Digital Image Tips: Adjusting Brightness And Contrast In Micrographs Using Adobe PhotoshopÆ

Bradley R. Johnson, Pacific Northwest National Lab*
Bradley.Johnson@pnl.gov

Recently on the microscopy list server, a question came up as to how to compensate for charging in SEM micrographs. Several good answers were suggested. Some of them were 1) recoating the sample to prevent the build up of charge on the sample in the first place, and image it again, 2) use an environmental SEM (ESEM) in the "wet" mode to eliminate the charging phenomena, 3) use a different detector, such as a backscattered electron detector, which is much less sensitive to charging phenomena, and 4) apply adjustments to the digital image using software such as Adobe PhotoshopÆ to minimize the effect of the charging problem. Sometimes there is not the luxury available to use a different microscope, such as an ESEM, or maybe the microscope is not equipped with a BSE detector, or perhaps it is not possible or practical to recoat and relocate a particular feature of interest. In such a situation, working with a digital image to improve its esthetic appearance might be the preferred solution.

A digital SEM micrograph was collected from a glass-ceramic specimen to illustrate one approach to digitally adjusting brightness and contrast in an image. The micrograph is of a large void in the ceramic specimen, and the interior of the void was charging heavily. The objective was to simultaneously obtain information about the microstructure both in the void and on the polished surface, and since the specimen was charging, this was difficult to accomplish.

The first step in making digital adjustments to an image is to collect an image that contains information that can be improved on. If the micrograph contains charging artifacts where it is all white, then in most cases, very little if anything can be done to improve the field of view where the charging occurred. In order to avoid this problem for this example, the contrast and brightness were greatly reduced on the microscope to eliminate the charging problem. However, this resulted in a very dark image with very little contrast (Figure 1). In order to improve on the quality of this micrograph, a series of adjustments to the image will be illustrated and explained.

This micrograph was taken with a JEOL JSM 5900 SEM using the manufacturer's digital acquisition system. It was collected using the secondary electron detector (SED) on a scan setting of 4 (slow scan speed of approximately 160 seconds per frame). This produced an image that was 1280 X 960 pixels. The file was saved in tiff format, and image adjustments were made on an Apple MacintoshÆ computer using Adobe PhotoshopÆ 6.0.1. The user interface for Adobe PhotoshopÆ is essentially the same on a Mac as on a PC, and the commands for PhotoshopÆ 6.0.1 are very similar to those for PhotoshopÆ 5.0. Hence, the adjustments described should be broadly applicable to a fairly large audience.

After collecting the image and opening it up in PhotoshopÆ, the first thing to do is to set the image mode to gray scale (many imported images are identified as being in indexed color mode) and make a duplicate layer of the image. The following steps explain how to do this.

1. Open the image in Photoshop 5 or 6
2. Set image mode to Grayscale
   Image -> Mode -> Grayscale

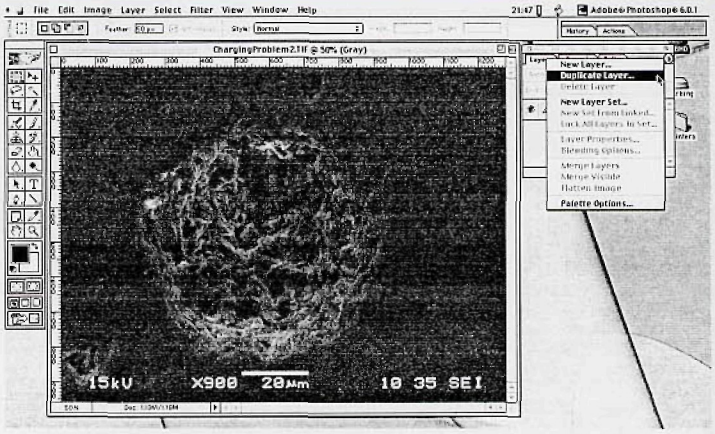


Figure 1. SEM micrograph of a void in a glass-ceramic specimen. The void in the center of the field of view was charging, so the brightness and contrast settings on the SEM were greatly reduced.

