Tutorial Example – Retina Imaging

In this worked example we will cover how to generate an image from raw LA-ICP-MS data. We will be working with 212 files of data that were generated from the analysis of Zn in a sample of retina tissue (see Figure 1). Each file contains the time resolved data for a separate line of the image. The laser ablation parameters (1 µm spot, 10 Hz repetition rate, 10 µm/s scan speed and no spacing between lines) were selected to enable the signal from each laser shot to wash out before the next, adjacent location was sampled. The data was acquired using an external board connected to the ICP-MS in order to remove the problem of blind time.[1] Data points were collected every 0.01s and are presented as raw counts. During processing we will sum the signal within the areas corresponding to each laser shot (i.e over 0.1 s periods) and convert these values into colour coded pixels in the image.

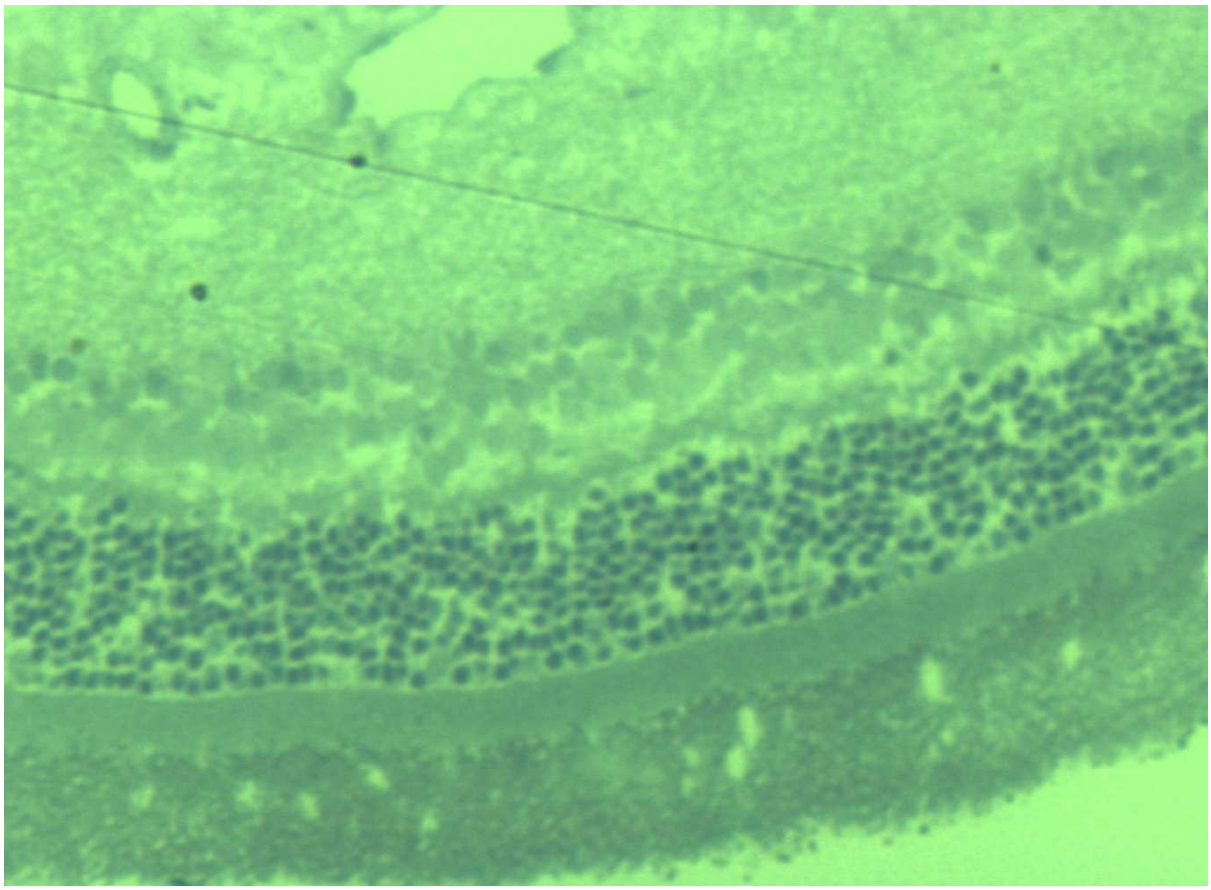


Figure 1. Pre-ablation microscopic image of the 290 µm x 212 µm sample of stained retina tissue. The scan direction was left to right, starting at the top left.

[1] A.J. Managh, D.N. Douglas, K.M. Cowen, H.J. Reid and B.L. Sharp, *J. Anal. Atom. Spectrom.,* 2016, **31**, 1688-1692.

1. Download a copy of the folder “Retina Imaging Example Data” from the download page and place it in unzipped format in a convenient location. Do not rename the files.

2. Open the LA-ICP-MS Image Tool app. A message will appear advising that this is a demo version that, whilst otherwise fully functional, is limited to a maximum of 250 files per run. Click OK. The main window will load as follows:

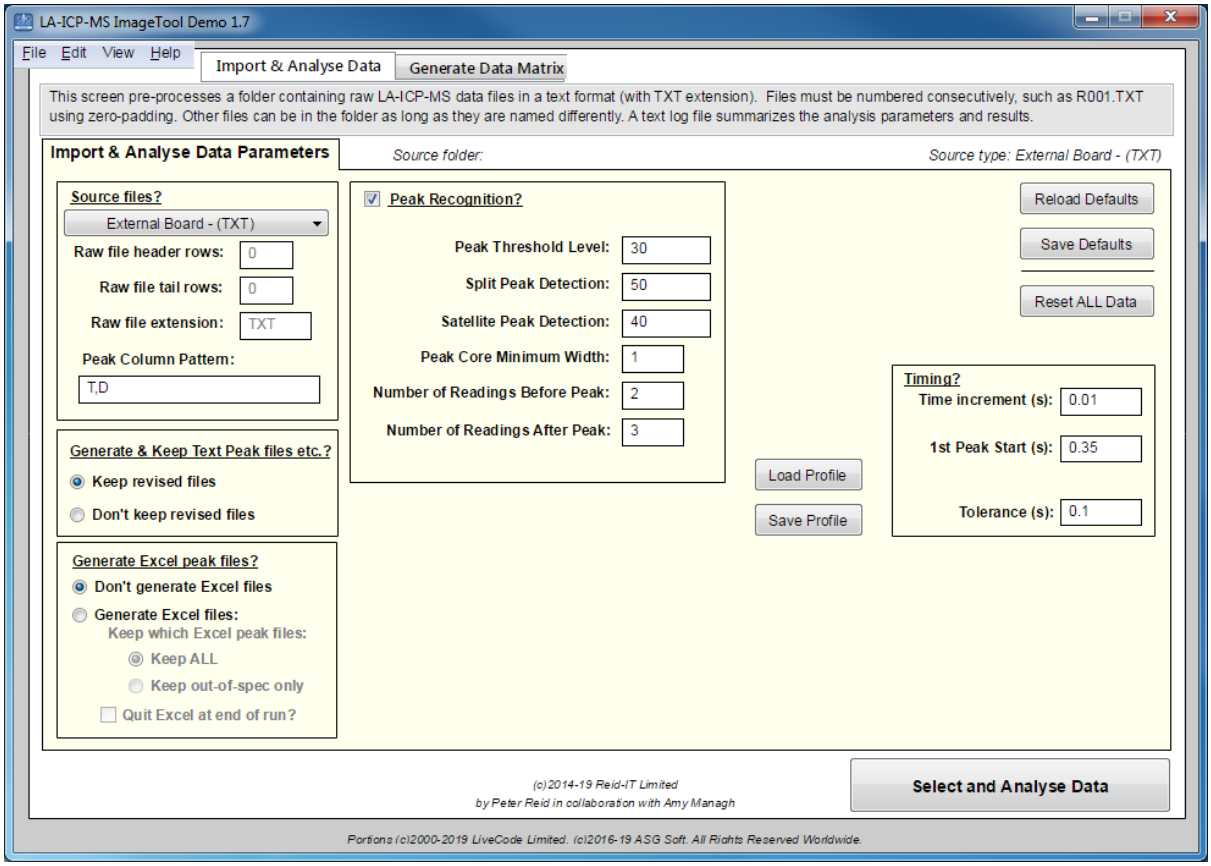


Figure 2. Screenshot of the user interface, showing the settings recommended for this analysis. These will be explained in the following sections.

3. Under *Source files?* select External Board – (TXT) from the dropdown list. This will populate the fields in this section of the interface. The example data was acquired using an external data acquisition board plugged into the Element XR, which produces a TXT file data output, so TXT is specified as the file extension. The data is in the first row of each file, with no supplementary information e.g. date or instrument settings written before or after the data, hence the raw file header rows and raw file tail rows both read zero. The files contain time data in the 1st column and data for Zn66 in the 2nd column, so in this instance T,D (i.e. time, data) should be entered as the *Peak Column Pattern*.

4. Under *Generate Excel peak files?* click Don’t generate Excel files. These files are produced for peak profiling applications and are not required for this imaging example.

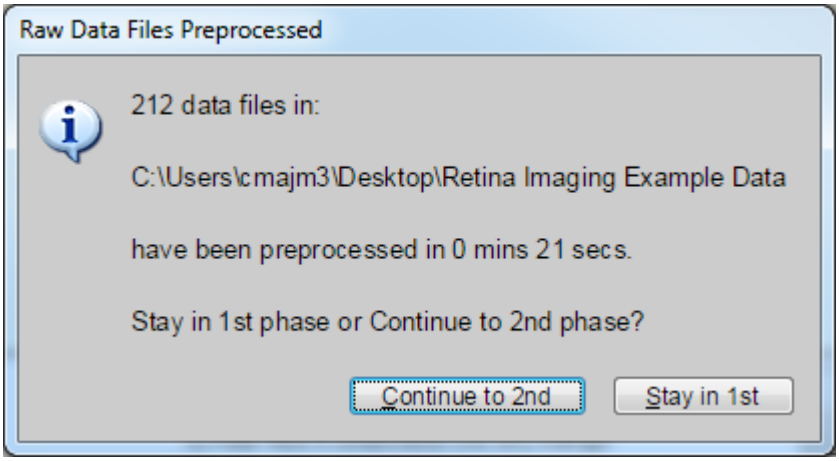
5. Although a trigger cable was used to start the analysis there is usually a few ms of electronic jitter in the sending and receipt of the signal. Although very small, this may affect the resolution of the image, so turn on *Peak Recognition* using the checkbox provided to correct for this. This feature will enable us to find the time point of the first peak within each file. Since we started each line on an area that was expected to produce a measurable signal, we can use the 1st peak location to align each line and make sure that peaks do not fall on the boundary between the regions that we are converting into pixels.

6. First we must choose a *Peak Threshold Level* value above which a signal is likely to be a peak. Select a value of 30 counts. We then select the *Peak Core Minimum Width* i.e. how many consecutive data points above this threshold are required to confirm that it is a peak. For this example we are dealing with very short duration peaks, so we will set a value of only 1 data point. Set the *Number of Readings Before Peak* and *Number of Readings After Peak* to 2 and 3 data points respectively. This accounts for any wings of the peak that are below the threshold. The other two parameters in this section are relevant to peak profiling rather than imaging, so we can simply set any value above the *Peak Threshold Level* here e.g. 50 and 40.

