

# UltraQuant 1D Gel Analysis Software

aplegen<sup>™</sup>

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## Introduction

Welcome to the UltraQuant ID Gel Analysis software. The software is designed for image processing and ID quantitative gel analysis. It allows you to view your data in real time via the status bar, which displays the (x, y) position of the mouse cursor and the intensity information at that location. You can make enhancements to the images for viewing and easily export and data to commonly used applications for publication. You can also print or save the data reports. The following sections will guide you through the various menus of UltraQuant.

## Basic Operations

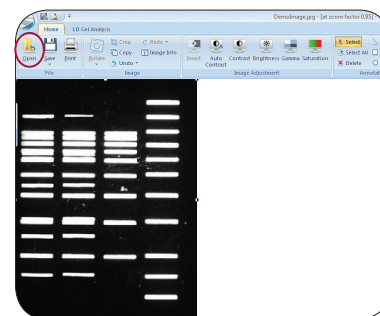
### File Management

#### How to open and save images

- Main File Menu – Open, save, or print images. Also contains Options, which allows you to manage analysis display options.

#### How to print images and reports

- Home Tab
  - File Menu – For file management.
    - Open – UltraQuant can be used to annotate and analyze most image file types including 8 bit and 16 bit tiff (.tif), jpeg (.jpg), and ulq (.ulq) files.

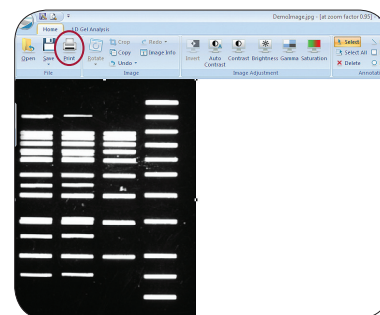
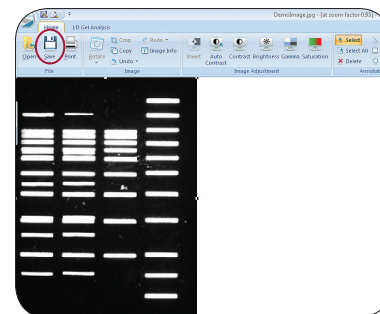


- Save – Files can be saved as either .jpeg, .tiff, or .ulq files. UltraQuant files save the images as well as the data from the analysis performed. Use this file type to save your analysis for future reference. To change the file extension either select the alternative file extension from the drop down menu or use the Save As option.

By default the first time you save, the file will be saved as a .ulq file. This prevents accidental overwriting of your original data file. Bear in mind, the software also prevents you from making changes to your image as a .ulq file to prevent altering the image the analysis was based on. For best results, open your image, make all image alterations and save, then perform your analysis.

Save As – Use the Save As option to save the file under a different name or file type. It is recommended that you save your native data with Save As before you start manipulating and analyzing your data. Some file manipulations, such as contrast adjustments made to your image, will be saved under the .jpg extension, but this can affect analysis.

- Print – Use the print option to print your image file and reports. If an analysis was performed, you will be asked if you want to print the image, graph, and/or table.



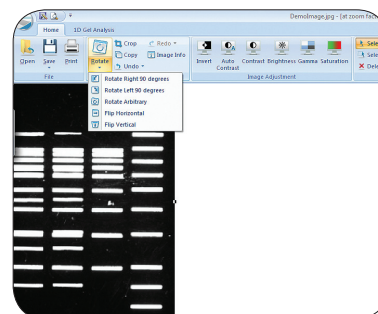
## Image Handling

### How to adjust the image size and shape

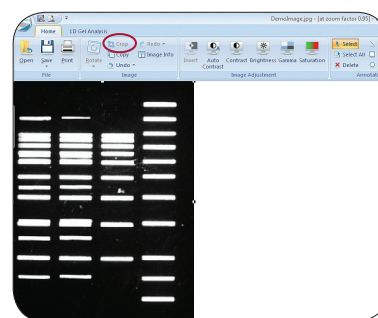
- **Image Group** – Under the image group there are multiple options for manipulating the image. These include Rotate, Crop, and Copy.

