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IEL isolation and image processing in MATLAB

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Submitted by 05/11/22, Washington University in Saint Louis

Abstract

The purpose of this study was to create a streamlined image processing protocol which isolates the internal elastic laminae (IEL) from an image stack taken on a confocal microscope. The projection of the isolated IEL can be used to investigate structural changes to the elastic laminae. Specifically, this process will be used in image analysis for a study on the effects of elastic laminae structure changes on mass transport.

Keywords:

Aneurysm, IEL, elastin, fenestrae, MATLAB, structure, projection, image processing

Introduction

In an aortic wall, the IEL serves as a boundary between the *intima*, the innermost cells in contact with the blood flow, and *media*, a thick middle layer with muscle cells and elastic fibers. The IEL structure influences the mechanical properties of the wall [1]. Included in this structure is fenestrae, small holes in the IEL, as seen in Fig. 1, which function to enhance the transport nutrients across the IEL. Having too few or small fenestrae can lead to complications that degenerate the aortic wall which can have fatal effects in the form of aortic aneurysms [2]. Aneurysms are defined as “a localized widening or ballooning of a portion of an artery, greater than 50% of its normal caliber, usually related to a weakness in the wall of the blood vessel” [3]

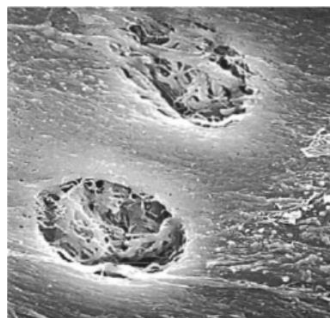


Fig. 1. “Internal elastic lamina (IEL) seen as smooth elastin sheet formed of fibrils with two fenestrae. Elastic fibrils can also be seen through the fenestrae, where they form an underlying meshwork or ‘wire fence’ like structure (3000 \times)” from Robertson et al. [1]

Previous work has been done in the Wagenseil lab and other labs in an effort to isolate and view the structure of the IEL. Lopez-Guimet et al used a computational model to analyze the injuries to layers of the aortic wall due to Marfan Syndrome. The 3D projection aided in the analysis of fenestrae in the wildtype and Marfan models. They found that fenestrae were more common in the IEL of the mice with Marfan syndrome [4]. In the Wagenseil lab, Shawn Pavey created a protocol that combined ImageJ and MATLAB functionalities that was effective in isolating the IEL and fenestrae, but it had a lot of moving parts which made it difficult to analyze a large quantity of images or make adjustments in the process.

The goal of this project was to streamline the process by transferring the code into MATLAB for all processing steps. The new processing code allows for intermediate steps to be viewed and nearly instant results. If adjustments need to be made to the image, it is possible to see the problem, adjust the image and reprocess it without significant time costs.

Image Processing

Because of the wavy nature of the elastin layers, the images taken on the confocal microscope have dark areas where the layer is out of plane and cannot be analyzed. As a solution to this problem the MATLAB script iterates through each XZ-slice of the image stack (Fig. 2a). Then using the MATLAB `imbinarize()` function created a binary mask of each slice (Fig. 2b). The white indicates the elastic laminae, while the black is other muscle cells.

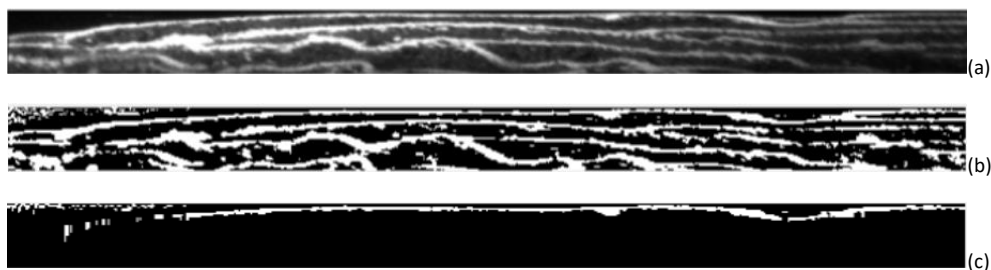


Fig. 2. XZ-slice of an arterial wall image. The original slice (a) is modified into the binary image (b), which is edited to form the skeleton (c).

This binary slice can then be iterated through in the x-direction to find the first instance of white at each Z coordinate. Once the first instance of white is found, the script continues iterating until it encounters a black pixel. These coordinates are used to isolate the IEL pixels and form a skeleton (Fig. 2c). As the image shows, there are areas where noise is picked up in the skeleton which slightly decreases the quality of the final image.

Multiplying the skeleton of the IEL with the original image stack, an image stack of only the IEL pixels is created. Manipulating the rendering of this image in the `volshow()` feature of MATLAB gives a clearer image of the enface IEL. The original and final images are compared in Fig. 3. More fenestrae are clearly visible in the second image which can be helpful for visual qualitative analysis and future image processing protocols which could isolate the fenestrae through thresholding.

