

DIGITAL IMAGE CAPTURE

POLAROID DMC VERSION

Introduction

This article describes the basic procedures for obtaining an image of your microstructure using an optical microscope equipped with digital camera and a PC running Windows. This procedure assumes that the following equipment is available:

- C Optical microscope with a trinocular head for attaching the digital camera
- C High-resolution digital camera, Polaroid DMC
- C PC running Windows 95/98 or NT.
- C Adobe Photoshop 6.0

Furthermore, it is assumed that the specimen is in place on the microscope and that the desired image can be viewed through the microscope's eye-piece.

General Guidelines

The following guidelines should help you get the best results in your digital capture and image enhancement work.

- C The resolution (related to the number of pixels per unit area or length) of the image should be suitable for final output. For instance, standard laser printers print at 300 or 600 dpi while some laser printers can print at resolutions up to 1200 dpi and most color printers print at 300 to 600 dpi. The resolution requirements for images to be displayed on the web or similar digital media are much less demanding.
- C A 600 dots per inch (dpi) laser printer does not print at 600 pixels per inch (ppi) because each pixel is represented by a number of dots to produce the different shades of gray. A typical 600 dpi laser printer, for example, actually prints at a resolution slightly under 200 grayscale ppi.
- C The generally accepted rule of thumb for minimum image resolution is that it should be greater than twice that of the output device. Experience, however, has shown that you can print a reasonable looking image on a 600 dpi printer at resolutions as low as 150 ppi and that the optimum results will be obtained around 180 ppi. Printing at higher resolutions produces no noticeable improvement. See figure 1.
- C Keep copies of the original images. That way you can always go back to the originals for future projects or in case something happens to your working copies.
- C Save your work in progress frequently. This will protect you from system crashes and will let you go back to an earlier stage of the project in case you spoil the image when trying to use

a filter or any other image enhancement tool.

- C Keep track of the magnification. The easiest way to do this is to capture an image of the stage micrometer right after you capture an image of your specimen. Afterwards, whenever you change the size or resolution of your image you'll have to do this with the image of the stage micrometer and when you print the image you'll have to print the image of the stage micrometer. Another approach is to paste a portion of the stage micrometer on the image. This way whenever you resize the image you'll also resize the image of the stage micrometer.
- C Make sure you save your image files in the correct folder. Files found in other folders tend to get lost.
- C Do not leave the only copy of your valuable work on any laboratory computer unless it has a secure operating system such as Windows NT, VMS or Unix. Copy your files to a diskette, a Zip disk or to another, more secure, computer on the network. And even if the computer you keep your files on is secure it is still a good practice to keep backup copies of your work.

Color Balance

If accurate color in your micrographs is important you will need to specify the color correction to apply to your image. The Polaroid DMS software offers two standard color corrections and the option to create your own color correction. These are:

- | | |
|--------------|--|
| Daylight | Colors are not altered. |
| Tungsten | Tungsten correction is similar to use of 80A filter on daylight balanced "outdoor" film such as Kodak's Kodachrome or using a tungsten corrected "indoor" film such as Kodak's Ektachrome. Indoor lighting is often provided by tungsten filament incandescent which gives the room a yellow cast. Indoor films are formulated with this in mind, and filters such as the Wratten 80A, can be used to correct daylight films by adding blue to the image. In the case of the Polaroid camera, the software will correct for the excess yellow by adding blue to the image. |
| User Defined | User defined allows you to create your own color correction. This can be useful when you want to correct for color shifts created in the microscope or illumination system and when neither of the two standard color corrections provide satisfactory results. Creating a user-defined color correction is simple. The Polaroid software's tools will guide you through this process. |

Micron Bar

The best way to indicate the size and/or magnification in a micrograph is to put a micron bar in the image. This way, no matter how much the micrograph is enlarged or shrunk in the future, possibly by the publisher of your paper or by a projection system used in the seminar you are giving, the correct size of features will be represented.

To make a micron bar one needs to know either the magnification or the precise size of a feature in the image. In practice, creating a micron bar usually involves first grabbing an image of a stage

micrometer, a precisely drawn micron scale image which, for optical microscopy, may be graduated down to 10 microns. Then, one can either draw a micron bar of desired length or one can mark two points on the image and tell the software what the dimensions are. (The Polaroid DMC software employs the latter method and can store any number of micron bars for future use.) Once a micron bar is defined for the optical and camera arrangement you are using, it can be pasted in the image.

