Progress In Design And Applications Of CCD Cameras For Electron Microscopy

Kenneth H. Downing, Lawrence Berkeley Laboratory

Over the last several years the long-awaited revolution in direct-digital readout systems has begun, with the introduction of efficient slow-scan CCD cameras. Earlier, the introduction of video cameras to electron microscopes had brought a quantum leap in the speed and efficiency of carrying out a host of operations. The high sensitivity of the video cameras provided the ability to see the image in much more detail and at a lower beam intensity than had been previously possible by viewing the fluorescent screen. The ability to assess, on line, characteristics such as specimen quality and image focus, even qualitatively, gave feedback to the operator that previously took hours to obtain. Due to the low resolution of these video systems, however, they were rarely useful for data recording.

The current generation of CCD cameras goes a long way toward meeting the needs of data recording in many, though certainly not all, applications. Immediate access to the data recorded in digital form has provided another quantum leap in efficiency. With their high dynamic range, linearity, and increased resolution, CCDs are capable of replacing photographic data recording in a number of applications. The on-line quantitative analysis of the image and imaging conditions, as well as avoiding the tedious processes of developing and printing or digitizing negatives, allow a tremendous improvement in data collection. Digital image information can be fed back to a control computer for automating a number of complex microscope operations, such as the long repetitive sequences of steps involved in tomographic data collection.

For some applications, such as electron crystallography of proteins, the limited number of pixels in the CCD still poses a serious problem. We discuss here some of these limitations, along with our approach to circumventing them by use of a larger-format CCD. Most present CCDs used for data collection consist of an array of 1024 pixels on edge. CCD cameras coupled to electron microscopes also suffer from a rather poor modulation transfer function (MTF) that limits the useful array size to something closer to 512 pixels. In contrast, arrays of 6000 or even 10000 pixels on edge are now frequently used when micrographs on film are digitized. Thus the transition from the use of film to CCD requires careful consideration of our requirements and the camera system limitations.

A recent series of experiments (Perkins, Downing and Glaeser, *Ultramicroscopy* in press) was aimed at determining the minimum array size that could be efficiently used for the crystallographic extraction of high resolution data from crystal images. We found that data from a set of 1000 x 1000 pixel images of purple membrane could be combined to provide the same quality as would be obtained from larger images of the same total number of unit cells. With smaller images, however, parameters such as lattice vectors could not be determined with sufficient accuracy to yield data of the same quality at high resolution. This experiment sets a lower limit on the actual image array size that is required for extraction of high resolution data from images of protein crystals.

In an effort to overcome the limitations of current CCDs, we did a careful study of ways to increase the number of pixels in the image and still have a system that would perform at least as well as film in terms of both MTF and detective quantum efficiency (DQE). As the system is intended primarily for low-dose work, we need to see how the DQE at low exposure will compare to that of film. We assume for the present that images are to be digitized at around one angstrom per pixel, either on film or on the CCD. At an exposure of 10 e/Å^2, the exposure is about 10 electrons per pixel. The DQE of the image on film is limited at low exposure by the fog level of the film. The fog has a typical optical density of around 0.1, which corresponds to an effective exposure of ~5 e/Å^2 when 10 µm pixels are used. The system noise for a slow scan CCD is on the order of one primary electron per pixel, so it is expected that the CCD will outperform film more and more as the exposure level is decreased.

The MTF is principally limited by the point spread function (PSF) of the scintillator. One needs a reasonably thick scintillator to produce enough photons from each electron to give an adequate signal. On the other hand, the width of the PSF increases with the scintillator thickness. Most present systems employ scintillators that have a PSF on the order of 50 µm in diameter. CCDs, on the other hand, have pixel sizes only up to around 25 µm. One approach to improving the resolution is to demagnify the image formed on the scintillator onto the CCD using either lens coupling or a reducing fiber optic. Either of these, however, has a light coupling efficiency roughly proportional to one over the square of the demagnification. To compensate for a two-fold demagnification, which would decrease the light incident on the CCD by a factor of four, the scintillator could be made four times as thick, but the resultant degradation of its PSF would more than offset the gain from demagnifying the image. Thus, the most efficient approach seems to be to use direct coupling (e.g., 1:1 fiber optics) of a thin scintillator to the CCD.

