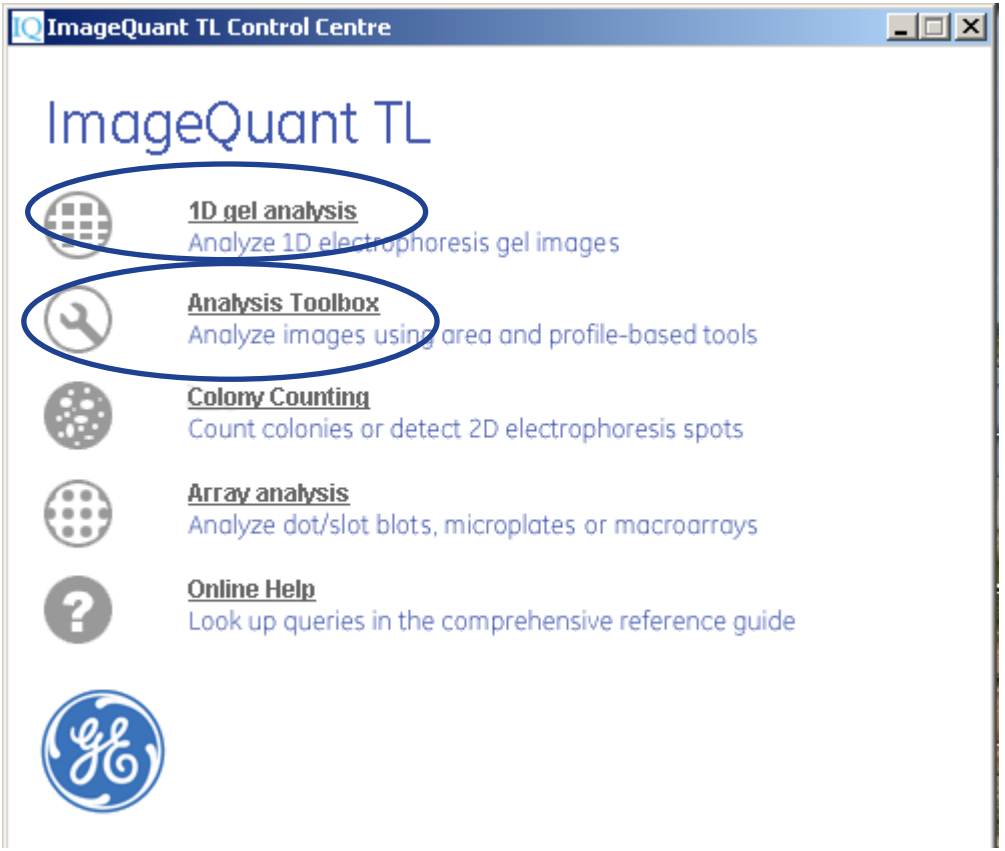
Overlay and Analysis of Multiplexed Images

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



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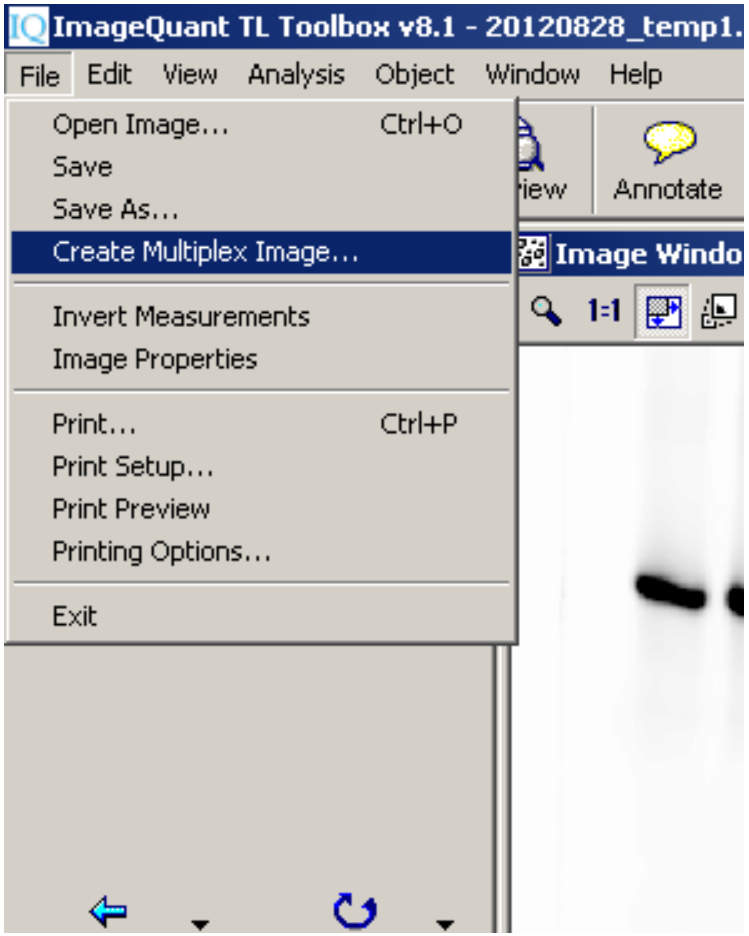
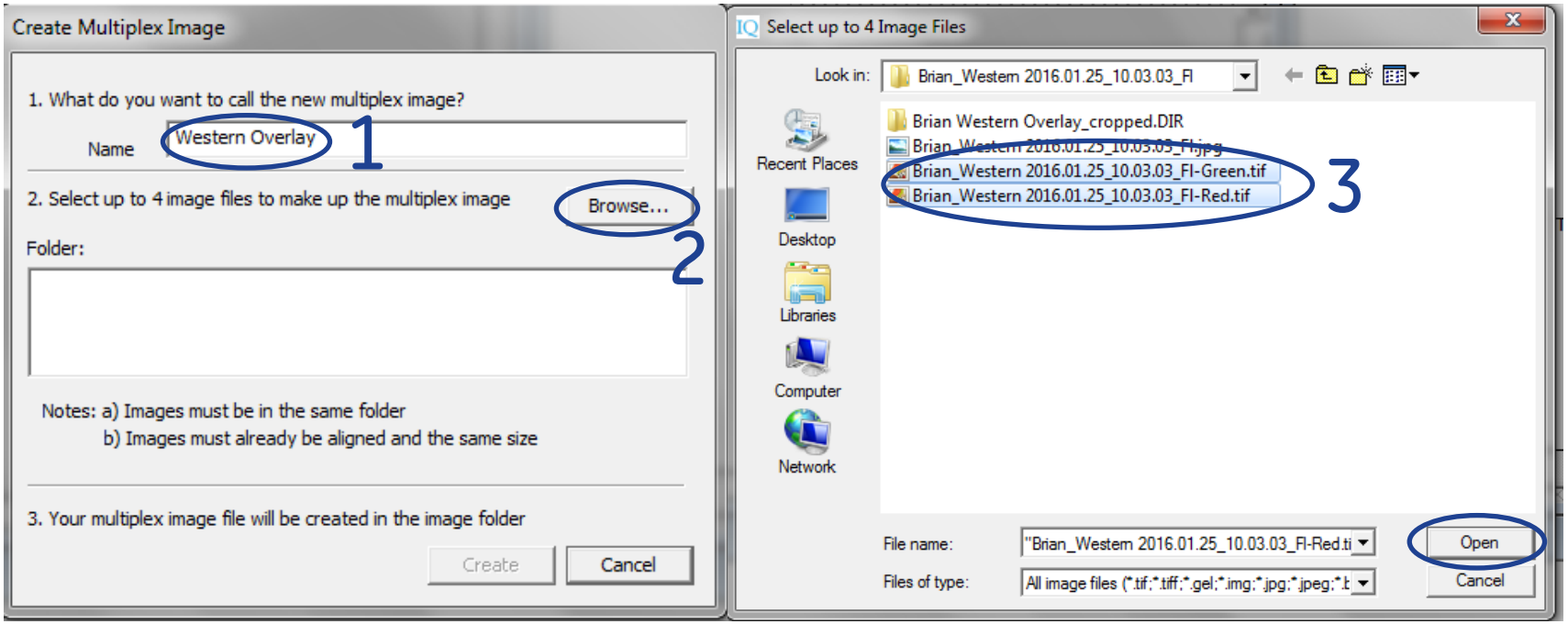
Create multiplex image

