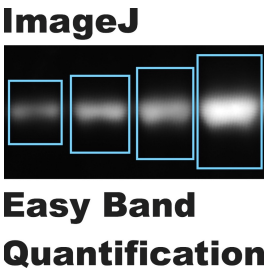
Oct 10, 2019

Quantification of Gel Bands by an Image J Macro, Band/Peak Quantification Tool



DOI
dx.doi.org/10.17504/protocols.io.7vghn3w

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DOI: dx.doi.org/10.17504/protocols.io.7vghn3w

Protocol Citation: Kenji Ohgane, Hiromasa Yoshioka 2019. Quantification of Gel Bands by an Image J Macro, Band/Peak Quantification Tool. **protocols.io https://dx.doi.org/10.17504/protocols.io.7vghn3w**

Protocol status: Working
We use this protocol and it's working

Created: October 01, 2019

Last Modified: October 10, 2019

Protocol Integer ID: 28296

Keywords: Image J, quantification, gel, western blot

Abstract

This protocol offers an Image J macro/plugin that enable easy quantification of bands on western blots, dot blots, and fluorescent gels etc., by simply selecting bands with rectangle or oval tools as region of interest (ROI), and running this macro. This macro automatically estimates background level based on mean or median intensity around ROI boundary, and should help you speed up your routine quantification tasks. Note that the quantification algorithm is similar to that used in Image Studio Lite software (freely available from LI-COR Biosciences), which is widely used for quantification purpose in many publications.

Guidelines

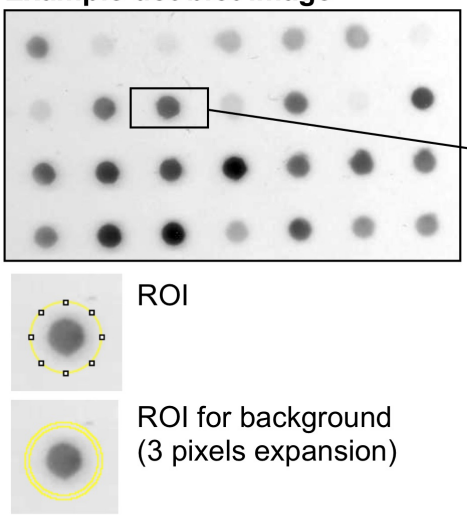
This macro allows you to quantify bands or peaks from gel/blot images, by simply selecting bands with rectangle or oval tools as region of interest(ROI),and starting this BandPeakQuantification macro. The algorithm is basically the same as that implimented in a free, commercial software Image Studio Lite(LI-COR Biosciences), which has been widely used in many publications.

The trick is that background signal is estimated from a slightly-expanded ROI, and the calculated background is subtracted from the total integrated signal within the ROI(seefigures below). By default, the background is calculated from simple expansion of the ROI, users can also select top/bottom or sides options to further define how background intensity is estimated. Also, users can select mean or median for the calculation of the background intensity from the expanded ROI.

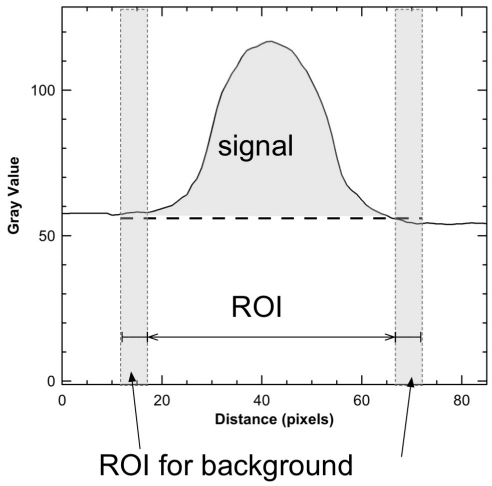
This macro is compatible with rectangle, circles including ovals, and free-hand selections(notethat top/bottom or sides options are only applicable to rectangles). By using ROI Manager tool in the Image J/Fiji, all the ROIs can be quantified in one go.

