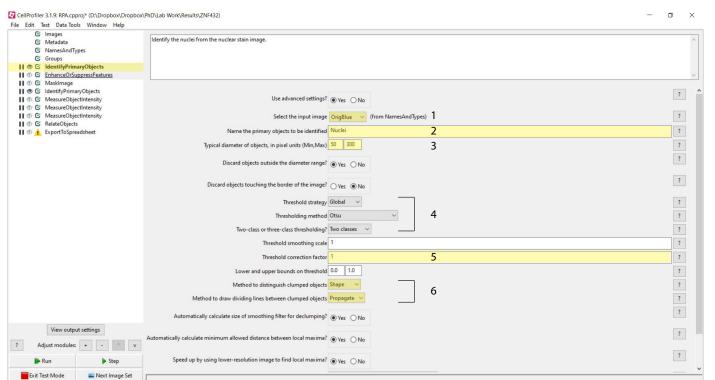
? Assign a name to Images matching rules V ? Process as 3D? O Yes No Match All v of the following rules ? Select the rule criteria ∨ Does ∨ Contain ∨ C03 · • ... Name to assign these images OrigBlue ? ? Select the image type Grayscale image ? Set intensity range from Image metadata ? Duplicate this image ? Match All v of the following rules Select the rule criteria File ∨ C01 ? Name to assign these images OrigGreen ? Select the image type Grayscale image ? Set intensity range from Image metadata ? Duplicate this image ? Remove this image ? Match All v of the following rules ∨ Does ∨ C02 View output settings Name to assign these images PCNA Adjust modules: + - ^ v Start Test Mode Analyze Images

Representing the DAPI channel

Representing the BrdU channel

Representing the PCNA channel

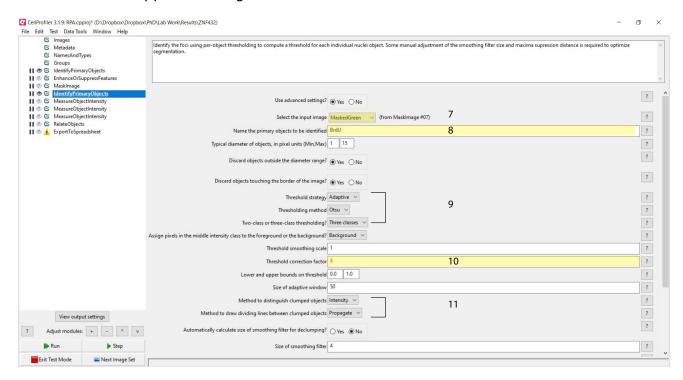


- 1) The channel used to identify the nucleus in this case is the DAPI
- 2) The label assigned the objects identified
- 3) The acceptable size range for the nuclei, which should be changed to best fit the cells in your images.
- 4) The thresholding strategy
- 5) The thresholding correction factor, i.e. how stringent the the thresholding strategy will be. This is the setting that will be most altered to fit cell shape and distribution.
- 6) How the cells are differentiated from each other by the program

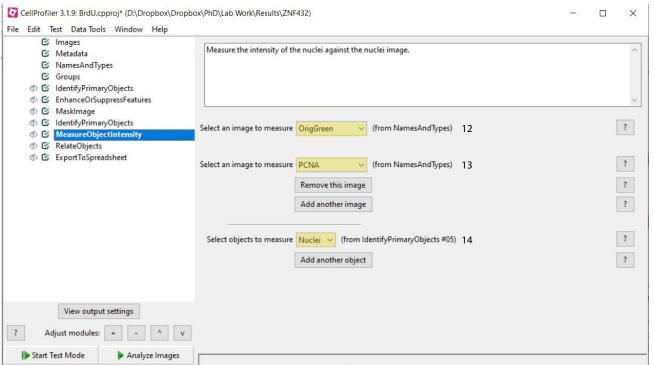
## O'Sullivan, Mersaoui et al. Supplemental Fig 2

A)

B)



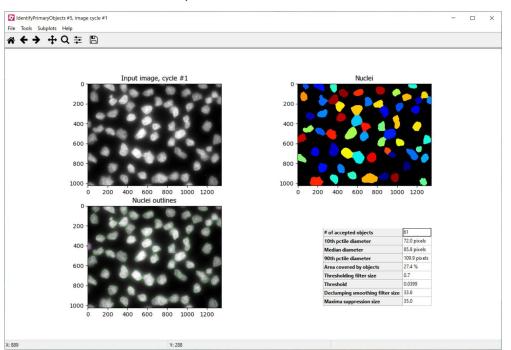
- 7) The channel used to identify the foci is the processed BrdU channel.
- 8) The name that will be given to data resulting from this section of analysis, which can be changed without issue.
- 9)The threshold strategy to identify and differentiate individual foci.
- 10) The threshold level, this will be changed to increase or decrease the number of objects identified as foci.
- 11) How the foci are differentiated from each other by the program



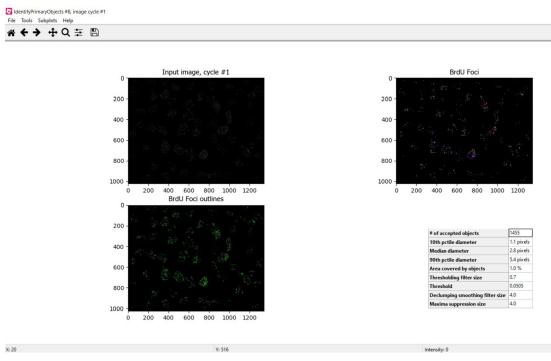
- 12) The unprocessed channel whose intensity will be measured, in this instance the green BrdU channel.
- 13) The unprocessed channel whose intensity will be measured, in this instance the red PCNA channel.
- 14) The area that is to be measured, either nuclei or foci.

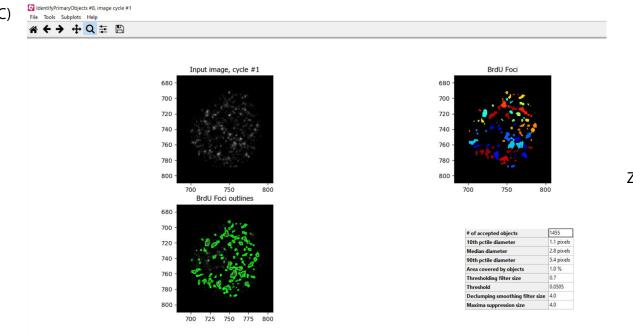
A)

## Example of nuclei identification



## Example of foci identification





B)

Zoom-in of foci identification

