AMT Camera Manual

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Getting Started

   Welcome to the AMT Image Capture Engine. This first chapter tells you what all new users want to know, how to prepare the scope and camera and start taking pictures.
The first four pages are about what you do before before any imaging, and the other three describe taking live and final images, and turning the camera off.

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Retract the Phosphor When Starting or Finishing

If there is a sidemounted camera, make sure that the phosphor is not in the beam. The phosphor can be damaged by an overly concentrated electron beam.

A previous user may have forgotten to remove the phosphor from the beam path.

Align the TEM

1) Align TEM and center beam.

<u>Note: It is easier to align the microscope using the standard TEM viewing screen and local alignment procedures.</u>

2) On side mounted systems, sure that the phosphor is retracted so that the beam is visible! Camera operation is easiest when the "current axis" is aligned and the intensity remains centered as magnification changes.

3) If the TEM supports "Brightness" "Intensity" Zoom, use it. The camera typically operates at intensities equivalent to about 2 sec of film exposure.

4) Spread the beam with the condenser (C2) control (clockwise) until the film exposure meter read at least 2 seconds. Another method is to just spread the beam until it is kind of dark. The camera is pretty sensitive.

Starting the Camera

<u>Camera Power On</u>
The power switch on the camera control box needs to be turned before starting the AMT Image Capture Engine. Most labs leave the camera power on all the time, except possibly for weekends or vacations. Turning the camera power off for 60 seconds is also one of the first things to try if there's image problems.

<u>Get a Beam</u>
A previous user may have forgotten to remove the phosphor from the beam path. The phosphor can be damaged by an overly concentrated electron beam. It is good practice to spread the beam to ~2 sec exposure before inserting the phosphor spread the beam.

<u>Launch the AMT program</u>
At this point, if the camera program is not open on the AMT computer, open it by clicking the AMT Icon in the Start menu.

Background Collection

Background correction compensates for inhomogeneities in the beam and phosphor and any other optical imperfections.

Image quality critically depends on having a good background image. We recommend that background images be collected each day or whenever there is a change in the gun high tension or mechanical drift.

1. View the beam on the TEM viewing screen. For side mount be sure that the phosphor is retracted.
2. Pull out specimen rod or find empty position or hole in the specimen.
3. Set the magnification in the mid-range for planned operation. Spread the beam with the condenser (C2) control for **2 sec** on the film exposure meter. The 2 second value is for a typical setup. Use higher or lower values if your camera is adjusted differently.
4. For side mounts insert the phosphor. (For bottom mounts lift the viewing screen.)
5. Select the Menu Item ***"Background - >Acquire Background"***.
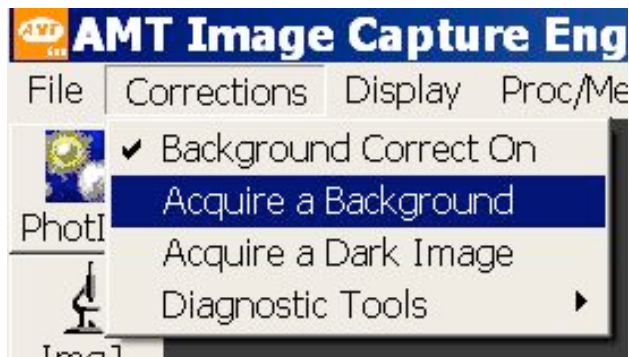
6. Adjust the second condenser (i.e. the TEM Intensity or Brightness knob) so that the histogram [red line in box] is <u>approximately centered</u> in the "box". When this is correct, click on the red command button.
7. You will get a chance to cancel. If you proceed, it will take a minute or so for the backgrounds for the various modes to be acquired. **WAIT until the "Click Live Imaging" button is re-enabled (gray letters return to black).**
8. You are now ready to collect images.
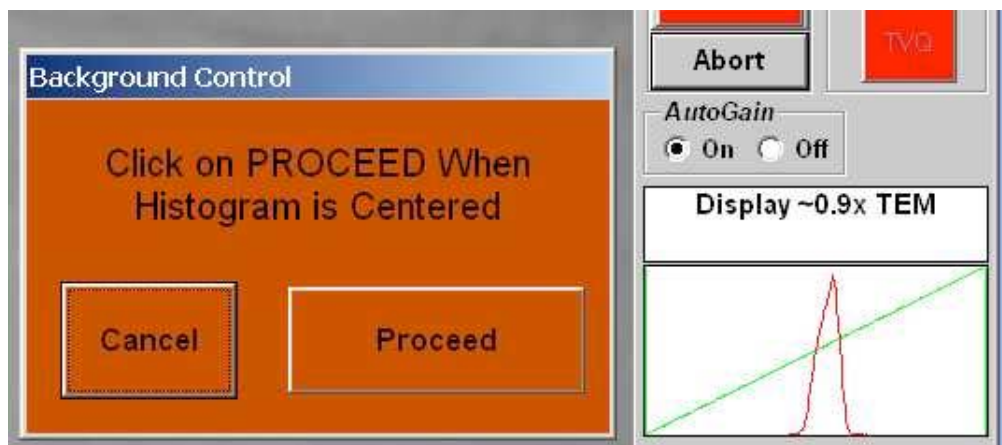
Live Viewing

<u>For Side Mount Systems</u>

Flip the phosphor retractor switch toward the column on retracting mechanism. (This is automatic on H7500/H7600.) Or
turn the wheel counter-clockwise to the end position *for manual system prior to 2001.*

For Bottom Mount Systems

Lift TEM viewing screen (This is automatic for systems with TEM communication.)

Camera Control

Starts camera then changes to "Click for Final Image"

Click for Live Image

Speed (red) is fast display
Quality (blue) is frame averaged live

Speed-Live

Aborts "Live" imaging -
Results is for display only
Not saveable!

Abort

○ Survey
○ Focus
○ FFT

AutoGain
● On ○ Off
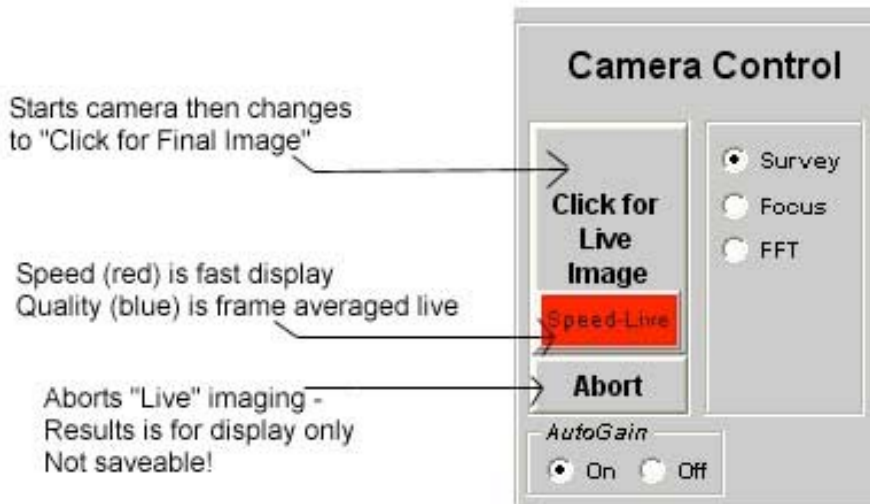
1) The **Survey** radio button is normally selected for full images at fast viewing rates.