   For a more detailed description of how peak recognition works and recommendations on the selection of settings, please see section 2.2 of the manual and the tutorial example “Peak Recognition and Extraction”.

7. Under *Timing?* enter the time increment, 0.01 s. A quick look at the data files reveals that the first peak within each file usually occurs at around 0.35 s, so enter a *1st peak start* of 0.35 s. The final box in this section allows users to set a tolerance within which the first peak of every file is expected to occur. As the present analysis used a trigger cable we would expect this point to be close to 0.35 s, but entering 0.1 s here will generate an alert on the subsequent screen if the first peak for any of the files is found more than 0.1 s either side of this point.

8. Finally click *Select and Analyse Data*. In the pop-up window, navigate to the folder containing the data and select it with a single click (don’t double click to open the folder), then press *select folder*. The Image Tool will start to process the data. Progress can be viewed in the bottom left side of the window. Upon completion the following message will appear:



9. Click Continue to 2nd.

10. The 2nd screen shows a table containing the names of all the processed files and the time stamp of 1st peak found within each file. We can see that for the majority of the files the 1st peak was within the ±0.1 s tolerance set, as indicated by the Y on the *OK?* column, but 4 of the files contain peaks outside of this range, marked by N.

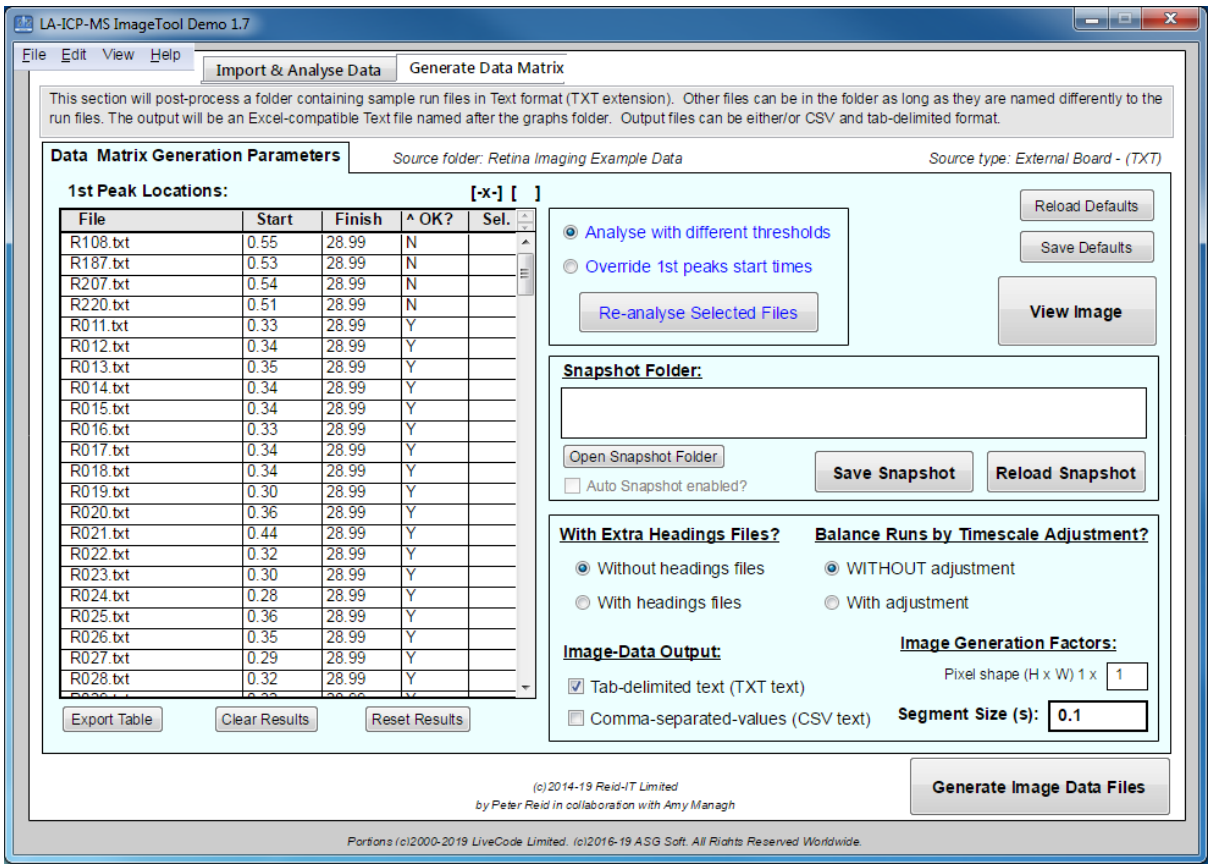


