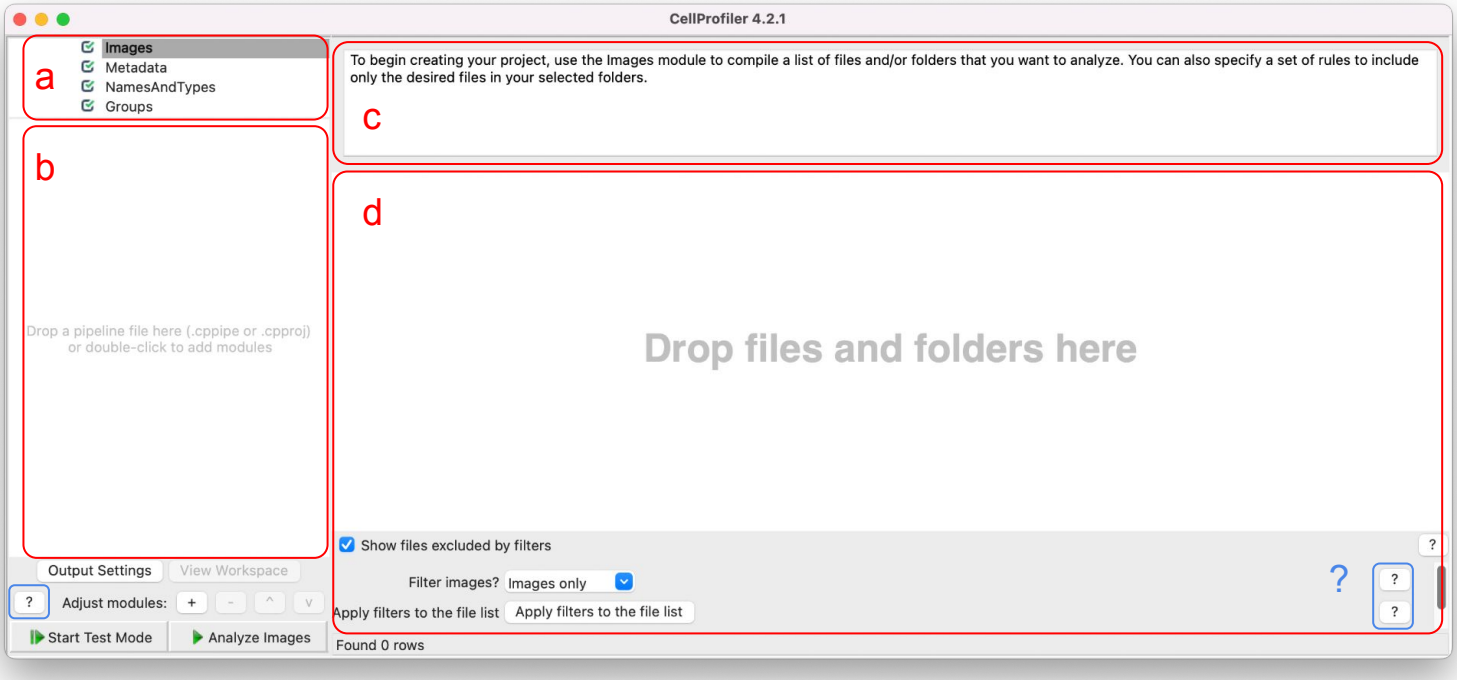
CellProfiler™
cell image analysis software

CellProfiler is a free, open-source software for image analysis, developed and supported by Anne E. Carpenter at the BROAD Institute. More information and software available at https://cellprofiler.org
For in depth tutorials and training, check videos published by the Center for Open Bioimage Analysis (COBA): https://www.youtube.com/c/COBACenterforOpenBioimageAnalysis