3. Open up the Layers palette
   Window -> Show Layers
4. Highlight the background layer, and make a working copy of it. (Figure 1)
   Click the little triangle in the upper right corner
   Choose "Duplicate Layer"
   Type in an appropriate name for the layer, or use the default name "Background copy"
   The next step to is to create an adjustment layer. This allows one to make changes to several different features of the image without permanently changing the raw data. Additionally, these changes can be tweaked and re-edited at any time until you obtain the desired result.
5. Click on the half-filled in circle at the bottom of the Layers pallet and choose "Levels" (Figure 2)

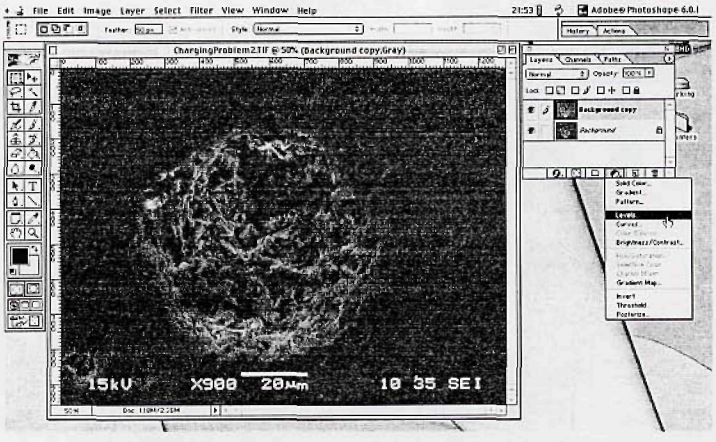
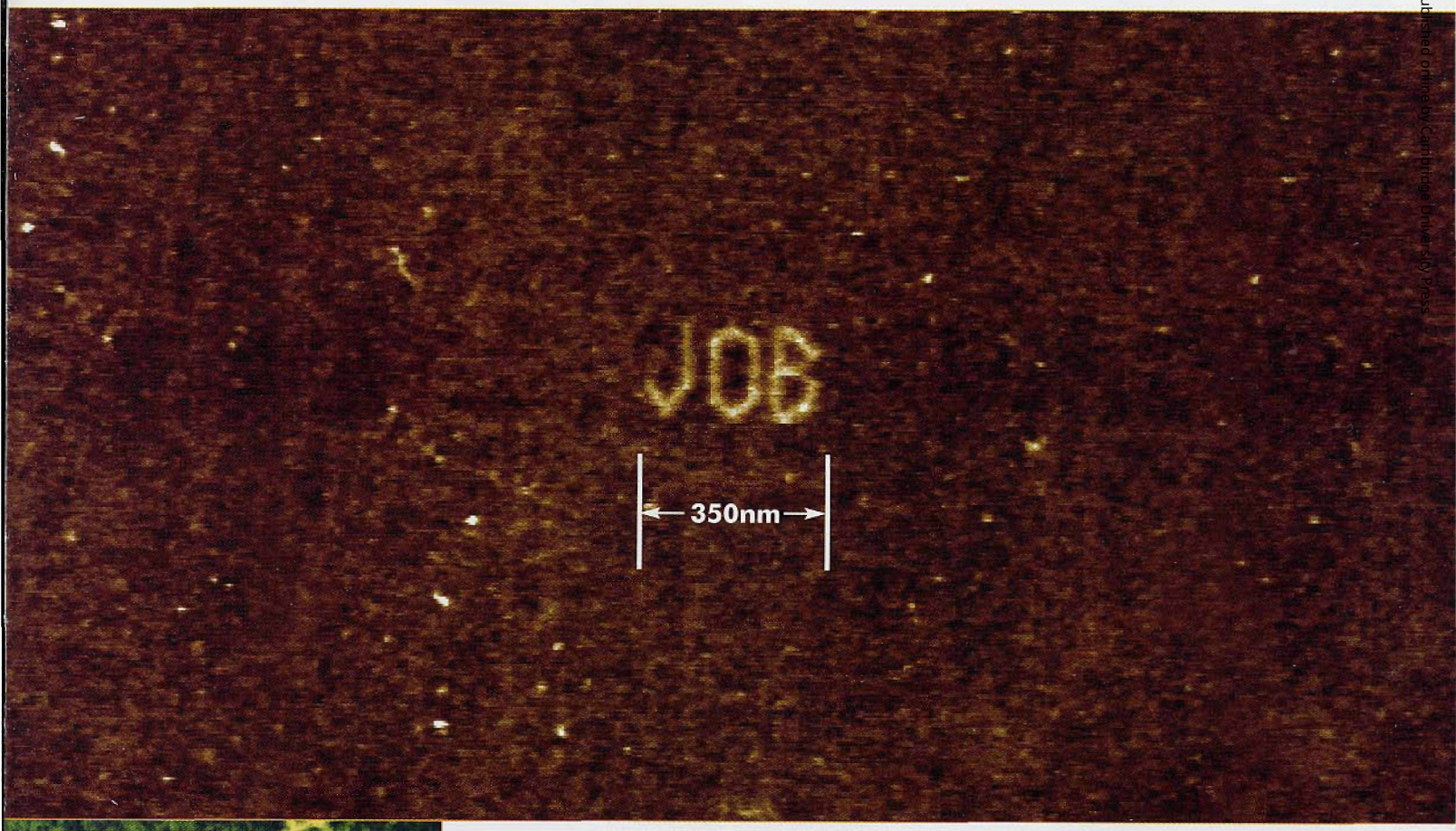


