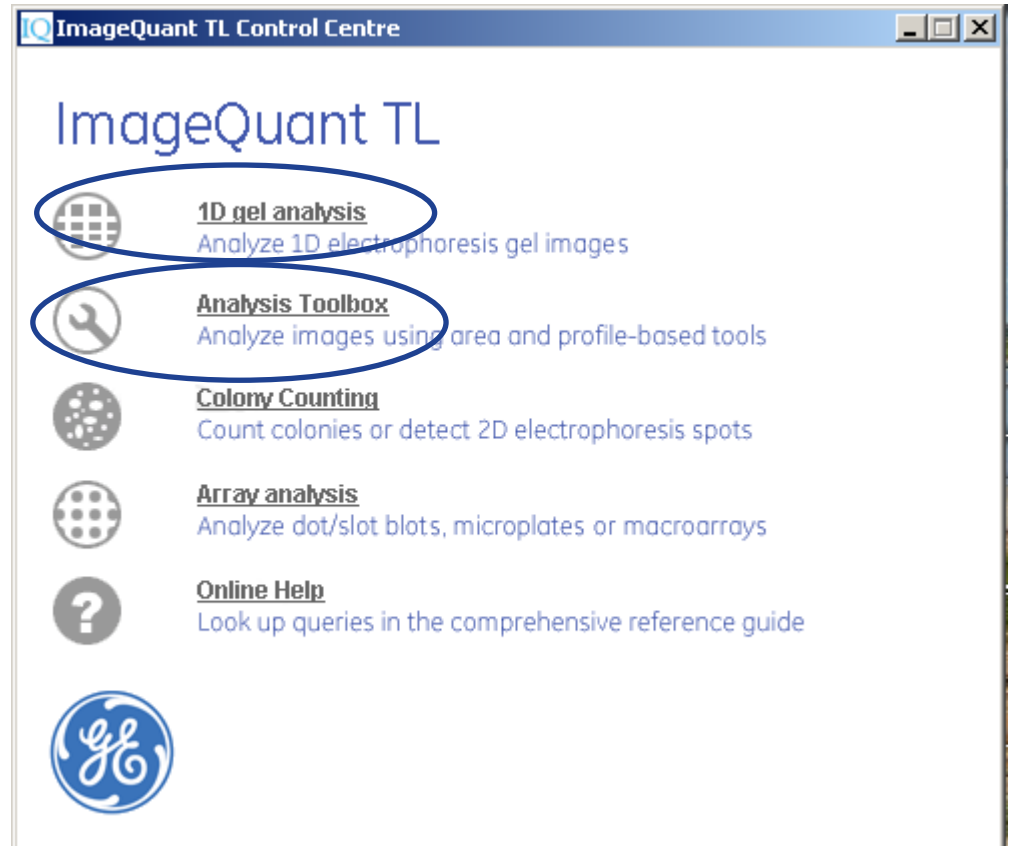


Overlay and Analysis of Multiplexed Images

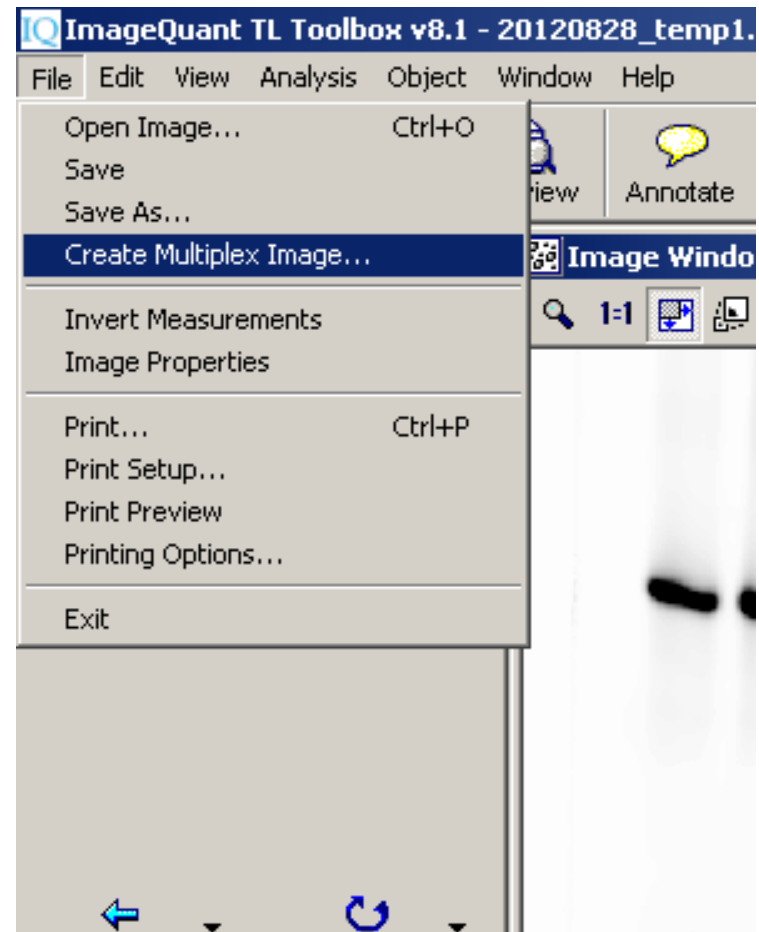
Open “1D gel analysis” or
“analysis toolbox”