We are led to the conclusion that the best approach to matching the scintillator PSF and the CCD is to bin adjacent pixels during readout, doubling the effective pixel size. This is a straightforward way to meet our requirements, although it is expensive, since it requires us to use a larger CCD in order to obtain the necessary field of view. The camera uses a Tektronix 2048 x 2048 CCD that has 24 µm pixels. There are a number of less expensive CCDs available with even larger pixel arrays, but they have smaller pixels that make them unsuitable for this application. As shown in fig. 1, the MTF of this camera at the Nyquist limit with 2 x 2 binned pixels is nearly the same as that of film with 10 µm sampling, as is frequently used for digitizing micrographs.

There are a number of additional benefits of using the larger format. A further enhancement of the DQE results from reducing the cross-talk between pixels that is caused by the PSF[2]. For electron diffraction studies, magnifying the pattern by a factor of two decreases the effect of light diffusion within the fiber optic that arises from the high intensity of the direct beam, and increases by a factor of four the exposure required to saturate the CCD, which reduces blooming from the direct beam. ■

1. K. H. Downing and D.A. Grano. *Ultramicroscopy* 7, 381 (1982).
2. K. Ishizuka. *Ultramicroscopy* 52, 1 (1993)
3. O.L. Krivanek and P.E. Mooney. *Ultramicroscopy 49*, 95 (1993)

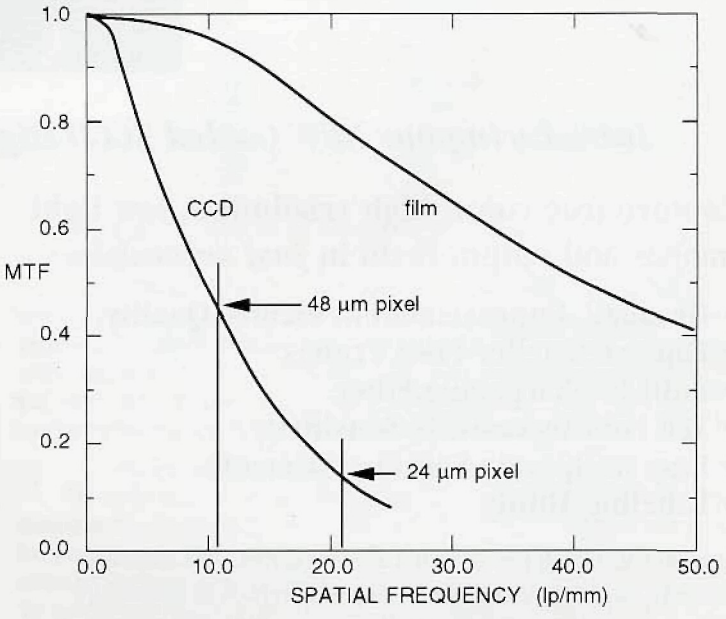
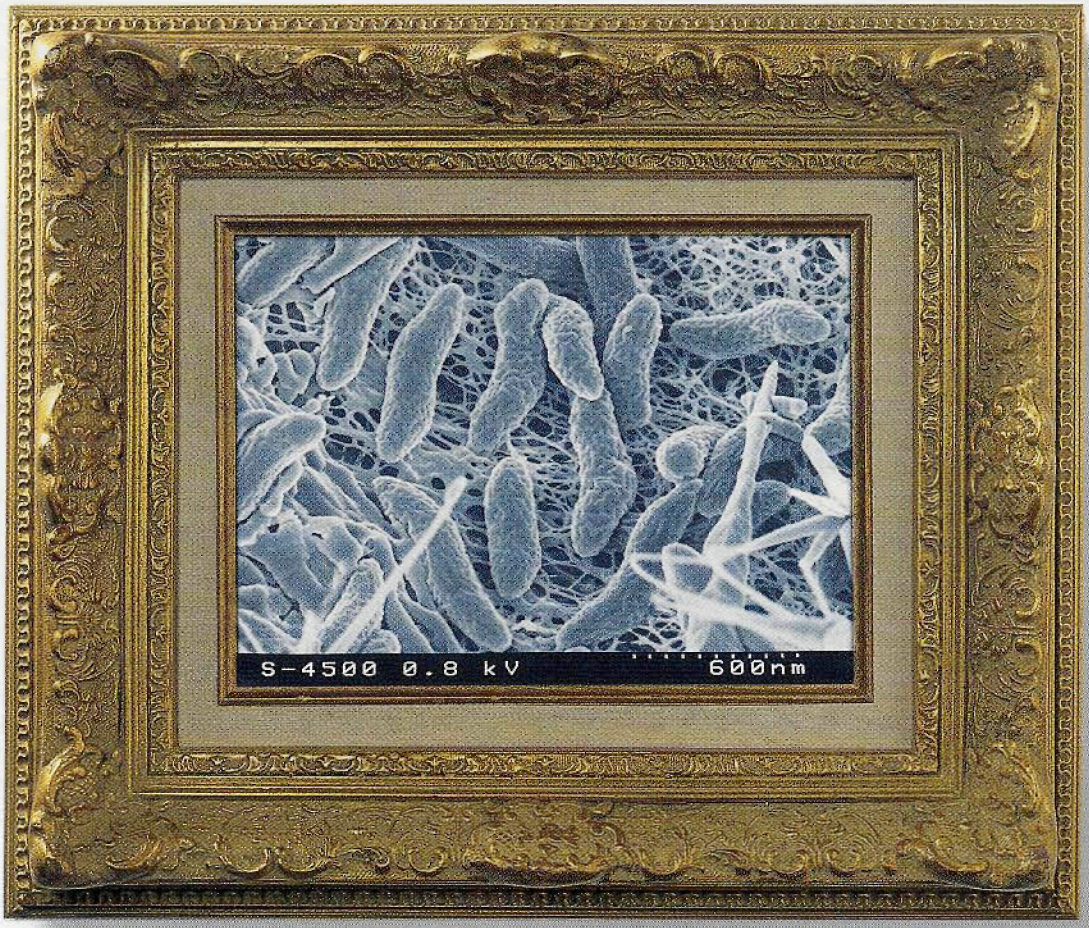


