

Image Quant TL (PSC 563, 537, NSC 338, 438)

Contact: Hyuk Kyu Seoh hseoh@gsu.edu

Rm: PSC 537

Tel: (404) 413-5379

This software can analyze 1D gels, dot and slot blots, microplates, other basic arrays and colony images captured by the Typhoon scanner. The Toolbox option allows you to select how you want to analyze the image using area and profile based tools.

Scan with the Typhoon or image with UVP (image must be converted to 8-bit)

Quantitate

1. You can either do Normalisation or quantity Calibration.
 - a. Normalisation – normalizes the volumes in a gel image that sets the normalised volume of a band or a group of bands to a specific value and then recalculates all the other volumes relative to that value. Can be useful when comparing the volumes across lanes or gels where the loading might be different.
 - b. Quantity calibration – relates band image volume/intensity to real world values. The real-world values are entered and a calibration curve of real-world volume against raw volume is created. The real-world volumes of the bands with unknown values can then be derived from this curve.
2. Size –the molecular size calibration. Using a known molecular weight standard (which can be added or edited), the size of any band on the gel can be determined.

Importing gel image

1. Open software. Or File/Open
2. Select the module you are going to use. If you went through File/Open, select the appropriate module analyze this image using.
3. All Image Quant TL image files.
4. browse until you find your image. Select it – the image should be visible in the preview window (lower right hand side). If it's not, you probably have the wrong type of file.
 - a. the image should be either .tif, .tiff, .gel, .img. All images from the Typhoon are .gel
 - b. UVP images. Capture image as usual. Edit/convert to/gray scale 8 . When window opens, don't change anything, just select convert. Save image to usb mini drive.

Image Options

1. Contrast. You can manipulate the gray scale so you have a clearer image or difference between bands

2. Invert display will change white to black and vice versa
3. Color scheme. You can change the color so that it looks like the stain you used (ex. Coomassie Blue).
4. Tables tab. This lets you add whatever data fields you might need. Be careful – some data fields are not available in all modules.

Data Choices:

Data Field Name	Description	ID	Array	Colony counter	Toolbox
Area	The area of the Band, spot, colony displaying the # of pixels quantified in the image in order to produce the measurements	Y	Y	Y	Y
Area>Background	Area > Background is the # of pixels which have an intensity greater than the background level	N	N	N	Y
Average Intensity	The volume of the image feature divided by its area	N	N	Y	Y
Background	The total background for the Band, Spot or colony or other image feature	N	Y	Y	Y
Background Level	The average background intensity in the Image Rectangle used in the background subtraction method of the Toolbox module	N	N	N	Y
Background Type	The method of background subtraction used	N	N	N	Y
Band Index	The # of the band in the lane	Y	N	N	N
Band %	A measure of the band's Volume divided by the Total Volume of all the bands in the lane	Y	N	N	N
Band % (Calib/Norm)	The band's calibrated/normalised volume as a portion to the sum of the calibrated/normalised volumes, of all the bands in the lane	Y	N	N	N
Calib/Norm Volume	The Calibrated/Normalised Volume of the bands	Y	N	N	N
Center	The X, Y position of the center of a given shape, relative to the top-left of the image (position 0.0)	N	N	N	Y
Circularity	See notebook; for Colony counter	N	N	Y	N
Colony #	See notebook; for Colony counter	N	N	Y	N
Comment	Displays the text you typed into the data field	N	N	N	Y
Coordinates	Equivalent to the center of the detected mass, with the coordinates	Y	Y	Y	N

