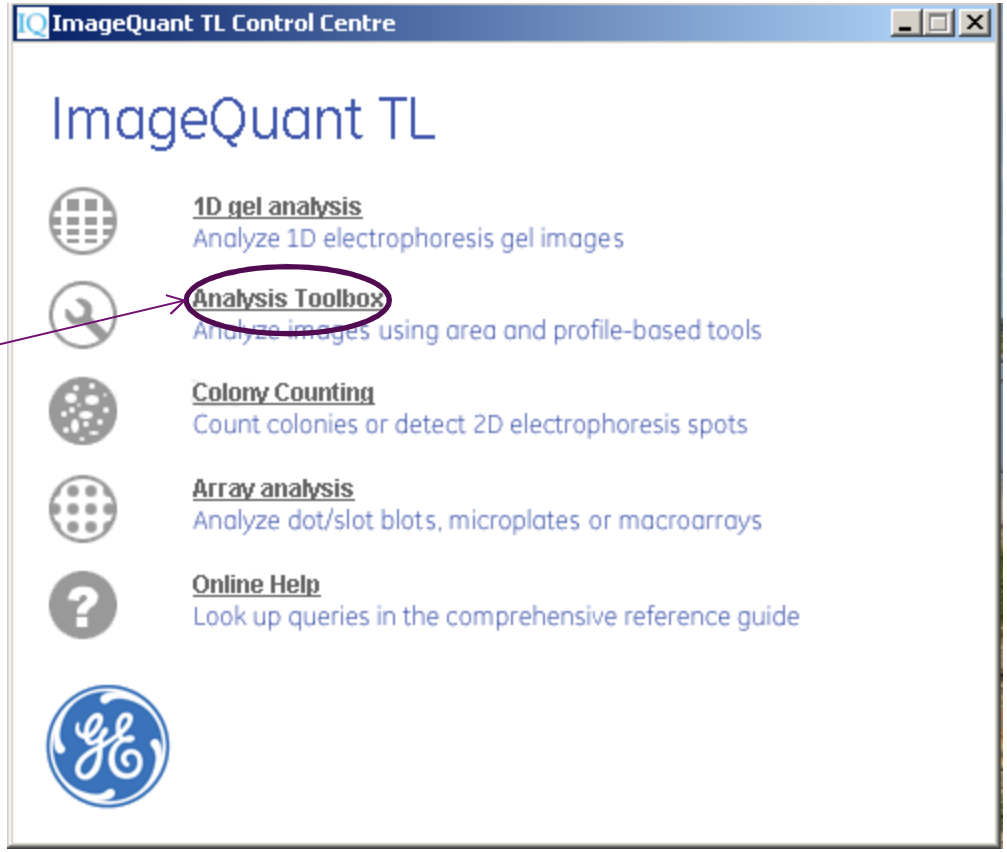
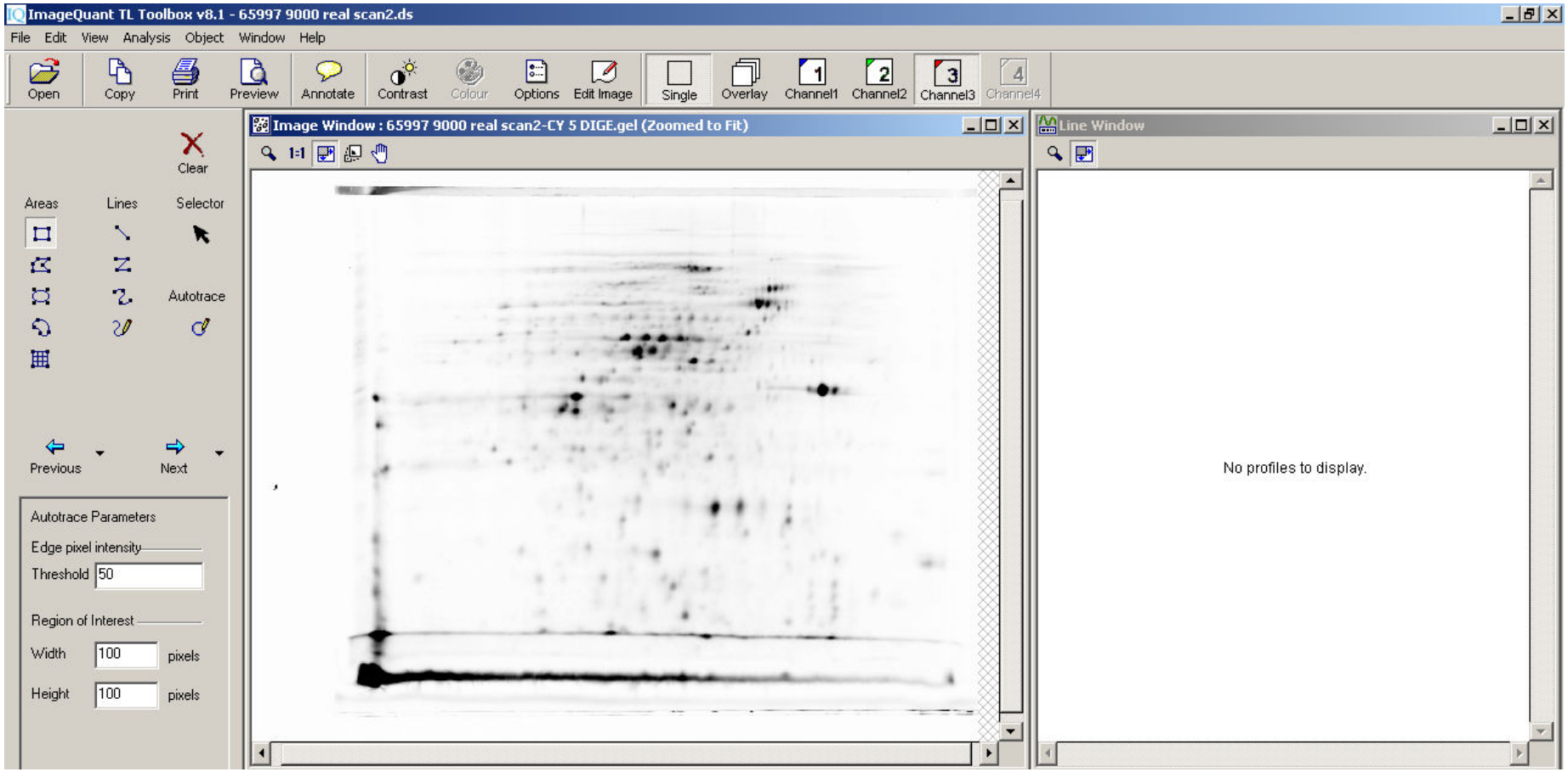
IQ TL – Finding Saturation…