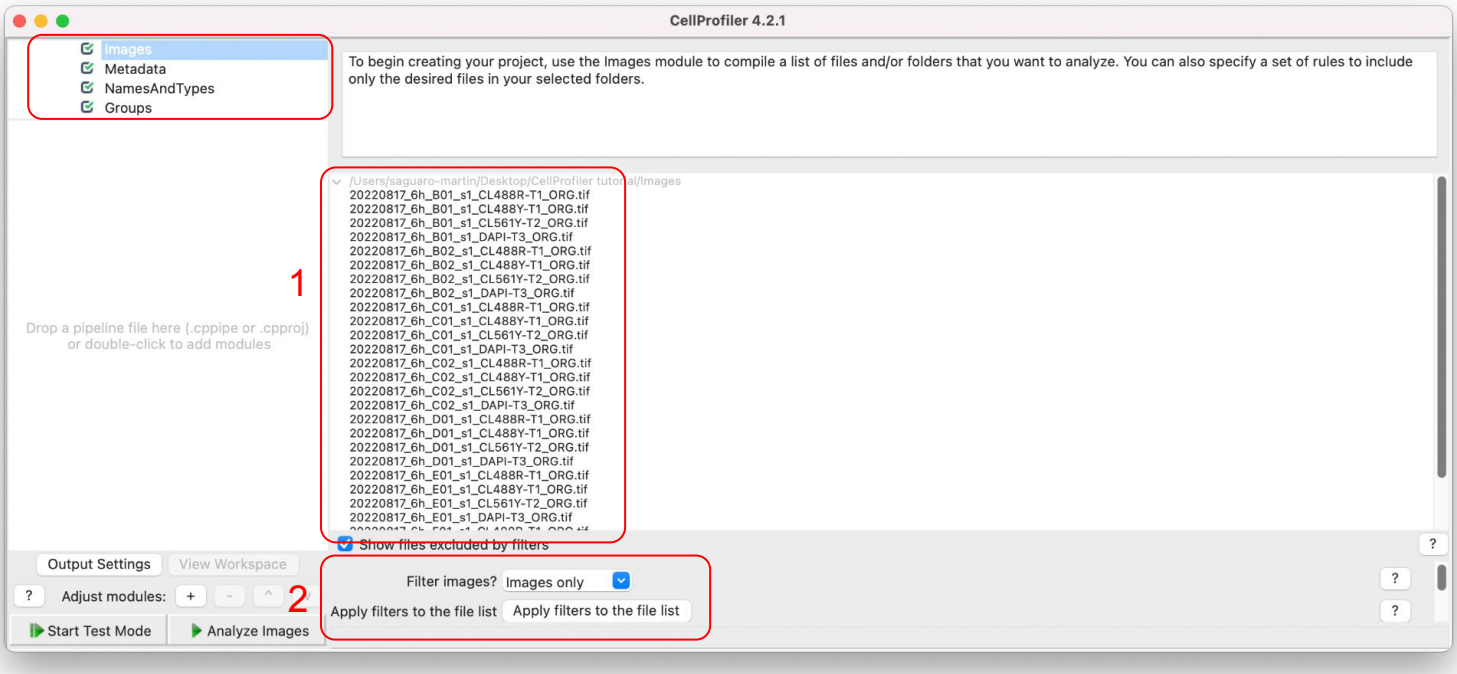
IMPORTANT NOTE: For proper image analysis and coherent measurement of features, images must be supplied as original files, free of value modification (brightness/contrast adjustment), filtering and other deconvolution calculations. Image values must be absolute and comparable inside a same data set (same bit depth, same acquisition parameters for instance)

1) CellProfiler user interface overview



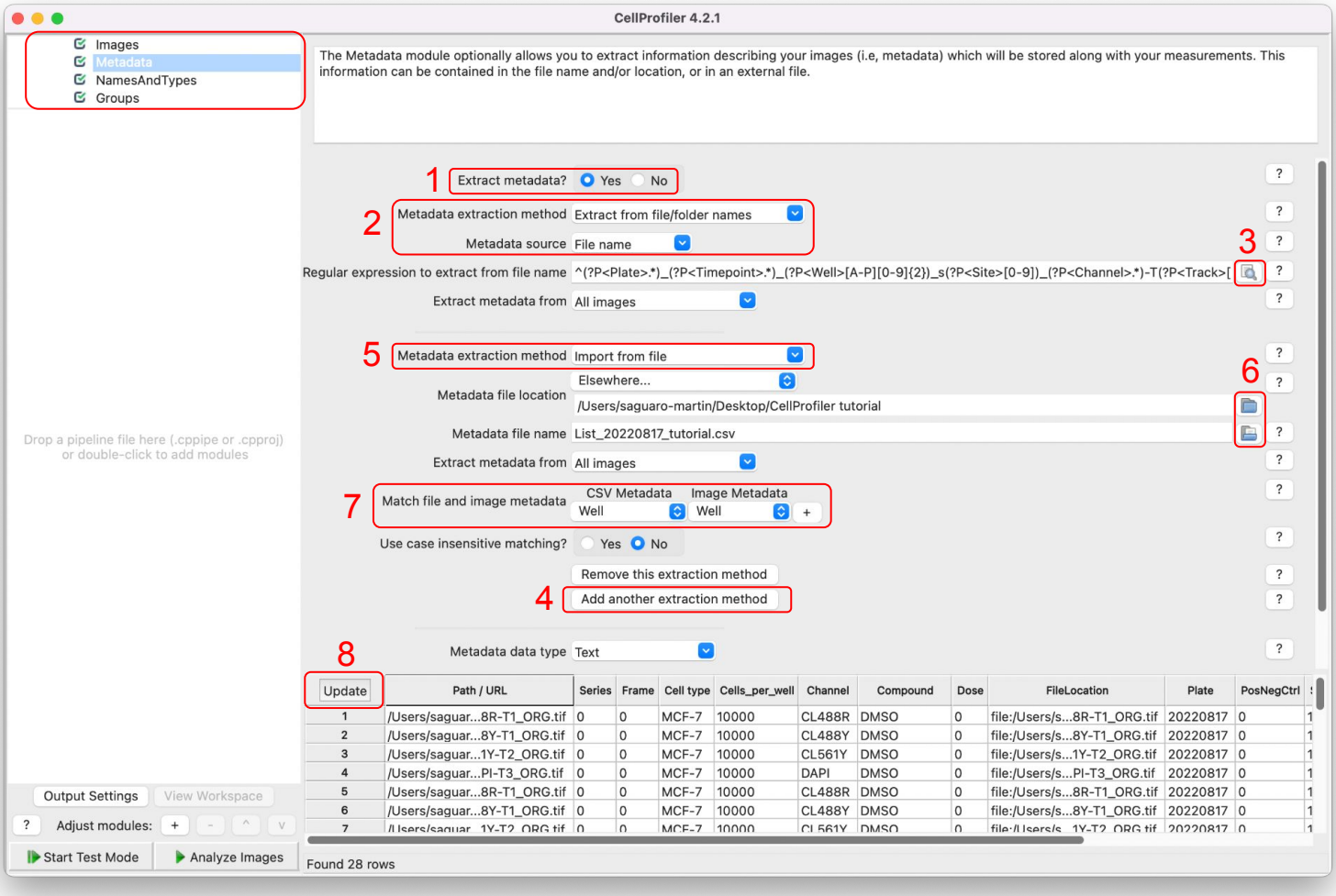
a- Input modules, for image selection, metadata retrieval and integration
b- Image analysis pipeline window. Add modules to build your image analysis pipeline
c- Brief description of the module in use, either provided by the software or the user. Very helpful to review past pipelines, indispensable when sharing/publishing pipelines.
d- Settings windows, specific to each module
?- “Help” buttons throughout the user interface. The “Help” button on the left provides global help for the selected module. To the right, a button is available for each setting, providing a more specific description of the corresponding setting. Help is never far, and always available!