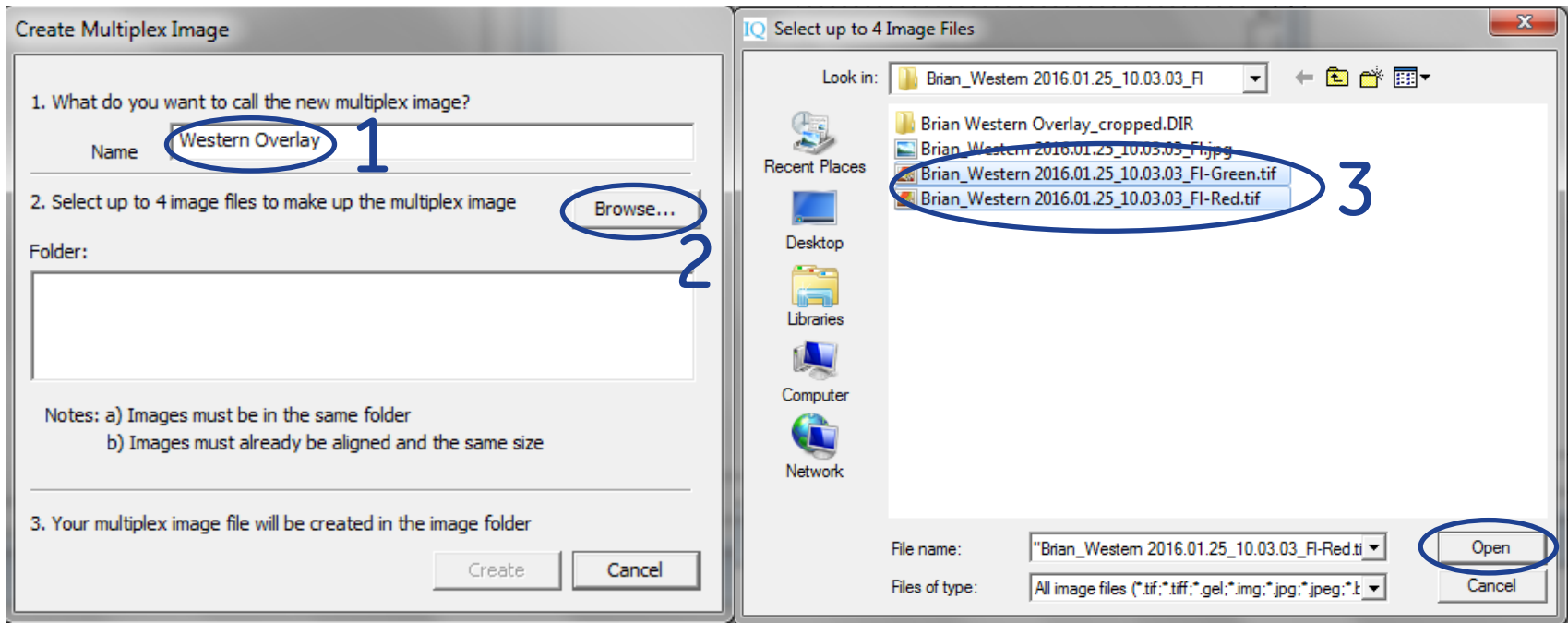
imagination at work

Create multiplex image

From dropdown select
“file” → “create
multiplex image”



Select images to overlay



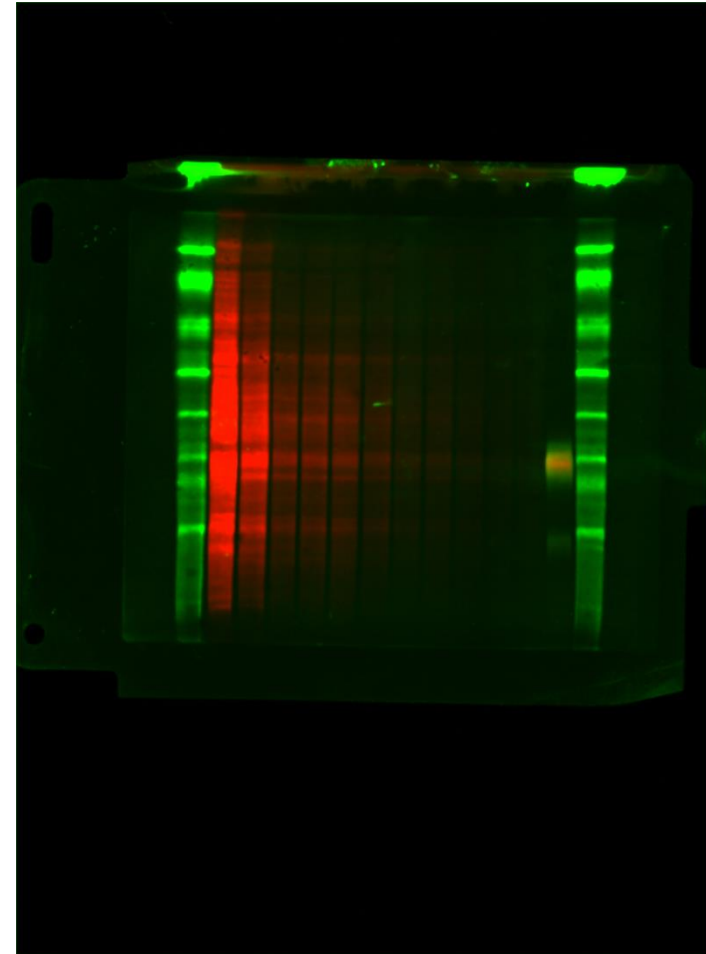
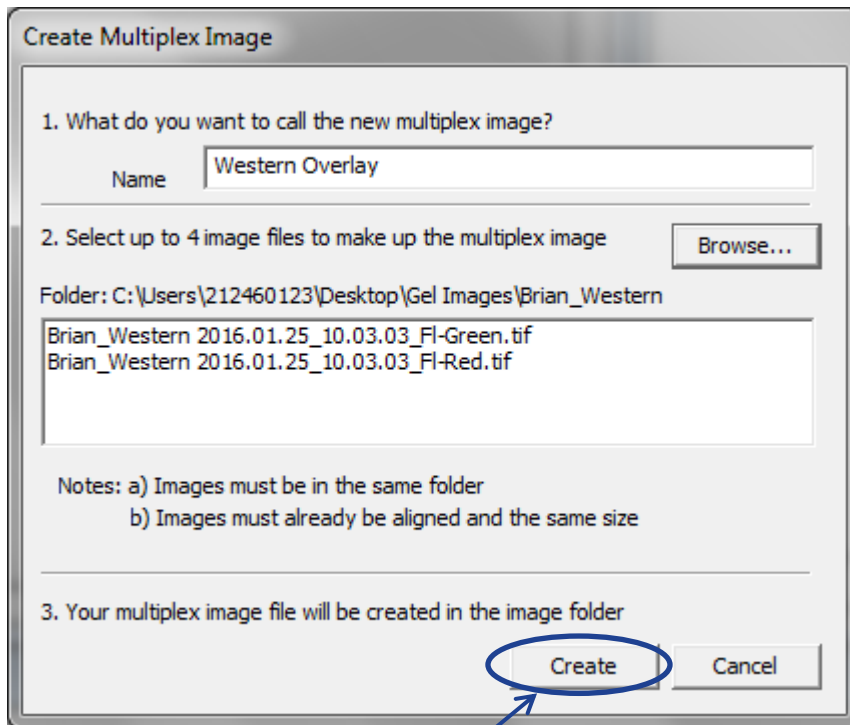
Step 1 – name new file

Step 2 – browse for location of images to overlay – ensure they are in the same source folder!

Step 3 - select associated digitized images (separate channels)

Step 4 – select “open”

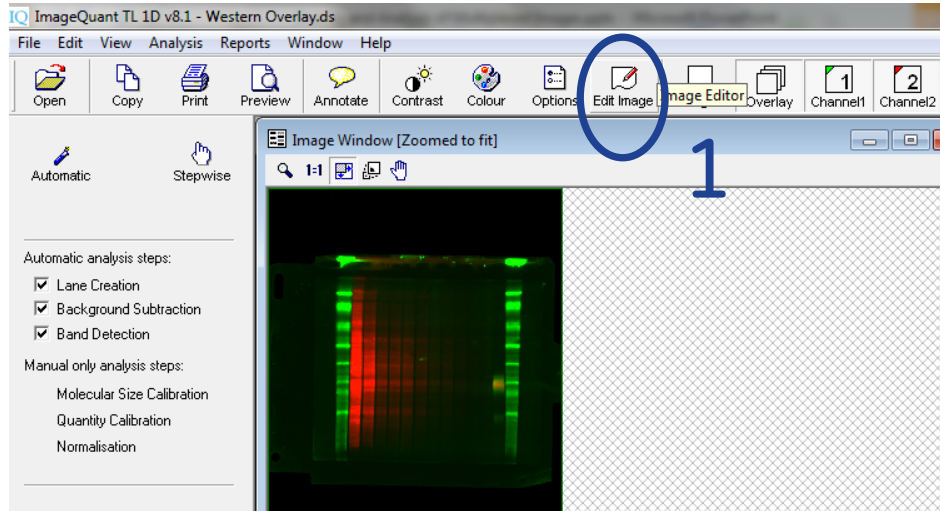
Create overlay



Select "create"

