Application Notes

2-Dimensional Gel Analysis Application

Background

Two dimensional electrophoresis is an important protein assay technique, combining SDS gel electrophoresis and iso electric focusing. Together, these methods of separating protein samples allow the resolution of more protein species than any other current analytical procedure. Researchers have also found two-dimensional gel electrophoresis to be an invaluable tool in the study of dynamic molecular pathways and regulatory mechanisms related to grwth, production and disease.

2-D gel electrophoresis combines high resolution isoelectric focusing in one dimension, in which proteins in solution are sepatated in an electric field on the basis of their net charge. Gel electrophoresis in the presence of a charged detergent is used in an orthogonal dimension. Proteins are seived in a polyacrylamide gel matrix approximately according to molecular mass in the second dimension.

Autoradiography is the most sensitive and quantitative of the methods available for detecting and analyzing separated proteins. In autoradiography, x-ray film is brought into contact with a radiolabeled gel where it essentially performs the same function as an electronic scintillation counter. In addition it preserves the spatial information of proteins separated by 2-D electrophoresis.

Cross-comparison of protein spots on different gels requires that a consisten system of coordinates be described in which to express the postitions of proteins whin the gels. Transformation to a coordinate reference system representing pH in one dimension and molecular weight in the second dimension, allows comparison of data from different gels.

Landmark proteins within the gel images are used for these coordinate transformations by measuring x and y position and calibrating to the known pH and molecular weights. Molecular weight and pH markers are commercially available which can be run in a gel along with a sample to provide accurate landmarks. Or, proteins familiar to the researchers, recognized by distinctive local spot patterns, may also be used as landmarks.

After density and coordinate calibration, individual protein spots are quantified by summing the counts per minute for all pixels corresponding to each spot. These summed values provide an index of individual protein abundance. Spot are segmented in one of three ways:

- hand drawn outline of each spot
- individual edge detection based on intensity threshold
- automated object finding based on intensity threshold

In some cases, hand drawn outlines are preferable to thresholded segmentation in cases of high level of unfocused background polypeptides which obscure spot boundaries, or overlapping protein spots.