Figure 3. Screenshot of the 2nd screen of user interface, showing a list of the files processed. The default shows 'N' files at the top of the list, but the list can be sorted by file name, start time or tolerance status by clicking on the respective header.

11. It is possible that the start time in the non-conforming files was genuinely outside of the tolerance range, but this requires further investigation to confirm. Opening the non-conforming files indicates that earlier peaks were in fact present, but the signal was very low at the start of these lines (presumably due to gaps in the thin tissue section or inhomogeneity of the stain). Setting a lower threshold may help the app to find these low peaks. Click the *Sel.* column of the respective files (which will mark them with a blue [x]), then click *Re-analyse Selected Files*. This will take you back to the first screen.

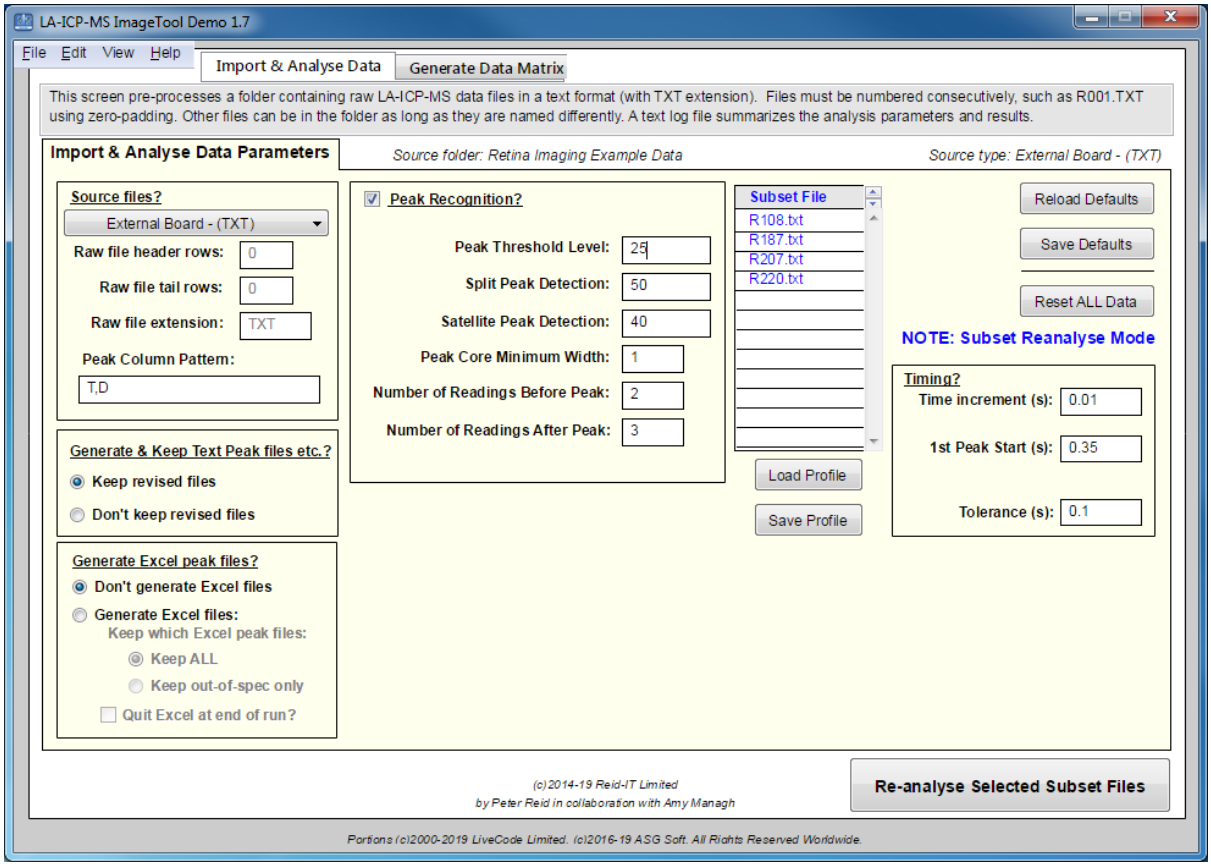
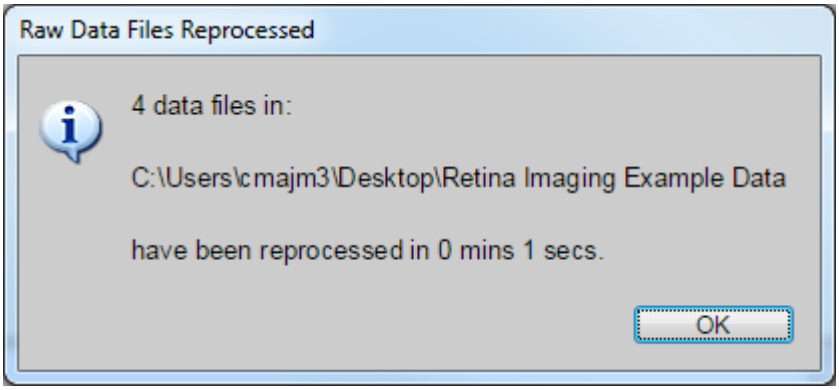


