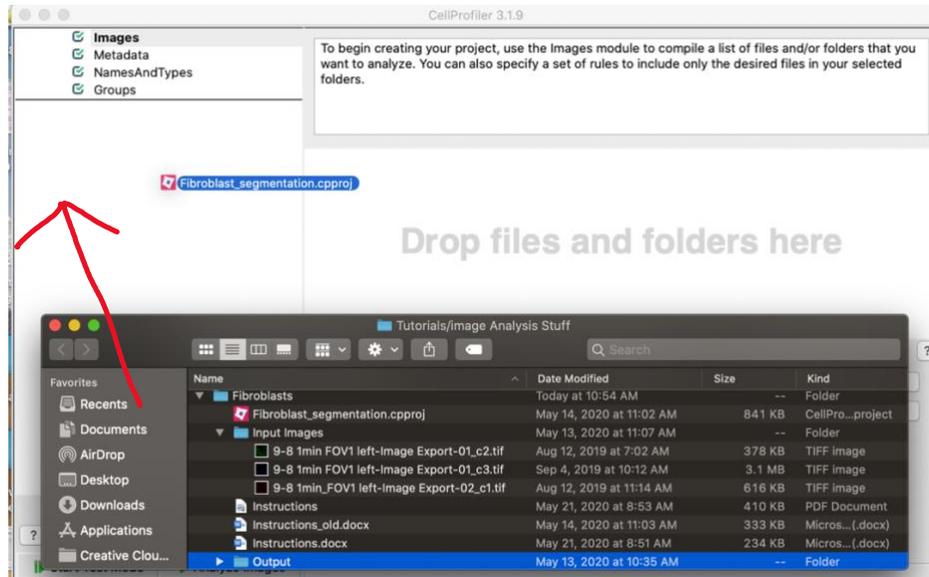


Getting Started with CellProfiler, The Basics

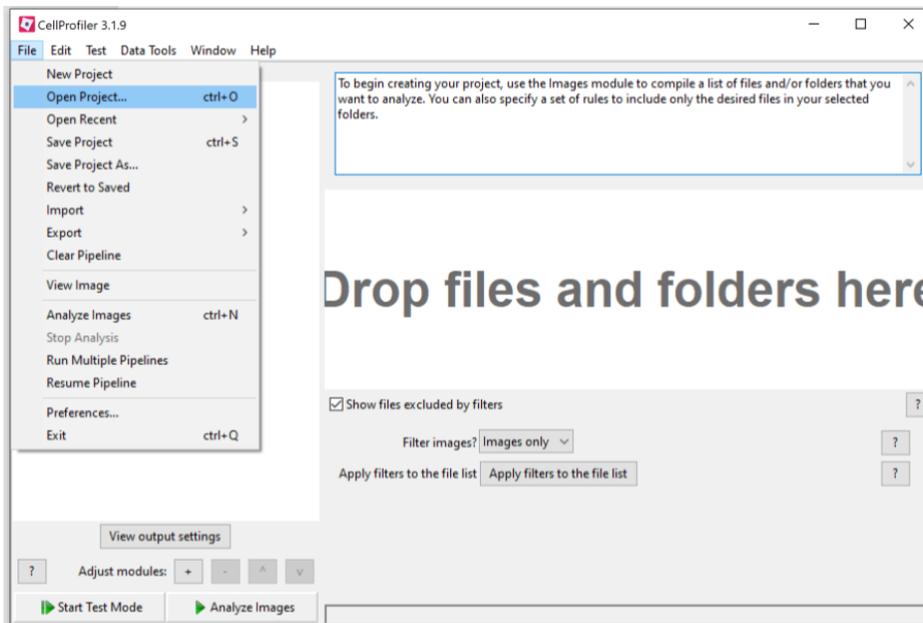
- Opening Previously Created Pipelines
- Clearing Images from a Pipeline
- Adding New Images to a Pipeline
- Viewing Images
- Metadata Module
- Adding or Removing Modules
- Changing the Default Output Folder Location
- Testing and Adjusting a Pipeline
- Start Analysis

Opening Previously Created Pipelines

Previously created pipelines may be opened by dragging and dropping them into the section of CellProfiler indicated by the red arrow below. This can be done even if a pipeline is already loaded into that section.



