2017

SFU FCReL

Manual stack alignment

USING IMAGEJ/FIJI

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Purpose

Given two image sets, say ‘wet’ and ‘dry’, degraded and beginning of life, sample1 and sample2 – how can you align them such that the same x,y,z coordinate represents the same location in both images sets (or even across multiple, indefinite number of sets)?

This is the purpose of the procedure below. It is ideal, and simply best, to be able to exactly align image sets in a way so that the user can be confident the same location is represented by the same x,y,z coordinates. This makes analysis easier, and quicker, and conclusions more accurate. In this same regard, it is also very useful to have the image sets not only have the same coordinate system, but also the same greyscale values (pixel values) for materials which are consistent between them, such as air, or solid which is unchanging. Only in this way can ‘wet’ be subtracted from ‘dry’ or observations on material composition be made accurately.

As an example of the end result once stacks are imported into a 3D viewer:

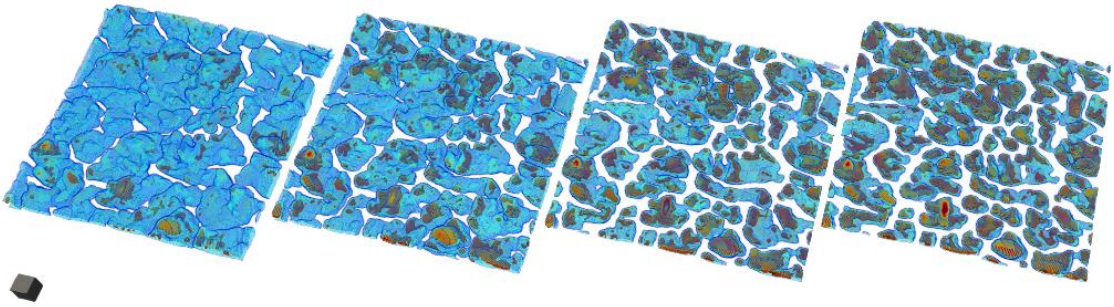


Figure 1: Same location tracking of catalyst layer following degradation. Colour changes are associated with increase in x-ray attenuation from material composition change following carbon corrosion – brighter regions are in brown/red

1. Coordinate Greyscale values

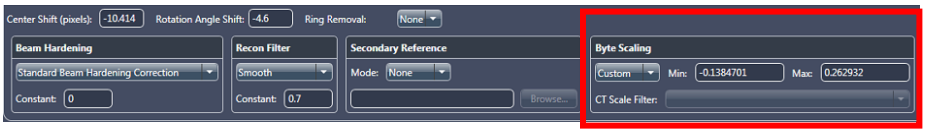
Typically for image sets of the same sample or for comparison of one sample to another. Requires exact same image settings (exposure, projections, pixel size etc.).

Must be done at time of reconstruction, worse comes to worst can do in ImageJ/Fiji but may have errors due to linear extrapolation. This method won’t be discussed here.

At time of reconstruction, choose one image set as base. Consider the image set with the brightest (most attenuating) material to copy settings from. This is the dataset all others will be set like, called the base image set from here on. If unknown, only issue will be that some pixels will be capped at 16-bit scaling.

Reconstruct this base image-set in usual manner; center shift, rotation, beam hardening etc.

