Documentation for MitoTouch software. Executable available upon request. Note that a valid MATLAB Compiler may be required.

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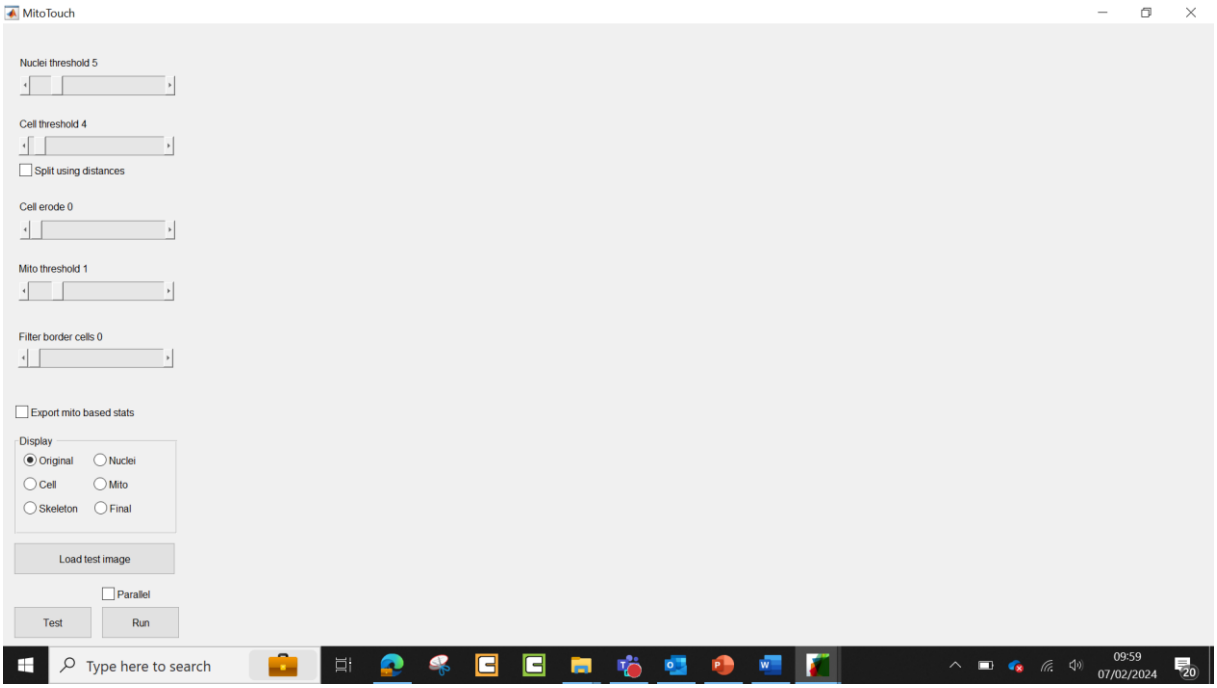
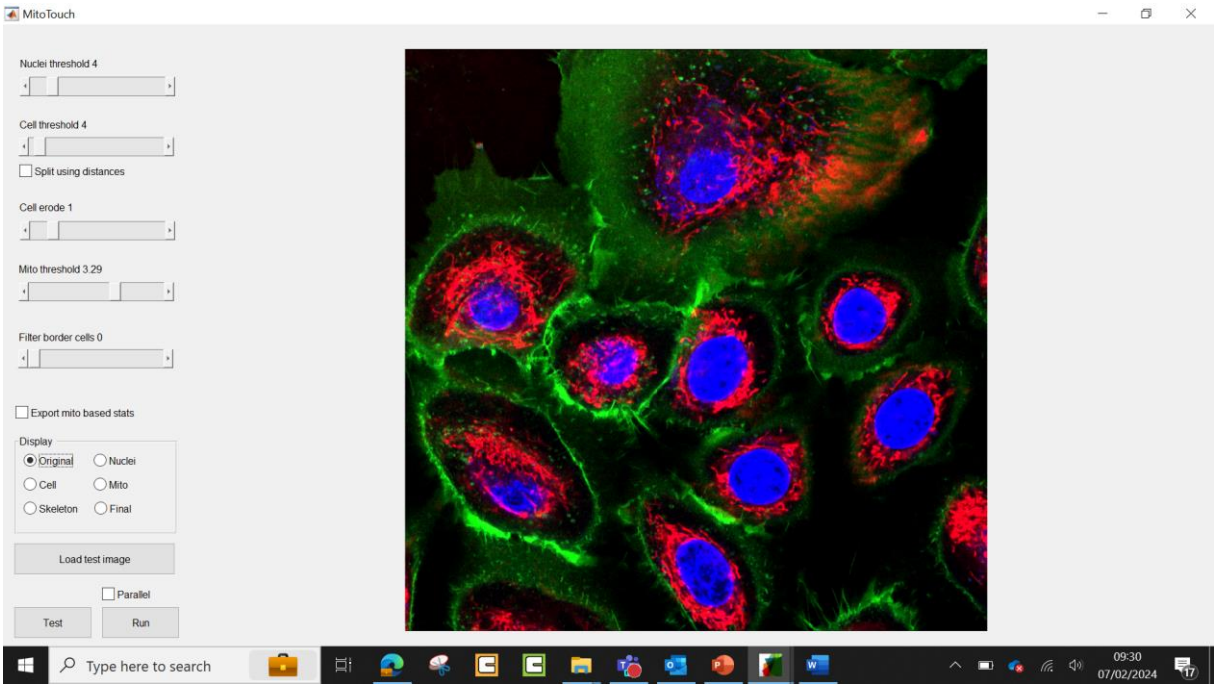
I. Pictures that will be analyzed by Mitotouch software must have 3 channels (blue, green and red) in TIFF format (16 bits), with mitochondria in red (C1), membranes in green (C2) and nuclei in blue (C3).