Expected result

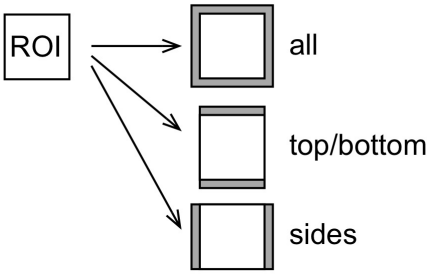
Example dot blot image



Background subtraction

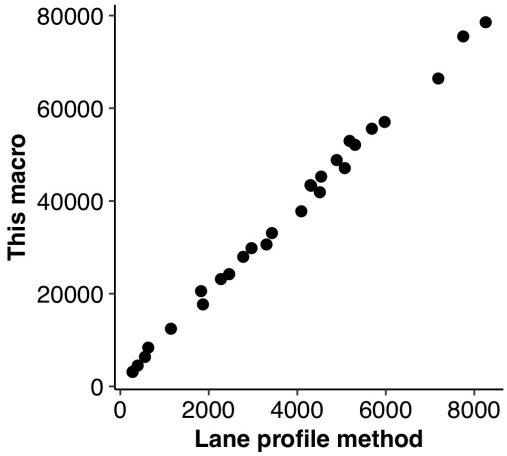


Background estimation



Background:
mean or median of the expanded area

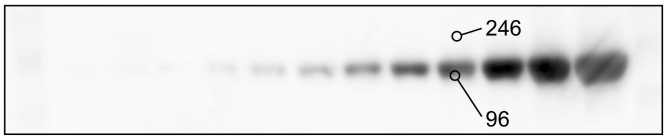
Consistency with the lane-profile method

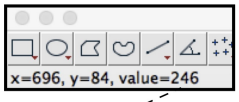


Expected result

Quantification of western blot bands

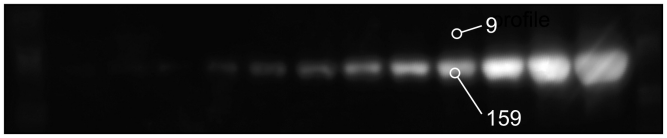
1. Open TIFF image
(in this case 8-bit TIFF)





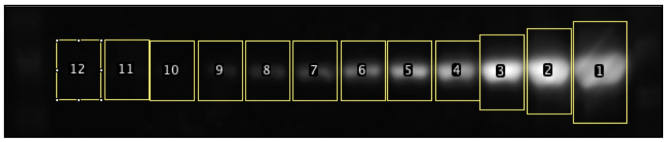
Note:
Image intensity at the cursole position can be read from the ImageJ main window

2. Edit > Invert
(if necessary)



3. Start ROI Manager
(Analysis > Tools)

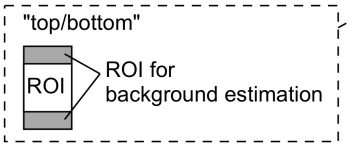
4. Register ROIs
(select then Cmd+t)

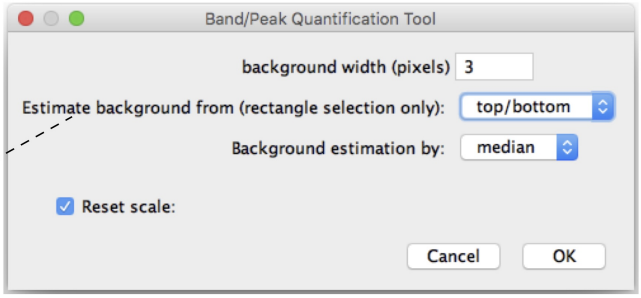


Note:
Size of the ROIs needs not to be the same with this macro.