2) Click on command button **"Click for Live Image"** Adjust the brightness of the electron beam (the TEM!) to center the histogram's peak. The verticle green line on the right hand side should also be visible and at least slightly away from the right side of the white box.
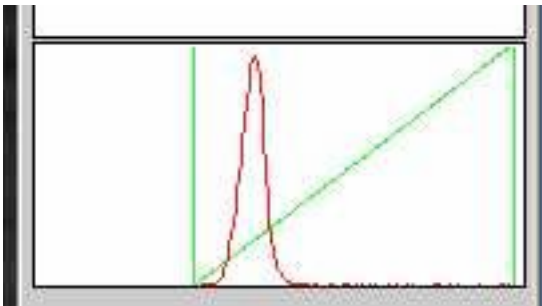
3) Clicking Speed Live, to make it **Quality Live**, averages frames in live imaging to reduce beam noise. The tradeoff is that the camera will be a little bit slower.

-- For faster imaging select **Speed Live.**
-- To reduce live noise chose **Quality Live.** The Quality setting has a slower update rate. *This button has no effect on the final image. You can fine focus using the Focus button, which enlarges the center region of the image.*

4) ***Focus*** Mode is an optional focusing tool. It zooms in on the image and removes binning, so you see more of the full resolution.

5) Note: Starting in version 5.4 there is a ***4 by 4*** mode option (with 4x4 binning) for most cameras. This setting runs very fast with near TV rates, but live image quality is less than survey mode.

Acquiring a Final Image for Saving

After the "Live" image is focused and illuminated properly, then "Click for Final Image" for an image that can be saved (i.e. a Final Image). This will take approximately 15 seconds. The camera is usually set to take a number of final images and average them to reduce noise. You can adjust how many are averaged (See "*Camera Settings And Control Window*").

Aborting Imaging and Closing AMT Software

The fastest way to stop imaging is to click the "Abort" button under the Live/Final Imaging button.
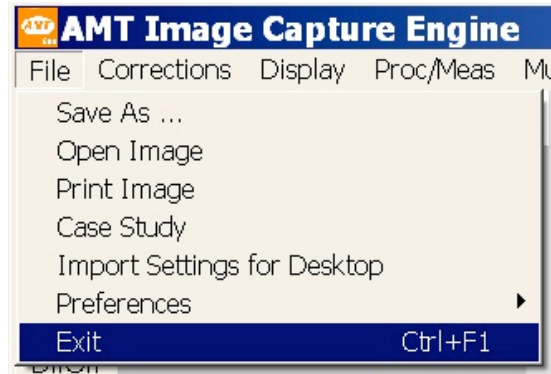

Aborting just turns off camera imaging, it doesn't collect a final image.

To close AMT Software click on the menu items:

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Saving Images and Cases

Now that you're experienced taking pictures, you probably want to save them. There's two methods in AMT for saving images. The first is the familiar **"File -> Save As"** method. For a more efficient method of collecting and saving multiple images quickly use the **Case Study** protocol.

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Microscope Information Window

Whichever method you choose for saving images, before saving you will encounter the Microscope Information window. This is a collection of information which will be stored with your image. The items you check will be displayed at the bottom of the image when it's opened in an image processor and in a print. A saved image's information can also be displayed in the AMT program by clicking (on the upper menu) "**Display**" and then "**Show Current Caption On Screen**".

If your scope has communications, the Magnification, and possibly the Voltage, stage X and Y and Tilt, will be automatically entered when you collect your final image. For scopes without communications you need to type in the correct values.

There is room for two optional Caption lines. The main reason for the Keywords is that all this information is saved to two databases, besides being saved with the image.   You can use Keywords to facilitate searches for images or data. If you check the Keywords they will also be displayed in a processor, but not on separate lines. The "Facility" name is set in **"Utilities->Special Options"** on AMT's upper menu.

If you're using the Case Study protocol the window will display a path and a filename, which is the case name plus an auto sequenced number.

Near the top of the window you choose whether you would like the saved image to display the "*Print Mag*" (e.g. "Print Mag: 19600x @ 7.0 in") or **Pixel Size** (e.g. "Cal : 0.005 microns/pix") or neither. "*Printer Units*" sets the print width, using either inches or millimeters. This number is used by whatever image processor you print from.

(Note: The "*Print Mag*" displayed under the image is calculated for a print of the width you set here. If, when printing, you click *"Fit to available space"*, the Print Mag displayed on the printed image will be wrong. If you want a wider print, it is best to set it wider here.)

The first item in menu at the top of the window, *"KeyboardCommands"* displays two keyboard shortcuts when clicked. The "*SwitchMode*" item changes the "Imaging Mode" from imaging to diffraction, which changes "Magnification" to "Cam Length". *"Add File Options"* allows one to add a suffix to a filename (case study only) or create a text file with all the image's information (Not available in Case Study).

When the information in the window is complete, click the "**Save With Caption".** This saves all the information in the window. For a shortcut back to live imaging, use the right button on the mouse for this click. This will automatically restart live imaging after the image is saved.

Saving via "File -> Save As"

Users with Windows computer experience are familiar with saving files this way. **"File"** is the leftmost item in the top menu. When you click **"Save As"** the Microsope Information Window discussed above will appear. After you click **"Save With Caption"** in that window the following window will appear:
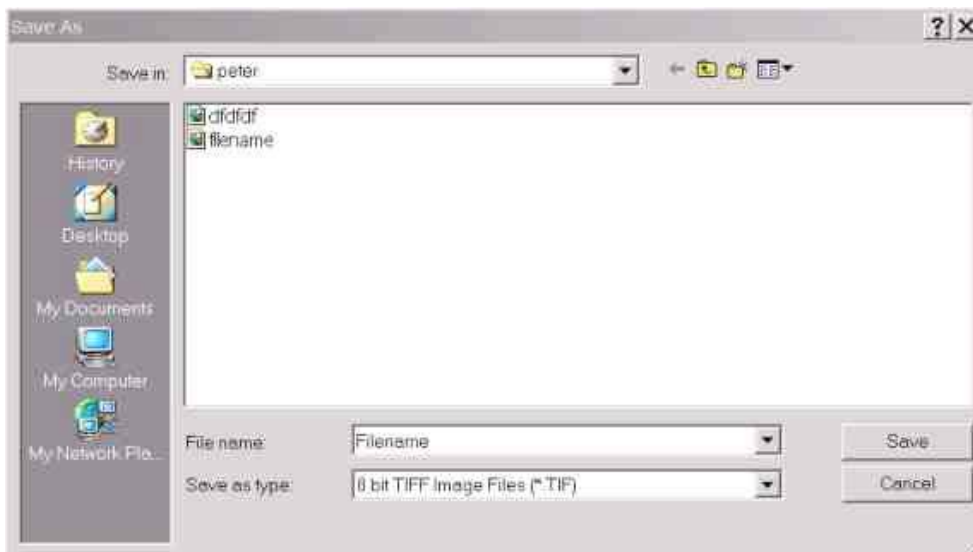
Here you are asked to choose a location folder for the image, and to give the File a name. You can also choose what type of file to save. The default type is a Tiff image file.