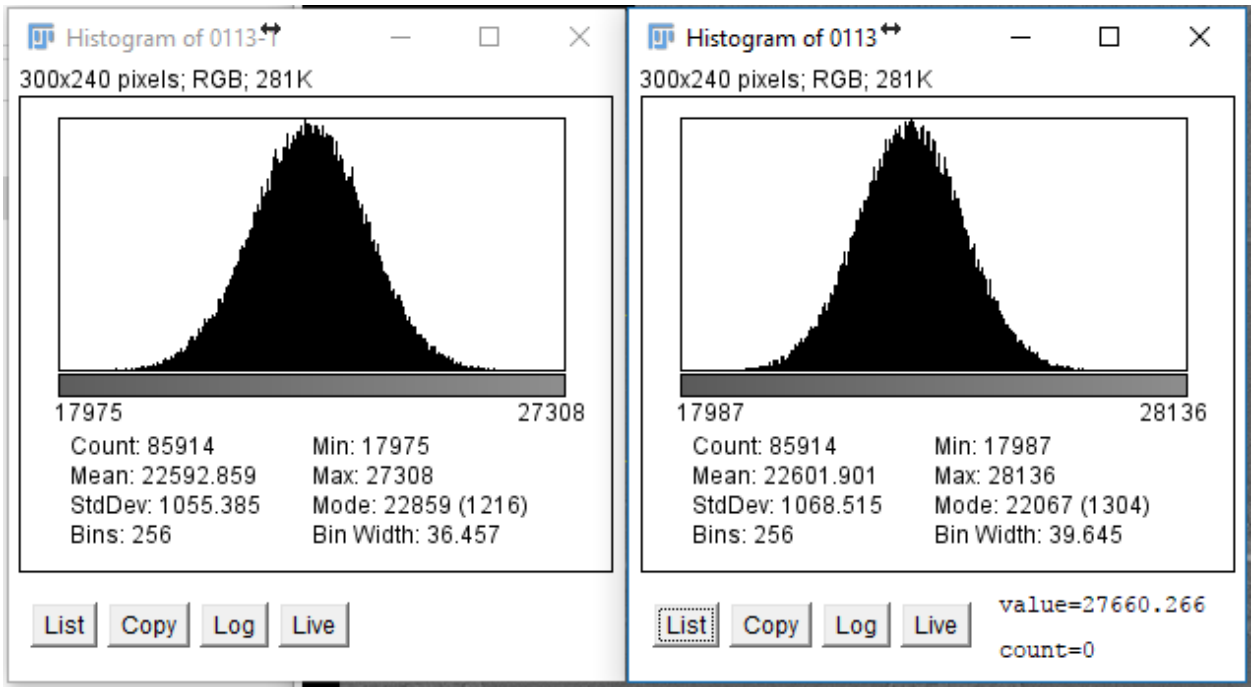
After reconstruction is completed, open second image set you wish to compare this base against. **Select the base image-set .txrm file in the section marked ‘Copy reconstruction settings from’**. This will update the center shift and all other values for your current reconstruction to what was done from your base image-set reconstruction. What is important is that the byte scaling will also be updated, as seen from the reconstruction settings tab.



Find center shift as usual. For this dataset, use the same artifact removal such as beam hardening or ring removal as was done in the base image-set. Only update center shift and rotation – all other values should be the same as the base image-set. This will ensure consistent greyscale values (gsv).

Reconstruct.

Check. After opening using 3DViewer or by tiffs in ImageJ/Fiji, greyscale values for air or other similar materials should be the same, within about ~100-200 gsv, although this will depend on the greyscale values. For example, air with gsv of ~22000 has a mean within ~10 gsv between two image sets as shown here.



This means any greyscale changes in catalyst layer or other material components should be due to composition or density changes. It is important to note however that the closer the two image sets are taken in time, the better – ideally the same day following each other such as during an operando run between wet and dry. This is due to slight changes in X-ray beam as the (X-ray) anode is rotated, aged or replaced.

2. Align tiff stacks

Performed for same location tracking when imaging 4D. In this tutorial example we will use dry (N_2 gas flow at room temperature) and wet (750mA/cm^2, H_2/Air) to then be used to subtract out non-water material from wet stack for water distribution calculations in the gas diffusion layer. Simply replace 'dry' and 'wet' with your own two samples. Here 'dry' will be used as the base, or reference sample and we will be aligning the 'wet' stack to it.

First, it is highly recommended that you have a text file open such as the one shown below to keep track of the changes made during alignment. This will make it easier when performing subsequent alignments, for example BOL to EOL. In that case, BOL dry will be the reference. All wet BOL image sets will be aligned to it. Then, EOL dry will be aligned to BOL dry, followed by aligning all EOL wet to EOL dry. As you can see, we would now have a completely aligned set of multiple image sets, all aligned to BOL dry.