5. Start the "Band/Peak Quantification" macro

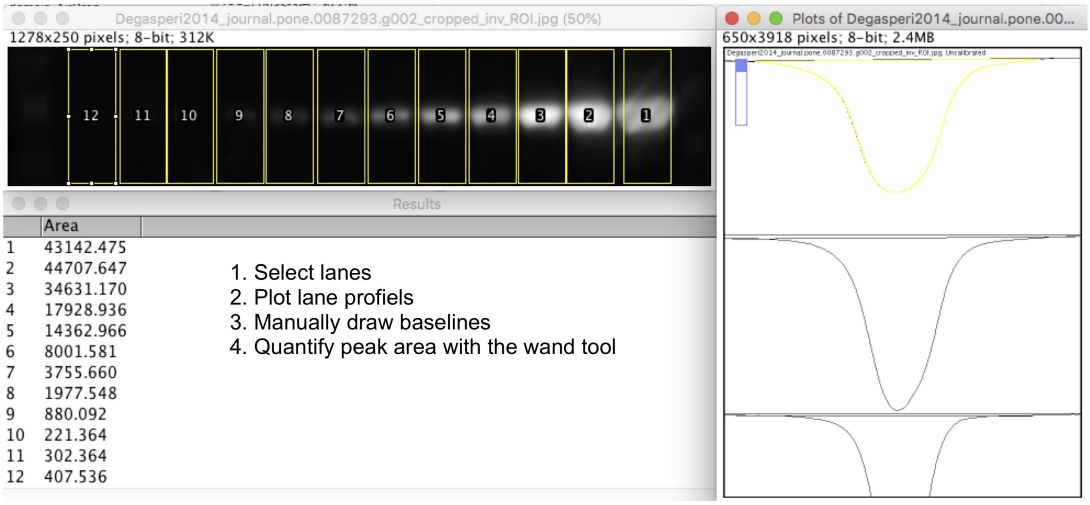
Note:
In this case, "top/bottom" option for the background estimation method is more suitable.





Example image from: Degasperi A, Birtwistle MR, Volinsky N, Rauch J, Kolch W, Kholodenko BN (2014) Evaluating Strategies to Normalise Biological Replicates of Western Blot Data. PLoS ONE 9(1): e87293.

Quantification by lane-profile method



1. Select lanes
2. Plot lane profiels
3. Manually draw baselines
4. Quantify peak area with the wand tool

Before start

This protocol requires a cross-platform image analysis software, ImageJ or Fiji.

Software	
ImageJ	NAME

Software	
Fiji	NAME

Installation of ImageJ and the macro

1 Down load the following ImageJ macro file (_BandPeakQuantification.ijm) and place in a directory under the ImageJ plugins directory.

_BandPeakQuantification.ijm

Note

For example, save the ijm file in“Applications> ImageJ > plugins”. With the“_”character at the start of the filename, ImageJ automatically recognize this macro file at startup, so that you can see the macro listed in the “Plugins” menu. Alternatively, you can do "Help > Update Menu" to make the newly installed macro in the menu.

