Focal Points

Application Note FP-116

UVP BioImaging Systems

Spot Densitometry Using LabWorks Analysis Software

Determining intensities of spots or bands of uniform or irregular shape is accomplished very simply and accurately with LabWorks. This is especially useful for Quantitative PCR and Western Blot Applications.

Acquire a gel, membrane blot or plate image (Fig 1).

Fig. 1. Typical Chemiluminescent Blot

Open the ***Area Density Tool*** with the ***Menu Icon*** (Fig. 2).

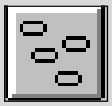

Fig. 2

This Tool (Fig. 3) enables the detection of intensities, background subtraction, calibration to a standard curve and statistical analysis of any bands, spots, cells or other objects.

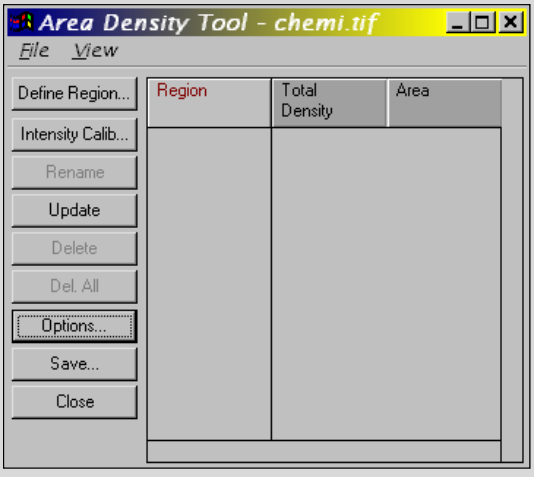

Fig. 3. Area Density Tool

Open the ***Options*** menu from the ***Area Density Tool*** (Fig. 4). Check the ***Keep regions shape*** box from the ***Measurements Options*** dialogue window if you would like to draw uniform shapes of the same pixel area around each object or uncheck the ***Keep regions shape*** box if you want to completely outline the objects. Also select what colors you prefer for outlining and labeling detected bands.

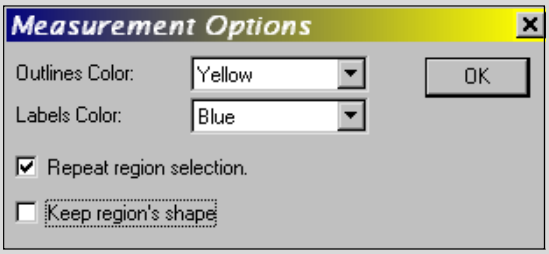

Fig. 4. Measurement Options Menu

Open the ***Define Region*** menu from the ***Area Density Tool*** and select a square, circle or irregular shape from the ***LabWorks*** main dialogue menu (Fig. 5).

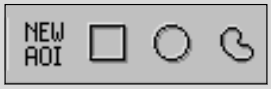

Fig. 5.

<u>*To draw uniform areas around the bands*</u>, drag and hold the left mouse button until you have an area that will fit around the largest band, but does not overlap into the intensity of a neighboring band. Once you are satisfied with the selection area, right mouse click on the area to save the detected band. To measure intensities of other bands, left mouse click on the detection area and drag it over the next band. Right mouse click to save. Repeat this procedure until you have outlined all the bands required for densitometry.

Fig. 6. Outlined Area of Uniform Shape

<u>*To completely outline objects of uniform gray scale intensity,*</u> select the ***Irregular Shape*** icon from the ***LabWorks*** main dialogue menu. The ***Magic Wand*** **window** should appear on screen (Fig. 7).

Fig. 7.

Place the mouse cursor over the first band and click the left mouse button. The band should be outlined in whatever color is selected in the ***Options*** menu. If the detected area is too large, adjust the ***Range*** control from the ***Magic Wand*** window to a smaller number and left mouse click on the band again. If the detection area is too small, increase the ***Range*** control. Once the proper threshold of gray scale is fine tuned and you are content with the detection ***Range***, left mouse click on each band followed by a right mouse click to save the detection. Repeat this for all the bands on the gel requiring densitometric analysis (Fig. 8).

