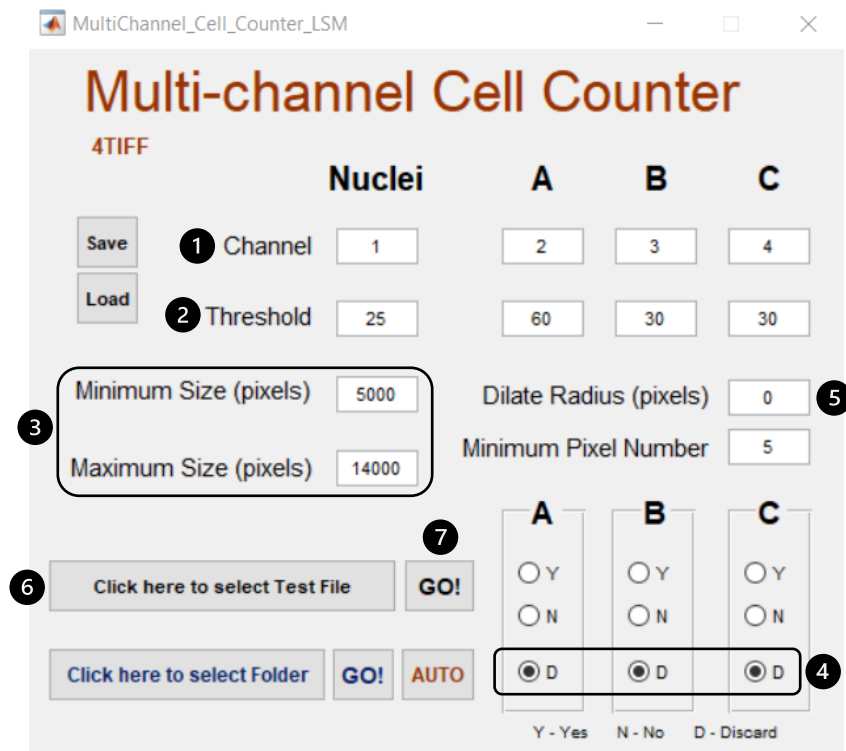


Multichannel Cell Counter 4TIFF automates cell detection and counting in multiple single plane **12-bit** TIF files series ending in `_c01.tif`, `_c02.tif`, `_c03.tif` and `_c04.tif`. For each series, single-cell nuclei are detected by thresholding and particle analysis. Each nuclear mask can be dilated 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.

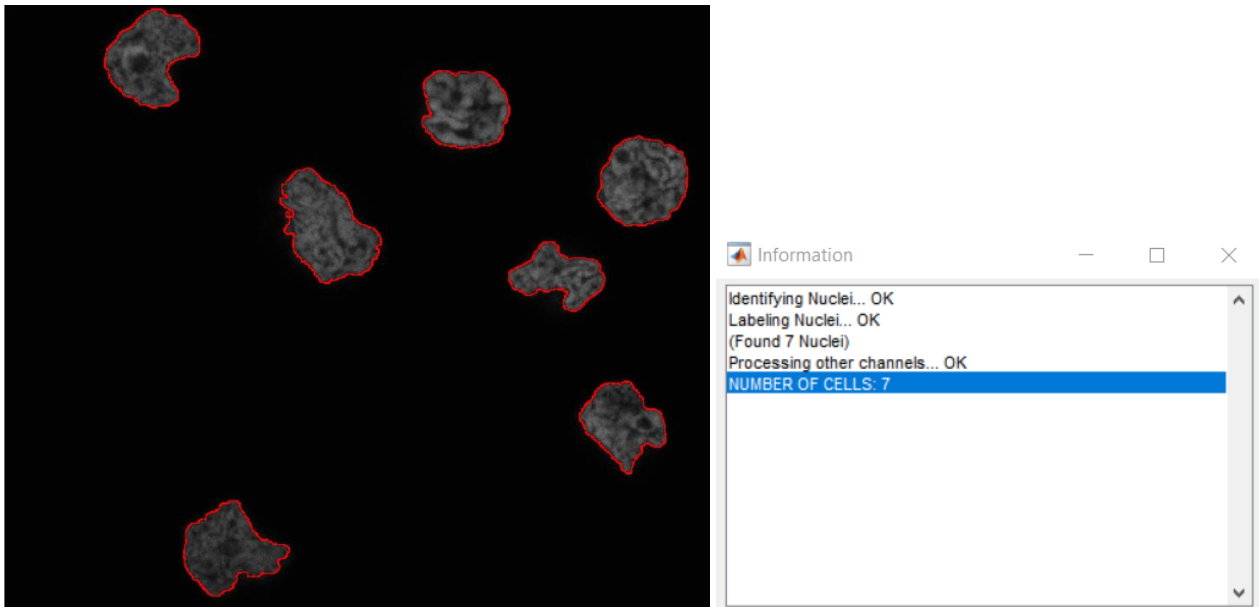
Step 1

Detect Nuclei

1. Set Nuclei **Channel** (e.g., 1 for TIF file ending in `_c01.tif`)
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** (can be any of the TIF files ending in `_c01.tif`, `_c02.tif`, `_c03.tif` or `_c04.tif`)
7. Press **GO!**