From dropdown select "file"⟶"create multiplex image" ⟶



Select images to overlay



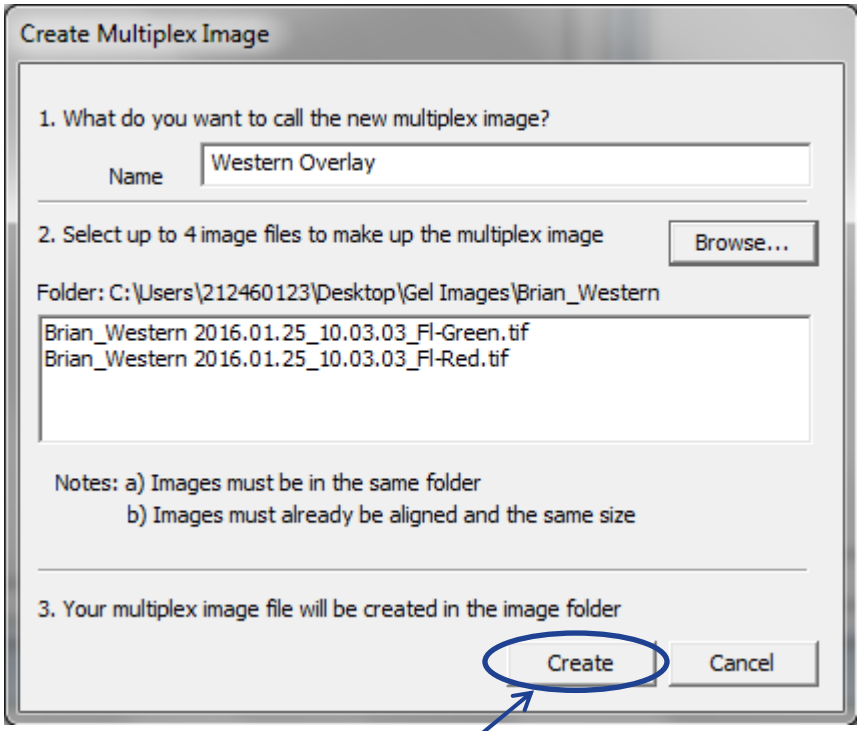
Step 1 – name new file

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

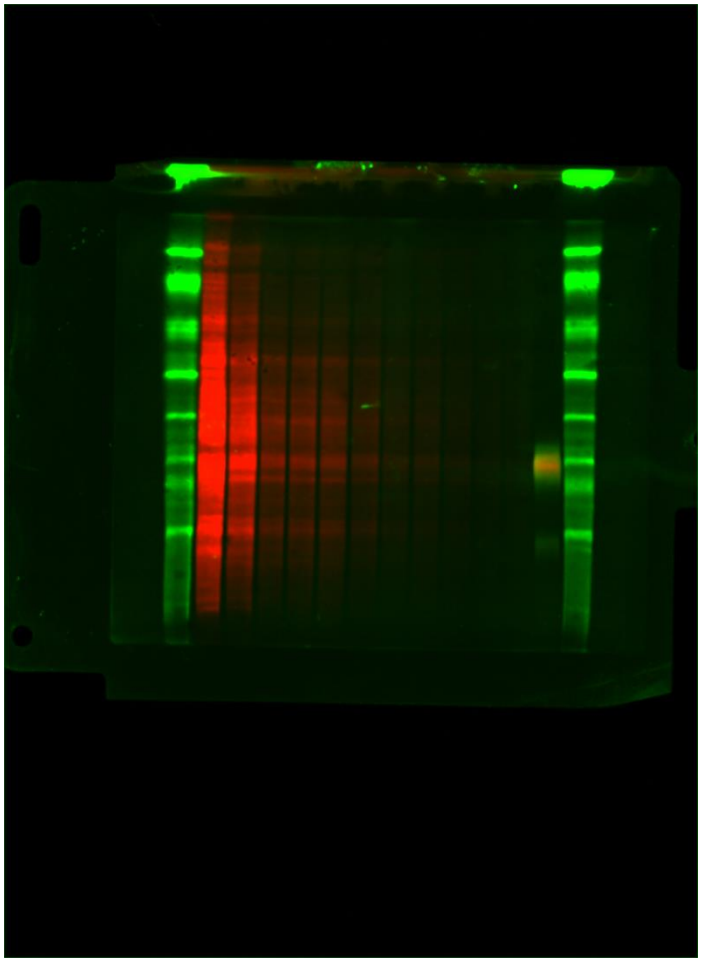
Step 3 - select associated digitized images (separate channels)

Step 4 – select “open”