Polaroid's Micron Bars - The Polaroid DMC software provides a simple procedure for both creating a micron bar and putting it in your micrograph. To create the micron bar ... To place this micron bar, or any other previously saved micron bars, in your image go to the ... and select the appropriate micron bar. It will be placed in the lower right corner of your image. One note of caution, you might want to verify the accuracy of predefined micron bars before using them in your images.

Custom Micro Bars - One may prefer to create a more attractive micron bar, perhaps one which is white with black drop shadow, or black with a white drop shadow, or placed anywhere on the image, or even on a rotated image. An article later in this manual describes how to create a custom micron bar that you can paste anywhere in your micrograph. The same basic technique can be used to create attractive labels, arrows and other annotations.

Capturing an Image

The following image capture procedure can be used with the Polaroid DMC digital cameras. The procedure is virtually identical whether using a PC or a Mac.

1. Obtain the best focus and image contrast as viewed through the microscope's eye pieces.
2. Start Adobe Photoshop.
3. Select *File: Import: Polaroid DMC* or *Polaroid DMC 1e* to bring up the Twain interface for the camera on your microscope.
4. Select the desired sensitivity (ASA), color/bit-depth option and color correction.
5. Divert the image path from the eyepieces to the camera.
6. Click the "Preview" button to see the image in the form.
7. Adjust the exposure time to obtain the desired image density (brightness/darkness).
8. Refocus the microscope if necessary so that the image on the screen is as sharp as possible. For the best possible focus mouse-click the image to bring up a small box on the image and a "focusing bar" on the plug-in's form. Make fine focus adjustments to obtain the highest value on the "focusing bar".
9. Click the "Transfer Image" button to capture the image and transfer it to Adobe Photoshop, or click the "Enhance Image" button to bring up a form that will allow you to add a caption and micron bar to the image.

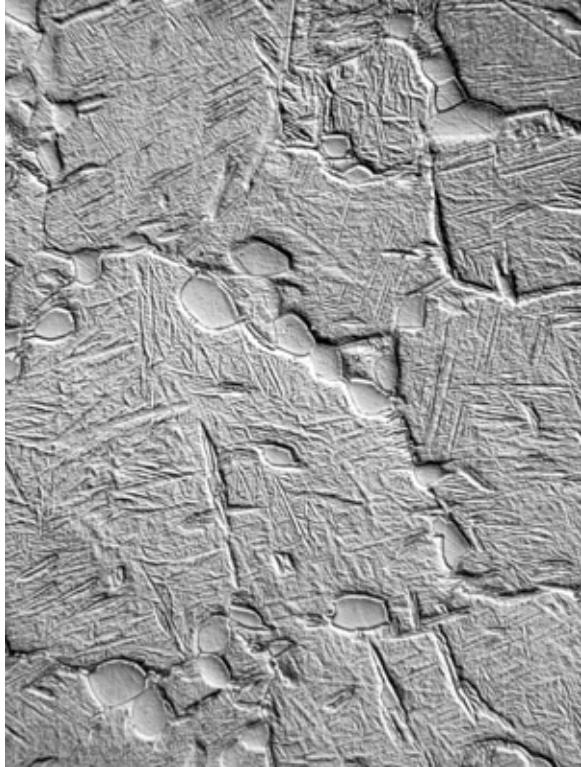


Figure 1a. A 1200x1600 pixel grayscale image resampled to 400x300 pixels so that it will print here as a 3"x4" image at 100 ppi. (File size= 122 kB)