Multiplex image displayed in IQTL

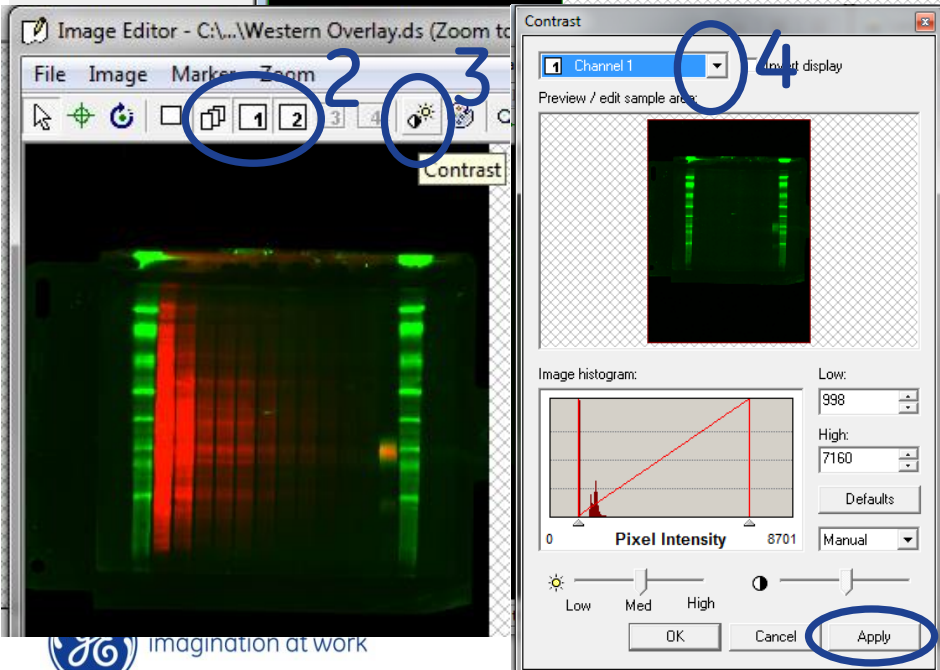
Adjust brightness and contrast



Step 1- select “edit image” icon

Step 2- select “overlay” with all channels selected

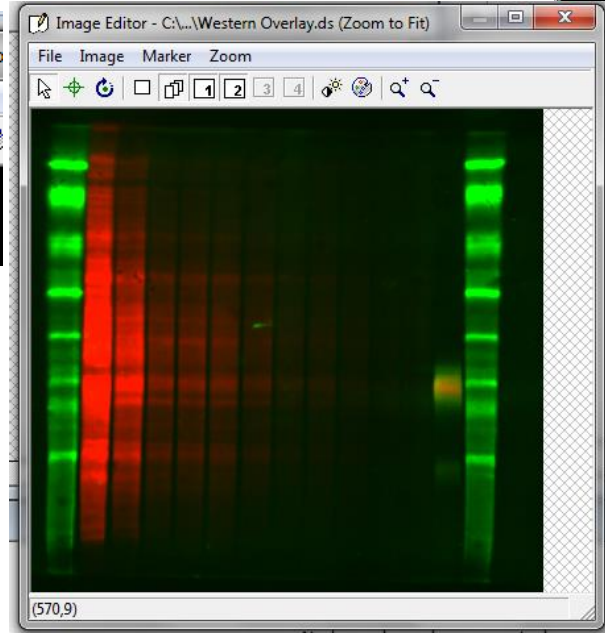
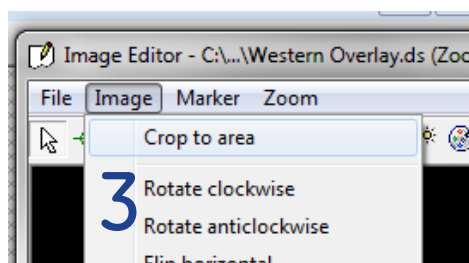
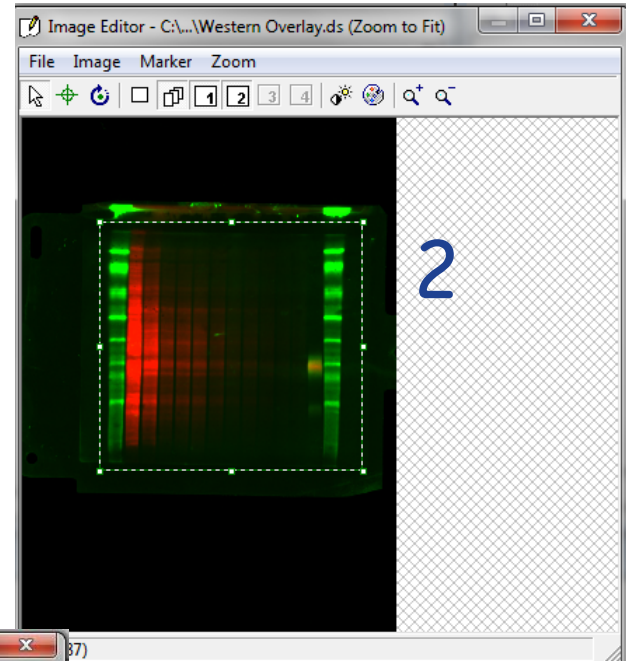
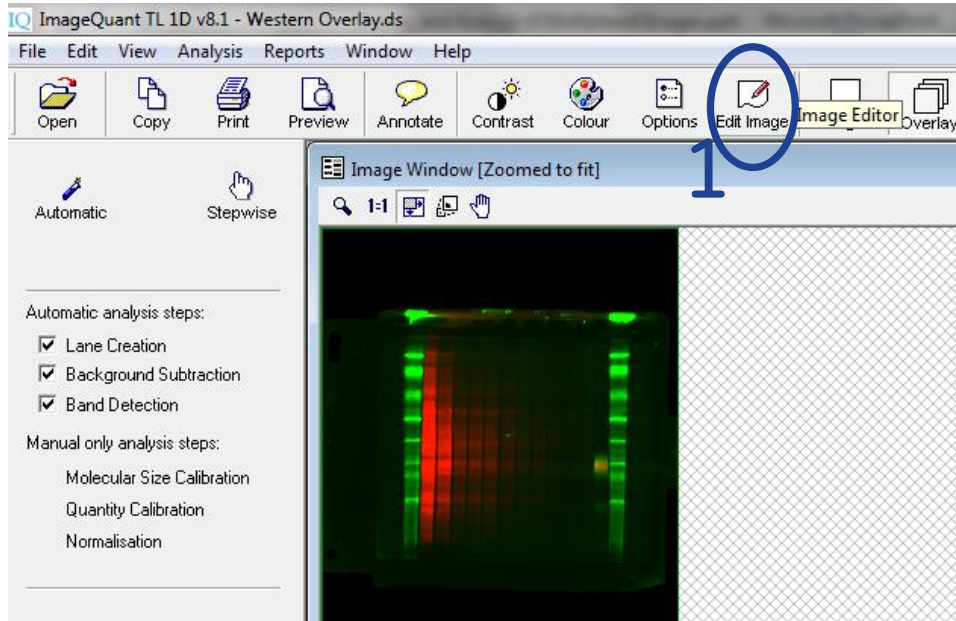
Step 3- select “contrast” icon



Step 4- select each channel from drop down menu and adjust each channel individually