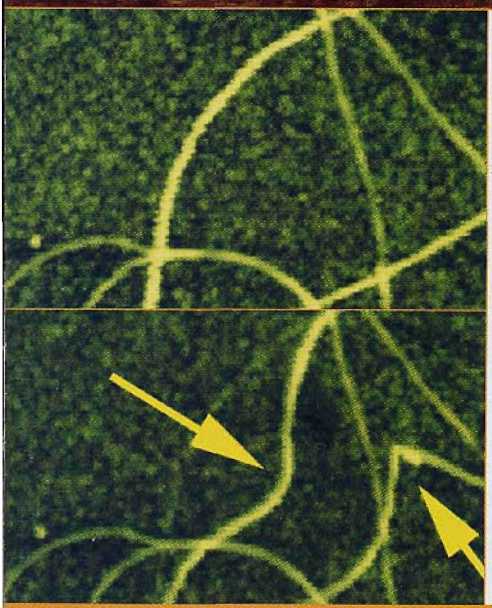
Figure 2. Illustration showing how to create a Levels Adjustment layer.

The Levels command shows the histogram of the image where the x-axis is the numerical value of the gray level (0 = black, 255 = white) and the y-axis is the number of pixels that have that particular gray level). Moving the left-most black triangle, and the right-most white triangle specifies which pixels will be mapped to pure black, and which pixels will be mapped to pure white, respectively. All pixels to the left of the black triangle will be mapped to black, and all pixels to the right of the white triangle will be mapped to white. The numeric values are shown in the boxes after the "Input Levels:" label. A transfer function is then used to

Continued on page 10

NanoMan for nanolithography and nanomanipulation: no job too small.





Introducing NanoMan: the most advanced nanomanipulation system.

With NanoMan™, you can easily perform nanolithography or nanomanipulation that was previously difficult, time-consuming, or impossible. Now, "writing" on surfaces with nanometer-level resolution and manipulating nanometer-sized objects is as easy as using a point-and-click interface. Veeco-Digital Instruments has combined the innovative NanoScope IV controller, the ultra low-noise, highly accurate Dimension CL closed-loop head, and NanoMan user interface to create the most powerful and versatile nanomanipulation system you can buy.

Images
Top: Nanolithography image with word width of 350nm and line width of 15nm.
Side: NanoMan nanomanipulation of carbon nanotubes, before (top) and after (bottom).

www.di.com/nanoman
1.800.873.9750 or
1.805.967.1400

Veeco

di Digital Instruments

Digital Image Tips: Adjusting Brightness And Contrast In Micrographs Using Adobe PhotoshopÆ

Continued from page 8

remap all of the pixels between the black and white triangles from their original values to their new values. An example adjustment applied to this image is shown in Figure 3. Due to the charging problem in this field of view, the white point could not be moved farther to the left without causing the interior of the void to appear all white.