Setting the Default Image Path

Whether you are saving using "*File -> Save As*" or are creating a case, you are presented with a default saving folder. The usual default folder is C:\Images (the Images folder on the C drive). But if you frequently save to a different location, say to a network drive, you can streamline the process by making that the default saving folder.

To change the default path, before you start saving an image, go to the "*File -> Preferences->Image Save Options->Set Default Image Path*".
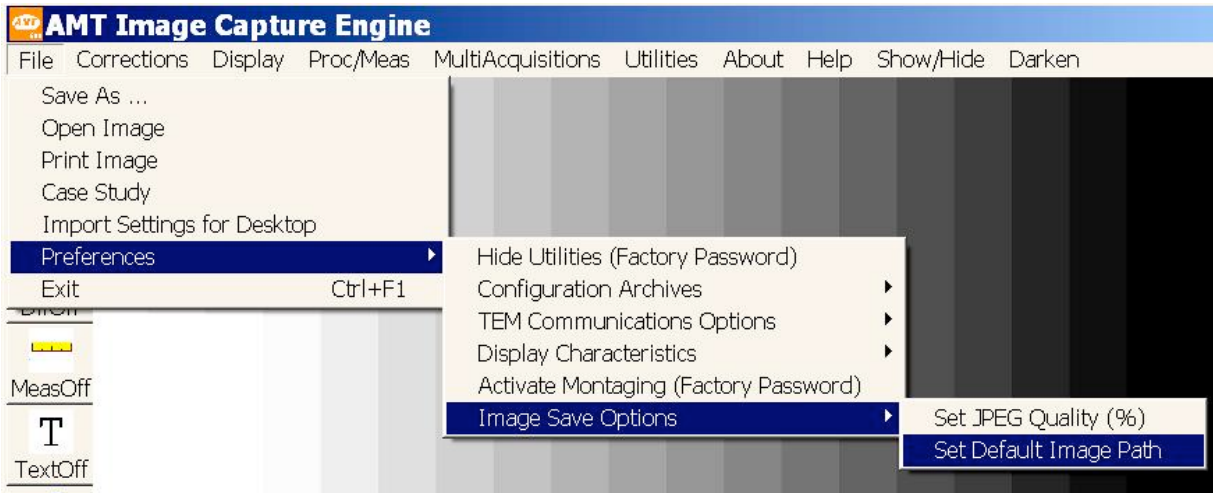
After this selection you will get the following window for typing in the default path:

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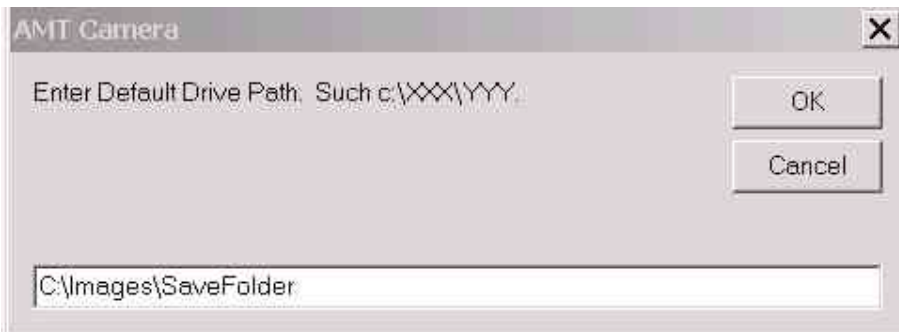
Saving via the Case Study Protocol

When you save an image to a Case it is automatically given a file name and a location, so it's the fastest way to save multiple images of a specimen. If you right click the save button in the Microscope Information Window, Live imaging is automatically restarted, saving a few more seconds.

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Creating A Case
Saving To A Case
Case Display Options

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Creating a Case
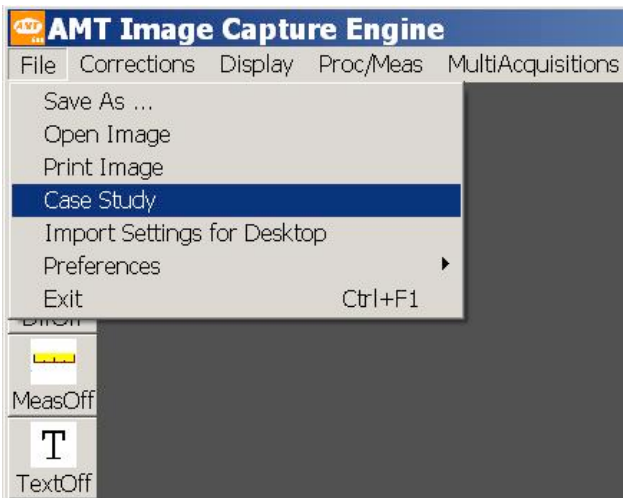
The first step in using Case Study is to create a Case. To do this go on the menu to "*File -> Case Study*".

After this selection the "*Case Study Control*" window will appear. It has a number of options for choosing, managing and displaying cases. Here we click the "*Create New Case*" button.
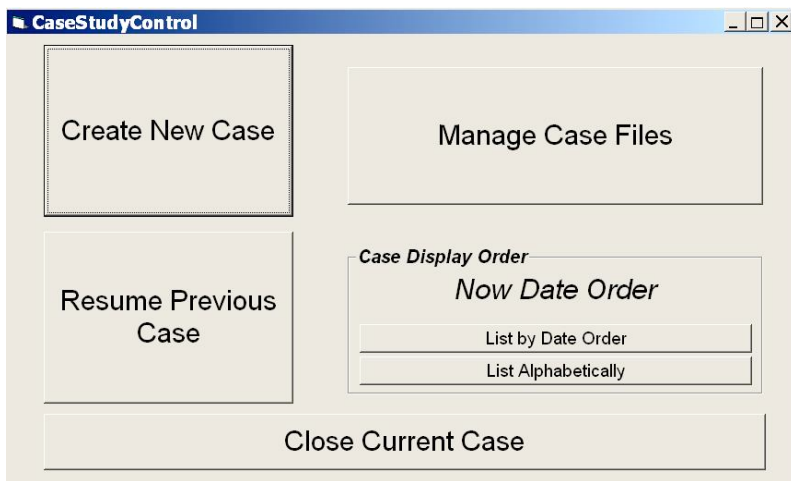
The "*Create New Case*" Window is where you set up the new case by choosing a case name, a file type and a location. The filename of images saved to a case will be the case name plus an automatically incremented number.

