



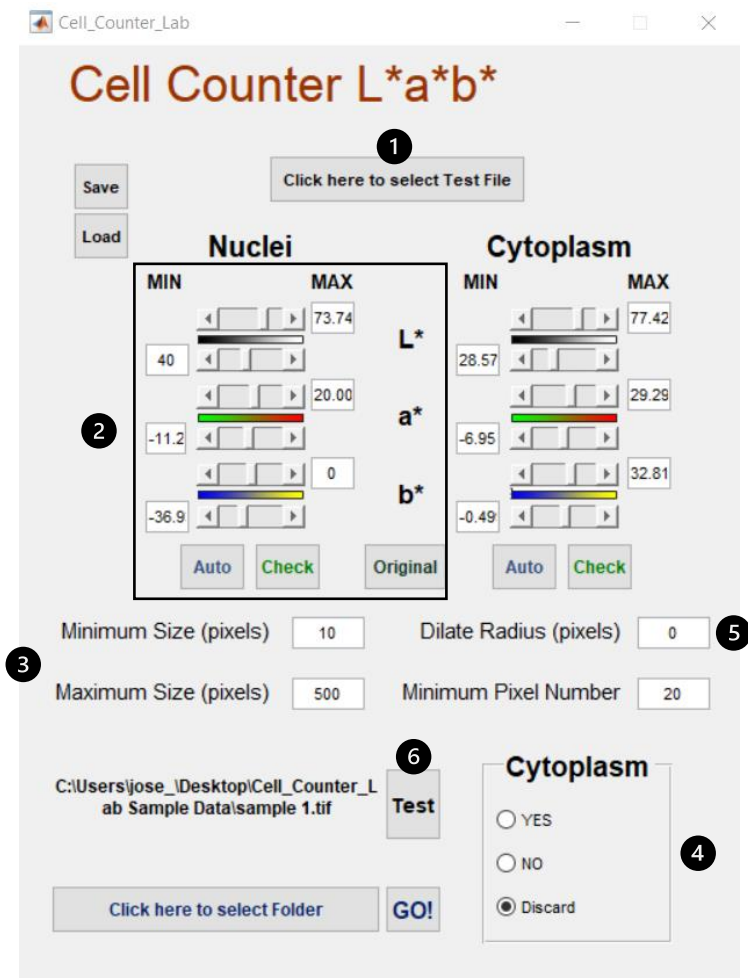
# Quick User Guide

**Cell Counter L\*a\*b\*** automates cell detection and counting in multiple RGB color TIF files with specific nuclear (blue) and cytoplasmic (brown) immunohistochemical staining. For each image, single-cell nuclei are detected by color thresholding using the L\*a\*b\* color space and particle analysis. Each nuclear mask can be dilated by a user-defined radius, defining the corresponding cytoplasmic area in which a staining is considered positive if a minimum number of pixels are within the specified color threshold. Cells can be detected based on the presence or absence of cytoplasmic staining. Parameters are best extracted from FIJI pre-analysis.

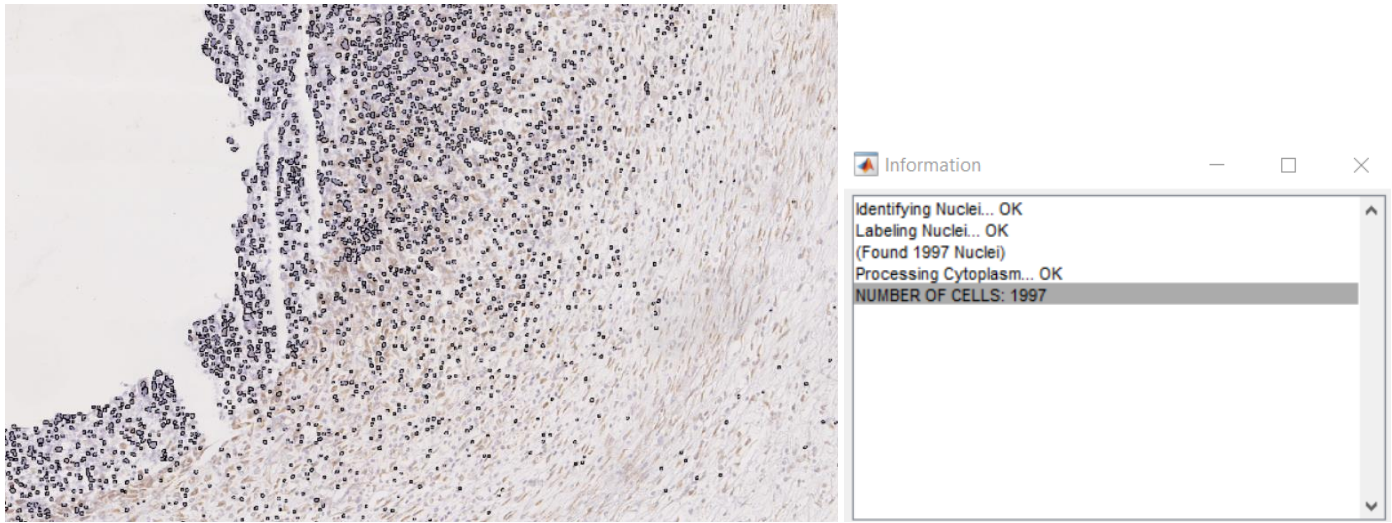
## Step 1

### Detect Nuclei

1. Select the Test file (crop the original image to a smaller area in FIJI if necessary)
2. Set Nuclei 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 Nuclei **Minimum** and **Maximum Size (pixels)** – use FIJI for nuclei area measurement in pixels
4. Set **Cytoplasm** to Discard
5. Set **Dilate Radius (pixels)** to 0
6. Press **Test**