Open analysis toolbox



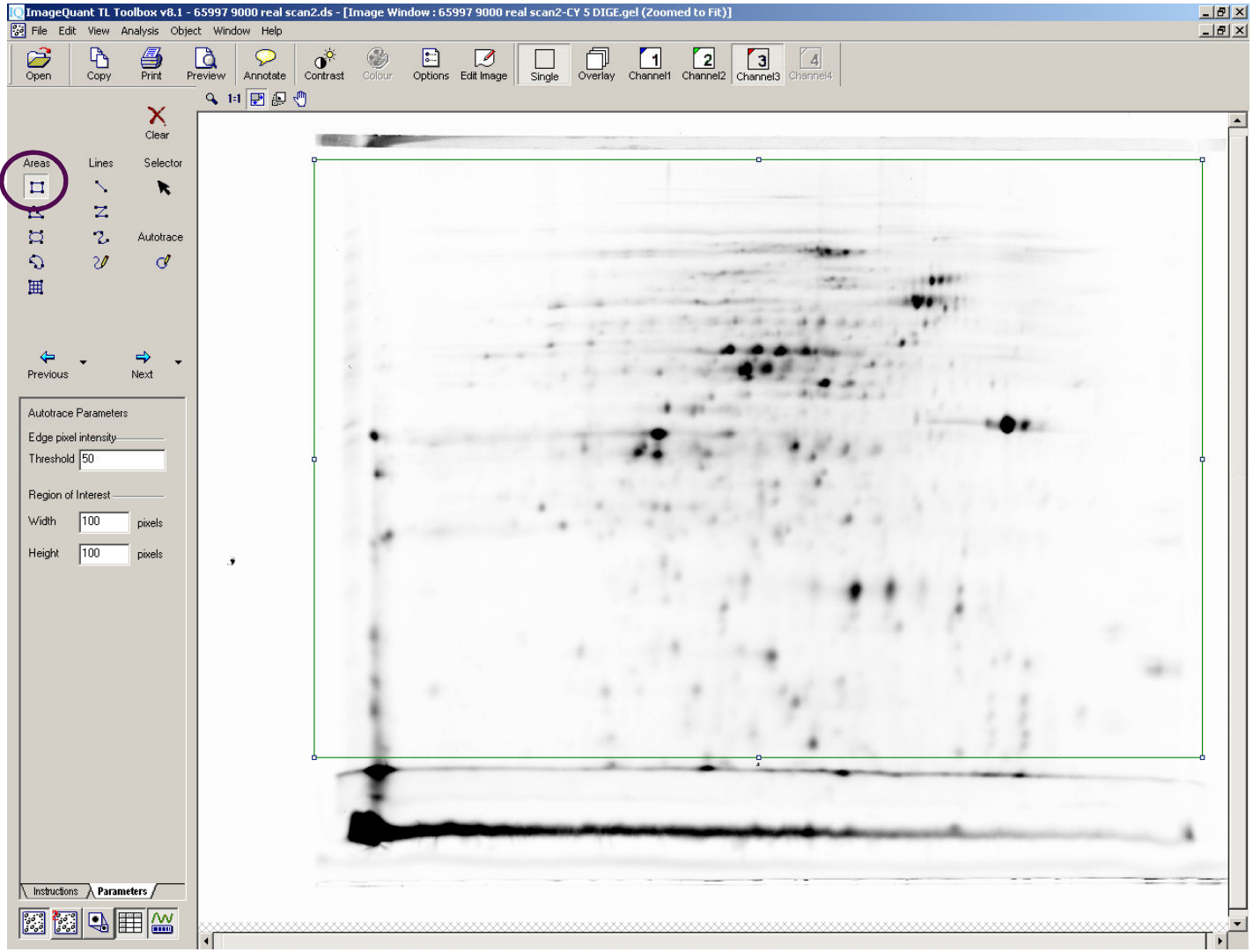
Open image of interest



Quick look

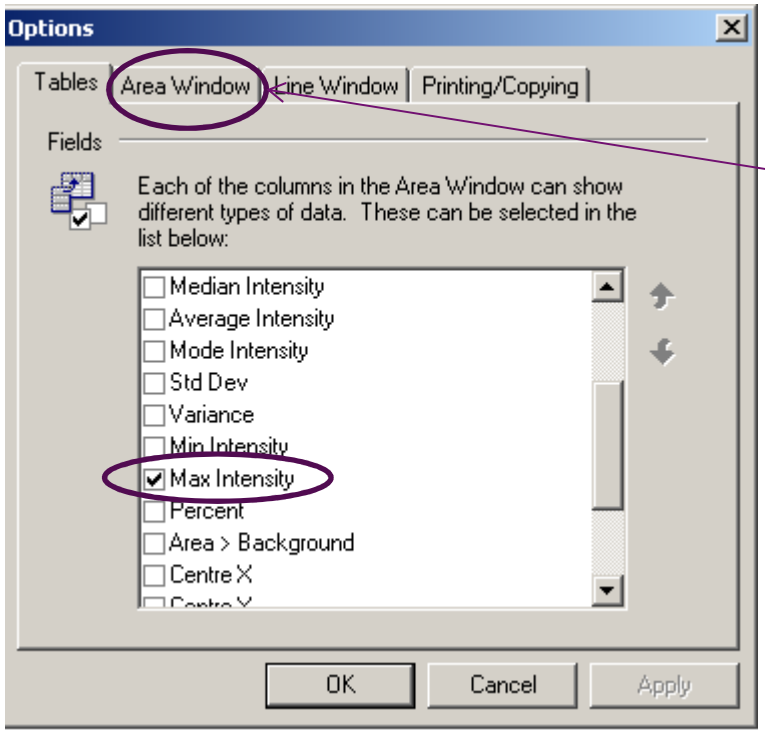
Draw box over area of interest (avoid “dye front”, reference markers, etc.) – usually better to work with the “cropped” image (not shown)

Use “area” tool



imagination at work

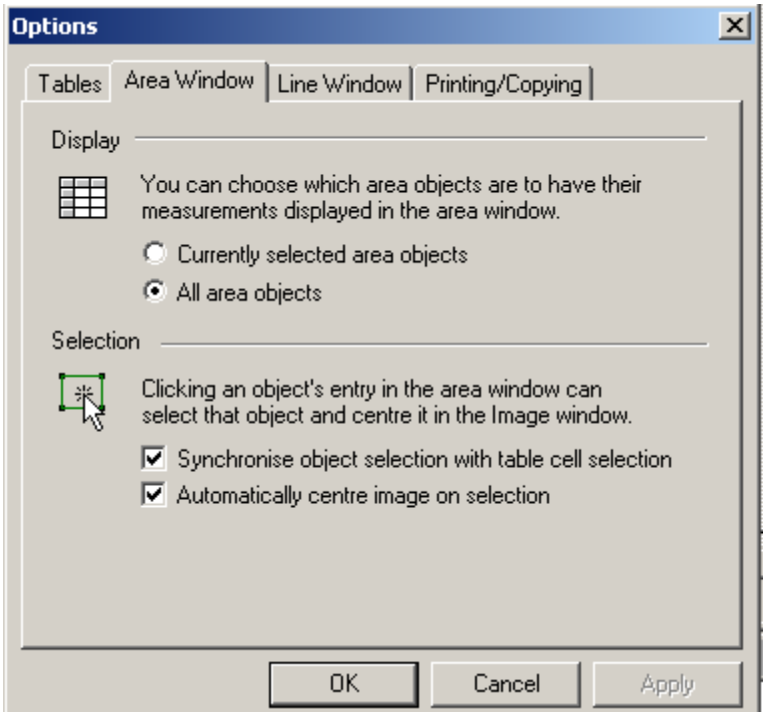
From View dropdown make sure “maximum intensity” is selected as one of parameters for “table view”



