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Cell Counter L* a* b* 2

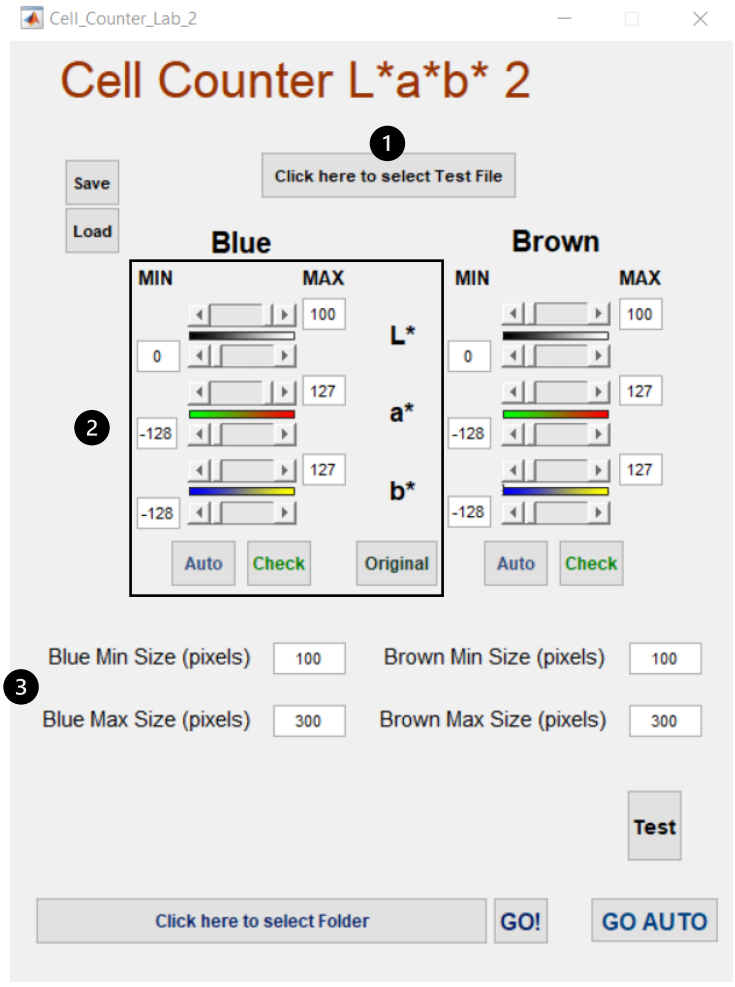
Bioimaging

Quick User Guide

Cell Counter L*a*b* 2 automates cell detection and counting for 2 different cell populations with blue and brown immunohistochemical staining in multiple RGB color TIF files. For each image, single-cell nuclei for each population are detected by color thresholding using the L*a*b* color space and particle analysis. Parameters are best extracted from FIJI pre-analysis.