Step 5- select “apply” for each channel

Crop overlaid image

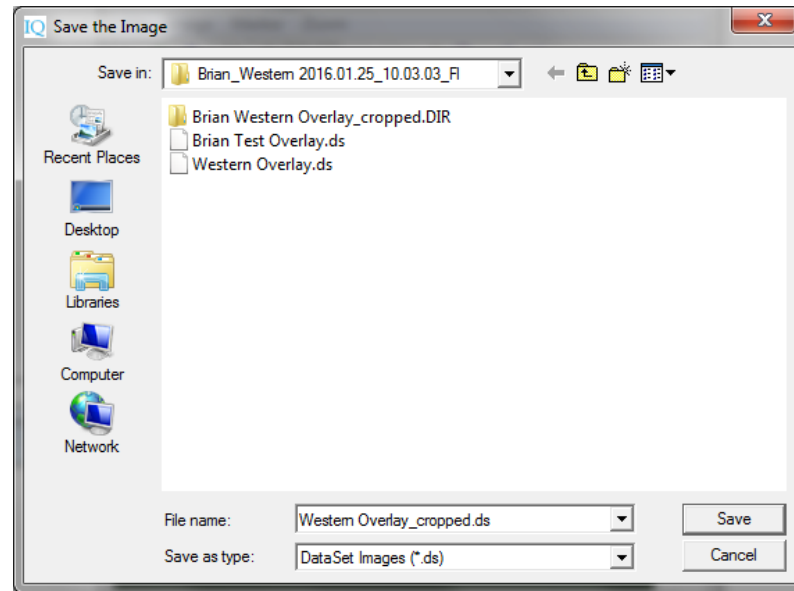
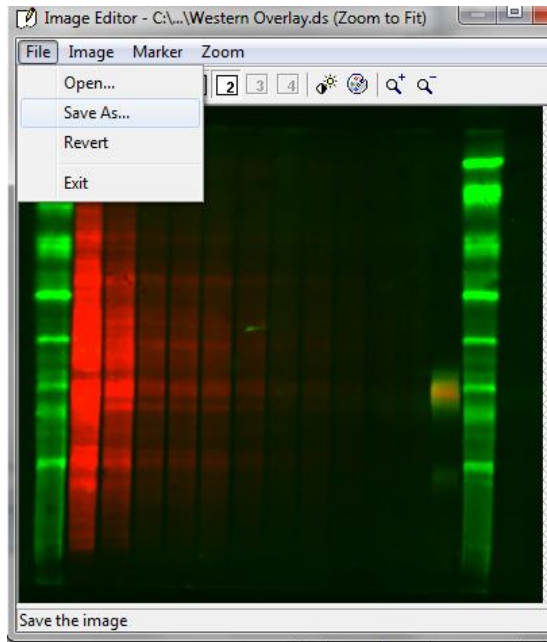


Step 1- select "edit image" icon

Step 2- create box around region of interest

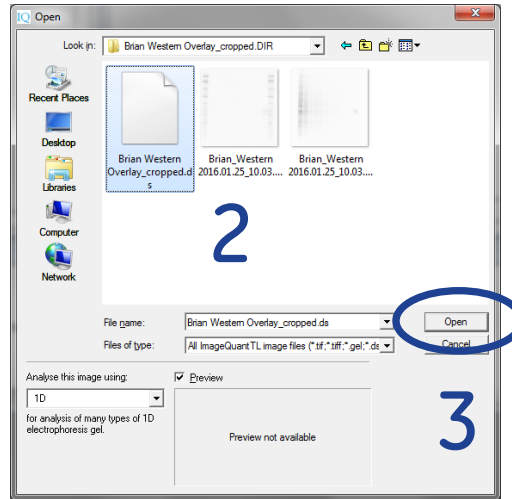
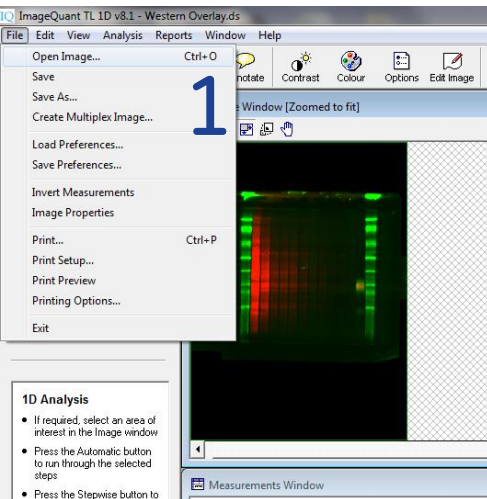
Step 3- select "image" → "crop to area"

Save cropped image as new file



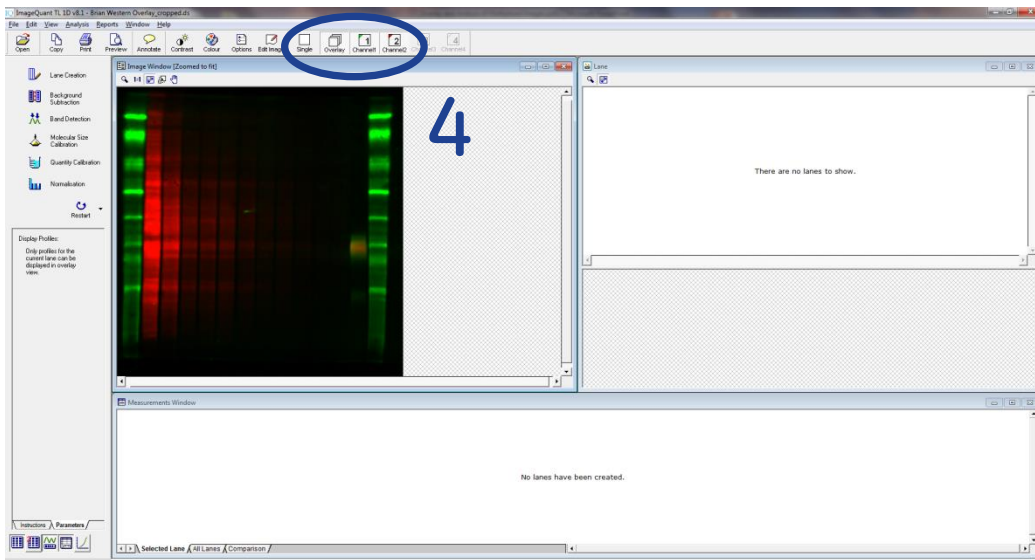
Select "file" → "save as" in the "image editor" window
Give cropped image a new name
This will automatically create a new .dir folder containing
cropped images from both channels and a cropped .ds file

Open cropped overlay image in IQTL



Step 1- select "file" → "open image"