- **Rotate** – This option includes four preset rotations, 90 degrees right, 90 degrees left, flip horizontal, and flip vertical. It also includes Rotate Arbitrary. Rotate arbitrary allows the image to be moved in one degree increments by sliding the bar to the right or left, or by entering a number in the box.

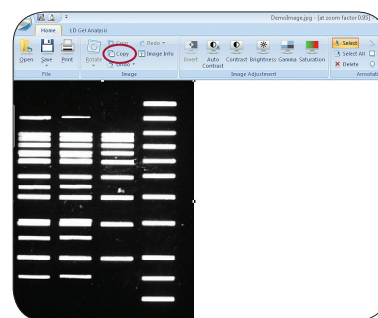
Selecting Rotate Arbitrary opens a dialogue box. Use the slide bar or type in the desired angle. The image will automatically re-size to retain the image data. If you do not want the image to re-size, then uncheck the re-size box



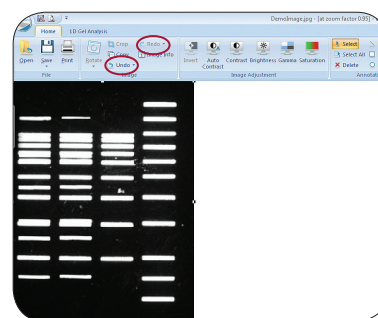
- **Crop** – Use the Crop function to select only a portion of the gel for further analysis. To crop an image click on the crop button. Move the mouse over the area you wish to select, hold down the mouse button, and drag. A dotted white select box will outline the area you are selecting. If you do not like the area selected and the resulting image, click Undo.



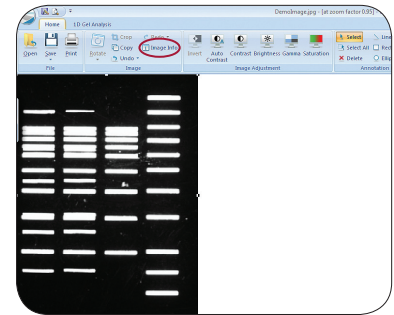
- **Copy** – Copies the image to the clipboard. This allows you to paste your image into other applications. If you have made annotations to your image, make sure they are selected by using "Select All" before you copy your image.



- **Undo** – Undoes the last action performed.
- **Redo** – Redoes an action that has been undone.

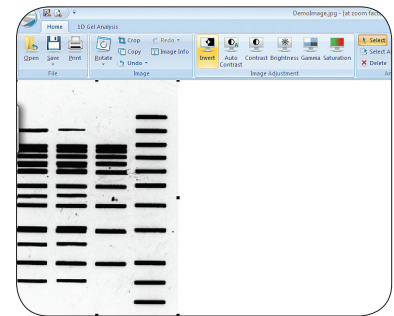


- Image Info – This includes Bits per Pixel, Image Size, Width, and Height.

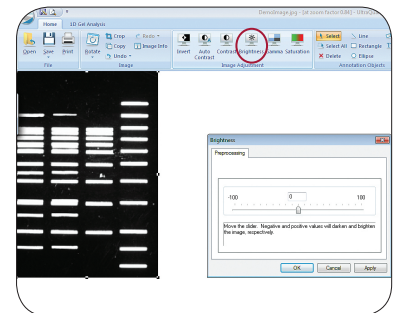
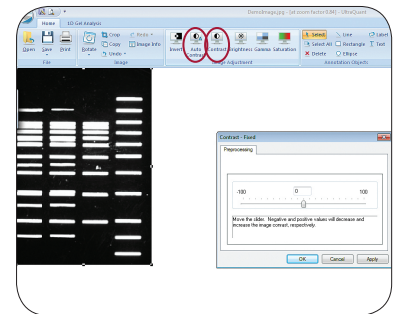