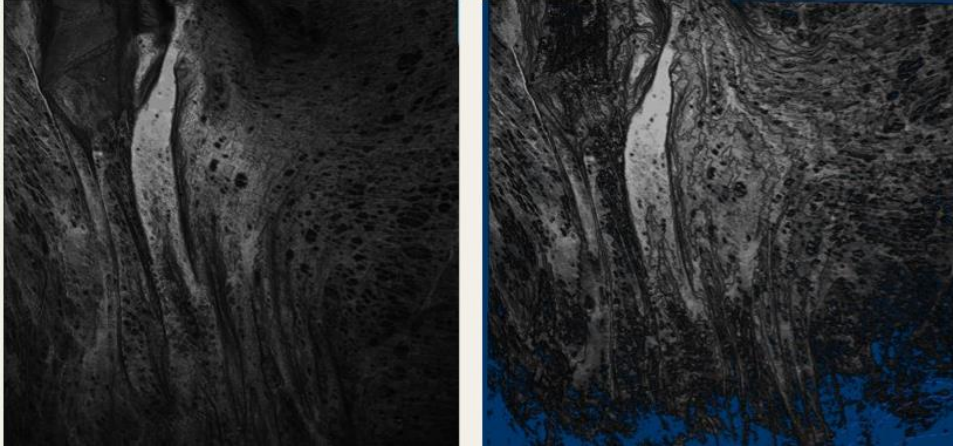


Fig. 3. The left image shows the enface view before processing and the right shows the enface view after processing.

A Second Example

The protocol was also tested using images from a different microscope. The second microscope imaged using red and black contrast and had 340 images in the stack compared to 42 in the image from the previous example. Images taken at each step of the protocol for the second image are shown in Fig. 4. The image is much clearer after the processing protocol and easier to analyze. The 3D rendering shown in Fig. 5. also gives clear and valuable information about the structure of the IEL.

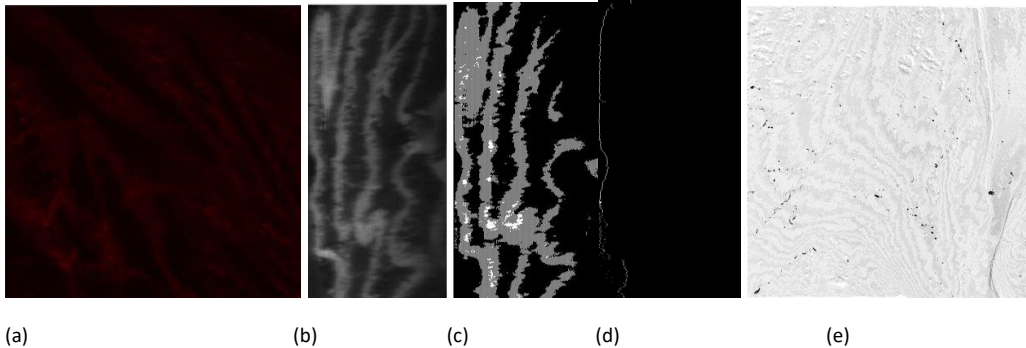


Fig. 4. The original enface view of the image (a), the XZ original slice (b), binary slice (c), the skeleton (d) and the final enface view (e) of the image taken on the second microscope.

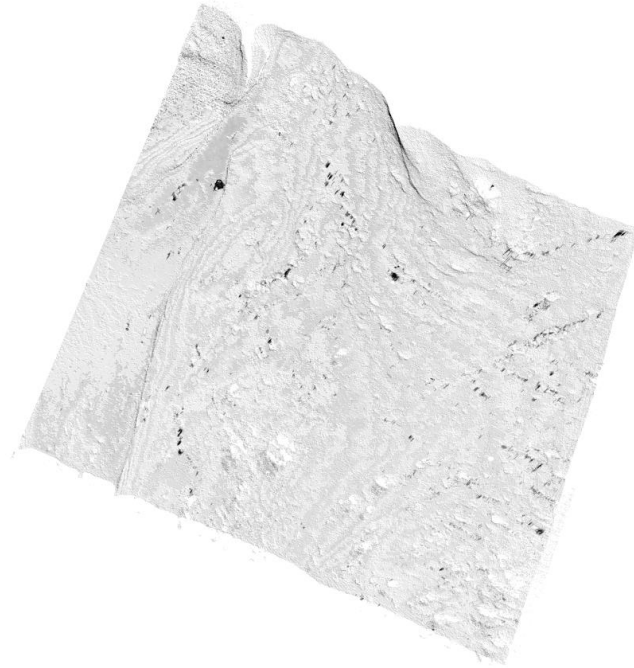


Fig. 4. The 3D rendered image of the IEL captured on the second microscope.

Problems and Next Steps

The aforementioned issue with noise effecting image quality may be alleviated with further image editing before binarizing the image. A suggested route for this in MATLAB is the `lim()`/`caxis()` function. Another future step would be to threshold the resulting images to isolate the fenestrae area and quantify the holes in the IEL. This result would help to quantitatively compare the aorta of mice with different mutations in the lab.

Conclusion

The structure of an imaged IEL is important to recognize patterns in mass transport and resultant deficiencies across the aortic wall. MATLAB protocol as seen above allows the IEL of a given aortic wall to be projected within seconds of loading the file into the code. This efficiency will allow for more samples and consequently more accurate results in subsequent experiments. Additionally, with more work on image adjustment and thresholding, the process could achieve higher results.

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