Auto-TT: Automated Detection and Analysis of T-Tubule Architecture in Cardiomyocytes

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Supplemental Materials

Figure Legends

Figure S1. Annotated AutoTT application graphic user interface.

Figure S2. About morphological operations.

For detailed definition of morphological operations, please refer to Gonzalez, R. C., R. E. Woods, and S. L. Eddins, *Digital Image Processing Using MATLAB*, Gatesmark Publishing, 2009.

Morphological operations are based on two fundamental operations: erosion and dilation.

Rule for Erosion: The value of the output pixel is the minimum value of all the pixels in the input pixel's neighborhood. The neighborhood is defined by the structuring element.

Rule for Dilation: The value of the output pixel is the maximum value of all the pixels in the input pixel's neighborhood. The neighborhood is defined by the structuring element.

A structuring element is a matrix consisting of only 0's and 1's that can have any arbitrary shape and size. The pixels with values of 1 define the neighborhood.

Morphological opening is erosion followed by dilation using the same structuring element. For binary images, the shape of large objects that can completely contain the applied structuring element will be captured, while the small objects that can't completely contain the structuring element will be removed. For a grayscale image, the values of pixels with higher contrast to neighborhood defined by structuring elements are less likely to be kept by morphological opening. As a result, morphological opening can be used to detect background when small structures are object of interest.

Morphological closing is dilation followed by erosion using the same structuring elements. For binary images, this operation can fill the gaps that can't completely contain the structuring elements.

In the following simplified example, the vertically and horizontal originated bands schematically represent Transverse elements (TE) and Longitudinal elements (LE) respectively. Both TE and LE are 5 pixels wide. Note that the background illumination is artificially made to be brighter on the right of the image than on the left. If the task is to subtract the spatially uneven background illumination, morphological opening with a structuring element in shape of 7 pixels * 7 pixels square is applied to the Image A. Given the TE and LE are 5-pixels wide, they cannot completely cover the 7 pixels*7 pixels square. Therefore these objects are not detected by morphological opening. Image B is the background detected by morphological opening. Note the spatial heterogeneity of the background detected by the algorithm. Subtraction of B from A results in objects of interest (C) with relatively even and low background.

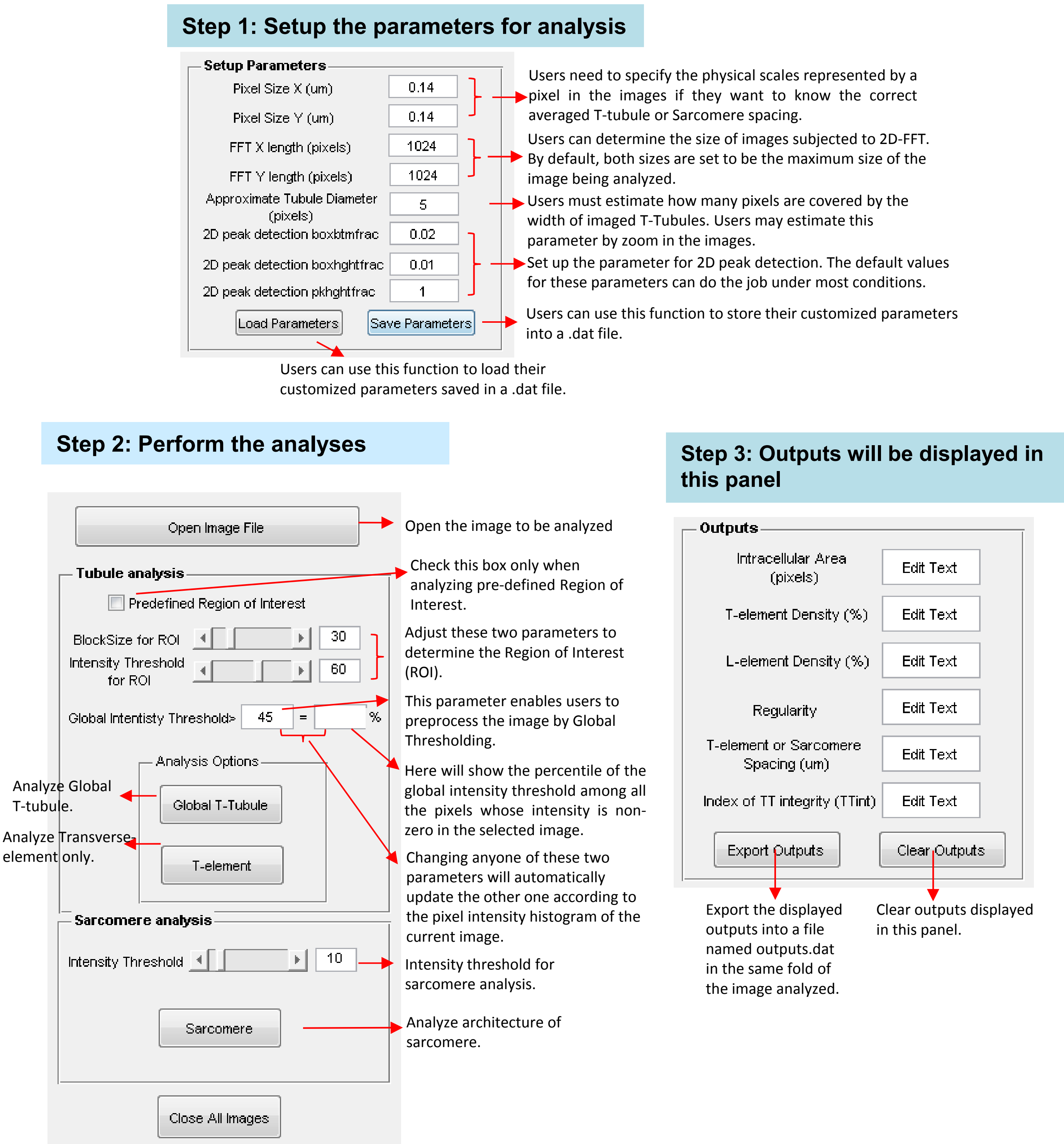
Subtraction of background while preserving both TE and LE can also be achieved by morphological opening with 'X' shaped structuring elements that cannot be contained in the objects of interest. The resultant images show the successful separation of background (D) and objects of interest (E).