The "*ChangeFileSeparator*" menu item give you the option of choosing what character separates the case name from the number. The default separator is an underscore

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Create New Case

File   ChangeFileSeparator

Set Case Path

🖳 c:

📁 c:\
📁 images
📁 16bit
📁 16BitCase
📁 8bit
📁 8BitCase
📁 8j
📁 8nj70
📁 all3
📁 av1
📁 Back
📁 BothBitCase
📁 dfdfdf
📁 j_8
📁 j100
📁 jj
📁 jon
📁 jonly2
📁 jpeg
📁 sa8
📁 saj
📁 test
📁 test5
📁 test6

Similar Case Names

Save Format

☑ 8 Bit TIF   ☐ 16 Bit TIFF   ☐ Save Also As JPEG@ 50%

Enter New Case

Images Will Be Saved In Case

c:\images

Accept New Case
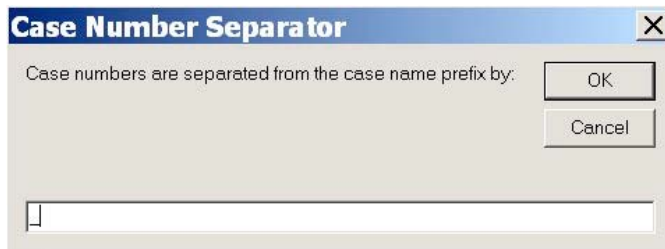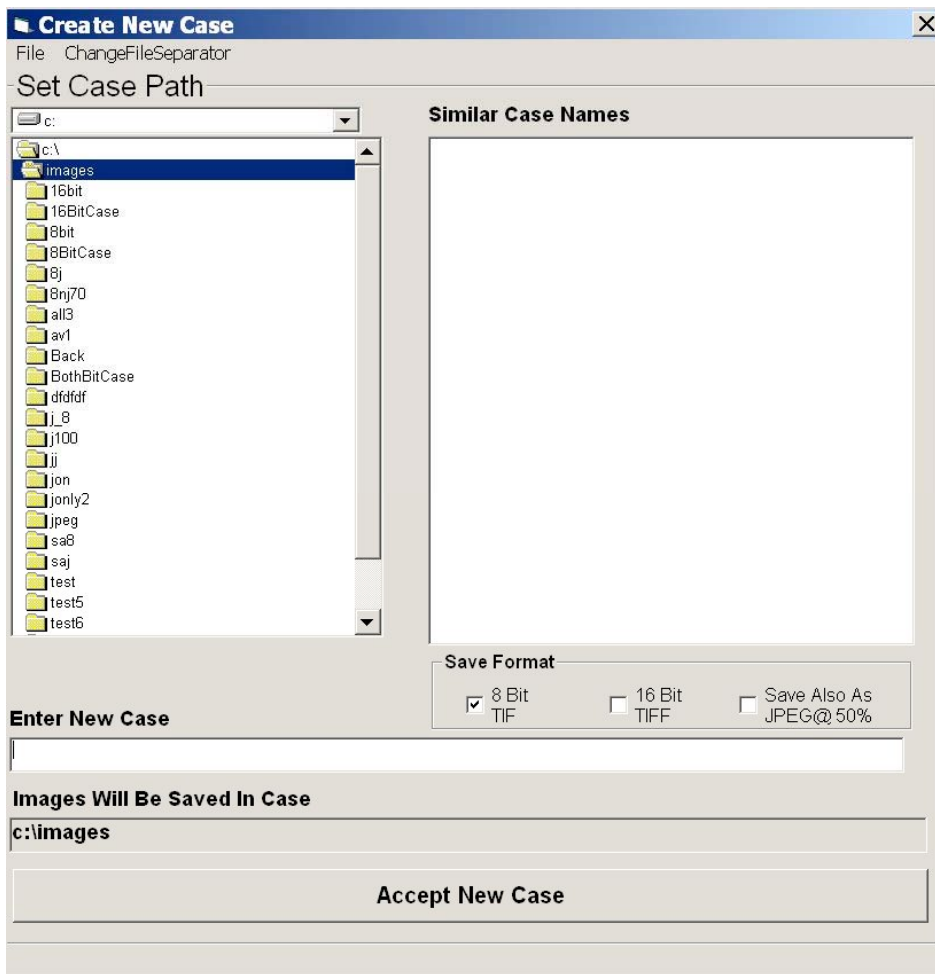
The normal file format is 8 Bit TIF. TIF images are "lossless" and are therefore the most stable. Eight bits is a big enough file for most purposes. A person might save 16 bit images if they were planning to do quantitative analysis (such as gray level comparisons or Fourier Analysis) of the final images after they're saved. There's an option to also save the images as JPEGs, which are smaller and therefore easier to send by email.
 After you've entered a name for the case, click **"Accept New Case"** and the window will close.

JPEG quality (%) is set in the main AMT window's "Preferences".

AMT Image Capture Engine

File   Corrections   Display   Proc/Meas   MultiAcquisitions   Utilities   About   Help   Show/Hide   Darken

Save As ...
Open Image
Print Image
Case Study
Import Settings for Desktop
Preferences   ►   Hide Utilities (Factory Password)
Exit   Ctrl+F1   Configuration Archives   ►
                                    TEM Communications Options   ►
                                    Display Characteristics   ►
MeasOff   Activate Montaging (Factory Password)
                                    Image Save Options   ►   Set JPEG Quality (%)
T   Set Default Image Path
TextOff

Saving to a Case

After you've collected a Final Image to save, click on the Case Study Icon (the File Cabinet), at the bottom of the left toolbar.

If you're in a case, for instance if you've just created a case, the Microscope Information Window, described next, will appear. If you're not in a case a list of your active cases will appear for you to choose from.
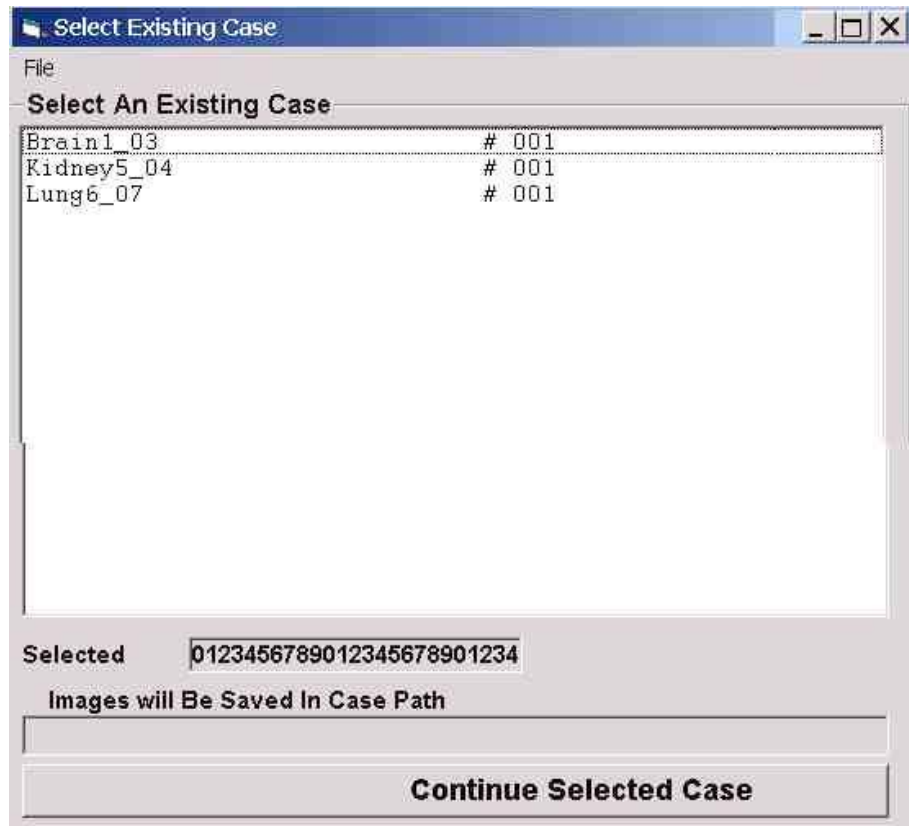
Select one of the cases and click **"Continue Selected Case"**.   The window will close. Now you're in a case, but the image is not yet saved, so click on the File Cabinet Icon again to save.