Create overlay

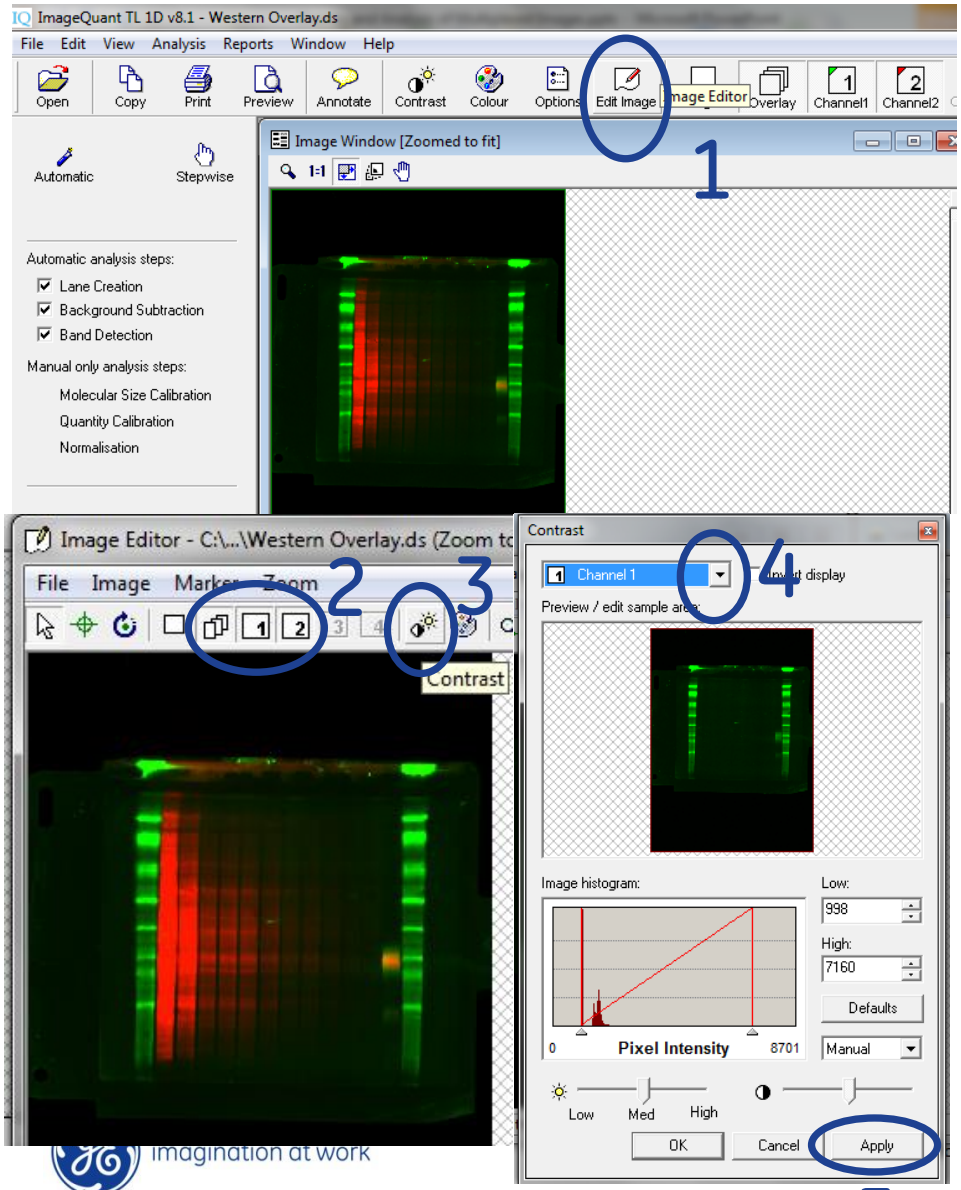


Select “create”



Multiplex image displayed in IQTL

Adjust brightness and contrast



Step 1- select “edit image” icon

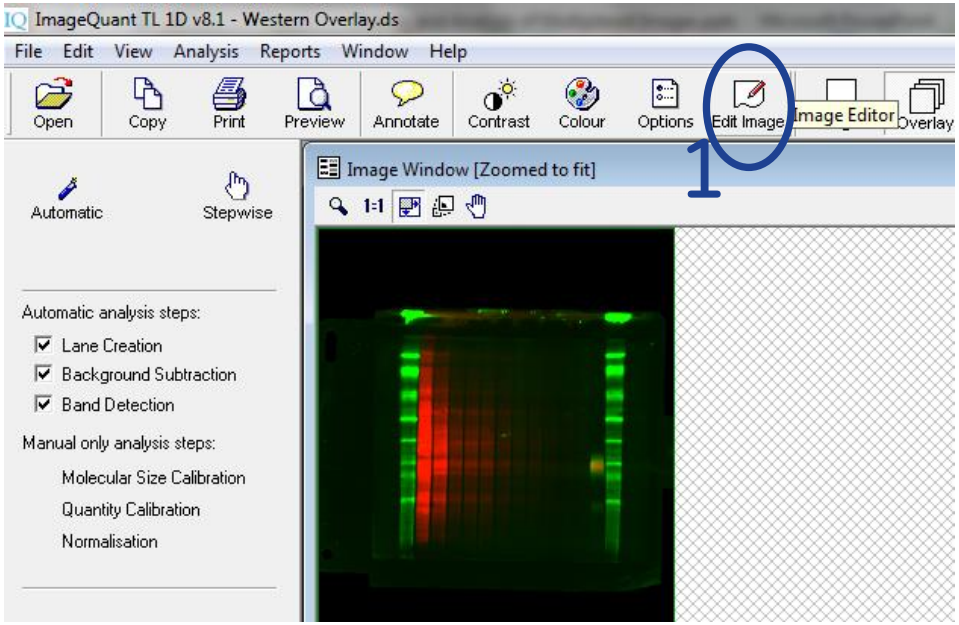
Step 2- select “overlay” with all cannels selected

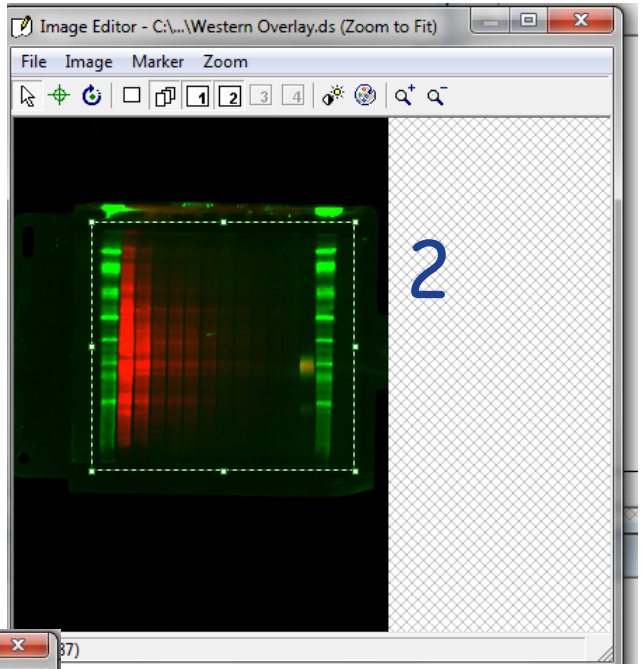
Step 3- select “contrast” icon

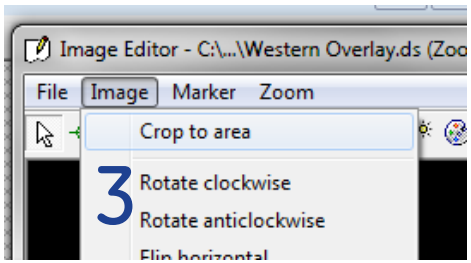
Step 4- select each channel from drop down menu and adjust each channel individually

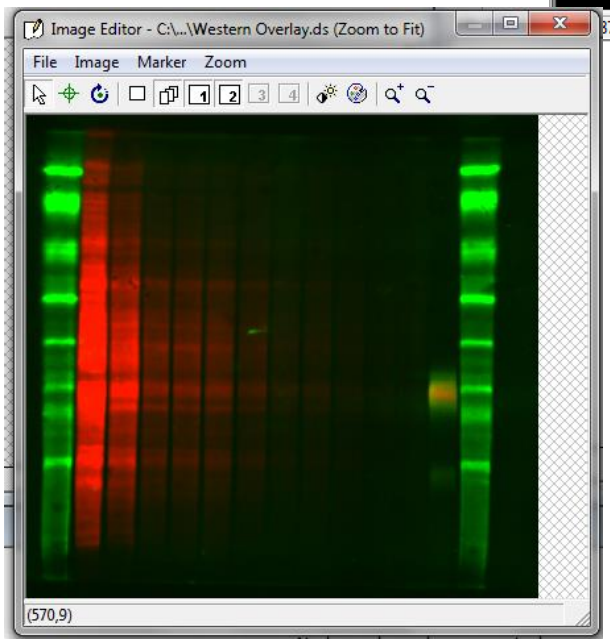
Step 5- select “apply” for each channel

Crop overlaid image









Step 1- select “edit image” icon

Step 2- create box around region of interest

Step 3- select “image” ⟶ “crop to area”

