Training for Axon Scanner

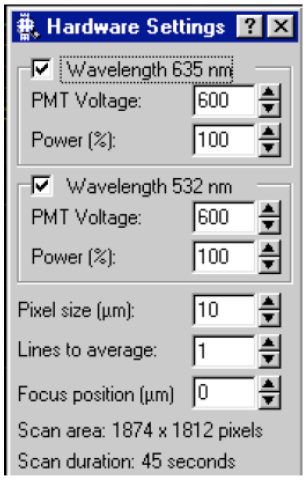
You need a computer account with the school of medicine to use the scanner computer; if you don't already have an account go to the Expression Analysis website for Scanners and there will be instructions on how to do this: http://genomecenter.ucdavis/expression_analysis/Agilent_scanner1.html

IMPORTANT, in this order: you must turn on the scanner first. Then turn on the computer. Log in and start up GenePix Pro 6.0 to allow 20 minutes for the lasers to warm up. If the scanner is inactive for more than an hour the lasers automatically turn off and you will have to restart the GenePix Pro software to turn the lasers on again to begin warming up.

After 20 minutes of warm up, load the array into the scanner. Follow the picture diagram instructions in the drawer and remember to have the array facing down since the lasers are reading from the bottom of the machine.

First Time Scans

1) Click on the Hardware Settings button  to open the Hardware Settings box.



Click the box to _ for the color(s) you will be scanning. Set your PMTs to an educated guess for the wavelengths you will be using: 600—800 is a good starting range. Focus position is 0 um for most glass slides, exceptions are listed in the suggested Hardware Settings for company arrays located in the scanning desk area. Lines to average and pixel size are adjusted following the initial adjustment scans.

2) Click the Preview Scan button  to start a lower resolution scan. This will help set the scan area, insure focus distance area is correct, and give some idea of whether initial PMT settings are appropriate. Initial PMT settings should be high enough to see your features, but not so high that you image has a lot of saturation. You will do more with PMTs during the actual data scans, but you can begin to get an idea with the preview scan, through the Histogram window (see below for a description of what you're looking for as far as intensity goes, but keep in mind Preview scans will only give you a rough idea).

3) Once the area of interest is scanned in Preview Scan you may click the Stop Scan button. 

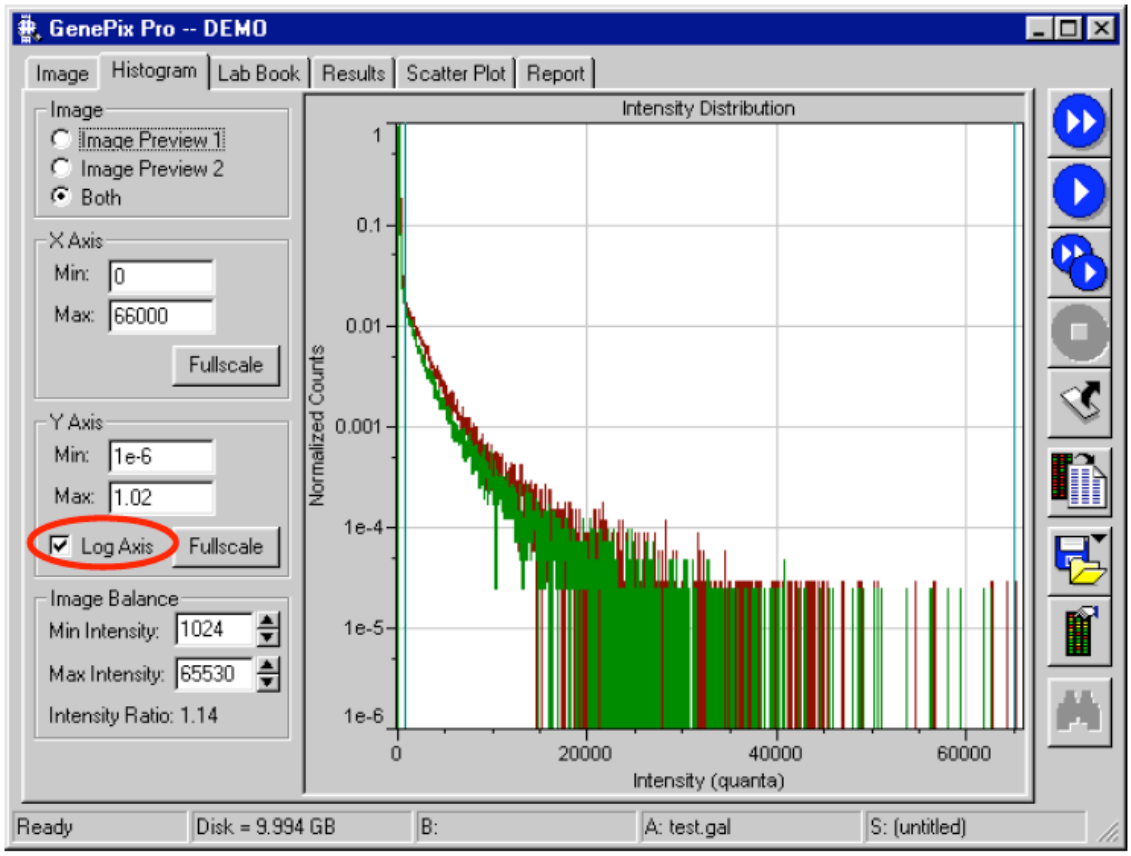
4) Click the Zoom Button  to adjust the image so that you can easily see where your array is on the slide. Click the Set Scan Area button  and select the array area that will be scanned for data.

