

Automatic quantification of nuclear staining colocalization

This pipeline is for analysis of LSM files of nuclear staining.

- 1) **CellProfiler** from <http://cellprofiler.org/> available for OSX Windows and Linux

Carpenter, Anne E., Thouis R. Jones, Michael R. Lamprecht, Colin Clarke, In Han Kang, Ola Friman, David A. Guertin et al. "CellProfiler: image analysis software for identifying and quantifying cell phenotypes." *Genome biology* 7, no. 10 (2006): R100.

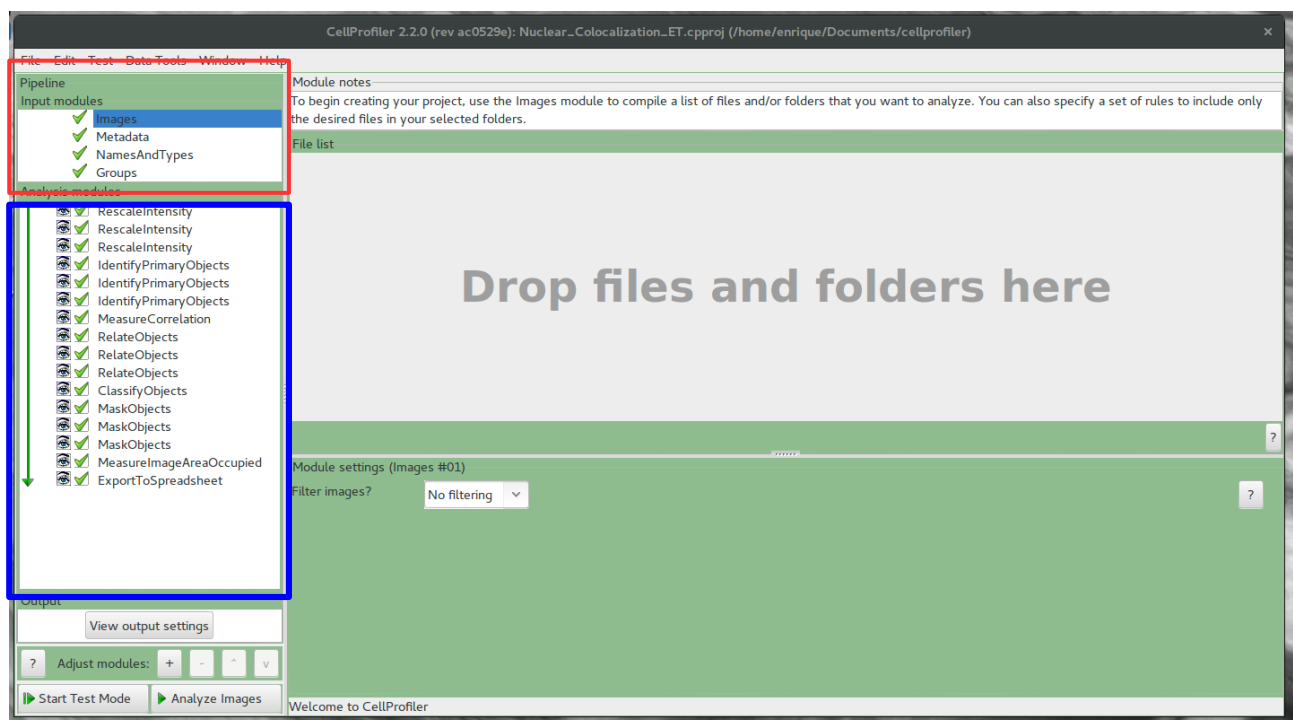
- 2) **Configure CellProfiler**

In File → Preference, set the default folder for the output

- 3) **Load Pipeline file.**

This file contains the instructions for the whole procedure

File → Import → Import Pipeline from File... → *Nuclear_Colocalization_ET.cppipe*



In Red are the input modules that load the files and extract the metadata

In Blue are the analysis modules for the pipeline

- 4) **Load Images (Input module - Images)**

- a) Right click where it said “Drop files and folders here”, you can also drag and drop them.
- b) Select to show all files, not only image files.
- c) Select all images to quantify and press open.