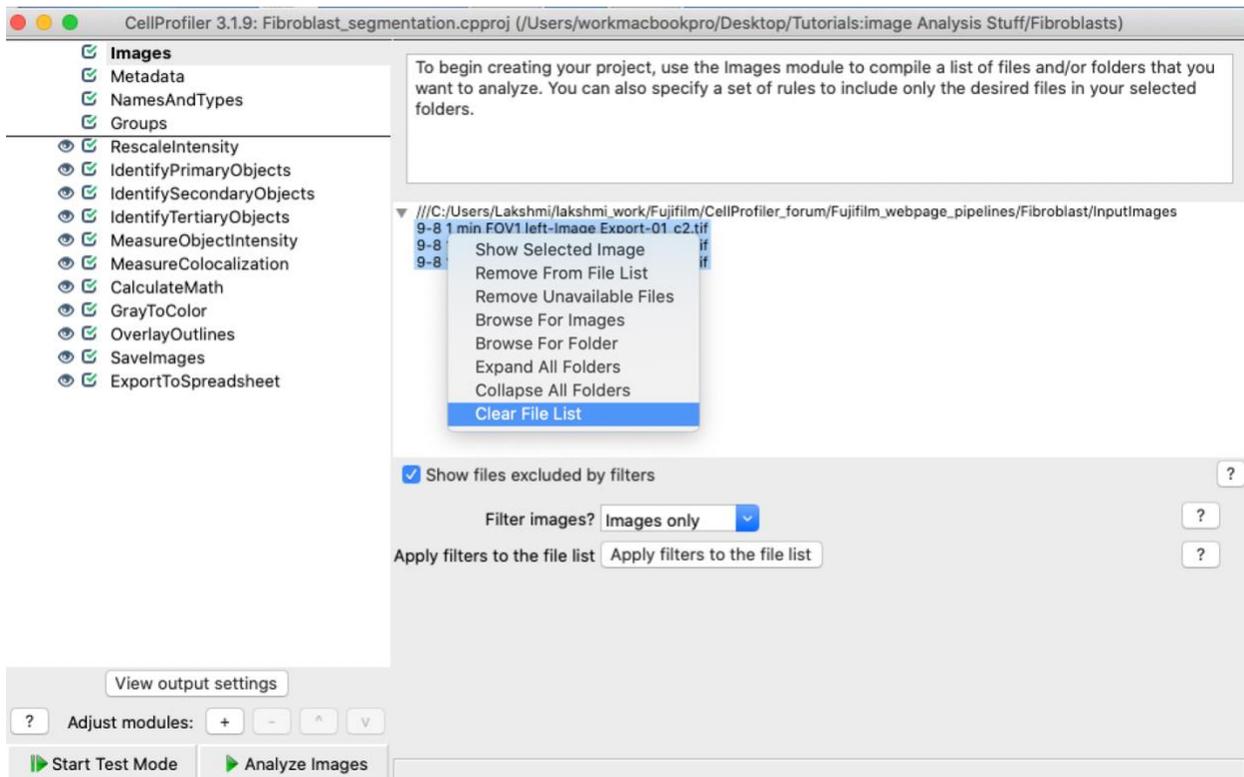
An alternate way to open a previously saved pipeline is to use File>Open Project



