User Guide for NADH Image Processing

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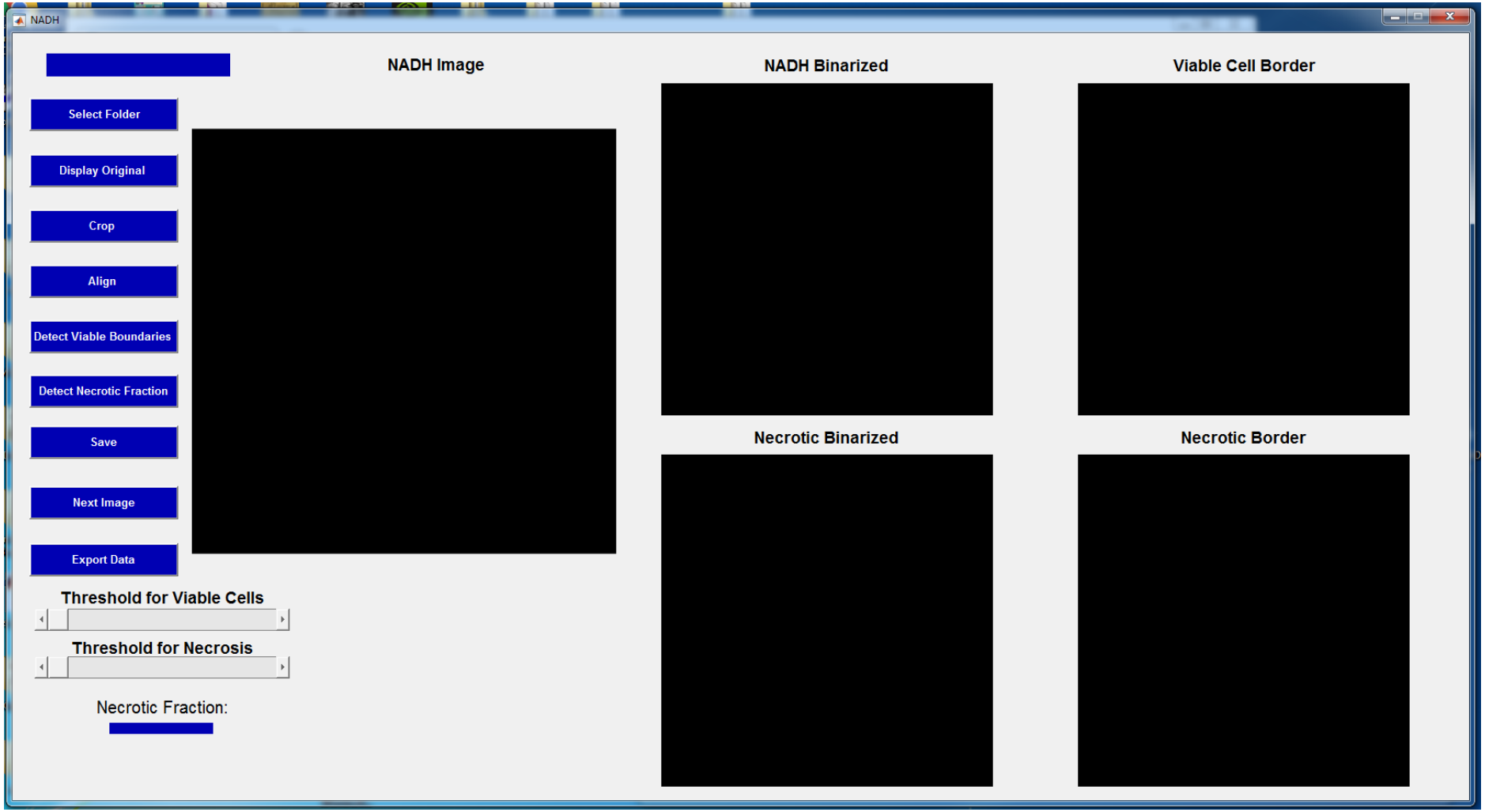
General Explanation of Code

- The code functions by having the user first detect the tissue sample, then removes the excess pixels in the image (which typically confound the detecting due to the gelatin on the slides used for mounting the coverslip and preservation of the sample), and then allowing the user to detect the necrotic section of the sample.
- Some of the processing of the images is a bit slow (I am still optimizing this and open to suggestions if you have any---your coding experience greatly excels mine)
- Depending on the resolution of your samples and the size of the pixel groups you are detecting, the dilation and erosion functions may need to be adjusted from the way I have optimized them for my samples. (see last slides for step by step process of the code)

