Please visit our website at http://www.owlstonenanotech.com/ for further information.

The Support tab:

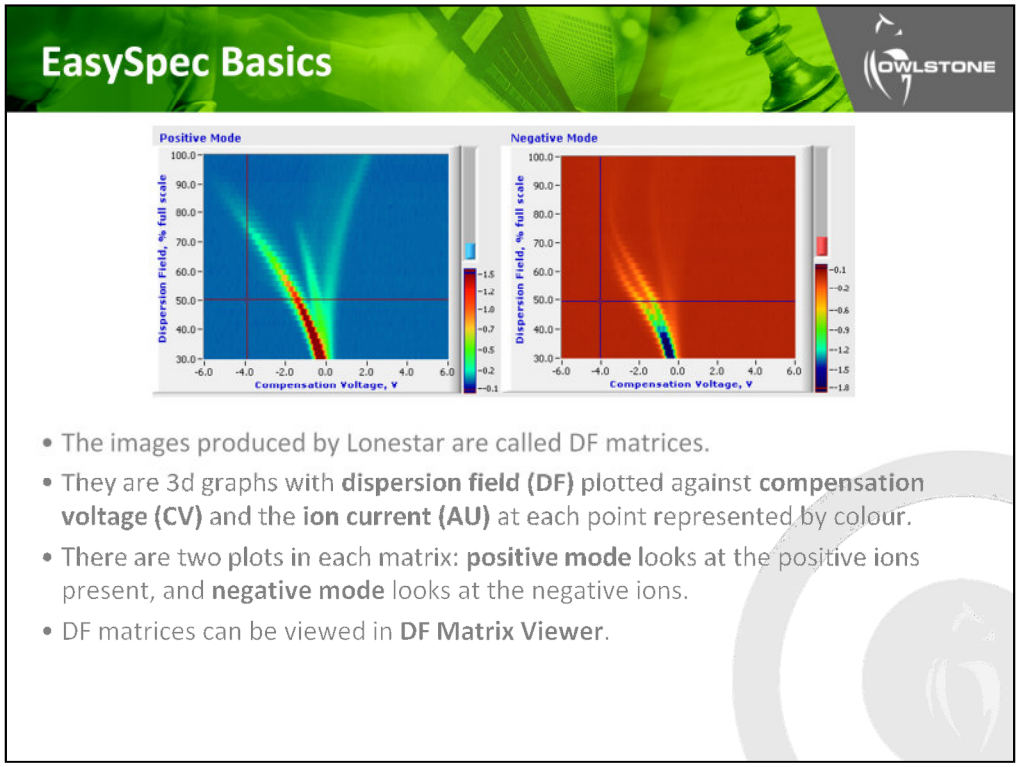
(http://support.owlstonenanotech.com/home) contains Lonestar manuals and information.

The Training and Resource Centre forum:
(http://support.owlstonenanotech.com/forums) contains product manuals, video tutorials and software downloads.

The Submit a Request Forum:
(http://support.owlstonenanotech.com/anonymous_requests/new) enables Owlstone customers to submit questions and seek troubleshooting advice from our Technical Support Department.

Software session 1: topics covered

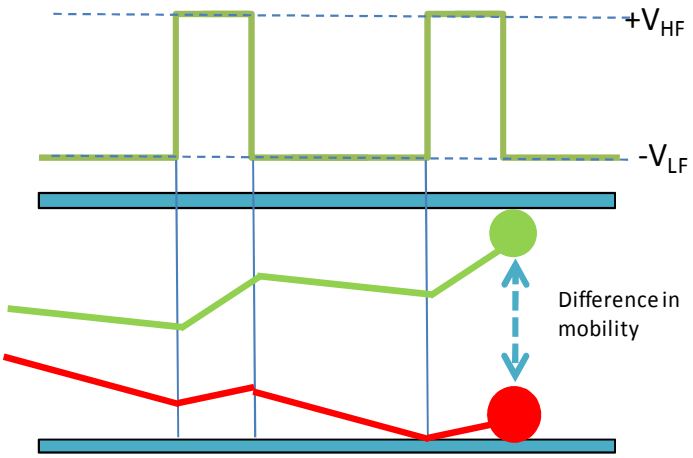
- Lonestar: building up a DF matrix (spectrum)
- DF Matrix Reviewer features
- Rule Builder features
- Rule Builder: producing Rules for multiple analyte peaks
- Extracting data with EasyExtract



