Multichannel Cell Counter CZI

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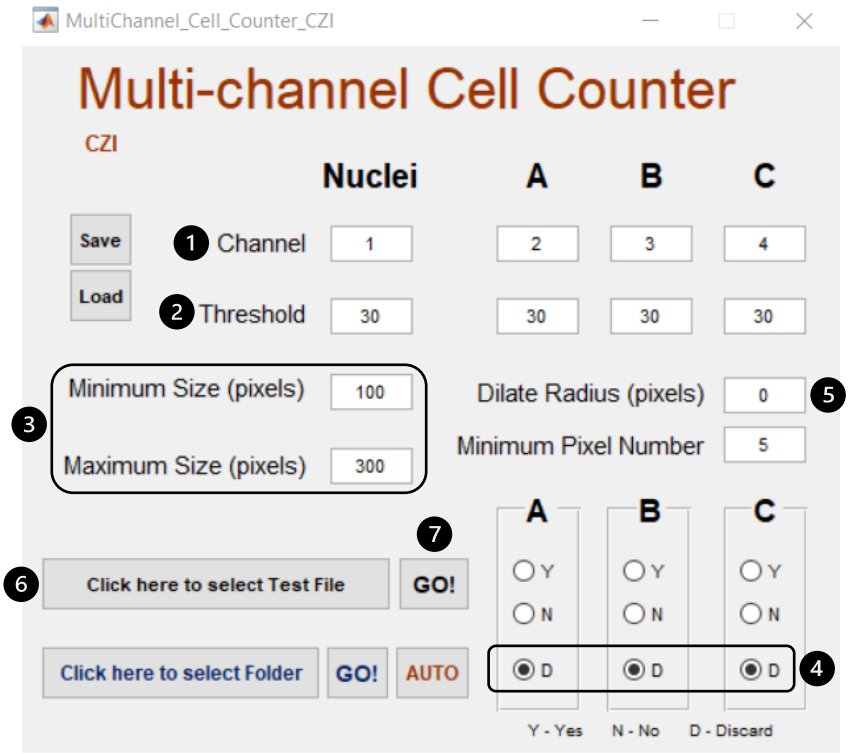
Bioimaging

Quick User Guide

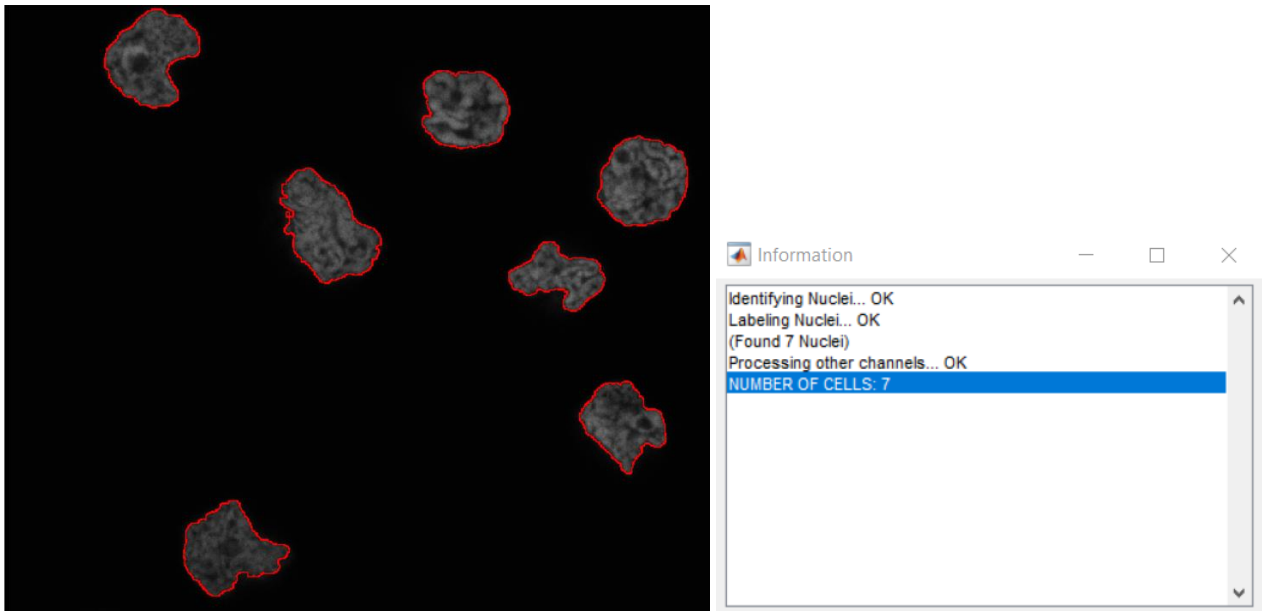
Multichannel Cell Counter CZI automates cell detection and counting in multiple single plane files (no z-stacks). For each image, single-cell nuclei are detected by thresholding and particle analysis. Each nuclear mask can be dilated by a user-defined radius, defining the corresponding cellular areas for the other 3 channels. For each channel and cellular mask, a staining is considered positive if a minimum number of pixels are above a given threshold. Combinatorial filters for cell counting can be defined based on staining (e.g., A+ B+ C-). Parameters are best extracted from FIJI pre-analysis.

