Tutorial Example – Peak Recognition and Extraction

In this worked example we will cover how to recognise peaks within raw LA-ICP-MS data and we will extract the located peaks into an Excel file. We will be working with 10 files of data, each acquired during the ablation of a gelatine standard reference material at 10 Hz. Each file contains approximately 50 separated peaks.

1. Download a copy of the folder "Peak Recognition Example Data" from the download page and place it in unzipped format in a convenient location. Do not rename the files.

2. Before opening the Image Tool we will first examine a file of the raw data to determine the features of a typical peak. Plotting a quick graph of the data in Excel or a similar package may be helpful, as shown below.

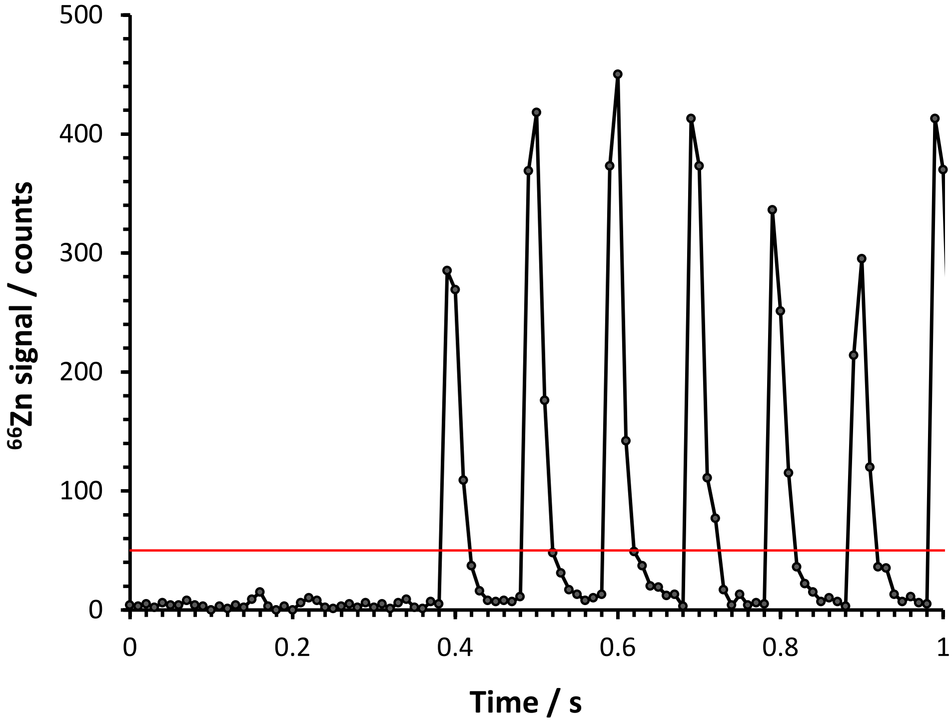


Figure 1. The first second of data from the file "R001.txt", with a suggested Peak Threshold Level of 50 counts (red line). We can see that the data points are spaced at 0.01 s intervals and the first peak appears at 0.39 s.

3. 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:

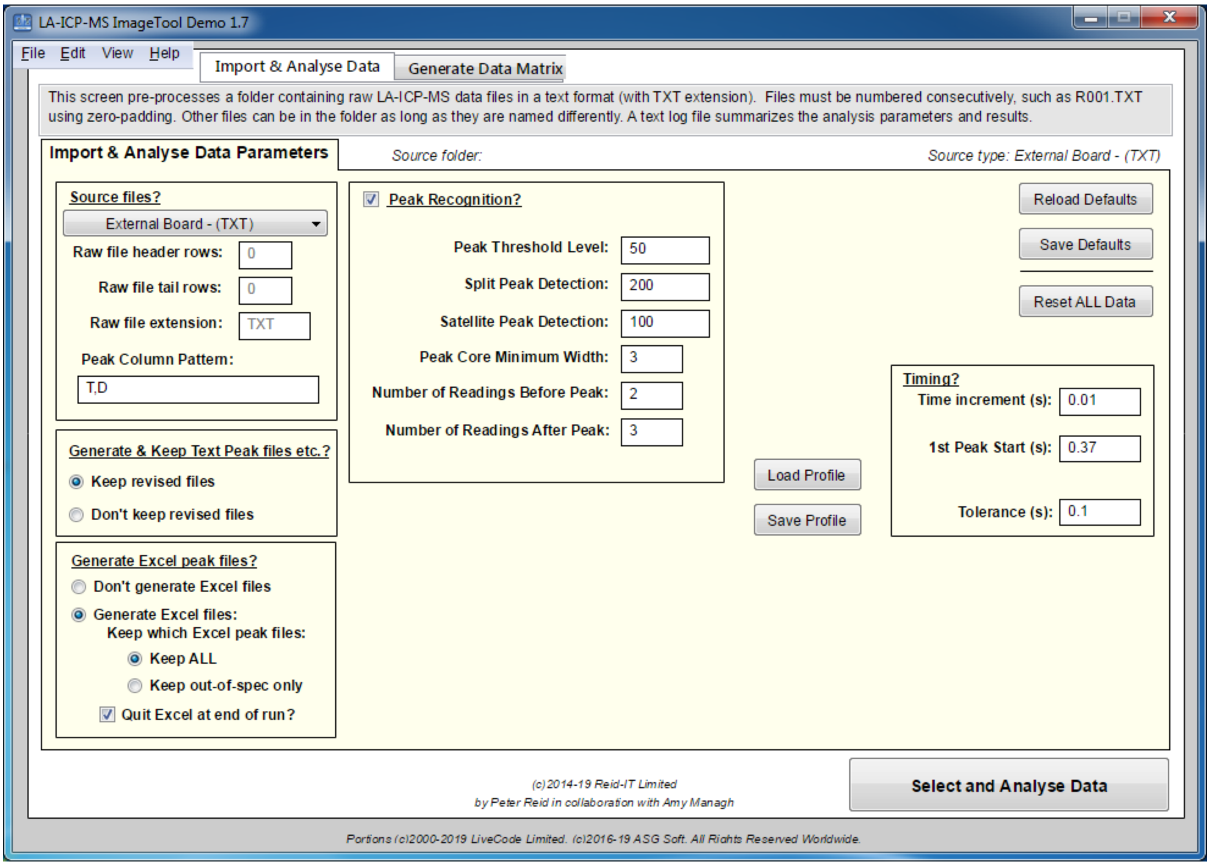


Figure 2. Screenshot of the user interface, showing the settings recommended for this analysis. These will be explained in the following sections.

4. 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*.

5. Text files containing the peaks found within each file will be automatically created within the folder containing the raw data and these will be named '*filename*-peak.txt.' However, it is possible to create a more functional Excel version of these files. Under *Generate peak Excel files?* click Generate Excel files and select Keep ALL. Note that using the Excel feature is not generally recommended for imaging work because it slows the app down, but it can be useful for applications such as single particle work that use a small number of files.

6. In this example we are looking for peaks, so tick the box labelled 'Peak Recognition?' to enable this feature. The settings in this section can be determined from our observations in step 2:

   Peak Threshold Level: This is the point above which we would consider a signal to be a peak. Users may wish to use a statistical method to determine the threshold, such as the average of the blank signal plus 10 standard deviations of the blank. For this example we will use a value of 50 counts for the *Peak Threshold Level*.

   Peak Core Minimum Width: This is the number of consecutive data points above the *Peak Threshold Level* that are required to classify that the signal is a peak (rather than a random spike in noise). From Figure 1 we can see the peaks have either 3 or 4 consecutive data points above the threshold value chosen, so we will choose a value of 3 here to ensure that we capture all peaks.

   Number of Readings Before Peak and **Number of Readings After Peak**: These settings account for wings of the peak that may be below the *Peak Threshold Level* i.e. these are the number of data points before and after the peak core that should be taken to be part of the peak. From Figure 1 we can see that there is a small tail that is below the threshold, so we will use a setting of 2 and 3 data points for the before and after settings respectively to allow for this.

