Three-dimensional, marker-based watershed segmentation of MSCs encapsulated within hydrogel materials as implemented in Matlab

READ ALL OF THESE INSTRUCTIONS BEFORE TRYING TO RUN THE CODE

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Last updated: 04/29/2024

The following sources were used to generate the code:

https://www.mathworks.com/help/images/ref/watershed.html

https://www.mathworks.com/help/images/marker-controlled-watershed-segmentation.html

Note on data format: The code is designed to work on z-stack image datasets separated into .png files. An intuitive image numbering scheme is encouraged (e.g., image001, image002, …). The code uses nuclei positions as markers to perform maker-based watershed segmentation of cellular bodies. Therefore, images of both nuclei (NUC) and cell bodies (CELL) are needed. The .png files for the nuclei should be stored in a folder named NUC and the .png files for the cell bodies should be stored in a folder named CELL. For ease of use, ensure the Matlab script is in the same folder as the folders NUC and CELL.

The script is split up into 4 main sections:

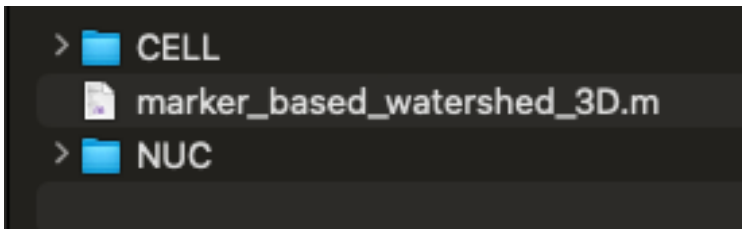
1) **Load .png images into Matlab**
    a. This section allows the user to set the pixel resolution and the step size for their images. It also reads in the .png files for the nuclei from the folder NUC and the .png files for the cell body from the folder CELL. The files in the NUC and CELL folders are then represented as 3D arrays in Matlab.
    b. Note that many stains can be used for the nuclei (e.g., DAPI, Hoechst) and cell bodies (e..g, phalloidin, CellMask) and the code should be adaptable for all stains.
    c. The code includes an algorithm to identify and ignore pseudofiles which begin with '._' that may appear in your directory.
2) **Segmentation of NUC**

a. This section allows the user to segment the nuclei from the images contained in the folder NUC. First, a max intensity z-projection is produced. Then, a user-defined threshold is used to binarize the nuclei and the result is compared side-by-side to the max intensity z-projection. The script is written to allow iterative manual refinement of the threshold value.

3) **Segmentation of CELL**
   a. This section allows the user to segment the cell bodies from the images contained in the folder CELL. First, a max intensity z-projection is produced. Then, a user-defined threshold is used to binarize the cells and the result is compared side-by-side to the max intensity z-projection. The script is written to allow iterative manual refinement of the threshold value.
4) **Watershed segmentation**
   a. This section performs the maker-based watershed segmentation. First, a distance transform is performed on the segmented cell bodies. Then, regional minima are suppressed to prevent over-segmentation. Next, the pixels comprising nuclei are set to -Inf to denote them as markers for the watershed segmentation. Finally, the watershed segmentation is performed. A figure is generated to compare the composite fluorescent images to the final segmentation. Each identified cell is colored a random color. The cell and nuclear segmentations are saved as 3D label matrices in .mat files.

Instructions on how to run Matlab script with screen shots:

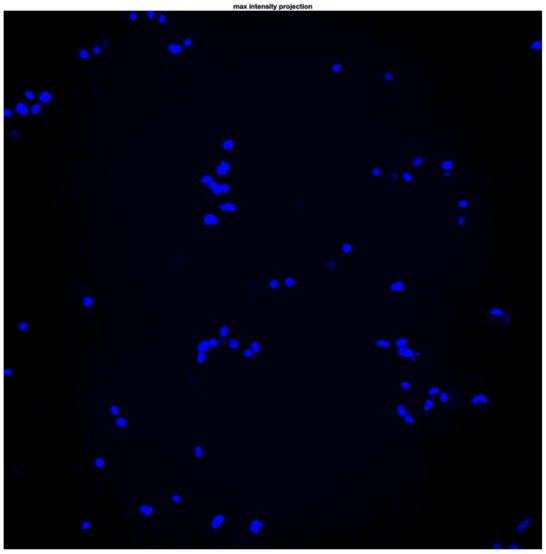
1. Ensure the Matlab script titled ‘marker_based_watershed_3D.m’ is in the same folder as the folders NUC and CELL which should contain Z-stack image sets for nuclei and cell bodies, respectively.

