

# Digital Imaging, an introduction

All Electron Microscopes in the Research Resources Center allow users to collect images in a digital form. Scanning instruments (SEM, STEM) have an analogue to digital converter (ADC) which is used to digitize the signal from the detector. In the case of the TEM a Charge Coupled Device (CCD) camera is used to collect the image.

## Scanning Electron Microscopes

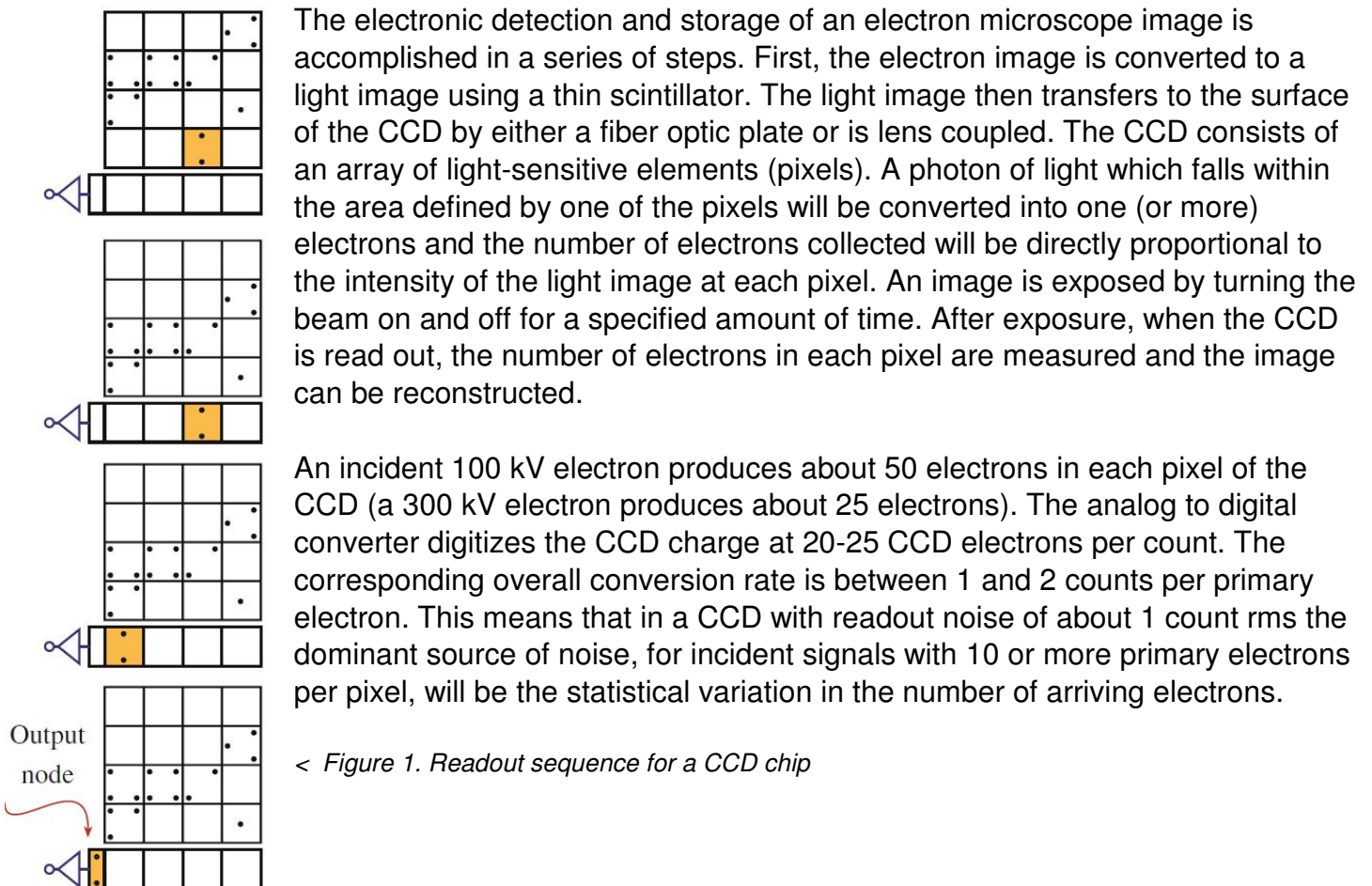
On the Hitachi S-3000N and JEOL JSM-6320F Scanning Electron Microscopes images are saved from PCI and Orion respectively in Tagged Image File Format (TIFF). Images can also be acquired and saved through the XEDS system on the S-3000N and again should be saved as TIFF.

