Digital Imaging: When Should One Take The Plunge?

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The current imaging trend in optical microscopy, scanning electron microscopy (SEM) or transmission electron microscopy (TEM) is to record all data digitally. Most manufacturers currently market digital acquisition systems with their microscope packages. The advantages of digital acquisition include: almost instant viewing of the data as a high-quality positive image (a major benefit when compared to TEM images recorded onto film, where one must wait until after the microscope session to develop the images); the ability to readily quantify features in the images and measure intensities; and extremely compact storage (removable 5.25" storage devices which now can hold up to several gigabytes of data).

The problem for many researchers, however, is that they have perfectly serviceable microscopes that they routinely use that have no digital imaging capabilities with little hope of purchasing a new instrument. The question for them is: should I wait until I can get funding to purchase a new instrument at a later date, or should I try and take advantage of the benefits of digital imaging now? The answer to that question depends on several criteria; the dynamic range of the images to be recorded, the desired resolution, whether real-time acquisition is desirable and, of course, how much one can afford to pay for a system.

Digital imaging requires the connection of a TV camera to the microscope and a computer system to capture the image that is output by the camera (SEM digital imaging is slightly different and discussed below). Since they are light, compact, offer the capabilities of slow-scan capture, and on-chip integration, CCD TV cameras are the most popular type of camera used. Prices range from several hundred dollars to tens of thousands of dollars and are commensurate with the number of features. The lower priced cameras typically will deliver images at regular TV rate, i.e. 30 frames per second, and offer no cooling of the CCD chip for noise reduction or on-chip integration to improve low intensity images. Higher priced cameras will offer digital slow-scan output direct to a computer with 8, 10, 12 or even 16 bit intensity resolution, on-chip cooling and integration, and will be packaged with a controller card that is inserted into a personal computer or workstation.

Optical microscopy's transition to digital imaging may be a very straightforward process with the purchase of a TV adapter for the microscope, a suitable TV camera and a computer system with an image acquisition board or frame-grabber. If 8-bits of information (256 grays or colors are sufficient for recording and analyzing the images in question) then such a system could cost less than $4000, however, if more than eight bits are required and/or a slow scan camera is necessary, the price can quickly climb into the tens of thousands of dollars.

TEM has the added complexity that the camera must be interfaced with the column of the microscope. Initially there were only one or two vendors who were able to offer such systems, but, as the market has expanded a number of vendors now offer systems. This competition between vendors coupled with a reduction in cost of the basic hardware has driven prices down. It is possible to buy a basic slow scan imaging system for a TEM for less than $35,000.

Digital image acquisition in SEM is performed in one of two ways. Passive recording involves synching the computer based acquisition system with the raster of the SEM probe, whereas active acquisition requires the computer system to take control of the SEM probe. In either system the secondary electron signal is digitized by an analogue to digital acquisition board in the computer and displayed on the computer screen. X-ray Energy Dispersive Spectroscopy (XEDS) systems manufacturers have been offering this capability for decades, but, with the introduction of the inexpensive desktop computers, and provided that the scanning system of the microscope in question can be controlled remotely, such systems can be purchased for less than $20,000 (including the price of the computer).

The Electron Microbeam Analysis Laboratory (EMAL) at The University of Michigan is a good example of where low-cost digital acquisition systems have been added to existing scopes. The EMAL analytical electron microscope, a

JEOL 2000FX, has a Gatan model 673 TV-rate CCD camera mounted on the 35mm camera port. This relatively inexpensive camera (less than $20,000) was purchased to allow the video-taping of dynamic experiments conducted with the hot, cold and straining stages of the microscope. Connection of this camera to a Scion LG-3 video frame-grabber in an Apple Macintosh computer running the NIH-Image software has allowed a number of groups acquire digital images with this camera for grain and particle size measurement and dislocation density determinations.

The JEOL 2000FX can be used to acquire digital images in the scanning transmission (STEM) and secondary electron (SEM) modes. The XEDS system on this microscope is a Tracor/Noran TN5500 with the digital imaging option. Although this system is far from state-of-the-art, it performs flawlessly for the acquisition of digital images. The images are transferred via Ethernet to external workstations for manipulation and analysis. The Tracor/Noran system does not support such networking and so a secondary operating system and file transfer software have been installed on the TN5500 PDP-11. Networking the TN5500 cost approximately $2500, and it was the most cost effective way of accessing the digital images recorded on the 2000FX.

The EMAL high resolution electron microscope, a JEOL 4000EX, has an image-intensified Gatan model 622 TV camera mounted beneath the camera chamber. This camera has also been connected to a Scion LG-3 frame grabber and Macintosh combination. Moderate quality images may be recorded with this system, particularly when one uses the real-time capture capability of the LG-3. With 16 megabytes of on-board memory it is possible to perform frame averaging from 32 consecutive video frames. Users are able to check the scope alignment by Fourier analysis of the digitized images before committing their data to film. The 32 video frames can also be used to study dynamic events in the microscope, Martin *et al* have used the system to study the *in-situ* polymerization of their polydiacetylene samples[1].

EMAL also houses an Environmental Scanning Electron Microscope (ESEM), an ElectroScan Model E3. While this instrument is computer controlled, it is one generation removed from having a completely integrated image acquisition, manipulation and storage system. Digital images and XEDS maps are acquired with a 4Pi Analysis Spectral Engine, installed in an Apple Macintosh computer. The 4Pi system drives the scanning system of the ESEM directly and can record 8-bit images up to 4096 by 4096 pixels in size. Since such images are 16 megabytes in size, routine images are recorded at 512 by 384 pixels or 1024 by 768 pixels.

A single microscope user may be able to generate tens or even a hundred images in a single session. If each image is between 0.25 and 1.0 megabytes in size, then even the 1 gigabyte hard disks of today's typical desktop computers cannot store more than about 20 days worth of data for a single user. Serious thought has to be given to off-line storage and archive options. Since EMAL is a multi-user facility serving twenty to thirty departments on campus for their microscopy and microanalysis needs, efficient storage of data has been absolutely essential. Each of the data acquisition computers in the laboratory not only has a removable storage option (Iomega ZIP drives), but also has access to the campus computer network and is capable of communicating via a variety of network protocols. The principle here is that the users may transfer their data to their local PCs or workstations where it is their responsibility to archive their data or save it directly to a removable disk. The data storage space on the laboratory hard disks is viewed as temporary and is purged frequently.

It is clear from the experience of The University of Michigan EMAL one can use digital imaging effectively without having to purchase all new instrumentation. While it is still necessary to use film for high quality publication images, much of the routine data may be recorded on the low cost systems described above. ■

1. J. Liao & D.C. Martin, Science 260 (1993) pp1489-1491.

Reprinted from The Proceedings, Microscopy & Microanalysis '96, by the Microscopy Society of America, the Microbream Analysis Society and the Microscopical Society of Canada.