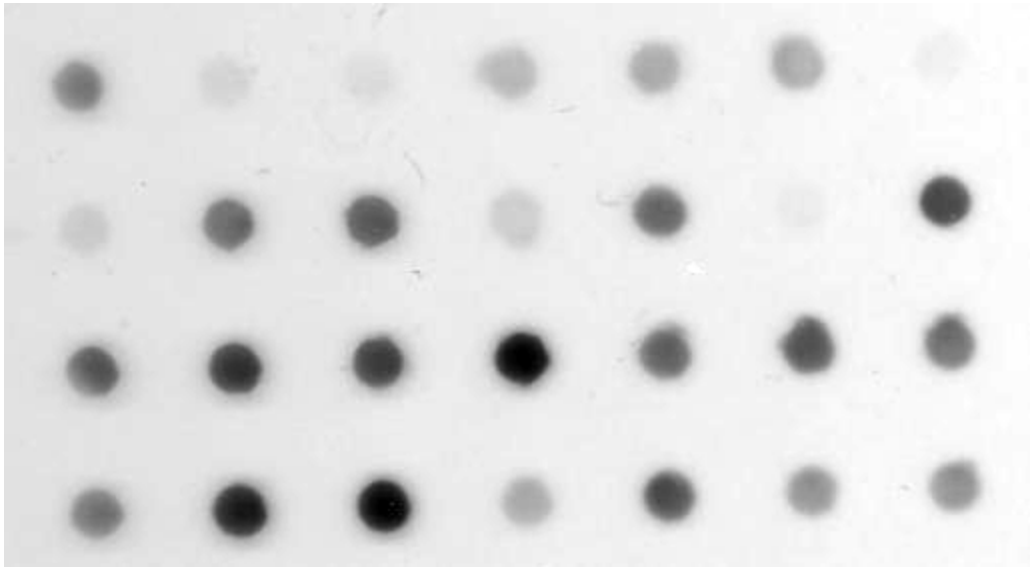
Note

Within ImageJ's plugin directory, you can use subdirectory (or folder) to store multiple macros in an organized manner.

Quantification of dot blot with this macro

2 Open your image. Here, in this example, we use a sample dot blot image provided with ImageJ software. Open the image from "File > Open Samples > Dot blot". 1m

Expected result



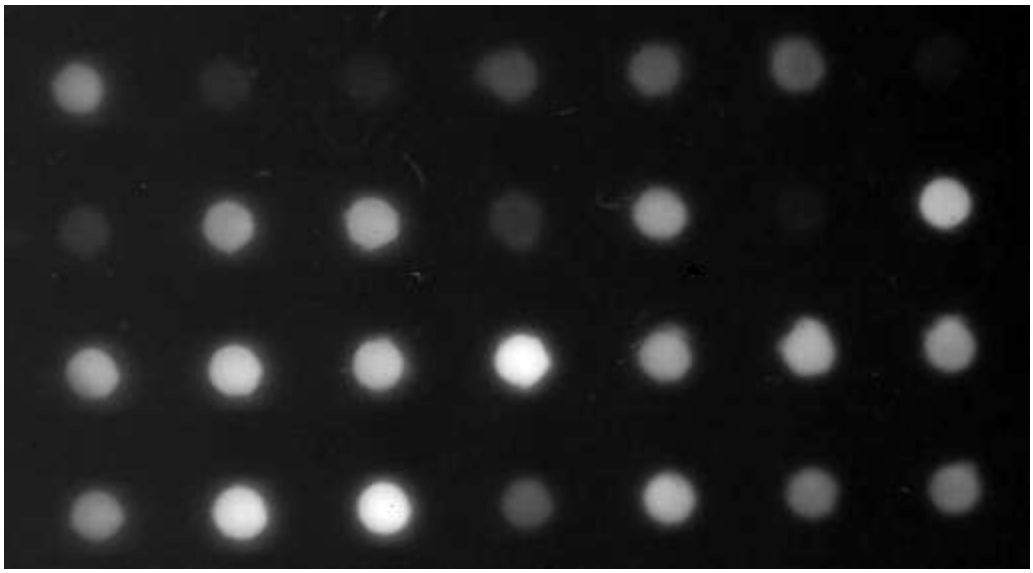
An example image (dot blot) from ImageJ sample images.