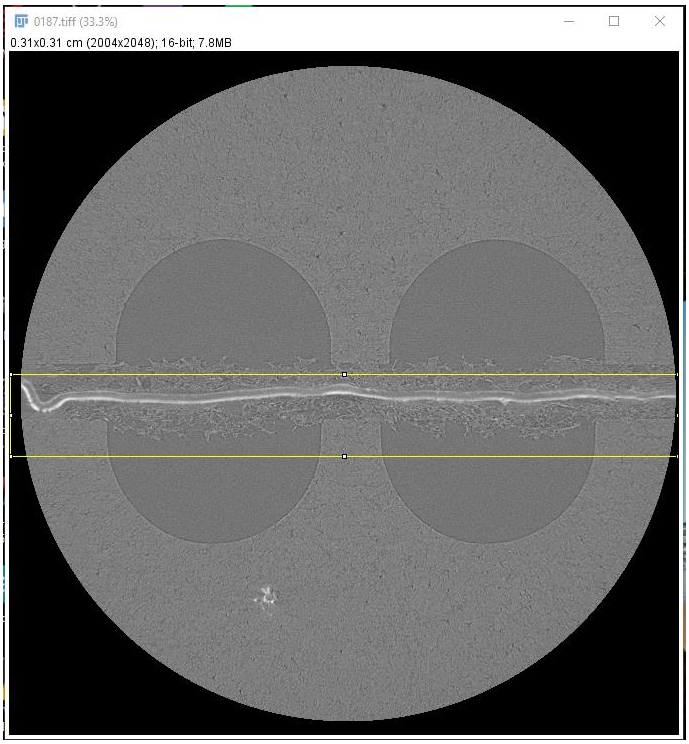
```
BOL_dry raw                          pixel size: 1.531

makeRectangle(0, 968, 2004, 248);
ytranslation shift:-0.01257
reslice
saved as tiff

BOL_750
makeRectangle(0, 968, 2004, 248);
ytranslation shift:-0.01191
reslice
saved as tiff
zshift=13 (dry116 wet103)
move x=32px
move y=-15px
slice70-220
```

2.2 Crop

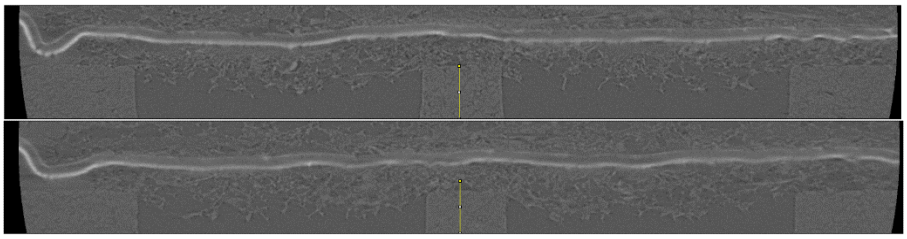
First import tiff stack into ImageJ/Fiji. Select a region of interest to crop using the rectangle select tool, in this case we are only interested in the MEA and so can greatly minimize our file size by only focusing on that. (Above we run 'makeRectangle(0, 968, 2004, 248)' which is just a macro command for ImageJ to create a rectangular box at x,y 0,968 and width,height 2004,248). We will crop both tiff stacks to the same size, and use a rough approximation for a common area. This is where obtaining multiple image sets without having to move the sample pays off since they will already be somewhat closely aligned. **It is important if you are using the small fixture to keep at least one side of the landings visible during the crop step, this will be helpful with rotation correction later**. If you are not using the small fixture, you will need something that is consistent between the two samples that provides a similar horizontal



reference.