The middle gray triangle on the Levels command dialogue

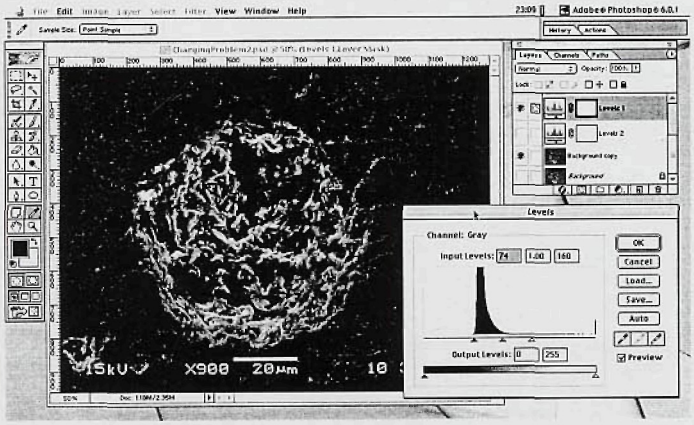


Figure 3. Illustration of how to set the black point and white point for a micrograph using the Levels command.

box controls how the new white and black points are mapped to 0 and 255, respectively. This is sometimes called the gamma function. A value of 1.0 corresponds to a linear transfer, a value greater than 1.0 generally lightens the image overall, and a value less than 1.0 generally darkens the image. This adjustment can be very helpful in an image where there is too much contrast. (Note: To evaluate the effect of the changes, click the "eye" icon on the left side of the Levels Adjustment Layer to turn the effect on an off.)

The settings chosen for this example (Figure 4) allowed the

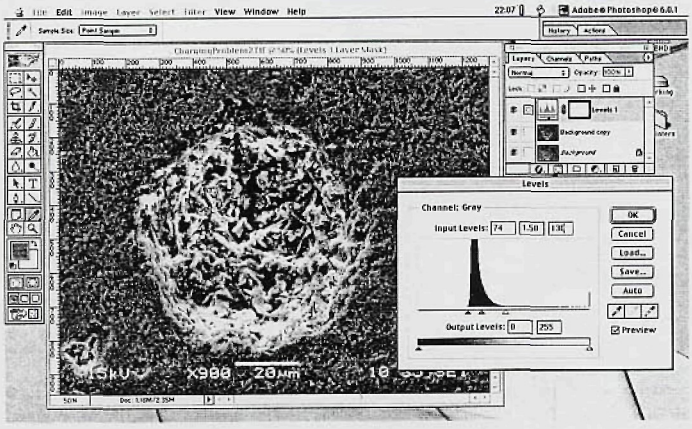


Figure 4. Illustration of how to use a Levels Adjustment Layer to set the white point, black point and the value of the gamma function to compensate for charging in a micrograph.

morphology of the interior of the defect to be seen as well as the microstructural features of the surface. However, these gains were obtained at the expense of contrast on the surface region. Nonetheless, the effect of this simple adjustment made a dramatic improvement to the esthetic quality of the micrograph. It is important to point out that it would not have been possible to collect this type of micrograph on this specimen while on the microscope. That is because this particular microscope has a fixed, linear transfer function from black to white, whereas an image analysis program like Photoshop allows one to apply a non-linear transfer function to an image. Consequently, this enables a person to display meaningful and important features that might not otherwise be visible. No new data was created, this approach merely allowed the author the ability to display the data that was already present in the micrograph, but which was obscured by the charging problem.

Another approach that could be used with an image where there is a contrast problem is to use multiple adjustment layers, such as a Levels Adjustment Layer as well as a Curves Adjustment Layer, or even two different Levels Adjustment Layers. A Curves Adjustment Layer allows more direct control of the transfer function from black to white, and can be used to set the black and white points as well. This approach can sometime be useful to minimize contrast in one region of the sample (the defect in this case) while emphasizing contrast in another part of the specimen (the surface in this case). An example of how to use a Curves Adjustment Layer is described below.

6. Click on the half-filled circle on the bottom of the Levels pallet, and select "Curves". Adjustments to the transfer curve are made by clicking on the diagonal line to adjust its position. This establishes fixed points on the transfer curve. The points can then be moved by clicking and dragging them with the mouse, or by using the arrow keys when they are highlighted. An example transfer curve is shown in Figure 5.

Adjustment layers affect only the image data for those layers positioned below them in the layers pallet. One can experiment to determine whether it is easier to use the Curves Adjustment Layer located above or below the Levels Adjustment Layer. In this example, the Curves Adjustment Layer was applied below the Levels Adjustment Layer so that the image data was modified by the new curves layer first, before the effect of the level adjustment was applied. Consequently, after applying the new transfer curve to the image data (with the curves layer), the histogram for the image data "observed" by the Levels Adjustment Layer was different than for the raw image. Thus, in order to optimize the image appearance, the set points in the Levels Adjustment Layer were edited (the new values for the black point, gamma function, and white point were 74, 0.60, and 156, respectively).