Once you're in a case and click the File Cabinet, the Microscope Information Window, described above, will appear. The image now automatically has a File Name, which is the Case Name plus an automatically sequenced number.
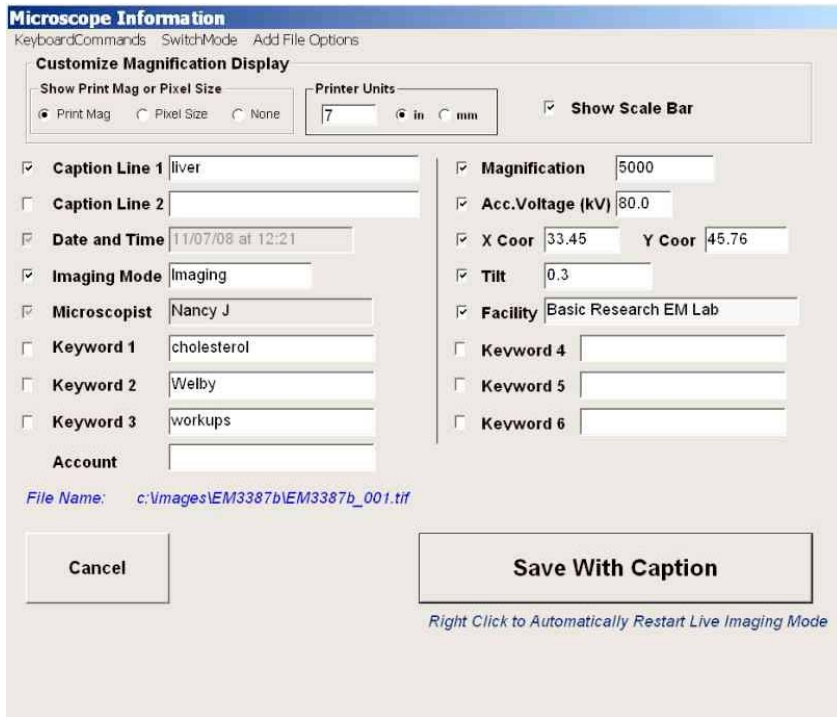
You don't have to type anything in (except the Voltage and Mag if your TEM doesn't have communications). You have the option however of appending the filename with a suffix, to facilitate finding the file in Windows. To do that click **"Add File Options"** in the upper menu and then click "*Add Suffix to Filename*"
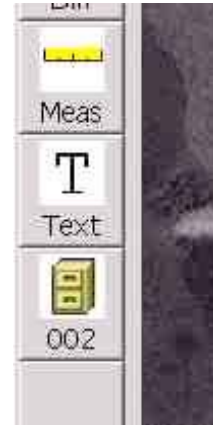
When you're in a case the Case Study Icon (File Cabinet) will display the number of the next image to be saved there.

Case Display Options

The Case study control window allows you to choose how the cases are ordered in the active case list - by date or alphabetically.
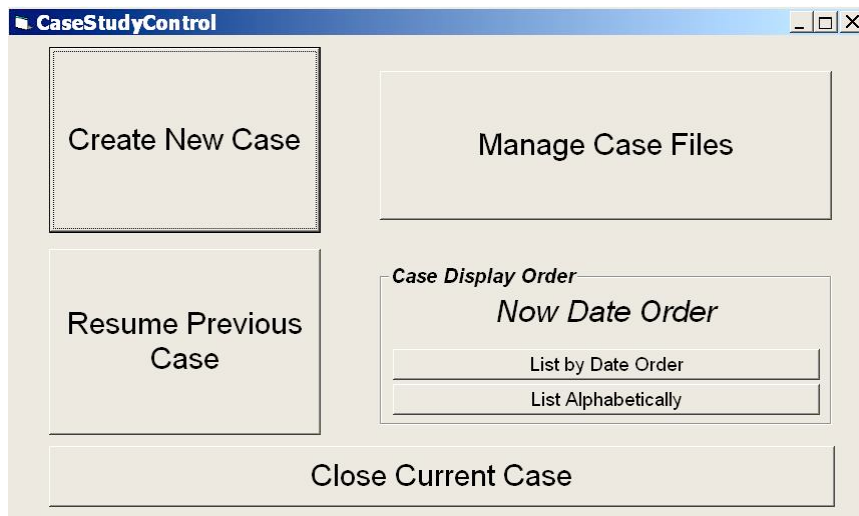
Clicking "*Manage Case Files*" will bring up the Case Archive Manager. Moving a case into archive does not move any images, it just takes the case out of your active case list to make it shorter. A case can be restored to the list just as easily.

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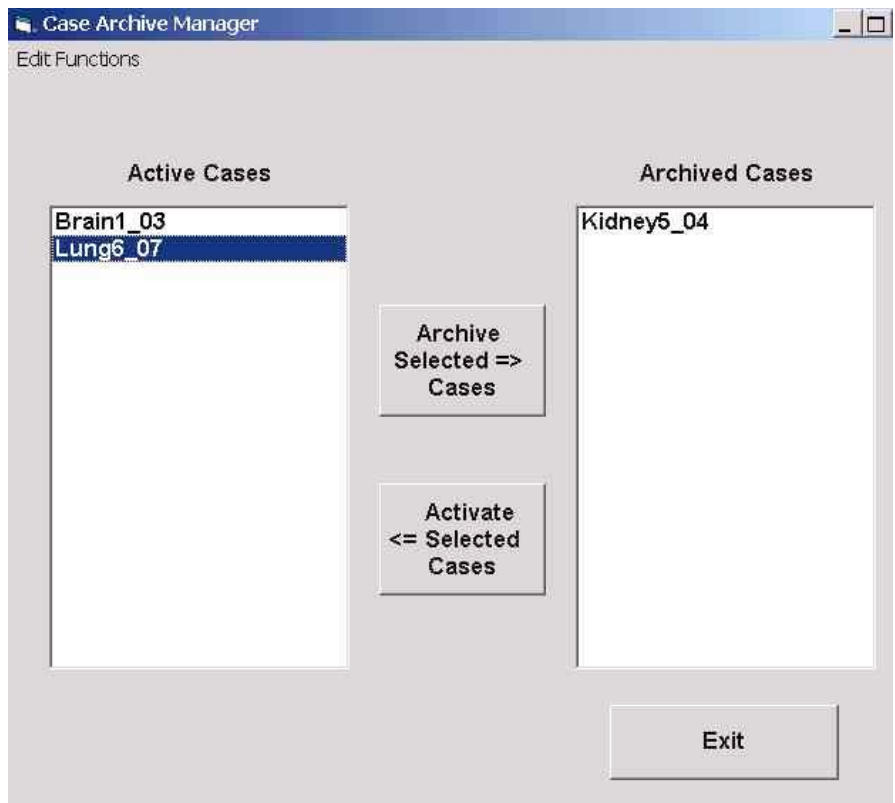
Camera Settings and Control Window

Your system has a multi-mode camera: So it is possible to adjust gain, exposure, binning conditions and record integrations. During installation a general purpose set of default conditions are set up. However, to optimize results under a variety of conditions, other settings are often needed. This section describes how to make changes to the camera and save them for use later on.