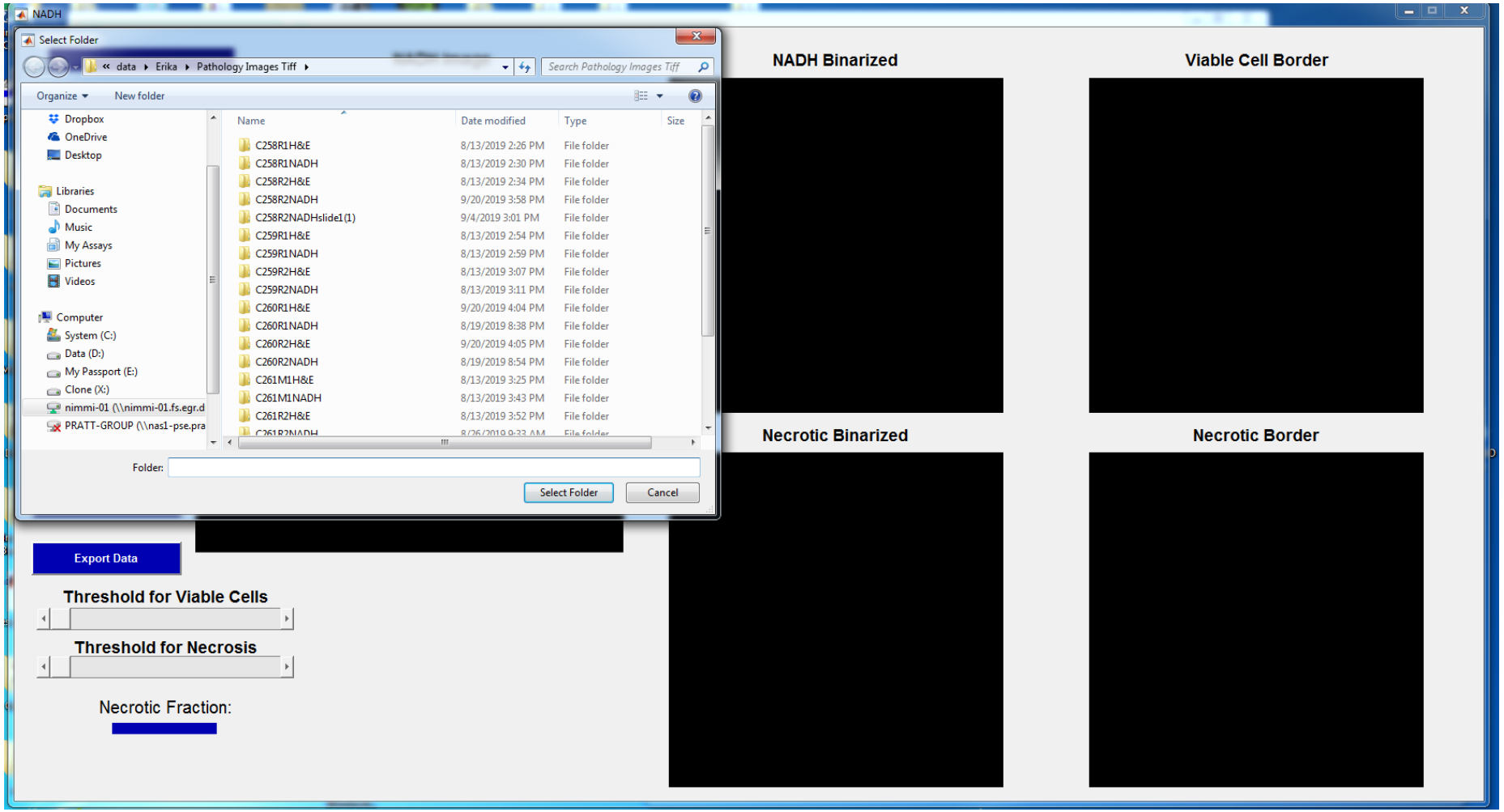
Pre-Steps

1. Organize the NADH tiff files from your sample into a folder. The program functions by selecting a folder and the user segmenting the stain in each of the images in the folder. Images should have unique names, as the output data will correspond to the file names.
2. NADH.m, NADH.fig, and the excel output sheet should all be in the same folder. Excel output sheet should be named ‘NecroticFractions.xls’
3. Create a Folder ‘NADH Save Space’ in the same folder as the code files
4. Update directories in the code to match your computer (lines 505 and 632)

