2018

Cathode Separator

USING IMAGEJ/FIJI MACRO TO SEGMENT CATHODE CATALYST LAYER

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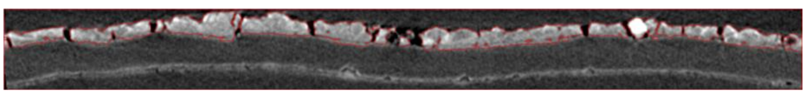
Purpose

The need to segment the cathode (or anode, which can be done with the same following steps but different parameters) is used often in failure analysis of polymer electrolyte fuel cells. To quantify the changes following degradation and corrosion, accurate segmentation is required, as well, when wanting to analyze membrane changes, first removal of the cathode (and anode) is required (for this, see membrane segmentation).

An example output of running the following procedure is shown below for both 4x imaging (1.5um pixel size) and 20x imaging (0.67um pixel size) with the Zeiss Versa 520 XCT. Any pixel size can be used, provided that the spatial resolution is sufficient to isolate for the catalyst layer of interest. This is performed in 3-dimensions over a full stack of images, although only one slice is shown below.

1.5um pixel size



0.69um pixel size

Average thickness Green: 11.6 ± 3.8um (9mm^2 area)
Average thickness Red: 11.04 ± 3.9um (0.42mm^2 area)
SEM thickness: 11.8 ± 3.3um

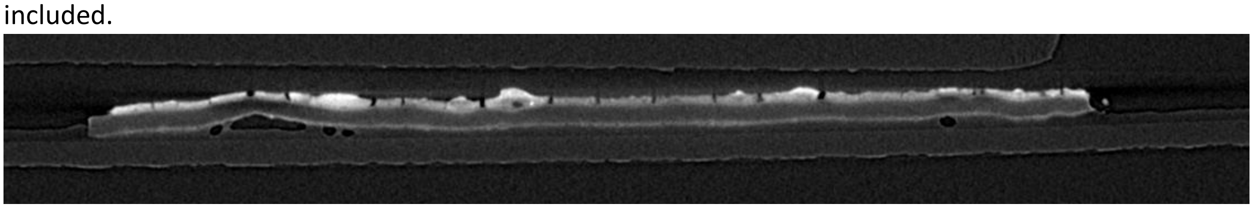
As is observed, the cathode is isolated from the anode and GDL fibres, although much of the greyscale values are similar. The below procedure improves accuracy and reduces the need for significant user input allowing for improved repeatability.

1. Preprocessing image stacks

Although it is not required, running noise removal such as 3D median filter can be used to improve results, this will need to be determined by the user and should be consistently applied to any subsequent image sets which are used for comparison. Rotation correction in the X and Y planes (Tilt) is also not completely necessary but will help with segmentation and analysis later.