Clearing Images from a Pipeline

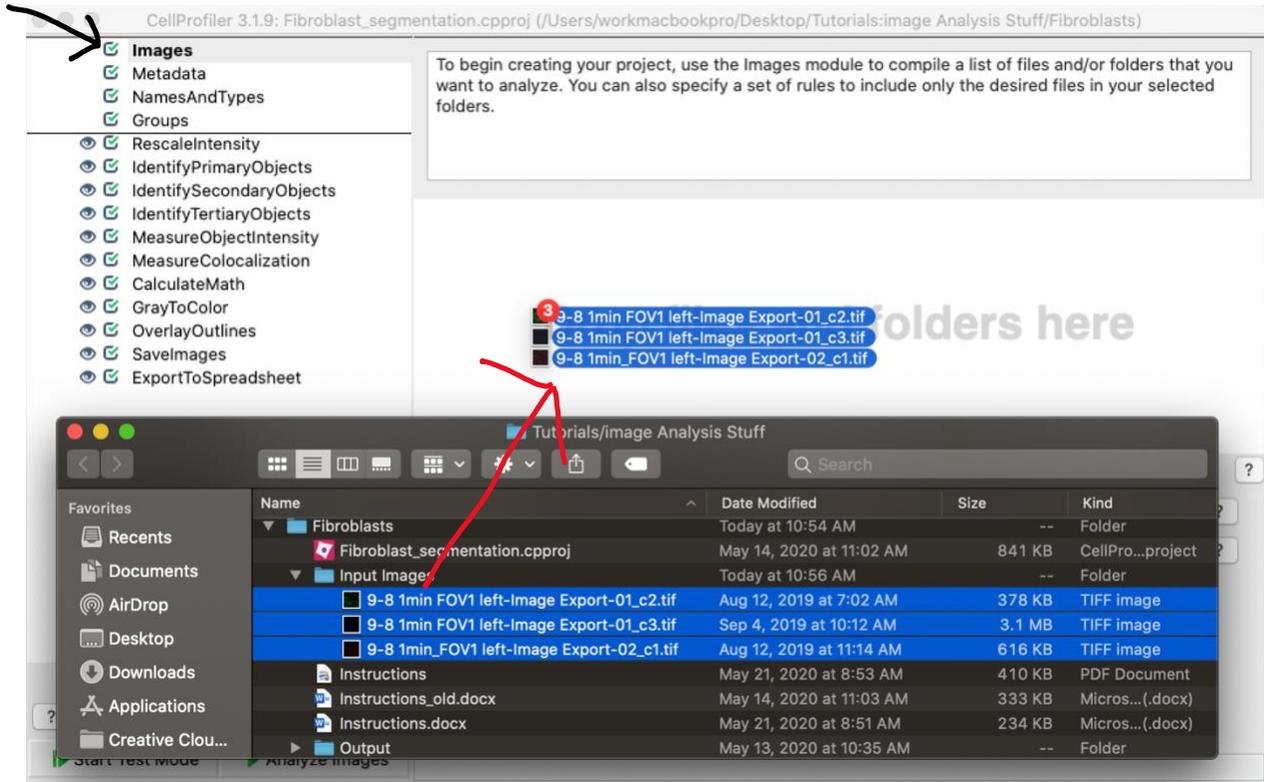
If a previously created pipeline was created on a different computer, the files structure indicating the location of images loaded into the pipeline will not match the new computer. They must be removed and loaded from the new computer.

Drag over the images or click the first and last image in the list while holding the shift key to highlight them all. Right click over the highlighted area and select “Clear File List”



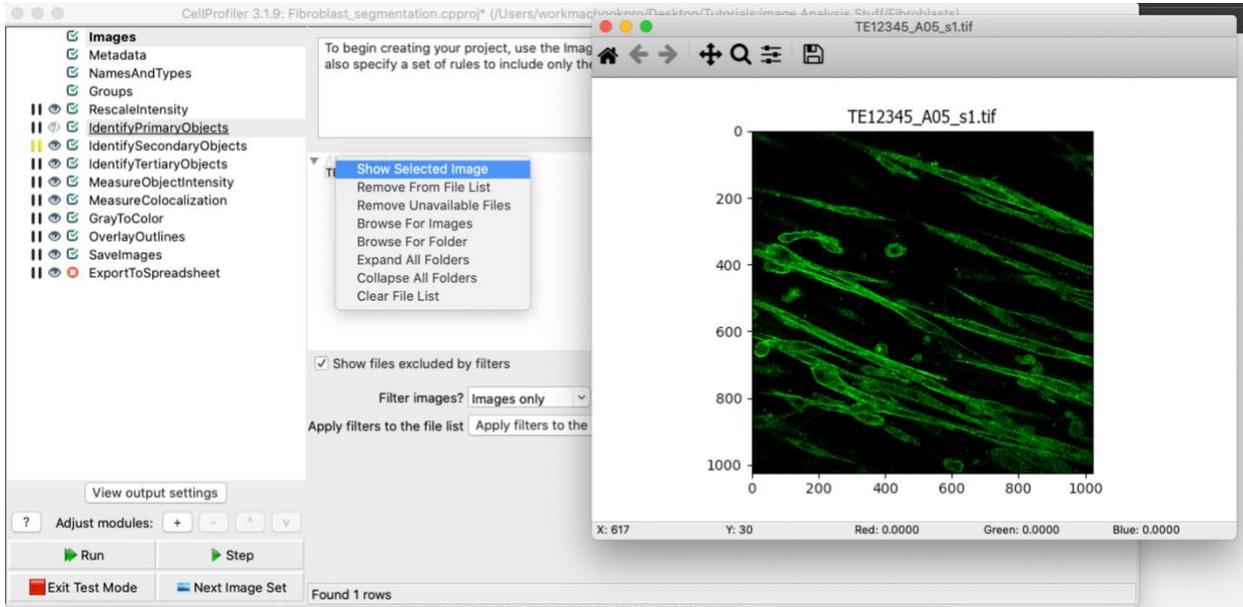
Adding New Images to a Pipeline

Images may be loaded by dragging and dropping them into the proper CellProfiler window. Click on the “Images” module located in the top left and then drag images to the location indicated by the red arrow below.