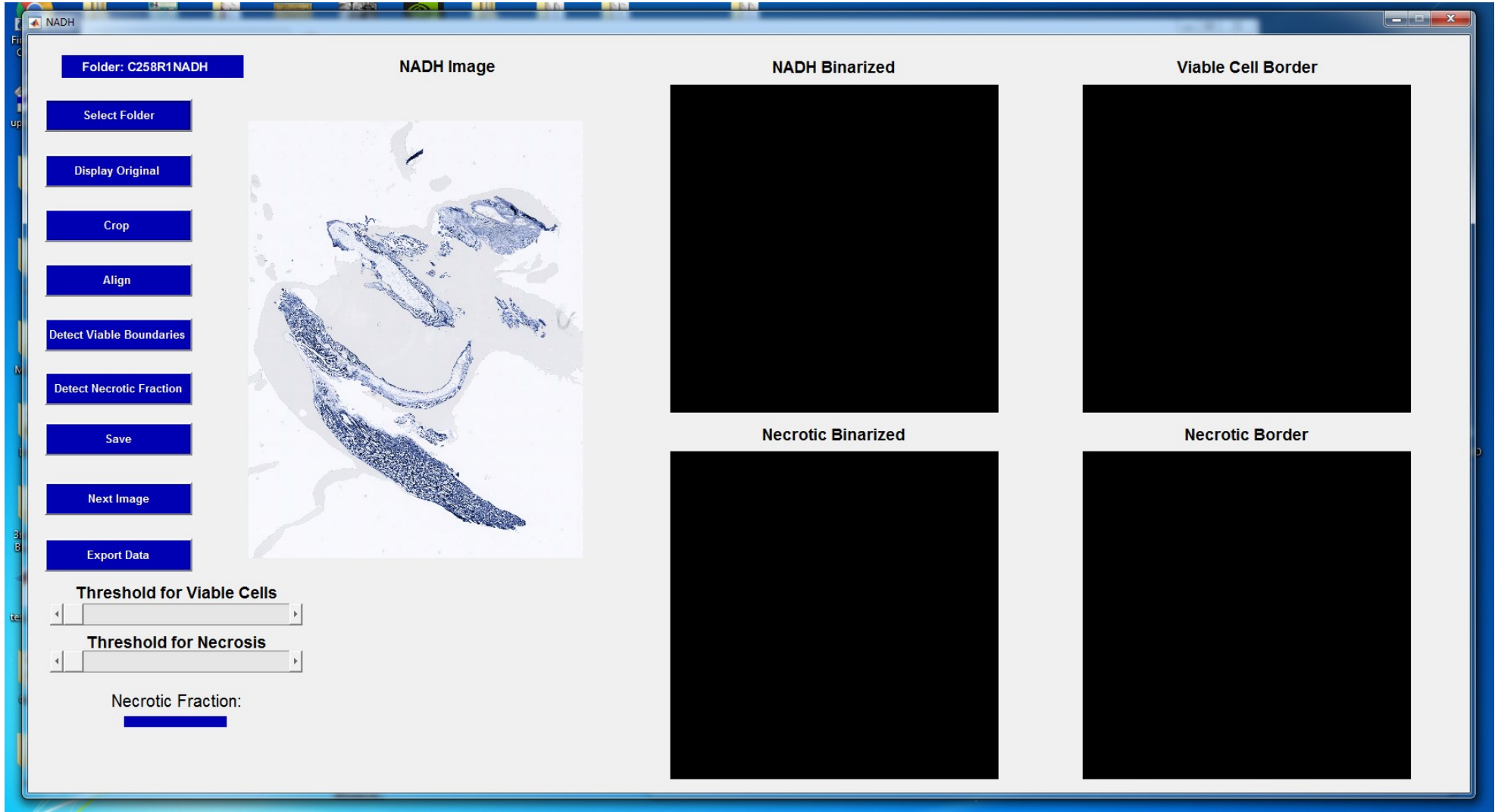
Initial Launch Screen



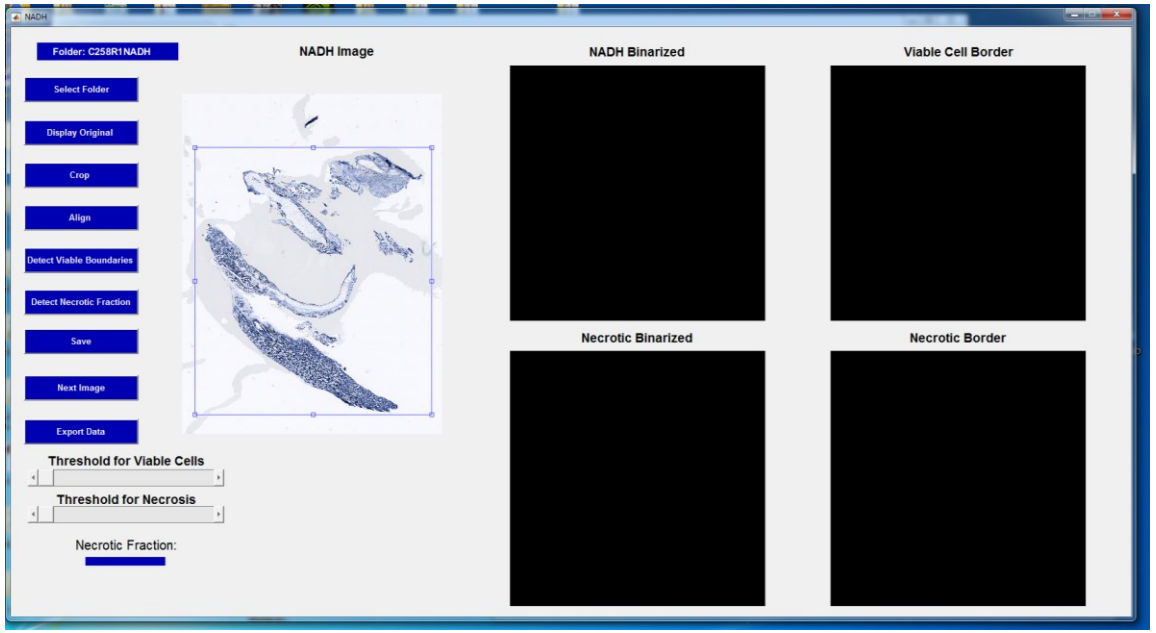
Click ‘Select Folder’ button (top left) and select the folder of NADH tiff files to process