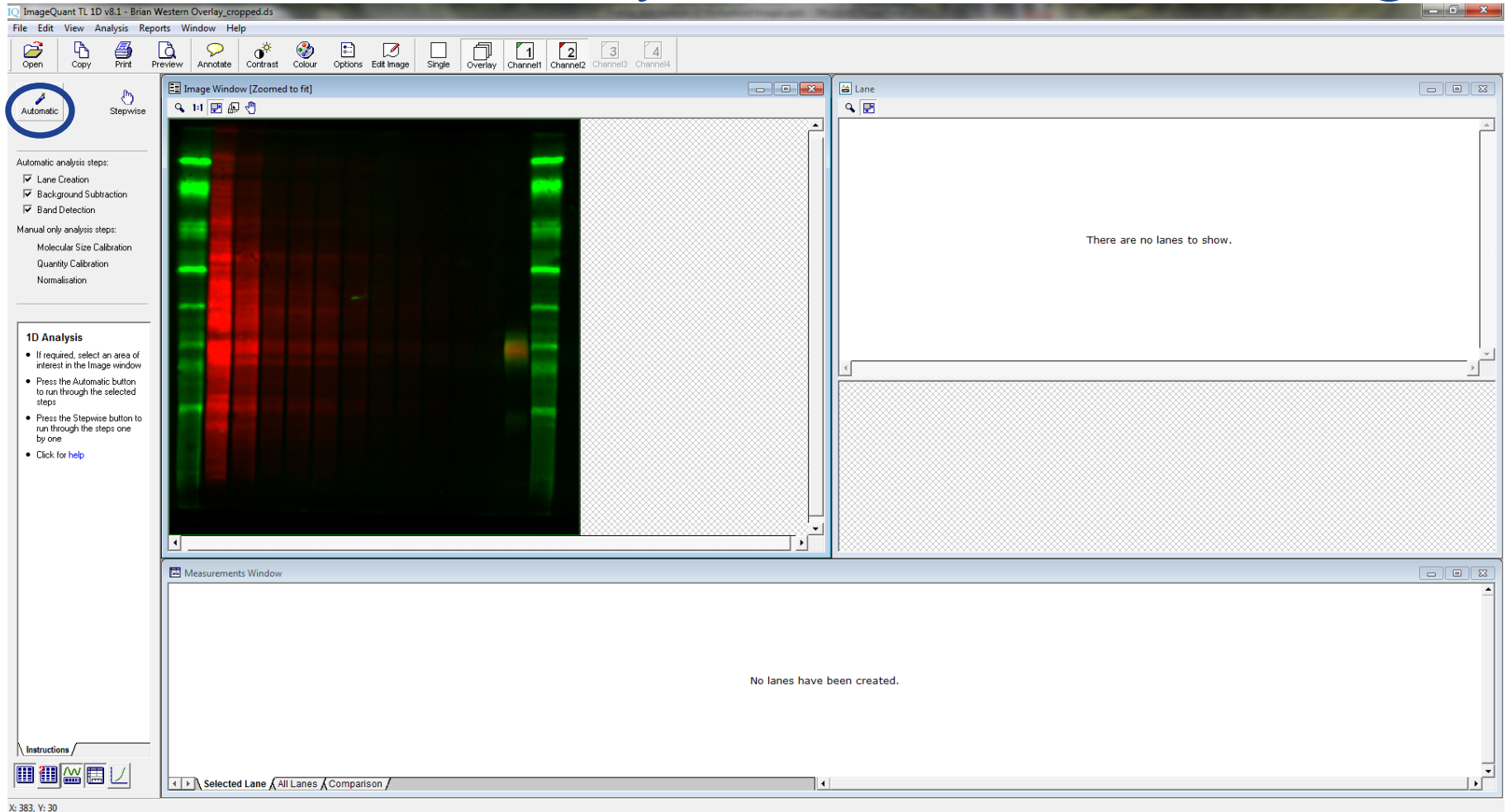
Step 2- navigate to new cropped .dir folder and select .ds file



Step 3- select "open" to open overlaid image in IQTL

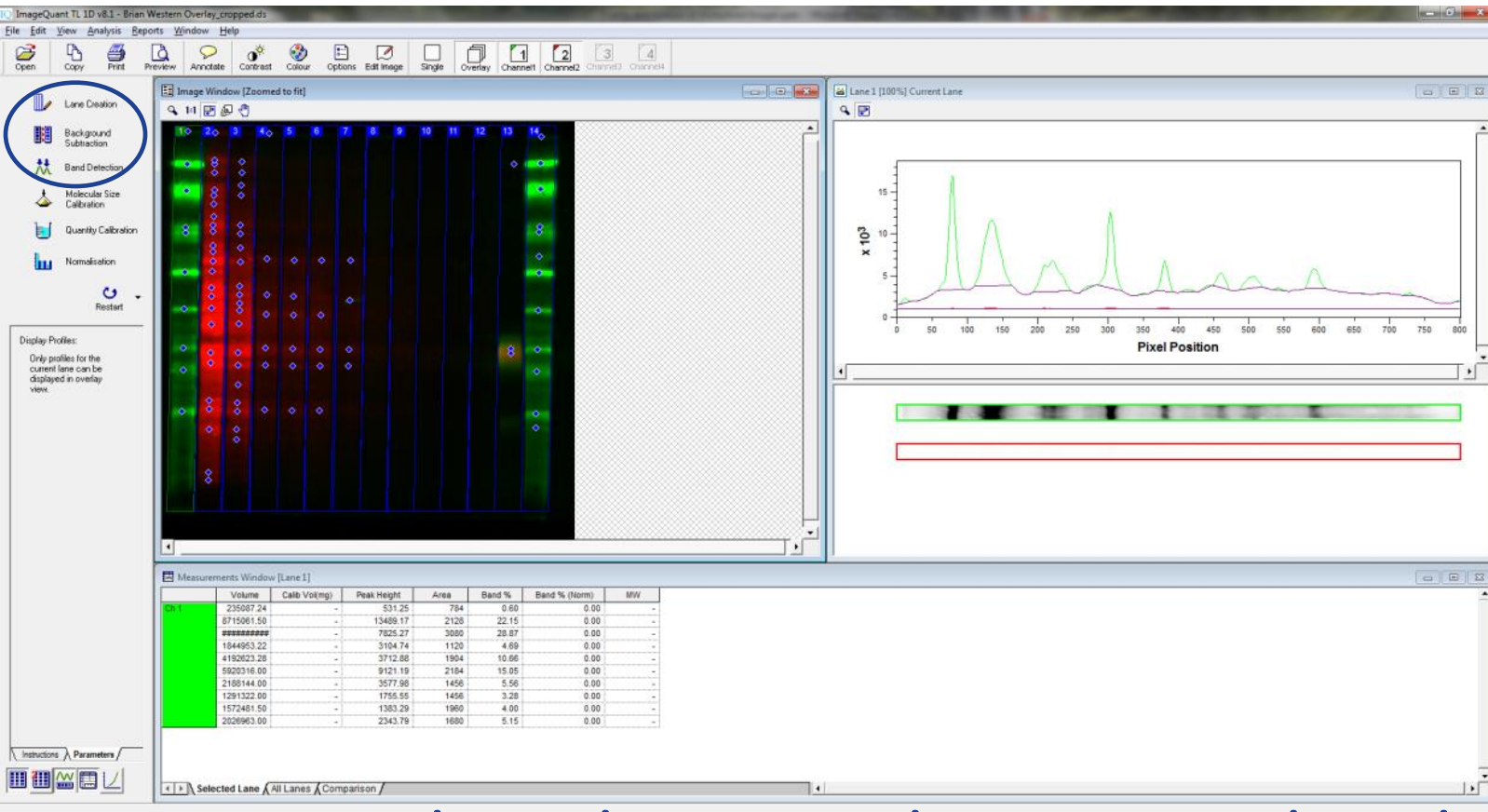
Step 4- you may not toggle the display of each channel using the "channel icons"

Automated analysis of overlaid image



Using the overlaid image, select “automatic” for lane creation, background subtraction and band detection