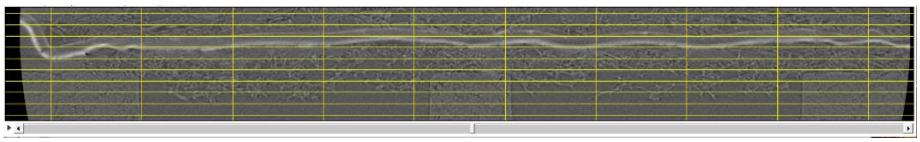
2.3 Tilt correction

This is done as a pre-alignment step to both wet and dry image sets. After cropping we need to correct for the tilt in the dataset. This is the amount by which the region of interest (ROI) moves in the y-direction between the start slice and the end slice, as shown below. To correct this, run TranslateMacro_.ijm or if you have it installed into ImageJ, go to Plugins>Macros>TranslateMacro. Follow the instructions and wait for the macro to finish. Reslice the dataset from top to bottom (Image>Stacks>Reslice, set to Top and avoid interpolation). You should now check that moving through plane the horizontal reference (in this case the channel lands) come into view evenly. If top and bottom are off, reslice and repeat tilt correction. If left and right are off, see rotation correction. If satisfied, save as a tiff stack *_dry_raw_slopeadjusted.tif and *_750mAcm2_slopeadjusted.tif.

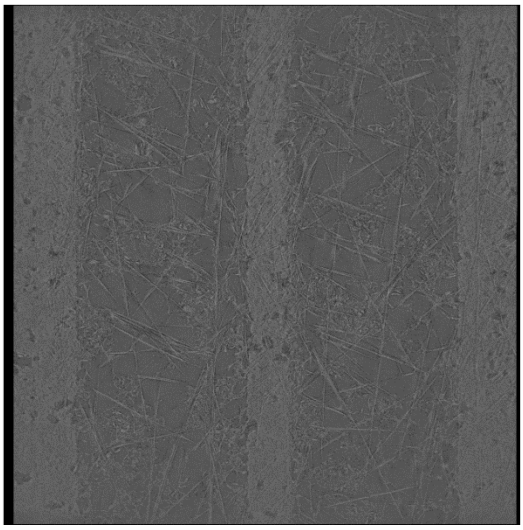


2.4 Rotation correction

Ideally rotation correction is applied during reconstruction – this is to minimize interpolation errors, however it is often still needed to be finely tuned which can be done in ImageJ. From a through plane view in ImageJ, select the ~center image in the stack and run Image>Transform>Rotate… Select about 10 grid lines (up to the user), bicubic interpolation and select Preview. Adjust the rotation until the horizontal reference is flat and select ok. Reslice for through-plane (top) and repeat checks described in Tilt correction. Save when satisfied.



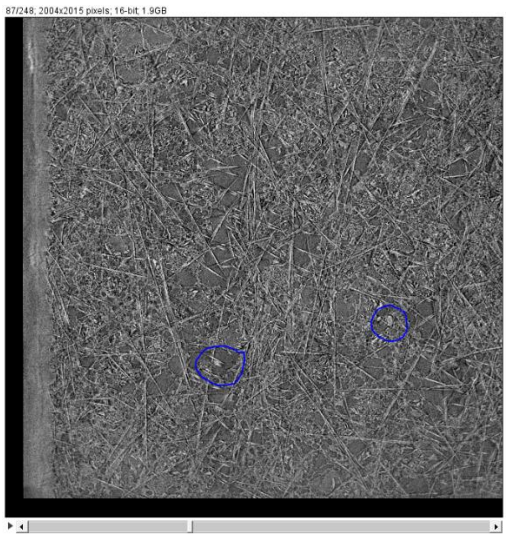
Both image stacks should now be corrected for tilt or rotation as shown below, where top,bottom and left,right lands appear in through-plane at the same slice.