5) Click the Full Scan button  to begin scan for further Hardware Settings adjustment.

6) You should adjust PMTs while the full scan is going for the most accurate representation of image intensity. To adjust PMTs you must zoom onto an area in the scan that represents a "good" portion of the scanning area, and view it through the Histogram window. If you zoom in on a blank or oversaturated area (eg, on the outer perimeter of some slides) your settings will be biased. If you view the Histogram window once the scan is complete, you get an overall average of the intensities. It is recommended that PMT settings be in the range of 300 to 900 for full scans. After zooming in on an area that would represent your image well follow the directions for your color(s).

Single-Color: Adjust your PMTs so that a majority of your features fall between ~15,000 and ~50,000 "intensity units" in the Histogram. Avoid over saturation (intensity units >65,000) without sacrificing good data into background.

Two-Color: Adjust your PMTs so that a majority of your features fall between ~15,000 and ~50,000 in the Histogram. You also need to balance the two colors so that the Count Ratio is close to 1 (count ratio can be adjusted during the Preview, but total intensities can't). The Count Ratio is calculated by using the Minimum and Maximum Intensity in the Image Balance located in the lower right. The Minimum default settings is 1024, but you may vary this for your background. An acceptable Count Ratio value is generally between 0.8-1.2.



The key for both single and two color intensity adjustment is to minimize over saturation without pushing good data (of low expressing genes) into background. Sometimes it's best just to save mulitple scans at different settings and sort it out in the analysis. Keep in mind that you will get dye bleaching following multiple scans, though.

Note: if you adjust the PMTs during a scan to get your settings in the right range, don't try to use this image for subsequent analysis. Also, while 15,00-50,000 is optimal, you can get good data on either side of these values

8) Once you feel confident in your PMT settings, select your other final hardware settings. Pixel size should be set to 5 um for any features < 80 um in size. Lines to average is how many times the laser passes across the same region, averaging the intensity for all the passes. Setting this to 2 is a good place to start. Finally, click the Full Scan button  to obtain an array image with these settings.

9) After your full scan is complete, click the File button  and choose to Save Image. When saving your image consider the following:

If you will be using **GenePix Pro 6.0** software to analyze data, in the Save Image box specify to
Save as Type: Multi-image TIFF Files (*.tiff)
Check Compression: _ use TIF LZW compression (lossless)

If you will be using other software to analyze data then in the Save Image box specify to Save as
Type: Single-image TIFF Files (*.tiff)
Check wavelengths scanned with: _ Wavelength 635nm and/or _ Wavelength 532nm
Uncheck Compression, save as an uncompressed file.
Each wavelength image will be saved in a separate file.

9) If you will be performing scans on many similar arrays for a particular project it may be beneficial to save your Settings (Pixel size, Lines to average, Focus distance, PMTs) for this type of array. Click on the File button and go to Save Settings As. Save under an appropriate name with the .gps extension. With future similar arrays you can then have a good starting point. To protect your .gps file from accidental changes by other users simply find the settings file (.gps) you saved and right click to get to Properties and choose to make the file Read-only.

10) Close all programs.

11) Shutdown computer.

12) Sign up on sheet with the number of official scans you obtained (do not include scans taken while adjusting Hardware Settings).

13) Turn off Scanner.

Returning for Similar Scan on Another Array

1) Follow protocol as before until you are ready to start clicking on buttons in the software. Click on the File button  and go to Open Settings. Chose the Settings that you had saved previously with a similar array and adjust and scan accordingly.