2) Adding image files/folders



Add files and/or folders by simply dragging and dropping (1). Here the “Images” folder was selected. Additional files/folders can further be added to the list. Right-clicking on elements of the file list allows you to select or delete them (from the list). By default, only image files are selected. Custom filters can further be added to select specific files (2).

3) Extracting metadata



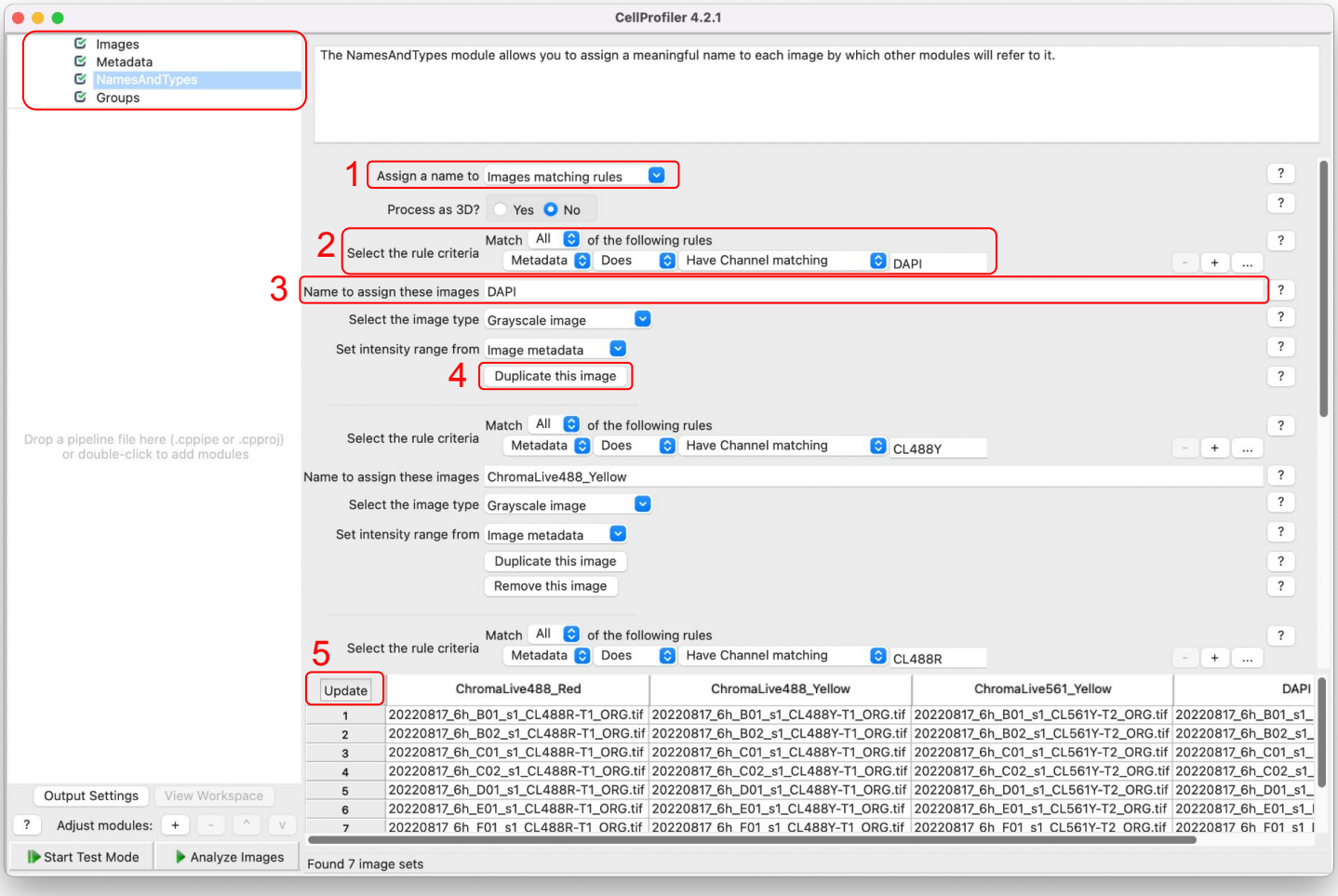
In this example, metadata (more specifically, information regarding the plate, well and channel) is extracted (1) from the filename(2) via a regular expression (3) (see Note at the end of this section). Additionally (4), a .csv file (5) containing further information has been provided (6) and matched well to well (7). This provides metadata for each image, which will help direct the right analysis modules to the right images, and complement the extracted measurements. The extracted metadata can be previewed at anytime by updating the bottom table (8).

