Image Lab™ Software for the GS-900™ Densitometer

Quick Start Guide

Catalog # 170-9690

BIO-RAD

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Procedure for Image Acquisition and Analysis

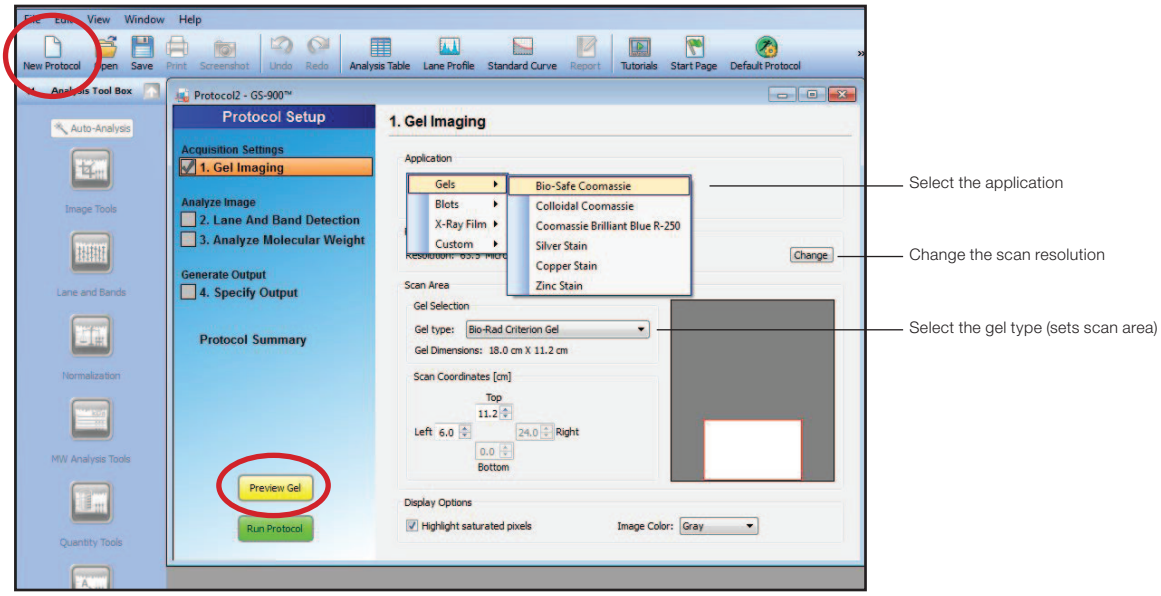
Overview

The procedure described here is for first-time gel imaging and analysis with the GS-900™ densitometer. It involves the following five steps (click for details):

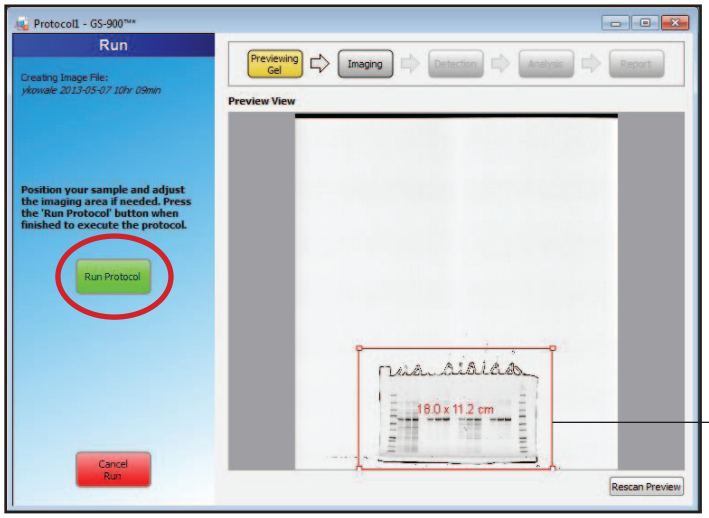
1. Protocol setup and acquisition
2. Image adjustment
3. Lane and band detection
4. Molecular weight analysis
5. Result and report generation

1. Protocol Setup and Image Acquisition

1. Click **New Protocol** to open the Protocol Setup window. Set the acquisition settings, then click **Preview Gel** to preview your gel image.



2. In the Preview view, select the region to scan by dragging the edges and corners of the red box. Click **Run Protocol** to scan your gel.



3. The gel image appears, and the options in the Analysis Tool Box (at left) are activated. Use these options to adjust the gel image and to detect and analyze gel lanes and bands.

Note: At any time during image adjustment and analysis:

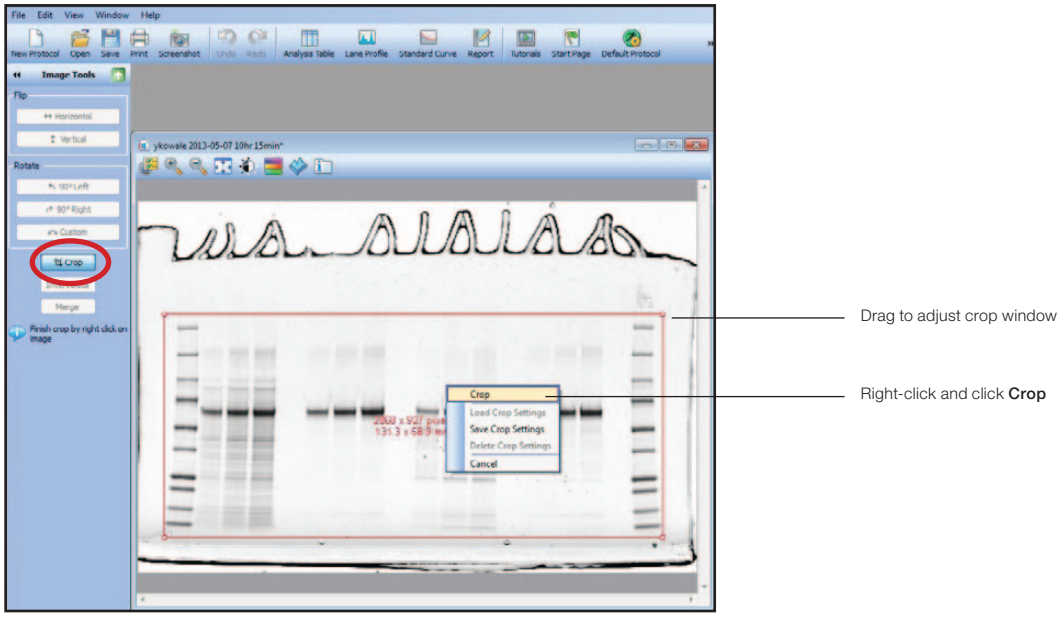
- Click **Undo** in the header to undo any changes.
- Click **View > Operation History** for a list of all actions performed during image adjustment and analysis. To revert to a previous state, click on the action.

The Operations History and Undo capabilities are cleared each time the file is saved.

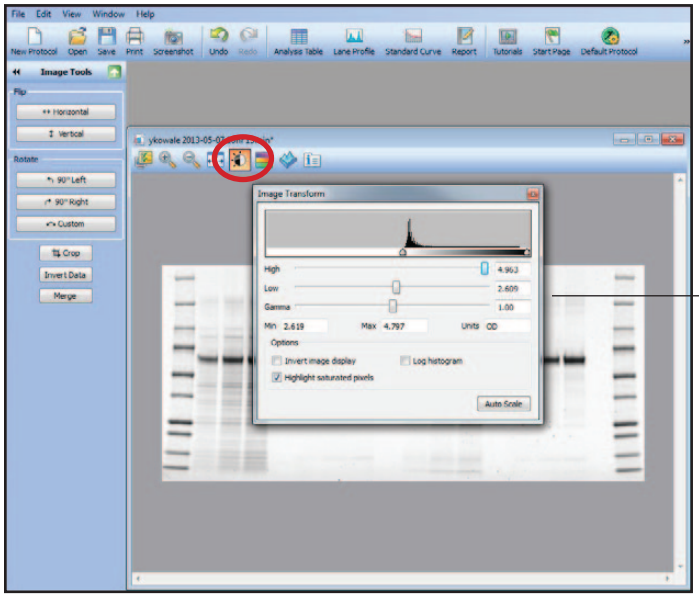
2. Image Adjustment

In the Analysis Tool Box, click **Image Tools**. Use these tools to flip, rotate, or crop the gel image. To return to the Analysis Tool Box, click the arrow.

1. To crop a gel image, click **Crop** and adjust the red box to the desired crop window. Right-click within the box and click **Crop** to crop the image.

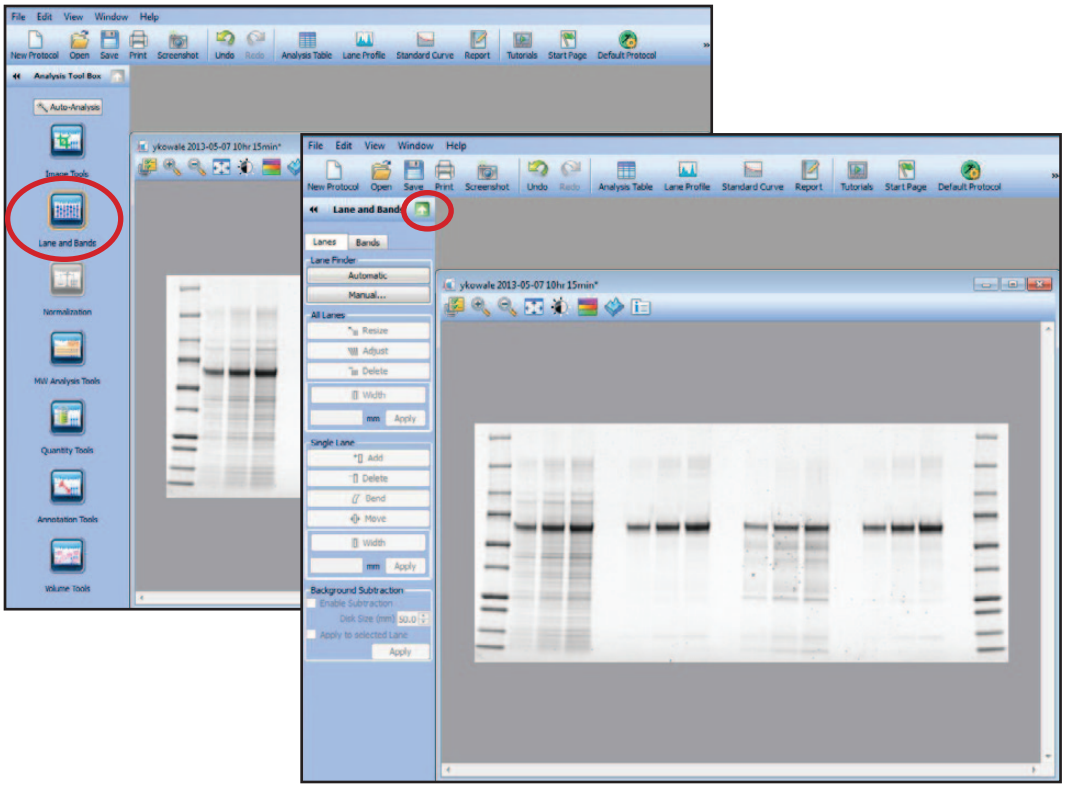


2. To adjust the image contrast or brightness, click the **Image Transform** icon. Use the High, Low, and Gamma sliders to optimize the image for presentation. The image transform has no effect on the data; it changes only the appearance of the image.