	rounded to the nearest integer				
Height	Shows the height in pixels of the bounding rectangle of the shape	N	N	N	Y
Lane %	A measure of the Bands Volume divided by the Volume of the whole lane	Y	N	N	N
Max Intensity	The maximum pixel intensity in a given area	N	N	N	Y
Median Intensity	The median pixel intensity in a given area	N	N	N	Y
Min Intensity	The minimum pixel intensity in a given area	N	N	N	Y
Mode Intensity	The most common pixel intensity for a given area	N	N	N	Y
Molecular Size	Shows a band's molecular size with the units shown in the table column header	Y	N	N	N
Name	The name of the shape object	N	N	N	Y
Normalised Volume	The Normalised Volume of the spots	N	Y	N	N
Peak + Background	The maximum value of the Profile of the Band with the background value added	Y	N	N	N
Peak Height	The maximum value of the Profile of the Band not including the Background	Y	N	N	N
Percent	The % of total volume for all area objects represented by a given object	N	N	N	Y
Position	The distance, in pixels, of the band's peak from the start of the lane	Y	N	N	N
Presence/Absence	The Current Grid of Array displays a 1 for spots tht contain a sufficient quantity of material to be considered present and a 0 for spots not considered as present. The threshold volume can be set in the Presence Flagging dialog box	N	Y	N	N
Rf	Rf (Retardation Factor) is a measurement of position along the lane, relative to its length. By default, the first position in each lane has an Rf of 0 and the last has an Rf of 1. There is a linear	Y	N	N	N

	increase in Rf from start to finish				
Std Dev	The standard deviation of the pixel intensities within a given area	N	N	N	Y
Spot Label	Use in reporting and identification of spots in the image. The label for a spot can be edited in the Spot Properties dialog box, and in the table when you select the Spot Label field	N	Y	N	N
Spot Quality	Used to highlight any spots of dubious quality that can affect the measurements results. Spots thought to contain only noise and spots thought to contain a spike are highlighted	N	Y	N	N
Spot Radii	Displays the X and Y radii for the given spot. These will be identical for square and circular spots	N	Y	N	N
Variance	The variance of the pixel intensities within a given area. Population variance as opposed to the Sample variance	N	N	N	Y
Volume	The raw volume of the uncalibrated quantity of material in the image feature after the background intensity has been removed	Y	Y	Y	Y
Volume + Background	The uncalibrated quantity of material detected before the background intensity has been removed	Y	Y	Y	Y
Width	The width in pixels of the bounding rectangle of the shape	N	N	N	Y

Image Window. This is the main window that displays your image.

1. Zoom 1:1. Button on toolbar that shows 1:1. Adjusts the magnification of the image to its original size.
2. Zoom to Fit. Button has arrows pointing to right and bottom. Fits the image to the size of the window.
3. Zoom. Button has a magnifying glass.
 - a. Click on the zoom button.
 - b. Position the pointer on the area you want to zoom.
 - c. Drag the pointer over the desired area.
 - d. Or, click the zoom button then left click to zoom in and right click to zoom out.

- e. Press ESC cancels the zoom mode.
4. Magnify. Button has white square in middle. Allows you to magnify specific parts of an image. This is good to use if you are having to manually add lanes and bands to make sure they don't overlap and cover the area you want.
 - a. Click the magnify button.
 - b. Position the pointer over the part of the image you want to magnify. The pointer changes to a small magnifying glass.
 - c. Hold down the mouse button. The area under the pointer appears in a magnified box.
 - d. Drag the pointer to any area you want to magnify.
5. Panning. Button with an open hand. Allows you to move the image around the Image window when the image is zoomed.
 - a. Click the Panning button.
 - b. Position the pointer over the image, it changes to a small hand.
 - c. Drag to change the view.

Measurements window. Displays the analysis data results. The types of data fields displayed depends on the analysis module and also on what data fields have been selected. Go to Options/Tables tab/Data Fields to add or delete whatever you want.

1D gels

1. Window Selector Bar contains buttons for the Image, Second Image, Analysis, Measurements, Graph and Report windows.
 - a. Image Window. This displays the image you opened and are working on.
 - b. Analysis window (2 panes). The upper pane (Lane Profiles) shows the intensity at each point along the length of the selected lane. It will number the bands in that lane. This will help when you are adding/deleting bands to make sure you have what you want. You can display a selected lane as well as additional lanes (Options/Analysis Tab). The lower pane (Lane Images) displays the image of your selected lane/s. It is displayed horizontally so the bands align with the calculated lane profile in the upper panel.
 - c. Measurements window. This shows whatever data field you have selected in View/Options/Tables tab. You can display either the selected lane, All lanes or Comparisons (this lines up the bands of a similar position in the lanes and displays them in the same row).
 - d. Graph window. The graphical information calculated by the molecular size calibration and quantity calibration modes. It also shows which curve type was used.
 - e. Report window. Displays reports about the analysis of the gel images. You can print these reports, but can't copy to the clipboard. Export to Excel if you want to manipulate the report more.
2. Automatic or Stepwise (Manual) Analysis. Select automatic. This will go through all the steps (lane creation, band selection, background Subtraction). If it has too many lanes/bands, select Clear
3. Stepwise/Lane Creation.
 - a. You can automatically create lanes. Select the Create button. If you don't