II. Mitotouch window after loading the software:

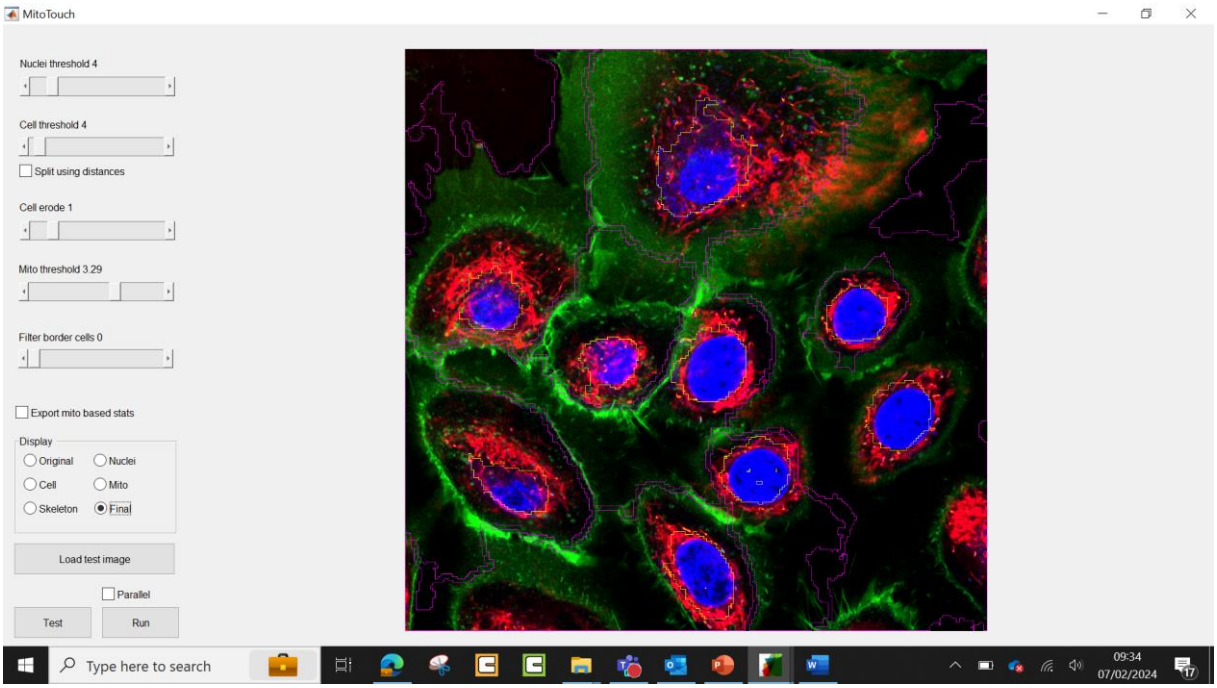


➪ Load the most representative assay picture via the "load test image" button.