## How to adjust contrast and brightness

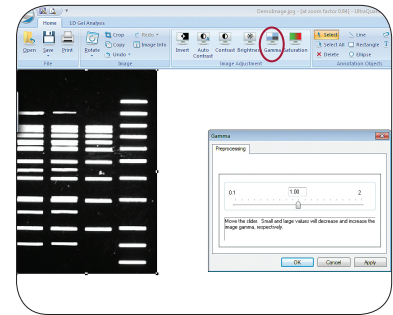
- Image Adjustment Group – Allows you to visually enhance the image.
  - Invert – Reverses the black and white levels in the image.



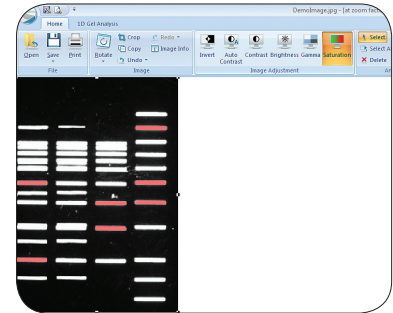
- Auto Contrast – Auto contrast uses an algorithm to adjust the contrast level across the range of the image. Note: if autocontrast is selected, you will not be able to adjust the contrast, brightness or gamma.
- Contrast – Allows you to manually adjust the contrast to the preferred levels.
- Brightness – Adjust the brightness level of the image.



- Gamma – Adjust the fit to the curve of the image data.

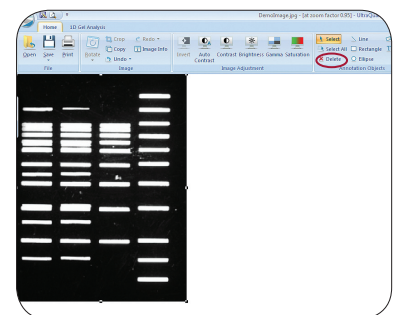
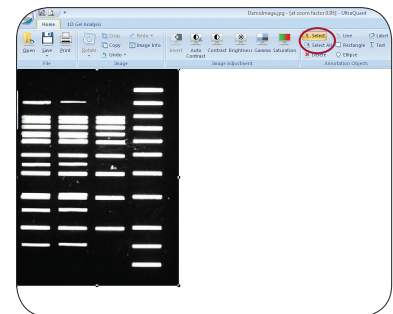


- Saturation – Shows areas of saturation by highlighting the pixels that are saturated in red.



## How to mark up and annotate the image

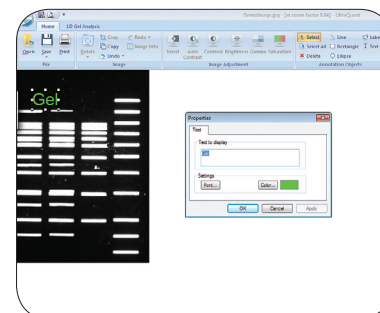
- Annotation Objects Group – Tools for adding and editing annotations on the image.
  - Select – Click on this button to return the mouse to the Arrow Select function. When you have completed adding objects such as Line, Ellipse, or Label, use this function to return to the select arrow.
  - Select All – Select all annotation objects that have been added to an image. This makes it simple to change the color of the objects, or delete the whole series if desired.
  - Delete – This tool deletes selected annotation objects. You can also select the annotation object and hit the “delete” key on your keyboard.



- Annotation Objects include:

- Line
- Rectangle
- Ellipse
- Label
- Text

The label tool allows you quickly numerically label objects or lanes in your image. Select label, and then click on your image where you would like to place the first label to be placed. The label settings dialog box will pop up. To label an object starting with 1, and in increments of 1, hit “ok” to use the default settings. To start at a number other than 1, change the “From” value. After hitting “ok” your first label will appear on your gel, and the Properties dialog box will pop up. If you want to make changes to the appearance of your label, you can do so in the Properties box. Hit “OK” and your label design will be updated. To continue to use label, simply click on your image where you want the label to be displayed. When you are done labeling, hit “esc” to get out of label, or hit the “label” button again. If you select the annotations you have made, and right click on your image, you will be able to align your labels to the right, left, top or bottom.