## Transmission Electron Microscopes

On all the Transmission Electron Microscopes Gatan Digital Micrograph software is used to collect and display the data. The Digital Micrograph format (DM3/DM4) is very powerful but can only be read directly outside Digital Micrograph by using NIH Image J freeware with the DM3 plug-in installed. For image analysis with other software packages it will be necessary to convert the image needs to a more common lossless format. We recommend TIFF.

On the JEM-ARM200CF Images are acquired using the Digiscan plug in to Digital Micrograph. These images should be saved in DM4 format to preserve the original data.

## CCD based systems (*JEM-1220, JEM-3010, JEM-ARM200CF (TEM)*)



### **JEM-3010 - Gatan Orius SC200**

The Orius SC200 (Model 830) camera is a retractable water cooled CCD camera. This is a fiber optically coupled CCD camera with 2048 x 2048 pixels at 7.4 $\mu$ m giving an active area of 15x15mm. The signal is digitized to 14 bit resolution (16384 grey levels) at readout speeds up to 30MHz allowing readout rates as fast as 30fs (4x binning, 0.0625s pixel time - TV rate).

### **JEM-1220 - Gatan Erlangshen ES1000W**

The Erlangshen ES1000W (Model 785) camera is air cooled. This camera has 2672 x 3608 active pixels with a pixel size of 9 $\mu$ m giving an area of 24mm x 32.5mm on the camera. Due to the optics and scintillator the effective pixel size is 20 $\mu$ m. The signal is digitized to 12 bit resolution (4096 grey levels) at a readout rate of 30MHz allowing readout speeds as fast as 12fps (with 4x binning).

On the JEM-1220 there are two parts to the camera system. On the left hand side of the column is a pneumatic retractable prism and scintillator, which is inserted by mean of a switch, and on the right hand side of the column is a lens coupled CCD camera and its electronics.

### **JEM-ARM200CF (TEM imaging) - Gatan Orius SC200D (top), SC1000 (bottom)**

There are two water cooled retractable CCD cameras on the ARM200CF. There is an Orius SC200D (Model 833) lens coupled CCD camera above the viewing screen which has 2048 x 2048 pixels at 7.4 $\mu$ m giving an active area of 15x15mm. The camera has a robust scintillator to minimize electron beam damage and deliver streak-free diffraction patterns. There is also an Orius SC1000 (Model 832) fiber optically coupled camera below the viewing screen which has 4008 x 2672 pixels (11MP) at 9 $\mu$ m giving an active area of 36mm x 24mm. For both cameras the signal is digitized to 14 bit resolution (16384 grey levels) at readout speeds up to 30MHz allowing readout rates as fast as 14fps (SC1000: 4x binning) and 30fs (SC200D: 4x binning).

The CCDs linear response is better than 1% with a geometric distortion of less than 0.1%. Hence the numerical intensity value displayed in a pixel is proportional to the electron-beam intensity at the scintillator. (This was not the case for film). The CCD also has a large dynamic range of greater than 10,000:1.

The position of the camera will determine the final magnification of the image. On the JEM-1220 and JEM-ARM200CF the magnification of the image at the upper CCD camera position is about 0.33 times that on the viewing screen. On the JEM-3010 and JEM-ARM200CF the magnification of the image at the lower CCD camera position is 1.5x that on the viewing screen. In all cases the image displayed on the computer monitor are typically 10x the size of the CCD sensor if displayed at full monitor size (JEM-1220, JEM-ARM200CF (upper camera) 3.3x screen; JEM-3010, JEM-ARM200CF (lower camera) 15x screen).

### **Digital Micrograph**

The images collected are displayed on various versions of the Digital Micrograph software. A typical window is shown in figure 2.