III. The window will be like this:

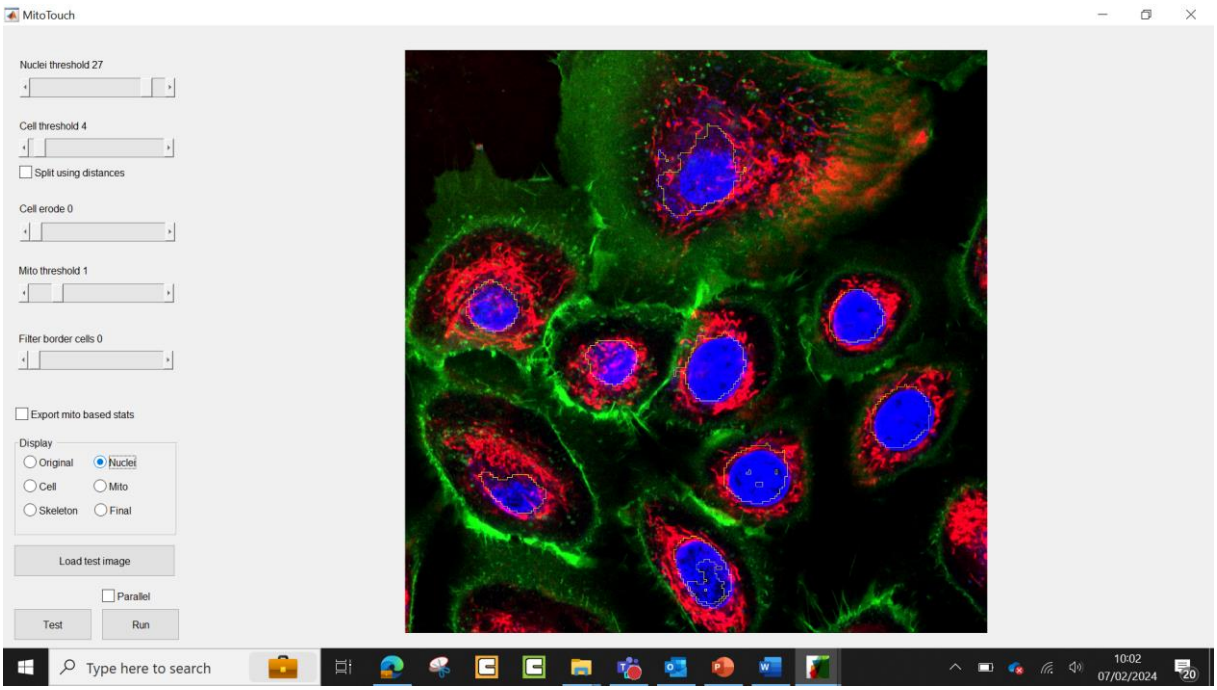


➪ To see how the software will identify the 3 parameters i.e. 1. nuclei; 2. Cell membrane; 3. Mitochondria/Mitochondrial network, press "final" in the display window (the 3 parameters are surrounded).



➪ Adjust Nuclei identification (blue colour) by clicking on "nuclei" in display window (only the objects identified as nuclei are surrounded).