NOTE: Extracting metadata from the filename requires the use of a regular expression. More information can be found on this subject, but here is the regular expression for this particular example:

Filename: 20220817_6h_B01_s1_CL488R-T1_ORG.tif

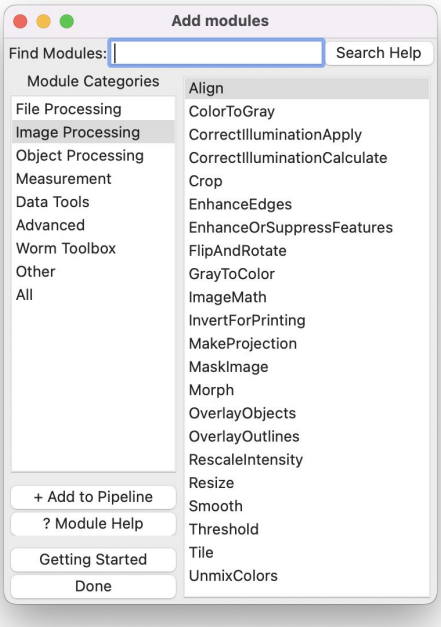
Regular expression: ^(?P<Plate>.*)_(?P<Timepoint>.*)_(?P<Well>[A-P][0-9]{2})_s(?P<Site>[0-9])
(?P<Channel>.*)-T(?P<Track>[0-9])(?P<other>.*)

4) Assigning Names and Types



This module allows you to assign names to specific images (1). For example, the previously extracted metadata (2) assign a desired name (3) to the selected images. By duplicating this filtering (4), image sets can be created containing multiple images. Again, the “Update” button (5) shows a preview of these image sets.