- like how it looks, select Clear.
- b. Draw Lanes Manually. Set the # of lanes in gel; use the default lane % width until you see if that width is OK. The cursor becomes a pencil. Hold pencil in upper left hand corner. Drag pencil to the right and then down to bottom of lanes. You should have a rectangle over each lane. Select Accept or Clear if you need to do it again.
 - c. You can edit multiple lanes or single lanes. In multiple lane edit, you can bend/resize lanes, move the edges of the lanes or add lanes. In Editing single lanes, you can bend/resize, move and add grimace. Also at the top and bottom of each lane are handles that can be used to bend the lanes. You can add more handles if you need to bend more.
 - d. Bend/Resize, move lane edges, add lanes, move lanes are easy to understand.
 - e. Adding a grimace. This compensates for bands that are smiling. Click on the Add Grimace button. Position the pointer at the center of the band and click. The software will add a grimace to the lane. This grimace can be adjusted using the handles.
 - f. Once you have the lanes looking right, click on Accept.
4. Automatic Background selection. To do the automatic background subtraction, select the background button. There are several types of background subtractions.
- a. Rubber band. Like stretching a rubber band underneath the lane profile. If the values at the ends of the profile are lower than the rest of the profile or the bands are badly separated, do not use this method.
 - b. Minimum profile. Sets the background level as the lowest value found in the in the profile. Don't use this method if the lowest point is found at the extremities of the lane since the result is dependent on where the lane is drawn. Also it is hard to repeat between analyses.
 - c. Rolling Ball. Requires you to enter a value for the size of the rolling ball. Calculations of background are like a disc, with the radius you have entered, rolling underneath the lane profile. The larger the radius of the disc, the less the background rises with the profile.
5. Manual Background selection. Best reproducible results should use automatic background subtraction. Manual subtraction could produce erroneous results.
- a. Image Rectangle. The average intensity within a rectangular area on the image is taken as the background intensity.
 - i. Click the Image Rectangle button
 - ii. Position the pointer over the image window you will see a small white box attached to the pointer.
 - iii. Draw a rectangle in a lane, from the top left to the bottom right.
 - iv. Release the mouse button.
 - b. Manual baseline. Allows you to define the shape of the background line using the profile graph in the Analysis window. Using this method produces a straight line with four movable handles along the bottom of the lane profile graph. You can edit these handles – move, add or remove.
6. Band Detection.

- a. Select Detect button. This will detect the bands automatically. You can change the band detection parameters (minimum slope, noise reduction and % maximum peak) and the Edge Detection parameters (automatic detection and fixed width).
 - b. You can manually edit the bands – add, resize and delete.
 - c. Adding a band. Position the pointer where you want to add a band. The pointer changes to a pencil with a + sign. Click to add band.
 - d. Resize. Position the pointer over a band edge. Up and down arrows appear. Drag the edge to resize. You can't resize a band past the lane edges.
 - e. Delete bands. Position the pointer over the band edge or the over the diamond shape. Right click the band and the band will be removed. You can also delete a group of bands. Position the pointer to the top corner, right or left, of the area you want to clear of bands. Drag the pointer to the opposite corner of the area. The pointer changes to an arrow with an X and an area of red crosshatch appears over the image. Release the mouse button to delete the bands covered by the box.
 - f. You can add, resize and delete bands in the Analysis window also. You just can't delete a group of bands.
7. Molecular Size Calibration. Using molecular weight or pI standards, the software will calculate the molecular weight or pI of the bands.
 - a. Select the Molecular Size Calibration button or by clicking Molecular Size Calculation from the Analysis window.
 - b. Make sure you have the Molecular size field selected in the Measurements window. If not, go to Options select molecular size.
 - c. In the Parameters tab, select the mappings list from the list that represents the values of your standard. You can create a new standard or edit an existing one.
 - d. After you select the standard, the list of its mapping values appears in the list box below the selector. These values can be selected or de-selected by clicking the check boxes.
 - e. Select the lane that you want to designate as a standard by clicking on it. This lane should have all its bands detected by this stage. The gel image should now show the selected standard with its mapping values attached to the bands.
 - f. The standard values can be re-positioned on the bands by holding down the left mouse button and selecting the band where the values has been assigned and dragging it to the required position. Standard values can be individually added or deleted from the gel image by selecting or deselecting them in the mapping values list. To remove all the assignments from a lane, right click on the lane.
 - g. Select the curve type from the list.
 - h. Select whether to use Rf to propagate. If selected, the molecular weight contours will conform to relative lane position, not absolute.
 - i. Click the Compute button.
 - j. Standard Mappings list can be edited, renamed and new standards created.