Fine tuning of automated analysis



Automated analysis can be revisited and modified using the respective icons

Lane creation on overlaid image

ImageQuant TL 1D v8.1 - Brian Western Overlay_cropped.ds

File Edit View Analysis Reports Window Help

Accept Clear

Edit mode:
Edit Multiple Lanes (1)
Edit Single Lanes

Band / Resize
Move
Add Gimmaces

The whole image

Previous Next

Half lane width: 28 pixels Apply

Delete Current Lane

Instructions Parameters

Chnl	Volume	Calc Vol(mg)	Peak Height	Area	Band %	Band % (Norm)
235087.24	-	531.25	784	0.60	0.00	0.00
8715061.50	-	13489.17	2128	22.15	0.00	0.00
1844953.22	-	7625.27	3080	28.87	0.00	0.00
4192823.28	-	3104.74	1120	4.69	0.00	0.00
5920316.00	-	3712.88	1904	10.66	0.00	0.00
9121.19	-	9121.19	2184	15.05	0.00	0.00
2188144.00	-	3577.88	1456	5.56	0.00	0.00
1291322.00	-	1765.55	1456	3.28	0.00	0.00
1572481.50	-	1383.29	1960	4.00	0.00	0.00
2026963.00	-	2343.79	1680	5.15	0.00	0.00

Selected Lane All Lanes Comparison

X:0, Y:99

ImageQuant TL 1D v8.1 - Brian Western Overlay_cropped.ds

File Edit View Analysis Reports Window Help

Accept (3) Clear

Edit mode:
Edit Single Lanes (3)
Edit Multiple Lanes

Band / Resize
Move
Add Gimmaces

The whole image

Previous Next (4)

Half lane width: 28 pixels Apply

Delete Current Lane

Instructions Parameters

Chnl	Volume	Calc Vol(mg)	Peak Height	Area	Band %	Band % (Norm)
235087.24	-	531.25	784	0.60	0.00	0.00
8715061.50	-	13489.17	2128	22.15	0.00	0.00
1844953.22	-	7625.27	3080	28.87	0.00	0.00
4192823.28	-	3104.74	1120	4.69	0.00	0.00
5920316.00	-	3712.88	1904	10.66	0.00	0.00
9121.19	-	9121.19	2184	15.05	0.00	0.00
2188144.00	-	3577.88	1456	5.56	0.00	0.00
1291322.00	-	1765.55	1456	3.28	0.00	0.00
1572481.50	-	1383.29	1960	4.00	0.00	0.00
2026963.00	-	2343.79	1680	5.15	0.00	0.00

Selected Lane All Lanes Comparison

X:19, Y:238

Lane creation can be done using "overlay" view looking at both or by toggling individual channels

Step 1- edit all lanes using the "edit multiple lanes" function

Step 2- edit individual lanes using the "edit single lanes" function

Step 3- select "accept" to apply changes

Step 4- select "next" to proceed

Background subtraction on overlaid image

ImageQuant TL 1D v8.1 - Brian Western Overlay_cropped.ds

File Edit View Analysis Reports Window Help

Open Copy Print Preview Annotate Contrast Colour Options Edit Image Single Overlay Channel1 Channel2 Channel3 Channel4

Subtract 3 Clear

Radius 86

2

Method: Rolling Ball

Previous Next

Background method:

- Rubber Band
- Minimum Profile
- Rolling Ball 1
- Image Rectangle
- Manual Baseline
- None

Intensity profile for selected lane shown for both channels

Step 1- "rolling ball" is recommended for quantifiable background subtraction

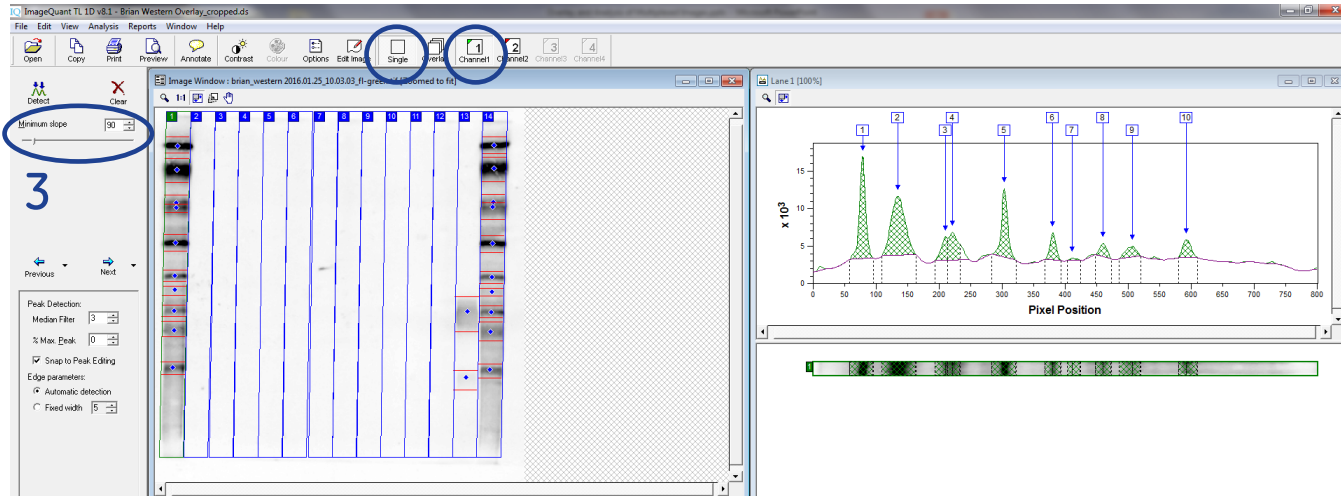
Step 2- adjust "radius" until desired background is subtracted shown in intensity profile

Step 3- select "subtract" to subtract background pixel intensity

Step 4- select "next" to proceed

Band detection on overlaid image

1 2

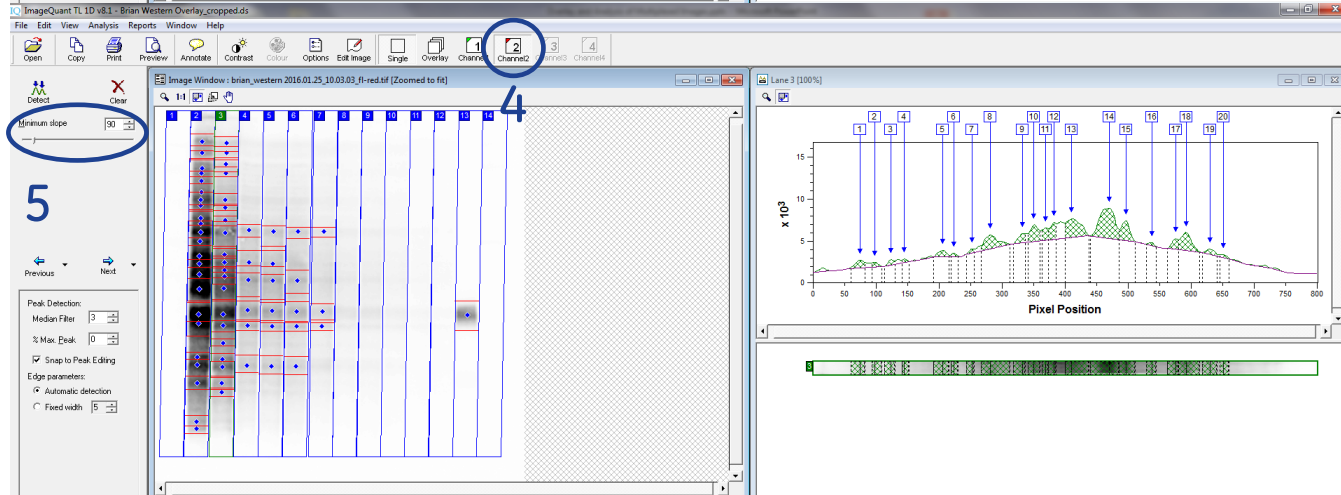


Must detect bands in each channel individually

Step 1- select "single channel view"

Step 2- select "channel 1"