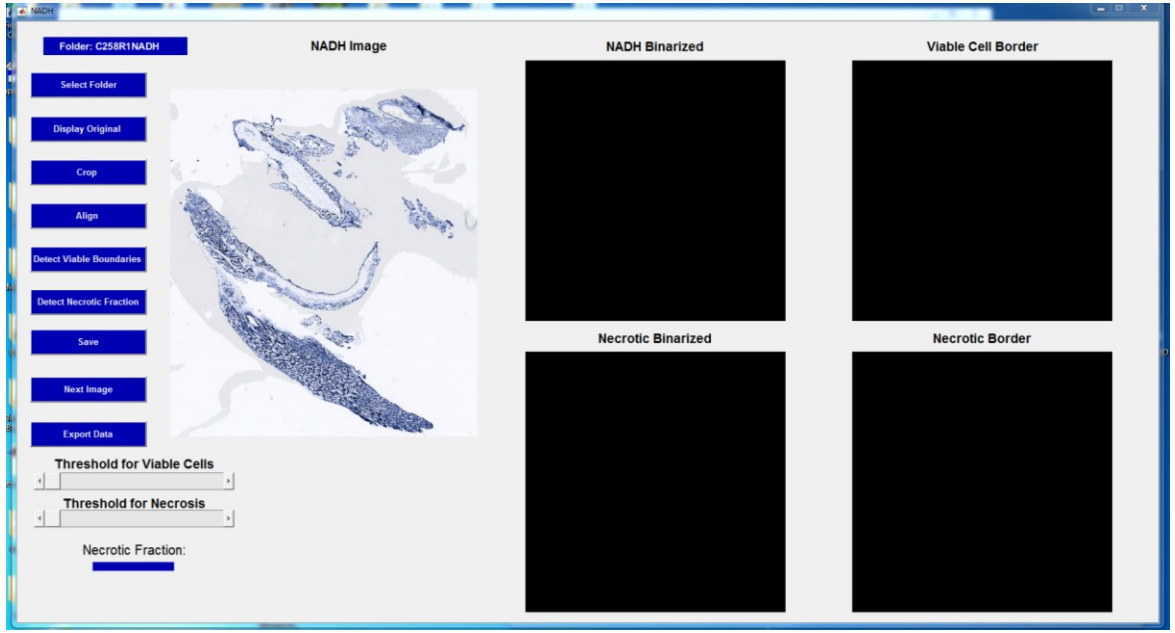


The first image file in the folder will pop-up in the GUI shown below

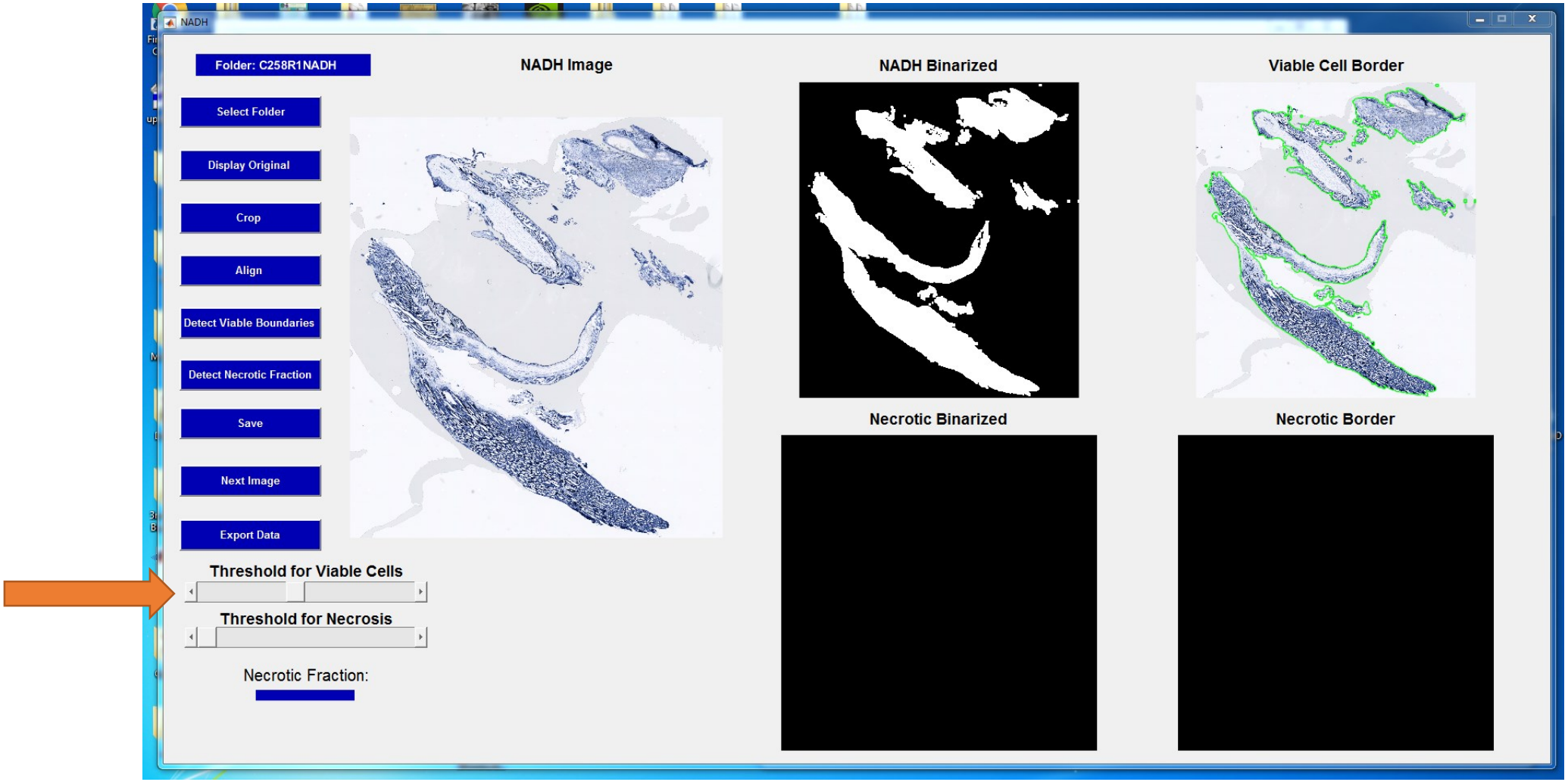


Crop the image if necessary: click the ‘Crop’ button, a blue square will appear on the image. Drag the square or its sides/corners to select an area to crop, and double click within the square to crop, the image should adjust to the cropped area

