Digital Imaging in a Multi-User Electron Microscopy Facility: Progress since 1995

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INTRODUCTION

In 1995 we wrote an article for Microscopy and Analysis entitled: Digital image processing (DIP) in the TEM: is it viable in biological morphometry?, in which we described our experience of using one of the best digital cameras available at that time (a Kodak Megaplus slow-scan digital camera with 1317 x 1035 pixels) attached to the wide-angle (35 mm camera port) on a JEOL 1010 transmission electron microscope (TEM). It utilized a phosphor screen, converting the electron signal to a photon signal which interacted with the CCD-TV. Our images were analysed using a software package, NIH Image, running on an Apple Macintosh computer. We carried out a cost-benefit analysis on using images obtained via the Kodak Megaplus versus traditional film images of brain cells and synapses, which is the main topic of research in our group.

We concluded that there was a time-saving in digital image processing versus conventional print photography (about 40%) but the main saving was in financial cost, because once the equipment for DIP had been purchased there was essentially no expense unless one wished to print images. The biggest drawback that we highlighted related to image quality. Digital images fell somewhat short of the quality achieved by use of negatives and printed photographs. Our final conclusion was that DIP had some way to go before we could consider dispensing entirely with film and print.

Some 12 years on how has the situation changed? The answer quite simply is that DIP has surmounted use of film in transmission electron microscopy just as it has in popular 35-mm and professional camera photography. The market for digital imaging cameras for use in electron microscopy is now impressive with several very good cameras available at prices ranging from £30k to over £100k.

METHODS

We have used two new (for us) high-resolution digital cameras, an Advanced Microscopy Techniques (AMT) XR40 on our JEM 1010, and an AMT XR60Z attached to our recently acquired JEM 1400 electron microscope. The purchase of these two cameras followed an intensive analysis by us of the market for digital cameras suitable for use in transmission electron microscopy. Our comments are not intended to imply that these cameras are necessarily superior to others which we examined, only that, for our purposes, they proved by far the most suitable. Perhaps one of the best pieces of advice we can give to people considering purchase of a digital camera for their electron microscope is: don’t pixel count! Look at the evidence of the image quality with your specimens on your own microscope, and examine how the camera interfaces with the acquisition software to capture images from the microscope.

So what are the features and qualities of our cameras? On our 100 kV JEM 1010 we have the AMT XR40 attached to the 35 mm port (commonly known as side mounted) (Figure 1). Mounting in this position gives a wide field of view of generally 20-30% more area than captured with conventional film. The camera is thus ideal for looking at biological samples.

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The AMT XR40 has a resolution of 2048×2048 pixels (4 megapixels), and is commonly known as a 2K camera. The image quality is very high and has enabled us to entirely dispense with photography for our main research techniques of synaptic stereology involving examination of images at a screen magnification of 12,000 and post-embedding immunolabelled sections at 25,000.

RESULTS
Because we have the AMT XR40 at the side-mounted port we decided to position our higher resolution camera (an AMT XR60Z) in the bottom-mount position on our 120 kV JEM 1400. The AMT XR60Z has a resolution of 4000×2624 pixels (11 megapixels). It has a zoom interface which can be positioned for either wide angle in the mid-mount position similar to film (∼×3 magnification) or high magnification (∼×10) imaging. Having the option to change magnification gives good flexibility in a multi-user environment. We currently use our camera in the mid-mount position.

All AMT cameras are coupled to custom-designed finite conjugate lenses and high-resolution glass-mounted phosphor detectors that have been developed specifically for TEM imaging. This configuration provides a spatial performance comparable to fibre optics but without the drawbacks of complex vacuum feed-throughs and background patterning. Acquisition software is PC-based operating under the Windows environment and is intuitive and very easy to use even for those not familiar with digital imaging.

For more advanced users there is a comprehensive list of features such as live fast Fourier transform display for astigmatism correction, feature measurement, montaging and automatic image stitching. A key feature of the camera is the interface with the electron microscope. The in-column lens has an exceptionally high specification: custom engineered F/1.4 with distortion of <2%, lens resolution as determined by the modulation transfer function is above 70% at 100 line pairs mm⁻¹ over the entire field.

On our JEM 1400 we have three monitors (Figure 2); one for TEM control, the second for the acquisition software AMT 5.42 with a live image, and a third for displaying saved images. Thus, we are able to make a direct comparison between a live image and a previous saved image; for example, images from two serial sections for stereological analysis or a series of images from 100 serial sections for 3D reconstruction. Once the images have been processed we can transfer via our network to a server, burn to CD/DVD or transfer to a memory stick, and these images can be analyzed in detail at a convenient date.

In our last report on DIP in 1995 we commented on the advances in computational power which made image storage a much more feasible option than had been the case previously. In the last 12 years this has become an even more realistic option with PCs having terabyte storage or more at a price a fraction of that in 1995.

DISCUSSION
Most of our recent research [1-8] has involved three-dimensional reconstruction of portions of neuronal dendrites, spines and synapses using software developed by John Fiala and Kristen Harris (http://synapses.bu.edu/) that required negatives to be scanned (Figure 3). Whilst our published research using stereology has involved the use of older digital cameras for image analysis only, high quality images for publication were obtained by scanning negatives.

For the first time in our research, images from the AMT cameras are of sufficiently high resolution for publication; this also increases the throughput of our research, without the need for film at any stage. The ability to view and manipulate images in realtime means that fiducial marks in the section can be lined up with ease before image acquisition using a rotating specimen holder, which is much faster than aligning saved images on the computer. The quality of images from the AMT XR60Z has surpassed even the expectations based on our survey prior to purchase. Two examples are shown in Figure 4 a,b. Figure 4a shows a photograph of the imaging monitor on our JEM 1400 with an image of an immunoreacted glial cell. This image indicates clearly the silver-intensified gold immunolabelling in the cell and the AMT software interface. However, the more impressive image is shown in Figure 4b. This is from tissue embedded in Lowicryl HM20 resin following light fixation in paraformaldehyde and glutaraldehyde, slam freezing and freeze substitution with methanol and uranyl acetate. The tissue has not been postfixed in osmium and is therefore of low contrast and was not easily visible on the viewing screen of the TEM; the digital image (Figure 4b) shows clearly the tissue structure without any counter staining.

We have also used the montaging ability within AMT 5.42 and thus far have obtained 5×5 images at a final magnification of 15,000 of an area selected at 2,500 (Figure 5a,b). This A composite to demonstrate 3D reconstruction from serial sections. Two serial sections are shown that form part of a series from which the longitudinal fragment of neuronal dendrite and axon have been reconstructed and rendered.
TEM Digital Imaging

CONCLUSIONS

Although we have yet to explore the full capabilities of our two new cameras it is quite obvious that we are consigning our film plate cameras to the garbage bin. In fact, our JEM 1010 is now housed in the room that was formerly our darkroom!

REFERENCES


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