Detailed Description

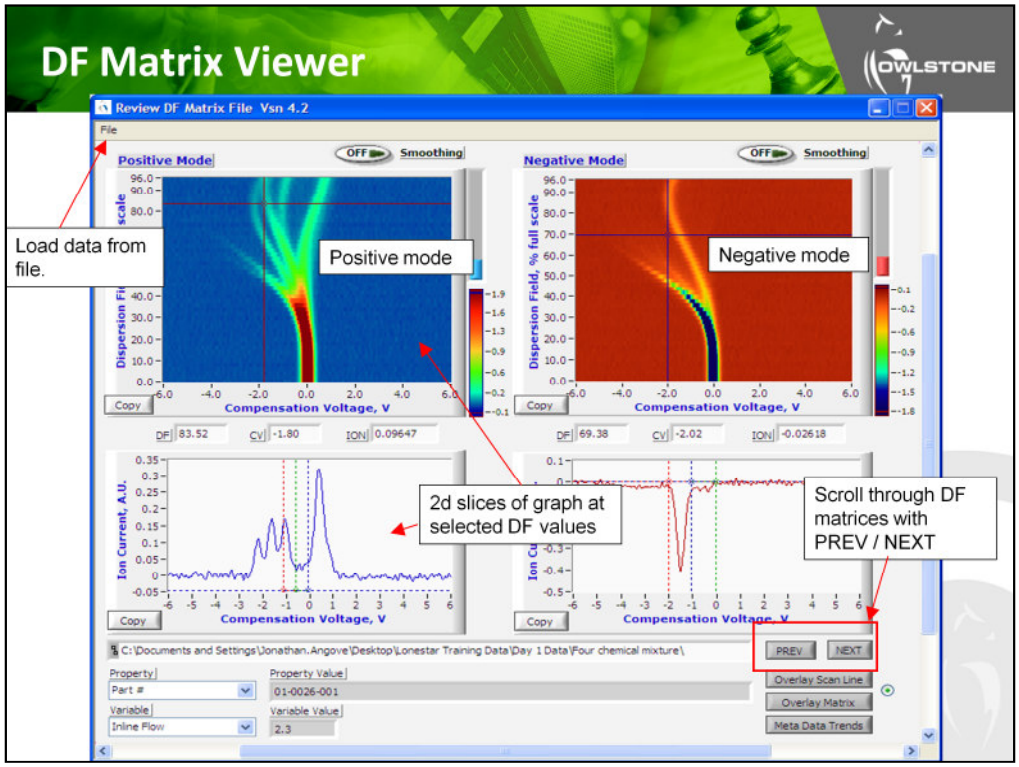
The Lonestar scans from -6V to +6V CV (typical setting) in positive mode, then in negative mode from -6V to +6V CV.

If we have a system scanning between 0-100% DF in 51 lines, The first line scans at 0% DF, then 2% DF, 4% DF ... 100% DF



IF the shown voltage between the $+V_{HF}$ and $-V_{LF}$ is 100% DF, then at 50% DF, the voltage would be half that shown.

The ions enter the electrode channel and the applied voltages create oscillating regions of high ($+V_{HF}$) and low ($-V_{LF}$) electric fields as the ions move through the channels. The difference in the ion's mobility at the high and low electric field regimes dictates the ion's trajectory through the channel.

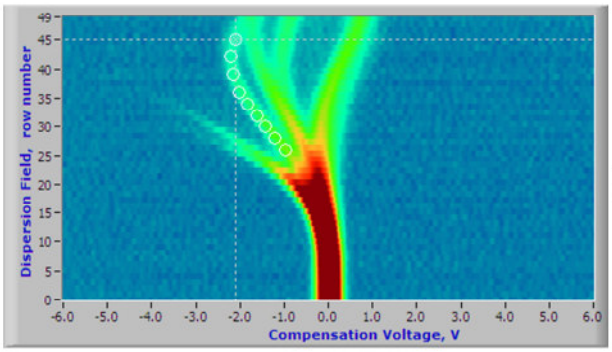


Tasks:

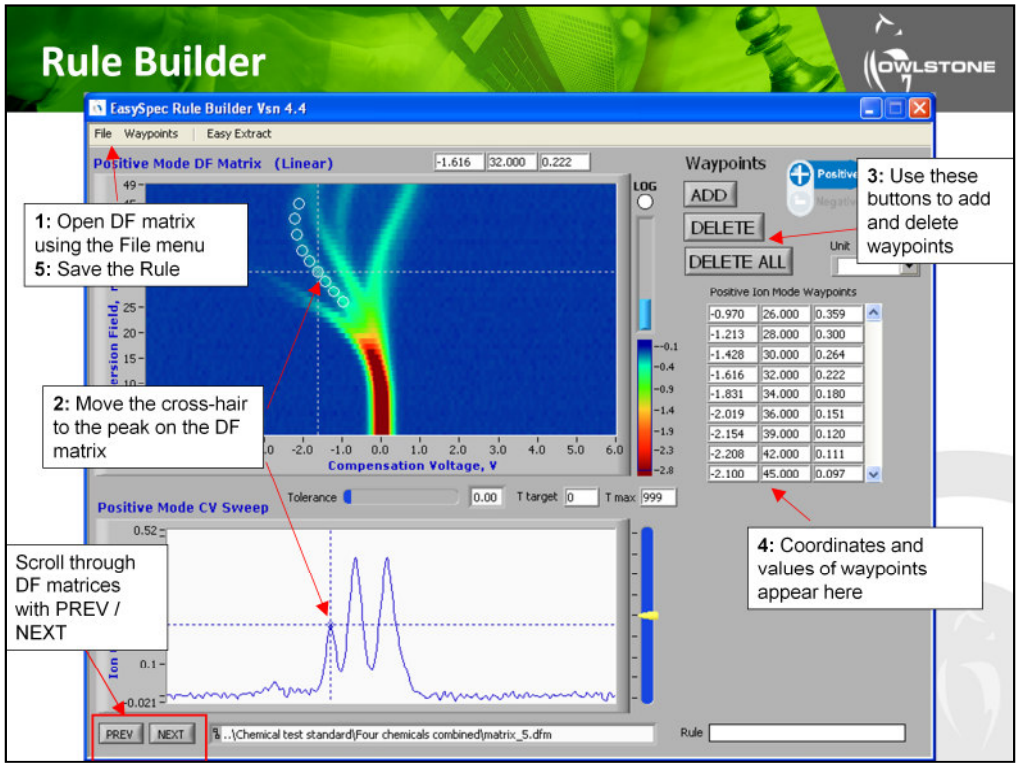
1. Open Review DF Matrix File software
2. Select “File”, “Load Data” and browse to Desktop\Training Day Example Data\Chemical test standard\Four chemicals combined. Open “matrix_1”
3. Change contrast bars to see the effect this has on the displays.
4. Move top horizontal bar - this shows the scan at the displayed DF, the readout below the matrix shows DF
5. Move top vertical bar - the readout below matrix shows CV and AU at this point
6. In the bottom display showing AU vs CV, place the crosshairs over the two analyte peaks
7. Scroll through matrices in folder using the “NEXT” button. Note the AU of the analytes build up and die away
8. Export file by selecting “File”, “Export File”. Open the created text document and review the peripheral readings

Rule Builder

- EasySpec works by creating **Rules** made up of one or more **waypoints**. Each waypoint records the coordinates of a point on the 3d graph (DF, CV and AU).
- Rules can be created in **Rule Builder** to identify a peak or peaks.



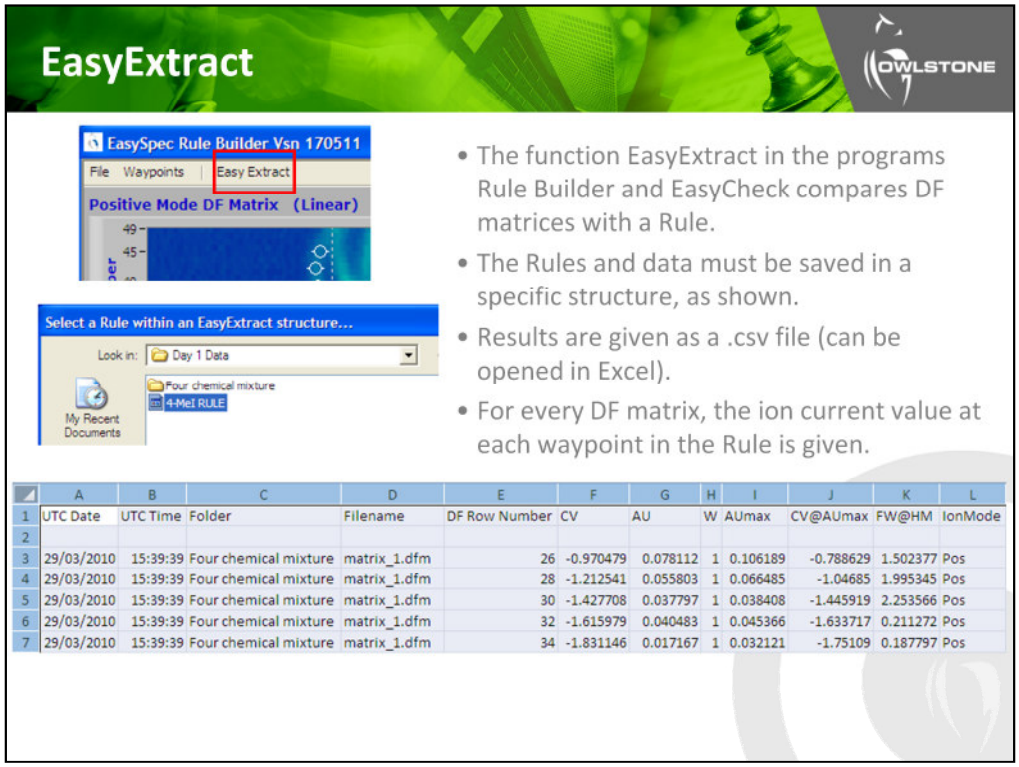
In the matrix shown here, the peak identifies the chemical of interest. This can be traced out using waypoints.