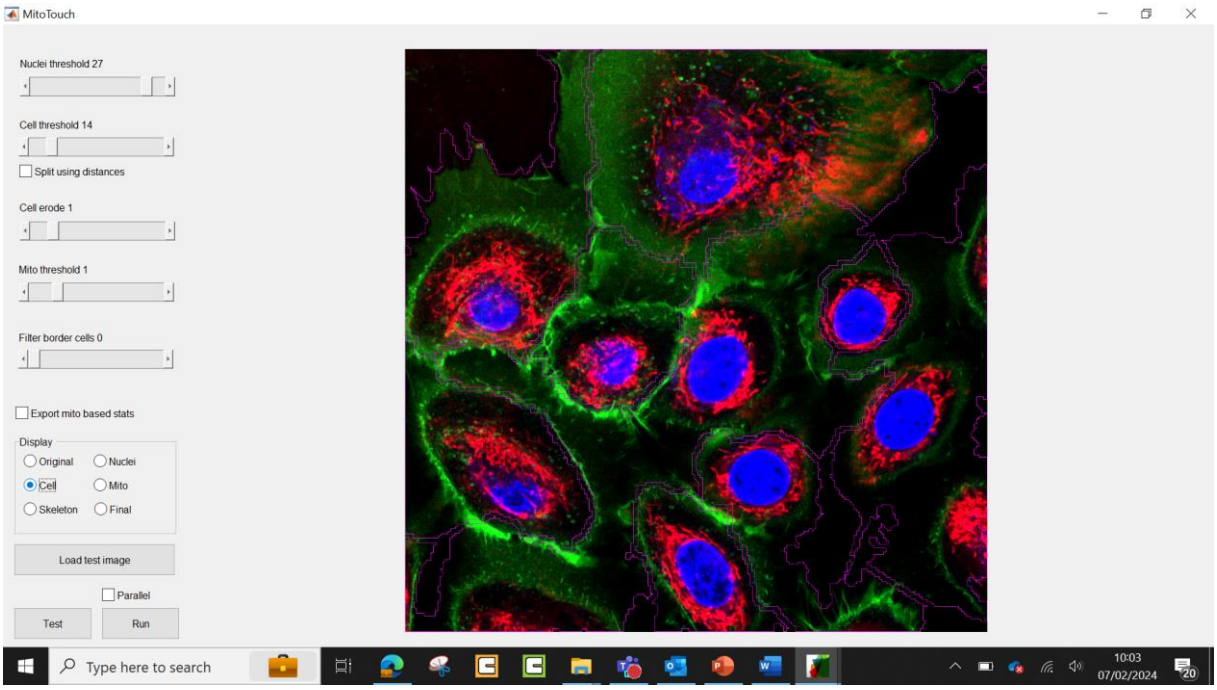
Nuclei detection and size can be adjusted thanks to the "nuclei threshold" cursor.



➪ Adjust Cell identification (green colour) by pressing "cell" in display window (only the objects identified as cells are surrounded).

Cell detection can be adjusted with the "cell threshold" cursor.

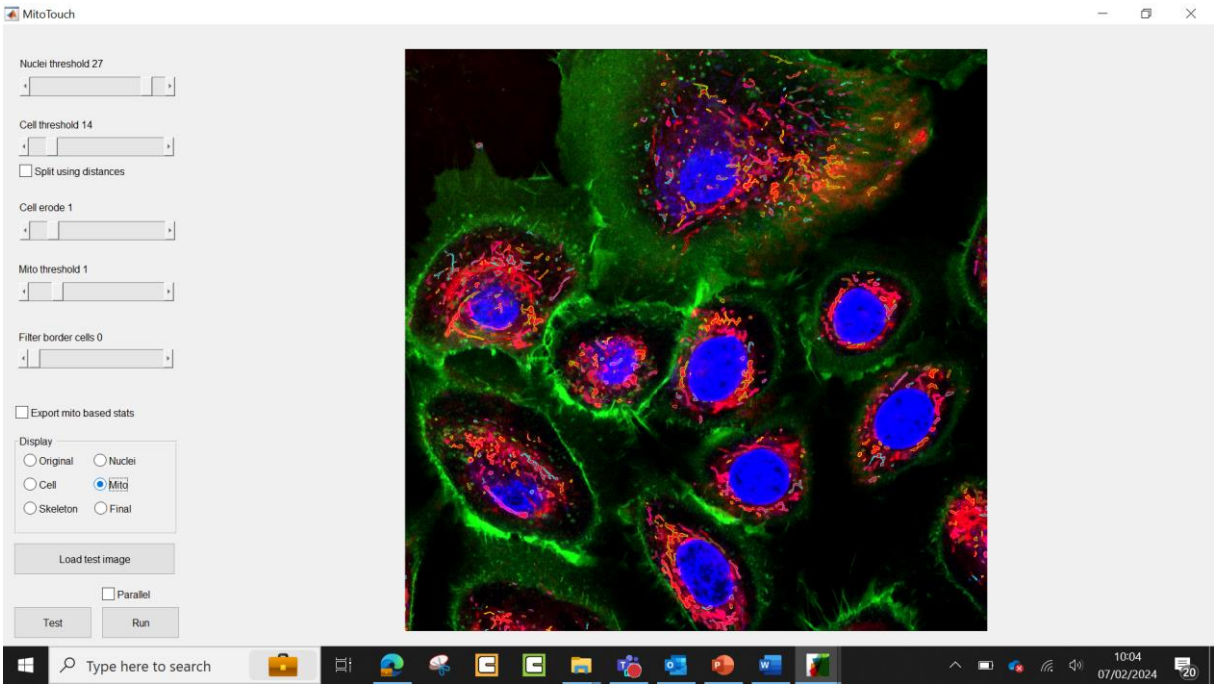
Cell size can be adjusted with the "cell erode" cursor.