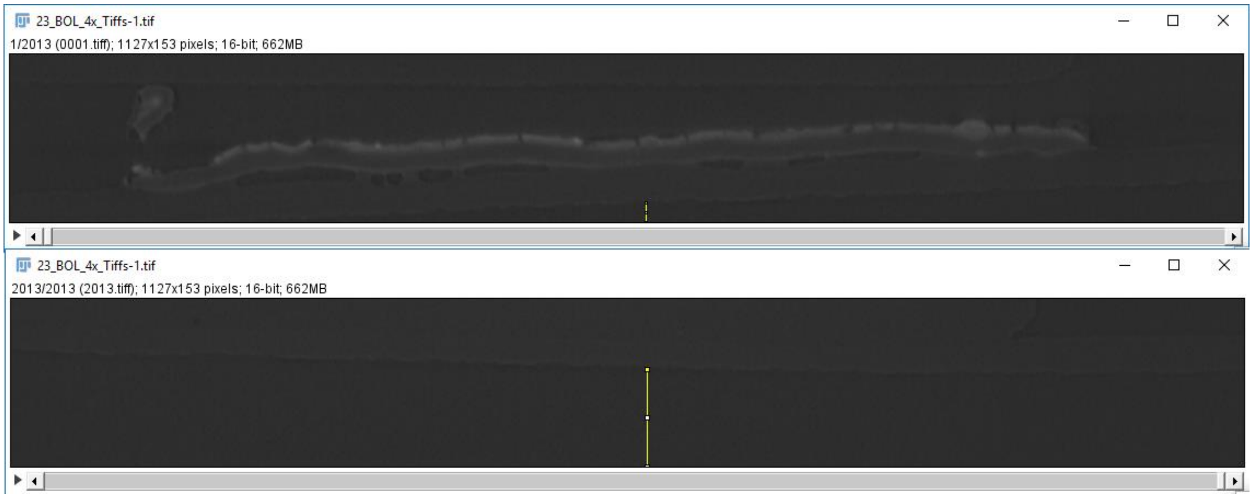
1.1 Crop

As is discussed in 'Manual Stack Alignment' (https://github.com/robintwhite/NXCT/blob/master/ImageJ_macros/ManualStackAlignment.pdf) first cropping is performed to reduce image size. Refer to 'Manual Stack Alignment' for details on this step. In this example we will use a sample which is not housed in the small-scale fixture for comparison. A region of interest (ROI) is cropped and shown below; where a reference side used in tilt/rotation is also included.



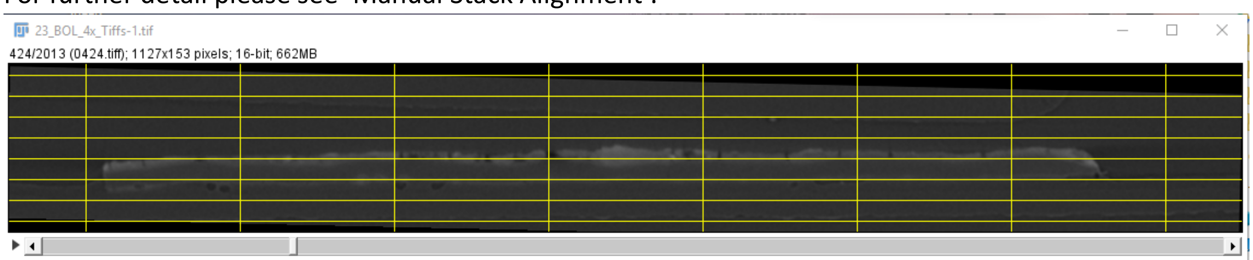
1.2 Tilt correction

For further detail please see 'Manual Stack Alignment'. After cropping, correction for the tilt is applied using TranslateMacro as shown below. It is important to remember when running this macro that the image unit of length properties are first changed to pixels in all directions (Image>Properties).

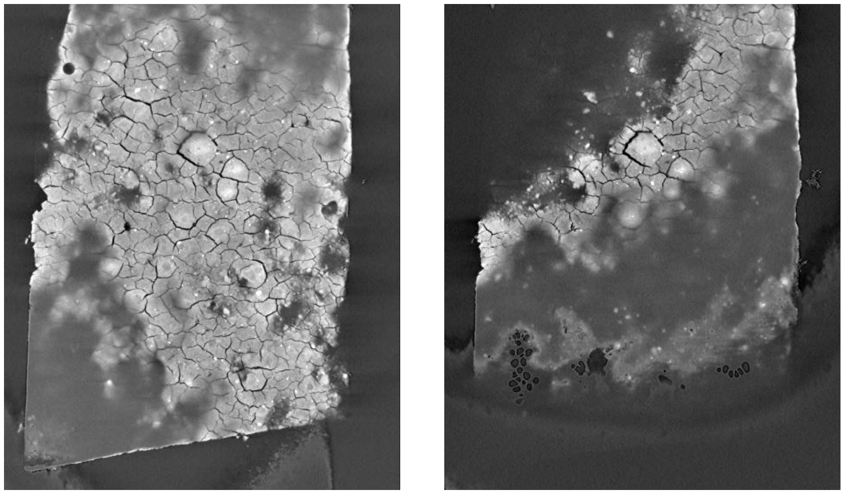


1.3 Rotation correction

For further detail please see 'Manual Stack Alignment'.



