Step-by-step instructions for creating a 3D Origin plot from a MassLynx continuum file

Your mass spectrum will be saved as a series of files in a folder called SAMPLE.raw (where SAMPLE is your filename).

DataBridge

Open DataBridge:



You will be greeted with the following screen:

🖳 DataBridge	
Source - MassLynx	Target - ASCII
Directory:	Directory:
F:\MASSLYNX\Default.pro\DATA	F:\MASSLYNX\DEFAULT.PRO\
Filename:	Filename:
SAMPLE	SAMPLE
Select	Directory
Close Convert Options.	Help

Hit "Select..." and browse to your spectrum:

Source file sele	ct	X
File Name: SAMPLE.RAW PEST03.RAW SAMPLE.RAW STANDRD1.RAW STANDRD2.RAW STANDRD3.RAW STANDRD4.RAW STANDRD5.RAW	Directories: f:\masslynx\default.pro\data	OK Cancel File info
,	Drives:	Network

Check that under "**Options...**" the conversion is set to Source = Masslynx, Target = ASCII:



Hit "Convert". The file will be saved as a .txt file, which may now be treated with EDit.

<u>EDit</u>

EDit may be downloaded freely as a .zip file (<u>http://web.uvic.ca/~mcindoe/edesi.html</u>). The file should be unzipped and saved to disk (EDit.exe). Upon running the program, users will be greeted by the following dialogue box:

🖀 EDit - ED-MS Data Manipulation 📃 🗖 🔁							
				💌 Integrate m/z			
Start	End	Number		🔲 Normalise			
0	0	0	m/z Ratio	()			
			C	Dimension			
U	ļu	U	💌 Scan Number				
0	0	0	C Retention Time	Process			
,	,						
Waiting							

To begin, hit "Dimension", and browse to the .txt file previously generated from DataBridge.



The program then fills out the boxes according to the data found in the file:

📽 EDit - E	🛿 EDit - ED-MS Data Manipulation 📃 🗖 🔁							
Start	End	Number		✓ Integrate m/z ✓ Normalise				
49.7303	700.846	651	m/z Ratio	Dimension				
0.052	7.848	151	C Retention Time	Process				
Dimensioning	I Complete							

The top row indicates the start and end values of m/z. "Number" indicates the number of m/z units between these two values (in this case, 701 - 50 = 651). If a higher resolution than 1 m/z is required, increase the "Number" value accordingly (e.g. for a resolution of 0.5 m/z in this example, change to 1302). The second row similarly computes the number of scans – there is no advantage in increasing the "Number" setting here (you can't increase the number of scans unless you repeat the experiment) but in some cases it may be useful to lower the number (e.g. a very weak sample may have had 4 scans collected per increment in the cone/collision voltage, in which case dividing the "Number" value by 4 is necessary). Finally, the third row gives the actual retention time rather than scan number, which is useful if chromatographic information is to be manipulated instead of a variable set to change linearly with scan number (as is the case for ED spectra).

The two "Number" settings give the size of the matrix, in this case 651×151 . Hitting "**Process**" allows you to choose the same .txt file and to perform the conversion to .csv format with the appropriate settings. Save the .csv file; it is now ready for processing using your scientific graphing package.

The "Integrate m/z" option sums up all of the intensity samples collected for every m/z within the range of the output "bin". When the m/z data are not equally distributed, this can result in more samples being integrated in one region (e.g. at low m/z) than another (e.g. at high m/z). This is usually the desired result, this conserves all data within the range. However, for some applications it may be desirable to divide by the number of samples collected within the m/z "bin", thus an averaging rather than an integrating behaviour. In this case deselect the "Integrate m/z" option. Note that in all cases, scans are averaged if there is more than one scan in an output "bin".

The "Normalise" option simply scales all of the output data to the highest (absolute) value observed in the data (after integration or averaging described above). Thus the data lie on a known scale from 0 to 1.