3. Lane and Band Detection

In the Analysis Tool Box, click **Lane and Bands** to access a set of lane and band detection tools. To return to the Analysis Tool Box, click the arrow.



3.1. Lane Detection

1. In the Lane tab, under Lane Finder, click **Automatic** to automatically detect lanes.

   a. To add a lane, click **Add**. The new lane is highlighted in red. Drag the lane to its new position, then right-click to set its position.

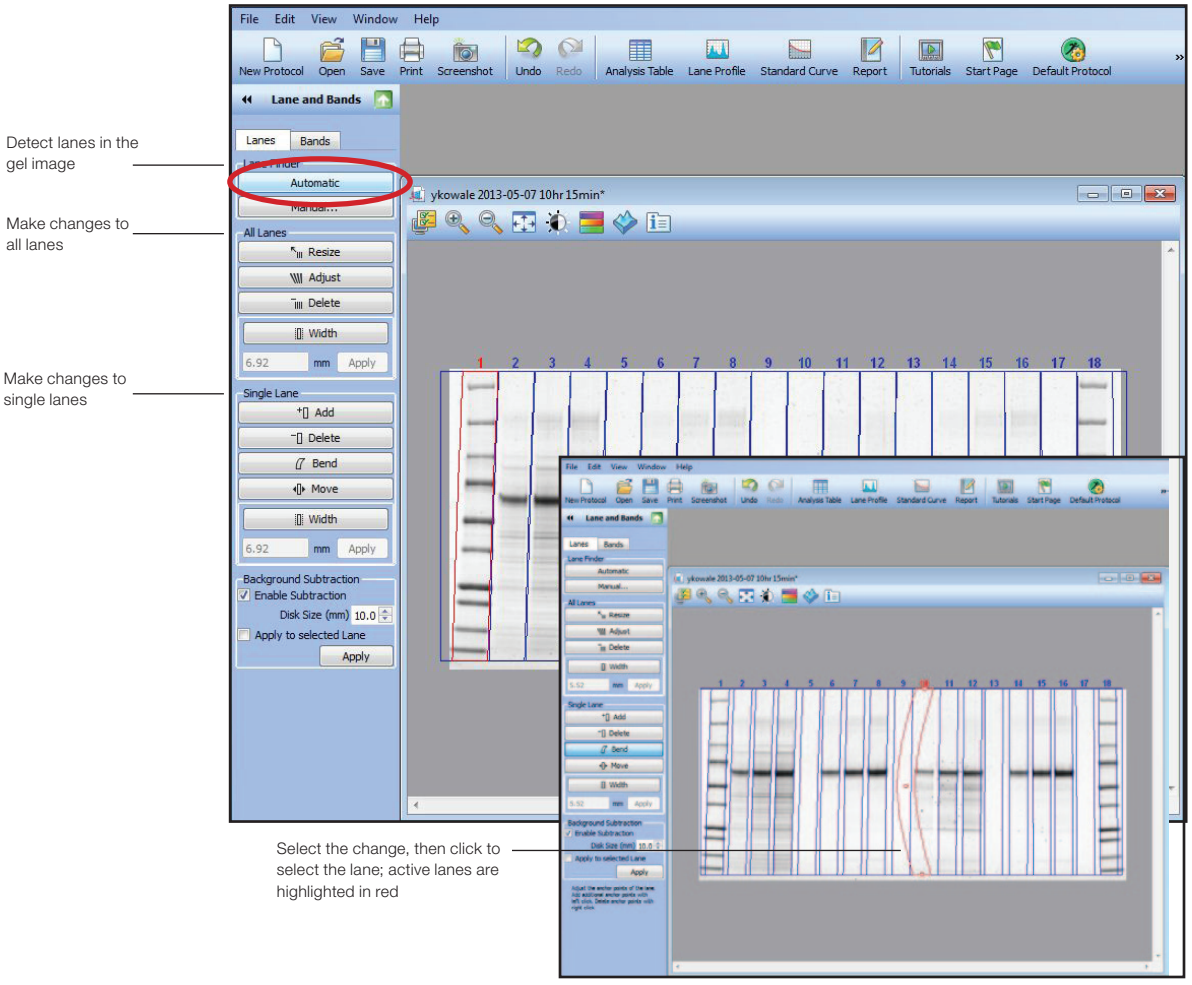
2. Options to adjust All Lanes or a Single Lane are enabled. Click outside the gel image to set any changes.

   a. To resize or adjust all lane frames proportionally and at the same time, click **Resize** or **Adjust** (under All Lanes). Drag a corner or side of the frame to make the desired changes.

   b. To adjust the position of a lane, click **Move** (under Single Lane). Select the lane and move it to its new position.

   c. To adjust the curvature of a lane, click **Bend** (under Single Lane). Click within the lane and drag points at the top, bottom, or center of the lane to adjust its curvature.

   d. To adjust the width of lanes, click **Width** under (All Lanes or Single Lane) and use the boxes to define the lane width. Alternatively, define the lane width in the mm box and click **Apply**.