   The next two settings involve detection of unusual features within peaks:

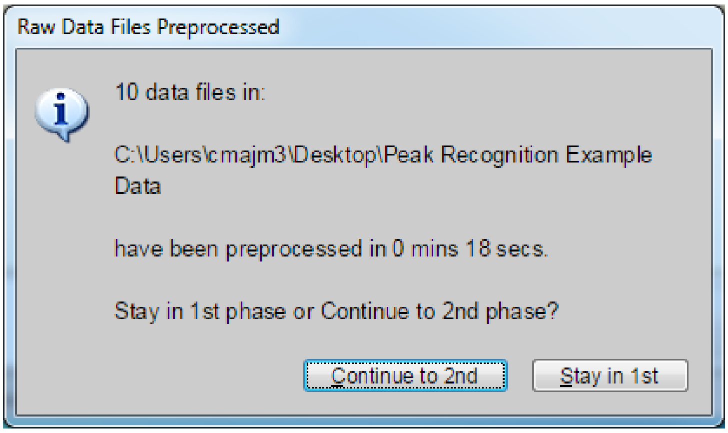
   Split Peak Detection: This is a setting to identify potential overlapping peaks. If a dip in a peak falls below the *Split Peak Detection* level then rises again, the peak is flagged as a potential split peak. For this example, set this value at approximately half the value of a typical peak height, 200 counts.

   Satellite Peak Detection: This setting allows for the characterisation of small signal spikes that occur in the peak tail (within the set number of *Readings After the Peak*). For this example, set a value of 100 counts.

7. Under *Timing?* enter the time increment, 0.01 s. From step 2 we determined that the first value above the threshold occurs at 0.39 s and in step 6 we specified that we would like to include 2 readings before the peak core, so enter a *1st peak start* of 0.37 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 the 0.37 s found in the first file, but we will allow for a bit of jitter in the trigger signal. Entering a value of 0.1 s will generate an alert on the subsequent screen if the first peak is found outside of the 0.27 - 0.47 s range.

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. For non-imaging applications it is not necessary to continue to the 2nd screen, but we will do so here for interest. Click Continue to 2nd.