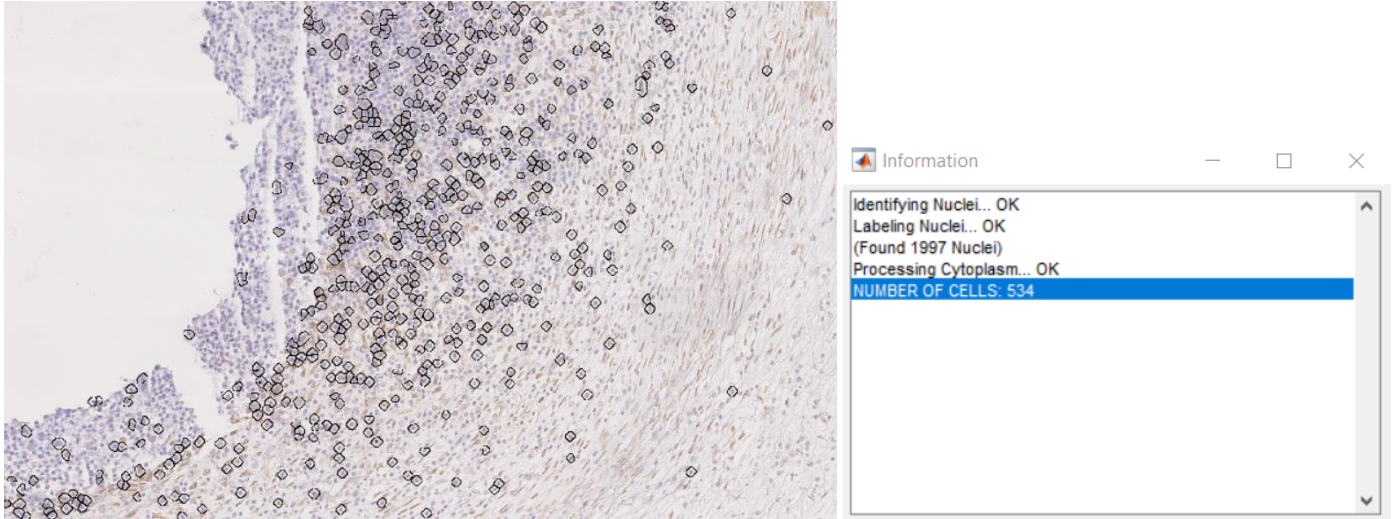
1 - Nuclei detection in blue color range: output image with nuclei masks outlined in black

## Step 2

### Detect Positive Cells

The screenshot shows the 'Cell Counter L\*a\*b\*' software interface. It features two main sections: 'Nuclei' and 'Cytoplasm'. The 'Nuclei' section has sliders for MIN (40) and MAX (73.74) with a 'Check' button. The 'Cytoplasm' section has sliders for L\* (28.57), a\* (-6.95), and b\* (-0.49) with 'Original', 'Auto', and 'Check' buttons. Below these are input fields for 'Minimum Size (pixels)' (10), 'Dilate Radius (pixels)' (10), 'Maximum Size (pixels)' (500), and 'Minimum Pixel Number' (20). At the bottom, there is a 'Test' button, a 'GO!' button, and a 'Click here to select Folder' button. A file path is shown: 'C:\Users\jose\Desktop\Cell\_Counter\_Lab Sample Data\sample 1.tif'. Numbered callouts 1 through 9 point to various UI elements: 1 points to the Cytoplasm sliders, 2 to Dilate Radius, 3 to Minimum Pixel Number, 4 to the Cytoplasm selection radio buttons, 5 to the Test button, 6 to the Save button, 7 to the Load button, 8 to the Folder selection button, and 9 to the GO! button.

1. Set Cytoplasm color range (**MIN** and **MAX** for **L\*** luminosity, **a\*** red-green and **b\*** blue-yellow axis) using **Auto** or manual adjustment. Use **Check** to view segmentation results. Compare with **Original**.
2. Set **Dilate Radius (pixels)** – set 0 for intranuclear staining; increase for cytoplasmic regions
3. Set **Minimum Pixel Number** – the minimum number of pixels above threshold for positive staining
4. Set **Cytoplasm** to **YES** or **NO**
5. Press **Test**



2 - Positive Cells with cytoplasmic staining in the brown color range outlined in black - using Dilate Radius 25

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

### Step 3 Process Folder

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

*RGB color TIF files in the folder will be processed with the option set in **Cytoplasm**. An Excel file named `MCC_Lab_results_[option].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** 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](mailto:joserino@medicina.ulisboa.pt)