3. Perform background subtraction using the options in the Background Subtraction box.

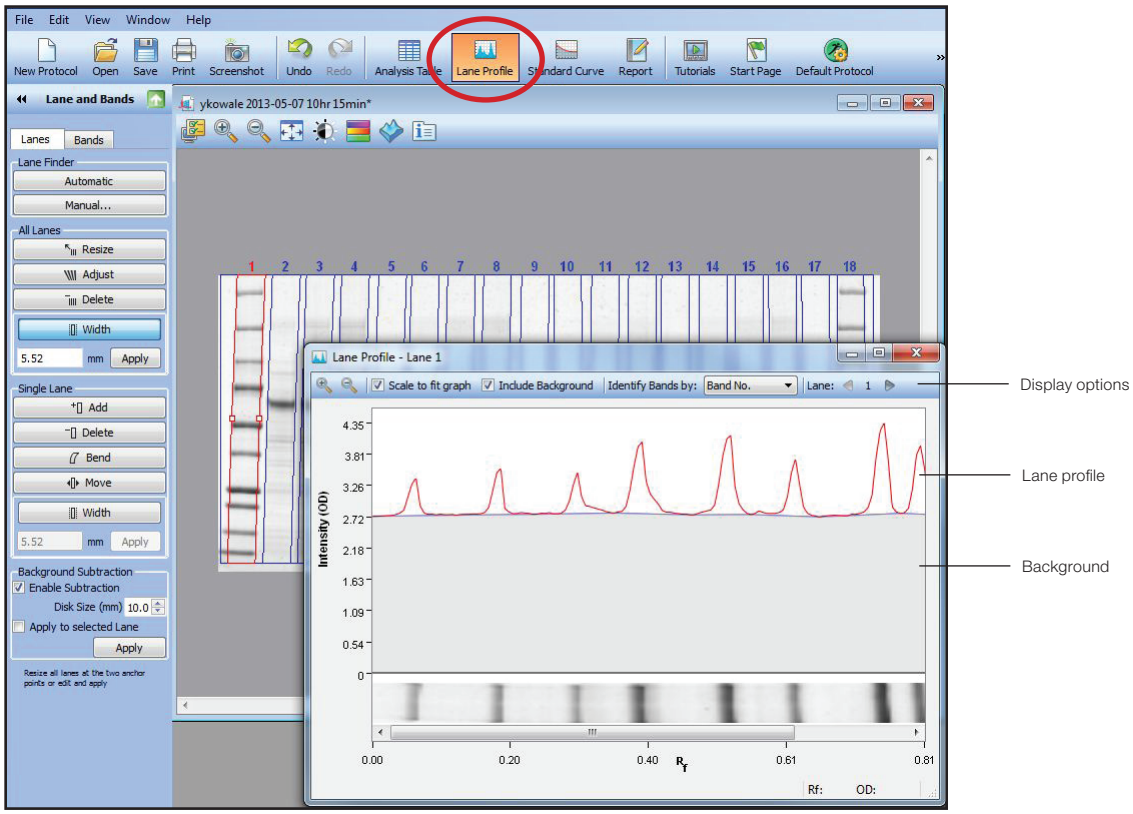
   a. Define the size of the rolling disk used to determine the amount of background subtracted from your lanes. Set the disk size in mm (1–99; reduce the size to subtract more background). Click **Apply**. To adjust the background for a single lane, select the **Apply to individual lane** checkbox.

   b. To visualize the background in a lane, click **Lane Profile**. In the Lane Profile window, the profile appears in red (OD, intensity) and the background in gray. Use the options in this window to include/exclude the background and view other lanes.
   Click to select a lane within the gel image; the selected lane is highlighted in red.

   c. To convert disk size between Image Lab (in mm) and Quantity One® (in pixels) software, use the following equations:

   Image Lab disk size = (Quantity One disk size x 4) / (1,000/Image Lab scanner resolution)

   Quantity One disk size = (Image Lab disk size x 1,000 / Image Lab scanner resolution) / 4



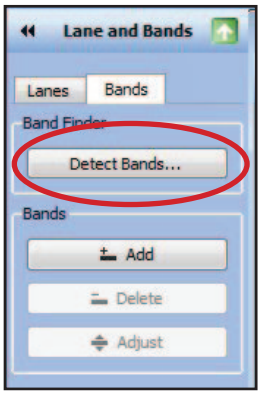
3.2. Band Detection and Analysis

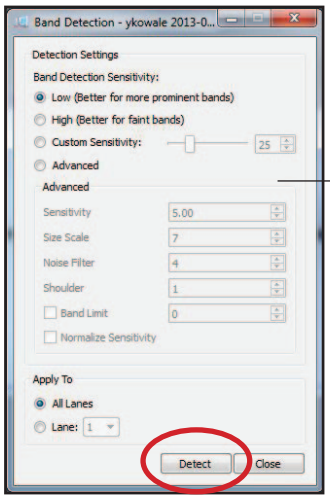
With lanes and background determined, use the options under the Bands tab to detect and analyze bands.