See manual.

8. Quantity Calibration. Relates the band volume in terms of image intensity to real world values. Entering the real-world volumes for bands with known values does this. From these values the software computes a calibration curve of real-world volume against raw volume. You can do either Quantity calibration or Normalisation – not both. All the bands included in the quantity calibration are shown in the Image window with yellow band diamonds, those that are not included have blue diamonds.
 - a. Ensure that you have the desired table fields selected to display your Quantity Calibration results in the measurements window. Options/Tables tab
 - b. To select a band in the Image window click on the small diamond on the band. The pointer changes to an arrow with a box above it. An in place edit box appears.
 - c. Type the band volume in this box and press Enter to accept it.
 - d. The band in the Image window displays the value you have entered. The software also adds the band with the volume to the table in the Parameters tab.
 - e. Repeat the steps to select more bands. Every time a band is added the software recalculates the curve and updates the Graph window.
 - f. TO change a value of a band, click the diamond; the in place editing mode highlights the text and allow you to enter a new volume. Press Enter.
 - g. Click the Calibrate button to calibrate all the bands with unknown real-world volume.
 - h. You can remove a band from the calibration curve, by selecting the band and right click.
 - i. Select curve type, Force through Origin and calibration unit. These settings can be changed at any time and the software will recalculate the values.
9. Normalisation. Normalises the volumes in a gel image that sets the normalised volume of a band or a group of bands to a specific value and then recalculates all the other volumes relative to that value. All the bands used for normilisation are shown in the Image window with yellow band diamonds, those that are not included have blue diamonds.
 - a. Ensure that you have the Calib/Norm Volume and Band % (Calib/Norm)table fields selected in the Options dialog box. The Measurements window will then show the Normalised volume and the Band % (Norm) values.
 - b. Select a band in the Image window by clicking on the small diamond on the band.
 - c. Repeat the above step as required.
 - d. Type the Normalised Volume parameter, in the left box, of the dialog box in the Parameters tab.
 - e. Select the unit of measurement.
 - f. If normalising a group of bands, select either the group average or collective option by clicking the button in the Parameter tab.

- g. Click on the Normalise button.
- 10. Reports. There are different report options available. You can also add Lane notes if needed. You can have notes for any of the lanes in your image.
- 11. File/Export to Excel if needed. Only available when the Image or Measurements window is active.