Step 1 Detect Nuclei

1. Set Nuclei **Channel** (check ZEN for DAPI channel)
2. Set Nuclei **Threshold** (use FIJI for threshold adjustment)
3. Set Nuclei **Minimum** and **Maximum Size (pixels)** – use FIJI for nuclei area measurement in pixels
4. Set **A**, **B**, and **C** to Discard (D)



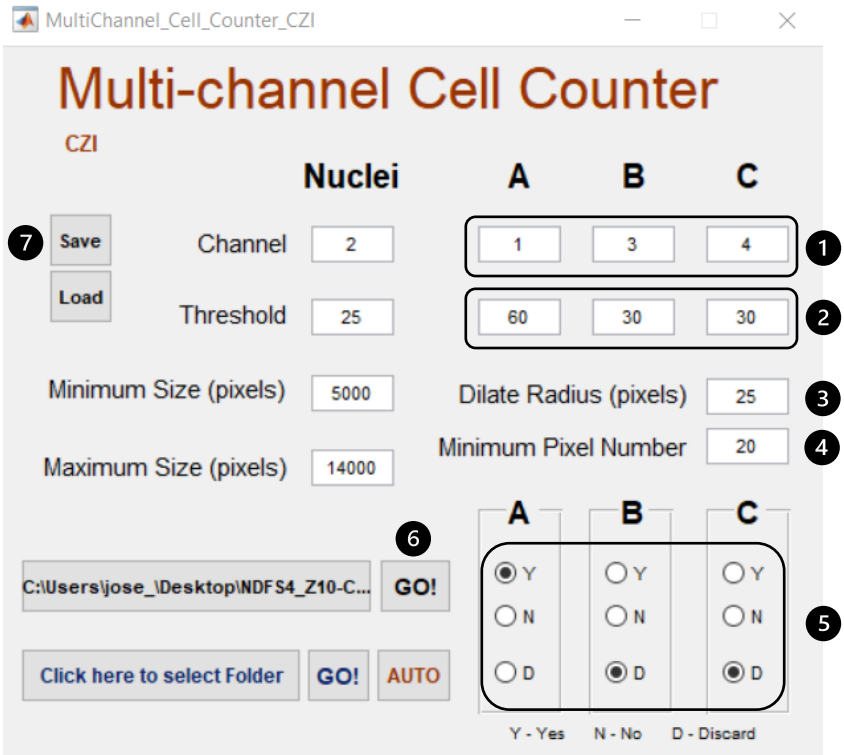
5. Set **Dilate Radius (pixels)** to 0
6. Select **Test file**
7. Press **GO!**