1. Click **Detect Bands...** to open the Band Detection window:

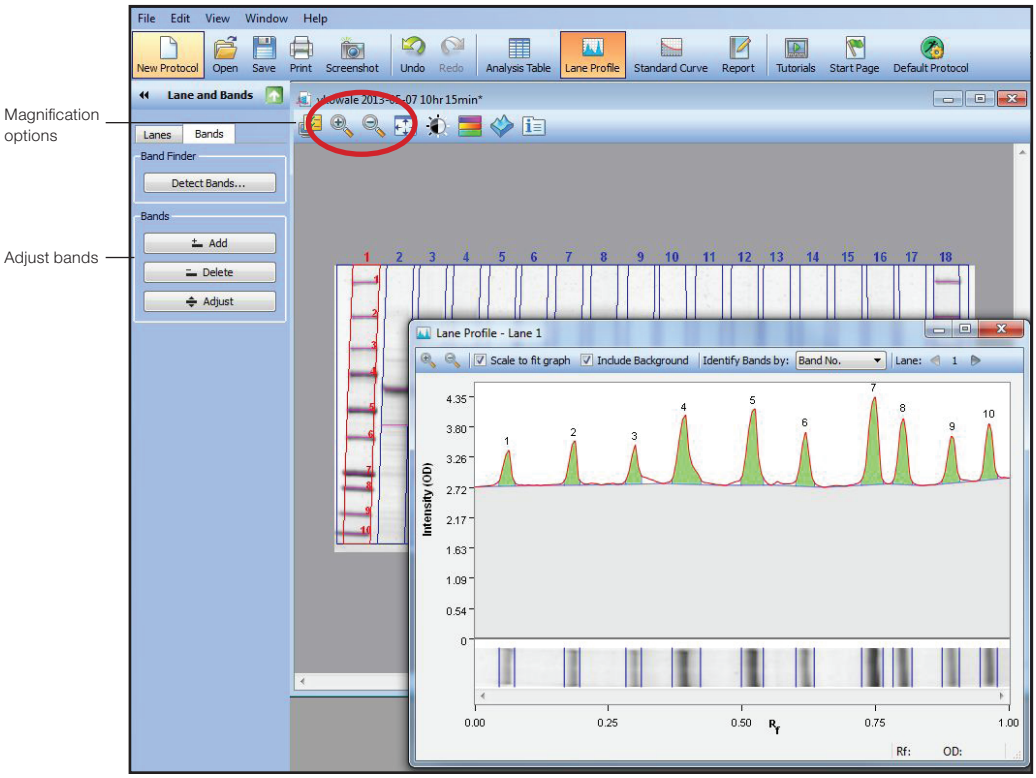
   a. Set detection sensitivity using preset or custom options. Band detection in Image Lab is controlled by a single sensitivity parameter (1–100), which is a composite of the various parameters from Quantity One.

   b. Apply the sensitivity setting to all or individual lanes using the options under Apply To, then click **Detect**.





Set detection sensitivity



Magnification options

Adjust bands

2. The detected bands are highlighted by red lines on the gel image and as green areas in the Lane Profile window. Use the options in the Bands field to make adjustments to the detected bands. These adjustments affect the areas used for determining the peak volume and subsequent quantification.

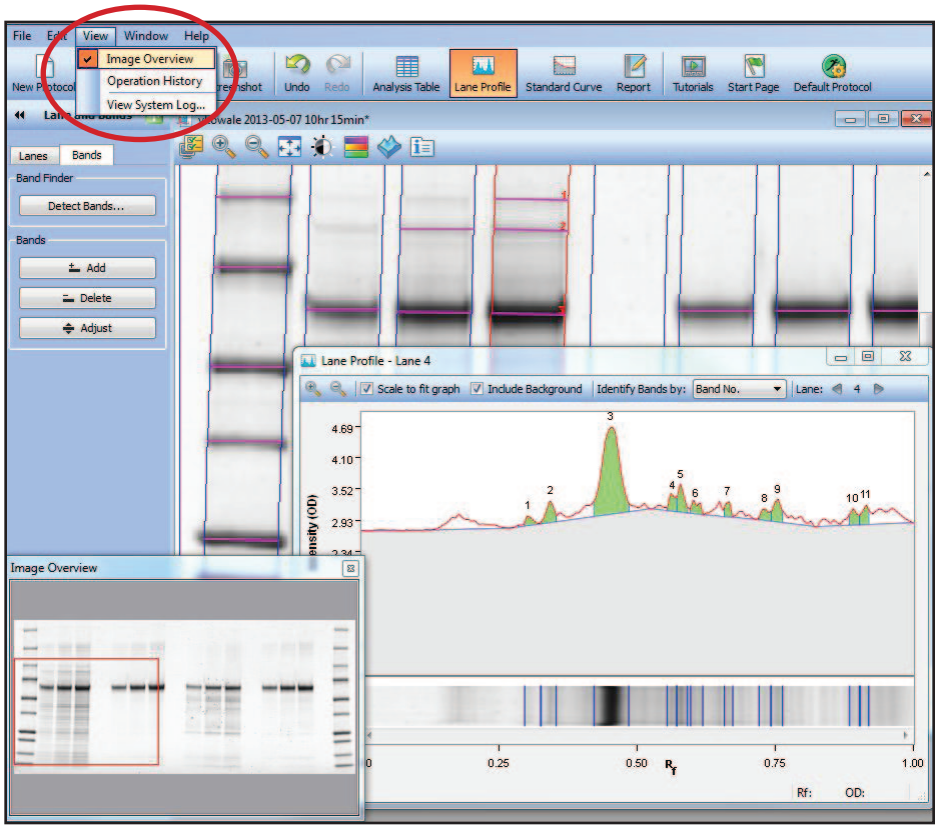
   a. To add or delete bands, use the **Add** or **Delete** options. Click on the desired region on the gel image to add or delete a band.

   b. To adjust the regions selected as bands:

      1) Zoom in on the region in the gel image or lane profile. Use the magnifying glass options in the header, or select a region by right-clicking and dragging to define the area (highlighted in blue). Right-click inside the image to revert to the original view.

