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.

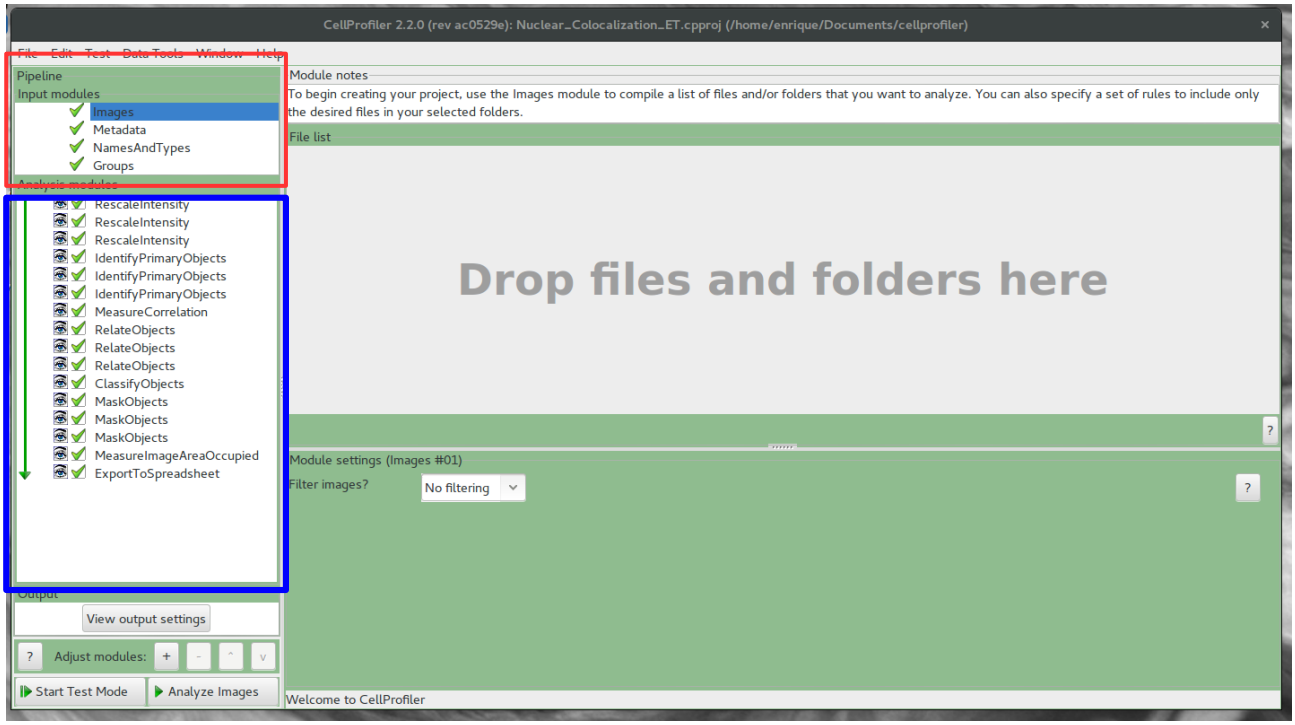
2) **Configure CellProfiler**

   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*

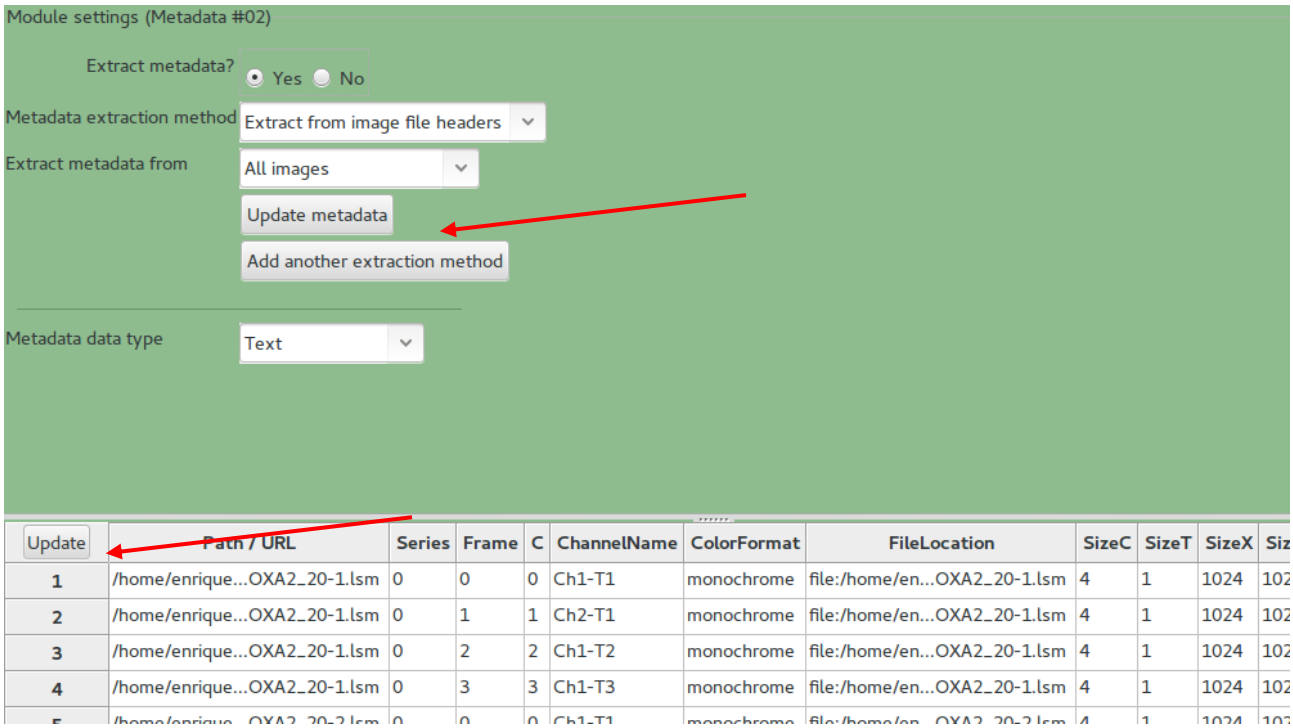


In Red are the input modules that load the files and extract the metadata
In Blue are the analysis modules for the pipeline