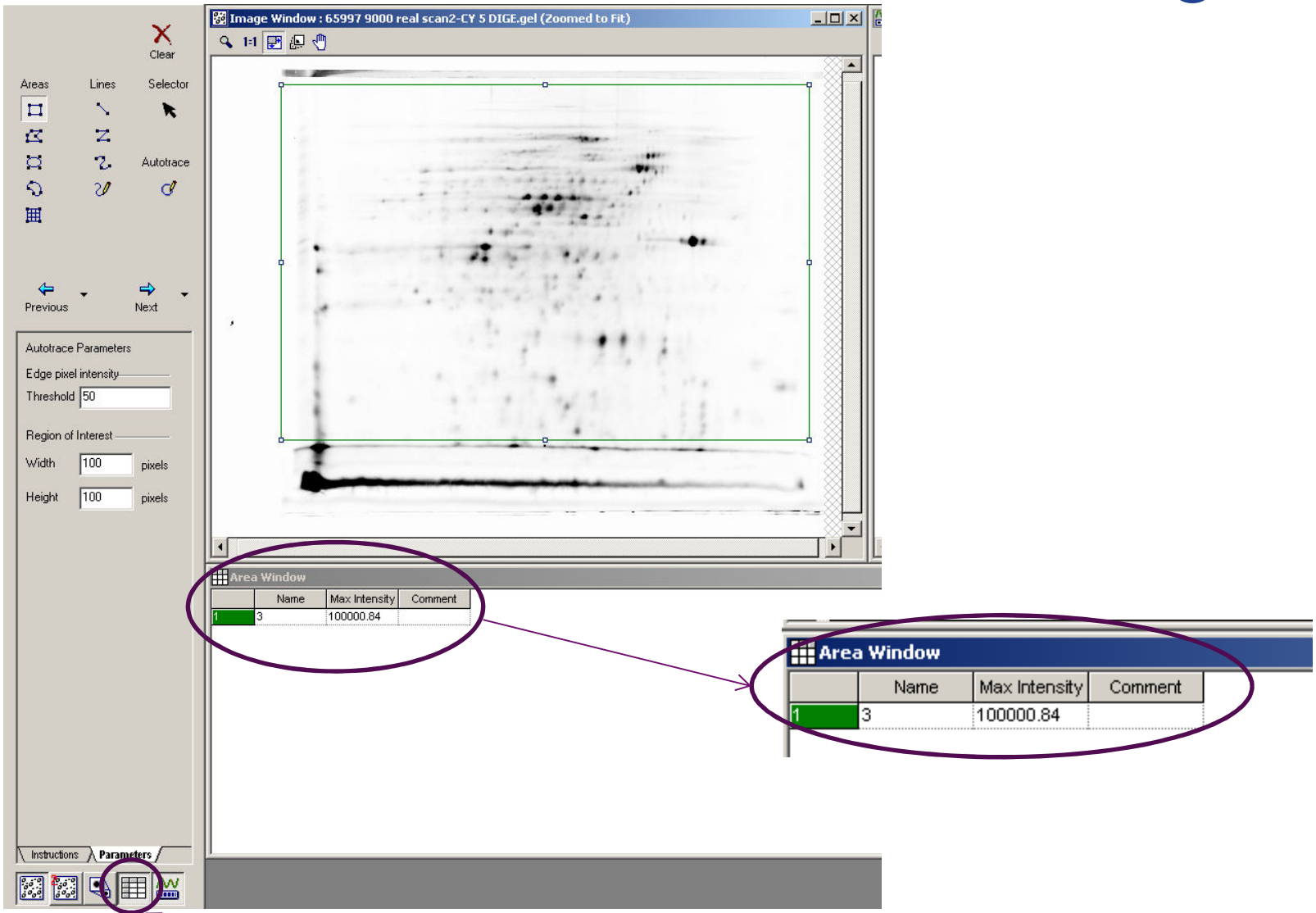
See next slide!

You may wish to display other parameters as well!

Also make sure “area window” displays “all area objects”



View “area window” box below image



Toggles area window display

Saturation!

Depending on the type of instrument and image file used the saturation point can be reached at different levels

Saturation level:
With a 16 bit .tif this is usually displayed as approx. 65,500 and with a 16 bit .gel as approx. 100,000 (N.B. some 16 bit .gel files only have the approx. 65,500 gray levels)

