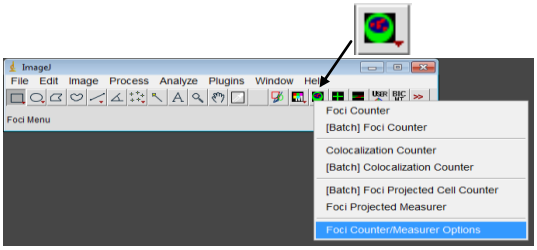
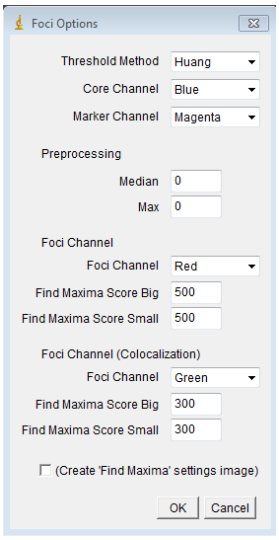
Manual „ FociCounter“



1. **Open an image…**
2. **Define your channels…**

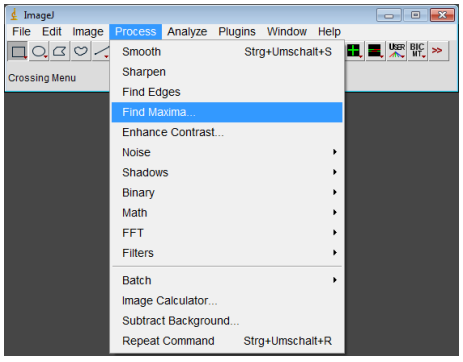
- In the Foci Counter tool, open “Foci Counter/Measurer Options”



- Set parameters:
  - **<Threshold method>** – used in batch processing
    - “Huang” is usually fine
  - **<Core Channel>** – Channel of the nuclear staining
    - used for sectioning
  - **<Marker Channel>** – Nuclear marker for classification
    - the presence or absence of a marker is noted in the results file (e.g. Cyclins for cell cycle, …) If not needed, set to white.
  - **<Preprocessing>** – Adjust settings if you have problems with noisy images, otherwise the default setting is fine.
  - **<Foci Channel>** – Here the settings for the foci counting are defined
    - Define the channels used for counting in one channel (Foci Counter) or two channels (Colocalization Counter). The different values to score big and small foci are set in the next step.

- Tick **<Create “Find Maxima settings image>** and click OK
  → Preprocessed images of the foci channels are calculated

- In the **<Find Maxima…>** tab adjust the noise tolerance, until only foci are detected. Toggle **<Preview point selection>** to show/hide the markers. If needed, identify different values for big and small foci. Test your values on several images from all conditions used.



- Enter the determined noise tolerance values in the “Foci Counter/Measurer Options” tab. If only one size of foci should be evaluated set the same maxima score for big and small foci.

3. Count the foci…

- Depending on the project foci can be counted in a single channel (Foci Counter), in two separate channels and determine their colocalization (Colocalization Counter) or count foci in projections from stacks. You can process one image at a time or process many images via batch processing. In the following the steps for a single image are described.

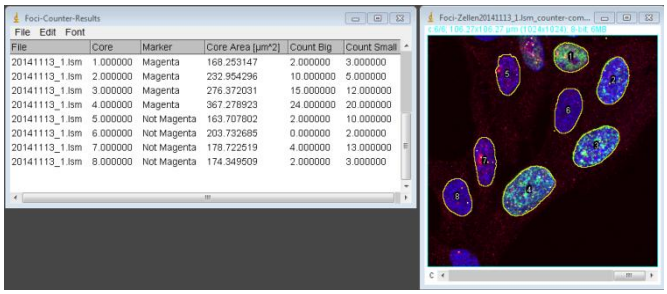
- To start counting foci in a single image, open the image and start the Foci Counter for a single foci channel or Colocalization Counter for two foci channels.

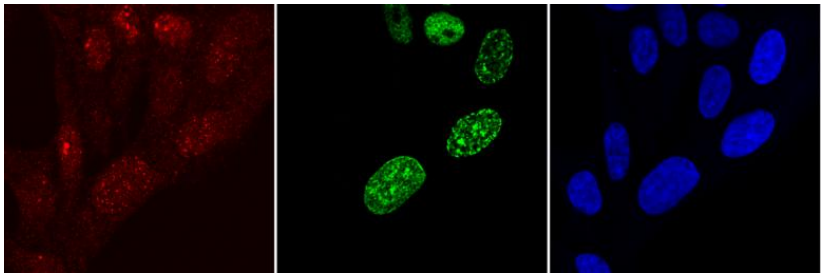
- Watch out for the "to do" instruction in the "BIC-MT-Log".
    - In the threshold window, adjust the threshold so that a clear identification of the nuclei in the "select" window is achieved. (e.g. "Huang B&W" → <Auto> → fine adjustment by sliders)

    - Refine the detected nuclei as indicated in the log. (e.g. remove out of focus nuclei)

    - Repeat the thresholding for the marker channel. Adjust threshold so that only cells which are "marker positive" are identified.

    - A result table and image are generated. In the case of the Colocalization Counter, also number colocalizations is saved.





Foci channel | Marker channel | Core channel

Big foci | Small foci | Core outlines

- Using the batch modes allows you to automatically process all files in a directory and its subdirectories. Using the prefix and postfix options, you can choose to process only parts of the file list. The result list should be checked against the images to ensure only properly imaged nuclei are taken into account.