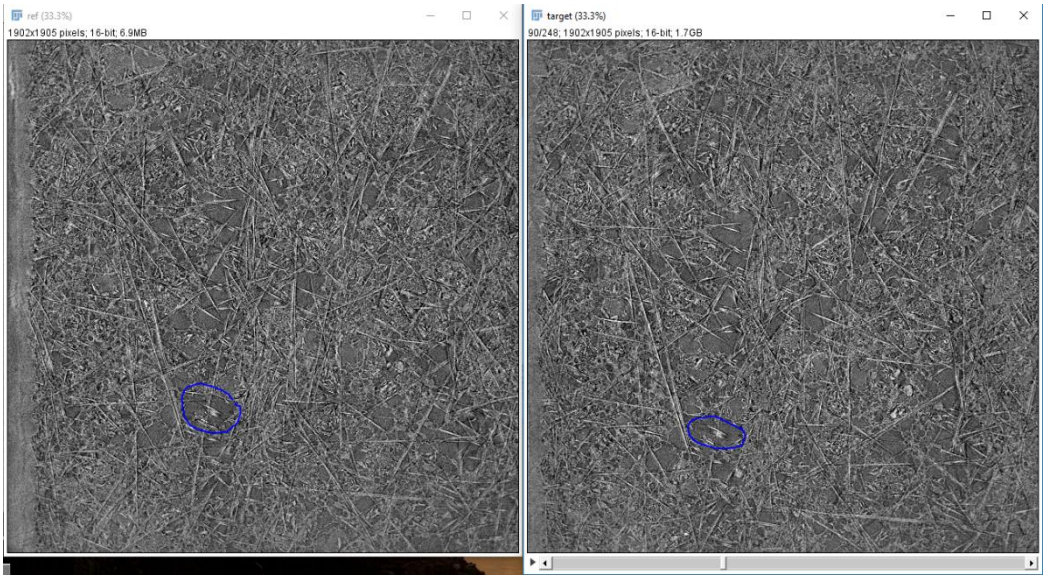
Alignment

2.5 Z-shift correction

You should now have two image stacks, in their through-plane direction which are very similarly 'flat'. We are now ready to start aligning the two stacks. First we will obtain the z-difference, that is the offset in the through-plane direction of the two stacks. To do this we will use a macro called ZShiftCheck, found in ImageJ macros on github or if you have it installed Plugins>Macros>ZShiftCheck. First select a slice from the 'dry' image stack this is approximately in the center but ideally has a feature which you could identify by eye in the 'wet' image stack as we are going to need an approximate range to check. I have circled two features which I was paying attention to and my selected slice, shown below.