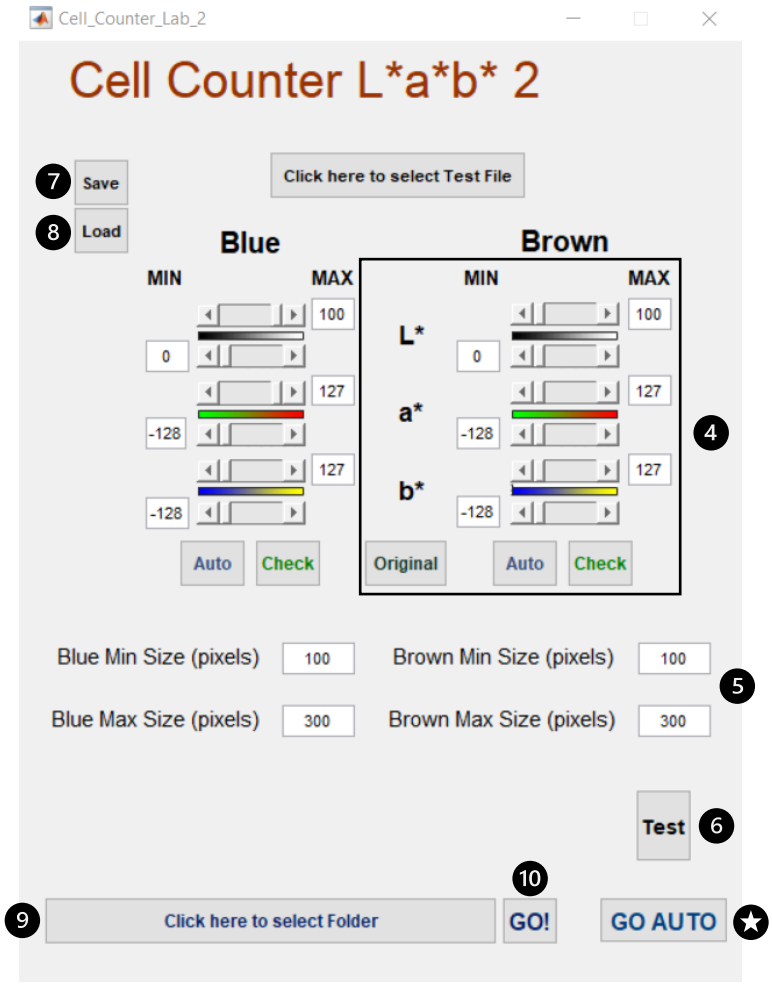
Step 1 Detect Blue cells

1. Select the Test file (crop the original image to a smaller area in FIJI if necessary)
2. Set Blue color range (**MIN** and **MAX** for **L*** luminosity, **a*** red-green and **b*** blue-yellow axis) using **Auto** or manually adjusting values. Use **Check** to view segmentation results. Compare with **Original**.
3. Set **Blue Min** and **Max Size (pixels)** – use FIJI for cell area measurement in pixels

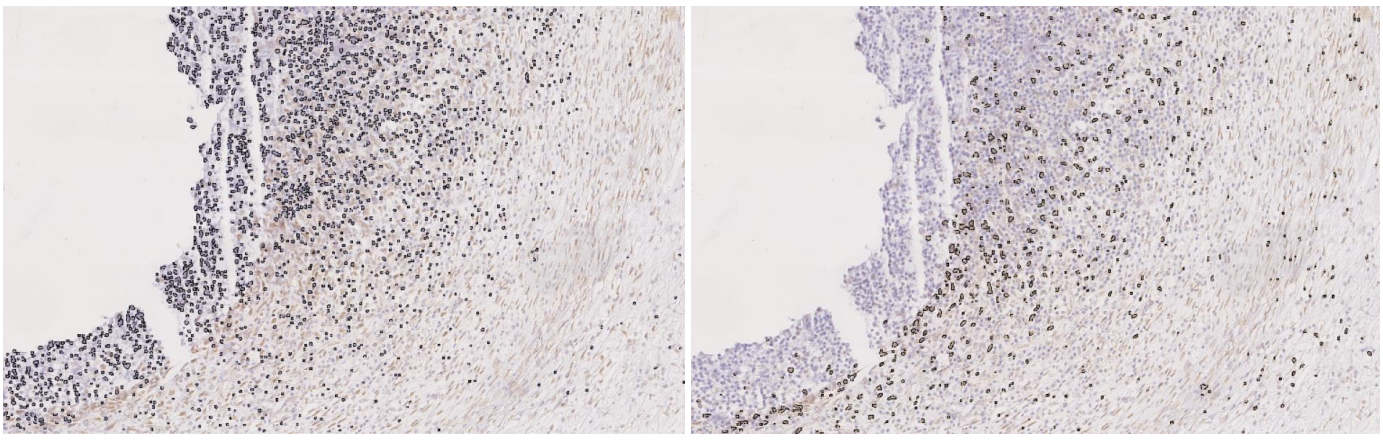


Step 2 Detect Brown Cells

4. Set Brown color range (**MIN** and **MAX** for **L*** luminosity, **a*** red-green and **b*** blue-yellow axis) using **Auto** or manually adjusting values. Use **Check** to view segmentation results. Compare with **Original**.
5. Set **Brown Min** and **Max Size (pixels)** – use FIJI for cell area measurement in pixels



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



1 - Positive Cells with blue staining (left) and brown staining (right) outlined in black

Step 3 Process Folder

8. (Optional) Load processing parameters from Excel file
9. **Select Folder** with RGB color TIF files to be processed
10. Press **GO!**

    RGB color TIF files in the folder will be processed. An Excel file named Cell_Counter_Lab_2_results.xls and individual JPG files with outlined positive cells will be created.

★(Optional) Press **GO AUTO**

RGB color TIF files in the folder will be processed with automatically determined Blue and Brown color thresholds. An Excel file named Cell_Counter_Lab_2_results.xls and individual JPG files with outlined positive cells 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 **Cell_Counter_Lab_2** 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