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Set/Save
Recall
Sample Region
Exposure Times and Gains
Thresholds and Tails

****

Set/Save

The "**Set/Save**" button at the lower right of you AMT display window opens the **"Camera Settings Control**" window. This window is where most of the parameters of your camera are set. You can experiment with parameters without changing anything permanently if, after you change a parameter, you click **"Apply For Now Only"** to close the window**.** If you find some settings you want to save permanently click on "**Apply And Save Conditions To File**"**.** If saving permanently you can give the settings a new name, and create as many sets of settings as you like. Changing the default conditions requires the password.

Among the items controlled here are **Exposure**s times (in milliseconds), **gains**, **record integrations**, live frame averaging (**Quality Setting**), Autogain **Sample Region**, contrast settings (**Thresholds** and **Tails**) and **Display Size**.

Exposure and Gain Control The exposure time (given in milliseconds) and gain settings are analogous to exposure time and film sensitivity on a film camera. They control the speed and sensitivity of your camera. For more on this subject, including balancing sensitivities between modes, see the page *"Exposure Times and Gains"*

Record Integrations and Quality Settings These are similar operations where multiple frames are averaged together to improve signal quality. The **Quality** setting work on the live image and the **Record Integrations** settings apply to Final images only. Drifting Specimens: If specimen drift is a problem, try lessening the Record Integrations.

Contrast Control The *AutoGain* function manages camera's contrast and uses it in the grayscales where you actually have sample. It uses the Black and White *Thresholds* to determine the contrast window. Increasing the Thresholds can make the image more contrasty. Then *Tails* are added to each side of the windows. Tails use is more subtle, but they have an effect opposing that of Thresholds (See also *Grayscale Manipulation*.)

Sample Region The Autogain's contrast control sets its range (the width between the vertical green lines on the histogram) by sampling a rectangle within the image. Its size is determined by the **Sample Region** setting. The default setting is usually 95%. If white or black features, such as grid bars, on the periphery of your image, are from "hogging" the contrast, choose a smaller sample region, say 40%. The Autogain will then set itself looking only at the center 40% of the image, and so will ignore the periphery for setting contrast. It is helpful to use a non-zero value of the Tails when the sample region is less than 100%.

Auto-Sharpen Level If you do sharpen images, AMT sharpening is probably better than doing it in an image processor, because it is native. You can choose sharpening levels of 1, 2 or 3.

Apply For Now Only This applies the changes until the program is restarted, or until the user Recalls a set of settings. It does not permanently change any parameters.

Save and Apply This saves the changes you have made in this window to be loaded later. You can create settings with a new name or overwrite one of the standard ones. The Default Settings are password protected. Each set of settings has its own background and darkfield. If you use settings other than the default ones, you need to acquire a background while in them. Creating settings with new exposure and gain values also requires acquiring a new darkfield.

Recall
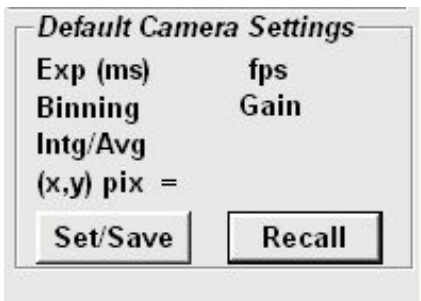
The **Recall** button is right next to the **Set/Save** one. When you click it you will be given a menu of the stored configurations of settings, each of which have been created using the **Set/Save button.**

The Default Settings are what your program opens with, and they're the ones you use most of the time. The MediumMag and HighMag have more sensitivity, but are a little slower and noisier. Recall these settings when you don't have enough light (beam) for normal imaging in the default settings. They can also be used at low mags if you have an especially sensitive sample you want to protect. The more sensitive settings usually have the number Record Integrations set to one, because stage drift is more of a problem at higher mags. If your sample is not moving you can bump up the Integrations to reduce noise.
The LowMagSettings have less sensitive settings in case they're needed for low mags. On many systems the default settings are fast enough for low mag work, so the LowMagSettings have the same exposures and gains. The LowMagSettings make it possible to store a separate background for low mag work. In some scopes the shape of the beam changes a lot between say 1.2KX and 6KX, and they find themselves taking new backgrounds when they navigate between these mags. They find that having a separate stored background for low mag saves them time.

Default Camera Settings

Exp (ms)   fps
Binning   Gain
Intg/Avg
(x,y) pix =

[Set/Save]   [Recall]
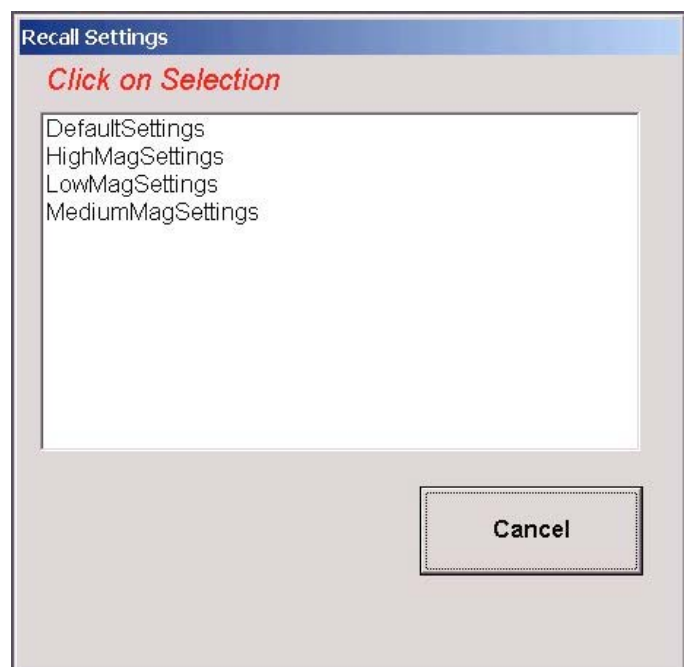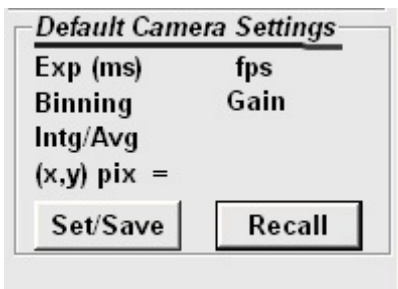
Each set of settings has it's own darkfield and background. When you take your daily background it will be in the Default Settings. If you use other settings they will need to have the background refreshed also. (The darkfields are good for a year or two.)

The set of settings you're using is displayed on the main user display, above the **Set/Save** and **Recall** buttons.

Sample Region

The "*Sample Region*" is the area which the AutoGain samples to set the contrast parameters. Pixels in this region are the make up the red graph in the histogram display. Normally this subregion is set between 80% and 95% the field-of-view as specified in the *Camera Setting Control Window*. The user can select values ranging up to 100%. This subregion is centered in the field of view.