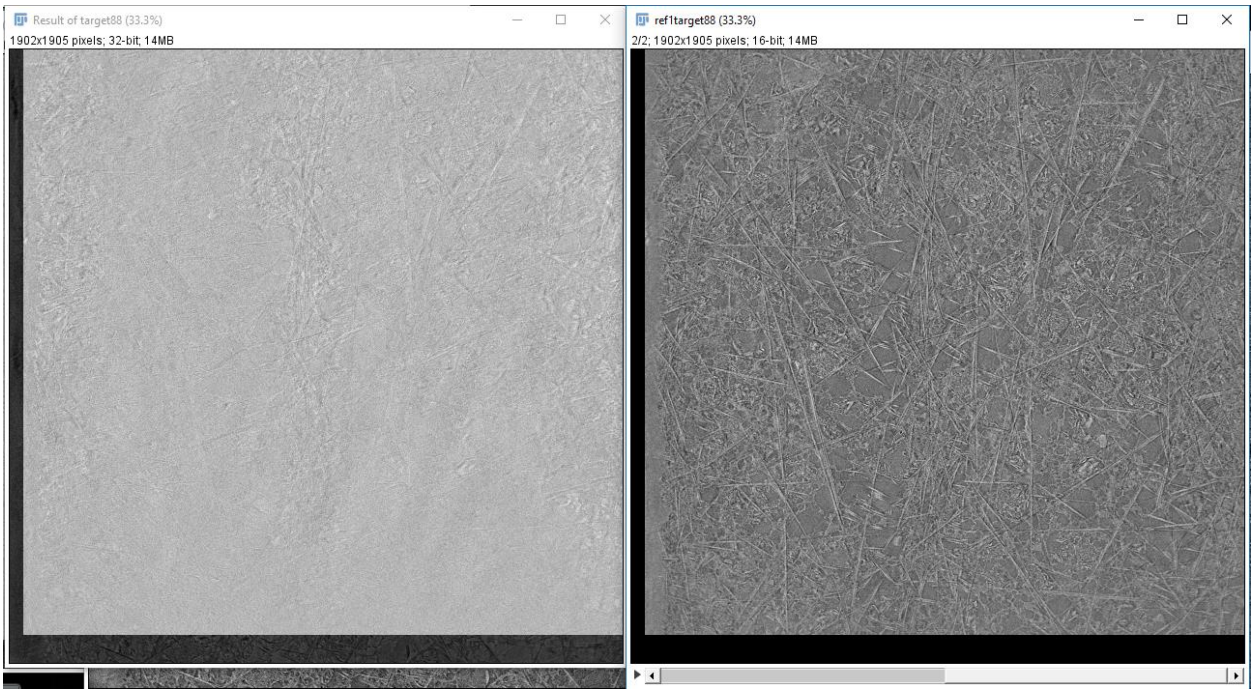


The z_shift macro uses StackReg to help with alignment, this macro however has problems with black boundaries as in the image above. Select an arbitrary box that fills most of the image not including the black borders. Duplicate just this slice, and call it ‘ref’. This will be the reference slice. Next, try to find the slice with same features in your ‘wet’ stack, at approximately the same x,y location as the reference, note the slice number. Hit CTRL+SHIFT+E while on this slice – this should redraw the same rectangle used for the dry image. If this rectangle still has black in the ‘wet’ image stack, adjust and re-duplicate the ‘dry’ stack using this same rectangle. Duplicate the full ‘wet’ image stack with the rectangle drawn and name it ‘target’. You should now have a single reference image ‘ref’ and an image stack ‘target’, which have the same image dimensions in x and y. This is shown below with the same feature highlighted. Note the difference in slice number from 87 for ‘dry’ and 90 for ‘target’ (‘wet’).

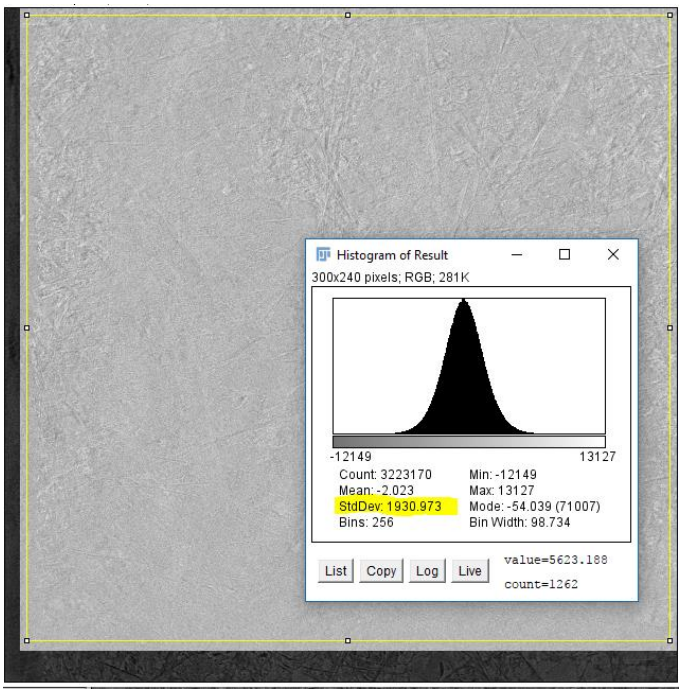


We are now ready to run the z_shift macro on these two images. Select the center slice (best guess as to which ‘wet’ slice matches the ‘dry’ slice) as well as a range to check over. Usually ± 5 slices is sufficient.