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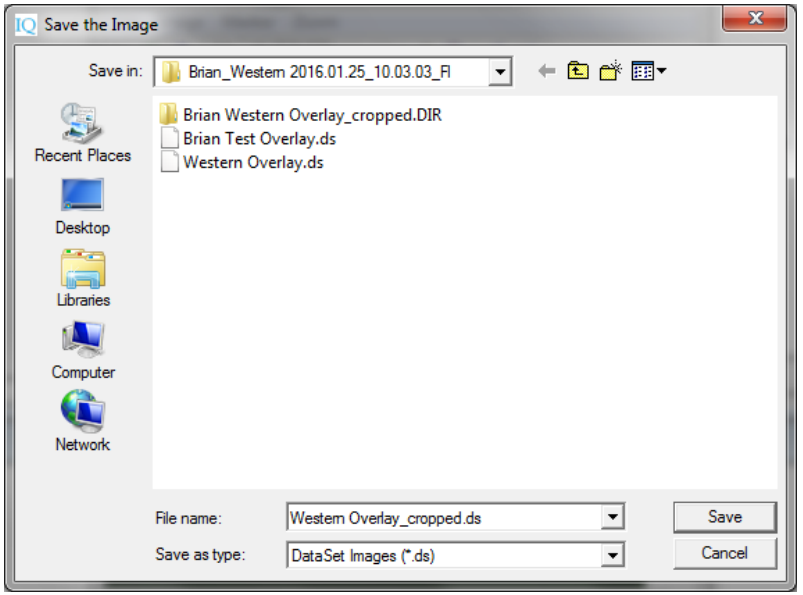
Save cropped image as new file

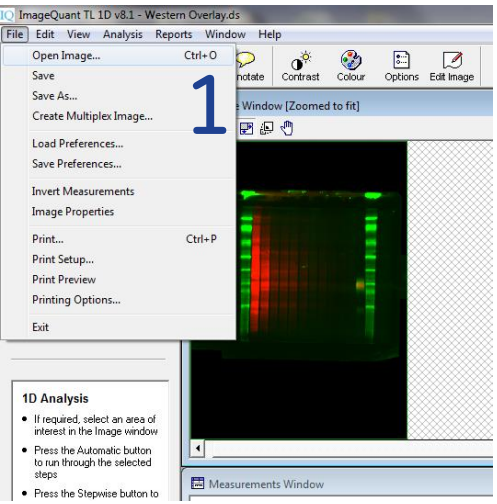


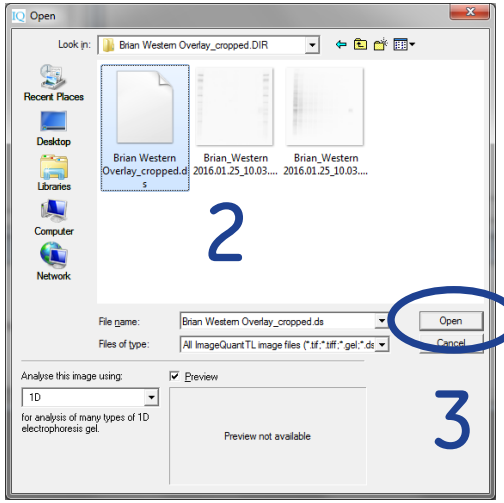
Select “file” → “save as” in the “image editor” window

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





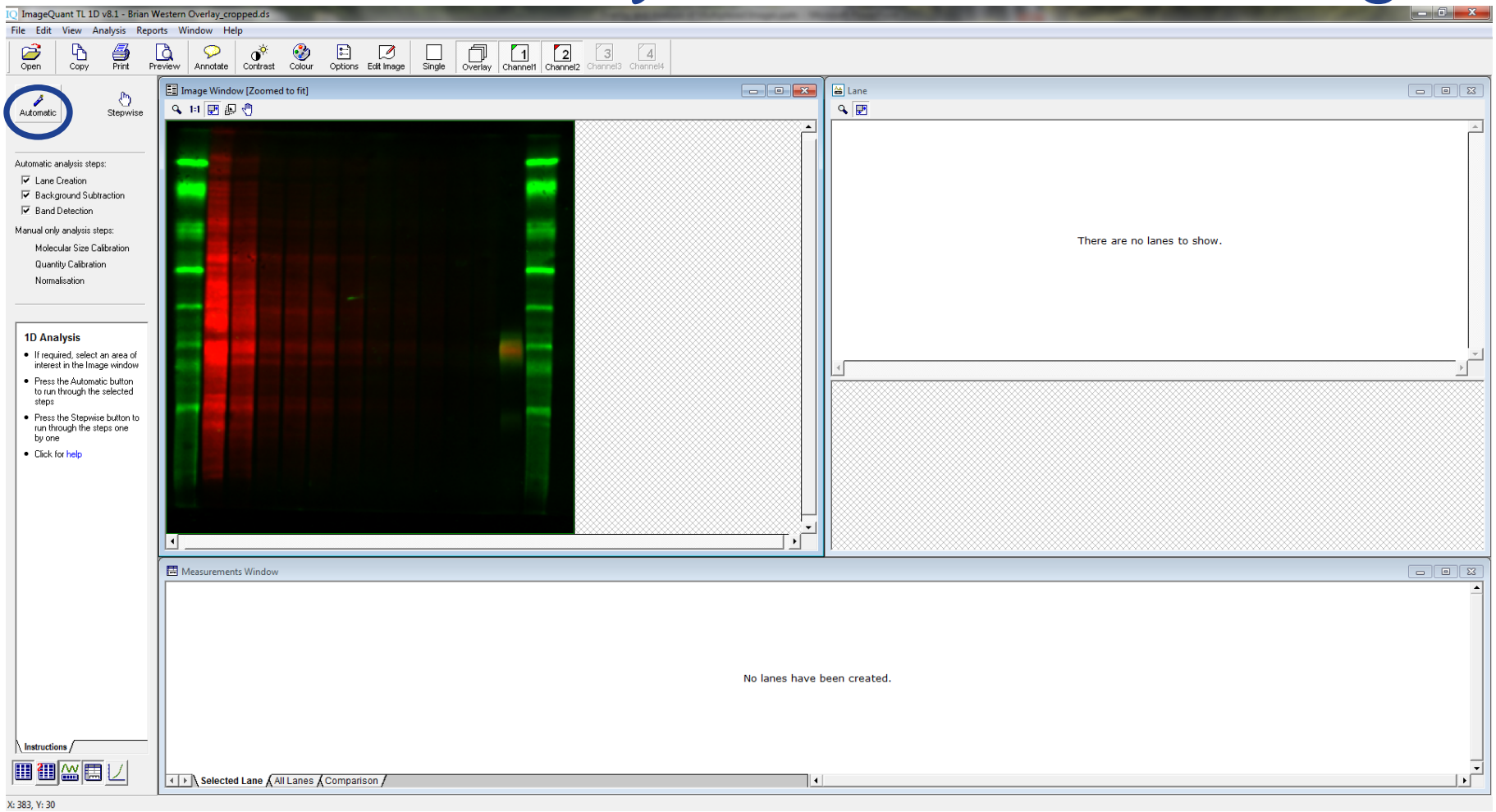
Step 1- select “file”→“open image”