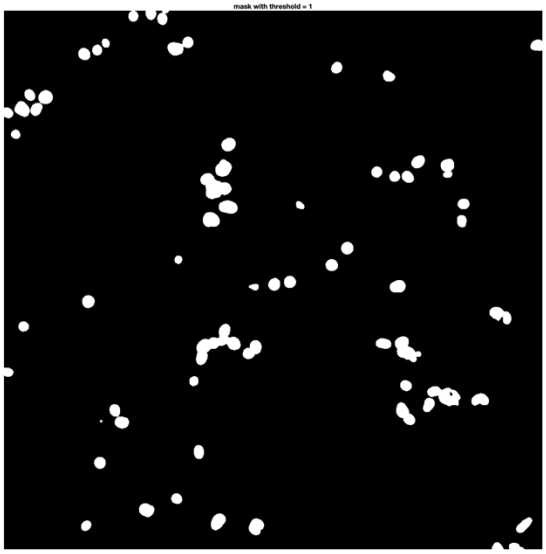


2. Update the pixel and step resolution for your images in the Matlab script accordingly.

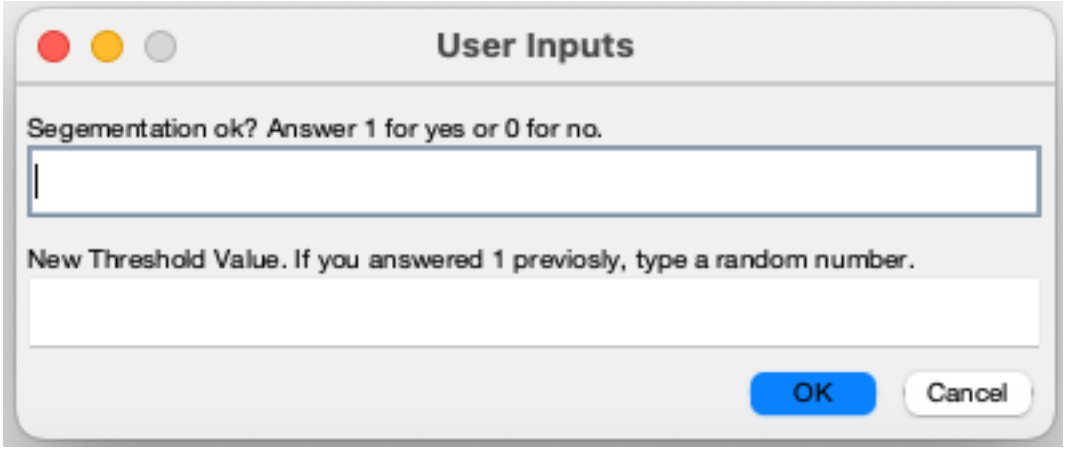
```
% pixel information
pixell=0.5979761; % microns per pixel
pixelw=pixell; % microns per pixel
pixelarea=pixell*pixelw; % microns squared

% stepsize
stepsize=2.0; %microns
```

3. Press Run.
4. A figure window will appear with a max intensity projection of the nuclei shown next to an initial binarized segmentation done with the initial threshold of 1.