3 If needed, invert the image ("Edit > Invert").

Note

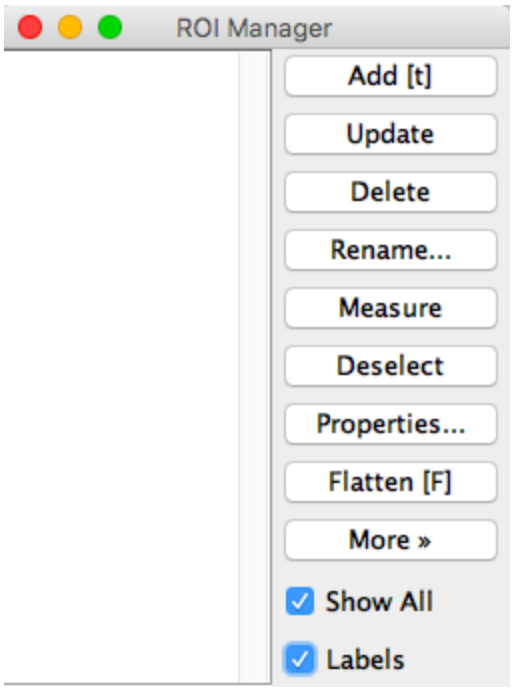
By placing the cursole on the image, you can see intensity value on the Image J main window. Background should be low intensity and signals should show high intensity. Basically, ImageJ shows background as black and high-intensity region as white.

Expected result



4 Start ROI Manager, which you can find at "Analyze > Tools > ROI Manager", and check “Show all” and “Labels” options.

Expected result



5 Place a rectangle (or circle) on the band, and register the ROI (regionof interest) to the ROI Manager by “Command + t” (forMac) or “Ctrl + t” (for Windows) or click "Add" on the ROI Manager window. Note that you can display the ROIs with numbering by checking .

Note

Note that you can change image contrast ("Image > Adjust > Brightness/Contrast" or "Process > Enhance Contrast") to make it easier to select ROIs. However, do not click "Apply" in the Brightness/contrast window, or do not check "normalize" in the Enhance Contrast window, as these operations alter image itself.

Note

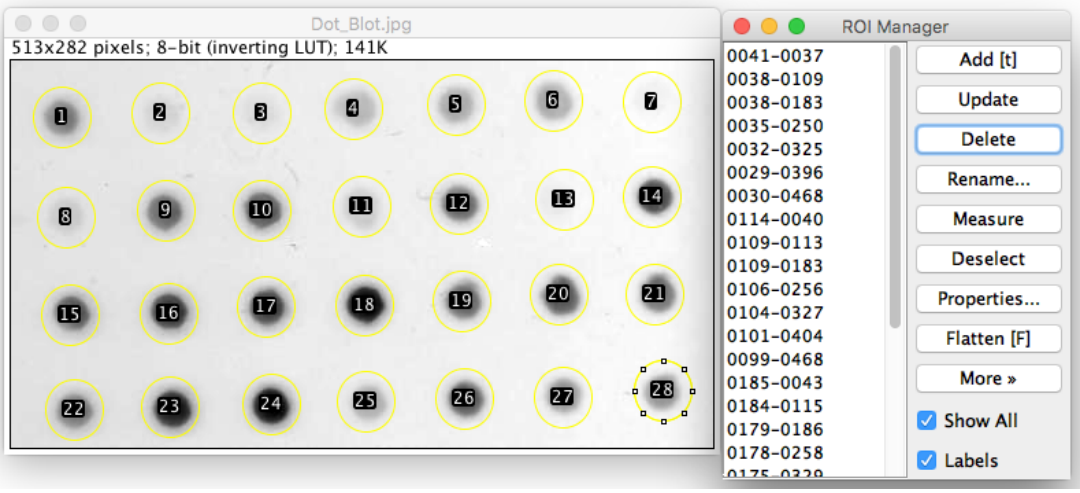
Note that this macro can quantify single selection of a band without using ROI Manager. Simply select your band, and run the macro, and you can get the quantification result in the Results window.

6 Select next band, and register the ROI again. Repeat for all the band you want to quantify.

Note

With this macro, the size of the ROIs needs not to be the same, as this macro quantify integrated intensity above the background. So you can define appropriate ROI of different size for each band.

Expected result



7 Run the “BandPeakQuantification”macro from Plugins > BandPeakQuantification on the menu bar. Within the Band/Peak Quantification Tool window, you can set several options, as described in the following notes.