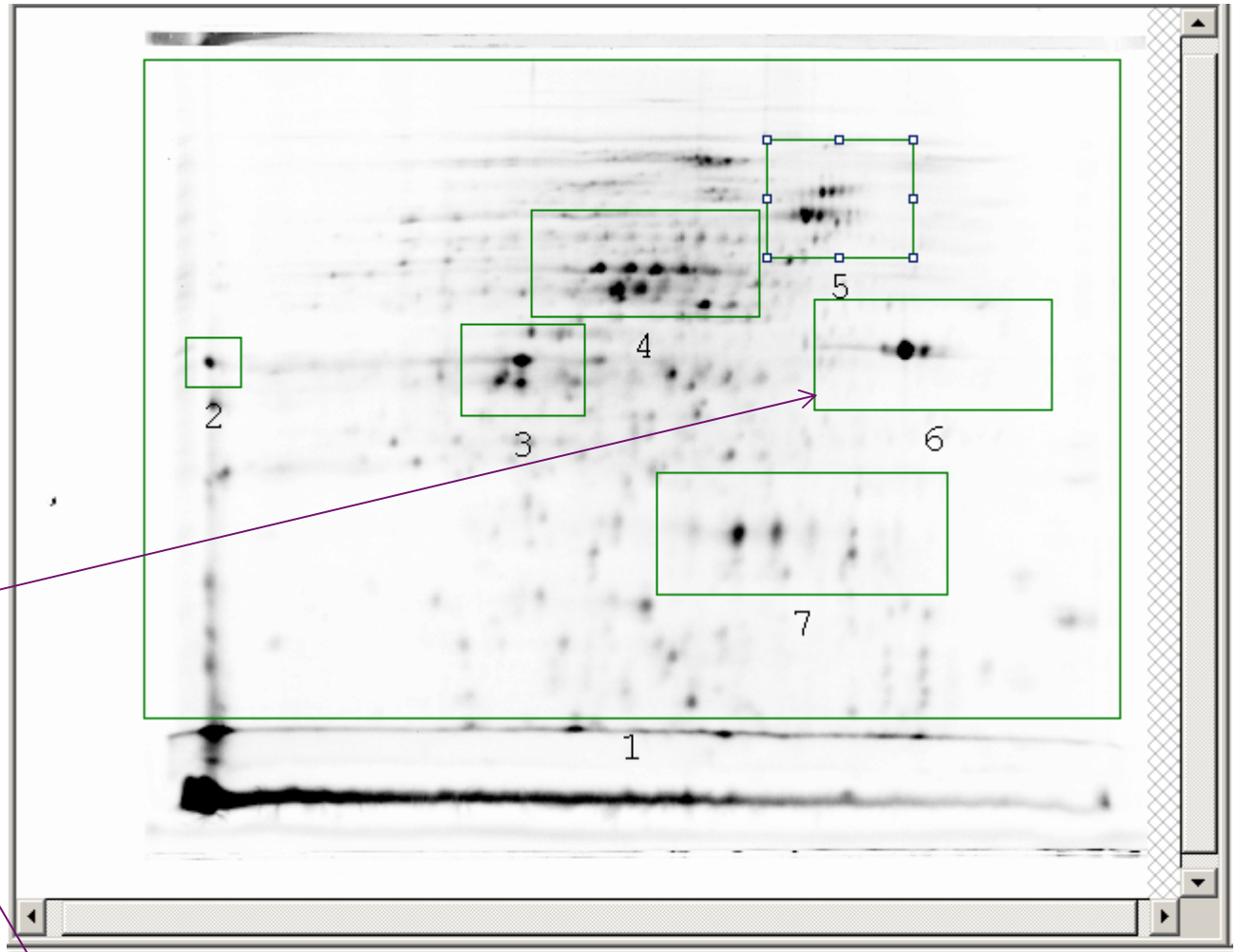
Where is the saturated signal in image ?

In previous image the maximum intensity is approx. 100,000 so we know we have a saturated feature.

One approach is to draw multiple boxes on the image, covering suspected features that appear to be the darkest on the image – (not very efficient)

Approach 1

Box 6 has the saturated feature



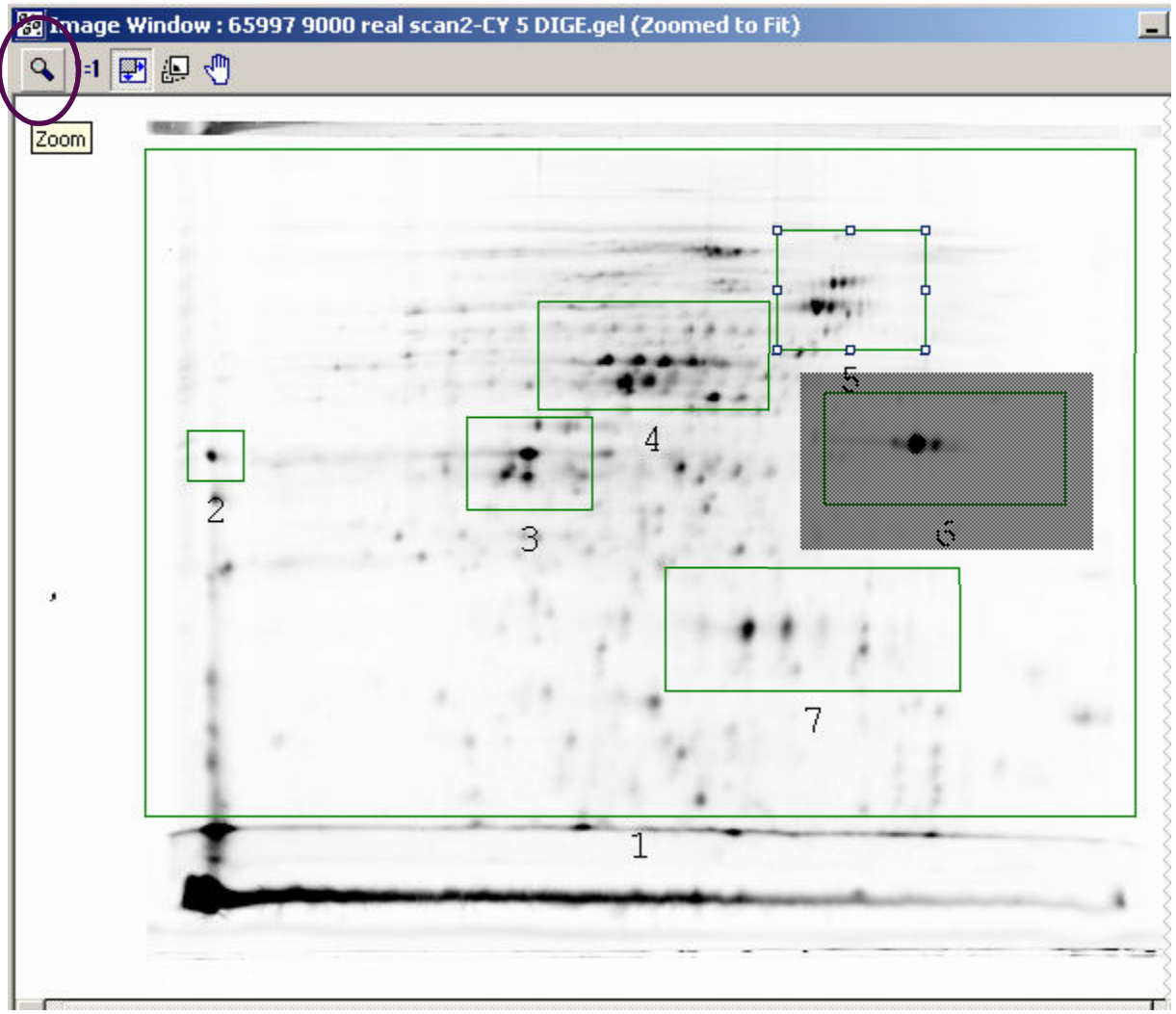
Area Window

	Name	Max Intensity	Comment
1	1	100000.84	
2	2	14212.22	
3	3	27927.84	
4	4	24069.83	
5	5	27765.18	
6	6	100000.84	
7	7	11475.27	