Figure 1: Modulation transfer functions for the Gatan Slow Scan CCD camera with gadolinium oxysulfide scintillator[3] and for Kodak SO-163 film[1]. Frequencies are indicated that correspond to the 24 µm pixel size of the Tektronix 2048 CCD and to the effective pixel size with 2 x 2 binning. With 48 µm pixels, the MTF is nearly the same as that of film with 10 mm pixels.

Reprinted from Proceedings: Microscopy and Microanalysis 1995 from the Microscopy Society of America.



This is the original. This is what you get with Hitachi's passive capture imaging system.

Sad, but true. Ordinary digital-image-capture systems, relying on digital beam control, just can't preserve the image quality offered by your microscope. It's your loss. And a big one, considering all you could do with a high-quality digitized image.

Hitachi's passive capture imaging (PCI) system, on the other hand, gives you images identical to the originals. Because it detects and uses the microscope's own scan signals, actually duplicating the scan generator's resolution up to 4000 lines—while microscope operation continues as usual.

Next, our easily used, Windows-based PCI lets you do just about anything imaginable with that superior image. Annotate it. Store it as a TIFF file. Archive and combine it with data from other microscopes. Measure it. Resize it. Color it—with up to 16 million colors. Print it out. Place it in documents. Send it across the lab or over the Internet. And (try to find this elsewhere) even replay it to your microscope's photo CRT.

Oh, yes. PCI works with any SEM or STEM. Same with TEM and optical microscopes, where PCI uses a slow-scan CCD camera to acquire sharp, non-interlaced, square-pixel 1024 x 768 images.

Call now for a PCI demonstration. Because, if you'd like to do any of 1001 things with your microscope's image, you'll want to capture it perfectly. Without letting its quality get away.

HITACHI
SCIENTIFIC INSTRUMENTS
Nissei Sangyo America, Ltd.

755 Ravendale Drive
Mountain View, CA 94043
(415) 969-1100

25 West Watkins Mill Road
Gaithersburg, MD 20878
(301) 840-1650