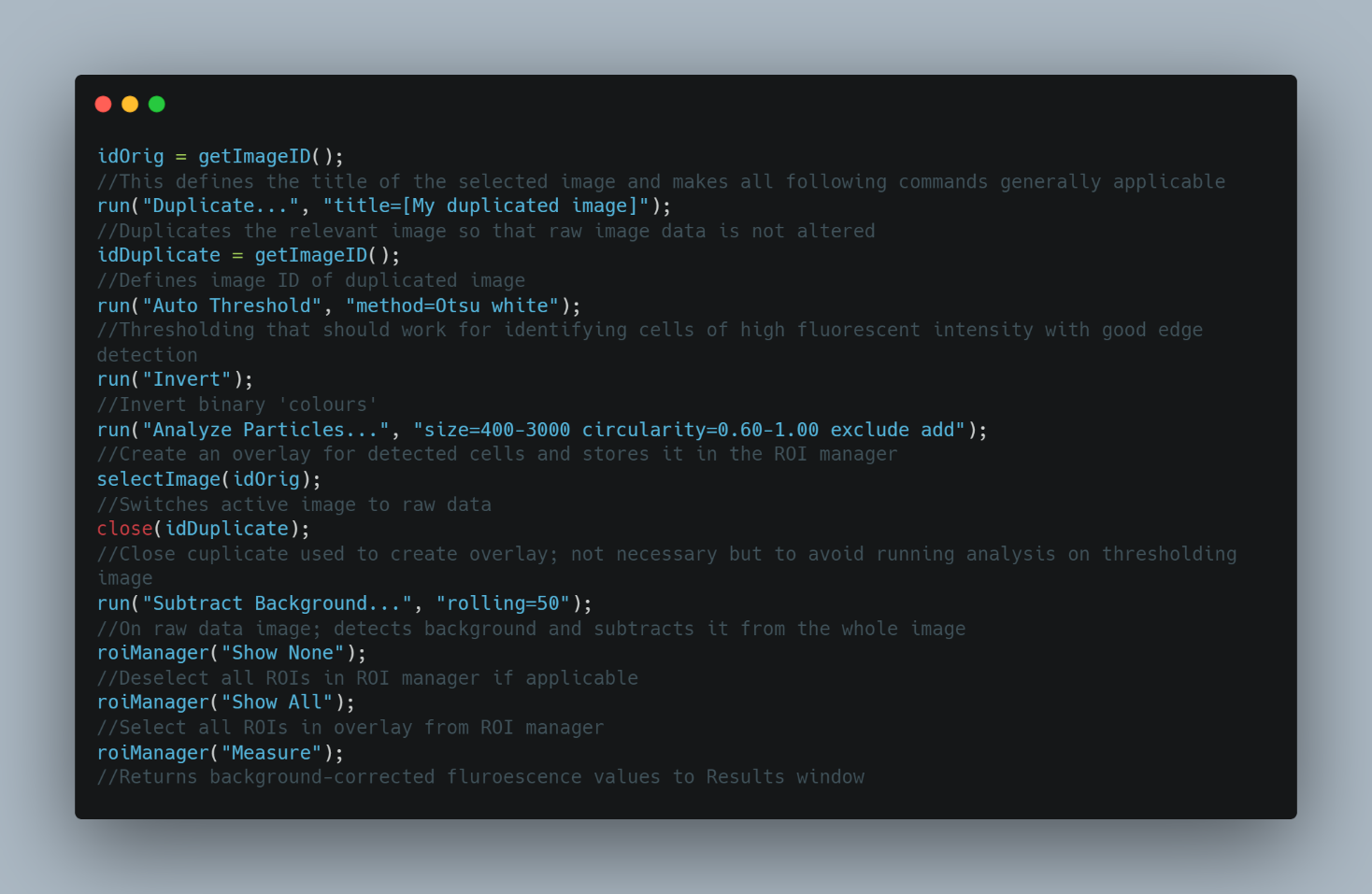
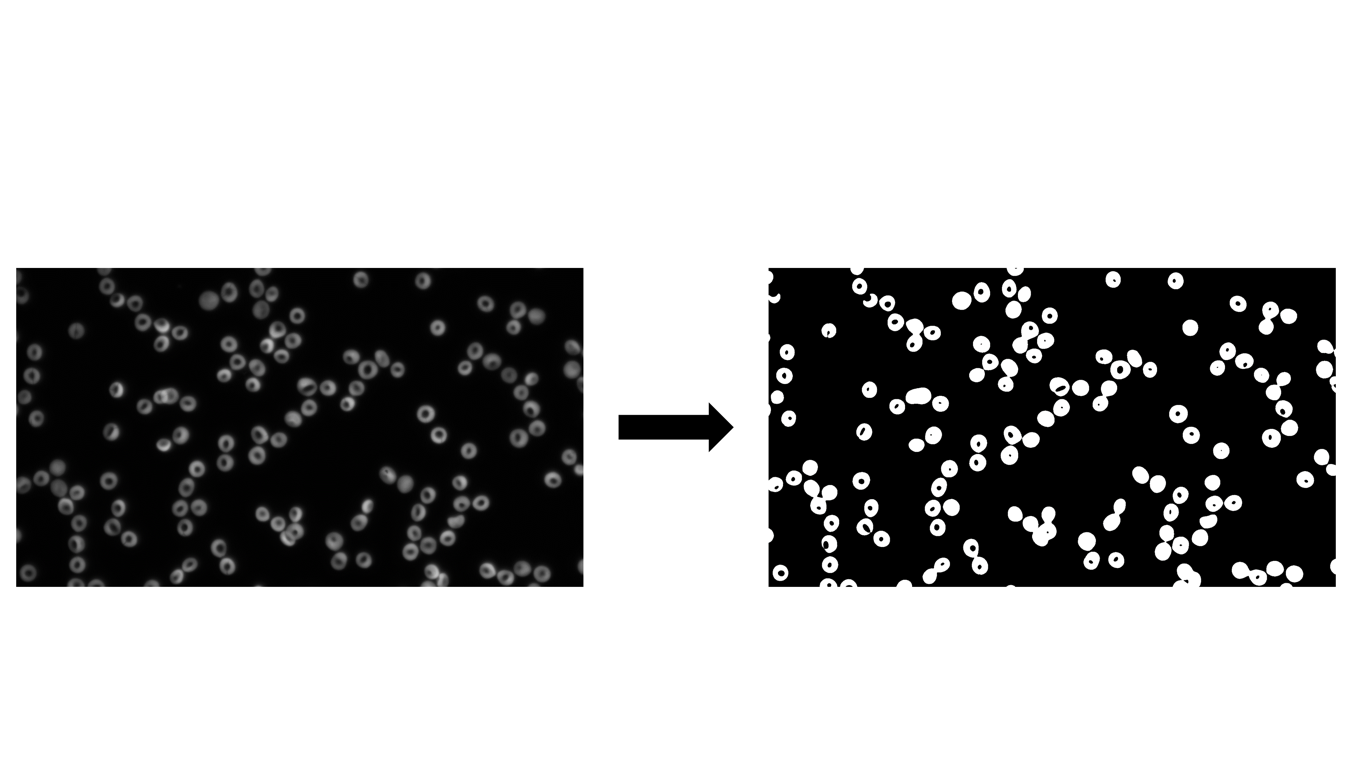
# Supplemental Material: Assessment of dynamic activation states of red blood cell proteins related to mechanotransduction using immunohistochemistry and immunofluorescence.

NOTE: This supplemental document provides a step-by-step description of the image analysis routine developed for the purpose of analyzing fluorescent red blood cells (RBC), as described in the main article. This routine uses automated thresholding to detect the cells present in a given image using a copy of the original image, produces an overlay that is imposed onto the original image to extract grey values of fluorescent RBC.