If the task is to separate TE and LE, morphological opening with horizontal line-shaped structuring element can be applied. The length of the line is set to 7-pixels, so LE completely contain the structuring element such that only LE are detected by this operation. Subtraction of LE (F) from input results in TE (G).

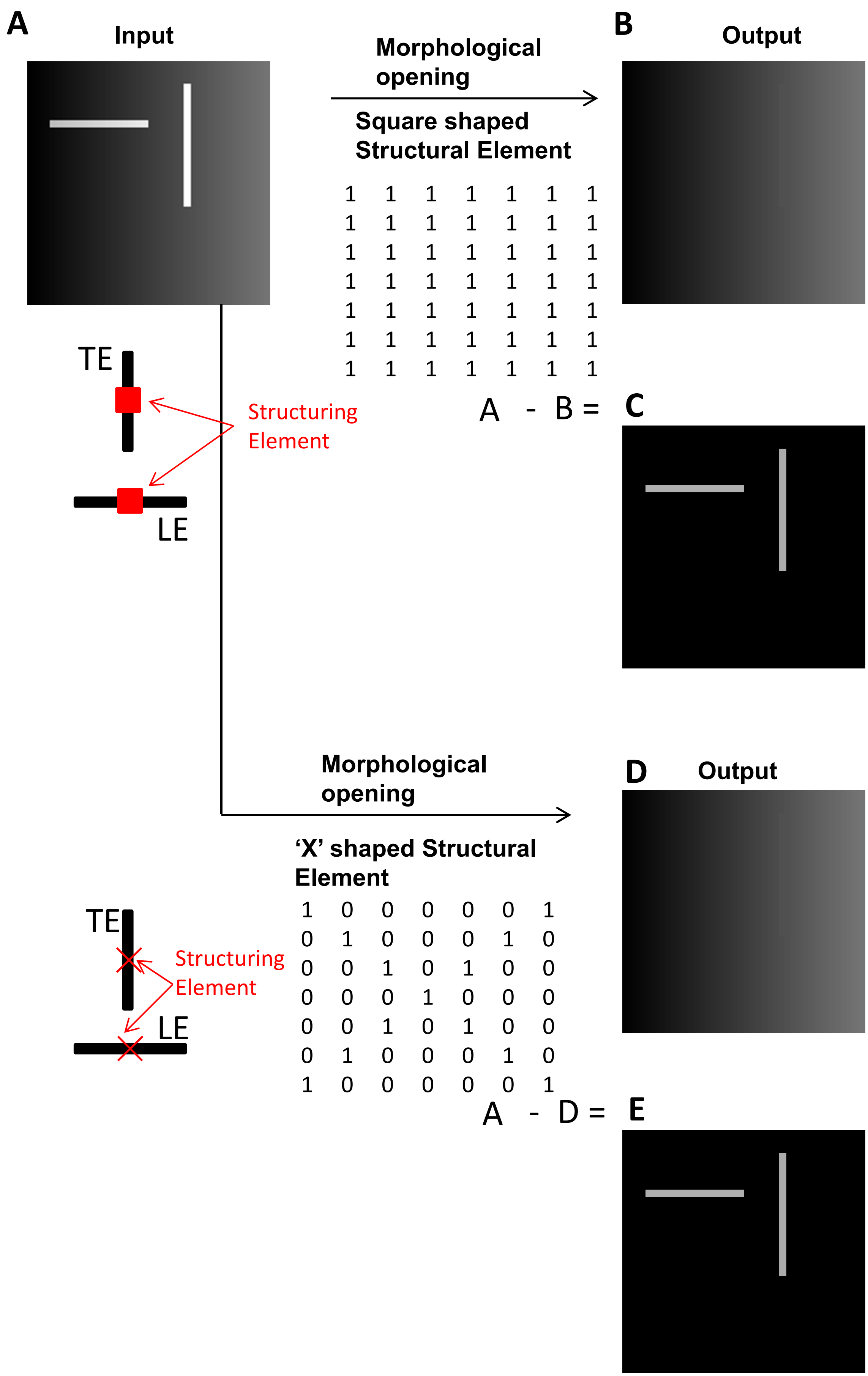
Figure S3. Several image processing steps not shown in Figure 1. **A**, Cell image before ROI selection. **B** and **C**, Separation of intracellular region (B) and other parts of a cell image (C). **D**, Extracted global T-tubule system by subtracting background. **E**, Skeletalized global T-tubules. **F** and **G**, Separation of Transverse Elements (**TE**, F) and Longitudinal Elements (**LE**, G).