2-Dimensional Gel Analysis Work Session Flow

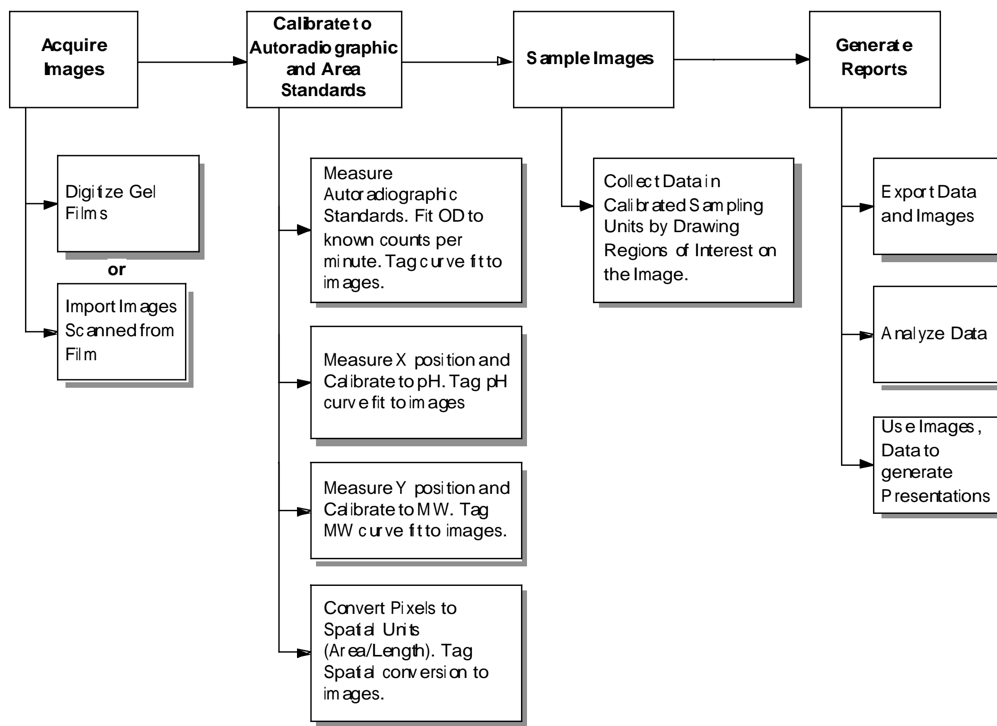


Figure 1 2-Dimensional Gel Analysis Work Session

The steps for measuring whole body autoradiograms or images from phosphor scanners are basically the same.

1. Images are brought into the system by digitizing equipment or by opening images from disk.
2. Standards are measured and calibrated to known counts per minute. The x postions of landmark proteins are measured and calibrated to known pH values. The y positions of landmark proteins are measured and calibrated to known molecular weights. A known area or length is measured for calibration to real world units.
3. Images are sampled by manually or automatically drawing regions of interest on the image. Sampled areas are automatically fit through the linked calibration curve fits and counts per minute, pH, molecular weight, and area are derived for each protein spot.
4. Data can then be exported to statistical programs for analysis, and pictures exported for reports.

Setup 2-D Gel Analysis Application

Configure the Tables

- Select File-Table Define-Calibration table to load a calibration table template with the appropriate rows (sample mean, input, known values, and calculated columns).

This step is repeated for each of the three calibrations we do for 2-D Gel analysis: horizontal calibration to Molecular Weight, vertical calibration to pH, and density calibration to autoradiographic standards (dpm). Each different calibration needs different measures for the sample column: x position, y position, and sample mean.

- Select File-Table Define-Sample Table to load a sample table template with appropriate rows (sample mean, x position, y position, and area.)

Load and Display Images

- Select Images… from the main menu to display the Image Selection Window.
- Click the Open button and select the gel and ruler images to open.
- Click the image thumbnail to highlight and click the Display button to display in the Image Window.

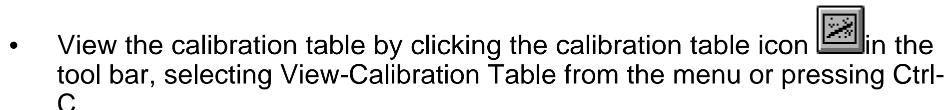
Calibrate Images

When working with 2D gels, we will be doing 4 separate calibrations:

- Standards are measured and calibrated to known counts per minute.
- The x postions of landmark proteins are measured and calibrated to known pH values.
- The y positions of landmark proteins are measured and calibrated to known molecular weights.
- A known area or length is measured for calibration to real world units.

We will then tag the image with the curve fit for each calibration, with the area conversion being combined with the tag for the molecular weight curve fit. Tagging images provides a facility for saving multiple curve fits with images. This allows us to tag the gel with curve fits for CPM, pH, molecular weight and area and derive all three values at one time in the sample table.

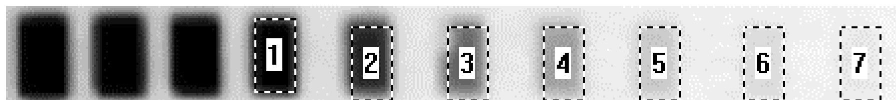
Calibrate Standards to Counts per Minute

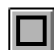
- View the calibration table by clicking the calibration table icon  in the tool bar, selecting View-Calibration Table from the menu or pressing Ctrl-C.

Sample the Standards

- Select the a sampling tool from the toolbar. We used the rectangle tool for this example.

For each standard:

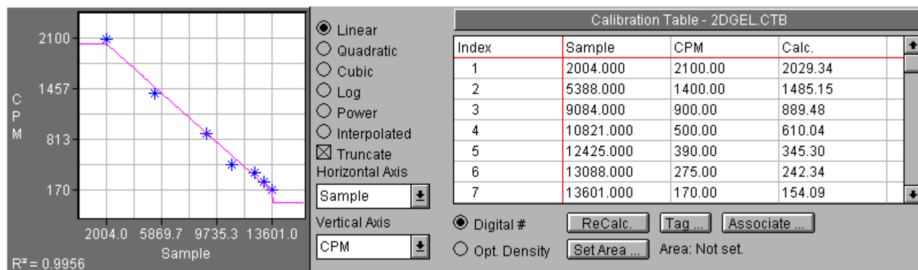




Click and drag the shape of the rectangle and click the right button to accept.