Note

"Background with (pixels)" means how much you expand the ROI for background estimation (see also Guidelines section).

Note

You can select all, top/bottom, or sides for the "Estimate background from" option. If set to "all", background ROI is made by expanding the ROI by background width pixels. If set to "top/bottom", background is estimated only top side and bottom side of the ROI (only applicable for rectangle ROIs). If set to "sides", background ROI is placed on both sides of the ROI.

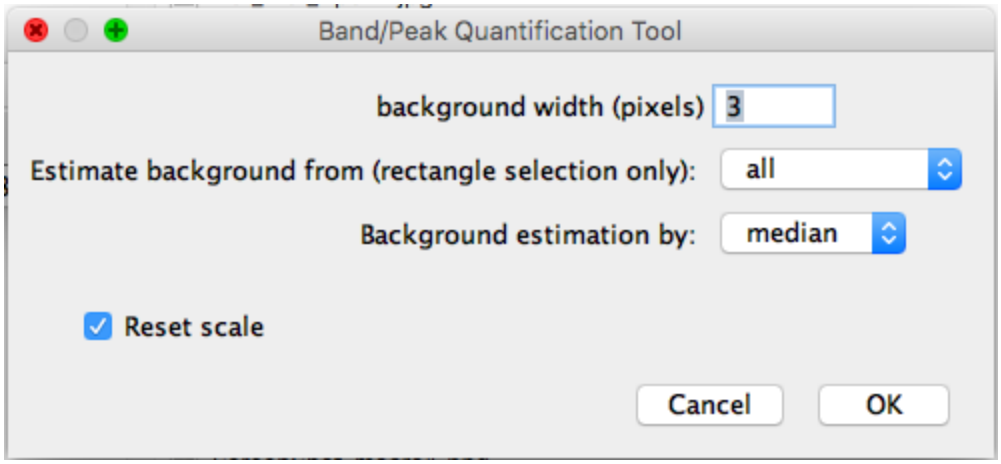
Note

You can select "median" or "mean" for how background is estimated from the region around the ROI. "Median" (default) can be more robust, as it is less sensitive to outliers.

Note

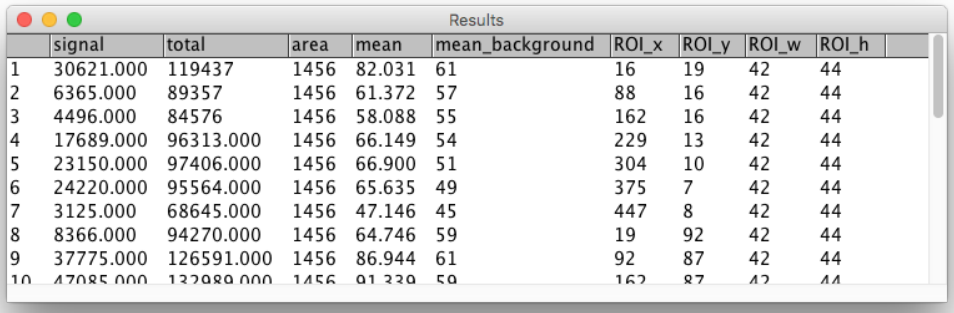
The reset scale option is checked by default. This option is required only when the image scale (µm/pixel) is set. Note that by running this macro, the scale will be reset.

Expected result



8 All the ROIs listed in the ROI Manager will be quantified. You can save the results as a CSV file from right click on the result window, or copy & paste to Excel or other softwares.