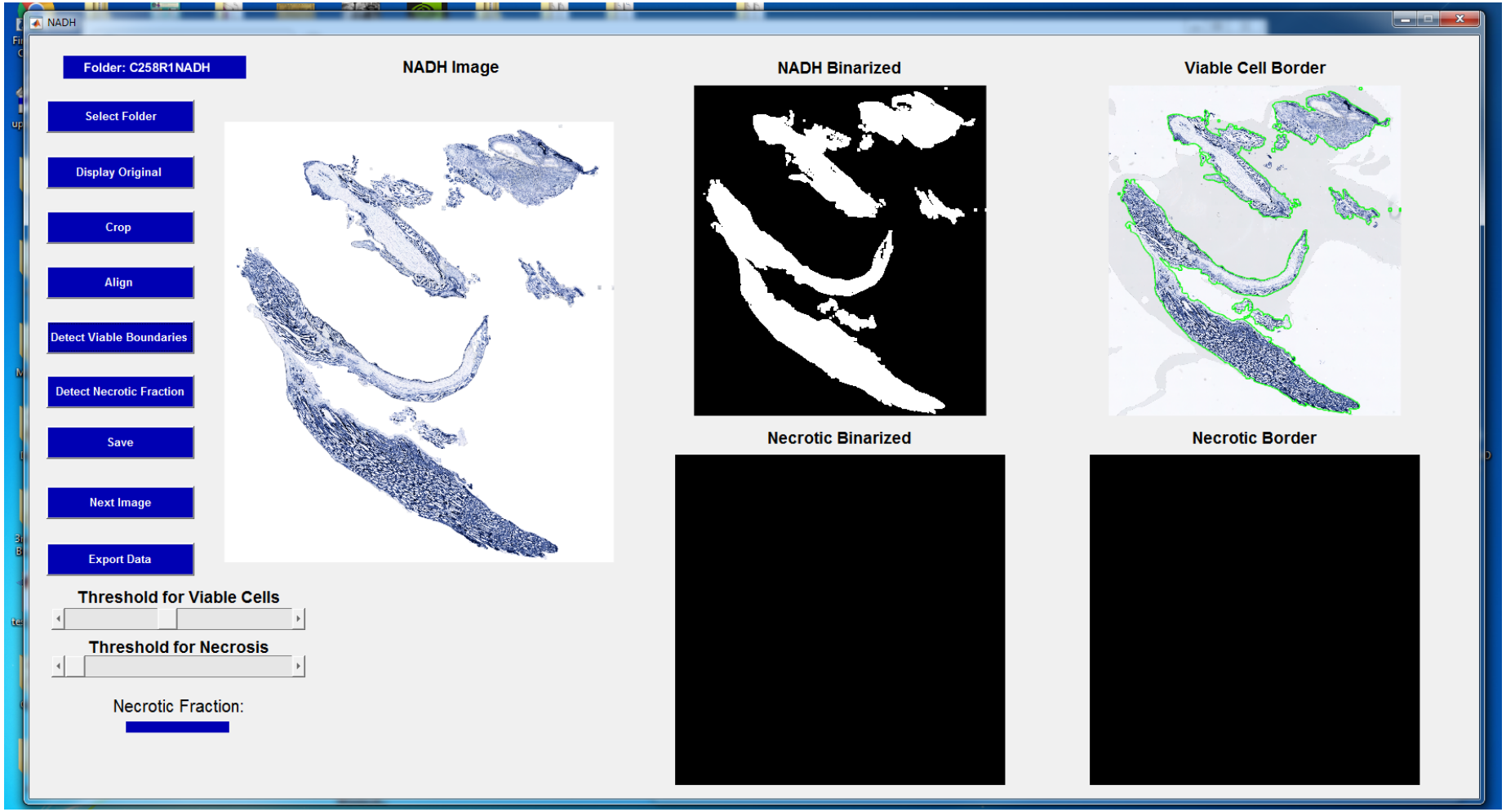




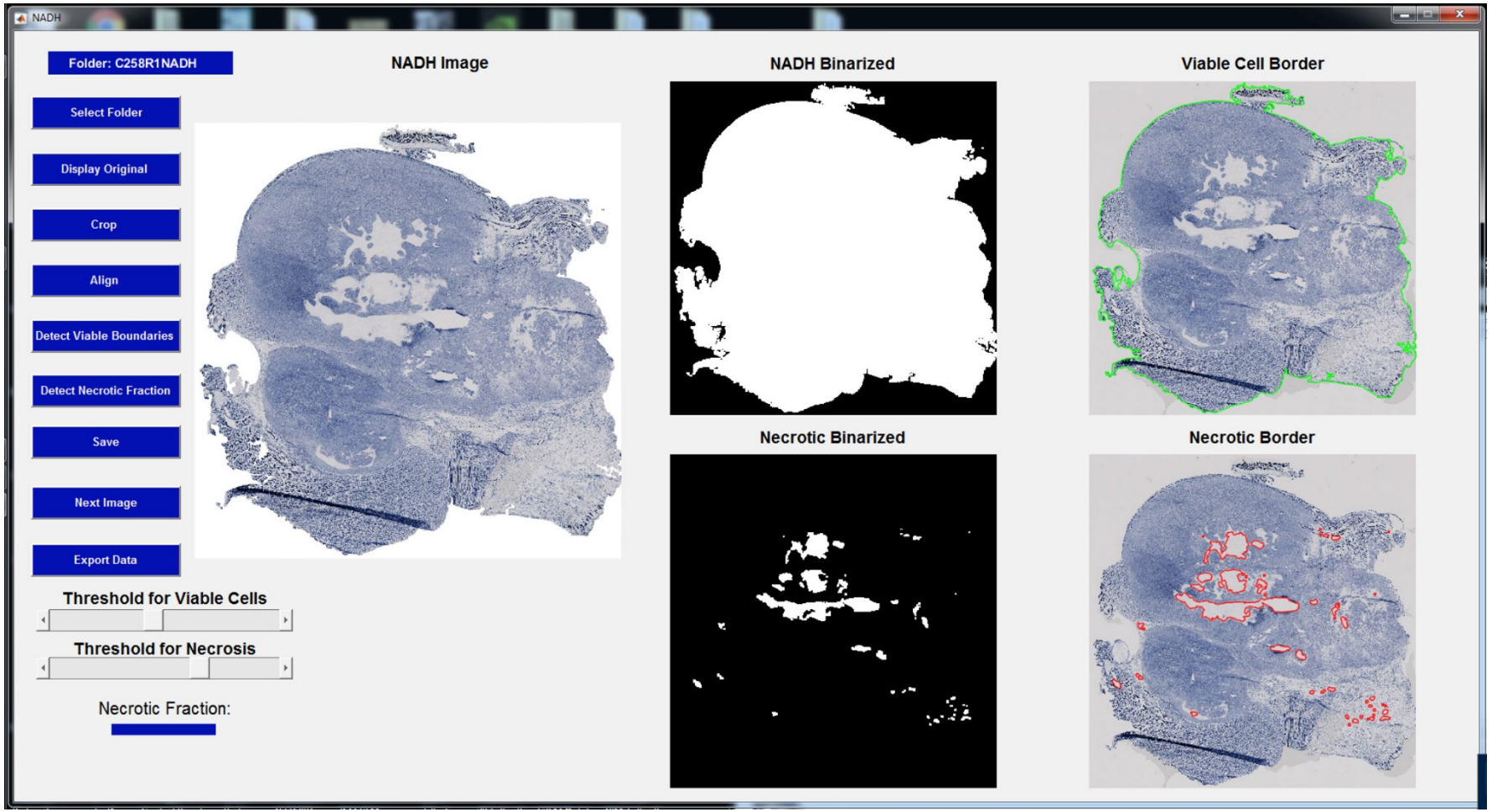
Drag the slider bar for the threshold for viable cells to segment the tissue sample from the background (this may take a second to update on the screen) and display the binarized map and detected borders.
Recommendation: grab and drag the slider in large increments first, then adjust using the arrows at the sides if necessary to maximize efficiency