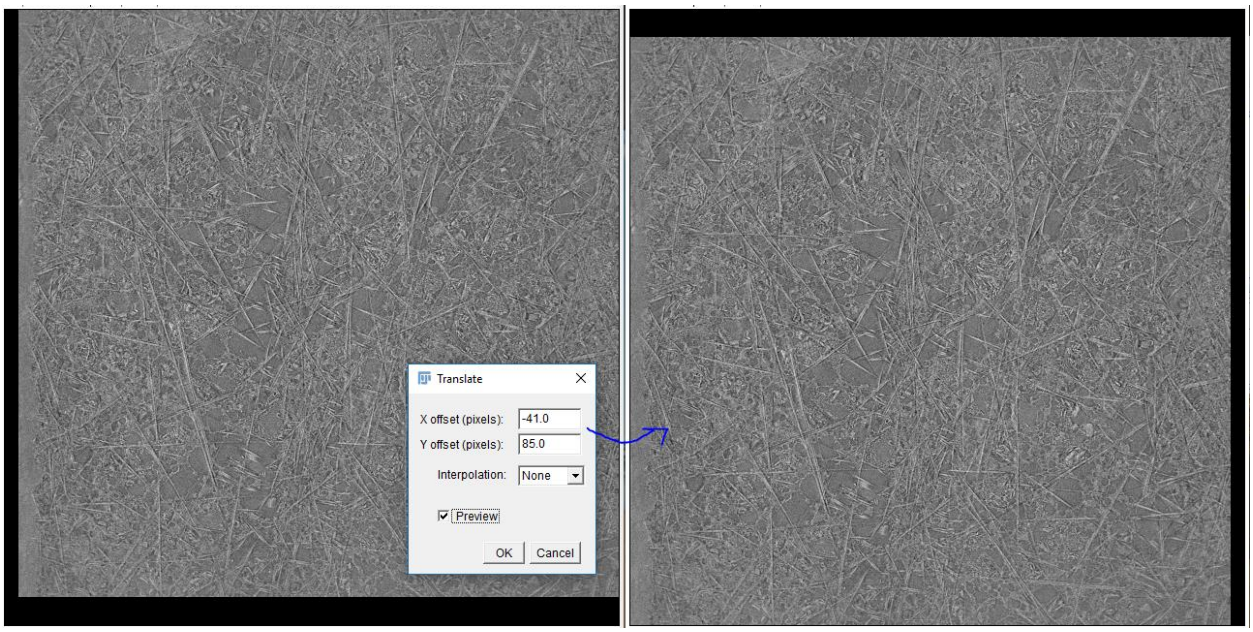
This will start creating several sets of windows such as the one shown below. It is important during the time that the macro is running, don’t select other windows in ImageJ/Fiji. The two image sets are a subtracted image (left) and the ‘ref’ reference image, and shifted ‘target’ image. The slice number is shown in the image titles. In this case it is slice 88 from the ‘target’ image set.



Judging by eye, as well as using the standard deviation of the histogram of the subtracted image (shown below) select the best slice that aligns best with the ‘ref’ reference image. In this case it is the one shown which is slice 88. For this example data set, we have a z-offset of just 1 slice.