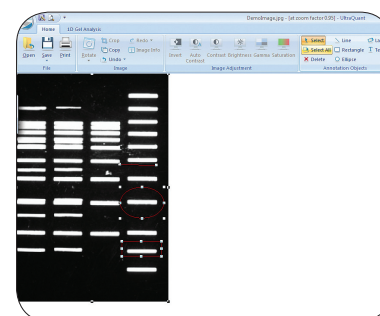
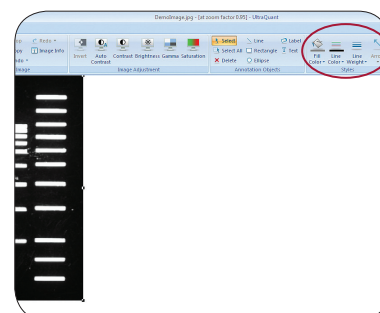
- Styles Menu – Adjust the linearity of the image data.

- Annotation objects can be modified in the following:

- Fill color – Fills inside an object.
- Line Color – Changes the color of the object outline.
- Line Weight – Changes the thickness of the object outline.
- Arrow – Adds an arrowhead to the selected line.

- To select an individual annotation, left-click on it directly. The eight handle boxes will appear around the annotation. These handles allow you to resize the annotation by selecting a handle and dragging it.
- To move an annotation click on it with the left mouse button and drag it to the desired location.
- To delete an annotation, make sure the annotation is selected and press the Delete key on the keyboard or choose Edit | Delete Annotation.
- Each type of annotation has specific features. To edit its features double-click or right-click on the annotation.
- To select multiple annotations, click and hold the mouse at one corner of the region, dragging the mouse to the opposite corner.

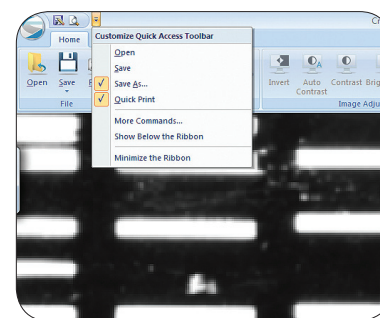
Groups of objects can also be selected by holding down the Shift key and left-clicking on the desired images, or by using the Select All tool.





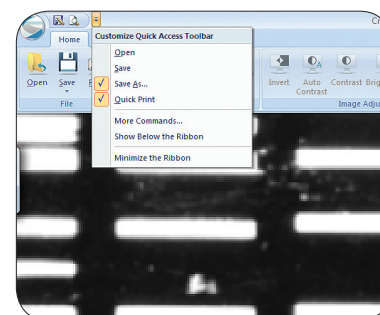
- Quick Access Tools

- The main menu has the options to Open, Save, Save As, and Print.



- The Quick Access Toolbar allows you to choose which options you would like to be displayed across the top. It includes the following:

Open  
Save  
Save As  
QuickPrint

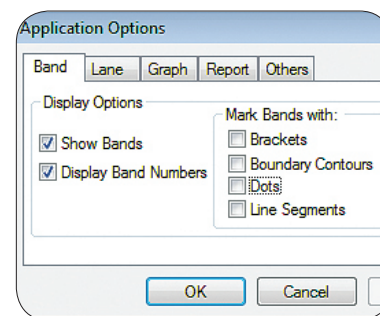


- Or choose More Commands to add any of the following:

Exit

Options, applications options for Band, Lane, Graph, and Report displays, or to create molecular weight marker files

Or create custom keyboard shortcuts for any file option

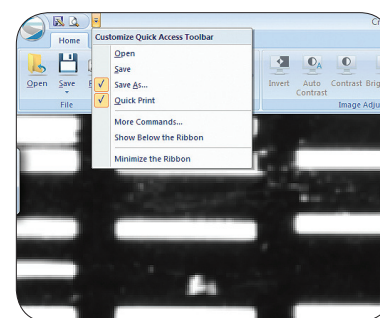


- Additional options include:

Show Below the Ribbon  
Minimize the Ribbon

- Use the +/- buttons in the lower right corner to zoom in or out on a particular image.