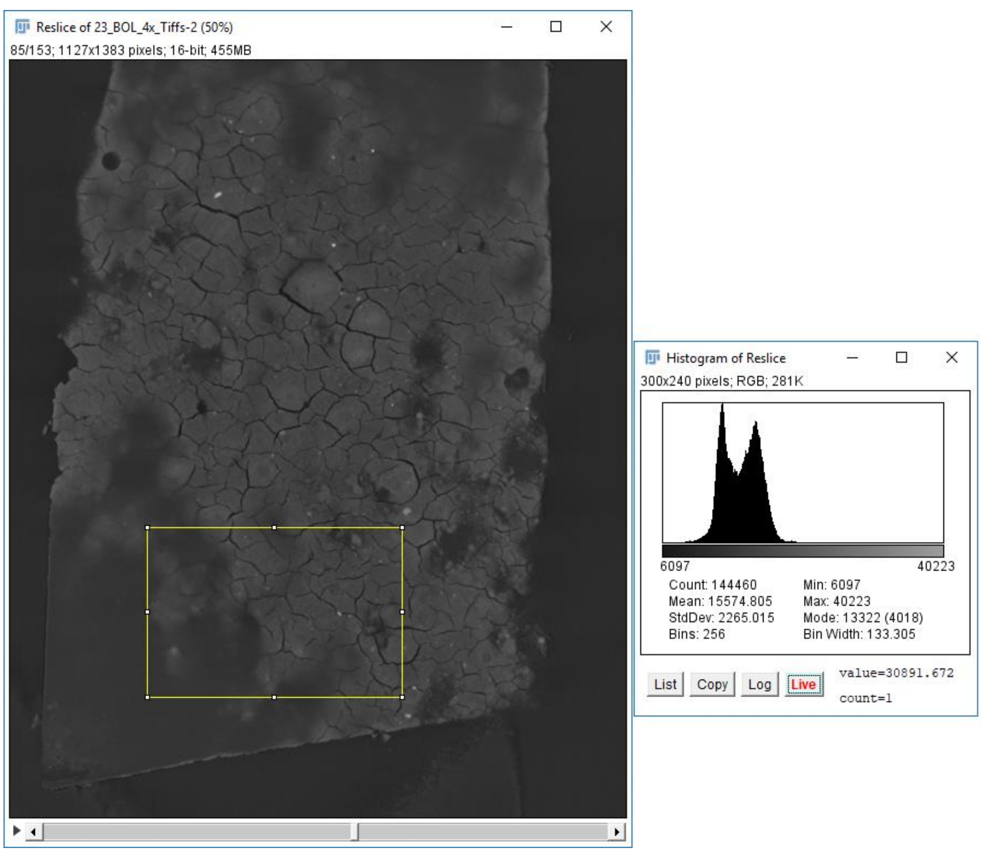
The image stack should now be corrected for rotation and tilt. The goal is to be able to have the full catalyst layer in a single slice. This however is often not fully achieved since parts of the catalyst layer may be differently rotated than others. It is up to the user to decide on how to get as much of the catalyst layer in a single slice as possible. As was mentioned, these steps are not absolutely necessary, and the separator macro will run well without them. Below shows a comparison with and without rotation and tilt correction of a single through-plane slice through the cathode catalyst layer.



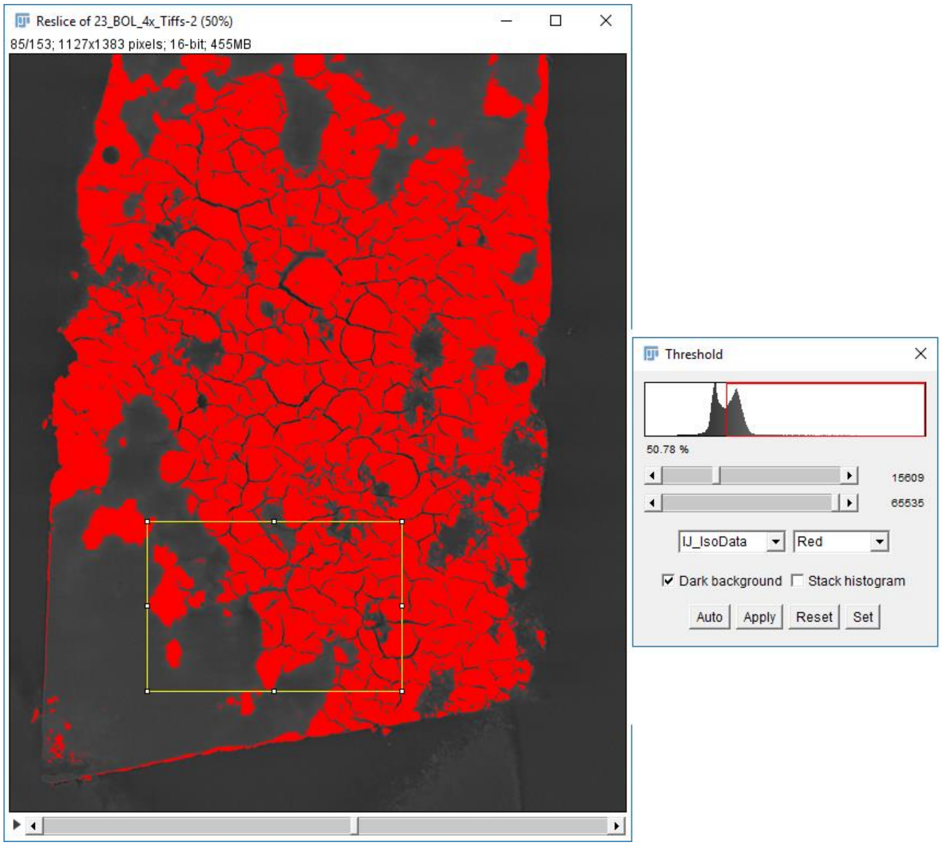
[Left] With tilt/rotation correction as outlined above [Right] Without correction. As can be seen a better view of the catalyst layer is achieved after correction.