Calibrate Measured Standards to Known CPM Values.

- Double click on cells in the CPM column to input the counts per minute for each standard.
- Select the sample value column to plot as the horizontal axis
- Select the CPM column to plot as the vertical axis
- Select a curve fit based on R^2 value and calculated values in the table.



Tag Images with CPM Calibration Curve

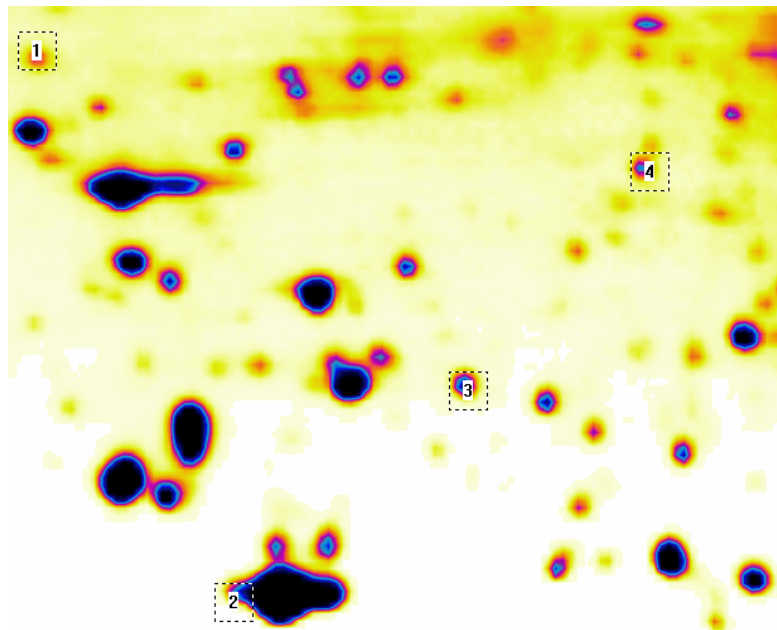
- Click the Tag button in the calibration window. Highlight the gel image to tag in the Image Selection Window and click Select.

Calibrate X Position to pH

Sample Landmark Proteins

- Select the a sampling tool from the toolbar. Fixed Box works well. Set the box size at 10 x 10.

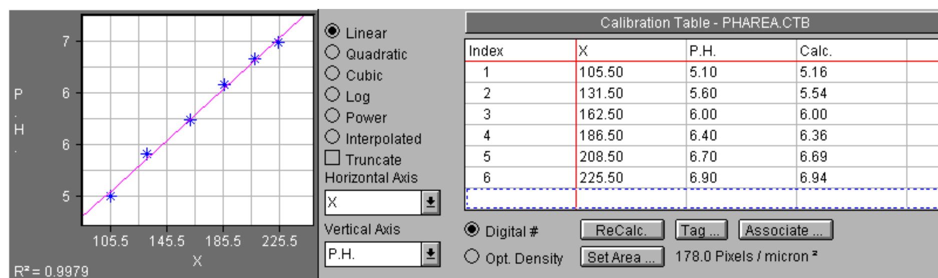
For each landmark protein:



Click and drag to place boxes at locations of known pH and click the right mouse button to accept.

Calibrate Measured X Position to known pH Values.

- Double click on cells in the pH column to input the known pH values for each standard.
- Select the sample value column to plot as the horizontal axis
- Select the pH column to plot as the vertical axis
- Select a curve fit based on R^2 value and calculated values in the table.