Tasks:

1. Open EasySpec Rule Builder software
2. Select “File”, “Load DF Matrix” and browse to “Desktop\Lonestar Training Data\Day 1 Data\Four chemical mixture”. Open “matrix_5”
3. Set contrast
4. Pick the shown peak and make an identification rule for it (use the values shown above)
5. Set the tolerance to 1.0, 0.5 and 0.2 and see the tolerance bars displayed
6. Save the rule as “4-MeI RULE” – “File” “Save Rule” and browse to Desktop\Lonestar Training Data\Day 1 Data
7. Scroll through matrices noting colour change of waypoints – white denotes lower AU than that used in the Rule, black denotes higher AU than that used in the Rule
8. T target is the time (seconds) between OK’ing the start of the Lonestar run and the detector actually starting. T max is the time (seconds) that the Lonestar will time-out if a DF matrix has not been collected

A good understanding of this process is essential for extracting reliable data!



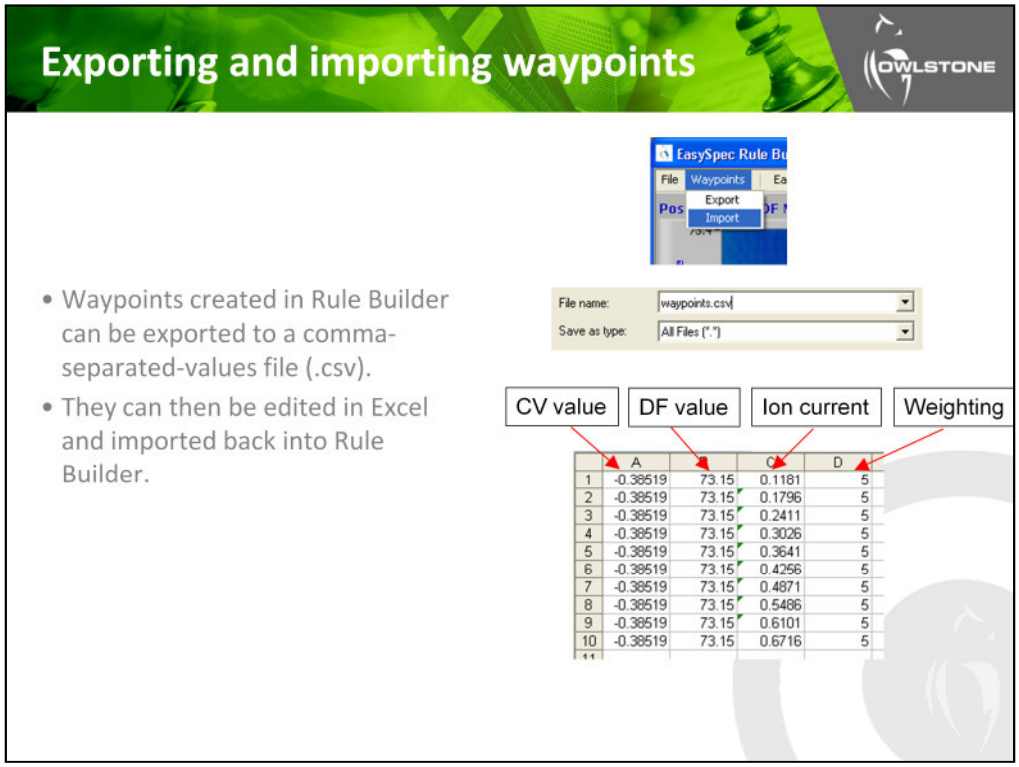
	A	B	C	D	E	F	G	H	I	J	K	L
1	UTC Date	UTC Time	Folder	Filename	DF Row Number	CV	AU	W	AUmax	CV@AUmax	FW@HM	IonMode
2												
3	29/03/2010	15:39:39	Four chemical mixture	matrix_1.dfm	26	-0.970479	0.078112	1	0.106189	-0.788629	1.502377	Pos
4	29/03/2010	15:39:39	Four chemical mixture	matrix_1.dfm	28	-1.212541	0.055803	1	0.066485	-1.04685	1.995345	Pos
5	29/03/2010	15:39:39	Four chemical mixture	matrix_1.dfm	30	-1.427708	0.037797	1	0.038408	-1.445919	2.253566	Pos
6	29/03/2010	15:39:39	Four chemical mixture	matrix_1.dfm	32	-1.615979	0.040483	1	0.045366	-1.633717	0.211272	Pos
7	29/03/2010	15:39:39	Four chemical mixture	matrix_1.dfm	34	-1.831146	0.017167	1	0.032121	-1.75109	0.187797	Pos