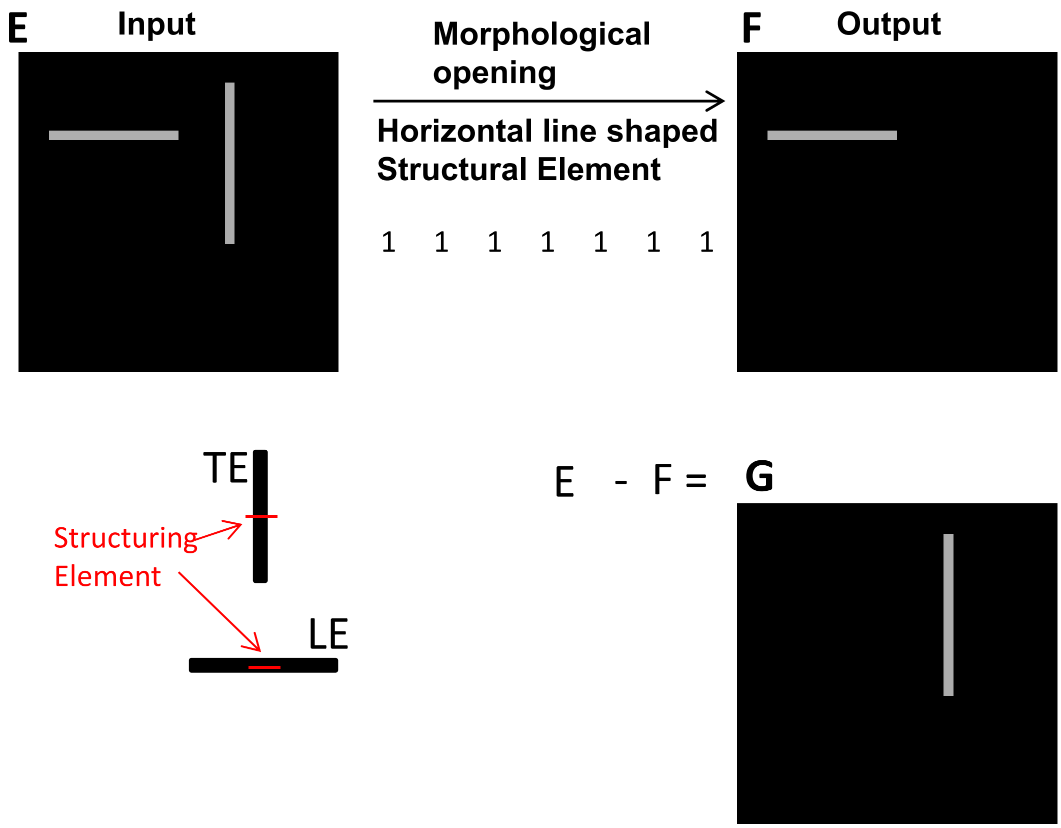
Figure S4. Global thresholding results in very different estimation of the T-tubule density when different thresholds are applied to a same image.