Array/Microplate

1. Window Selector Bar contains buttons for the Image, Second Image, Zoom and Measurements.
 - a. Image Window. This displays the image you opened and are working on.
 - b. Zoom window. This displays the whole image, overlaid with a rectangle representing the area of the image that is currently displayed in the Image window.
 - c. Measurements window. This displays the analysis results. Three different tables of data are available: Current grid, selected Spots and All spots. For each table you can select different data fields to display the various properties of the spots (View/Options/Tables tab). Data within a columns can be sorted by clicking the column header.
2. Spot Definition.
 - a. Defining the grid layout. You can store parameters used for creating grids as a grid type that you can quickly select from the Grid Type list.
 - i. Click the Parameters tab to display the Spot Definition dialog box.
 - ii. Select a grid type from the list in the Parameters tab.
 - iii. Click the Detect Button to create the grid automatically or drag a rectangle over the image to create the grid manually. The software automatically measures the spots and displays the data in the Measurements window.
 - b. Creating/Deleting a grid type.
 - i. Set values for the dimensions of the grid Columns and Rows (how many spots wide by spots tall).
 - ii. Select a grid type from the list in the Parameters tab. Select the spot shape from the list.
 - iii. Click the Save As button to save the created grid.
 - iv. Type a name for the grid and click OK.
 - v. To Delete a grid type, select the grid from the list and click on the Delete button below the list.
 - c. Repositioning spots.
 - i. Click the Create and Stretch Grid button.
 - ii. Position the pointer over any of the spots and then drag the grid to the new position.
 - iii. To move a small group of spots, click the Move and Resize button and drag from the top left to the bottom right to highlight the group of spots by drawing a green outline covering the spots. You can select more than 1 group by pressing the CTRL key when dragging over the additional spots. Position the pointer over the selected spots and drag the spots to the new

position.

iv. To move a single spot, click the Move and Resize Spots button. Position the pointer over the desired spot and drag the spot to the new position.

d. Adjusting a grid to allow for a skewed image.

i. Click the Create and Stretch grids button.

ii. Drag one of the grid's square corner handles to a new location. The spot positions are stretched uniformly between the 4 corner handles of the grid.

e. Resizing Spots. Spots can be resized in 2 ways: by stretching or by defining the spot radius value.

i. To change the radius by stretching a spot, click the Move and Resize button. Select the spots whose radius you want to change. Position the pointer over the spot edge. The pointer changes to a vertical or horizontal arrow. Drag the edges to the desired size for all the selected spots.

ii. To change the radius by defining the spot radius value, Edit/Spot properties. You can edit the size and also the spot labels.

4. Background subtraction.

a. Click the background subtraction method you want to use from the Selected method list in the Parameters tab.

i. Spot Surface Minimum. Locates the lowest pixel intensity within the spot and then removes this intensity from the volume for each spot. You can use this method to ensure that no part of the defined spot is below the background level for each spot. You should use this method for images with little noise and an even background intensity. Does not need additional parameters.

ii. Spot Edge Average. Locates the average intensity around the spot's outline and then applies this as the background for the spot. The background is calculated independently for each spot. This method is recommended if you have good localised background intensity and is tolerant of noise in the image. Does not need additional parameters.

iii. Negative Controls. Averages all the pixels inside the "flagged" spots in the grids and applies this average as the background intensity. This is used when certain spots have been set as controls. Select the spots you want to change by either clicking on them or drag a selection rectangle around the spots in the image window. Click either Set Negative control or Deselect negative control button. Does need additional parameters.

iv. Image rectangle. Requires you to specify a rectangle area of the image. This area is then averaged to calculate the background intensity. This is useful if your image contains a lot of noise and tightly packed spots. Position the pointer at the top-left corner of the rectangular background area. Drag the pointer to the bottom right of the rectangular background area. Does need additional parameters.

v. None. Allows you to turn off background subtraction. The raw volume calculated for each spot will be the sum of all pixel intensities in that spot. Does not need additional parameters. Provide any Parameters

required by the chosen method.

5. Normalisation.

- a. Select the spots that will be used to normalise the image.
- b. Type the normalised volume in the Parameters tab.
- c. Select the normalised volume unit.
- d. Click the average or collective group button.
- e. Click the Normalise button.

6. Presence Flagging. Allows you to set the level above the background at which the spots should be flagged as having a significant amount of material present. The presence or absence of spots can be seen in the Measurements window after you select the Presence/Absence data type.

- a. Make an initial estimate of the threshold. Right click a spot in the image and select either the Define Presence or Define Absence command. Or click the Estimate button to have the software calculate a threshold for the current image. The software evaluates the volumes of all spots in the image.
- b. Fine-tune the threshold level. After you make an initial estimate, you can manually adjust the threshold value so the presence or absence of the spots (as displayed in the Measurements window) reflects what can be seen in the Image window. Type a value between 0 and 100 in the Flagging threshold text box or drag the slider to the left or right.