**CellMAPtracer-FUCCI**

1. We call the new app: CellMAPtracer-FUCCI
2. The work-flow consists of 5 steps:
3. Loading the input data (tiff + tracks + cell diameter (um))
4. Labeling channels and cell-cycle phases [clicking on it will open new window that contains two main sections:

- **Channels**

In this section there will be three channels, for every channel the user should fill in the following fields: name - color - used for normalization]:

Name [text by the user]

Color [Red - Green or Blue]

Used for normalization [ Yes or No]

- **Cell-cycle Phases**

In this section there will be three phases G1 - S - G2. For every phase, the user should describe the intensity of each channel and select one of these four intensity options [High - Detected - Low - Absent]

I am not sure how you gonna determine the thresholds for being high or low. I think absence is easy and anything that is less than the high threshold and higher than than the low.

**Red Intensity Green Intensity Blue Intensity**

**G1** Absent Detected High

**S** Detected High Absent

**G2** Detected Detected High

After filling in all the needed info the user can click on NEXT button

1. “Inspecting the signals” clicking on it will open new window that something similar to what it opens after clicking on (Extent Track Example). Could you please change the "Enlarged cell" to be “Merged channels”?
2. “FUCCI analysis” clicking on it will open new window that shows the results.
3. “Exporting the results”
4. Since we are only interested here in the dividing cell, I am not sure if it is good from the very beginning to exclude all the cells that did not divide so we have a shorter list of genes or to have it as last thing before exporting the results. What do you think? Which way is easier for you?
5. The final results should include the original 5 columns (which are TiffFileName, CellName, ImageNum, X , Y) + 4 new columns (one column for the intensity of each channel [High - Detected - Low - Absent]. The final column is the predicted cell cycle phase.
6. I think the best way to predict the cell cycle phase is to depend on the channel where there is a big drop in the intensity, in our case it is the blue channel. By detecting the frame where the intensity suddenly drops, the phase will be G1 from the first frame till the detected frame. By looking at the frame where the intensity of the blue channel suddenly increases again then from this frame till the end the cell cycle phase will be G2. Between these two detected frames the cell cycle phase will be S.