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.

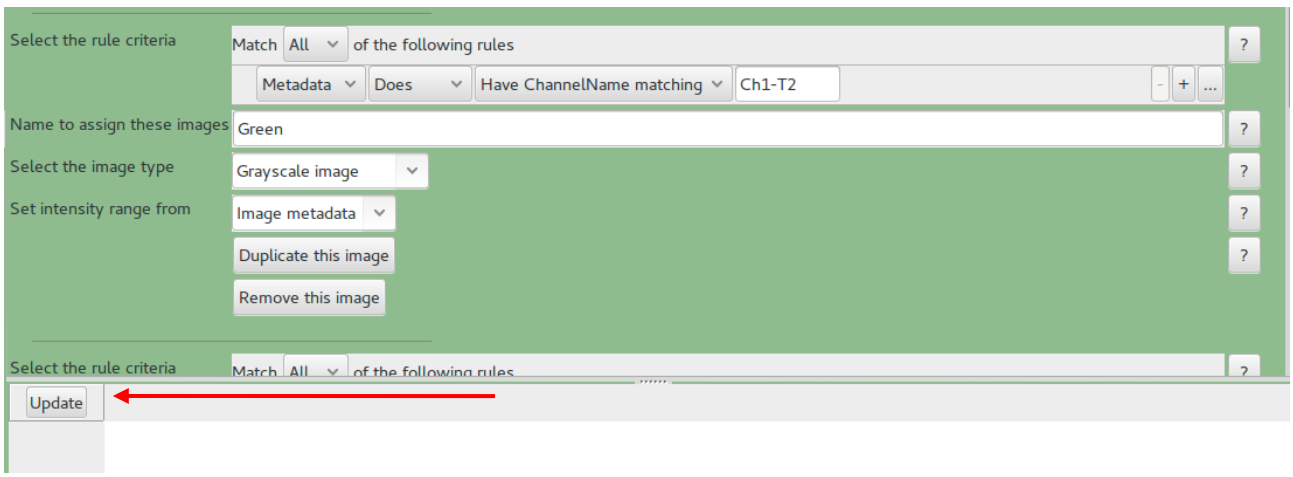
5) **Input Module -Metadata**



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

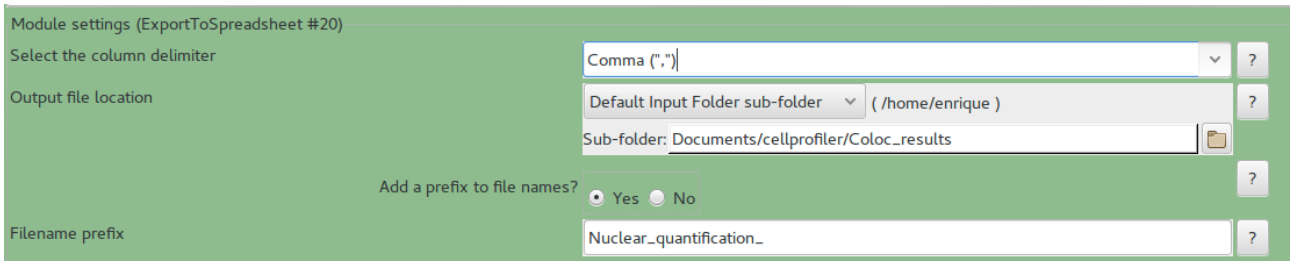
6) **Input Module Names and Types**

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



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