- Any preprocessing step you decide you do not like can be easily removed by selecting Undo.



## ID Gel Analysis Tab

### How to perform ID analysis

To analyze an image begin by opening the image file. We recommend that you only analyze .tif images. Before clicking on the analysis tab, it is important to rotate your image so the bands are horizontal, and then crop your image to the size of your gel. Then select the ID Analysis tab and click the Start button.

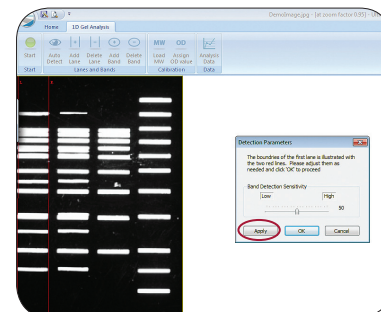
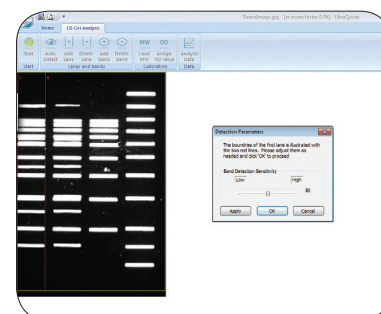
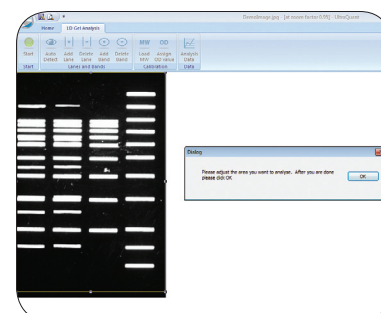
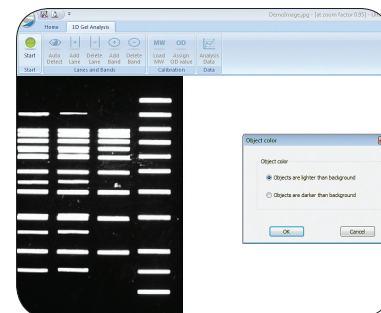
- The software first asks you to select the type of gel you are analyzing – are the bands lighter or darker than the background? Click OK on the dialogue box when done.
- A yellow box will appear around the gel. Adjust the size of the box to the area you are going to work on by clicking and dragging on the corners. Select OK when done. It is important that the yellow box does not overlap with any of your bands of interest.

Continue analysis by selecting Auto Detect.

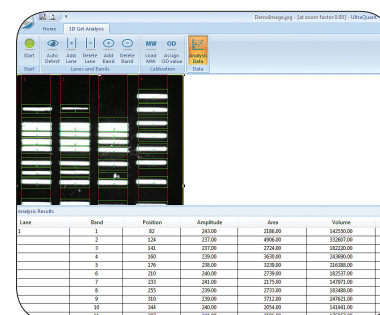
- Two red lane limit lines will appear. Adjust the lane size by clicking and dragging the lines to the width of the first lane in the gel. It is important that the limit lines accurately reflect the width of an average lane. The limit lines should be just on the edges of a lane, and should not overlap with the majority of the bands in the lane.

The “Detection Parameters” box will also appear. You can use this dialog box to set the band detection sensitivity. Hit “Apply” to see if the sensitivity setting picks up all of your bands. Band Detection Sensitivity: If many of your bands are faint, you may want to set the band detection sensitivity higher. If you have a lot of background in your lanes, you may want to lower the sensitivity so that variations in background are not detected as bands.