5) Input Module -Metadata

Module settings (Metadata #02)

Extract metadata? ☒ Yes ☐ No

Metadata extraction method: Extract from image file headers

Extract metadata from: All images

Update metadata

Add another extraction method

Metadata data type: Text

Update	Path / URL	Series	Frame	C	ChannelName	ColorFormat	FileLocation	SizeC	SizeT	SizeX	SizeY
1	/home/enrique...OXA2_20-1.lsm	0	0	0	Ch1-T1	monochrome	file:/home/en...OXA2_20-1.lsm	4	1	1024	1024
2	/home/enrique...OXA2_20-1.lsm	0	1	1	Ch2-T1	monochrome	file:/home/en...OXA2_20-1.lsm	4	1	1024	1024
3	/home/enrique...OXA2_20-1.lsm	0	2	2	Ch1-T2	monochrome	file:/home/en...OXA2_20-1.lsm	4	1	1024	1024
4	/home/enrique...OXA2_20-1.lsm	0	3	3	Ch1-T3	monochrome	file:/home/en...OXA2_20-1.lsm	4	1	1024	1024
5	/home/enrique...OXA2_20-2.lsm	0	0	0	Ch1-T1	monochrome	file:/home/en...OXA2_20-2.lsm	4	1	1024	1024

Press “Update metadata” and “Update”. This will populate the channels for each image in to the pipeline.

6) Input Module Names and Types

In ImageJ or Fiji open an image and check that the channel name (Ch1-T1, Ch1-T3, Ch2-T1... etc) and stain

Select the rule criteria: Match All of the following rules

Metadata Does Have ChannelName matching Ch1-T2

Name to assign these images: Green

Select the image type: Grayscale image

Set intensity range from: Image metadata

Duplicate this image

Remove this image

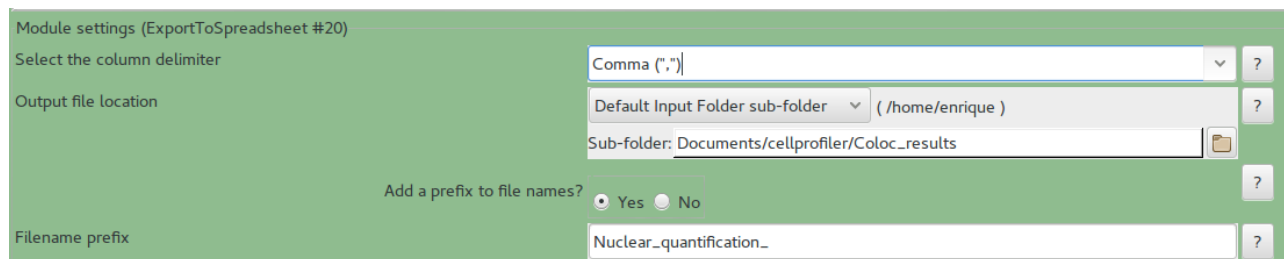
Select the rule criteria: Match All of the following rules

Update

In here, each channel and stain should have a name. You should only change the channel name if it is needed.

Press Update to populate the table below.

7) Analysis module ExportToSpreadsheet



In here, select the right Output subfolder to save the results.

In Filename prefix_ you can change how the files are going to be called, by default all files are called *Nuclear_quantification_(something).csv*. Which can be opened in excel.

8) Analyze Images

Default channels and object names

Channel	Color	Objects	Colocalization with nuclei	Colocalization with
Ch1-T1	Blue	Nuclei	---	---
Ch1-T2	Green	GreenObjects	GreenDAPI	RedGreen
Ch1-T3	Red	RedObjects	RedDAPI	

Press Analyze Images

This will take a few minutes, depending of the images size, number and the computer.

On the output folder focus on the one called *Nuclear_quantification_Image.csv*

On the file the columns of interest are:

Column name	Object counts
Count_GreenDAPI	Green objects with nuclear staining
Count_GreenObjects	Total Green objects
Count_Nuclei	Total Nuclei
Count_RedDAPI	Red objects with nuclear staining
Count_RedGreen	Objects that are Red and Green positive
Count_RedObjects	Total Red objects