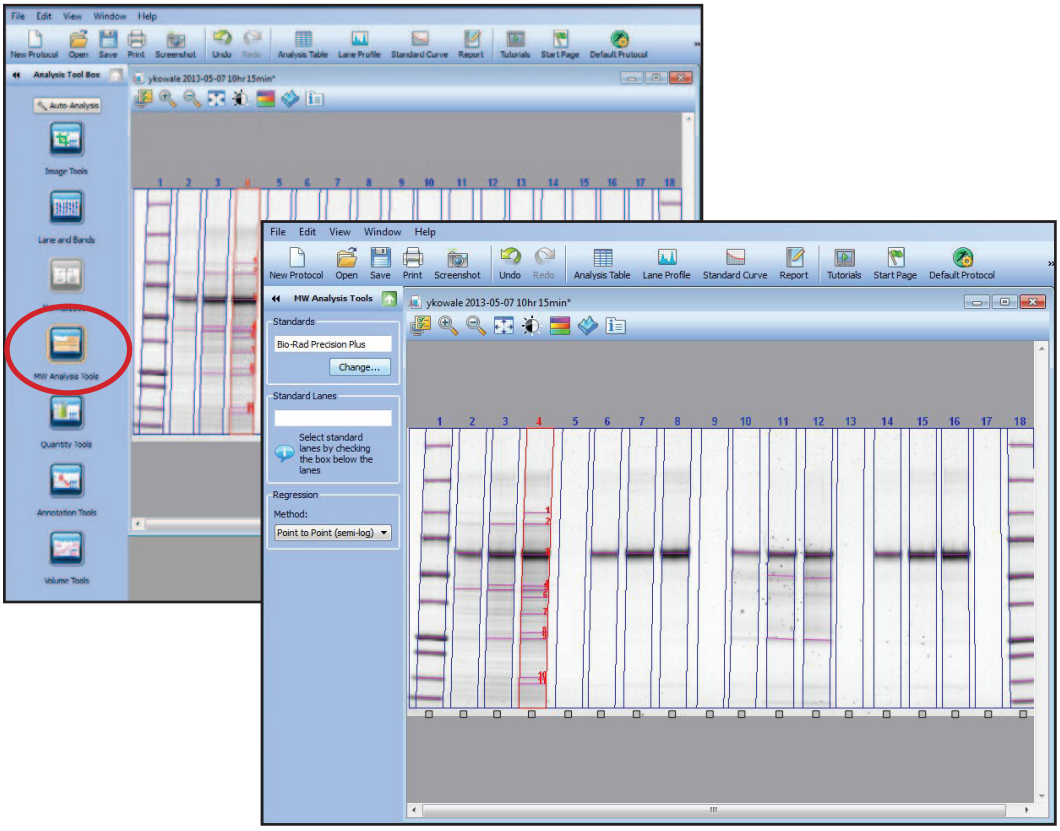
      2) Click **Adjust**. Adjust the band by selecting and dragging either the dashed line on the gel image or the blue line in the lane profile window.

   c. Click **View > Image Overview** to open an overview of the entire gel image in a separate window.

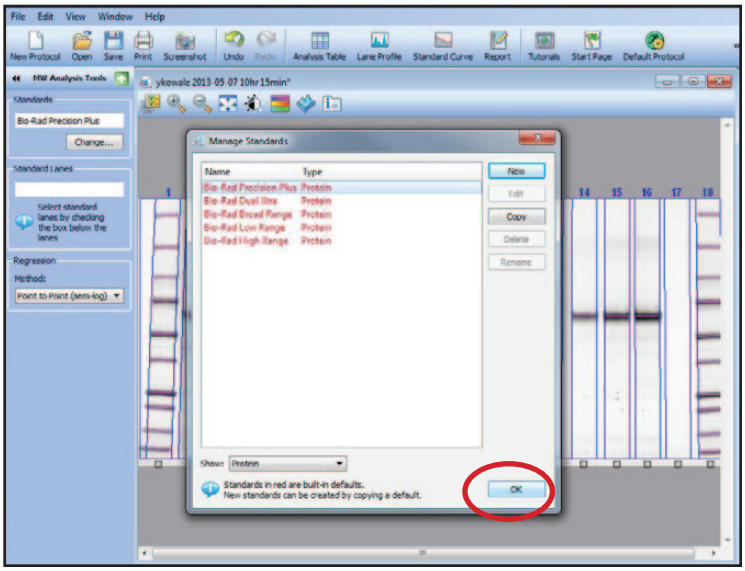


4. Molecular Weight Analysis

In the Analysis Tool Box, click **MW Analysis Tools**. Use these options to select the protein standard and regression method used for mass determination.