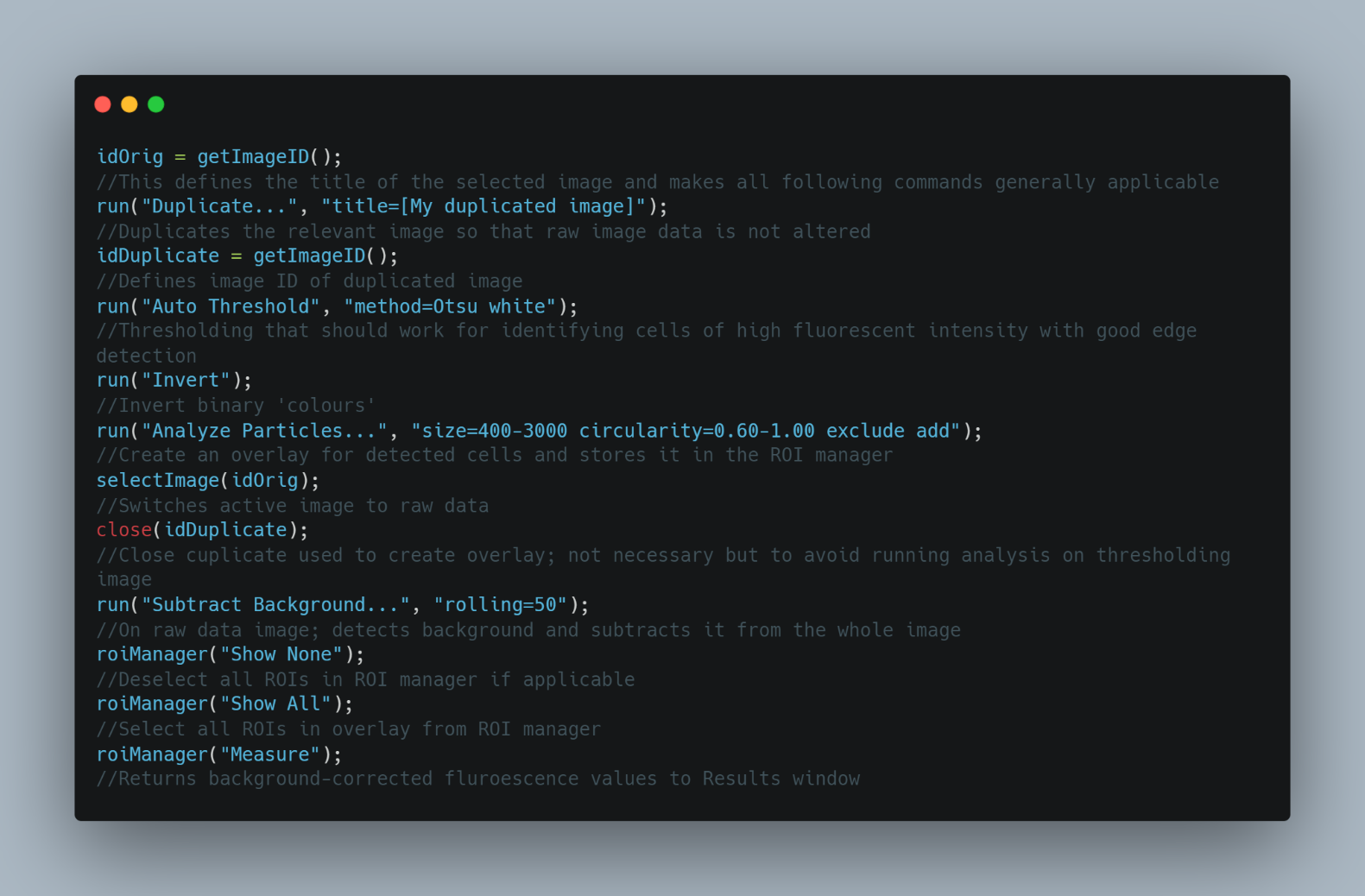
### Cell detection, thresholding, and binarization

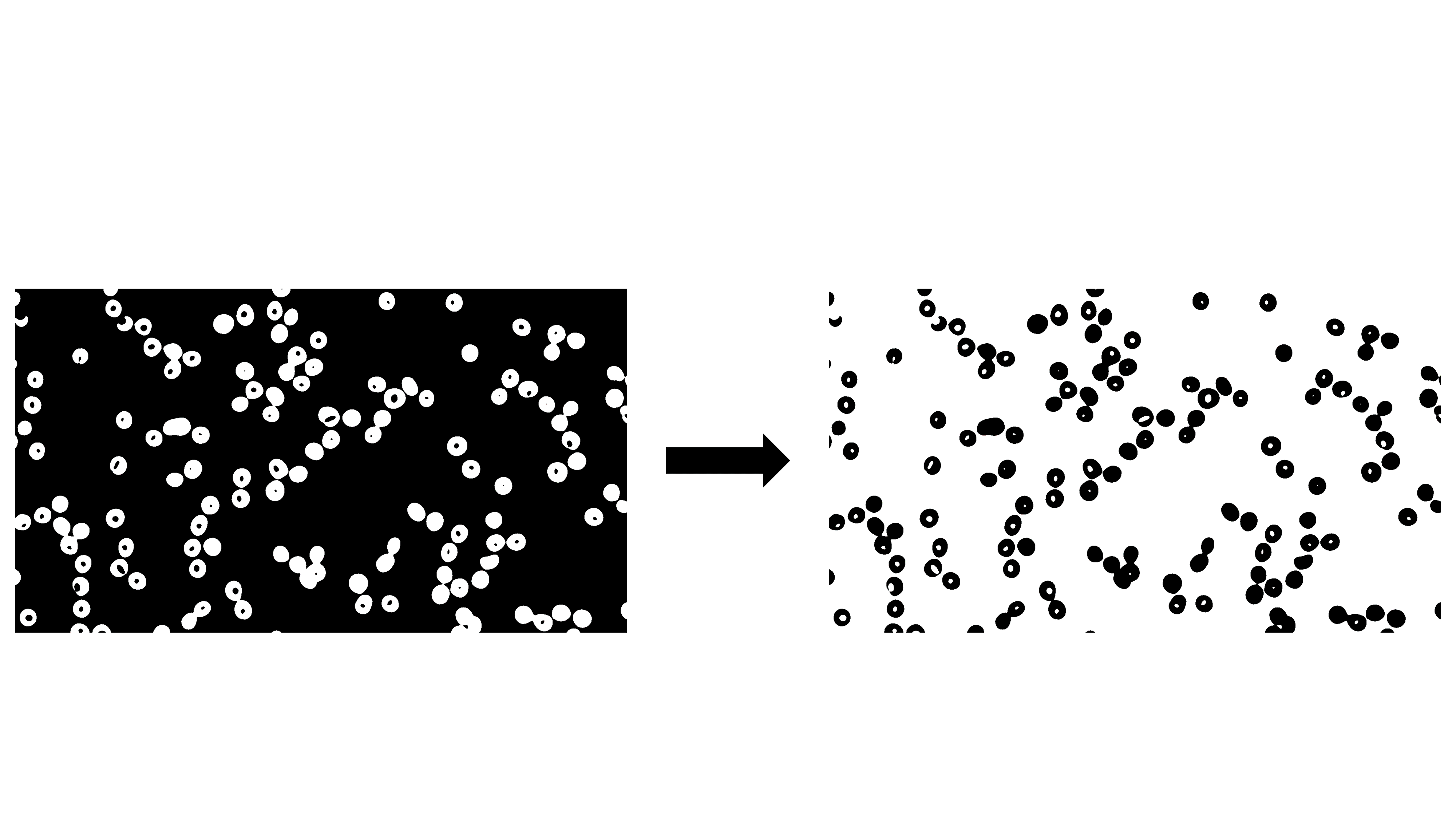
* 1. Perform cell detection on a copy of the original image to preserve the captured grey values. Use automated thresholding to generate a binary image (i.e., rather than grey values of varying intensities, the image only contains white or black pixels), which enables edge detection of cells, wherein every single pixel is either part (white) or not part (black) of the signal.