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

Step 3- select “open” to open overlaid image in IQTL

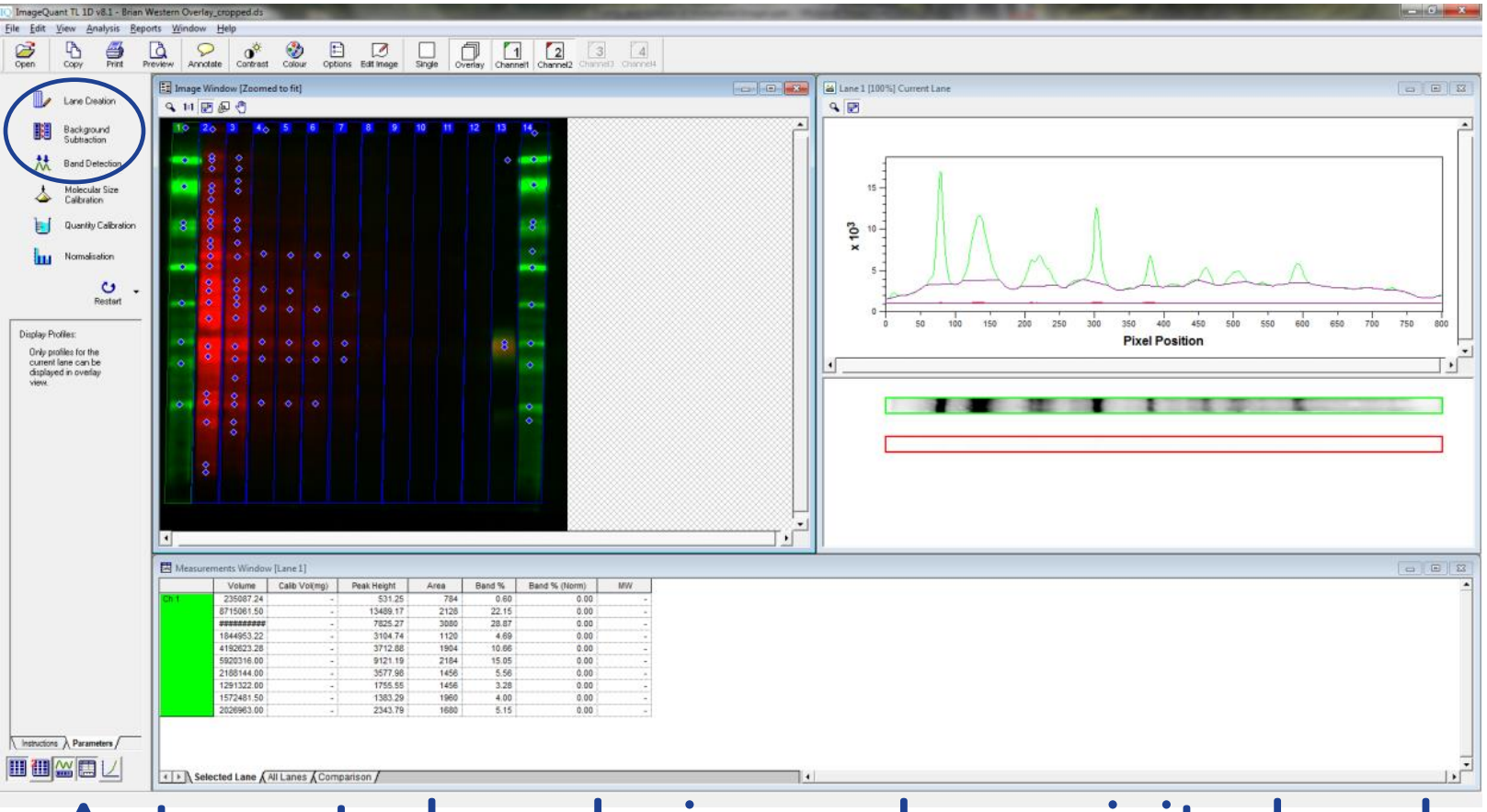
Step 4- you may not toggle the display of each channel using the “channel icons”

Automated analysis of overlaid image



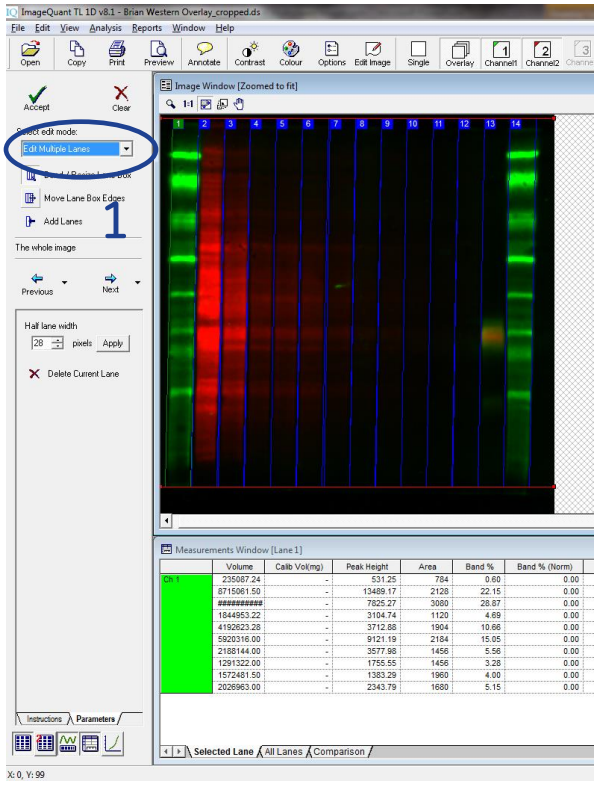
Using the overlaid image, select “automatic” for lane creation, background subtraction and band detection