Expected result



	signal	total	area	mean	mean_background	ROI_x	ROI_y	ROI_w	ROI_h
1	30621.000	119437	1456	82.031	61	16	19	42	44
2	6365.000	89357	1456	61.372	57	88	16	42	44
3	4496.000	84576	1456	58.088	55	162	16	42	44
4	17689.000	96313.000	1456	66.149	54	229	13	42	44
5	23150.000	97406.000	1456	66.900	51	304	10	42	44
6	24220.000	95564.000	1456	65.635	49	375	7	42	44
7	3125.000	68645.000	1456	47.146	45	447	8	42	44
8	8366.000	94270.000	1456	64.746	59	19	92	42	44
9	37775.000	126591.000	1456	86.944	61	92	87	42	44
10	47085.000	132989.000	1456	91.339	59	162	87	42	44

Note

Note that "signal" column denotes the background corrected integrated intensity. The "total" column is the integrated intensity within the ROI without background correction.

Quantification of a dot blot with traditional lane-profile method

9 Make ROI for the first lane of the gel in this case, I used horizontal rectangle). Press Cmd + 1 (or Ctrl +1 or Analyze > Gels > Select First Lane).

10 Select next lane and press Cmd + 2 (or Ctrl + 2 or Analyze > Gels > Select Next Lane). Repeat for the other lanes.

11 Press Cmd + 3 (or Ctrl + 3 or Analyze > Gels > Plot Lanes) to plot lane profiles.

12 Draw lines at the bottom of the peaks, so that uneven background is accounted for.

Note

A limitation of this method is that baseline can not be drawn for small peaks, as the lane profile tool does not offer zooming function.

13 From Image J tool bar, select Wand tool. Click the peaks and the calculated peak area is displayed in the Results window.

Note

Note that this wand tool quantify area surrounded by lines (closed area). So you need to make the peak area closed by lines.

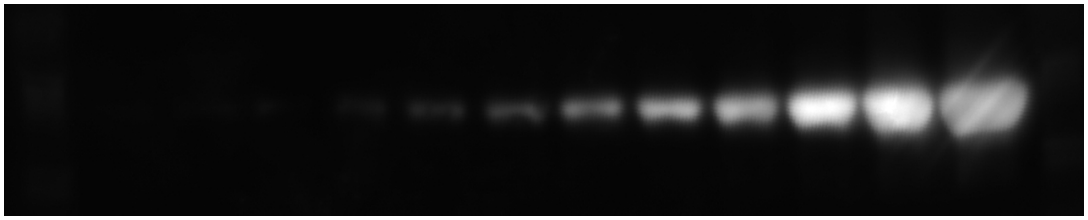
Another example: western blot

14 Open the image.

Note

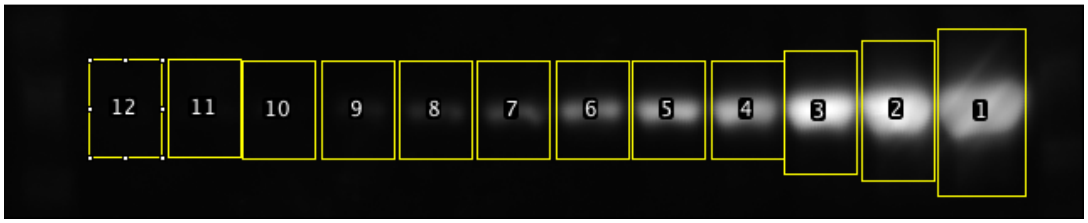
The example data was extracted from a published article(DegasperiA, Birtwistle MR, Volinsky N, Rauch J, Kolch W, Kholodenko BN(2014)Evaluating Strategies to Normalise Biological Replicates of Western Blot Data. PLoS ONE 9(1): e87293.). The data was from western blot of serially diluted BSA(2-folddilution) detected with an enhanced chemiluminescence reagent and a CCD camera system.