Tasks:

1. EasyExtract the Rule just created. The software should default to this Rule, otherwise browse to Desktop\Lonestar Training Data\Day 1 Data and select the Rule
2. Open results.csv in Excel
3. Important values are AUmax and CV@AUmax – understand the difference between AU and AUmax, CV and CV@AUmax
4. Note the ordering of the data

Additional Tasks:

1. Select one waypoint (line 36) and plot AUmax vs. Matrix number (time)
2. The initial increase in AU is due to the sample reaching its selected temperature. The drop in AU is due to the depletion of 4-MeI within the sample

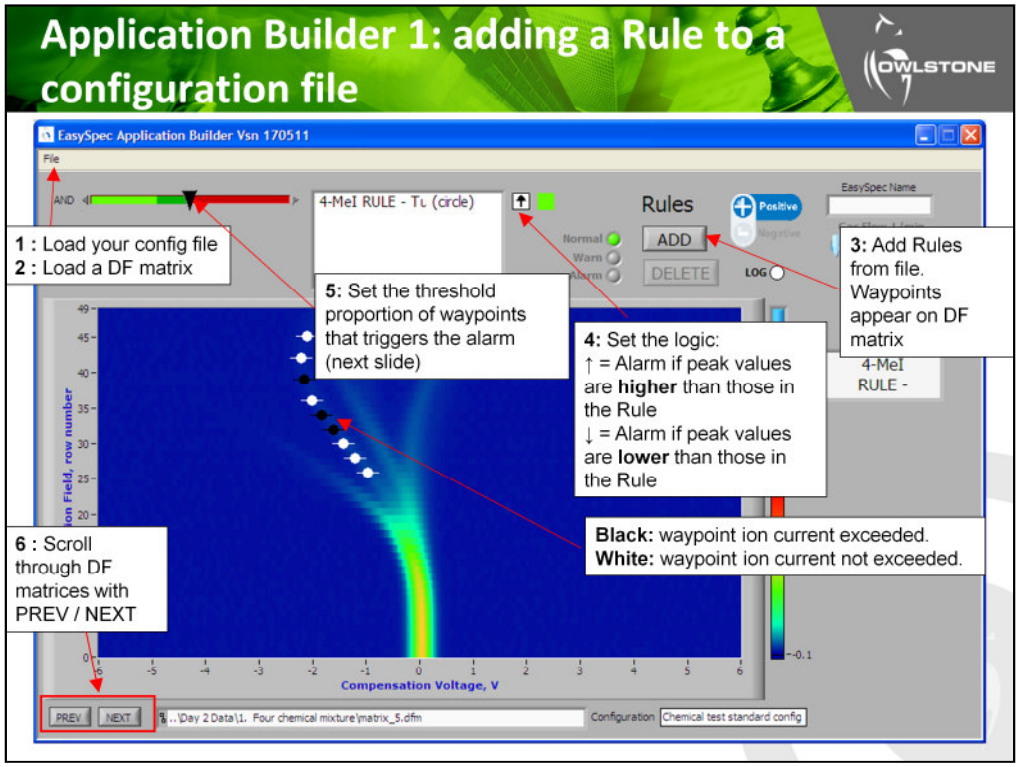


Tasks:

1. Export waypoints of the rule
2. Switch to negative mode and import them again

Software session 2: topics covered

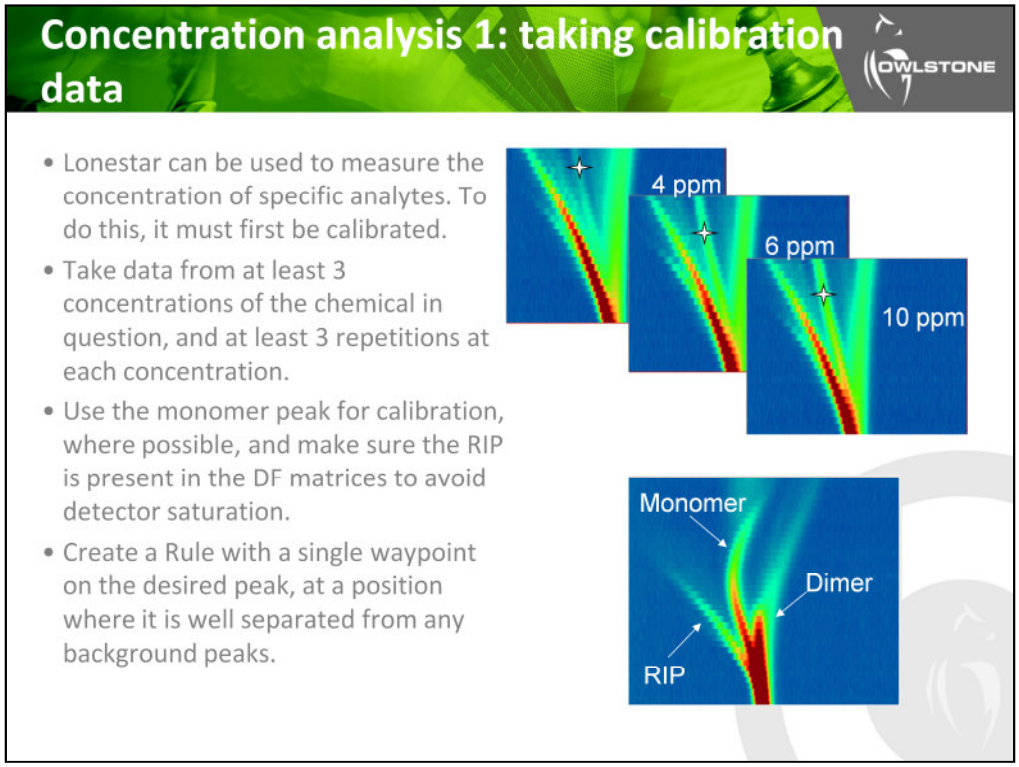
- Rule Builder 2: building a calibration curve
- Application Builder features
- Application Builder: loading a configuration file and adding Rules
- Overview of online concentration monitoring



Tasks:

1. Open Application Builder
2. Load the Chemical Test Standard configuration – “File” “Load Configuration” and browse to Desktop\Lonestar Training Data\Day 2 Data and select
3. Load a matrix – browse to “Desktop\Lonestar Training Data\Day 1 Data\Four chemical mixture”. Open “matrix_5”
4. Add the Rule “4-MeI RULE – Tutorial 1”
5. Using the “PREV” and “NEXT” buttons, see how the proportion of triggered to not-triggered waypoints change
6. Set the threshold proportions to 25, 50 and 80 and note which matrix triggers the alarm
7. Switch the alarm logic and set the threshold proportions to 25, 50 and 80 and note which matrix triggers the alarm

The setting of the threshold proportions and the alarm logic are important to understand for correct use of Application Builder



Tasks:

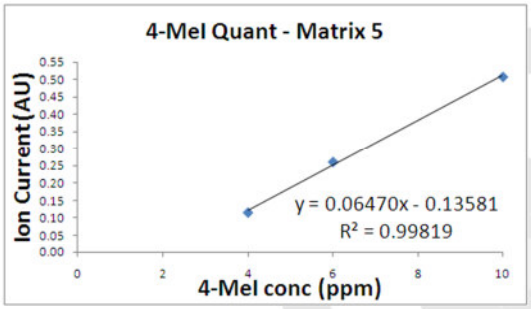
1. Open Rule Builder
2. Open the 4-MeI data files by browsing to Desktop\Lonestar Training Data\Day 2 Data\2. 4-MeI calibration. Select “matrix_5” of the “Sample 3 - 6 ppm 4-MeI” folder
3. Create a one-point Rule at line 21, with a tolerance of 0.4
4. Save the Rule as “4-MeI” in the folder Desktop\Lonestar Training Data\Day 2 Data\2. 4-MeI calibration
5. EasyExtract the Rule on the 4-MeI data

Concentration analysis 2: building a calibration curve

- Use Excel to plot ion current vs. concentration and find the equation of the calibration curve.