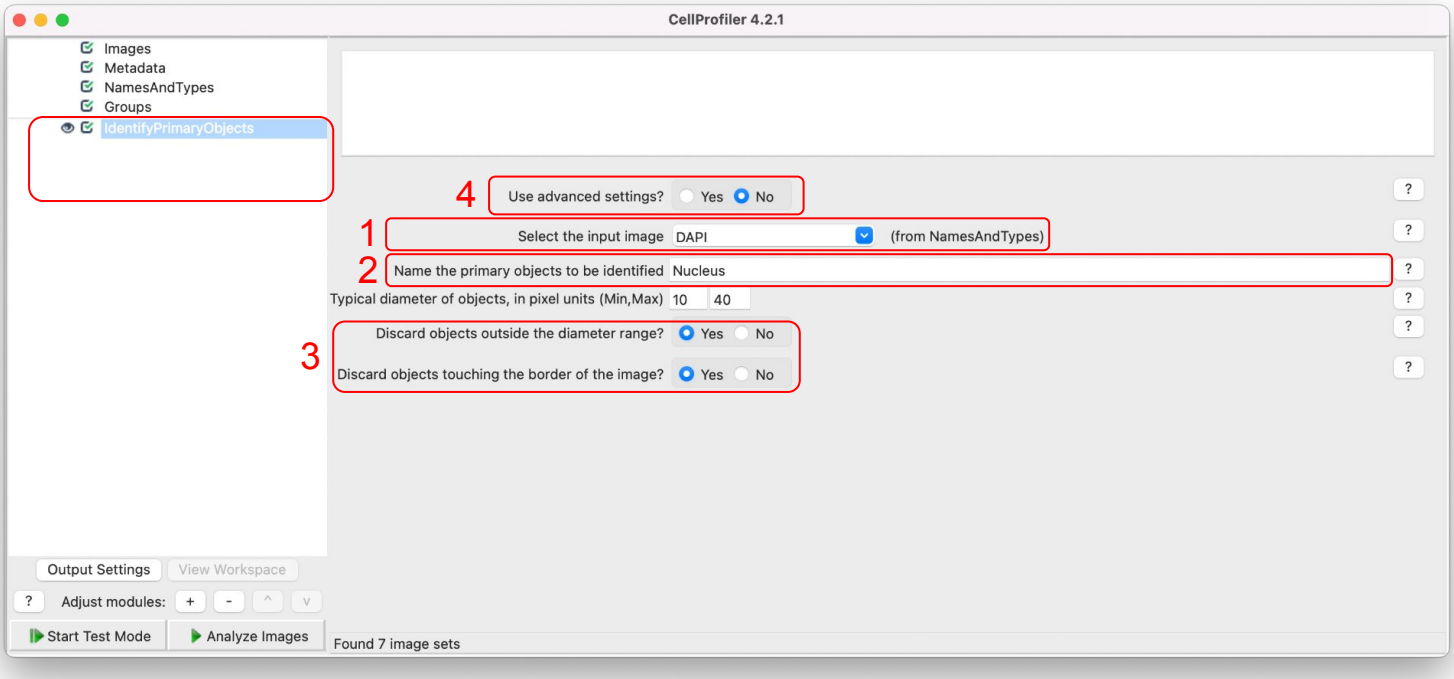
5) Adding modules to the analysis pipeline



Modules can be added to the analysis pipeline by simply clicking the “+” button by “Adjust modules:”, at the lower left of the screen. The popup window provides a search field to find modules, or they can be found in their corresponding modules category. Double-clicking a module adds it to the pipeline. Module can also be dragged inside the pipeline to reorder them. Again, help is also available to learn about each module.
It can be necessary to start an analysis pipeline with preliminary modules such as “Align” in case of shifted images, or “CorrectIllumination” in case of vignetting for example.