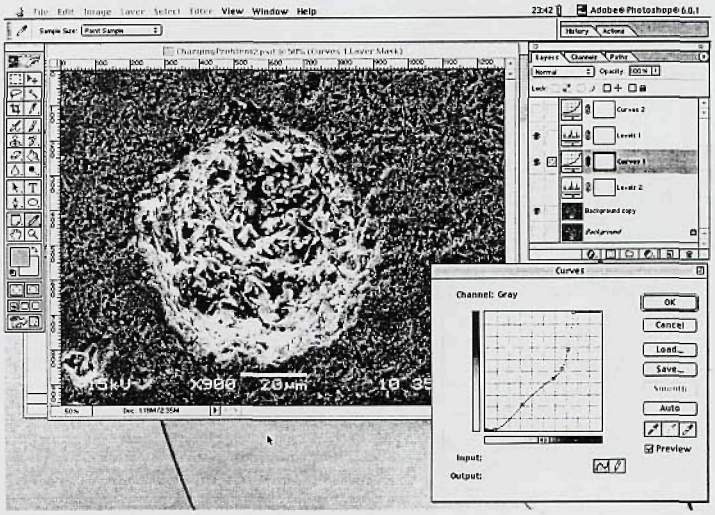


Figure 5. Illustration of how to use a Curves Adjustment Layer to control the transfer function from white to black.

Continued on page 12

Digital Image Tips: Adjusting Brightness And Contrast In Micrographs Using Adobe PhotoshopÆ

Continued from page 10

This adjustment does gain enhanced contrast on the surface region, but the interior of the void became more washed out. Both approaches (using only a Levels Adjustment Layer or a Levels Adjustment Layer along with a Curves Adjustment Layer) require a trial and error approach to find the settings that most suitably portray the data of interest. This is where the tremendous power of Adjustments layers becomes so valuable, because they allow the user to freely apply and edit numerous different adjustments without permanently changing the raw data of the image.

In summary, when faced with a difficult charging problem, meaningful and useful information can still be obtained from a digital micrograph by using Adjustment Layers in Photoshop to edit the black point, white point, and transfer function of the histogram for the image. These adjustments are reversible, and do not alter the raw data of the image. Additionally, this approach can achieve dramatic improvements to the brightness and contrast of an image with only modest effort, and can be used to emphasize valuable information that might be otherwise obscured. ■

*Pacific Northwest National Laboratory is operated by Battelle Memorial Institute for the U.S. Department of Energy under contract DE-AC06-76RL01830

Using the PMT in a Laser Scanning Confocal Microscope as a Digital Light Meter to Measure Detection Photon Efficiency