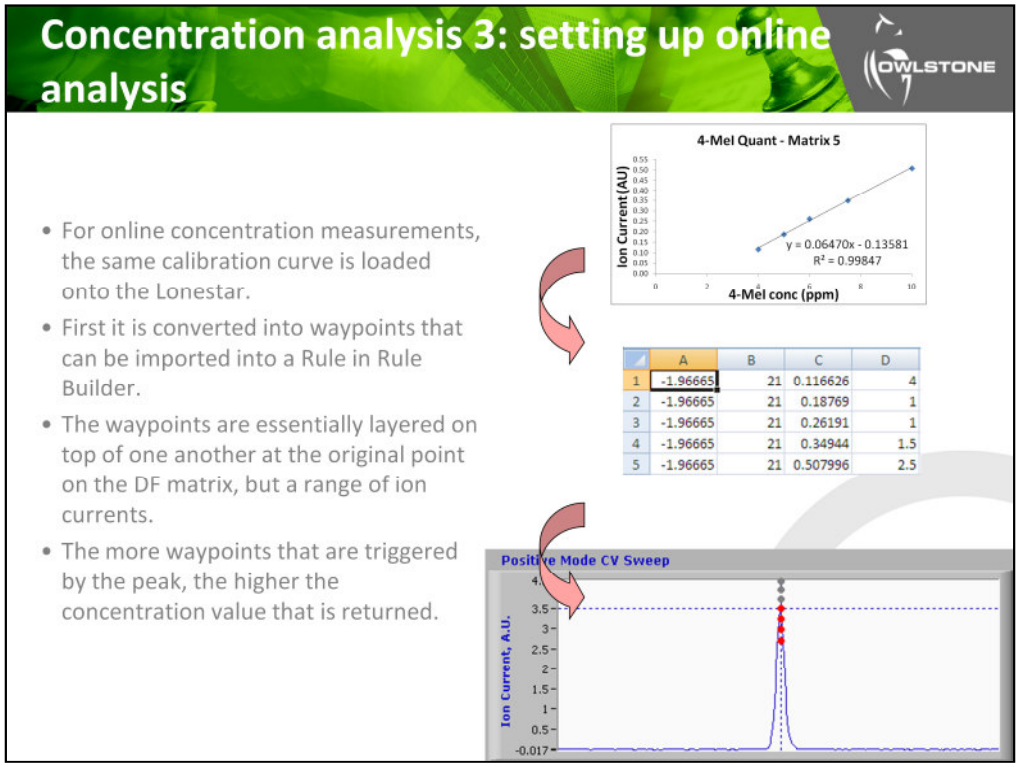
	A	B	C	D	E	F	G	H	I	J	K	L
1	UTC Date	UTC Time	Folder	Filename	DF Row Number	CV	AU	W	AUmax	CV@AUmax	FW@HM	IonMode
2												
3	18/05/2011	13:40:51	Sample 1 - 4 ppm 4-MeI	matrix_1.dfm	21	-1.966645	0.03766	1	0.05414	-2.338881	5.63249	Pos
4	18/05/2011	13:42:23	Sample 1 - 4 ppm 4-MeI	matrix_2.dfm	21	-1.966645	0.07683	1	0.07683	-1.963382	3.332557	Pos
5	18/05/2011	13:43:56	Sample 1 - 4 ppm 4-MeI	matrix_3.dfm	21	-1.966645	0.099048	1	0.099048	-1.963382	0.750999	Pos
6	18/05/2011	13:45:29	Sample 1 - 4 ppm 4-MeI	matrix_4.dfm	21	-1.966645	0.100619	1	0.114963	-2.080726	0.704061	Pos
7	18/05/2011	13:47:01	Sample 1 - 4 ppm 4-MeI	matrix_5.dfm	21	-1.966645	0.104114	1	0.116626	-2.104194	0.563249	Pos
8	18/05/2011	13:48:33	Sample 1 - 4 ppm 4-MeI	matrix_6.dfm	21	-1.966645	0.118381	1	0.120823	-1.986851	0.469374	Pos

- Use the calibration equation to calculate the concentration of the chemical in 5ppm and 7.5ppm samples.