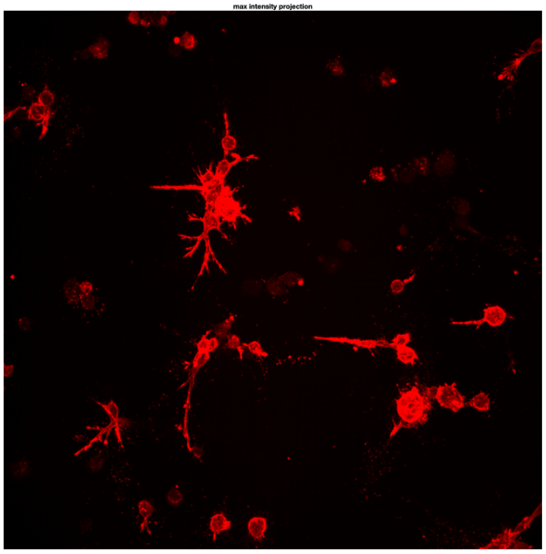


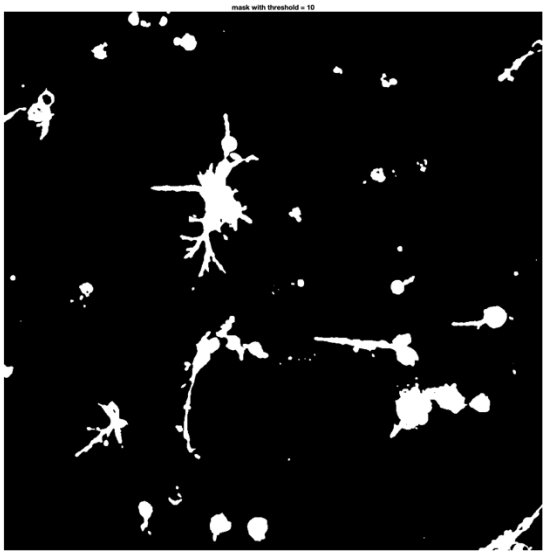


5. After a few moments, a window will appear asking if the segmentation is ok and prompting the user for a new threshold value if the segmentation is not satisfactory. If you answer 1 in the first box, a random number should be inputted as the new threshold value and will have no effect on the remainder of the script.

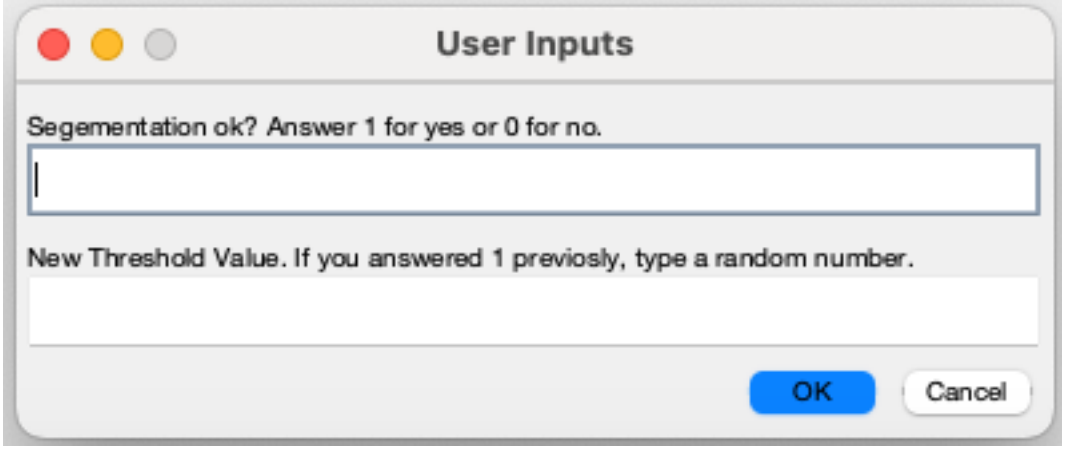


6. A new figure window will appear with a max intensity projection of the cell bodies shown next to an initial binary segmentation done with the initial threshold of 10.