Index	X	P.H.	Calc.
1	105.50	5.10	5.16
2	131.50	5.60	5.54
3	162.50	6.00	6.00
4	186.50	6.40	6.36
5	208.50	6.70	6.69
6	225.50	6.90	6.94

Tag Images with pH Calibration Curve

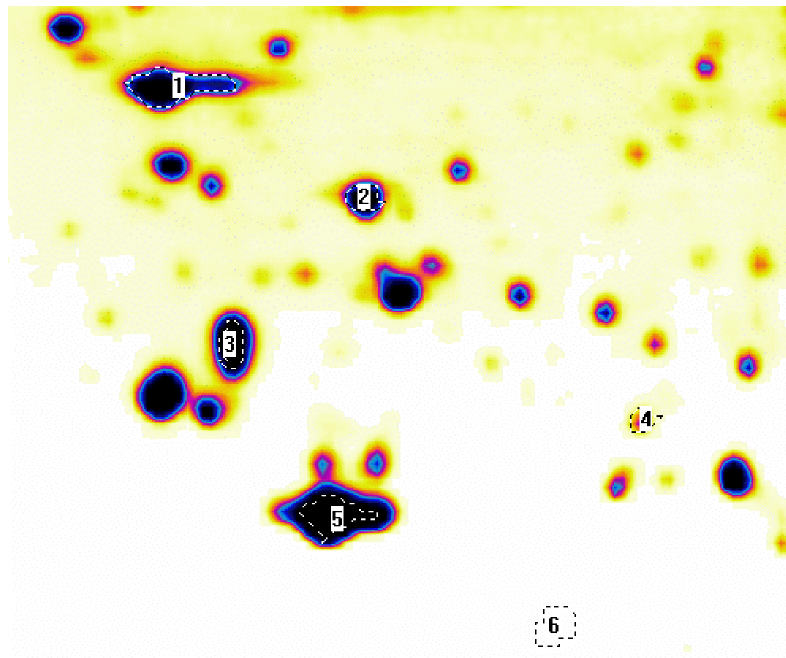
- Click the Tag button in the calibration window. Highlight the gel image to tag in the Image Selection Window and click Select.

Calibrate Standards to Molecular Weight

Sample the Landmark Proteins

- Select the a sampling tool from the toolbar. We selected the edge tool. Set the edge options to user-defined point and bright field image.

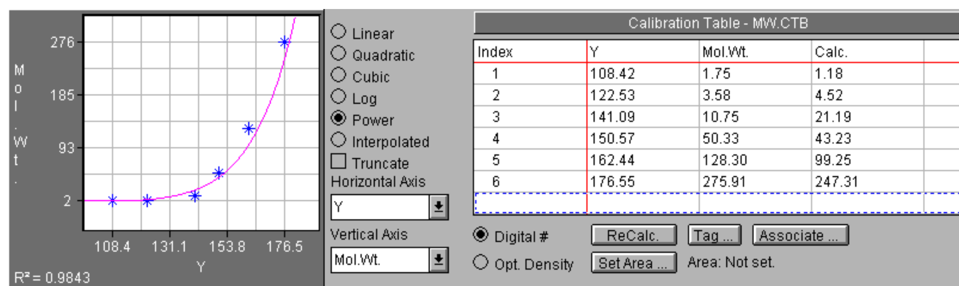
For each landmark protein:



Click to place points using the edge tool and click the right button to accept,

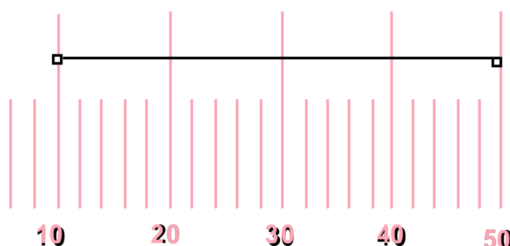
Calibrate Measured Standards to MW.

- Double click on cells in the MW column to input known molecular weight for each standard.
- Select the sample value column to plot as the horizontal axis
- Select the MW column to plot as the vertical axis
- Select a curve fit based on R^2 value and calculated values in the table.