1 - Nuclei detection: output image with nuclei masks detected in TIF file ending in _c01.tif outlined in red

Step 2 Detect Positive Cells

1. Set A, B and C **channels** (e.g., 2 for TIF file ending in _c02.tif)
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)
6. Press **GO!** to detect positive cells in test file

MultiChannel_Cell_Counter_LSM

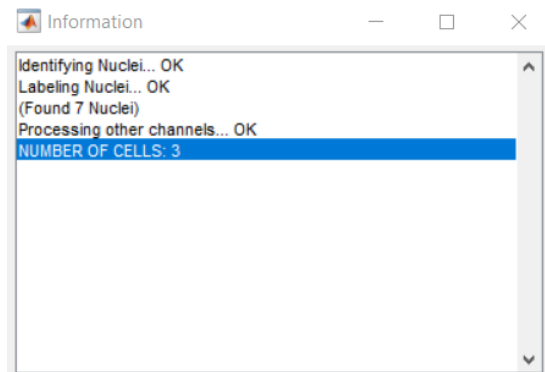
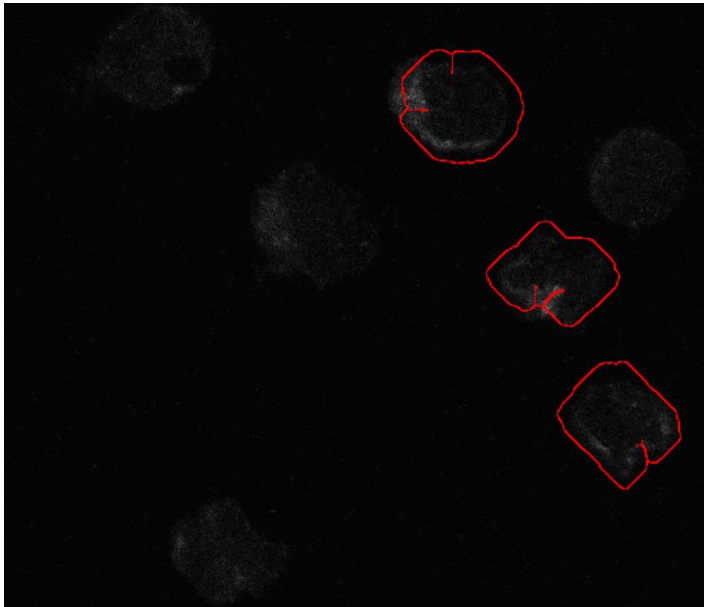
Multi-channel Cell Counter

4TIFF

	Nuclei	A	B	C
7 Save	Channel: 1	2	3	4
Load	Threshold: 25	60	30	30
Minimum Size (pixels): 5000	Dilate Radius (pixels): 25			
Maximum Size (pixels): 14000	Minimum Pixel Number: 20			
Click here to select Test File	6 GO!			
Click here to select Folder	GO!	AUTO	5	

A	B	C
<input checked="" type="radio"/> Y	<input type="radio"/> Y	<input type="radio"/> Y
<input type="radio"/> N	<input type="radio"/> N	<input type="radio"/> N
<input type="radio"/> D	<input checked="" type="radio"/> D	<input checked="" type="radio"/> D

Y - Yes N - No D - Discard



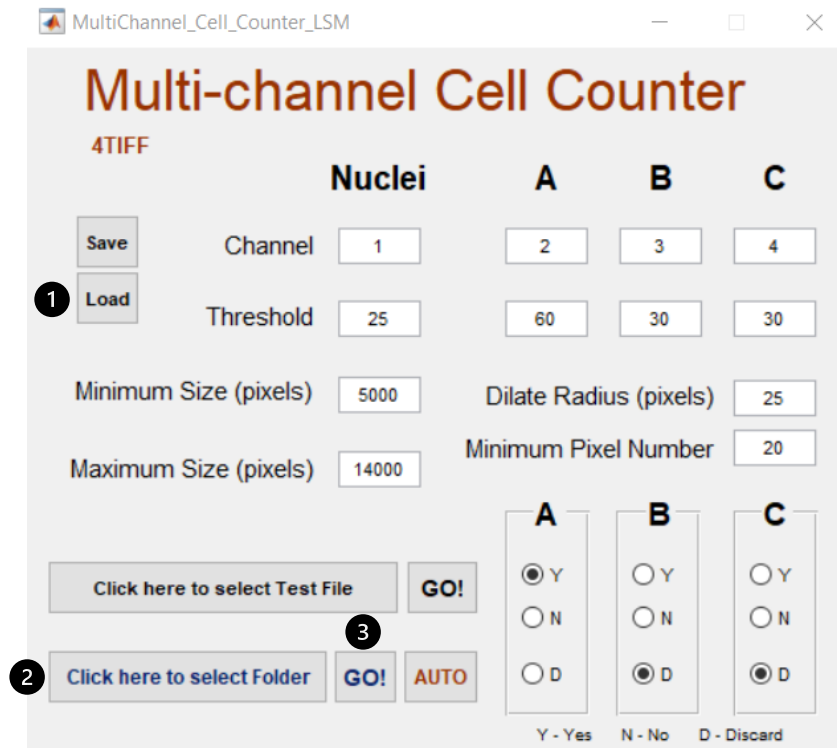
2 - Positive Cells for A channel (file ending in _c02.tif) - A(Y) B(D) C(D) - using Dilate Radius 25