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

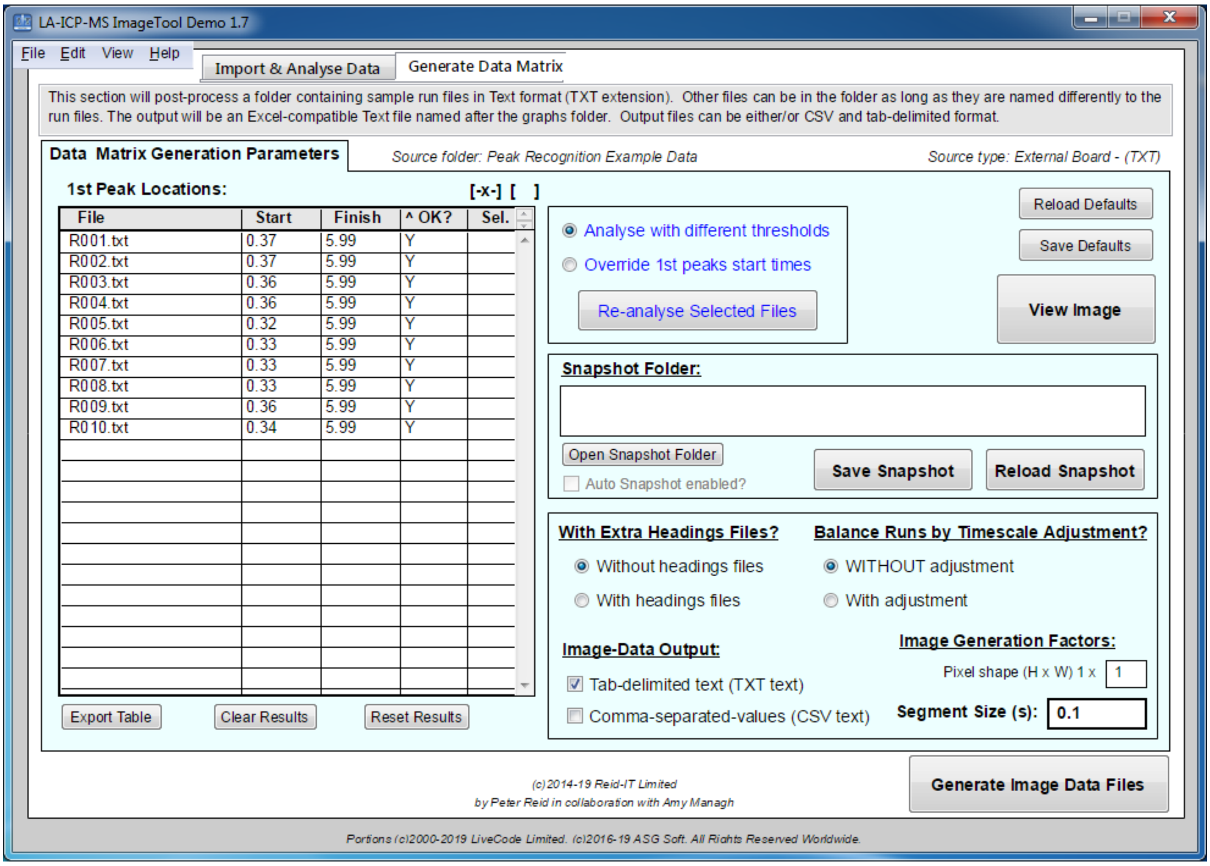


Figure 3. Screenshot of the 2nd screen of user interface, showing a list of the files processed.

Note if files are outside of the tolerance, indicated by N, it is possible to reprocess these using different conditions. This can be accomplished by clicking the *Sel.* column of the respective file (which will mark it with [x]), then clicking *Re-analyse Selected Files*.

11. Generation of an image is not required here, so you can now close the LA-ICP-MS Image Tool app.

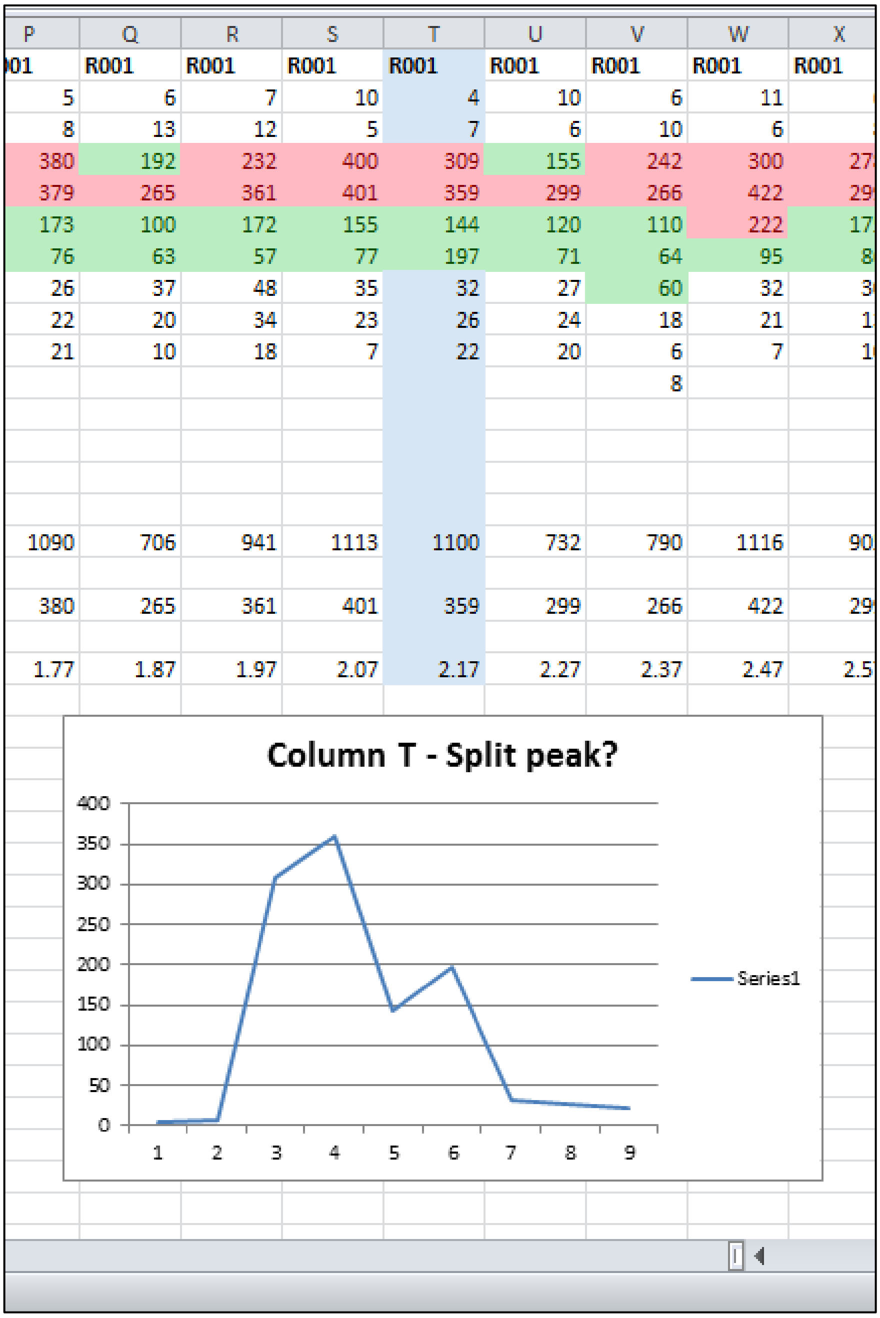
12. Navigate to the folder containing the raw data. A series of files named "*filename*-peak.xlsx" will have appeared. Open the first file "R001-peak.xlsx".