2. Thresholding

Although thresholding can be performed a number of different ways, a simple and repeatable method is to utilize some user input and automated assignment of threshold value. One method is to use the ROI tool in imageJ to select a region such that two gaussian peaks are distinguishable, i.e. as shown below.



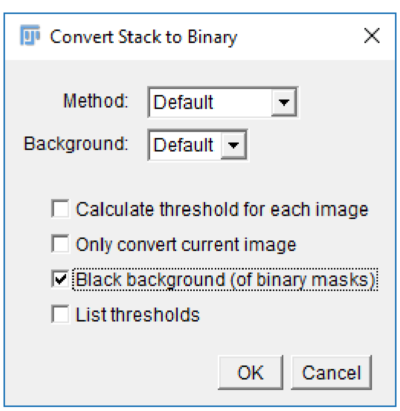
This can then easily allow for a thresholding algorithm to separate the two gaussians and give a good threshold value, shown below.



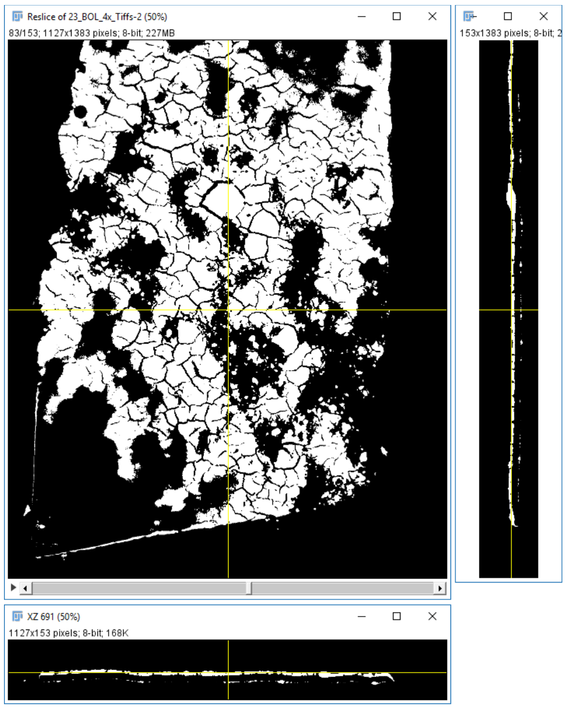
Here IsoData algorithm is used (Ridler, TW & Calvard, S (1978), "Picture thresholding using an iterative selection method", *IEEE Transactions on Systems, Man and Cybernetics* **8**: 630-632) however the result will not be sensitive to the algorithm used and can be determined from user preference, as long as it is consistently applied and stated.

Hit 'Apply' making sure Dark background is selected and the end threshold value is max at 65535 (for 16bit images) as shown above.

Use the following settings after being prompted:



The following shows the initial result following thresholding. As can be seen from the orthogonal views, poor isolation (segmentation) of the cathode catalyst layer is performed with much of the anode remaining in view. This will be even more prominent if GDL fibres are also present. Next, we will run the Cathode Separator macro to clean up the segmentation.



3. <u>Cathode Separator</u>

Provided you have followed the installation instructions, placing the CathodeSeparator_.ijm macro file in Fiji plugins folder you will have access to the macro from Plugins>Macros. Please see ReadMe if you have not yet installed the macro or BoneJ.

Start CathodeSeparator macro and follow the notice. It states that if the current image name ends in .tif, be sure to change it by Image>Rename to remove the filetype ending (this is to help with naming convention during the macro and will throw an error at the end). Sometimes naming can yield problems with the script, it is best to have the image name not end in -1 or -2 etc also since duplicates created during the script can cause problems. An example of naming used here is 23_BOL_4x_Tiffs_Thresh

If you select the Help button it will outline the input parameters.