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

Step 3 Process Folder

- (Optional) Load processing parameters from Excel file
- Select Folder** with TIF file series to be processed
- Press **GO!**

TIF file series in the folder will be processed using the combination set in A B C. An Excel file named MCC_results_[combination].xls and individual JPG files with positive nuclei outlines will be created.

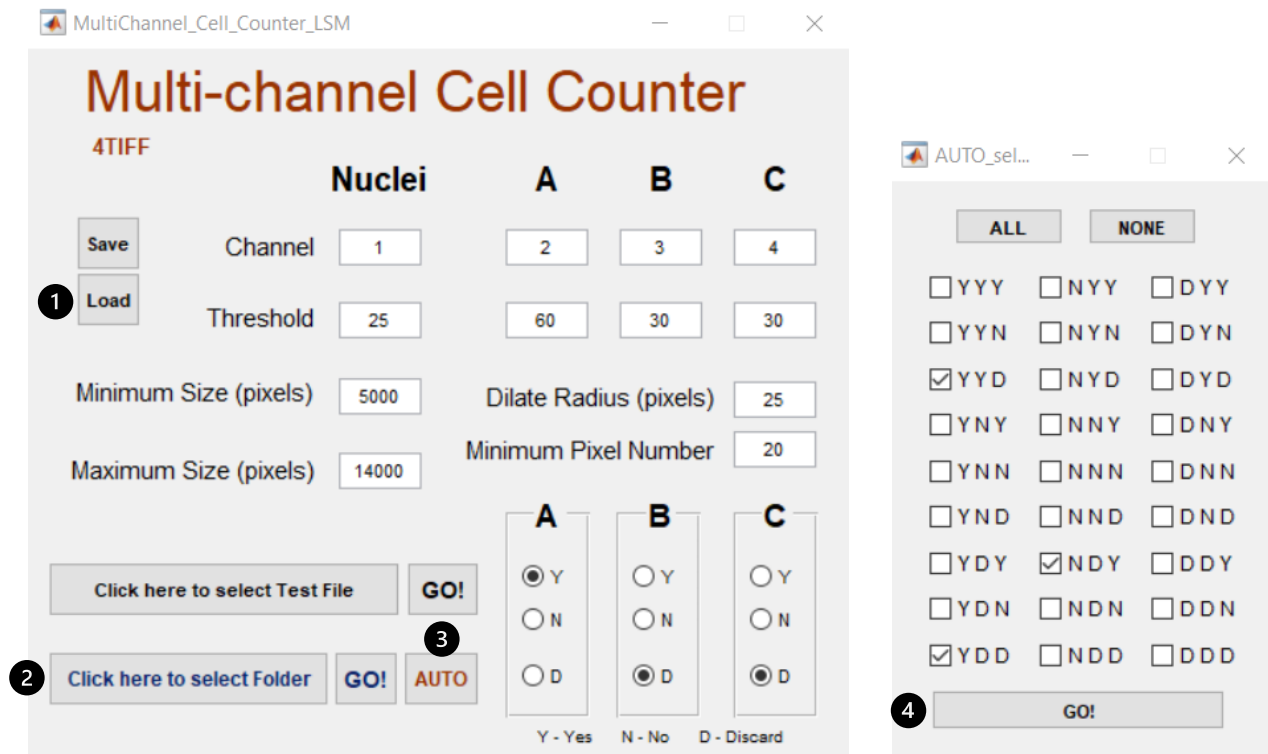


Step 4

Automatic Processing with different combinations

1. (Optional) Load processing parameters from Excel file
2. **Select Folder** with TIF file series to be processed
3. Press **AUTO**
4. Select combinations to be processed and press **GO!**

TIF file series in the folder will be processed using the combinations set in AUTO. Excel files named MCC_results_[combination].xls and individual JPG 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_4TIFF** 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