Expected result



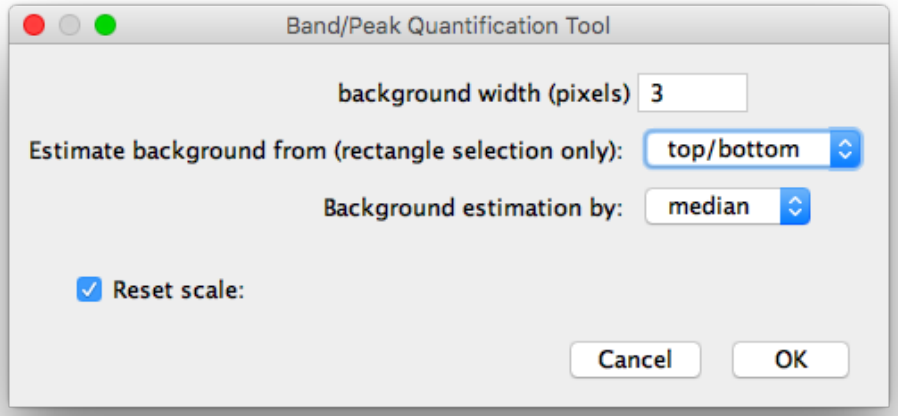
15 Select ROIs and add to the ROI Manager as described above.

Expected result



16 Run the Band/Peak Quantification Tool macro, and set the "estimate background from" option to "top/bottom".

Expected result



Note

In this case, it would be more reasonable to estimate top and bottom sides of the ROI. Also, the sizes of ROIs can be different, depending on the intensity of the band (generally, high-intensity band requires larger ROI).

17 The quantification results will be shown in the Results window. Save as CSV file or copy to Excel for further analysis.

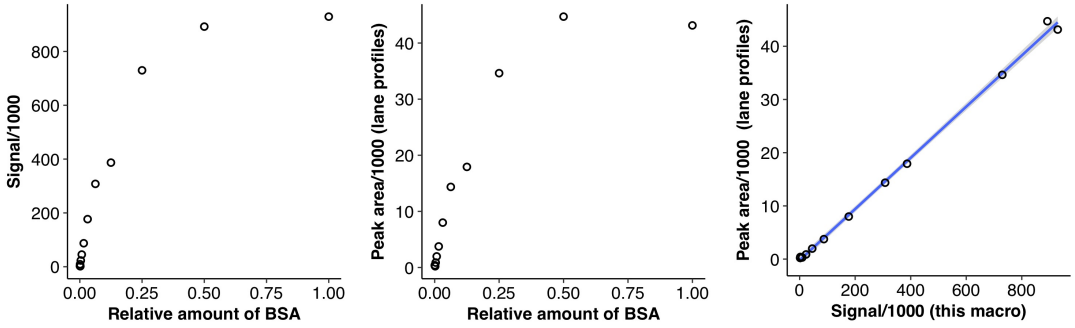
Expected result

Results

	signal	total	area	mean	median_background	ROI_x	ROI_y	ROI_w	ROI_h
1	1052093	1216829	20592	59.092	8	1106	26	104	198
2	937885.000	1080645	14276	75.697	10	1016	40	86	166
3	738535.000	838983	12556	66.819	8	924	52	86	146
4	392521.000	462353	9976	46.347	7	838	64	86	116
5	315443.000	375299	9976	37.620	6	744	64	86	116
6	185239.000	245095	9976	24.568	6	654	64	86	116
7	86028.000	145884	9976	14.623	6	560	64	86	116
8	45240.000	105096.000	9976	10.535	6	468	64	86	116
9	20907.000	80763.000	9976	8.096	6	376	64	86	116
10	2590.000	62446.000	9976	6.260	6	282	64	86	116
11	9527.000	69383.000	9976	6.955	6	194	62	86	116
12	2197.000	62053.000	9976	6.220	6	100	62	86	116

Expected result

Comparison of the "Band/Peak Quantification" macro and the lane-profile method



Both of this macro and the traditional lane-profile method gives similar quantification results, with high correlation. Note that the western blot intensity is not always linear against the amount of the protein in the input. For quantitative comparison, it is important to use linear range.