Fig. 8. Outlined Areas of Irregular Shape

To calibrate optical density pixel values, open the ***Intensity Calibration*** menu from the ***Area Density Tool*** (Fig. 9). You can use a standard 0-255 gray scale intensity curve to calibrate bright bands on a dark background by selecting the ***Free Form*** curve (a linear relationship), or calibrate dark bands on a light background with the ***Standard Optical Density*** curve (a 2nd degree polynomial relationship). In addition, you can develop your own standard calibration curve specifically for the imaging system by using a ***Step Tablet*** and saving it in the selection menu. Open the ***New*** menu to name each custom calibration curve.

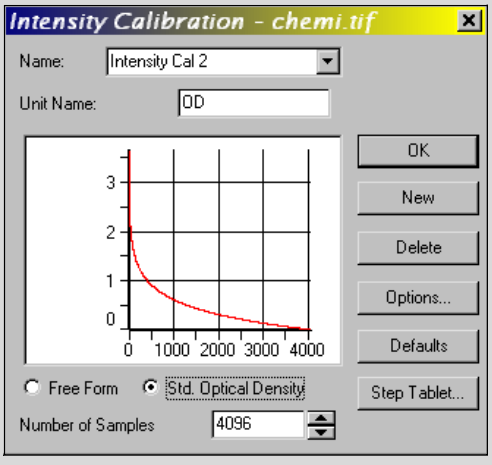

Fig. 9. Intensity Calibration Menu

After developing your calibration curve, click on the ***OK*** button and select ***Update*** from the ***Area Density Tool***. This will update the results for quick inspection of the relative band intensities.

Following are the results for the areas of ***Uniform Shape*** analysis (Fig. 10):

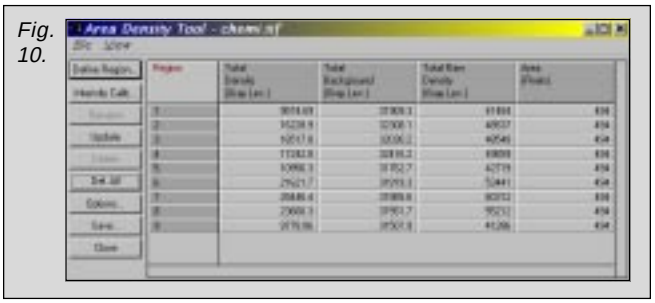

Fig. 10.

Following are the results for the areas of ***Irregular Shape*** analysis (Fig. 11):

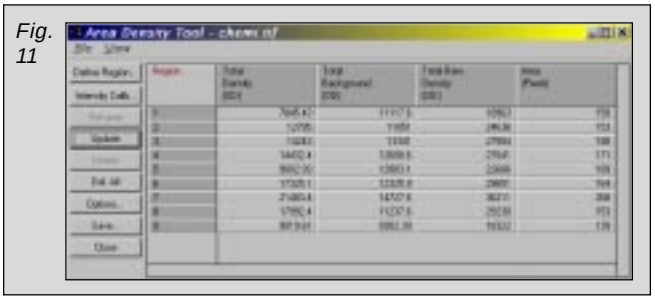

Fig. 11

Calculations are based upon these equations:

Total Raw Density *= Sum of All Pixel Values within the Defined Area*

Mean Raw Density = Total Raw Density / Area

Mean Background = Mean Raw Density – Mean Density

Total Background *= Mean Background x Area*

Total Density = Total Raw Density – Total Background

You can select which density values you which to display by opening the ***View*** menu (Fig. 12) of the ***Area Density Tool***, or you can display the ***Statistics*** related to the densitometry.

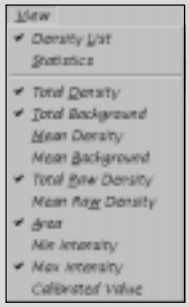

Fig. 12

UVP BioImaging Systems
Solutions for the Science of Life
UVP.com

UVP, Inc. • 2066 W.11th Street, Upland, CA 91786 • (800) 452-6788
(909) 946-3197 • Fax: (909) 946-3597 • E-Mail: uvp@uvp.com

Ultra-Violet Products Ltd. • Unit 1, Trinity Hall Farm Estate, Nuffield Road, Cambridge CB4 1TG UK
+44(0)1223-420022 • Fax: +44(0)1223-420561 • E-Mail: uvp@uvp.co.uk