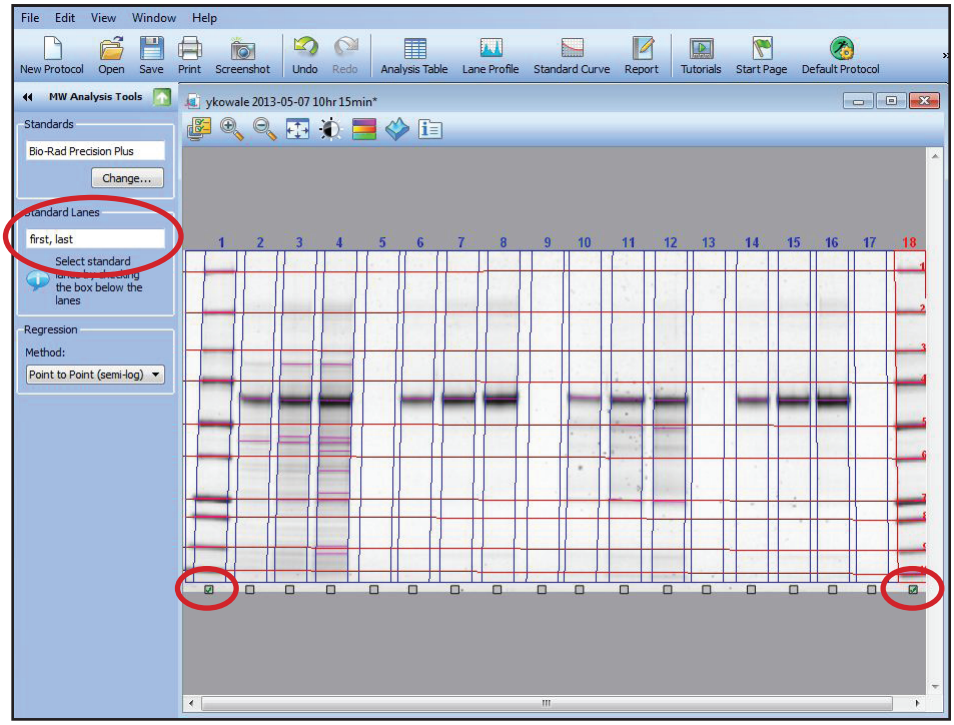
1. Select the protein standard used. Under Standards, click **Change...**. In the Manage Standards window, select the protein standard used in your gel and click **OK**. If the standard does not appear, click **New** to create and save the new standard.



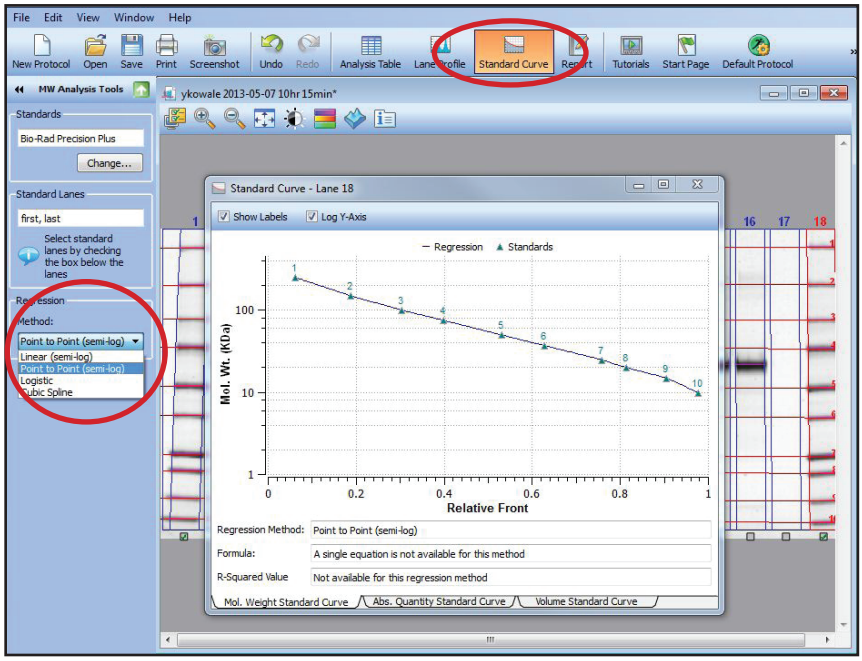
2. Identify the lane(s) containing the standard. Under Standard Lanes, either:

   a. Select the box at the bottom of each standard lane in the gel image, or...

   b. Enter the number of the lane(s) in the Standard Lanes window (for example, “first” to designate first lane, “last” to designate last lane, or numerals indicating the lanes).