Supplemental Figure S1

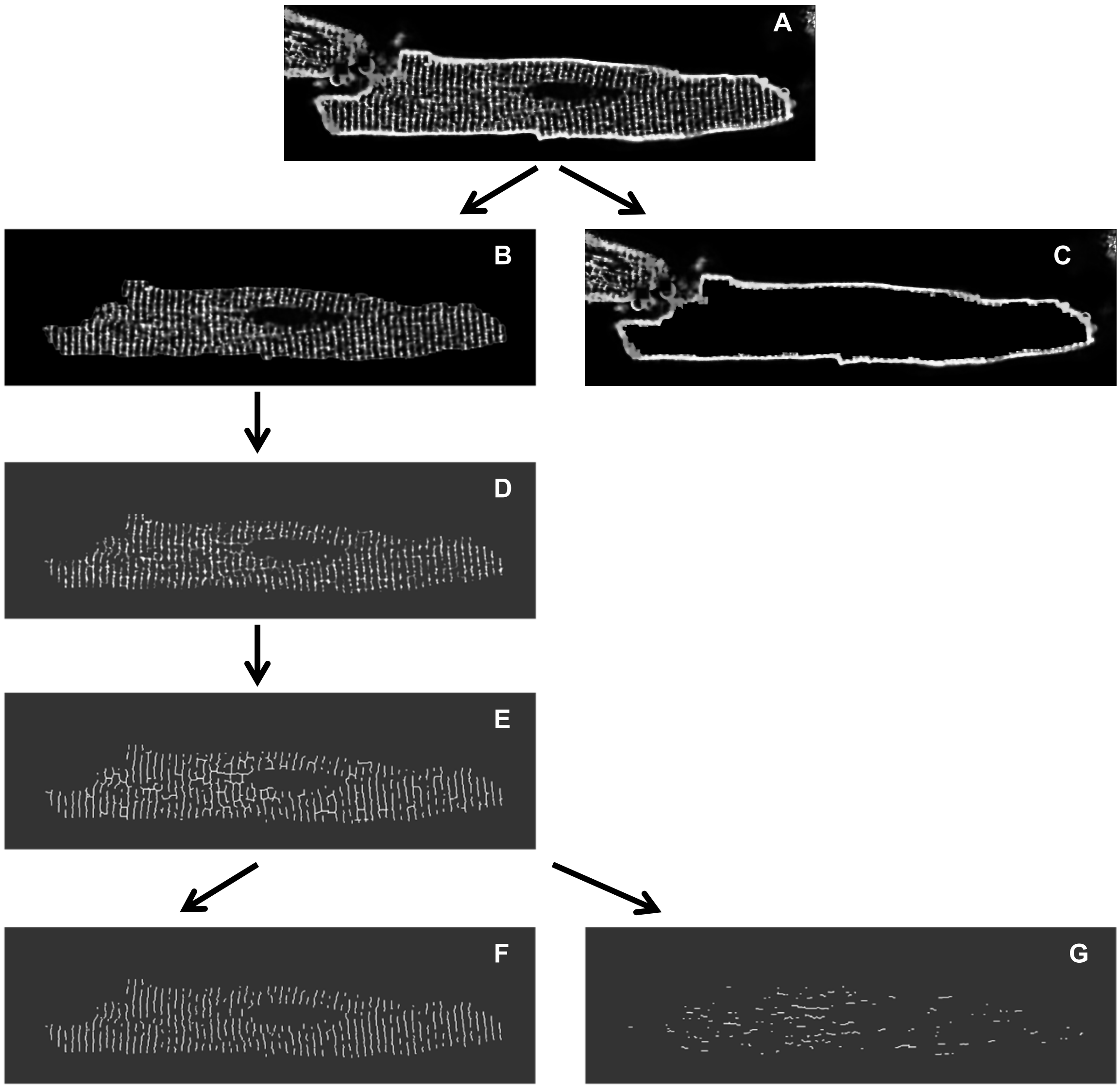


Supplemental Figure S2



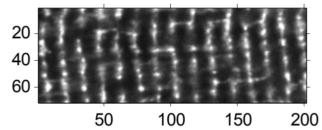

E
Input
Morphological
opening
Horizontal line shaped
Structural Element
1 1 1 1 1 1 1
F
Output
TE
Structuring
Element
LE
E - F =
G

Supplemental Figure S3



Supplemental Figure S4

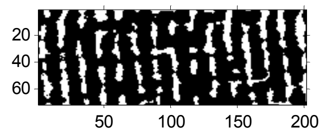
Original gray scale image



Binary image

Threshold =100

T-Tubule area=3450 pixels



Threshold =120

T-Tubule area=2442 pixels