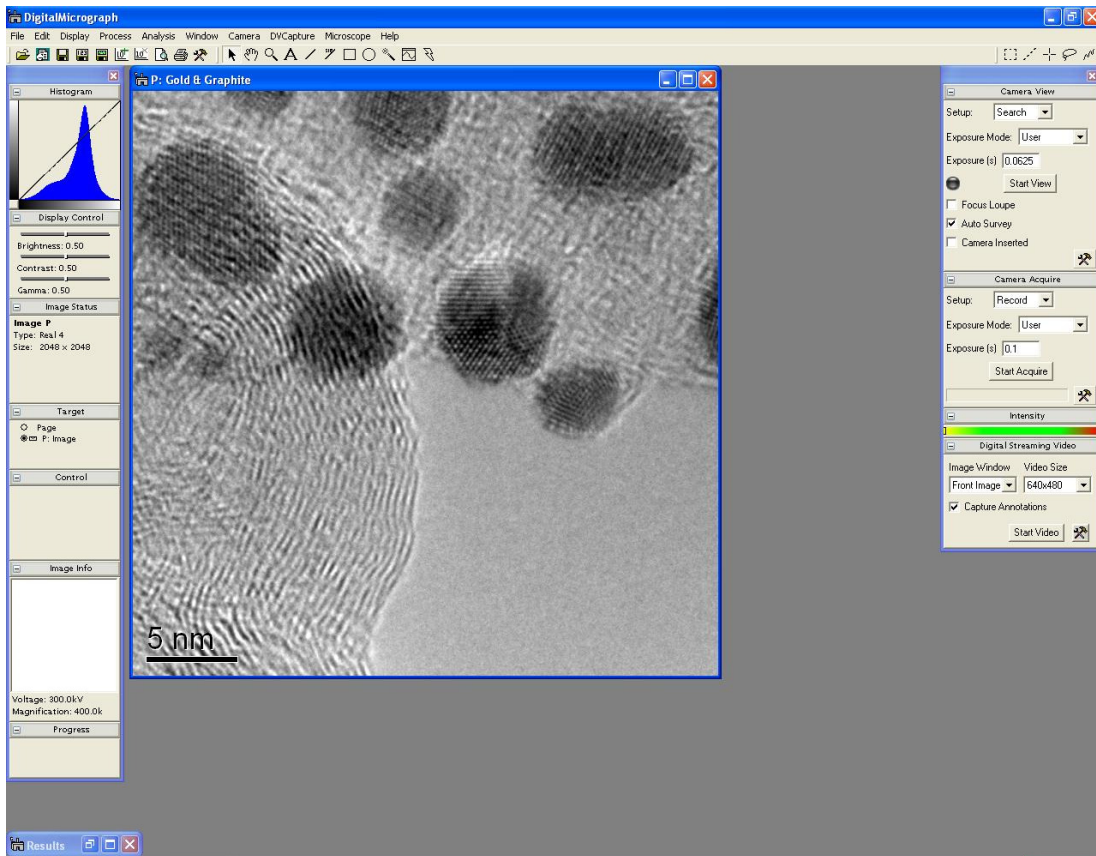
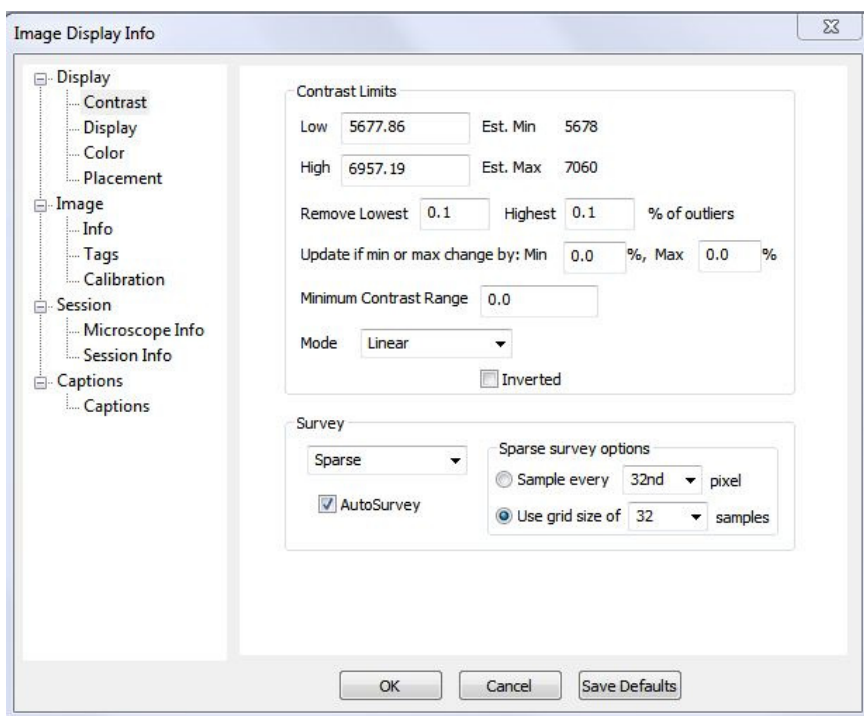