Area Calibration

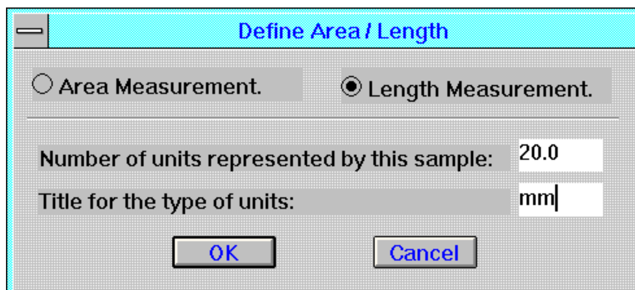
- Display an image with an object of known area or length. Highlight the image in the image selection window and click the Display button.
- Click the Set Area ... button in the calibration Window
- The instruction message is displayed beneath the image. Do not click the Cancel button unless you want to cancel the Set Area process.
- Select a sampling tool from the toolbar. In this example we selected the Line tool.
- Draw the shape of the known length or area. Here, we clicked the endpoints of the known ruler length. Click the right mouse button to finish the sample



Using one of the sampling tools, define a known area or length on the above image.

Cancel

- Select Area or Length measurement in the dialog box.
- Enter in known size and units of the sample and click Ok.

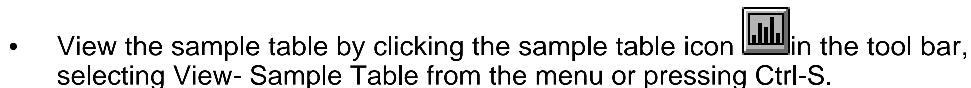


- The area conversion factor will be displayed next to the Set Area button.

Tag Images with MW Calibration Curve and Area Calibration

- Click the Tag button in the calibration window. Highlight the gel image to tag in the Image Selection Window and click Select.

Sample Images

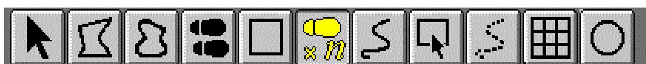


- View the sample table by clicking the sample table icon in the tool bar, selecting View- Sample Table from the menu or pressing Ctrl-S.

Sample the Image

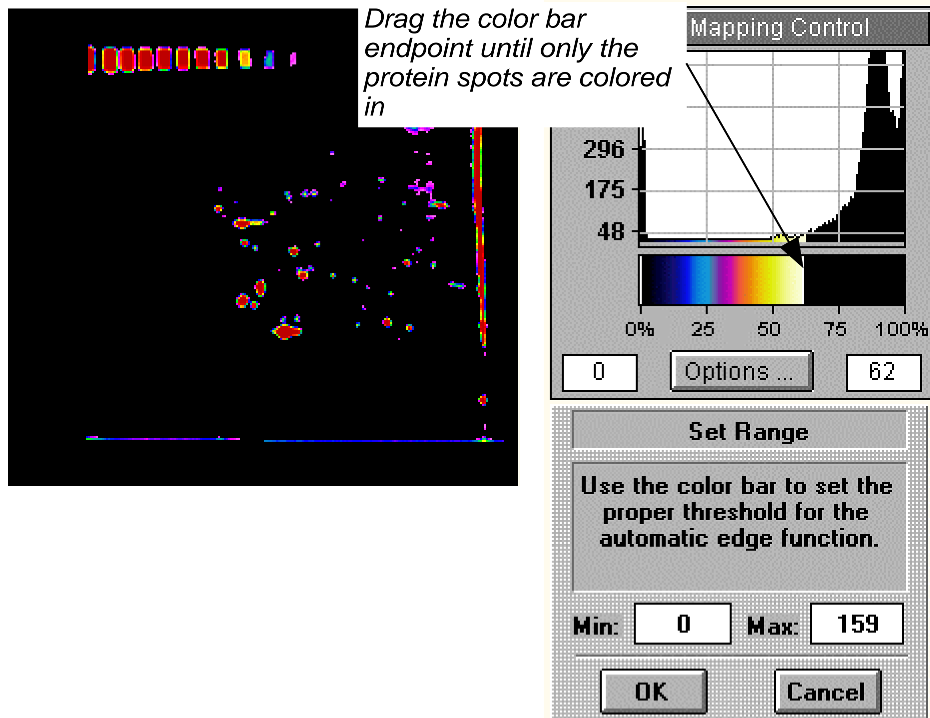
Select a Sampling Tool

Select the auto object finding sampling tool from the toolbar.