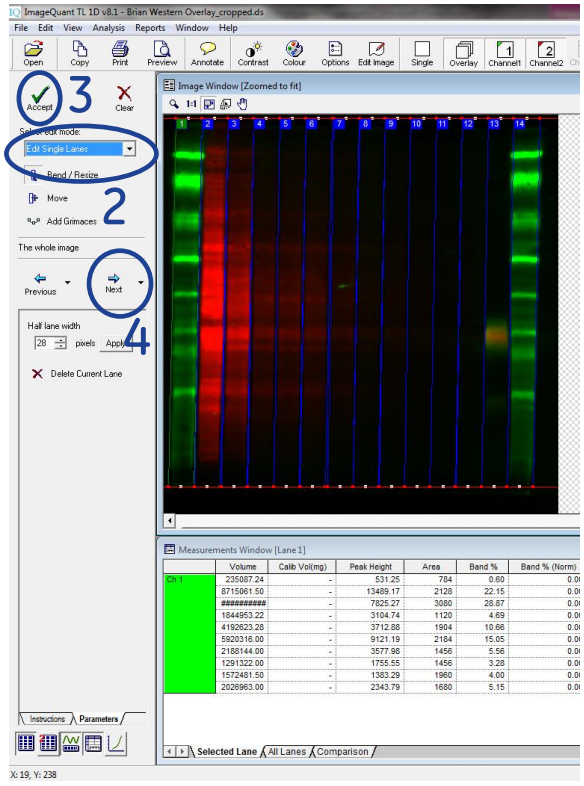
Fine tuning of automated analysis



Automated analysis can be revisited and modified using the respective icons

Lane creation on overlaid image





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

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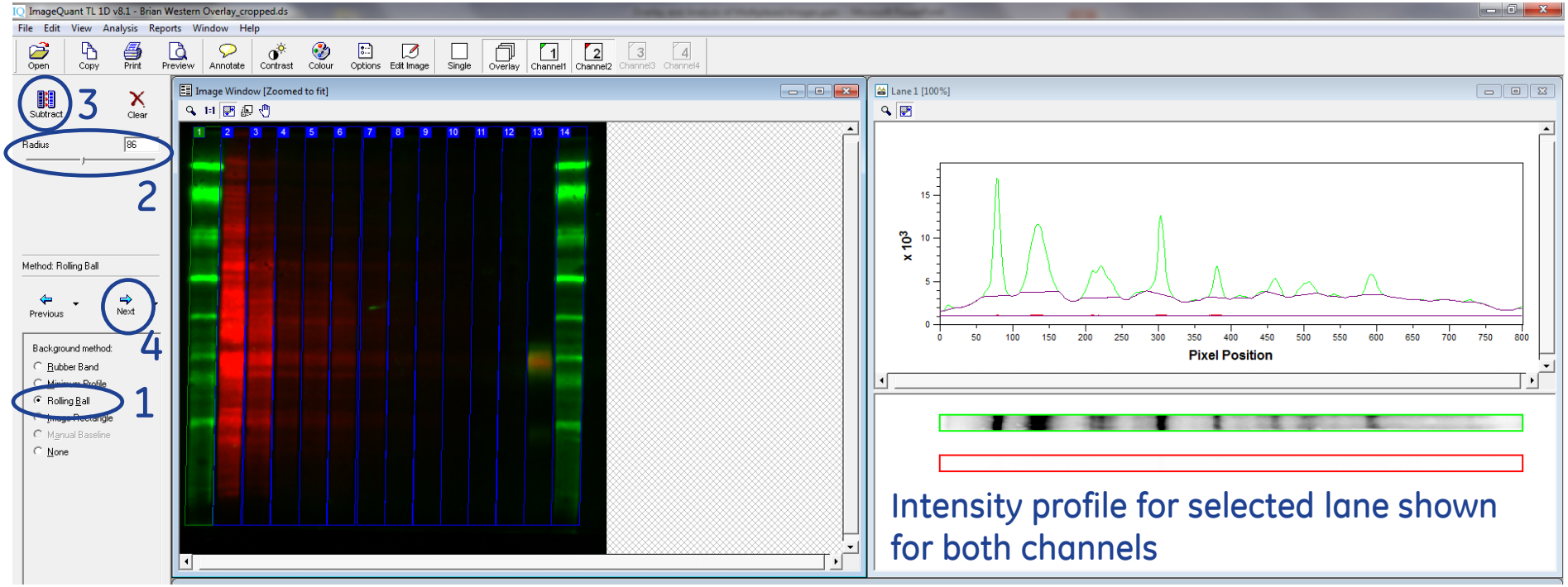
Background subtraction on overlaid image



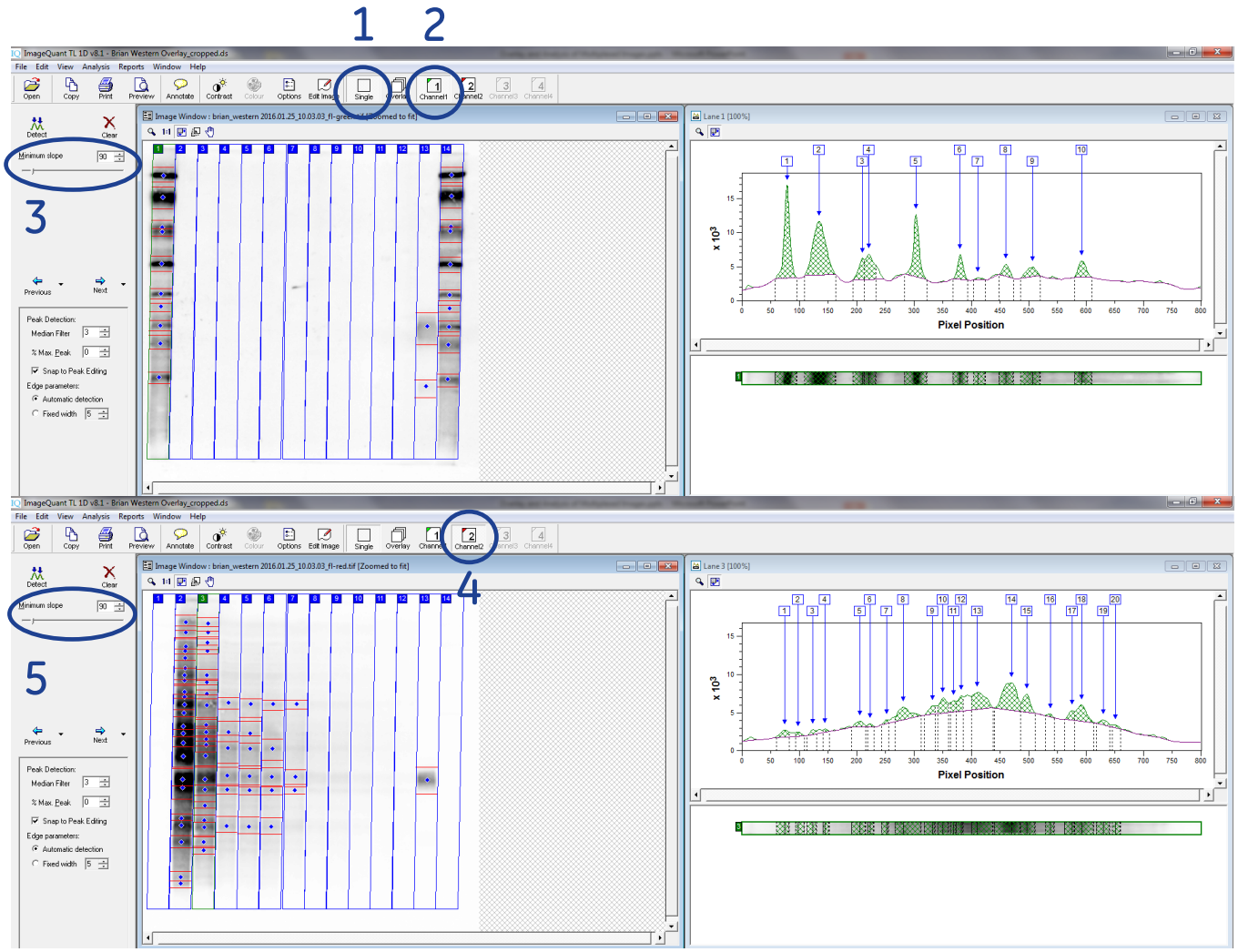
Step 1- “rolling ball” is recommended for quantifiable background subtraction

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



Must detect bands in each channel individually

Step 1- select “single channel view”

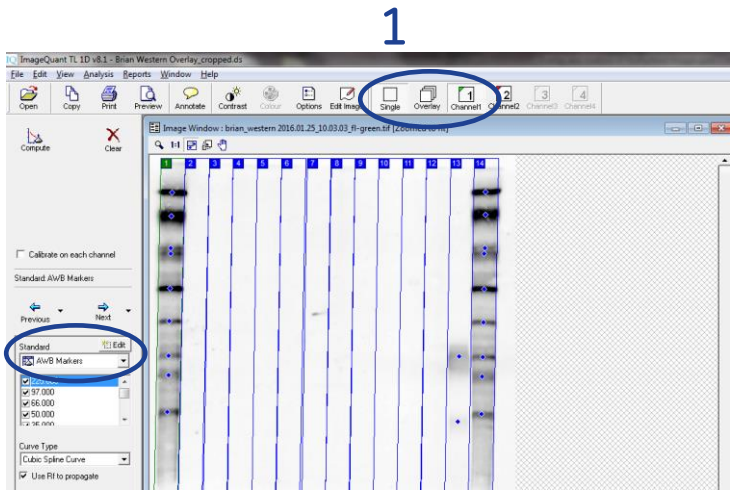
Step 2- select “channel 1”