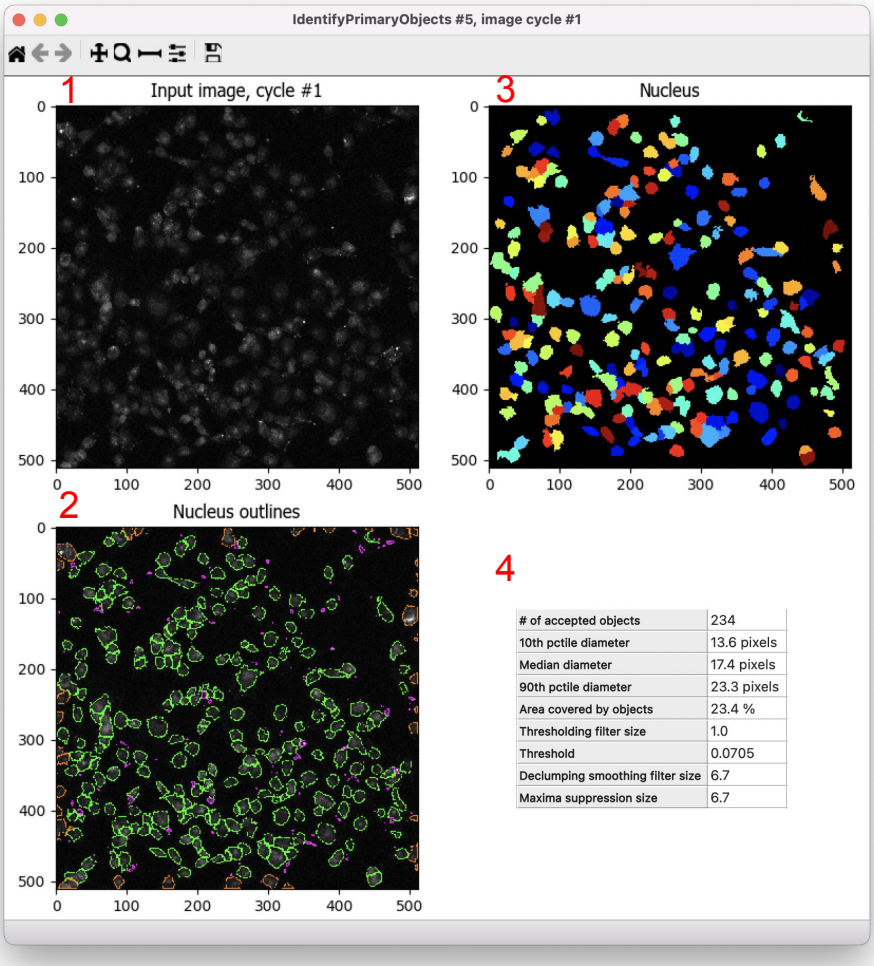
6) Identify primary objects

In our case, our first module of interest is the “IdentifyPrimaryObjects”, located in the “Object Processing” category. In this module, we segment the cell nuclei with the help of nuclear staining. This is done by selecting the proper image (1), as identified in the NamesAndTypes section, and naming the resulting object (2). It can be useful to discard objects outside the diameter range or touching the image border as they can generate false non-relevant objects (3). Advanced settings are also available in order to obtain more precise and thorough image segmentation and object identification (4).



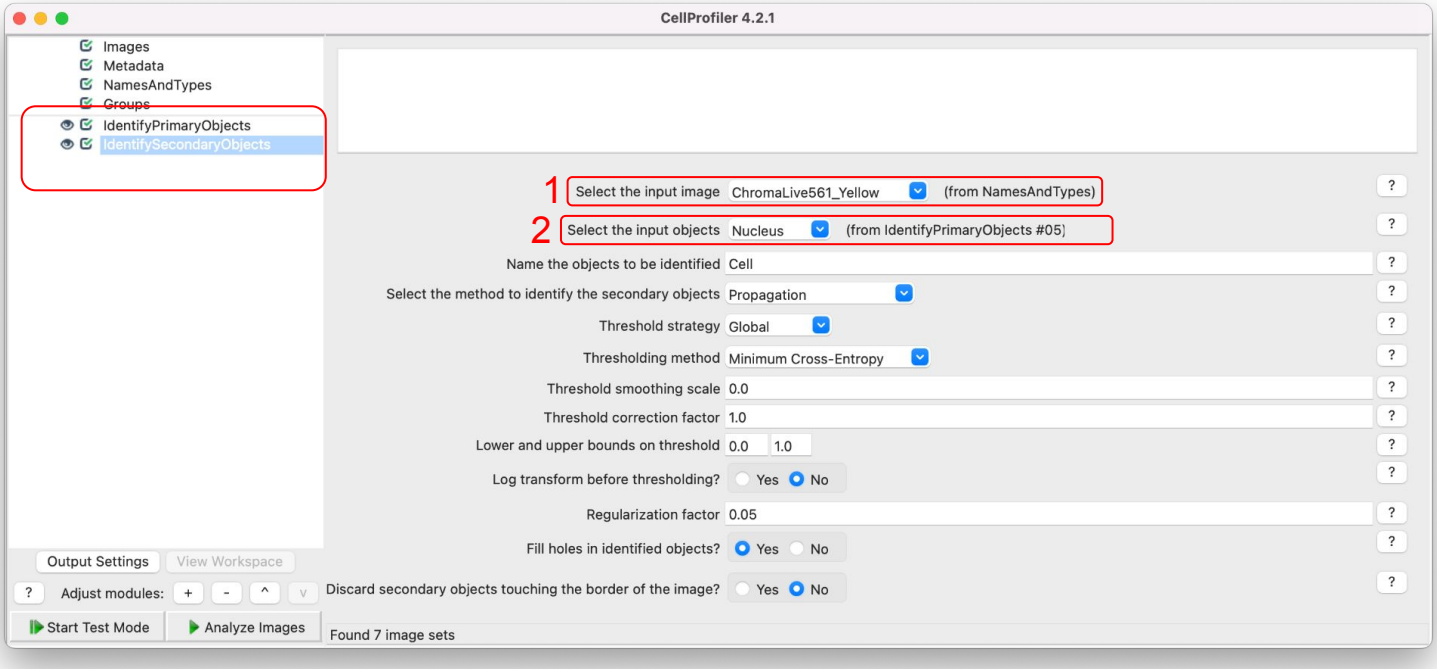
7) Test mode, to preview segmentation settings

Once preliminary settings are set in the module, it can be useful to use the “Start Test Mode” feature of CellProfiler, located at the bottom left. CellProfiler will then run through each module step by step (by clicking successively the “Next Step” button), and display the results of the ones selected (eye icon next to the module).
In the case of the “IdentifyPrimary Object” module, this will present the input image (1), as well as an overlay of the object outlines (2) and a final color image with the different objects represented (3). Finally a table presents some statistics of the segmentation (4).
“Next Image Set” moves through the image sets defined in the “Names AndTypes” module previously.
“Exit Test Mode” allows you to end the test run and return to the pipeline.