Set the intensity range for the auto object finding tool.

Highlight the image in the Image Selection Window and click the Range button



Drag the endpoints of the color bar in the image mapping control until only the protein spots, and not the background, are colored in. Click the Ok button when complete.

Set up Sampling Units

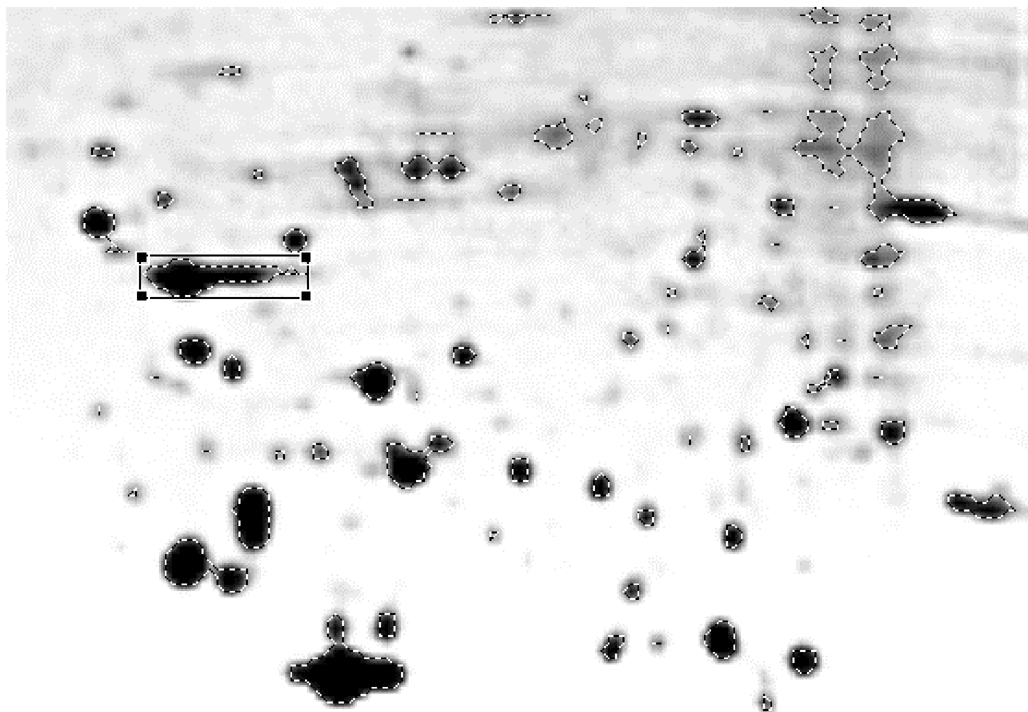
Select Calibrated Units and microns radio buttons in the sample table to use the calibration curve fits.

Image Values
- ○ Digital #
- ○ Opt. Density
- ◉ Calibrated

Area Units
- ○ Pixels
- ◉ microns

Sample Image

Point and click anywhere in the image window to start the auto object finding process.



Data is saved in a new row for each object found within the intensity threshold. Counts per minute, pH, molecular weight and area values are automatically derived based on the curve fits in the image tag.

Sample Table - Untitled

Index	Mean	P.H.	Mol.Wt.	area
48	120.46	6.36	0.83	13.00
49	167.50	6.51	0.75	2.00
50	134.61	6.73	0.89	18.00
51	60.97	5.37	1.16	74.00
52	167.50	6.30	1.46	4.00
53	157.75	6.72	1.46	4.00
54	153.57	6.50	1.66	7.00

Image Values
- ○ Digital #
- ○ Opt. Density
- ◉ Calibrated

Area Units
- ○ Pixels
- ◉ cm^2

ReApply ReCalc