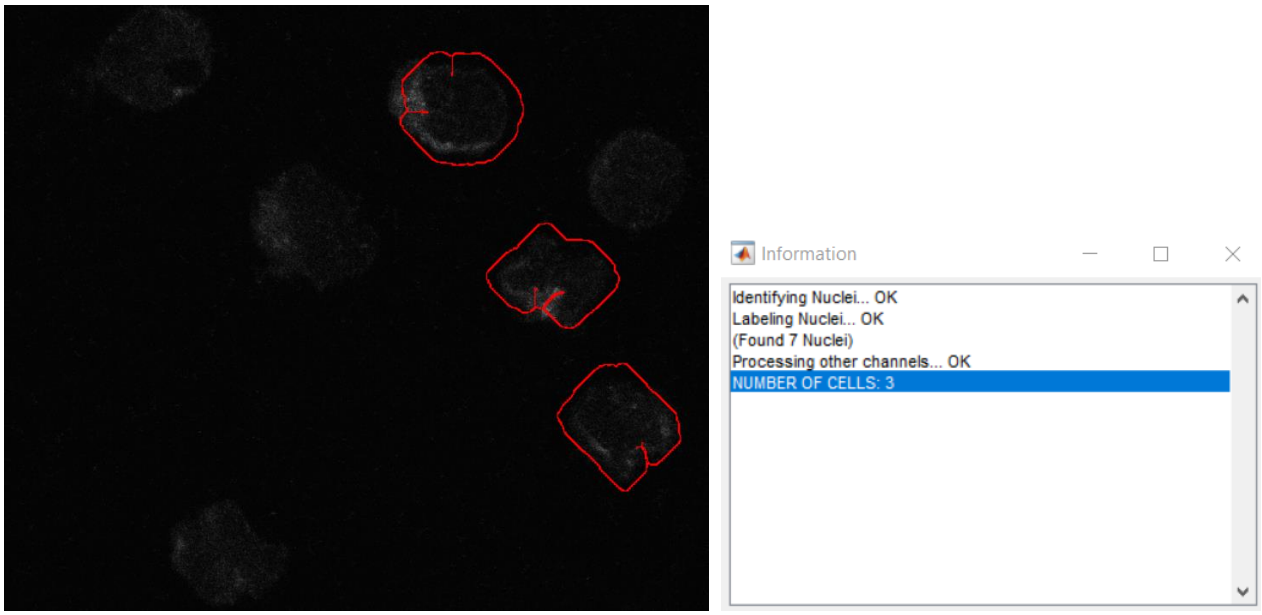


1 - Nuclei detection: output image with nuclei masks outlined in red

Step 2 Detect Positive Cells

1. Set A, B and C **channels** (check ZEN for correct numbering)
2. Set A, B and C **Threshold** values (use FIJI for threshold adjustment)
3. Set **Dilate Radius (pixels)** – set 0 for intranuclear staining; increase for cytoplasmic regions
4. Set **Minimum Pixel Number** – the minimum number of pixels above threshold for positive staining
5. Set **A**, **B**, and **C** to either Y (Yes), N (No) or D (Discard) – set Discard for non-existing channels
6. Press **GO!** to detect positive cells in test file