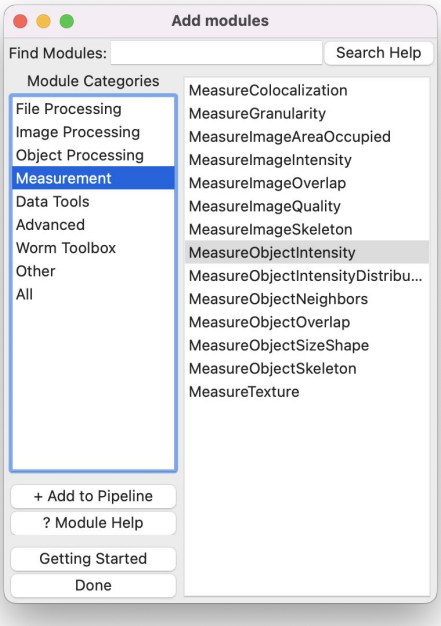


8) Identify secondary objects

Building upon our primary object (Nucleus), CellProfiler can then identify whole cells. This is done by selecting the input image (1), here ChromaLive561 shows the best whole cell staining, and selecting the object to build from (2) (here: Nucleus). Other settings can be adjusted for optimal cell segmentation. Again, perform test runs with the “Test Mode” feature optimise relevant settings.



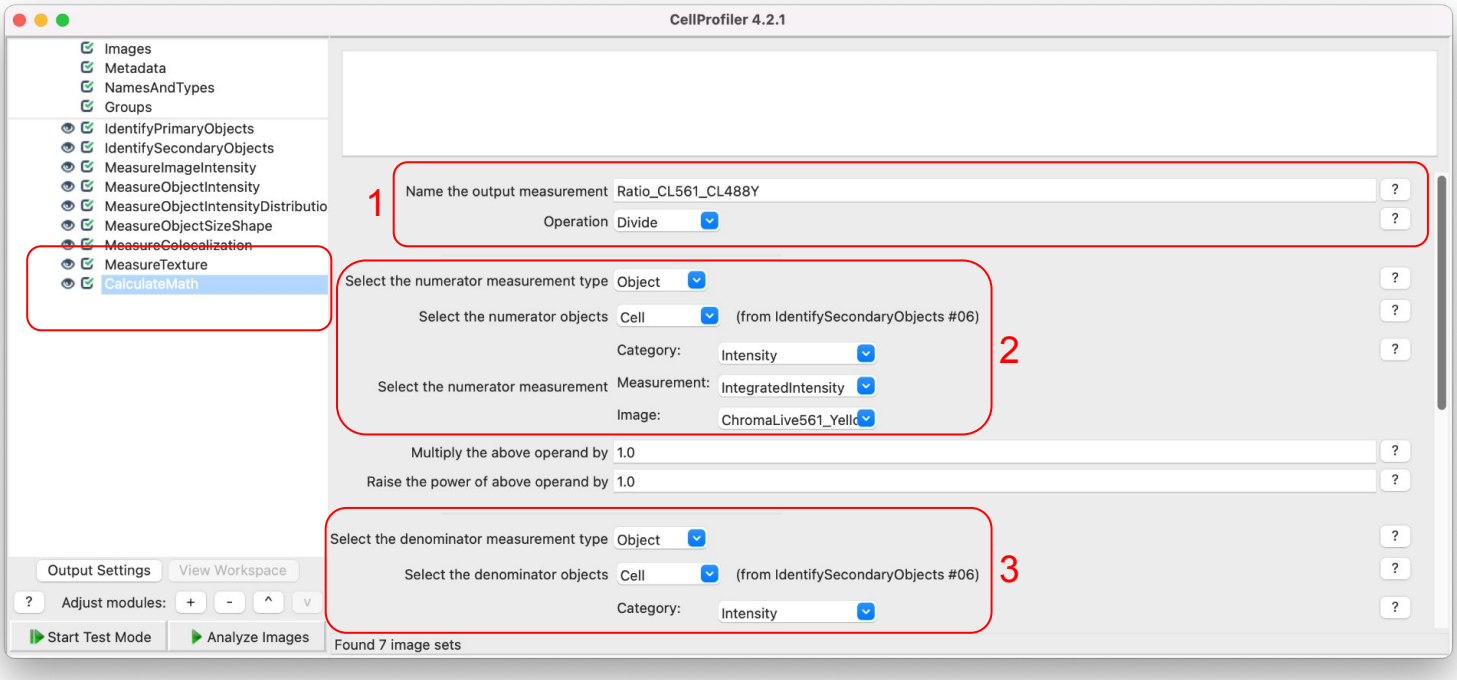
9) Adding measurement modules



Once all the objects of interest have been selected and identified, it is time to add measurement modules. The following screenshot shows a list of modules from the “Measurement” category. The guiding principle here is to gather as many measurements as possible for further data analysis.
In the case of ChromaLive dyes, we recommend measuring fluorescence intensity as well as shapes and sizes of objects as a bare minimum. Object texture (influenced for example by the presence of highly fluorescent vesicles in the cytoplasm, or condensing DNA in the nucleus) can also be a relevant measurement.