imagination at work

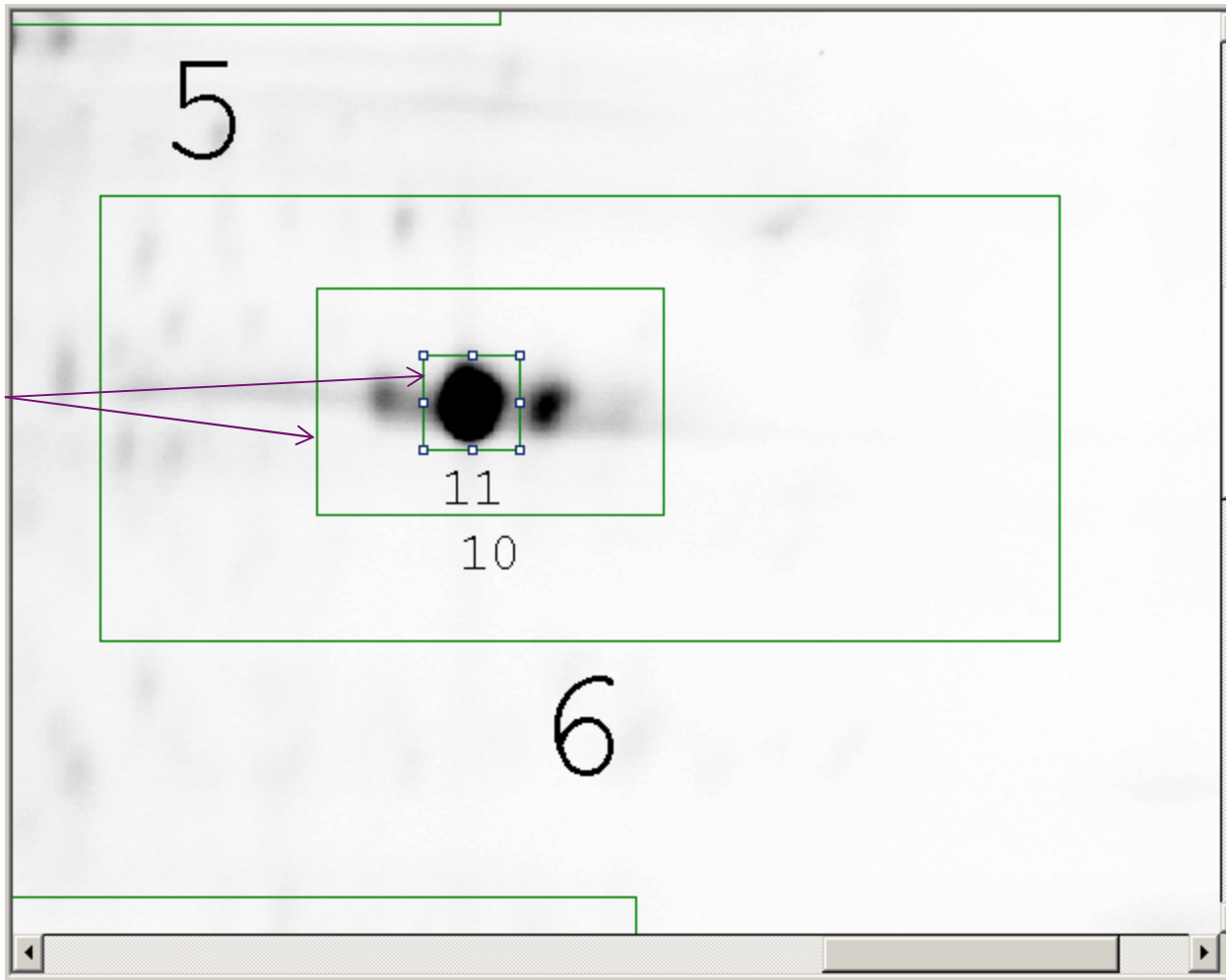
Use “zoom” tool to draw around “box 6”

Engage zoom tool



Confirm!

Draw further boxes to confirm saturated feature rather than an artifact (e.g. dust spike)



Area Window

	Name	Max Intensity	Comment
1	1	100000.84	
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4	4	24069.83	
5	5	27765.18	
6	6	100000.84	
7	7	11475.27	
8	10	100000.84	
9	11	100000.84	

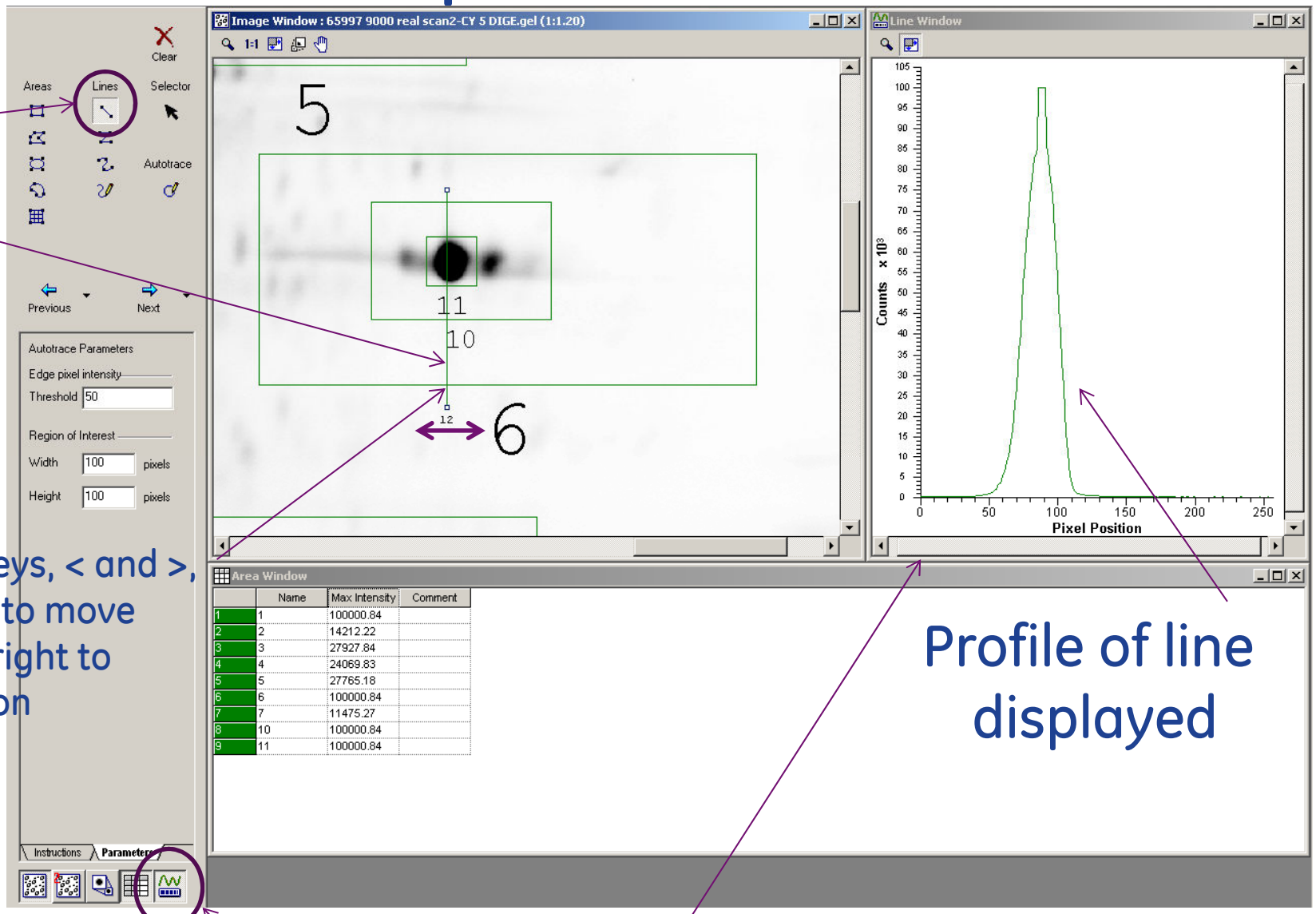
N.B. Further zooming in can be advantageous!