Figure 2. Typical Digital Micrograph window displaying an image and floating windows on the left of the screen and camera control on the right hand side

The image is displayed as an 8-bit (256 gray levels) screen representation of the collected data. Please note that the monitors are only able to display 256 gray levels. The image is automatically stretched to fill all available gray levels in the display by the software.



If we look at Display > Image Info window for the image in fig 2 (figure 3) we see, in the contrast limits section, that the lowest intensity has been assigned to an original intensity of 5678 and the highest intensity to 6957. Further down in the survey section is the technique used to find the maximum and minimum values – in this case sparse and to the right of that options for the sparse survey.

< Figure 3. Image Display Info window for image shown in fig 2.

The choice available for “survey” are cross wire (checks the maximum and minimum values on a cross going through the center of the image), sparse (checks the values on a distributed set of positions throughout the image), reduction (checks

the values by dividing the image into 16 equal sizes). and whole image (checks the values at every pixel). Whole image and reduction are slower methods and should not be used during acquisition, cross-wire or sparse are the best options during acquisition and are the normal defaults.

## Image Processing

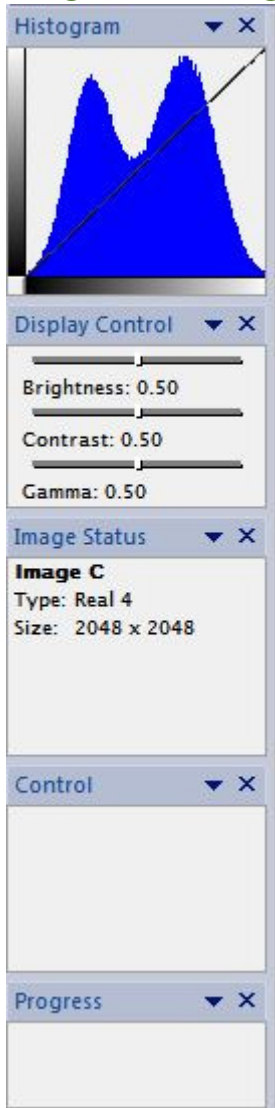


Figure 5. Floating windows with Histogram, Image Status and Display Control expanded.

The following section continues the description of Digital Microscope, however the comments are also relevant to any other image processing package that is used on the recorded data (Adobe Photoshop, Corel Photo-Paint etc.).

The histogram display on the floating window should be used to make sure the survey method has chosen representative values for low and high. The Image Status window allows you to measure the intensity at any point (X, Y) in the image. Out of the survey methods Sparse is usually better than Cross-wire, when available. Cross-wire has problems if the brightest (or darkest) area is not on the two lines through the center of the image.

Save the image in Digital Micrograph format before adjusting anything! This is the raw acquired data, which can be returned to if in subsequent processing something goes wrong.

Display control allows the Brightness and Contrast to be altered on all versions. The **only** controls that should be changed are the low and high values (a histogram stretch - select range on histogram or type in numbers to Image Display Info window) and Gamma. Contrast and Brightness **SHOULD NOT** be used. They are linear functions, which if altered will not show the full range of data satisfactorily, or a true representation of the image on the microscope. This is because a CCD camera is a linear detector of light intensities, whereas the eye (and film) has a logarithmic response, allowing both bright objects and dim, low amplitude objects in the same visual scene. Gamma is an exponential function that more closely matches the non-linear response of the eye or film. (On the Hitachi S-3000N histogram stretching should be done in PCI before

saving the file.)