Step 3- adjust "minimum slope" and/or manually select/deselect bands

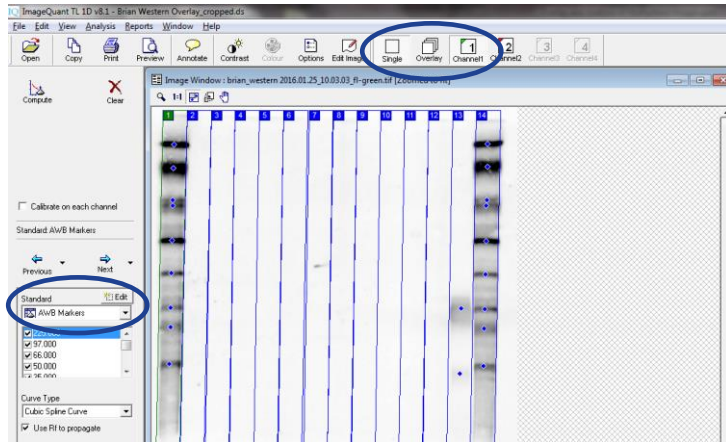


Step 4- select "channel 2"

Step 5- adjust "minimum slope" and/or manually select/deselect bands

Molecular weight calibration on overlaid image

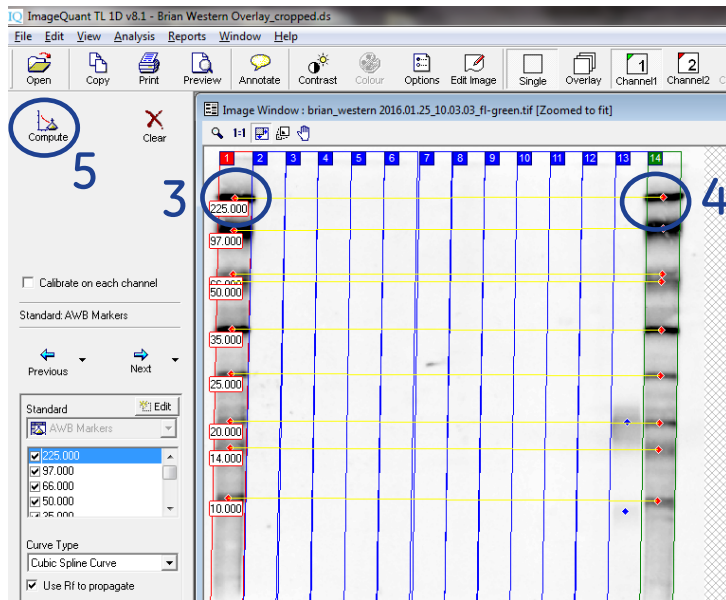
1



Step 1- select "single channel" view and the channel containing the mol wt markers

Step 2- select appropriate mol wt markers from dropdown

Step 3- select highest mw band from ladder lane



Step 4- if more than one ladder lane, select highest mw band in other lane to use both in mw calibration. Lines will form connecting the two corresponding bands

Step 5- select "compute"

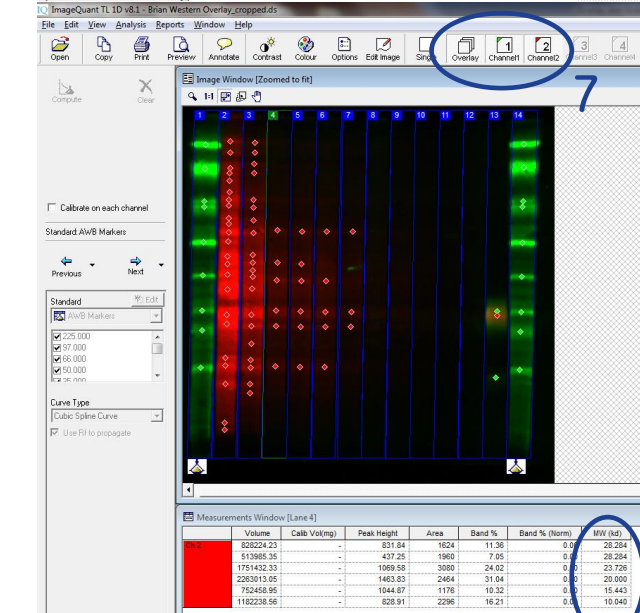
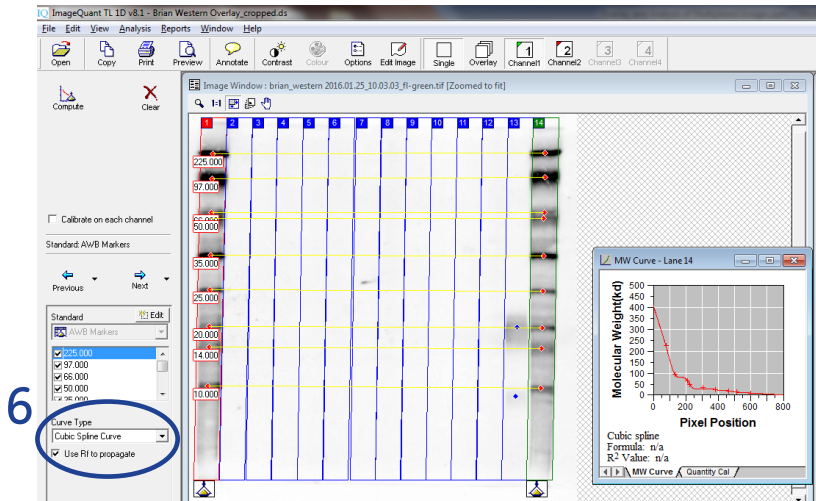
Molecular weight calibration cont...

Step 6- select "curve type" from dropdown menu

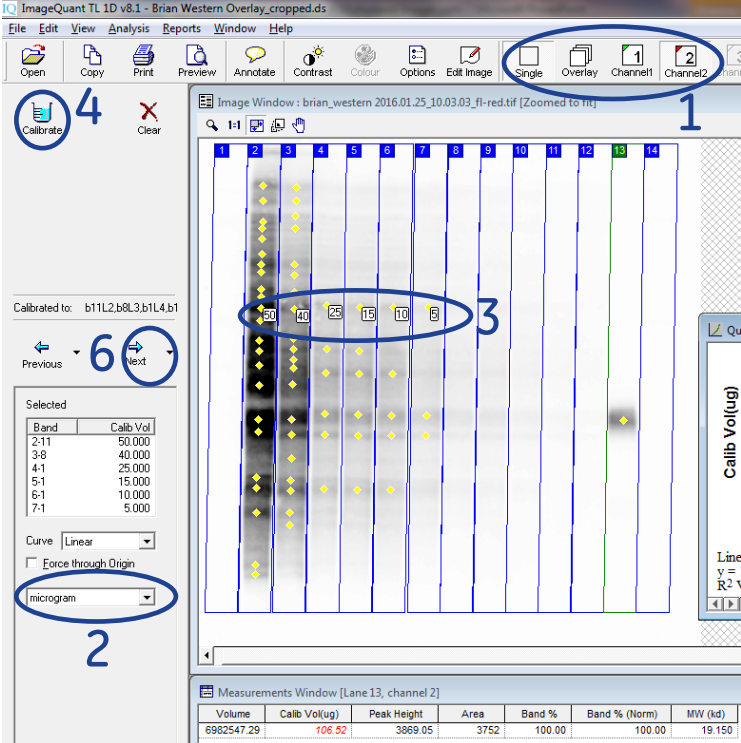
MW calibration curve will show curve fit