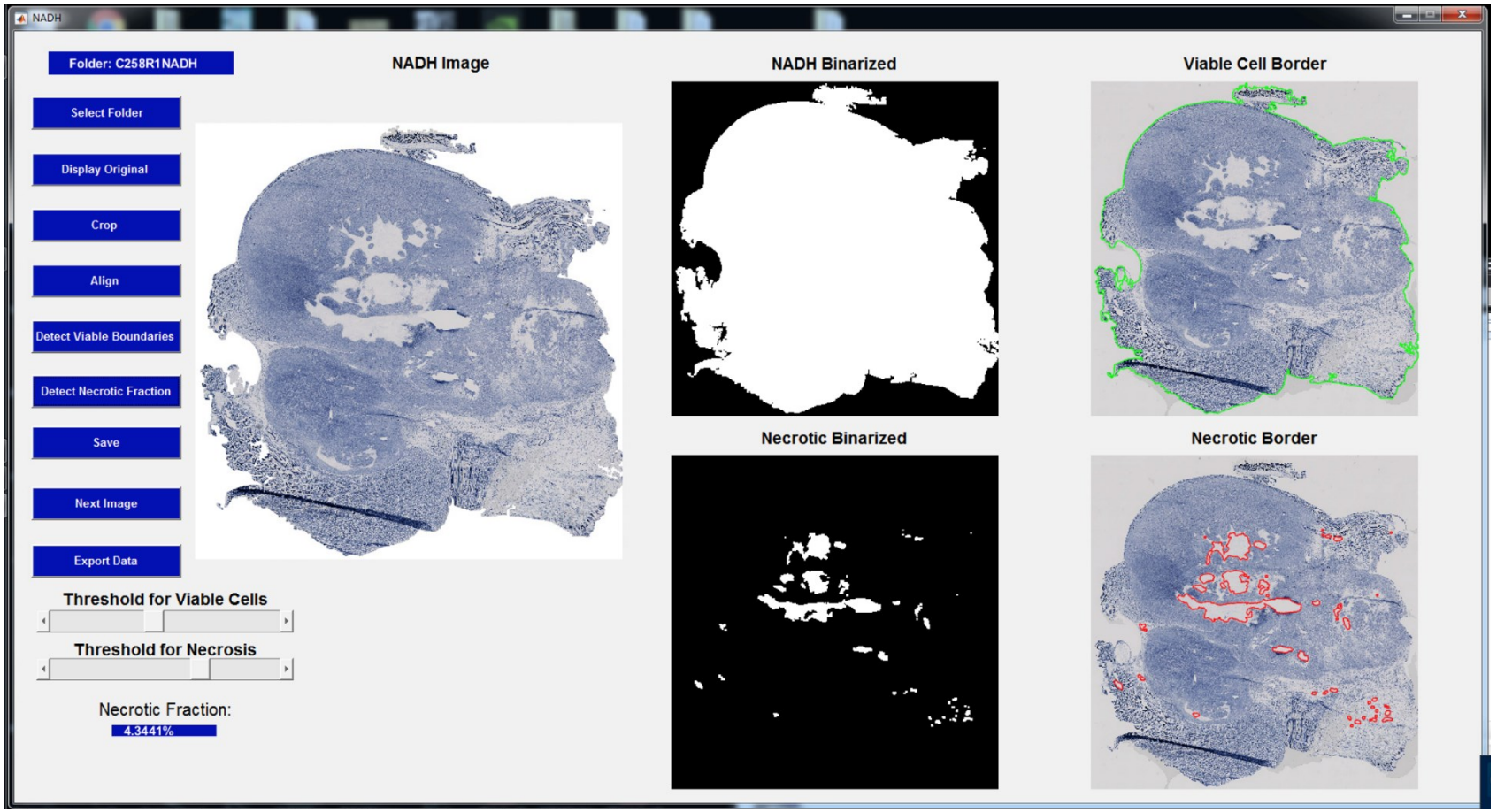
Click the ‘Detect Viable Boundaries’ button to save the data when you are satisfied with the borders of detection (you will notice a change in the large, original NADH image display---background pixels have been removed to improve processing of necrosis)



Drag the slider bar for the threshold for necrosis to segment the tissue sample from the background (this may take a second to update on the screen) and display the binarized map and detected borders.
Recommendation: grab and drag the slider in large increments first, then adjust using the arrows at the sides if necessary to maximize efficiency