Figure 4. Re-processing of non-conforming files. The selected files are listed in the window.

12. Set a new *Peak Threshold Level* of 25 counts, then click *Re-analyse Selected Subset Files*. Following the analysis, click OK to return to the 2nd screen.



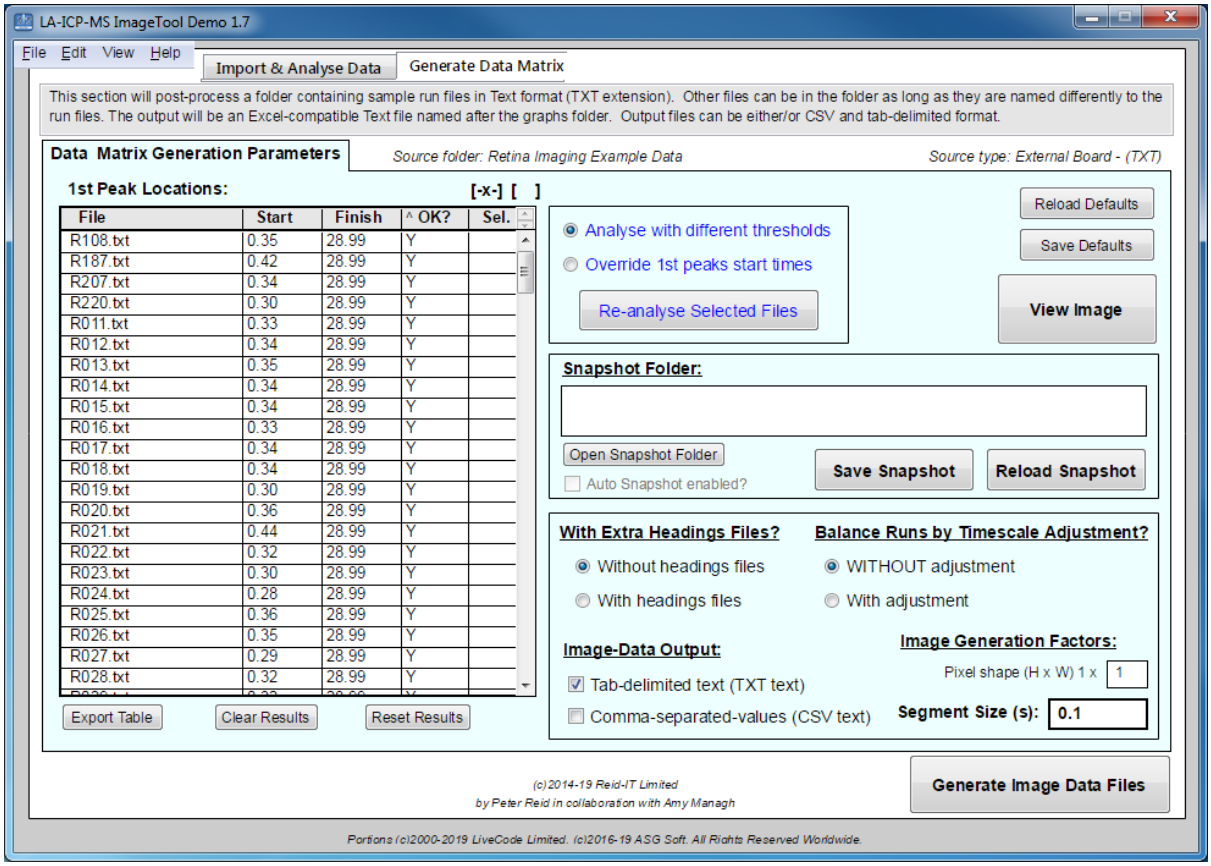
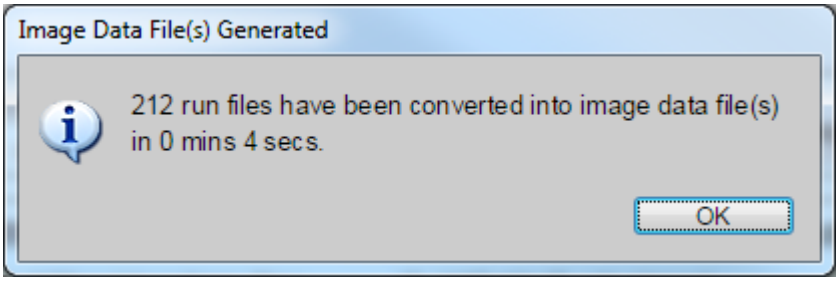


Figure 5. Screenshot of the 2nd screen of user interface, showing a list of the files after re-processing. All files now contain a 1st peak start that is within the tolerance level set.

Note that as an alternative method to the above it is possible to manually override the start times for a given line. In order to do so, switch the mode from *Analyse with different thresholds* to *Override 1st peaks start times*, then click the start time of a given line. This will prompt you to enter the desired start time. Finally, click the *Override Selected Start Times* button.

13. The bottom right hand side of the 2nd screen allows adjustment of the output file types. The default settings can be left as they are for this example. Enter 0.1 s in the *Segment Size* box. This is related to the repetition rate of the laser (firing at 10 Hz produces a laser pulse every 0.1 s). The method used for the analysis provides square pixels, so ensure that 1 is entered into the *Pixel shape* box. If spacing between lines were used, this value would need to be increased to provide rectangular pixels.

14. Click *Generate Image Files*. This will generate a matrix of pixel values, with each pixel containing 0.1 s of data. A copy of this matrix will be produced in the format specified (TXT or CSV). The file will be named according to the name of the folder that contained the raw data e.g. for this example we get 'Retina Imaging Example Data.txt'.