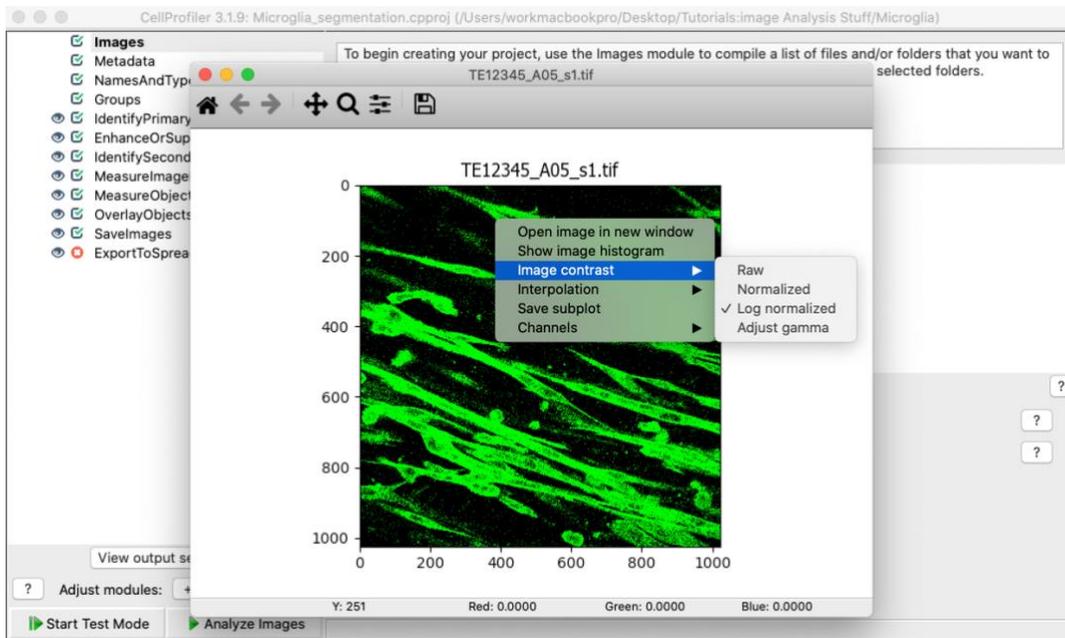


Viewing Images

To view an image, simply right click on the image file and choose “Show Selected Image”.