Step 7- select "overlay" with "channel 1" and "channel 2" to display overlaid image with all bands

Step 8- refer to data table and confirm the "MW" column has been populated for all bands in all channels



Quantity calibration/normalization on overlaid image



Quantity calibration/normalization are done for each channel independently

Step 1- select "single channel view" and select channel containing standards

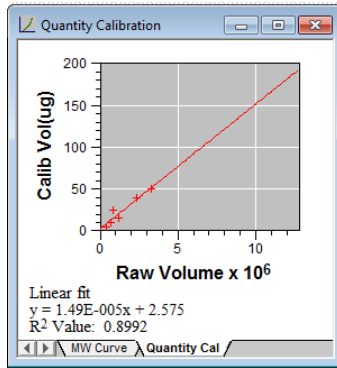
Step 2- select calibration units from dropdown menu

Step 3- select bands containing known amounts of sample and add amounts

Step 4- select "calibrate" and notice the "quantity calibration" standard curve appears and "calibrated volume" column populates in "measurements window"

Step 5- repeat process for other channel(s)

Step 6- select dropdown arrow next to "next" button and select "finish"



Exporting analysis data- option 1

	Volume	Calib Vol(mg)	Peak Height	Area	Band %	Band % (Norm)	MW (kd)
Ch 1	5824016.34	60.00	1868.44	4536	89.08	85.71	20.178
Ch 2	690597.16	10.00	424.67	2632	10.94	14.29	8.172
Ch 3	6982547.29	106.52	3869.05	3752	100.00	100.00	19.150



Lane Table : Channel 1

Lane	Events	Band Volume	Lane Volume
Lane 1	9	39102583.22	40323634.75
Lane 2	0	0.00	2521736.36
Lane 3	0	0.00	904875.23
Lane 4	0	0.00	617268.11
Lane 5	0	0.00	765602.27
Lane 6	0	0.00	73827.89
Lane 7	0	0.00	788629.87
Lane 8	0	0.00	691647.50
Lane 9	0	0.00	699654.62
Lane 10	0	0.00	954729.00
Lane 11	0	0.00	107298.98
Lane 12	0	0.00	119766.51
Lane 13	2	6314613.00	6337969.36
Lane 14	9	36264639.62	36934230.67

Lane Table : Channel 2

Lane	Events	Band Volume	Lane Volume
Lane 1	0	0.00	364186.84
Lane 2	23	60203077.62	62001936.50
Lane 3	20	24491129.01	26022630.49
Lane 4	6	729352.86	634876.61
Lane 5	6	6144178.00	1089152.23
Lane 6	6	6627613.66	7762363.45
Lane 7	3	1864149.71	466446.16

Displays all results from all channels

Page 1 of 25

This option will produce a .pdf file that summarizes all of your analysis
From top selection pane, select "reports" → "analysis report"

Exporting analysis data- option 2

The screenshot shows the ImageQuant TL 1D v8.1 software interface. The main window displays a gel image with 14 lanes. A dropdown menu is open, showing options like 'Copy to Clipboard', 'Export to File...', and 'Export to Excel'. The 'Export to Excel' option is highlighted. The 'Export to Excel' submenu is also visible, showing options like 'Export Lane Objects...', 'Import Lane Objects...', 'Export Lane Profile to Clipboard', and 'Export Lane Profile to File...'. The 'Export Lane Profile to File...' option is selected. The 'Export to Excel' submenu is also visible, showing options like 'Export Lane Objects...', 'Import Lane Objects...', 'Export Lane Profile to Clipboard', and 'Export Lane Profile to File...'. The 'Export Lane Profile to File...' option is selected. The 'Export to Excel' submenu is also visible, showing options like 'Export Lane Objects...', 'Import Lane Objects...', 'Export Lane Profile to Clipboard', and 'Export Lane Profile to File...'. The 'Export Lane Profile to File...' option is selected.

The 'Measurements Window [All Lanes] - channel 2' table is shown below the gel image. The table has columns for Volume, Calib Vol(log), Peak Height, Area, Band %, Band % (Norm), and MW (kd) for each of the 14 lanes. A red circle highlights the 'All Lanes' option in the 'Selected Lanes' dropdown at the bottom left of the table.

Lane 1							Lane 2							Lane 3						
Volume	Calib Vol(log)	Peak Height	Area	Band %	Band % (Norm)	MW (kd)	Volume	Calib Vol(log)	Peak Height	Area	Band %	Band % (Norm)	MW (kd)	Volume	Calib Vol(log)	Peak Height	Area	Band %	Band % (Norm)	MW (kd)
133917.00	29.96	1909.94	1668	3.06	3.14	243.286	607883.83	14.60	915.91	1238	2.30	2.49	234.1	182	172.1	1.88	113.1	1.26	88.1	
852028.02	15.26	1090.34	1008	1.42	1.80	172.108	338723.50	7.62	580.03	840	1.38	1.82	172.1	182	172.1	1.88	113.1	1.26	88.1	
1366728.00	22.92	1408.02	1456	2.27	2.40	98.190	355339.00	7.66	653.88	896	1.45	1.88	113.1	182	172.1	1.88	113.1	1.26	88.1	
438882.40	9.11	854.90	728	0.73	0.95	88.148	181048.55	5.27	409.36	616	0.74	1.26	88.1	182	172.1	1.88	113.1	1.26	88.1	
231959.48	6.03	500.61	896	0.39	0.83	82.362	650463.43	12.26	750.52	1344	2.66	2.93	70.2	182	172.1	1.88	113.1	1.26	88.1	
359590.13	7.93	619.30	1120	0.60	0.83	80.128	194587.71	5.47	420.05	672	0.79	1.31	47.5	182	172.1	1.88	113.1	1.26	88.1	
2272371.00	36.40	2210.59	1624	3.77	3.82	70.256	222406.63	5.89	490.40	672	0.91	1.41	29.1	182	172.1	1.88	113.1	1.26	88.1	
423013.33	8.87	1040.51	616	0.70	0.93	52.623	2326471.10	40.00	1652.39	2576	9.50	9.55	28.2	182	172.1	1.88	113.1	1.26	88.1	
923734.06	16.33	1427.78	1288	1.53	1.71	30.069	705550.70	13.08	1113.08	1064	2.88	3.12	28.2	182	172.1	1.88	113.1	1.26	88.1	
591506.55	11.38	1143.24	860	0.98	1.19	28.295	159489.50	26.32	1989.91	1008	6.51	6.28	28.2	182	172.1	1.88	113.1	1.26	88.1	
3297053.39	50.00	3312.48	1624	5.48	5.24	28.295	781921.17	14.21	1464.19	560	3.19	3.39	27.1	182	172.1	1.88	113.1	1.26	88.1	
619875.50	11.80	1149.54	1008	1.03	1.24	28.295	1145313.60	19.62	1987.94	672	4.68	4.69	24.7	182	172.1	1.88	113.1	1.26	88.1	
3248227.43	50.93	3310.69	1792	5.40	5.34	28.295	3268525.23	51.23	2181.47	2072	13.35	12.23	22.2	182	172.1	1.88	113.1	1.26	88.1	

Data tables must be exported in "single channel" view

- Step 1- select "all lanes" on data display options
- Step 2- from top selection pane, select "edit" → "export to excel"
- Step 3- you can also export images to excel file by clicking on gel or intensity profile and selecting "edit" → "copy to clipboard" then open the excel file and select "edit" → "paste"



imagination at work