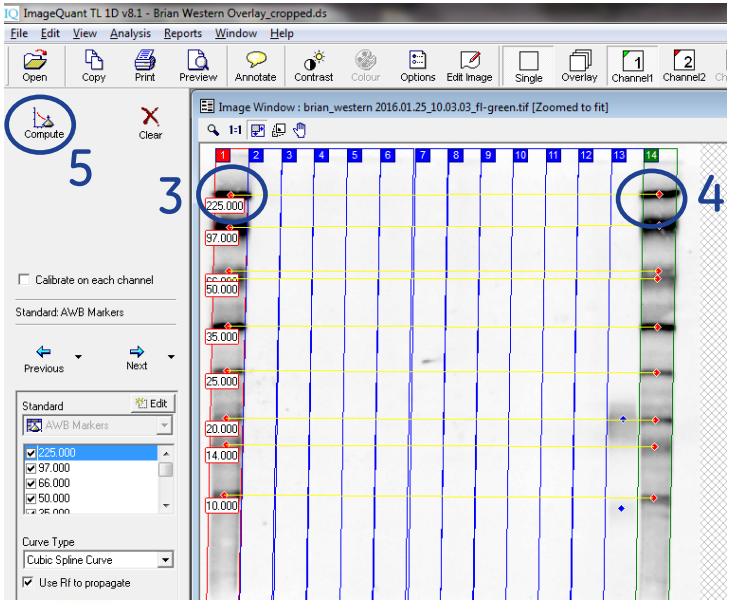
Step 3- adjust “minimum slope” and/or manually select/deselect bands

Step 4- select “channel 2”

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

Molecular weight calibration on overlaid image





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

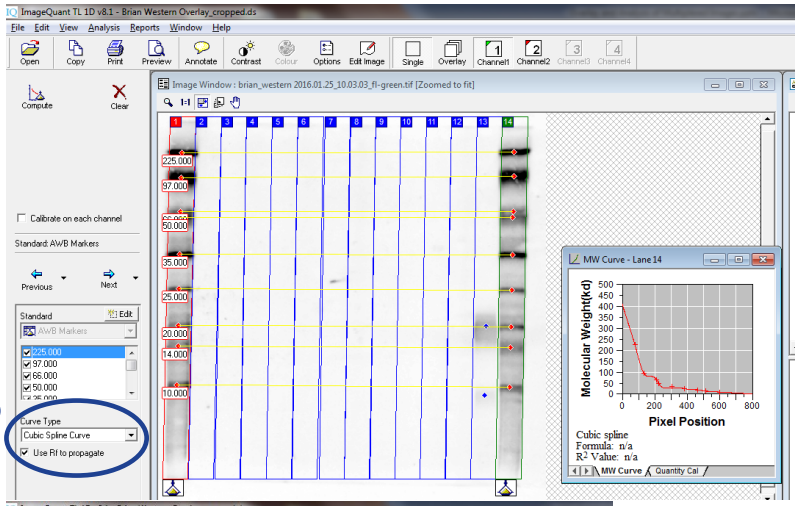
Step 2- select appropriate mol wt markers from dropdown

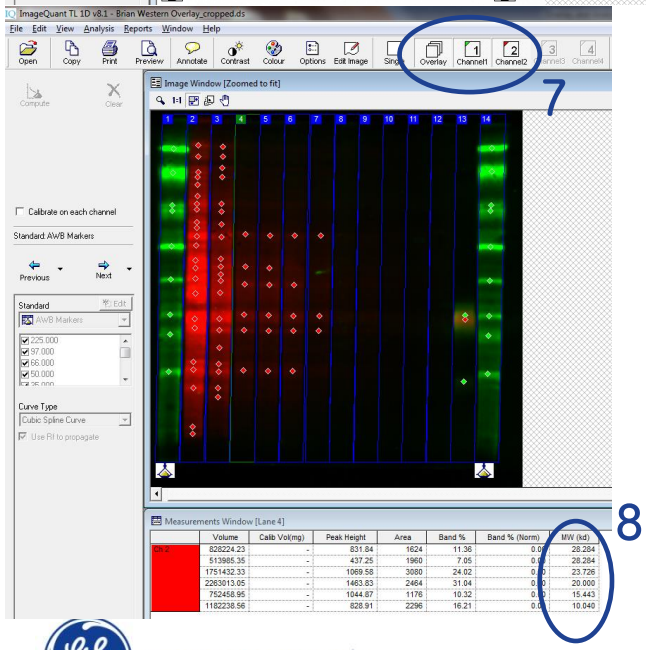
Step 3- select highest mw band from ladder lane

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…



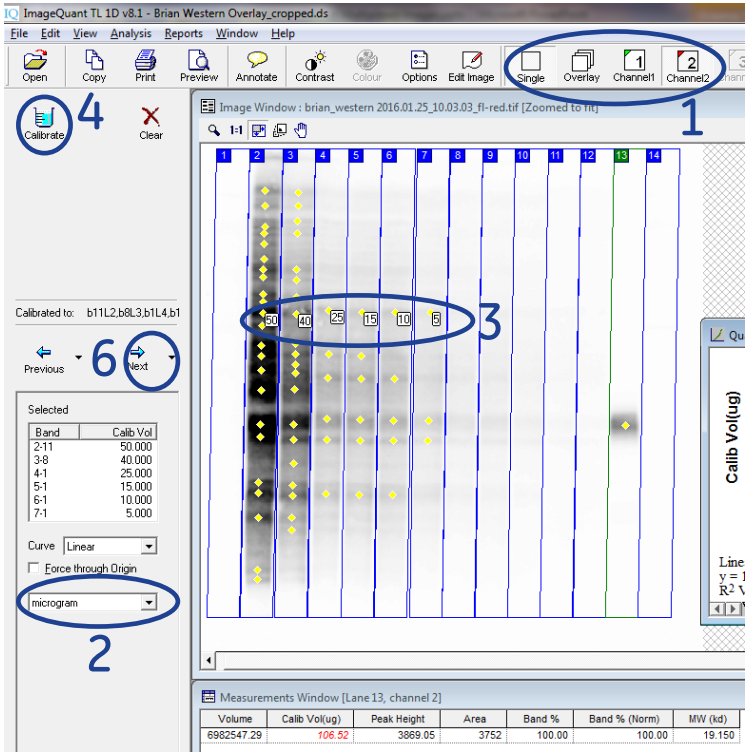


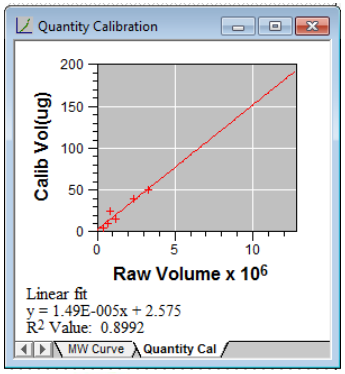
Step 6- select “curve type” from dropdown menu
MW calibration curve will show curve fit

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”