Image stack needs to already be segmented for cathode catalyst and in through plane view

GUI inputs:

Gaussian Sigma: The level of blurring to join cathode irrespective of cracks Select threshold: Manual selection of threshold after blurring. This should be chosen to be the lowest threshold value where the anode doesn't touch the cathode.

Gaussian threshold: If the above manual selection has already been determined and is unchecked
Get Thickness: Perform SUM Z-project and count pixels for local thickness calculation (in pixels)
Keep images: If unchecked, will close all windows except for AnodeRemoved stack and PixelThickness if Get thickness is selected. More for debugging purposes.

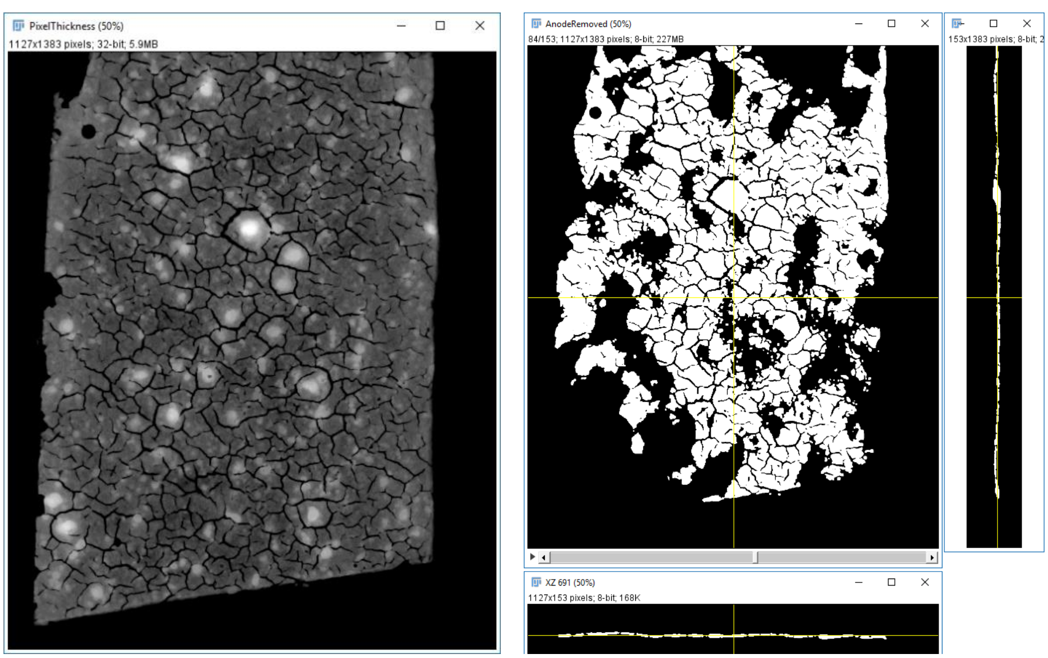
Using the desired values, select OK.

The script will first run through a generic noise removal by despeckle and median filters. Once this completes, the macro applies a gaussian blur which acts to connect the cathode into one object. The user is prompted to select a threshold and press **OK**. The threshold should be chosen such that the cathode is fully connected and not touching other components, an example is shown below:

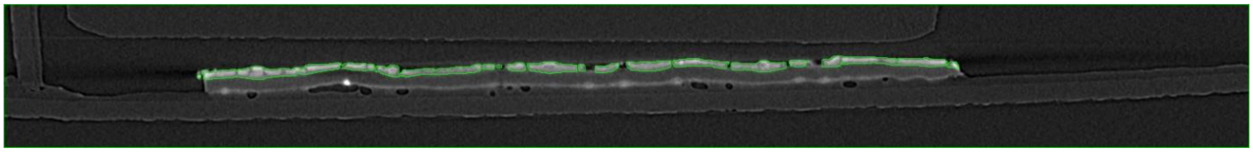
The user can step through the images in the stack to make sure the cathode is isolated when selecting a threshold value. This step can take some trial and error.

Following this the script will use particle labeler from BoneJ to select the largest volume object only. It will subsequently output the cathode isolated as well as a thickness image, which is the sum of pixels in the through-plane direction. This can later be multiplied by the pixel size in microns to yield a real thickness.

The following are example outputs:



As can be seen the anode noise has been cleanly removed and we have accurate segmentation of the cathode catalyst layer for further analysis.



Finally, the resultant AnodeRemoved image can be loaded into a visualization software such as DragonFly to produce a unique view of the cathode catalyst layer from XCT.