- You can increase or decrease the band detection sensitivity until you are satisfied, and then click “OK” when done.
- If bands are missed you can use Add Lane and Add Band functions to manually add the objects.

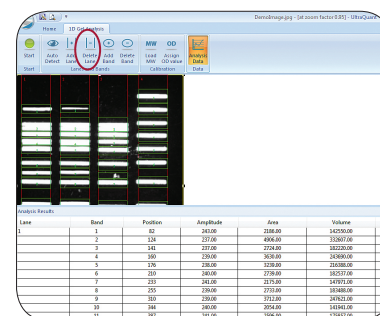
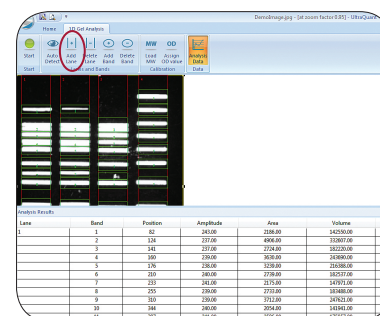


- Analysis Data examines your image and shows you where the lanes and bands are located. Lanes are indicated with a number at the top of the lane. A pair of red lines indicate how the beam runs down the center of the lane. The algorithm reads the pixel brightness information between the red lines. Bands are indicated with small green brackets around each band. Lanes are numbered from left to right, and bands are numbered from top to bottom.



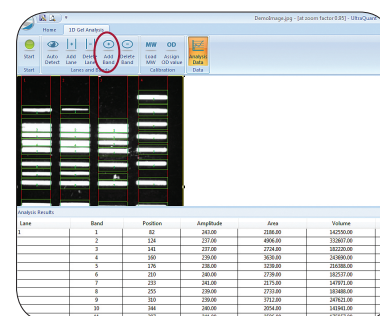
## How to add and delete lanes

- What if it missed a lane?
  - Use the Add Lane function to select the missed lane.
  - Add Lane – Select Add Lane, click on the center of a band in the lane you wish to add.
- What if there is an extra lane?
  - Use the Delete Lane function to delete the extra lane.
  - Delete Lane – Select Delete Lane, click on the center of the lane you wish to delete.



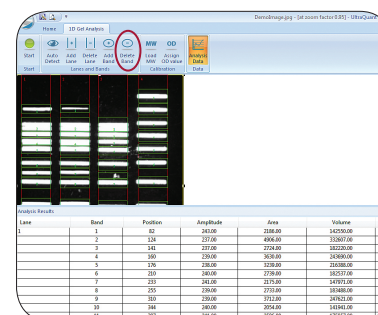
## How to add and delete bands

- What if it missed a band?
  - Use the Add Band function to select the missed band.
  - Add Band – Select Add Band, click on the center of the band you wish to add.



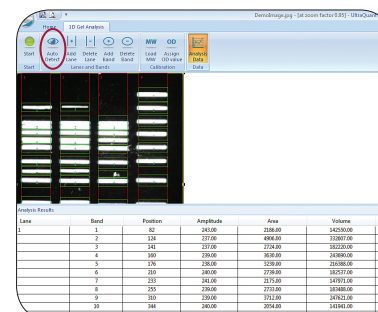
- What if I want to get rid of a band?

- Use the Delete Band function to delete the extra band.
- Delete Band – Select Delete Band, click on the center of the band you wish to delete.



- What if I want to change how lanes and bands are detected?

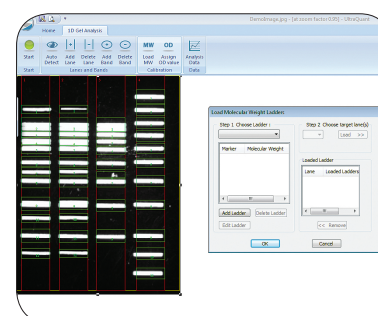
- Click on Auto Detect, adjust sensitivity, and click apply.