imagination at work

Show saturated peak!

Use draw line function to draw line

Use cursor keys, < and >, on keyboard to move line left and right to find saturation maximum



Profile of line displayed

Toggles "line window"

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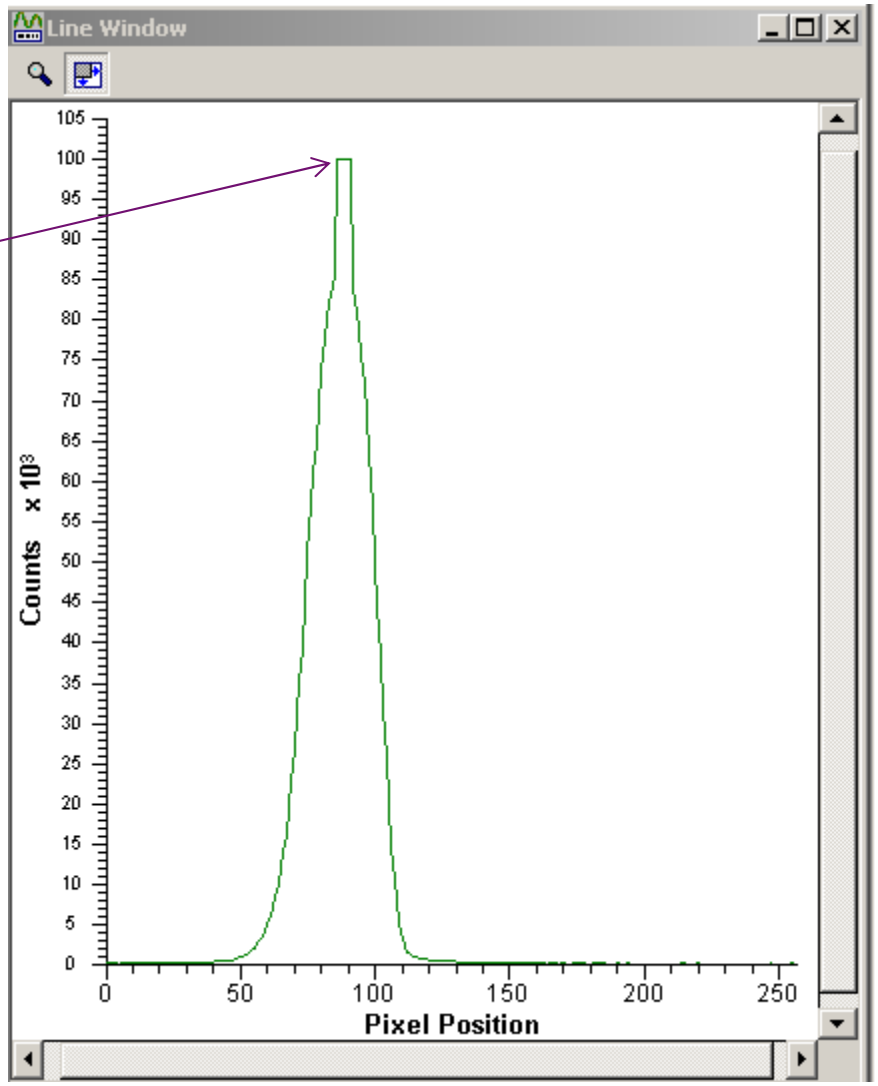
Saturation = “flat cap”

“Flat cap”



In this example the signal is only just saturated

Approach 2

This approach is less cumbersome and makes use of the pseudo color option.

Once the saturated spot has been located then approach 1 can be used on the grayscale image to look at saturation in more detail