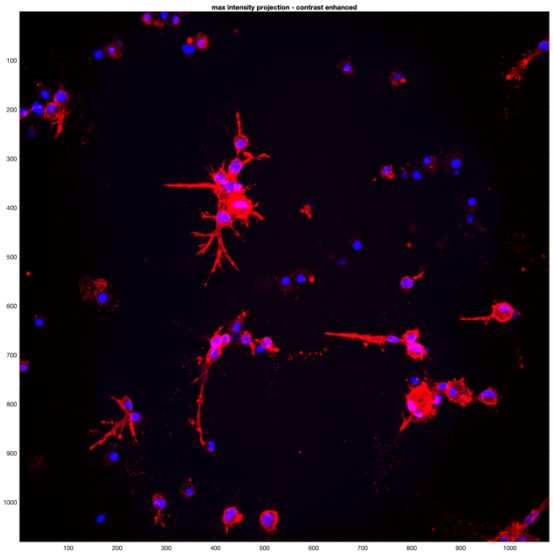


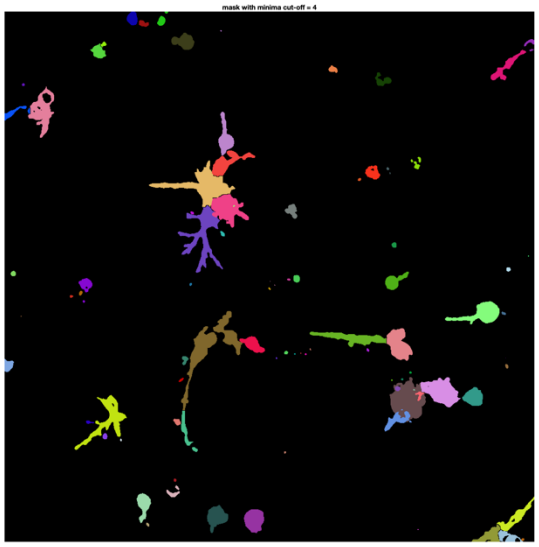
7. After a few moments, a window will appear asking if the segmentation is ok and prompting the user for a new threshold value if the segmentation is not satisfactory. If you answer 1 in the first box, a random number should be inputted as the new threshold value and will have no effect on the remainder of the script.



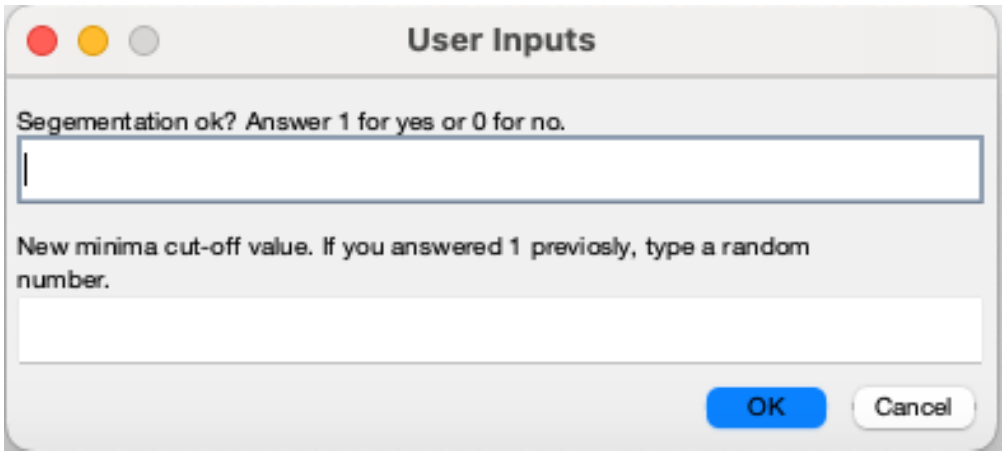
8. A new figure window will appear with a max intensity projection of a composite image showing the cell bodies and the nuclei next to a watershed segmentation

result with an initial minima cut-off value of 4.



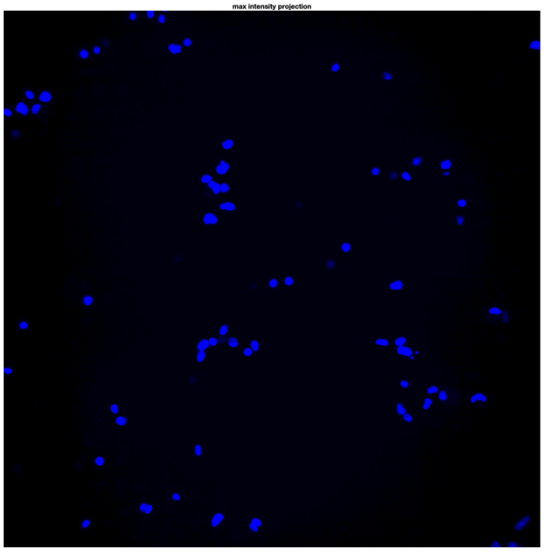


9. After a few moments, a window will appear asking if the segmentation is ok and prompting the user for a new minima cut-off value if the segmentation is not satisfactory. Setting a higher integer for the minima cut-off value will guard against over-segmentation. If you answer 1 in the first box, a random number should be inputted as the new minima cut-off value and will have no effect on the remainder of the script.