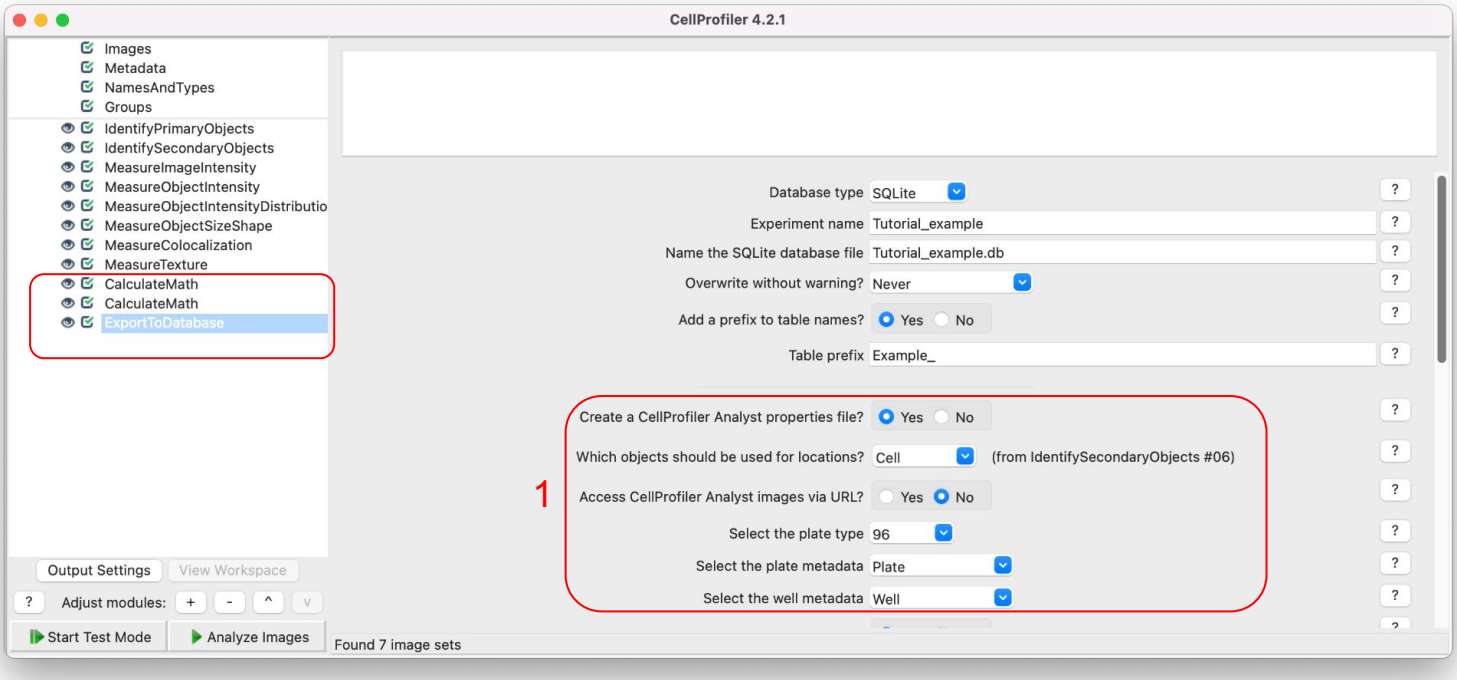
10) Calculating some math

Depending on the phenotype, and the ChromaLive dye used, it can also be relevant to include some extrapolated measurements. These measurements can be obtained via the “CalculateMath” module, in the “Data Tools” category.Here we are normalising fluorescence measurements, by dividing the intensity from one acquisition (ChromaLive561) by another (ChromaLive488_Yellow).
Name and define the desired calculation (1). From the previously performed measurements, select the type of measurement for the numerator (2) and similarly for the denominator (3).
Add additional modules if other calculations are needed.



11) Exporting measurements and saving files

By default, CellProfiler does not save any, it only offers to save the designed analysis pipeline. To export measurements or save images, additional modules have to be added to the pipeline. These can be found in the “File Processing” module category. Here is the example of the “ExportToDatabase” module which exports data compatible with the subsequent use of CellProfiler Analyst via a generated properties file (1).



12) Masking modules and running pipeline

Once the pipeline is designed and tested, it is time to run it on the entire image set. To avoid being saturated with popup modules, it is recommend to hide all the module (greyed eye icon next to each one). This can be achieved by the “Windows”>”Hide all windows on run” menu. Then click the “Analyse images” button in the lower left. Processing time will depend on the pipeline, the available computing power as well as the number and size of the images to analyse.