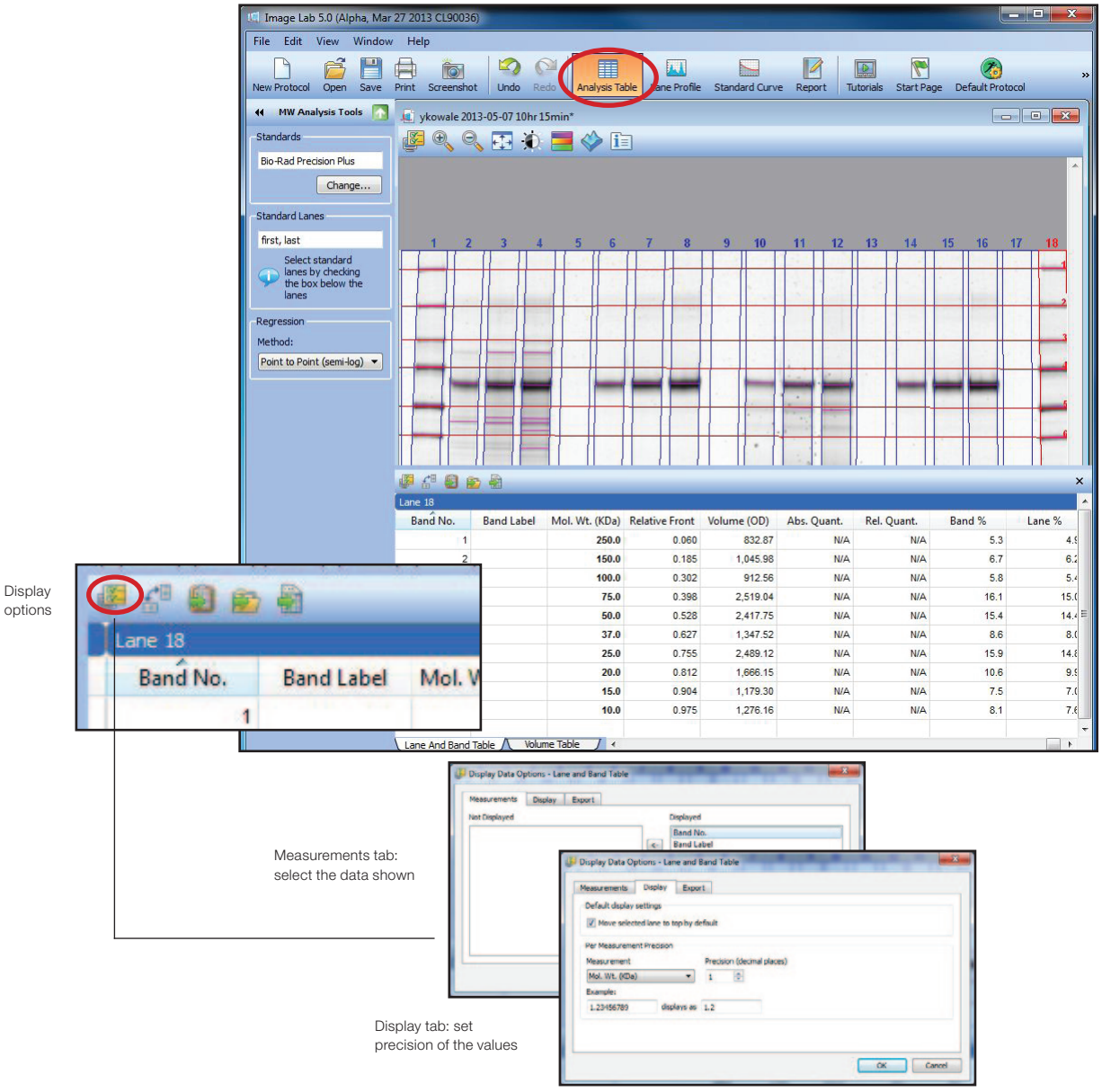
3. Select the regression method for estimating molecular weight. Review the results by clicking **Standard Curve** in the menu bar.



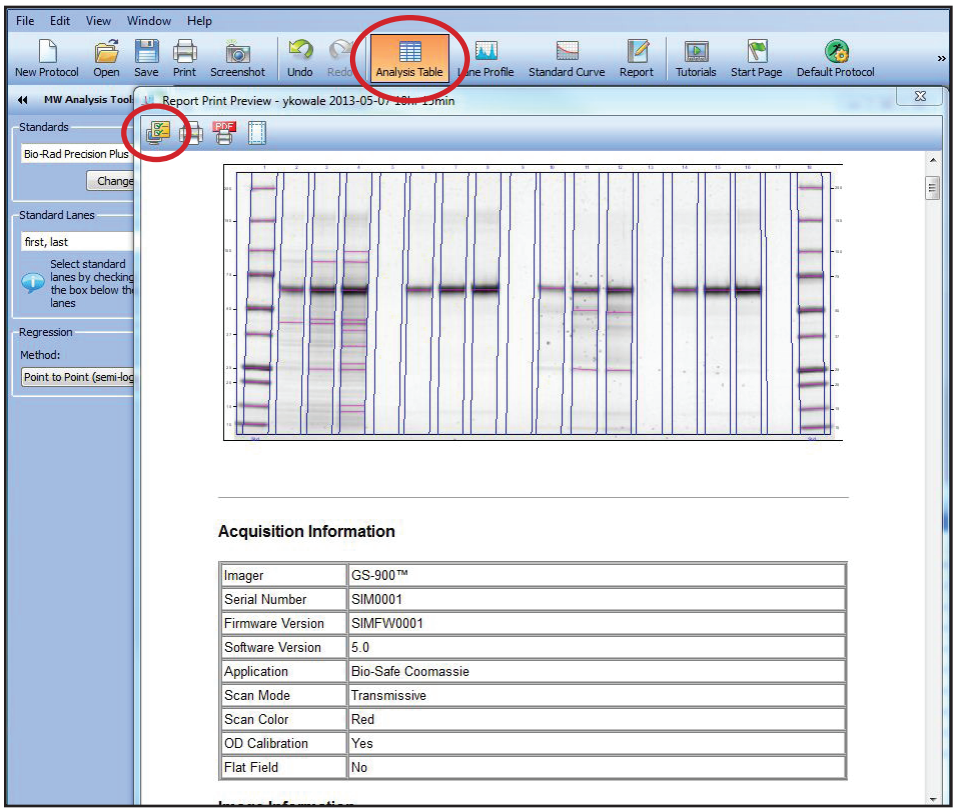
5. Result and Report Generation

1. Click **Analysis Table** in the menu bar to open a table of analysis results. Scroll through the data, or click in the gel image to view the data for a lane.
2. Click the **Display Options** icon to customize the data shown.
3. To export the data, use the other options in the table header.

Note: To obtain results with Image Lab software that are equivalent to those obtained with Quantity One (for example, a purity analysis defined by percent band), use the same background subtraction, band detection, lane width, and band integration as defined in the lane profile window (described above).



4. To generate a report containing the gel image and its associated data, click **Report** in the menu bar. Click the **Display Options** icon to select the items displayed in the report; click **OK** to regenerate the report with the new selections.



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Bulletin 6421 Rev A US/EG *0513 Sig 1212*