## How to load molecular weight markers

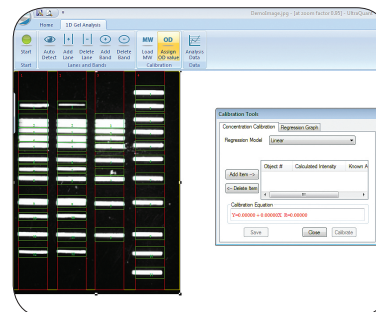
- Use of a molecular weight standard allows you to quantify your data in units such as base pairs or micrograms. You can select a molecular weight ladder from a library of standards previously entered. You can augment the library by creating your own molecular weight ladder.

- Before assigning molecular weight ladders to lanes, make sure that each of your standard lanes have the correct number of bands. Add or delete bands to correct any errors before assigning a molecular weight ladder to a lane.
- To assign molecular weight ladders to a lane, select a molecular weight ladder from the Choose Ladder drop-down list. Or, select Add Ladder to create your own.
- After selecting an appropriate molecular weight ladder from the library, you assign it to a lane by selecting a lane from the Choose Target Lane drop-down list.
- Click on OK to return to your image using the ladders you have selected or click on Cancel to abandon the operation.
- Sometimes certain molecular weight standard lanes have bands that are too close together and are difficult to distinguish. In such cases you should manually identify the bands for which sizes are known by deleting all other bands.



## How to assign an OD value

- OD is a measure of the opacity of a light absorbing medium (e.g. gel, filter, photographic transparency). Optical density is a logarithmic function representing the darkness (blackness) of a material.
  - Low density value (e.g.  $D = 0.04$ ) represent lighter areas, high density values (e.g.  $D=3.0$ ) darker areas.
  - Because OD is logarithmic, the perceived difference between 0.04 to 0.10 is much greater than the difference between 3.0 and 3.1. This matches the human eye, which can recognize subtle changes better in dark areas than in light ones.
  - Mathematically, density is defined as:  $D = \log(O) = \log I/T = \log I/R$ , where:  $D$  = Optical Density,  $(O)$  = Opacity,  $T$  = Transmission, and  $R$  = Reflectance.
  - To add OD values to your data, click “Assign OD value”. To add an OD value, click “Add Item.” Then, use your mouse to select your band of interest. Once you have selected a band, a dialog box will appear. In the “known amount” box, enter your OD value, then hit OK. Enter at least two values to use this tool. Select “Calibrate” when finished. The data will be updated in the Analysis Results table.



## Analysis data

- To view the analysis data click on the Analysis Data button. The Report will appear below the image.
  - Report
    - Right click and select All lanes to view all data.
    - Multiple Lanes opens a dialogue box where you can select just the lanes of interest.
    - Export to Excel. Copies the analysis data and exports it in Excel format.
    - Export to Text File. Copies the analysis data in text format to be pasted into Word or other text programs.
  - Graph – Shows the analysis data in graph format. To access the graph, click the “Graph” tab below the report table.

## Reports and graphs

- Position 0 corresponds to the top of the analysis area, while the largest position value corresponds to the bottom of the gel. Intensity is shown on the vertical axis. Each band appears as a peak on the graph, and the amount of signal from the gel is quantitated based on the area under each peak. For optimum accuracy, UltraQuant takes the background for each peak to be the average of the flat areas on either side of the peak.
- The baseline underneath each peak is the background intensity of the lane before and after each band; it is shown as a blue line for each peak. Although the software attempts to calculate the best baseline for each peak, the user has complete control over increasing or decreasing the baseline to appropriate levels. To do so, click and hold the left mouse on the red dots on either end of the baseline and drag the line up or down. It is important to visually inspect the baseline of each peak and modify them accordingly before generating the final report. Changes in the baseline movement and area under the curve calculation are automatically registered in the report.
- You may display multiple lanes simultaneously by right-clicking the mouse on any part of the graph to pop up a menu that lists all of the lanes. Select the Multiple Lane option and then the first lane you would like displayed. Repeat these steps for each additional lane you would like to display.