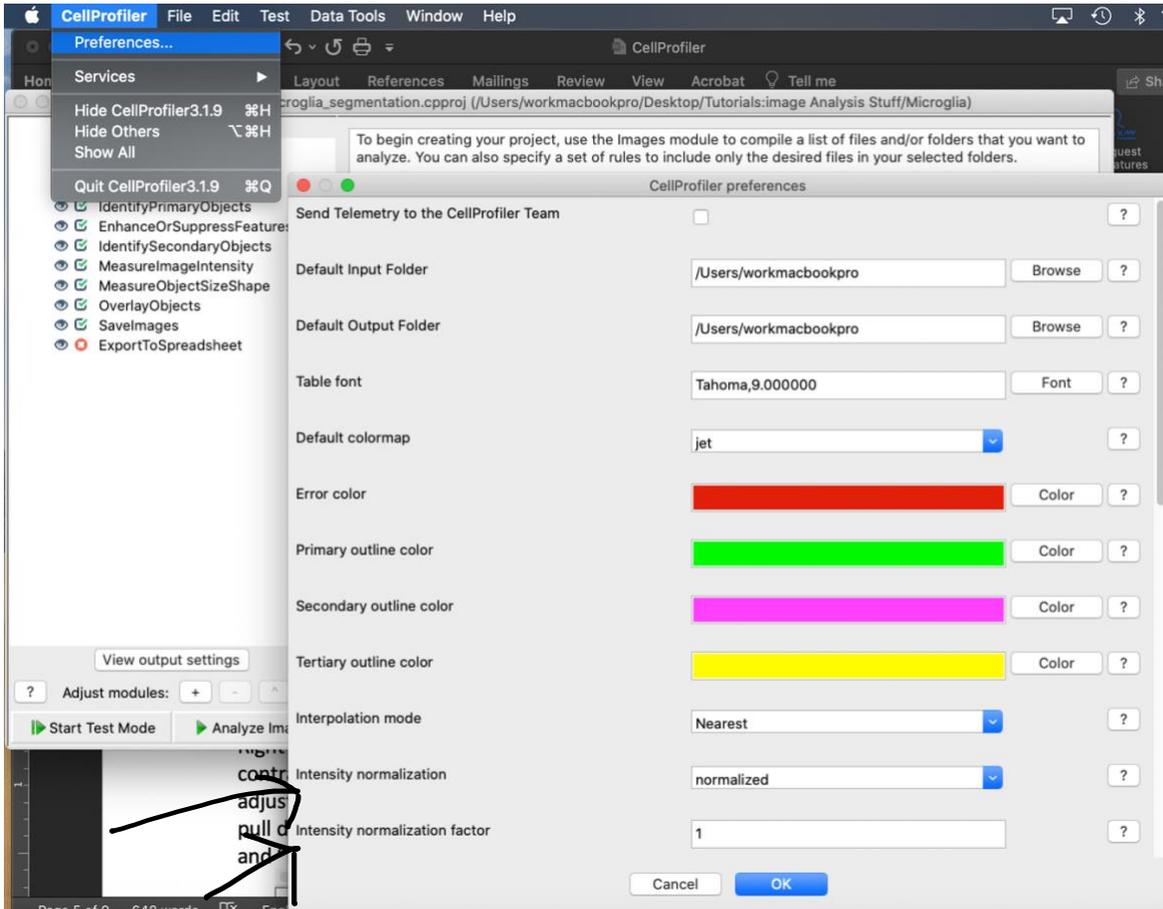


If an image appears dark, light, solid black, or solid white, try adjusting the image contrast. Right click on the image and choose “image contrast” and one of the options to adjust image contrast. You may also right click on images that are displayed when testing the pipeline to adjust contrast.



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To globally adjust contrast, so your settings will stick, select the “CellProfiler” pull down menu, select “Preferences” and adjust the “intensity normalization” and “intensity normalization factor” lines. The main CellProfiler window must be selected before selecting “CellProfiler”. If the focus is on one of the CellProfiler test popup windows, the “Preferences” option will not be shown. Please click on the main CellProfiler window first.



Metadata Module

This module is not required, and it is sometimes possible to select “no” for this module and still group data using the “NamesAndTypes” module.

To set an expression to enable CellProfiler to interpret metadata from a file name or folder click on the button indicated by the red arrow below. Typing directly into the field in the main window will sometimes cause strange behavior within CellProfiler.

When the expression is typed correctly the “Test text” will be displayed in the correct representative colors in the space indicated by the blue arrow below. In addition, after clicking “Submit”, when you hit the “Update” button the metadata will be extracted and displayed.

CellProfiler 3.1.9: Microglia_segmentation.cpproj* (/Users/workmacbookpro/Desktop/Tutorials:image Analysis Stuff/Microglia)

Regular expression editor

Regex: `(?P<Plate>.*)(?P<Well>[A-P][0-9]{1,2})_s(?P<Site>[0-9])`

Test text: TE12345_A05_s.tif

TE12345_A05_s.tif

Guess Submit Cancel

Metadata source: File name

Regular expression to extract from file name: `^(?P<Plate>.*)(?P<Well>[A-P][0-9]{1,2})_s(?P<Site>[0-9])`

Extract metadata from: All images

Add another extraction method

| Update | Path / URL | Series | Frame | FileLocation | Plate | Site | Well |
|--------|------------------------------|--------|-------|------------------------------|---------|------|------|
| 1 | /Users/workma...45_A05_s.tif | 0 | 0 | file:/Users/w...45_A05_s.tif | TE12345 | 1 | A05 |