Origin

Click on "File", and "Open...". In the first dialog box under "Files of type:" choose "ASCII Data (*.dat, *.csv, *.txt)". Browse to the .csv file you saved as output from the EDit program:

Open					? 🔰
Look in:	🚞 DATA		•	두 🗈 💣 🎫	
	SAMPLE.RAV	V 📸 sample.csv 🗒 SAMPLE.TXT			
	File name:	sample.csv		•	Open
	Files of type:	ASCII Data (*.dat;*.csv;*.txt)		•	Cancel
		🗖 Open as read-onlu			

The open file must be converted into the special Origin matrix format in order to plot 3D graphs. Select "Edit", "Convert to Matrix" and "Direct". Click on the radio button for "Y varies across columns" and check the boxes for "Y values in first row" and "X values in first column":

0	utput - o	utput.cs	v							×
	A[X]	B(Y)	C1[Y]	C2[Y]	C3[Y]	C4[)	1	C5(Y)	C6[Y]	^
1	ScanNum->	1	2	3	4	6	5	6	7	
2	49.7303	0	0	0	1	r	0	1	1	
3	50.73202	0	0	0	0		1	0	1	
4	51.73373	0	0	0	0		3	3	4	
5	52.73545	0	0	3	6		106	258	495	
6	53.73717	Diment	C	the second			5	13	20	
7	54.73888	Direct	convers	ion to M		<u> </u>	43	115	189	
8	55.7406	Selec	ted Range				2	4	11	
9	56.74232	- 🛃 loutor	# A[1]:C150[65	521		_	1	6	5	0
10	57.74403	(a) loorbo	Portbar (1):c120[032]						4	
11	58.74575	Data Form	Data Format						10	
12	59.74747	C X varie	e across colum	ns 💽 Y varie	ins –	1	2	1		
13	60.74918		s deress colum	ins - i vanc	s deross colum	-	0	4	9	
14	61.7509	□ × valu	es in first row	🔽 Y valu	es in first row		10	25	56	
15	62.75262	- TY valu	es in first colum	n 🔽 X valu	es in first colum	n –	1	5	5	
16	63.75433		karative with consider the fi	100			0	2	5	
17	64.75605			Convert			0	1	0	í.
18	65.75777			Convert		-	0	0	5	
19	66.75948		U				4	1	4	
20	67.7612	0	0	0	0		1	3	4	~
<									>	

The resulting matrix (the data appears with a yellow background) may be plotted in a variety of styles, but the best place to start is probably a simple contour map:

Plot	Matrix	Image	Tools	Forma	it	Window	He	lp		
🥭 З	D <u>C</u> olor F	Fill Surfac	e	1	8 →	₩ @	7		<u>a</u> 🖽 🛙	
<i>m</i> 3	Plot Matrix Image Tools Format Window Help Image 3D Color Fill Surface Image									
Plot Matrix Image Tools Format Window Help Image Stress 3D Qolor Fill Surface Image Stress Image Stress Image Stress Image Stress Image Plot Image Stress Image Stress Image Stress										
🥭 з	D Color <u>(</u>	<u>M</u> ap Surfa	ace	_						
🕅 З	D <u>B</u> ars					5		6	7	
/ Э	D <u>W</u> ire F	Frame 0 0								
a 3	Image: Supervision of the supervision									
				— L	3		0	0		
1 <u>I</u>	nage Plo	it			6		1	7		
C	ontour P	lot		- × 🔡	🕨 🧱 Contour - Color <u>F</u> ill					
n da H	listogram	1		Contour - B/W Lines + Labels						
🞽 E	rofiles/Ir	nage		::	Gŗ	ay Scale N	Мар			
P	rofiles/C	ontour		- 7	'50		34	305		
-				7	69		19	303		

The initial plot will probably appear nonsensical until Speed Mode is switched off (the matrix is usually a large one). Go to "**Format**", "**Layer...**", go to the "**Size/Speed**" tab and uncheck the box labelled "**Matrix data, maximum points per dimension**".

Origin automatically assigns contour lines to particular values; like all other features of the graph, these may be altered to suit. Generally, users will want to enhance the lower intensity peaks. Double-click on any peak in the spectrum, and you will be greeted by the following dialog box:

Plot Details	?	×
	Color Map / Contours Numeric Formats Label Click column headers to edit entire columns, click cells to edit individual properties. Level Fill Line Labels Level Fill Line Labels Image: Column col	
	Level Color Fill Control Missing values Insert Delete Fill to Contour Lines Fill to Grid Lines Rescale Mode Normal	
Plot Type: Contour	>> Matrix OK Cancel Apply	

Double-click on the value under "**Level**" and enter a smaller value. Other contour lines may be added, subtracted or adjusted to suit the desired picture. Maximising real signals while leaving out noise is the primary goal.

Relabel the x axis m/z, and the y axis with whatever parameter is appropriate, e.g. cone voltage, collision voltage, time, etc.