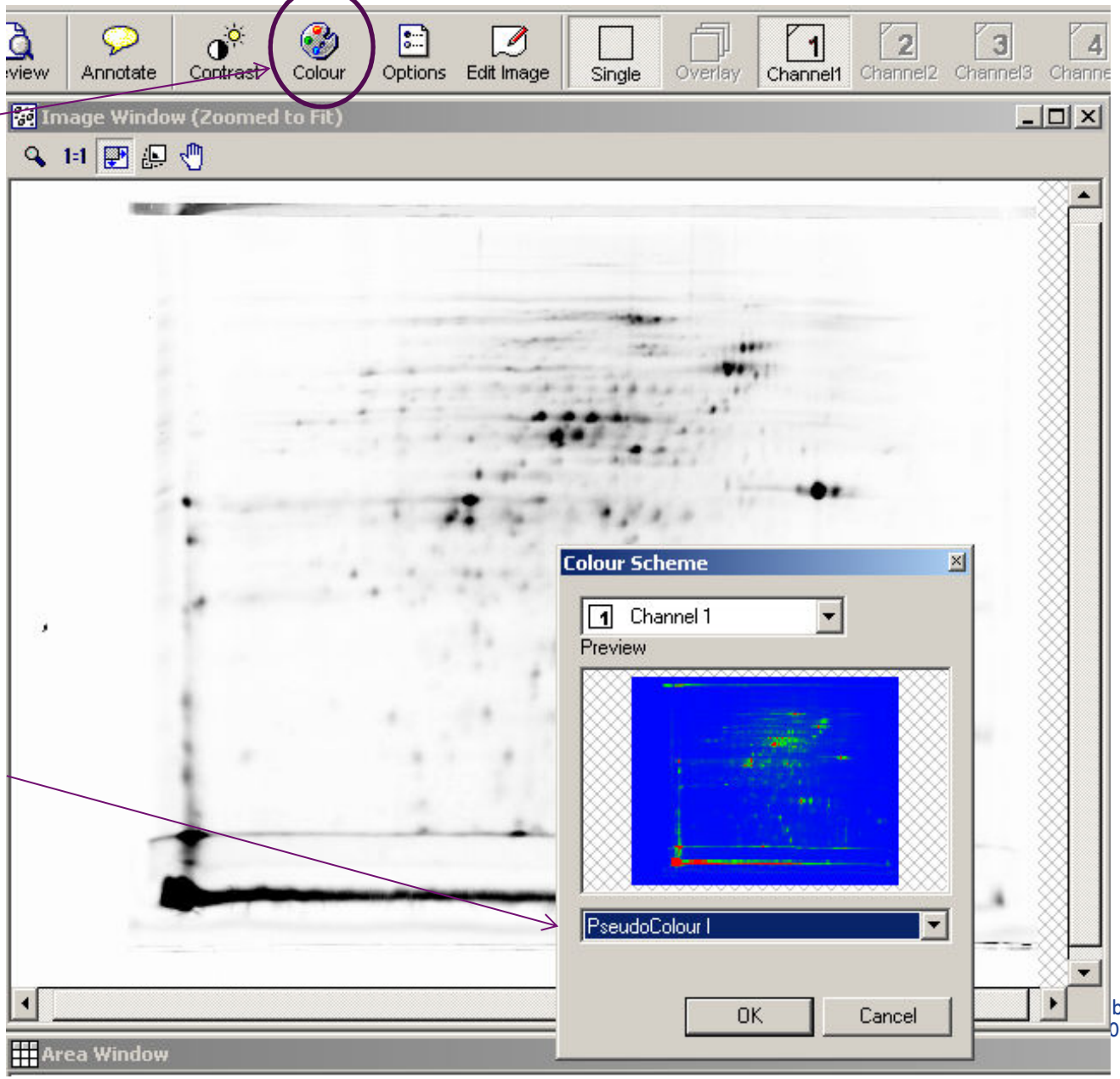
Approach 2

Make sure to select only a single image rather than a multiplexed image – do NOT use the .ds!

Pseudo color

Select “color”

Select PseudoColor I from options in splash screen



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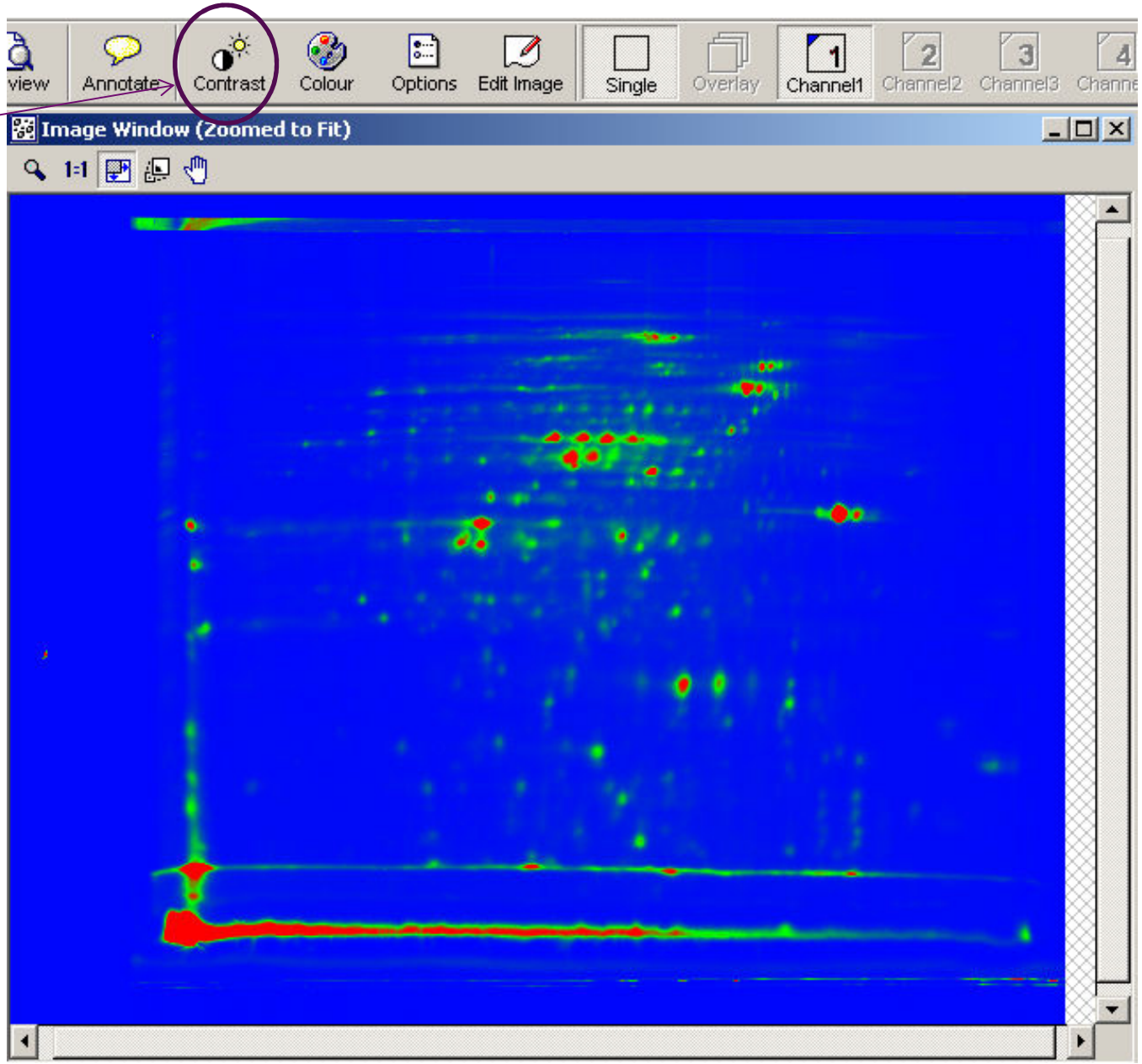
Display adjust