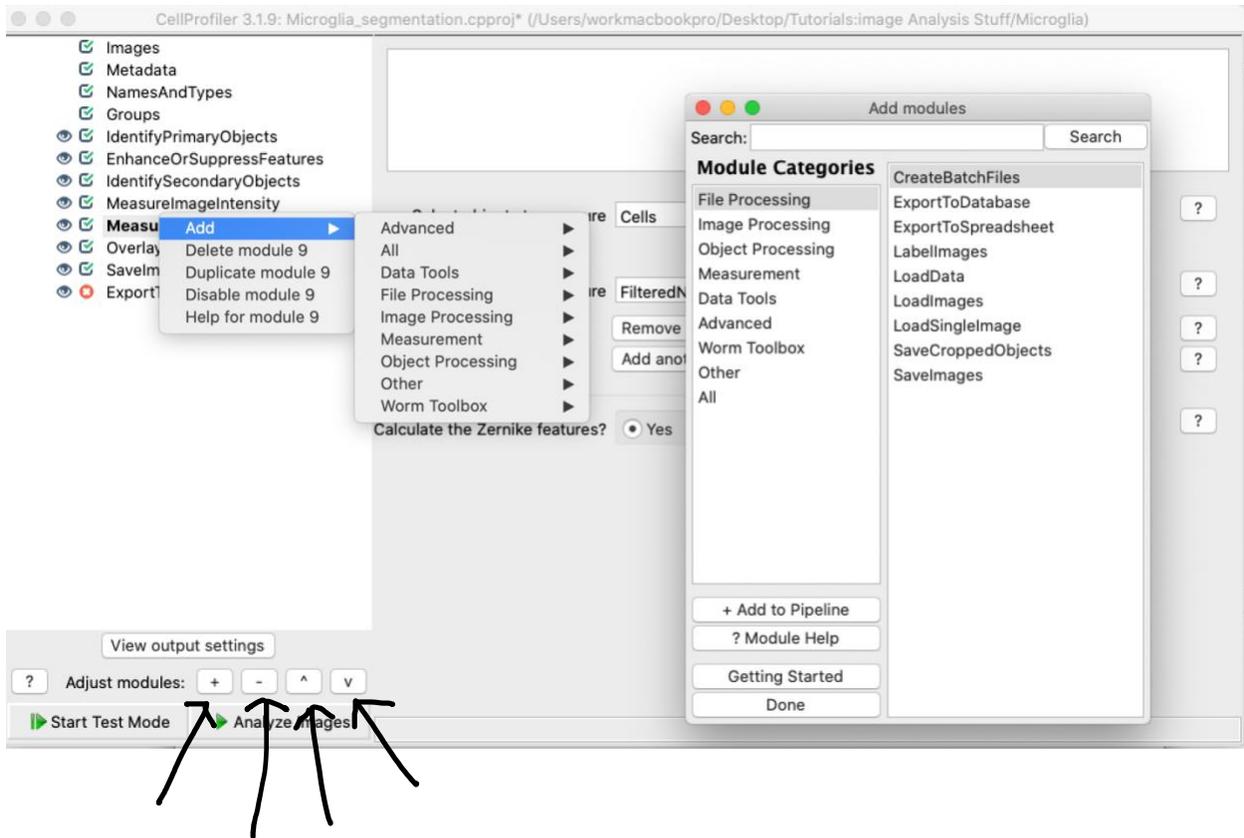
View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images Found 1 rows