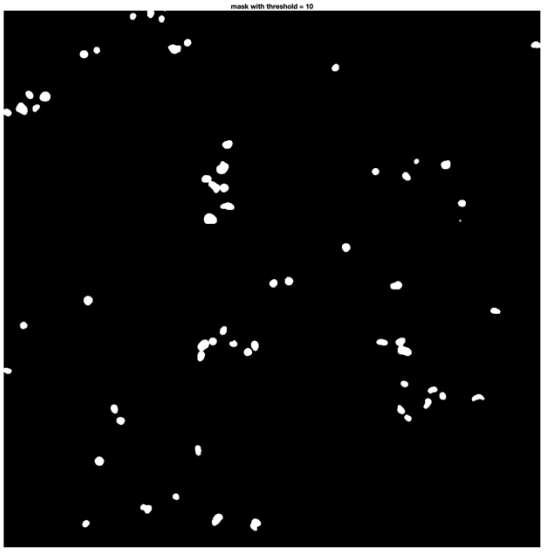


10. Below is an example of satisfactory segmentation of nuclei, cell bodies, and watershed segmentation generated with the example dataset by setting the nuclear segmentation threshold to 10, the cellular segmentation threshold to 10, and the minima cut-off value to 4.

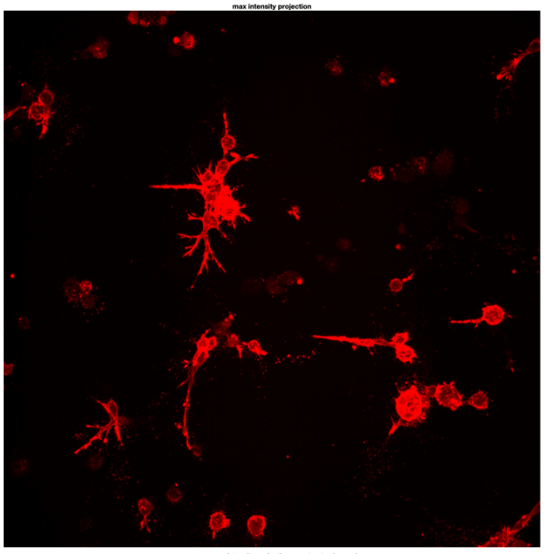
max intensity projection



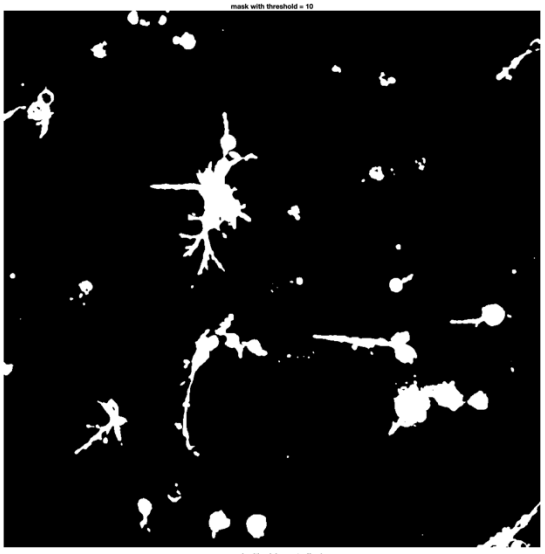
mask with threshold = 10



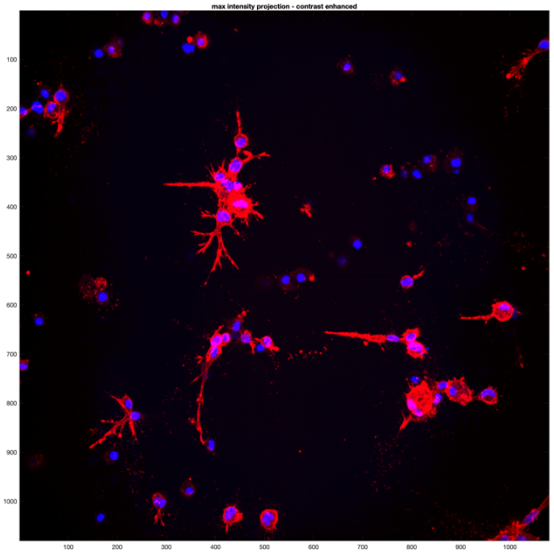
max intensity projection



mask with threshold = 10



max intensity projection - contrast enhanced



mask with minima cut-off = 4