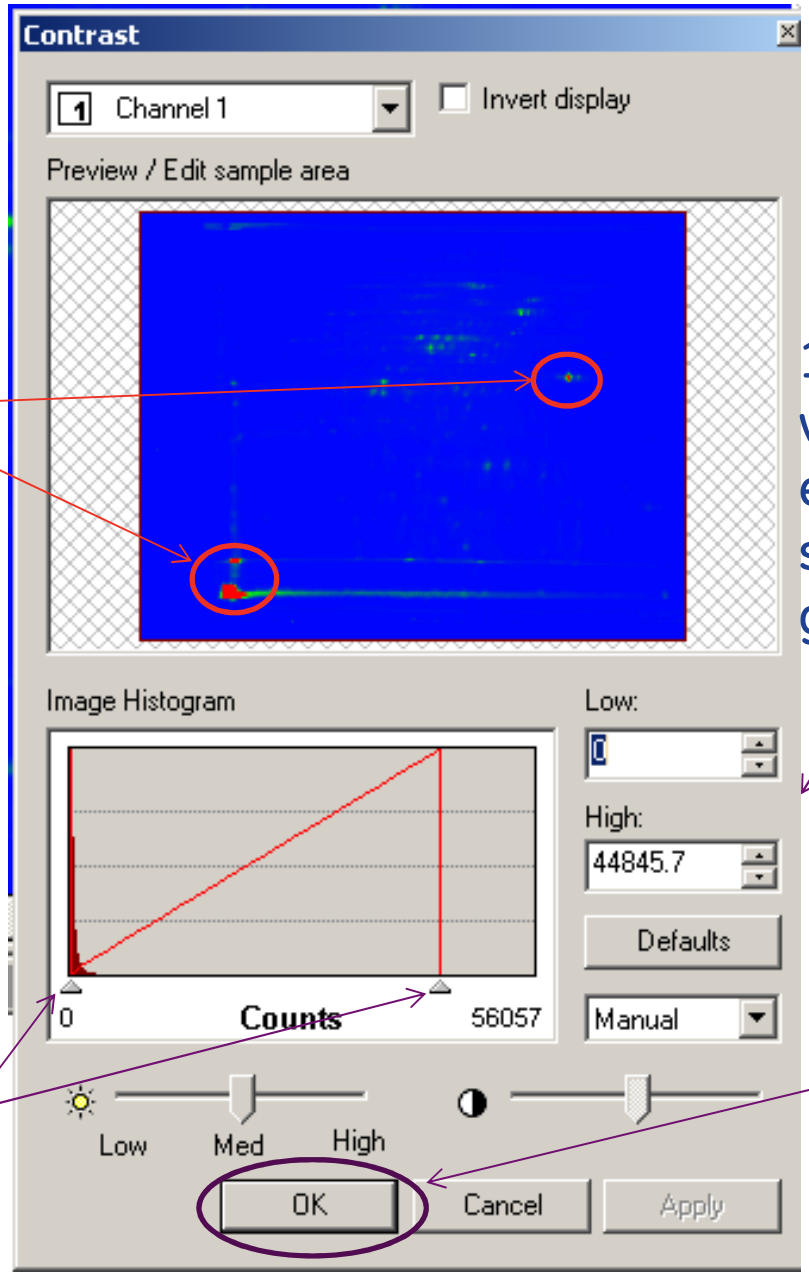
Select contrast



imagination at work

Display adjust

Red areas



1. Manually enter values for low and high e.g. 0 and 100,000 to start with (these are the grayscale levels)

2. Adjust ("fine tune") by using the slider bars until see red area/s on the image

"slider bars"

3. Click "OK"

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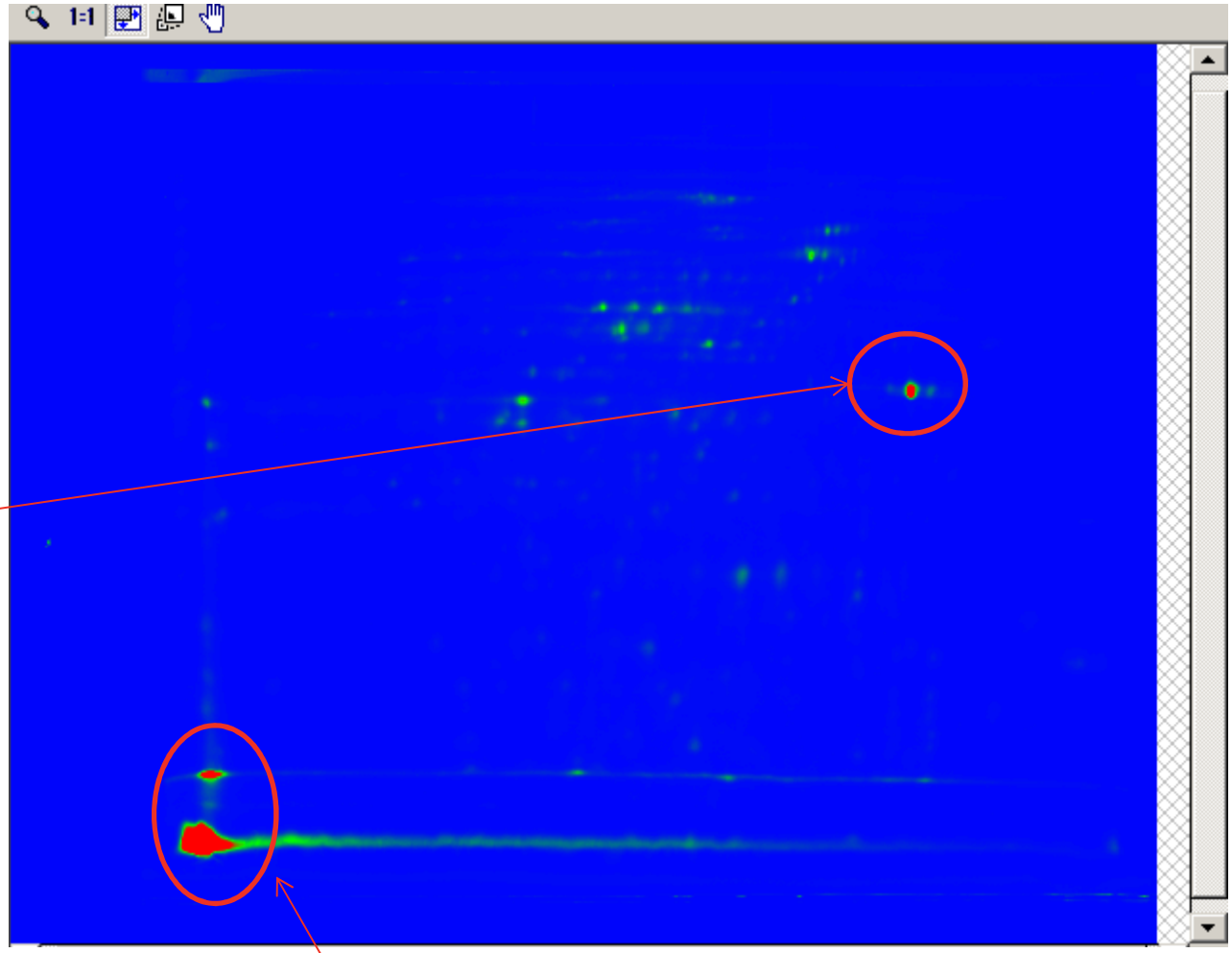
Look at full display

Visually confirm location of “red” features



Most intense feature

Outside area of interest

Confirmation

Can now draw box around this spot and analyze as in approach 1 – better to change from Pseudocolor I back to grayscale image for this analysis

Discussion

In this example the saturation is very slight but quantitation would still be compromised – usually saturation is shown as more extreme with more of the signal missing – the “flat cap” is more pronounced.

Also in this example the majority of the spots are below 40,000 grayscale intensity so a “high” of approx. 45,000 was used to emphasize the red color of the saturated spot.

You will need to optimize on a case by case basis!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