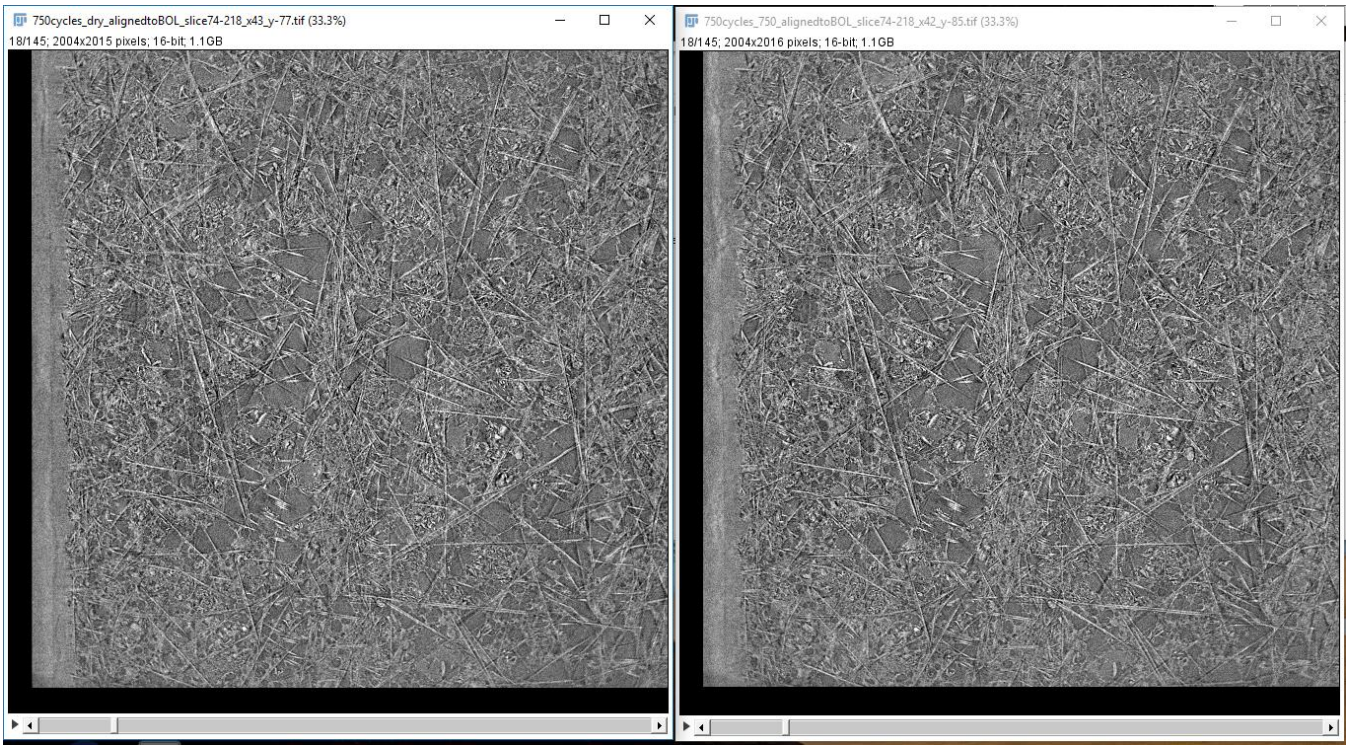


As you may have already noticed, there is a black border around the second image in the newly created stack ref1target88 shown above. This corresponds to the amount by which the 'wet' image stack needs to be shifted to align in the x and y with the reference 'dry' stack. There are several methods to find these values. The one I like is to use the measuring tool (draw a line and hit CTRL+M), which will give you a distance in pixels. I then check this value by going to Image>Transform>Translate, and using preview I double check the amount to shift the 'target' image by. For example, shifting to the left by (-)41 pixels to align the edge again. This means I must shift the entire 'wet' stack by **+**41 pixels to align to 'dry'. Similarly, I need to shift the 'target' stack down by (+)85 pixels, so I must shift the entire 'wet' stack by **-**85 pixels to align to 'dry'.



Following these steps you now have the amount to shift the 'wet' image stack to align to the 'dry' image stack. We found our values to be 1 in the z (slice number), 41 in the x and -85 in the y. Apply the x and y shifts to the entire 'wet' image stack by Image>Transform>Translate. It is important that Interpolation is set to none, and use only integer values. To align the z, choose the slices of interest, example '75-220' for 'dry', and duplicate this stack. Save the stack as *_dry_slice75_220.tif. After applying the x and y translate to 'wet', duplicate the stack including the offset i.e. from above, our slice 88 for 'wet' matched sliced 87 for 'dry' so for our 'wet' stack we choose slices 76-221. Save this stack as *_wet_alignedtodry_slice76_220_x41_y-85.tif.

An example is shown below where 750cycles_dry is aligned to BOL dry and 750cycles_750 was aligned to 750cycles_dry such that it is also aligned to BOL.


750cycles_dry_alignedtoBOL_slice74-218_x43_y-77.tif (33.3%)
18/145; 2004x2015 pixels; 16-bit; 1.1GB
750cycles_750_alignedtoBOL_slice74-218_x42_y-85.tif (33.3%)
18/145; 2004x2016 pixels; 16-bit; 1.1GB