15. Click on View Image. This opens a new pop-up window. The example image contains >60k pixels, so the image may take a few seconds to appear.

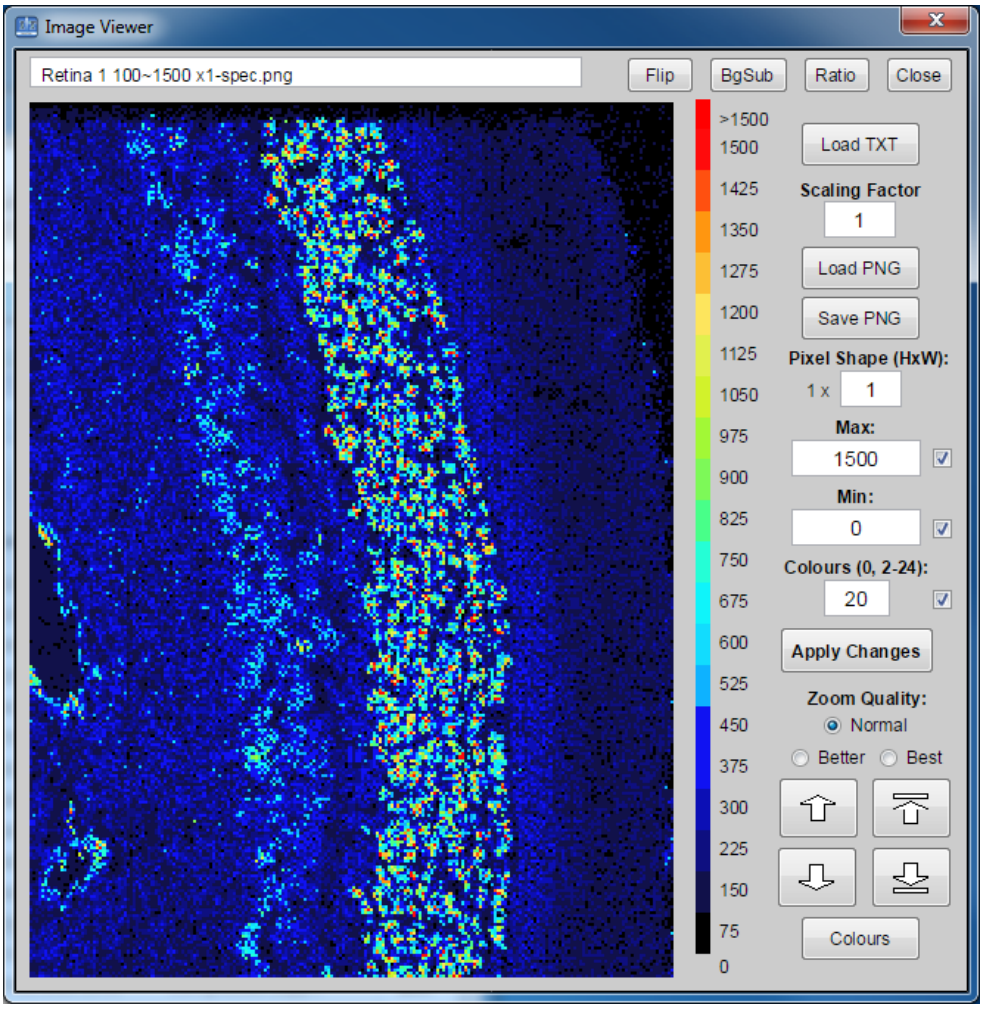


Figure 6. Screenshot of the Image Viewer window.

16. Enter a maximum value of 1500 counts and a minimum value of 0 counts, then click *Apply Changes.* The colour scheme uses rainbow as default, but this can be changed by clicking *Colours* and selecting an alternative palette (examples available on the download page). The image can also be expanded (zoom buttons) and flipped 180^{o} (Flip button).

17. The final image can be exported as a png file by clicking Save PNG.