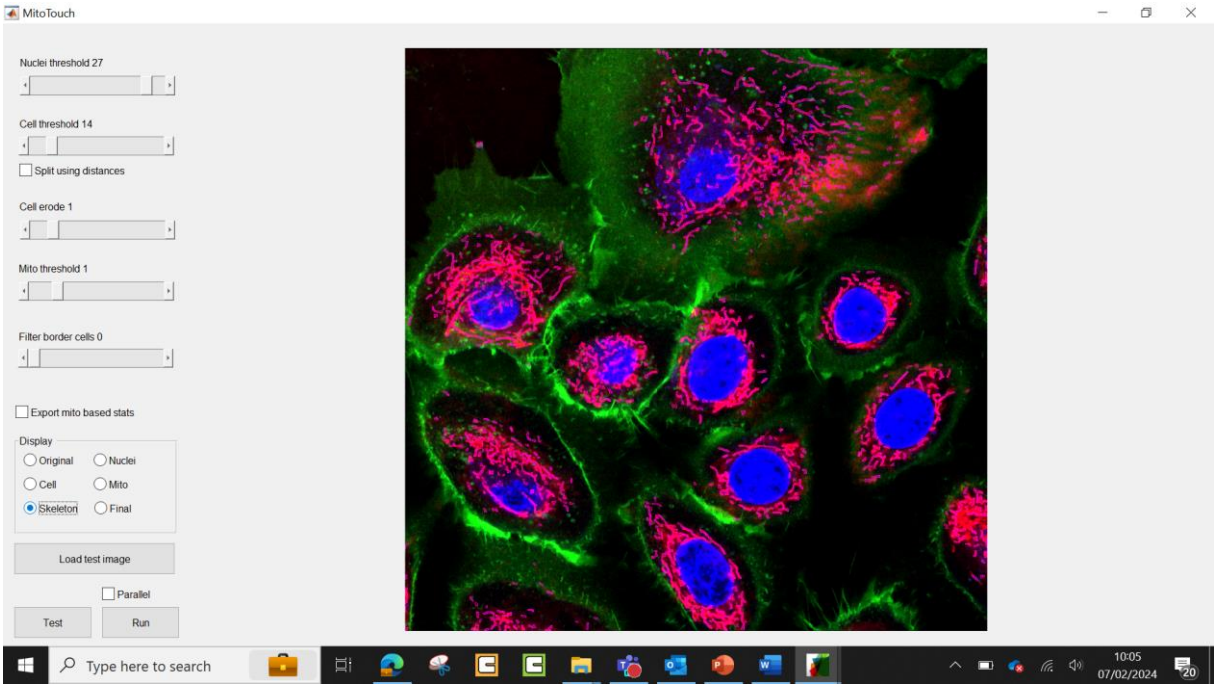


➪ Adjust Mitochondria identification (red colour) by clicking on the "mito" button in display window (only the object identified as mitochondria are surrounded).