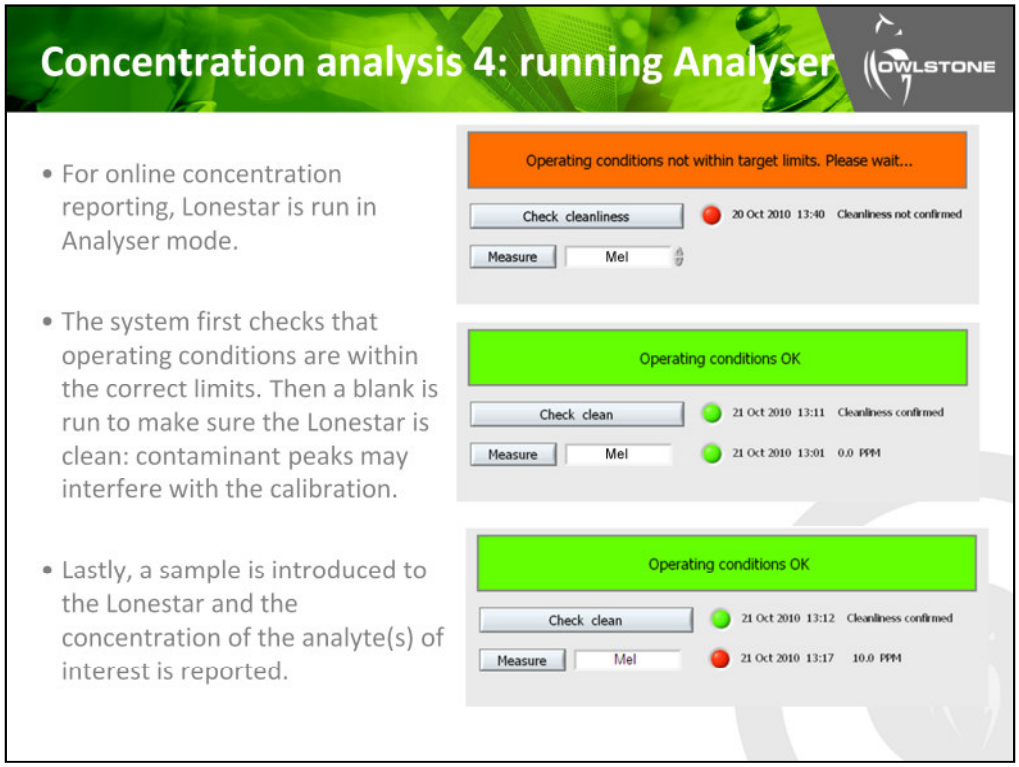
Tasks:

1. Open the results.csv file and plot the AUmax vs DF Matrix number for each of the three quant levels
2. Plot a calibration curve for the AUmax vs 4-MeI concentration for matrices_5 (also matrices_4 and matrices_6)
3. Show R^2 and equation on graph
4. Calculate the AUmax for a 5ppm and 7.5ppm sample
5. Include these points in the calibration curve. Note that the equation for the curve remains unchanged, but the r^2 improves.



After this slide:

1. Including the AU values for the 5ppm and 7.5ppm 4-MeI samples, we will be importing the waypoints into Rule Builder and creating a new online quantitation Rule and Configuration
2. Open the quantitation Rule in Rule Builder and export the waypoints into export.csv
3. Copy DF and CV values for the waypoints from A1 and B1 and paste down to A5 and B5
4. Add in the AUmax values for the 4ppm, 5ppm, 6ppm, 7.5ppm and 10ppm samples in column C (paste special, values)
5. Add the weightings in column D (4, 1, 1, 1.5, 2.5). The weightings equate to the concentration of analyte, summing the values for each triggered waypoint
6. Save file as a .csv file
7. In Rule Builder, delete the current waypoint and then import the .csv file saved above.
8. Set tolerance, scaling (1) and units (ppm) and save the Rule as 4-MeI Quant.
9. Open Application Builder afresh. Load Chemical test standard config file, load matrix_10 from the Sample 2 - Blank data and ADD the Cleanliness RULE and 4-MeI Quant Rule saved above and set the waypoint thresholds (Cleanliness 75%, down arrow, 4-MeI QUANT 10%, up arrow)
10. Save the Configuration as MeI QUANT config.
11. Re-load the MeI QUANT config.
12. Load matrix_1 of the 10ppm 4-MeI standard
13. Scroll through to see the reported concentration changing



The MeI QUANT config is loaded onto the Lonestar, which can now be used in Analyser mode to perform this analysis