Every image and every display media (monitor, printer etc.) will need a different gamma setting. The recommended procedure to get a good print out would start with a histogram stretch followed by gamma, check on printer, gamma, check, gamma etc... and finally a controlled histogram stretch at the end leaving the high value untouched and adjusting the low value.

Images should not be magnified more than x2 on the computer for the 1024x1024 cameras. If you need higher magnification than that the image should have been taken at a higher magnification in the microscope. Unlike film where post acquisition magnifications of x10 are not uncommon, the smaller number of pixels in a CCD image can limit the useful post magnification that can be used.

The following four images show examples of correct and incorrect histograms.

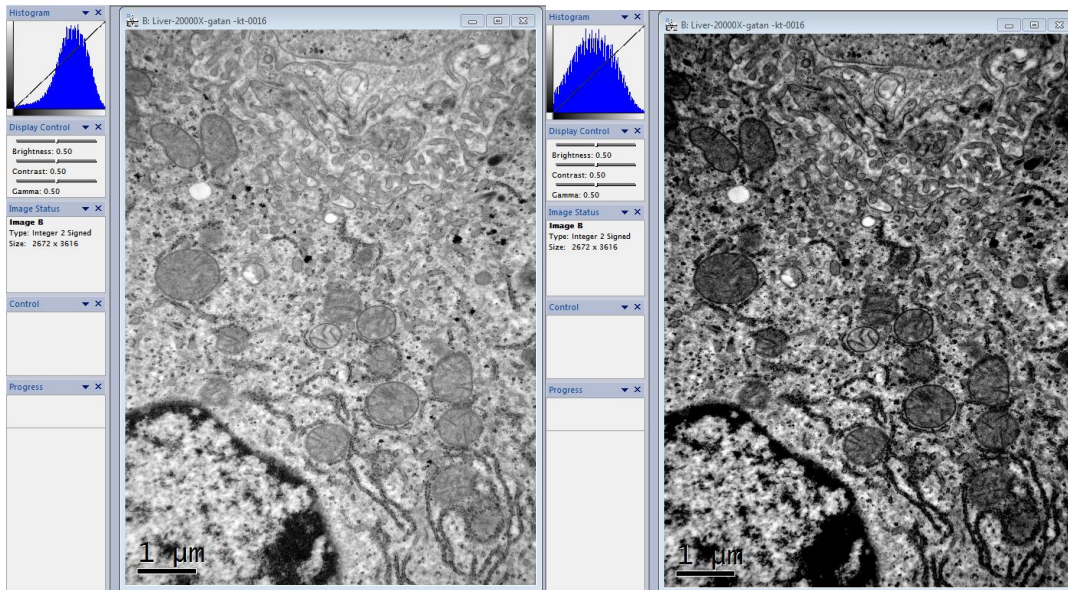


Figure 6a Correct representation

Figure 6b Range to small

In fig 6a the histogram fills the full range and is correct. In fig 6b the histogram is cut off at the lower end and some image information is lost as large areas are assigned a value of 0. If the histogram looks like this it is best to select Whole Image.

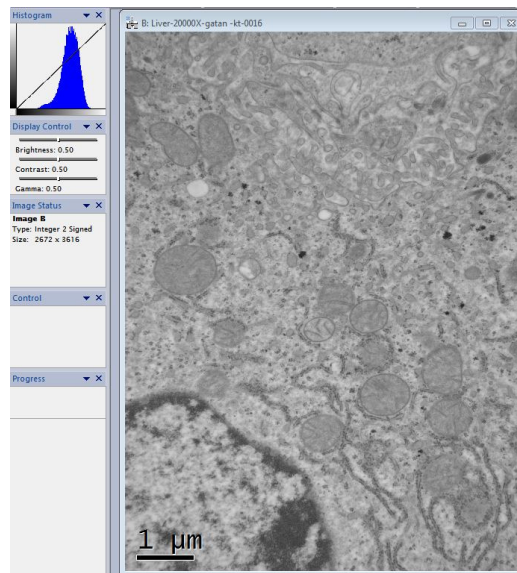


Figure 6c Range to wide

Typically if whole image is selected the histogram will end up as in figure 6c. Here we are not using all 255 grey levels to display the image. This can be corrected by adjusting low and high in the Image Display Info window, or use the mouse to highlight the range on the histogram.