➪ Adjust Mitochondria cluster identification (red colour) by clicking on "skeleton" in display window (only the object identified as mitochondria cluster are surrounded).

The mitochondria detection can be adjusted with the "mito threshold" cursor.





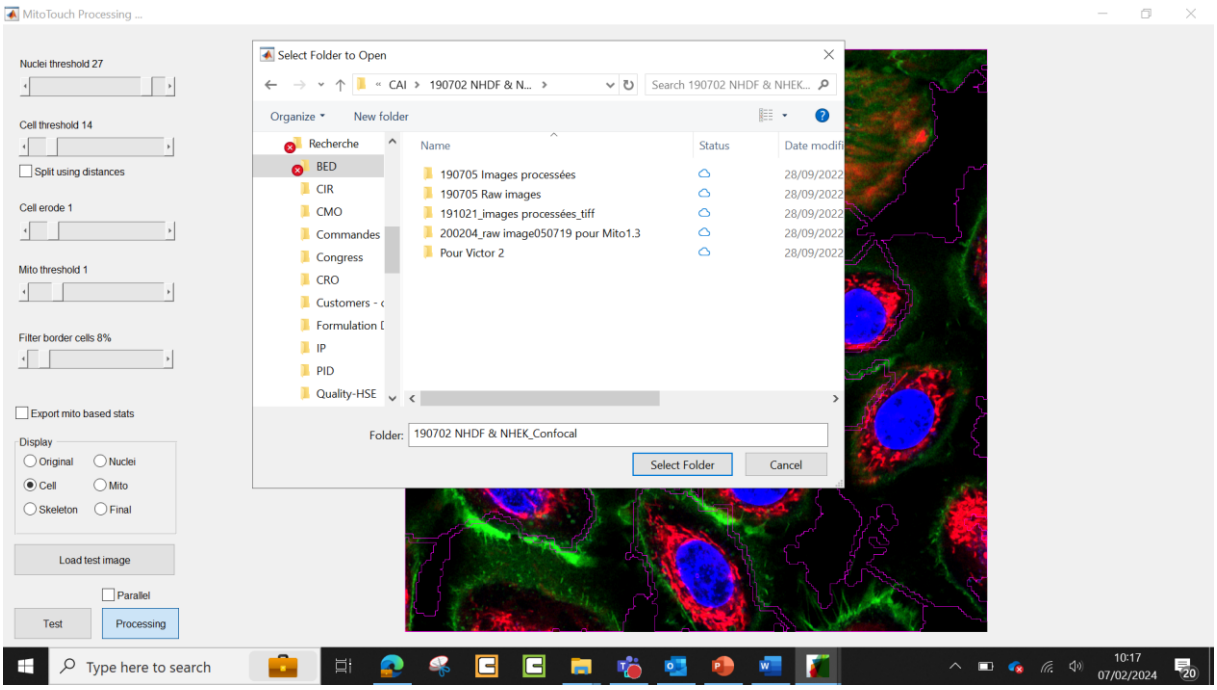
Please note: ***1. after each modification please "click" on test button the see on the screen the adjustment/modification.***

2 If faster analysis is needed then check the box named "parallel" (needs more computer RAM memories).

⇨Verify that the parameters are well adjusted by processing another picture.

IV. When the 3 parameters are well adjusted, select all the pictures by clicking the "run" button

⇨ A folder containing the all pictures should be selected.



At the end of the process, a folder named "Result" is generated containing:

- each analysed pictures in 8 copies 3 are the original channels (blue, green and red),

  4 corresponding to each "mask", i.e. for nuclei, cell, mitochondria and mitochondria cluster,

  and the original picture + overlay named "montage".
- an excel file named "final" with a .csv extension with all the descriptors and the data measured for each picture. Results are done cell by cell but the results are mean per cell.

Please note: If results are needed mitochondria by mitochondria, then check the box named "Export mito based stats". With this option selected, the Excel file named "final" generated at the end of the analysis with all the descriptors will be computed mitochondria by mitochondria.