Jim Pawley, University of Wisconsin
jbpawley@facstaff.wisc.edu

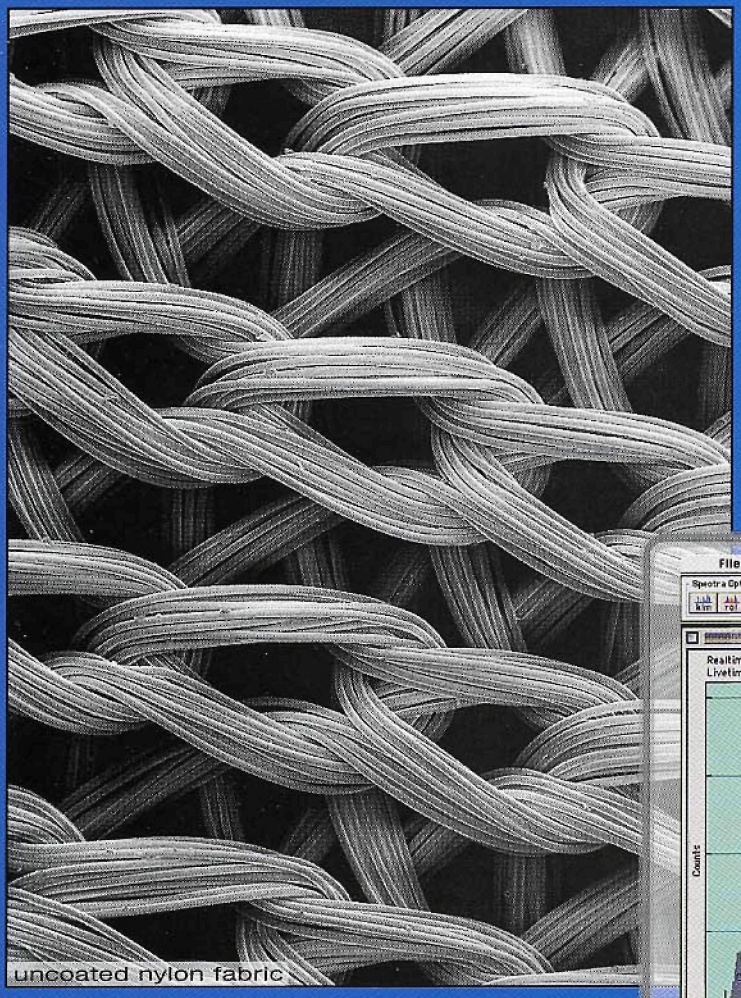
If we accept that the optical layouts of single-beam laser scanning confocals are generally quite similar, the most important variable is "Photon Efficiency" (PE): the fraction of photons leaving the focal plane that actually contribute to the number stored in the image memory. Unfortunately, this parameter is a function of a very large number of operator settings, design decisions and optical performance specifications (BioTechniques The 39 Steps: A Cautionary Tale about "quantitative" 3D fluorescence microscopy, April 2000 28:884-7). As a result, although all manufactures claim excellent "Photon Efficiency" none actually provide any specifications. We have to determine the PE ourselves.

Let us first agree that, because the loss of excitation photons can always be made up by using more laser light, Detection Photon Efficiency is what we are really interested in. Now the problem becomes, how can we create a "specimen" that has a known and repeatable brightness.

I suggest that the best plan is to use the transmitted-light illumination source as "the specimen." All light microscopes have one, and it is usually adjustable in intensity. With the laser off, any confocal microscope becomes a flying spot detector that only collects photons from one pixel in the image plane at a time. Each number recorded in the confocal image memory should be proportional to the number of photons passing through a particular pixel in the focused plane during the few microseconds that

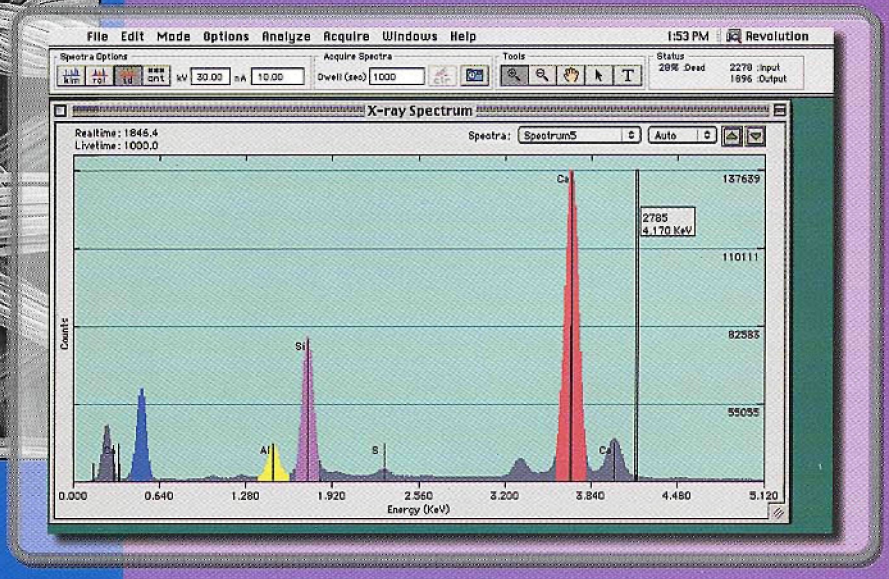
4pi

Digital Imaging & EDS Systems



uncoated nylon fabric

- *Easy-to-use software*
- *Light-element detection*
- *High-resolution digital imaging*
- *Qualitative and quantitative EDS analysis*
- *Simultaneous acquisition of SEM, WDS, SCA and X-ray images*
- *Multiple spectra windows with independent scaling and colors*



www.4pi.com • info@4pi.com

(919) 489-1757

3500 Westgate Drive, Suite 403 Durham, NC 27707-2534