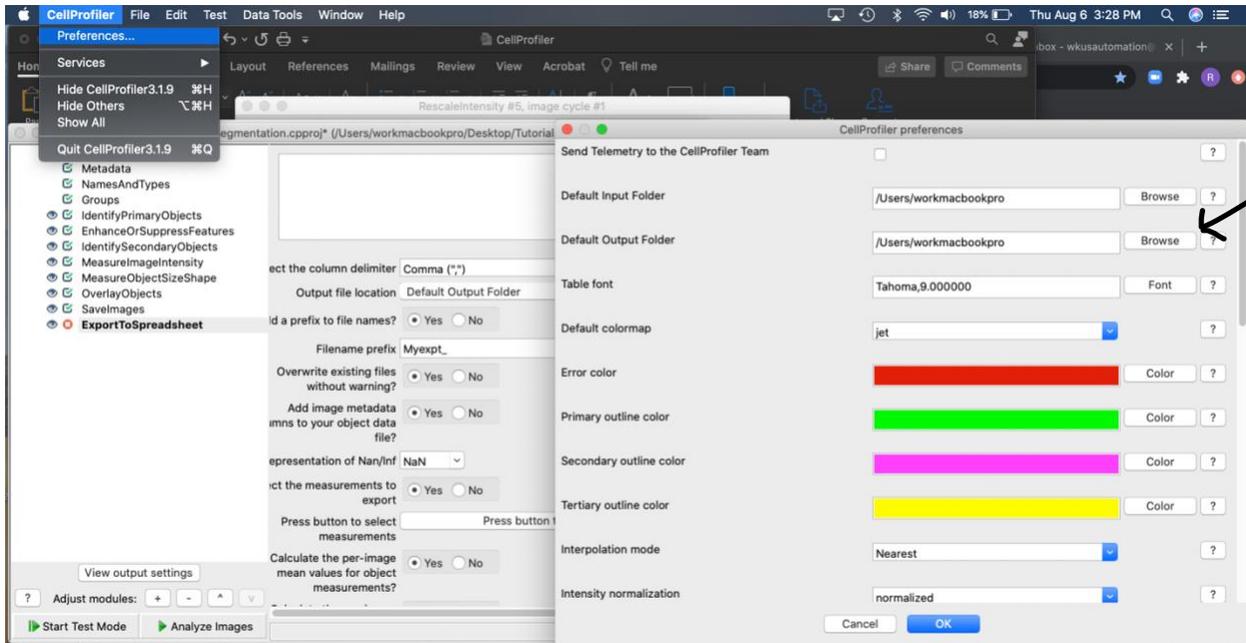
Adding or Removing Modules

To add or remove a module either hit the “+” or “-” buttons at the bottom of the window, or right click a module and select “Add” and then select the appropriate module, or “Delete module x” to delete the module. You may also move a module’s position in the pipeline by selecting a module (ensure it is highlighted in blue) and hitting the up or down buttons located next to the “+” and “-” buttons.



Changing the Default Output Folder

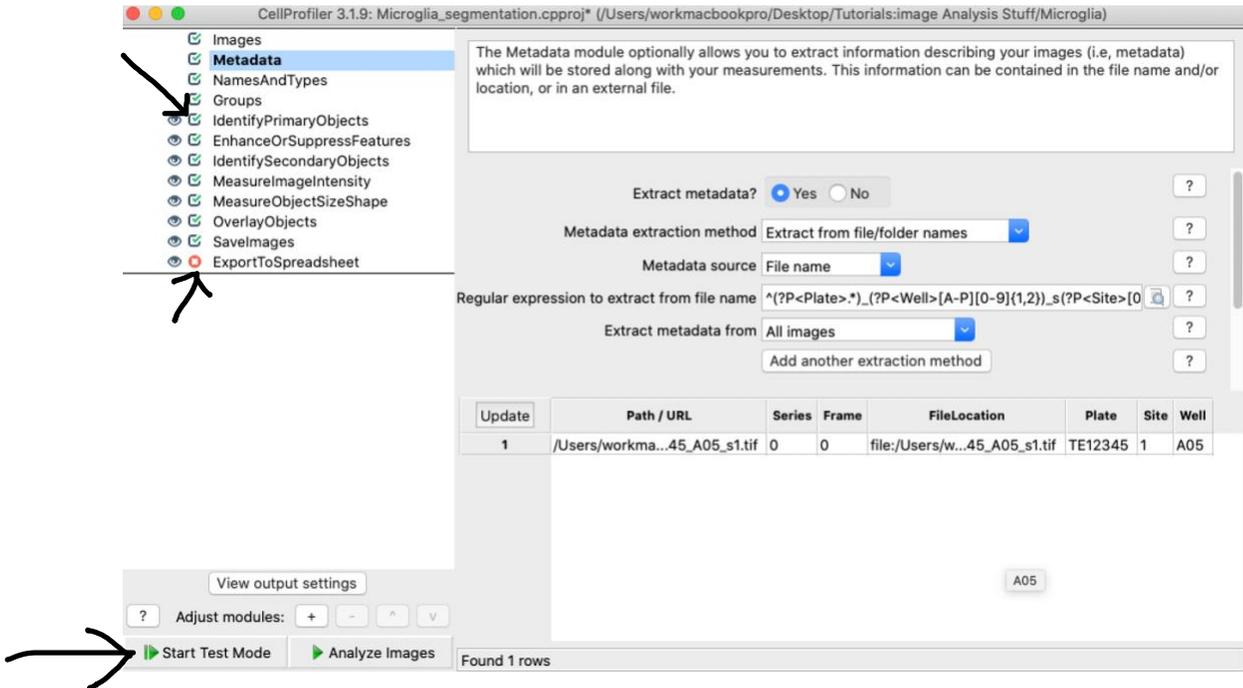
To change the default folder where data and images are output from CellProfiler, select the “CellProfiler” dropdown menu and select “Preferences”. Then select “Browse” next to the “Default Output Folder”.