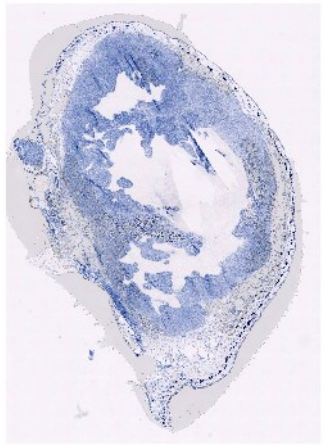
Click the ‘Detect Necrotic Fraction’ button to record the data when you are satisfied with the borders of detection, and a fraction as a percentage will be displayed in the bottom left corner



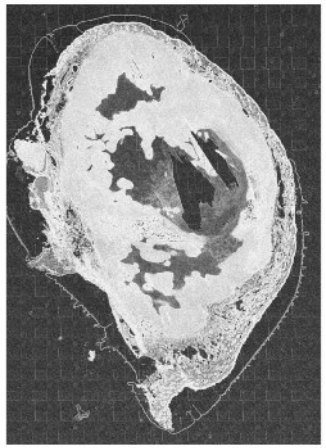
Next Steps

1. Click ‘Save’ to save the data in the memory of the MATLAB program before moving on to the next image. (Prior to saving the data, you can restart the process by clicking ‘Display Original’ at any time)
2. Click ‘Next Image’ to transition to processing the next image in the folder. When you click ‘Next Image’ the images of the borders overlaid on the NADH file will be exported to the save space. (Numerical pixel data will be saved in the excel file)
3. Repeat steps until all images are processed.
4. Click ‘Export Data’ to export the necrotic fraction data into the excel sheet (‘NecroticFractions.xls’)

NADH Processing Algorithm - Sample



Original Image



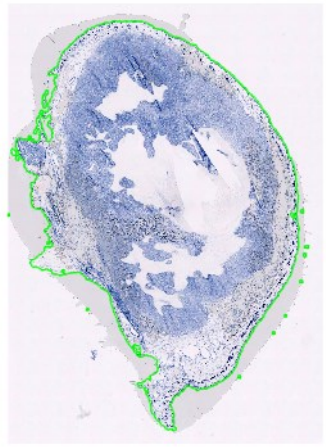
Apply Entropy Filter

Binary Image (based on a threshold value)

Remove Thin/Small Regions

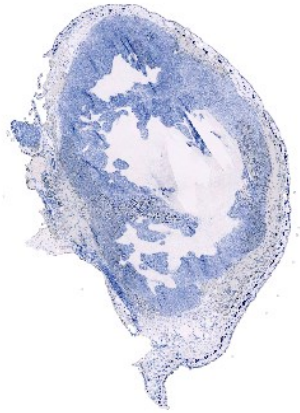
Fill in Necrotic Holes

Undo Thin Section Removal

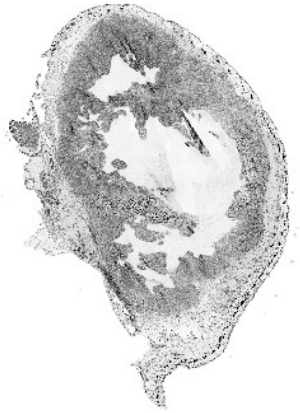


Detect Sample Area and Border

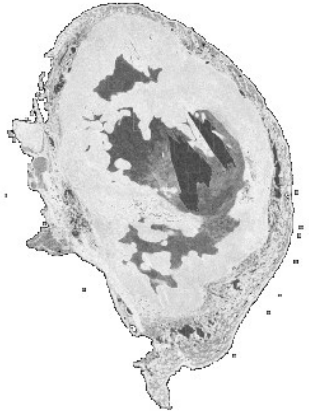
NADH Processing Algorithm - Necrosis



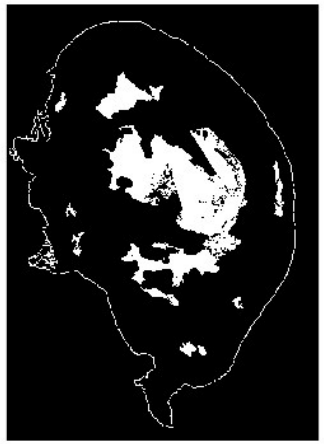
Remove Background from Original Image



Blue Channel Image



Apply Entropy Filter

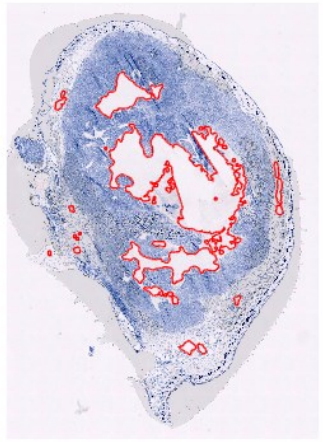


Binary Image (based on a threshold value)

Remove Thin/Small Regions

Fill in Holes

Undo Thin Section Removal



Detect Necrotic Area and Border

Example of entire sample processed (with corresponding H&E stains