Centered Sample Region

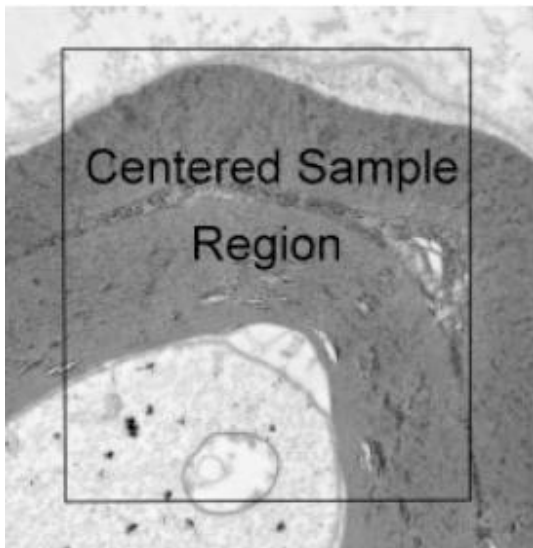A sample region which is centered allows image contrast calculations to ignore extreme values of light or dark at the edges of the image. If light or dark extremes, such as a grid bar, are entering into the sample region from outside it, and making the contrast washed out, you can eliminate its impact on contrast by choosing a smaller Sample Region.

Small window sizes tend to make harsher contrast, probably because of reduced statistics. Empirically, we find that increasing tails or using gamma helps.

Exposure Times and Gains

Exposure time and gain in a CCD camera are analogous to exposure time and film sensitivity in a film camera. They set the speed and sensitivity of your camera. The three live camera modes and the final (record) image mode, each has its own settings for exposure time and gain. Open them with the *"Set/Save"* button.

Camera Settings Control

ChangeCameraFormat (Factory Password)

Exposure/Gain/Integration Matrix for Camera

Modes	Exposures	Gains
Survey	100	1
Focus	150	2
Alternate	25	2
Record	700	1

Matrix Calculation

[Balance Camera Exposures]

Record Integrations [3]
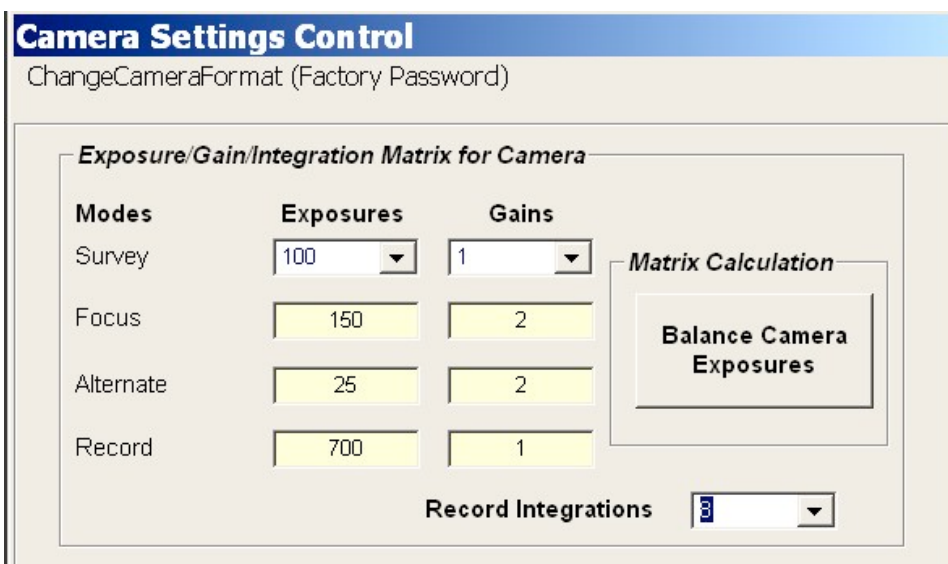
Speed vs Sensitivity
Speed and sensitivity are opposing ideals. A faster camera needs more beam intensity. Usually your default settings are set up to be fast, to facilitate scanning and focusing, but sensitive enough to cover your usual mag range without having the beam so focused that the edges are dark. For higher mags, or for very sensitive samples, **MediumMagSettings** and **HighMagSettings** are provided. They provide more sensitivity but run slower, and they may see a little more noise. **LowMagSettings**

usually have lower sensitivity (more speed) than your Default settings to facilitate a brighter beam at low mag. Sometimes LowMagSettings with the same sensitivity as the DefaultSettings are created which serve as a place to store a separate background for low mag.

Balancing Sensitivities

The three live modes and the final image mode need to have their sensitivities "balanced" so that your light level remains constant as you switch modes or go from live to final imaging. The sensitivities for your Default, Low, Medium and High Mag settings have been balanced by the installer. Balancing by the user might be needed if new settings are created, or the check balancing on the normal settings. A quick but imperfect, way to balance exposures and gains is with the "**Balance Camera Exposures**" button which is to the right of the exposure and gain values. This accepts the exposure time and gain of the Survey mode and adjusts those of the other modes to match it. For values close to the Default and MediumMag settings this method gives a pretty good approximation. For very fast or slow settings, or for precision balancing, one needs to use manual balancing.

More Sensitive Settings

To create a setting with more sensitivity start with the Focus mode, since that mode usually limits sensitivity. Using the current most sensitive settings, in focus mode, put the histogram to the left of center, say at 25% of the width of the box.
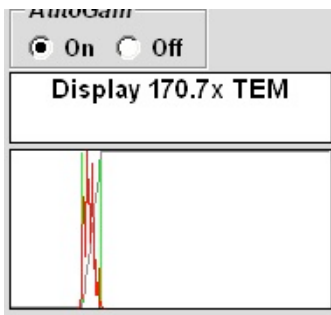
Then bump up the focus mode exposures and gain, click "**Apply For Now Only",** return to live imaging and see where the histogram is. If its near the center you're ready to do manual balancing, using focus mode as the standard. If you want super sensitive settings, and don't need to use focus mode during imaging, you can ignore focus mode and use Survey mode as you standard. If you are creating Default settings for a new camera setup, a Focus mode with Exposures of 150ms and a Gain of 2 (sidemount) or 3 (bottom mount) is usually a good standard to start with.

Less Sensitive Settings

Be careful not to set sensitivity so low that you burn the phosphor. If Survey Exposures gets below 45, check the beam with the camera out to make sure it is spread out past the TEM viewing screen, and that the condenser aperture is in place.