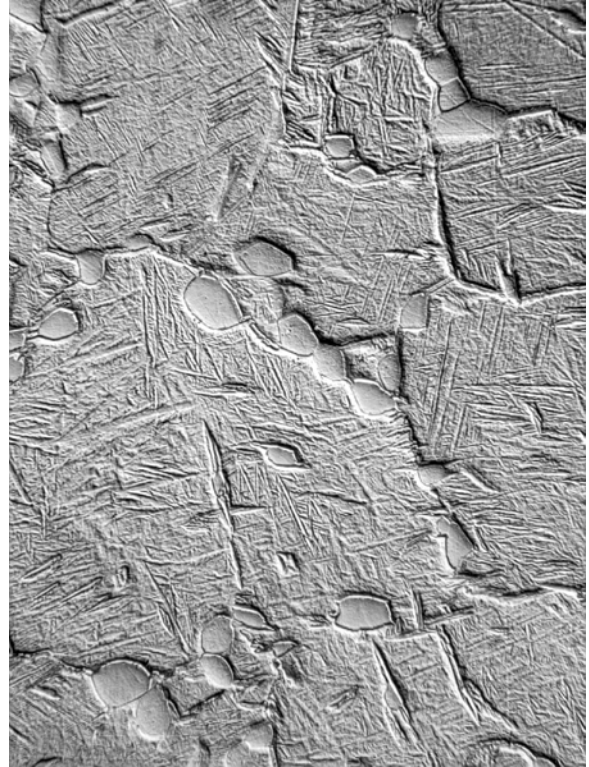


Figure 1b. Same as figure 1a but resampled to 450x600 pixels so that it will print here as a 3"x4" image at 150 ppi. (File size= 269 kB)

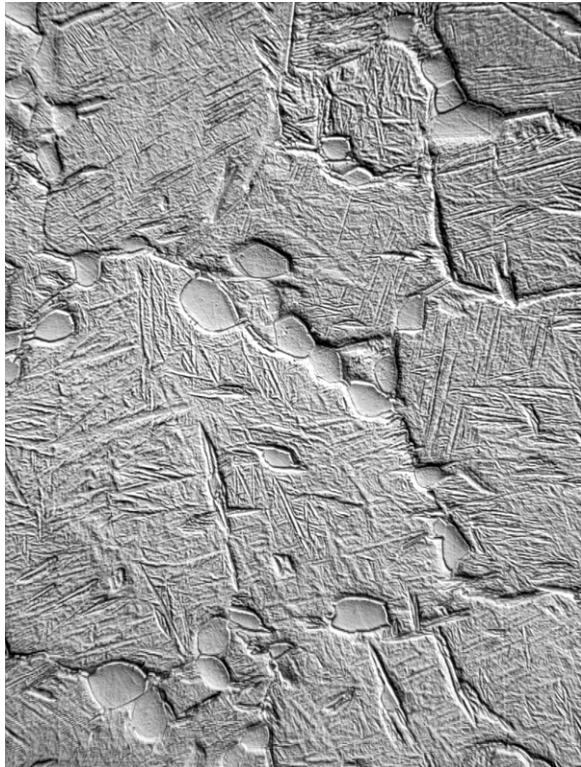


Figure 1c. Same as figure 1a but resampled to 600x800 pixels so that it will print here as a 3"x4" image at 200 ppi. (File size= 474 kB)

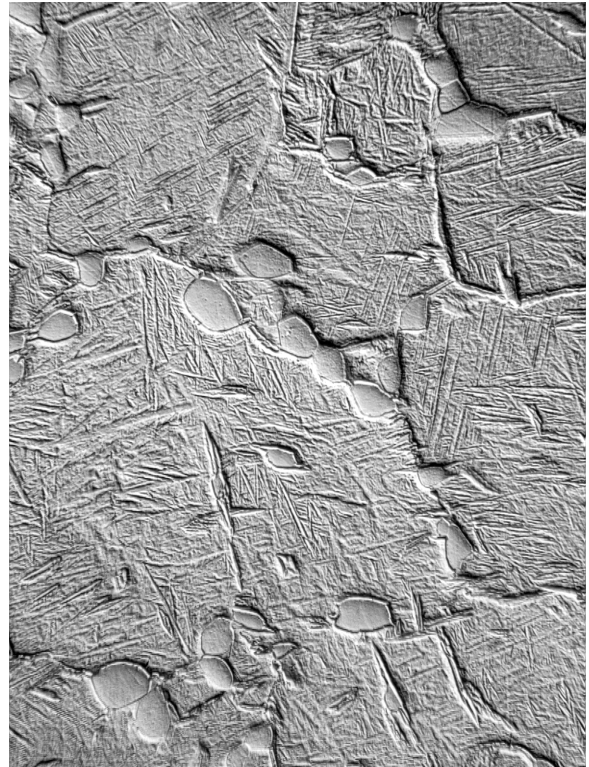


Figure 1d. Same as figure 1a but not resampled and printed here as a 3"x4" image at 400 ppi. (File size= 1.83 MB)