	A	B	C	D	E	F	G	H	
1	Time(S)	R001	R001	R001	R001	R001	R001	R001	R00
2	0	7	7	10	13	6	7	6	
3	0.01	5	11	13	3	5	3	5	
4	0.02	285	369	373	413	336	214	413	
5	0.03	269	418	450	373	251	295	370	
6	0.04	109	176	142	111	115	120	159	
7	0.05	37	48	49	77	36	36	67	
8	0.06	16	31	37	17	22	35	34	
9	0.07	8	17	20	4	15	13	20	
10	0.08				13			13	
11	0.09								
12									
13									
14									
15									
16	SUM/AREA:	736	1077	1094	1024	786	723	1087	
17									
18	MAX:	285	418	450	413	336	295	413	
19									
20	Peak Start(S):	0.37	0.47	0.57	0.67	0.77	0.87	0.97	
21									

Figure 4. An extract of the Excel file for R001-peak.txt.

13. The file contains the extracted peaks, with time data in column A followed by the extracted peaks in each subsequent column. In column B we can see that the Image Tool has identified at least 3 consecutive points above the threshold for each peak and has counted 2 points before these points and 3 points after them, as specified. Highlighting is used to label the values above the threshold. The sum of the area within the peak region, the maximum peak height, and the time location of the peak are calculated beneath each peak. We can see from the peak start times that there is a peak every 0.1 s, consistent with firing a laser at 10 Hz.

14. Scroll across to column T. The Image Tool has identified an unusually shaped peak (peak drops below the *Split Peak Detection* Level then rises again). The column has been highlighted and a graph has been automatically plotted to enable quick visual inspection.



P	Q	R	S	T	U	V	W	X
01	R001	R001	R001	R001	R001	R001	R001	R001
5	6	7	10	4	10	6	11	
8	13	12	5	7	6	10	6	
380	192	232	400	309	155	242	300	27
379	265	361	401	359	299	266	422	29
173	100	172	155	144	120	110	222	17
76	63	57	77	197	71	64	95	8
26	37	48	35	32	27	60	32	3
22	20	34	23	26	24	18	21	1
21	10	18	7	22	20	6	7	1
						8		
1090	706	941	1113	1100	732	790	1116	90
380	265	361	401	359	299	266	422	29
1.77	1.87	1.97	2.07	2.17	2.27	2.37	2.47	2.5

Figure 5. An extract of the Excel file for R001-peak.txt, showing a potential split peak.

15. Further processing can be done on the extracted peaks within Excel, as required.