### Saving and Transferring Images

Images should be stored in Digital Micrograph (DM3/DM4) format and as a full resolution, annotated TIFF file. The DM3 format saves the absolute data obtained from the camera in either 14 bits (16384 grey levels) or 12 bits (4096 levels) and also saves any annotation and the scale marker as a graphical overlay, using the final 2 bits, which can be moved later. If you need to do any quantitative imaging it should be done on the original data stored in the DM3 file. In TIFF format the annotations are burnt into the image and cannot be removed or changed afterwards and the image data is stored

as 8-bit. There is a Batch Convert window in Digital Micrograph that allows all files in a folder to be converted to a full pixel resolution TIFF Format. For files to be read by common imaging programs make sure Save Display As is selected as in Figure 7.

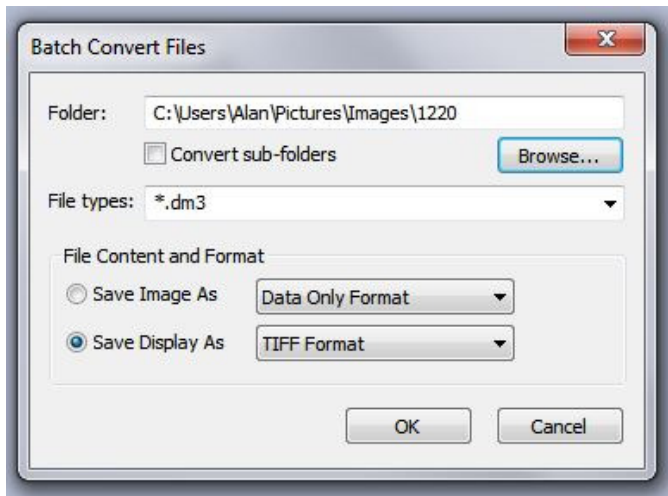


Figure 7. Batch Convert Files

Filenames must be letters, numbers and – or \_ only! We recommend that you use save numbered with specimen descriptor\_magnification\_index. See EZDM document for more information. Details of what each image is should be kept in your laboratory notebook and not in the filename!

It is important to move these off the microscope hard drives as soon as possible. Data from the JEM-3010 can be transferred to cloud storage via the network connection. All TEMS have CD-RW or DVD-RW

writers. Data can also be transferred to virus free USB drives (you must check your drive on a computer with up to date virus scan software before using them on a microscope computer), you MUST NOT transfer anything from your drive to the DM computer. In EMS-W there is a PC based imaging computer available in E-5F. This computer has a CD-RW drive, a network connection for network and USB connection and can be used to back up your data. It is strongly recommended that back up copies of all images are made preferably on CD-R discs (remember "if it has got a motor it will break" so do not leave your only copies on a hard drive!) Good CD-Rs have a lifetime of at least 15 years. If the data is important to you please remember to make another copy every 10 years! Under no circumstances save your only copy in JPEG format. Although this format saves space it does so by throwing away information. Every time a JPEG is opened and saved more information is lost.

### Image sizes for different file formats

Instrument	Camera Type	Active Size	DM3	TIF	JPEG
JEM-1220	Erlangshen ES1000W	3616x2672	18.8MB	9.23MB	2.04MB
ARM200CF	Orius SC1000	4008x2672	41.2MB	10.2MB	3.75MB
ARM200CF/ JEM3010	Orius SC200D, SC200	2000x2000	16.5MB	4.01MB	0.91MB

DM3 format files can be opened using NIH Image J (Image Processing and Analysis in Java) freeware with the DM3 plug in installed. This software also allows batch conversion from DM3 into many other formats and is recommended for image manipulation.

### Summary

Digital imaging in transmission electron microscopy has come a long way in meeting the high demands of recording images. CCD based cameras have become practical for all TEM applications and provide numerous advantages over film, including higher sensitivity, larger dynamic range, better linearity, lower noise and immediate access.