A Basic Introduction to Image Processing Using *NIH-Image* as a Model

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Advances in camera technology and digital instrument control have meant that in modern microscopy, the image that was, in the past, typically recorded on a piece of film is now recorded directly into a computer. The transfer of the analog image seen in the microscope to the digitized picture in the computer does not mean, however, that the problems associated with recording images, analyzing them, and preparing them for publication, have all miraculously been solved. The steps involved in the recording of an image to film remain largely intact in the digital world. The image is recorded, prepared for measurement in some way, analyzed, and then prepared for presentation.

Digital image acquisition schemes are largely the realm of the microscope manufacturers, however, there are also a multitude of "homemade" acquisition systems in microscope laboratories around the world. It is not the mission of this paper to deal with the various acquisition systems, but rather to introduce the novice user to rudimentary image processing and measurement. The platform for this introduction will be the public domain image processing program *NIH-Image*[1], which was, and continues to be, developed by Wayne Rasband of the National Institutes of Health in Bethesda, Maryland. This program runs on the Apple Macintosh personal computer (680XO or PowerPC based). While there is no one application that can fulfill all possible image processing functions, *NIH- Image* is an excellent program for basic acquisition, processing and measurement and, since it is also extensible, it has a good deal of advanced functionality. Novice users should not be concerned with the advanced functionality, but should explore the program by simply trying it out on a sample image[1], and if the need arises, there is a clear and concise manual.

For acquisition purposes *NIH-Image* supports a number of frame grabber boards that install directly into the Apple Macintosh. It is also able to use many of the "Plug-In Modules" written for Adobe System's commercial image processing package *Photoshop*[2], allowing direct access to a wide variety of input devices. *NIH-Image* currently performs all image processing functions on 8-bit data (256 colors or grays), however, it is possible to import a wide variety of image formats.

Once an image is read into *NIH-Image*, there is considerable scope for image enhancement. Digitally acquired images are often far from perfect and *NIH-Image* contains a suite of tools that perform many standard image processing functions, including contrast enhancement, density profiling, smoothing, sharpening, edge detection, median filtering, and spatial convolution with user defined kernels up to 63 x 63 pixels. The program also incorporates a Pascal-based macro programming language which provides the ability to automate complex, and frequently repetitive, processing tasks.

While the image processing tools may improve the appearance of the image to the human observer, they are also essential in the preparation of the image for quantitative analysis. *NIH-Image* allows the user to spatially calibrate images for the measurement of features in appropriate units. Density calibration can also be performed to allow for quantitative intensity measure- ments. Quantitative spatial analysis includes measurement of area, average gray value, center and angle of orientation of user defined regions of interest. It is also possible to perform automated particle analysis, measure path lengths and angles. The results of these measurements may be written to text files for importation into a statistical analysis application, or printed to any supported printer. Finally, the image itself may also be printed, the highest quality output is obtained with either a high resolution laser printer or dye-sublimation color printer. ■

1. *NIH-Image*, Wayne Rasband, NIH, Bethesda MD. Public domain software, copies (application, source and sample images are available by anonymous ftp from zippy.nimh.nih.gov in the directory /pub/nih-image.
2. *Photoshop*, Adobe Systems Incorporated, 1585 Charleston Road, Mountain View CA 94039-7900. Current version is 2.5.1.

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