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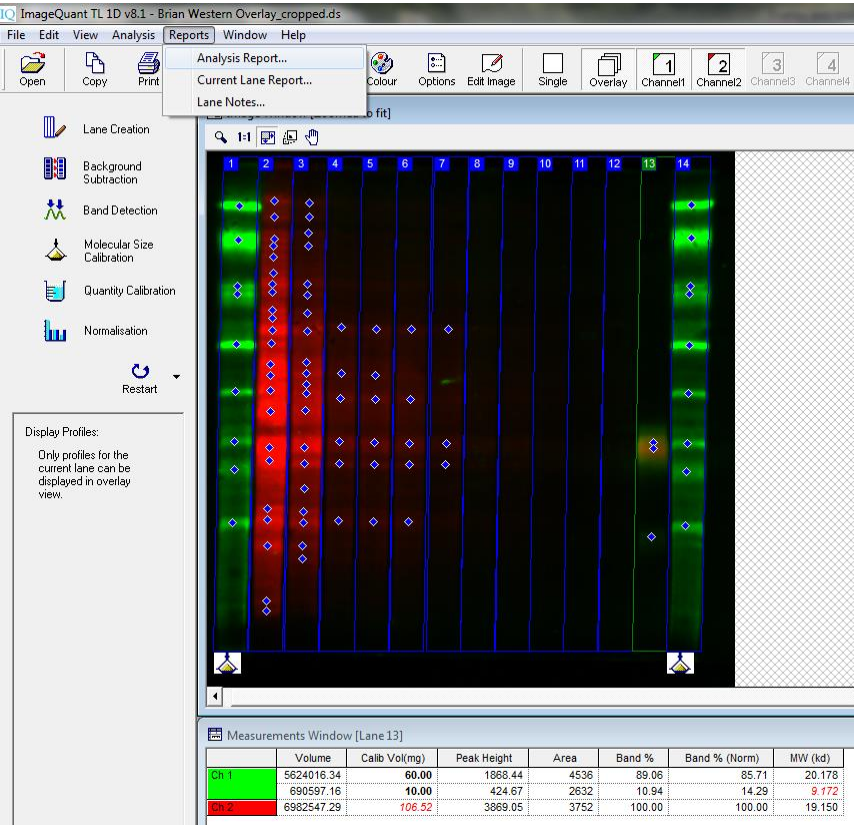
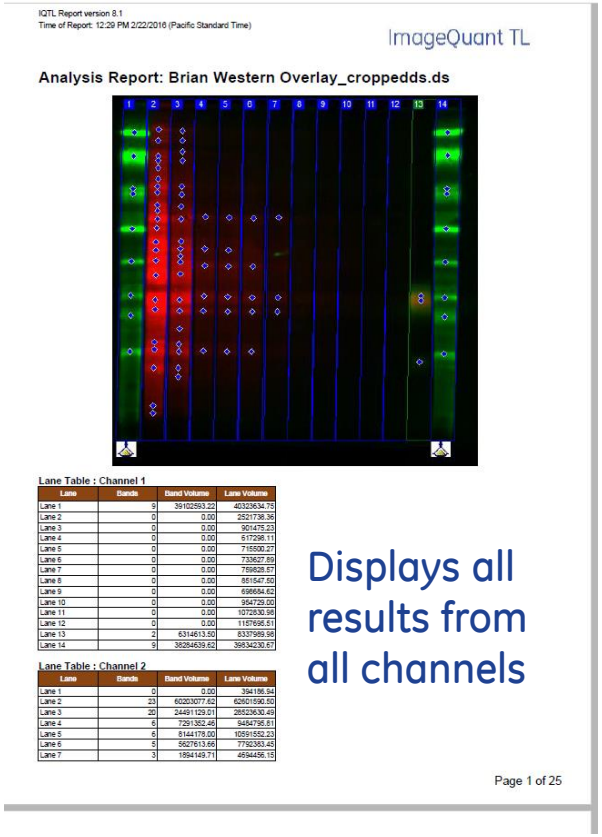
Exporting analysis data- option 1

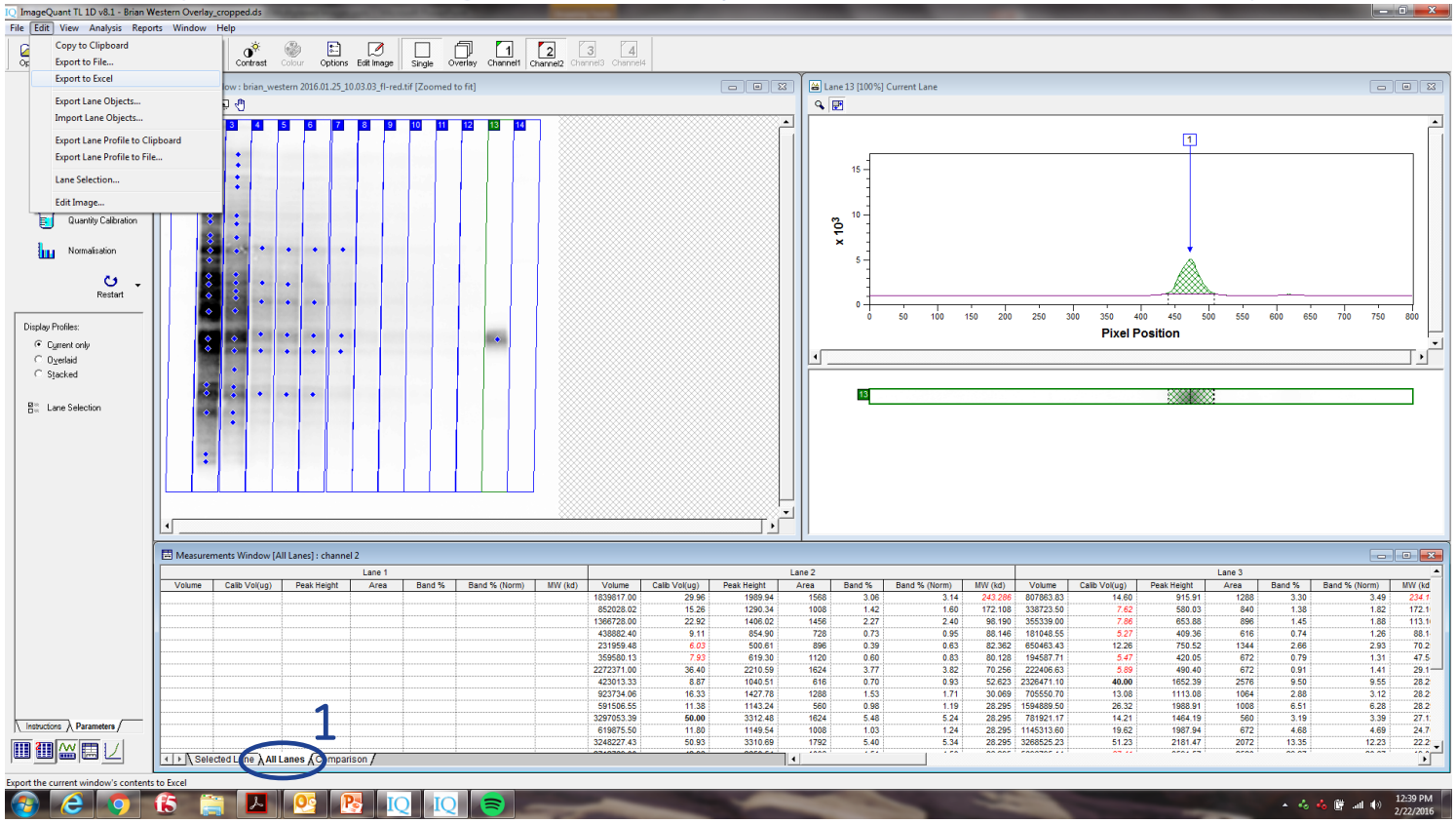




Displays all results from all channels

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



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”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