Testing and Adjusting a Pipeline

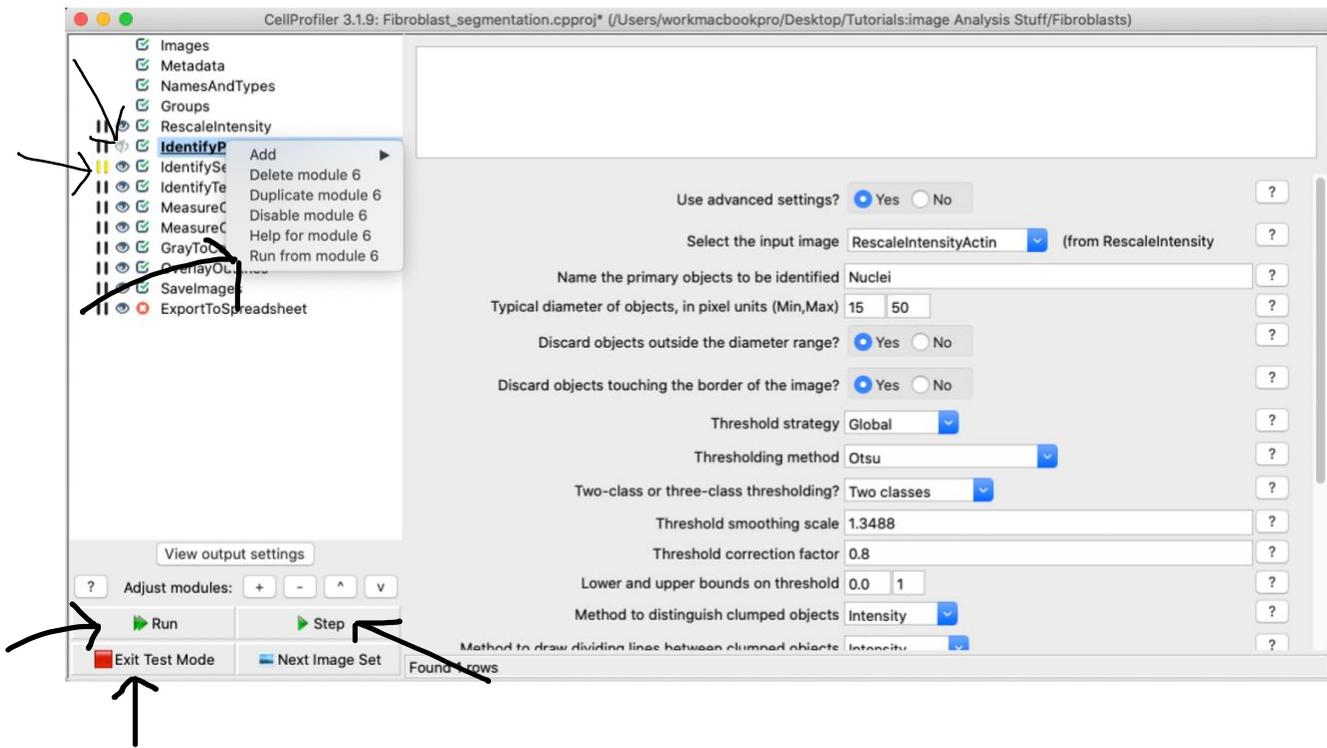
The only way a pipeline can be optimized is by trial and error, and testing the results of each change. In order to do this CellProfiler needs to be put into “Test Mode”. This is done by pushing the “Start Test Mode” button.

A green checkmark will display next to modules that have no errors. A red “x” will appear next to modules that contain an error. The ExportToSpreadsheet module always has a red “x” next to it. This is normal.



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Once in test mode the easiest thing to do is to use the “Step” button to step through each module. The last module tested will contain a black underline. Continuing to hit the “Step” button will only allow you to test the next module (the one under the module with the black underline). Right clicking any module at or above the module with the black underscore will enable you to run the pipeline from that step by selecting “Run from module x”. You may not start at a module below the one containing the black underline. If you try, the “Run from module x” choice will not appear. To control where CellProfiler stops, click the pause button (indicated by two vertical lines) next to the module you wish to stop at. The pause button will turn yellow. Clicking the “eye” icon next to a module will prevent that modules window from popping up during testing. It will turn grey. This can help prevent a clutter of windows from popping up on your desktop. You can also test modules by using the “Run” button located next to the “Step” button, but be sure the module you’d like to start at is **highlighted in blue** or CellProfiler may start at the module containing the black underline. When you are finished testing you may hit the “Exit Test Mode” button.



Start Analysis

To begin analysis, ensure you are no longer in “Test Mode” (Click “Exit Test Mode”), and click the “Analyze Images” button.