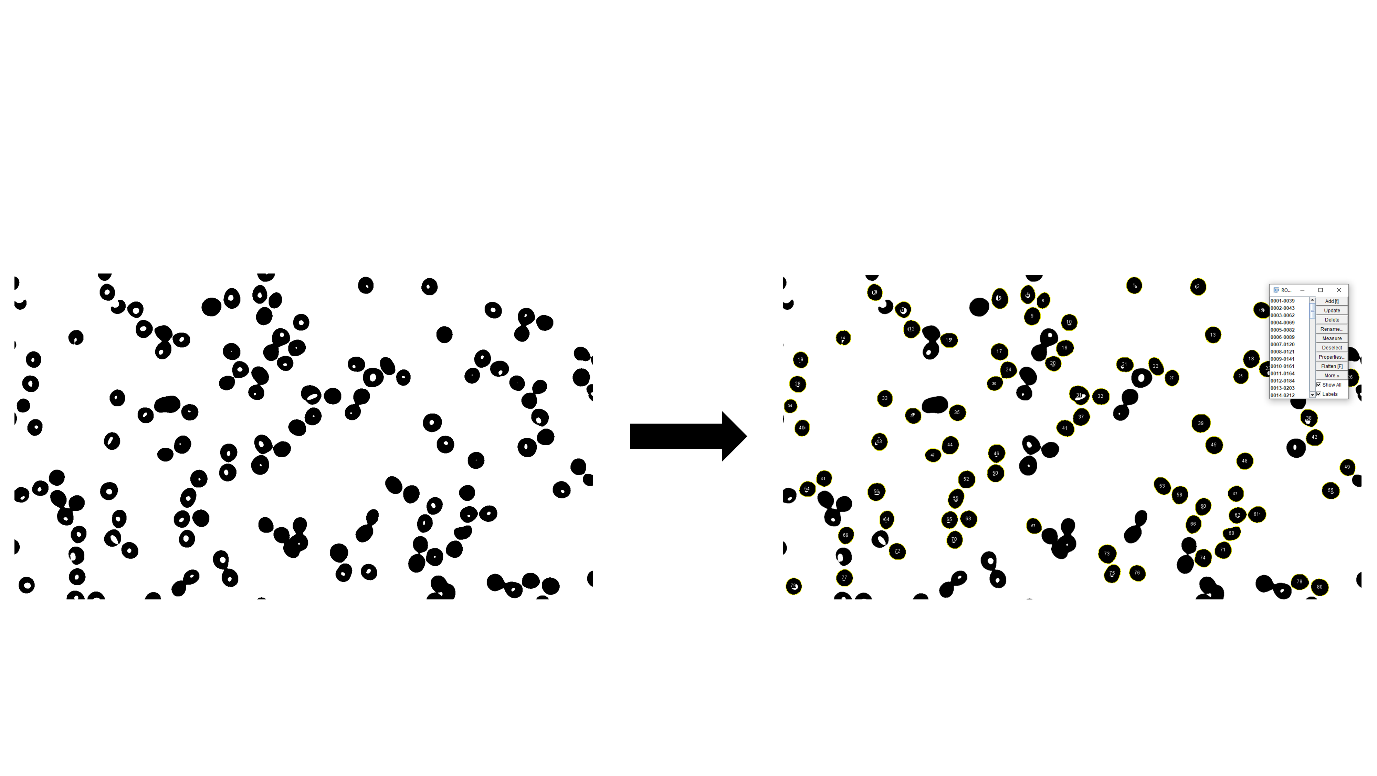
* 1. Invert the white and black pixel values to create an image that contains white background (i.e., pixel value has maximum grey intensity).





* 1. Create an overlay in the selected cells, where only those cells are included that match inclusion criteria of pixel sizes between 400-3000 and exhibit reasonable circulatory. While all cells should fall within these criteria and no other objects should be present in an image, these criteria exclude clustered doublets of cells that may have erroneous grey values due to overlap or cell fragments.





* 1. Store the overlay in FIJI’s ROI manager and project onto the original image. Subtract the background using a rolling ball algorithm and measure and display the background-corrected fluorescence in each ROI.