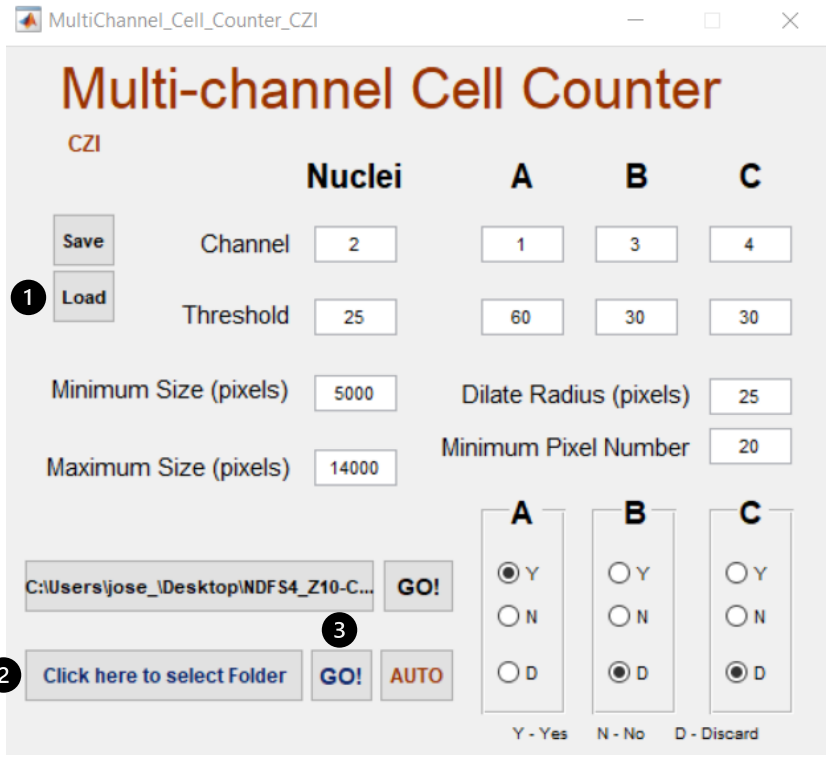


2 - Positive Cells for A channel - A(Y) B(D) C(D) - using Dilate Radius 25

7. (Optional) Save processing parameters in an Excel file to be reused later.

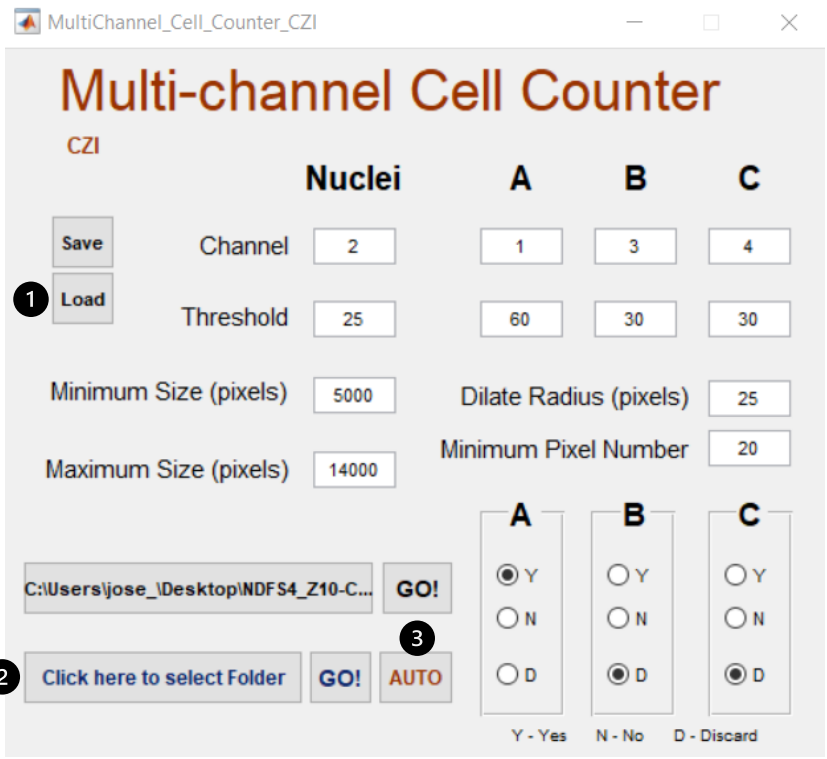
Step 3 Process Folder

1. (Optional) Load processing parameters from Excel file
2. **Select Folder** with CZI files to be processed
3. Press **GO!**
   CZI files in the folder will be processed using the combination set in A B C. An Excel file named MCC_results_[combination].xls and individual TIF files with positive nuclei outlines will be created.



Step 4 Automatic Processing with different combinations

1. (Optional) Load processing parameters from Excel file
2. **Select Folder** with CZI files to be processed
3. Press **AUTO**
4. Select combinations to be processed and press **GO!**
   CZI files in the folder will be processed using the combinations set in AUTO. Excel files named MCC_results_[combination].xls and individual TIF files with positive nuclei outlines will be created.



NOTE High DPI scaling issue

- If the graphical user interface (GUI) is not displayed as depicted in this Quick User Guide, you may need to override High DPI scaling in your Windows computer. To do so, right-click the **Multichannel_Cell_Counter_CZI** shortcut and select **Properties**. Click on the **Compatibility** tab and under Settings, select **Change high DPI settings**. In the High DPI scaling override section, select "Override high DPI scaling behavior. Scaling performed by:" and select **System (Enhanced)**.

HELP For support, please contact joserino@medicina.ulisboa.pt