Manual Balancing

After you have set a standard (one mode with the correct sensitivity for the new settings), the next step is to set the **Black Threshold** and **White Threshold** to zero to facilitate balancing. This keeps the AutoGain function from interfering with the histogram display. Don't change the **Tails.**
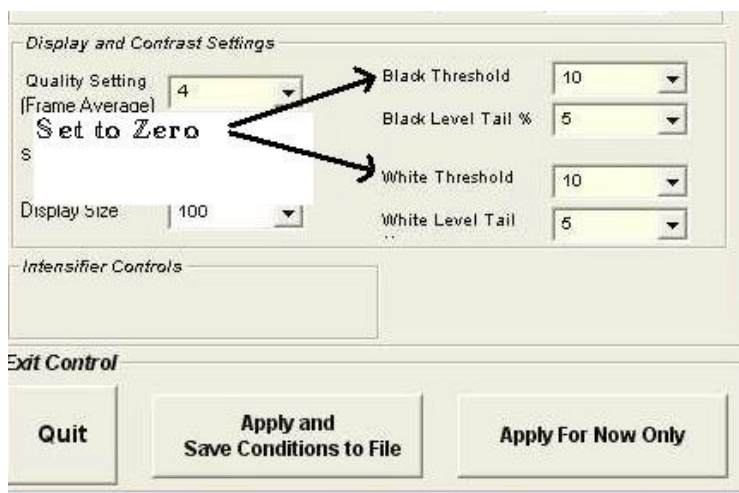
Take your sample is out and turn background corrections off.
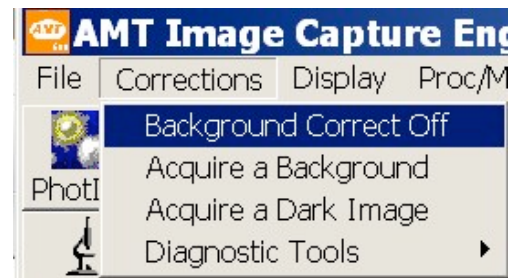
Using whichever live mode is your reference point (standard), put the histogram peak in the center of the display box, maybe under the "O" in "Off".
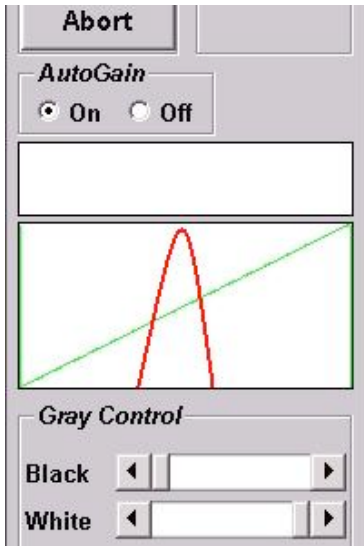
Now switch to the other live imaging modes and record mode (Final Image). Make a note for each mode of whether the histogram is higher, lower or about the same as where it is in your reference mode.

Then click **Set/Save** to go back to the camera control window and there make adjustments to the exposures and gains of your non-reference modes to get their histograms nearer to that of the reference mode. You can type in new values for the Exposures and Gains. The Gains can be changed in increments as small as one tenth, using a decimal point. It is usually best to limit **"Record"** exposure time to about 1 second (1000ms) and use the Gain to make increases after that.

After you adjustments click **"Apply For Now Only"** and repeat steps 3 through 6. When the histogram moves very little as you switch between modes set the Thresholds back to 10 and then click "**Apply and Save Conditions to File".** The settings can be saved with a new name or some current settings can be overwritten. After you create new settings you need to collect a new darkfield and background for those settings.

Thresholds and Tails

Black and White Thresholds
The AutoGain assigns pure black or pure white values to pixels at the lowest and highest values of a histogram. Those pixels under the red graph, but outside the vertical green lines are kicked out into pure black or white. How many pixels get kicked out depends on size of the **"Thresholds".** If thresholds are set to zero none get kicked out - all 256 grayscales that the camera receives are reported as is.
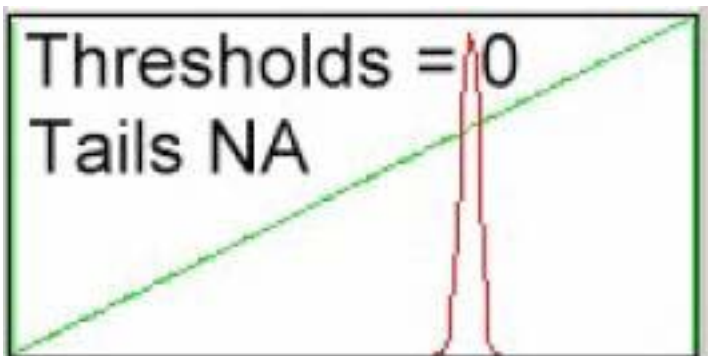
The white and black limits are designated by vertical green lines. All values below the black limit are assigned zero and all values above the white limit are assigned 65535.

The thresholds tell the green lines how far up the slopes of the histogram they should go. Higher thresholds move the green lines closer together, concentrating the camera's contrast on the grayscales where most of the pixels are, and kicking edges out into pure black or pure white. The default thresholds are 10, but some users like higher values, which gives a more contrasty image.
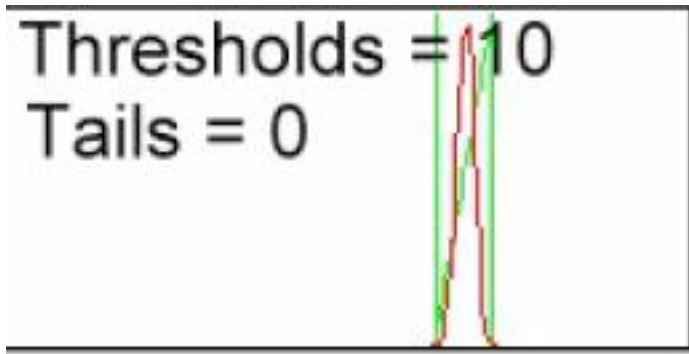
The mapping between black and white is shown by a diagonal green line drawn between the green vertical line. This line is straight for gamma = 1 and sigma = 1 in the live image.   For values of gamma or sigma, it is curved in live mode (See also *"Grayscale Manipulation->The AutoGain Function"*.
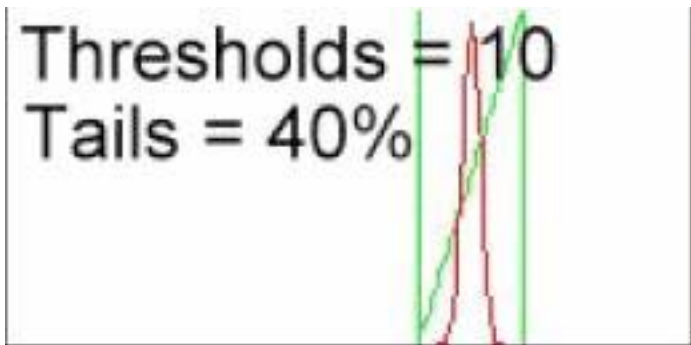
Note that the gamma during live operation is carried as the "base" for the final image.   So the diaganol line shown in the final image is always straight immediately after acquisition. The actual (based plus added) gamma value is shown in the gamma control screen, however.   The system remembers the base values for the session until it is changed by the user.

Tails
The ***"Tails"*** move the green lines away from the center by a percentage of the distance between them. This takes some pixels that were pure black or white and puts them into the gray. Positive Tails reduce the contrast of the image, and can be used to reduce "Blotchiness" caused by too many pure white or pure black areas.

Negative tails increase the number of pixels which are pure black or white. If you have some almost white features which you want to be pure white they will often show up on the histogram as small extra peak, such as in the picture below. Use a negative tail to move the right green line to the left, making the features pure white.

****
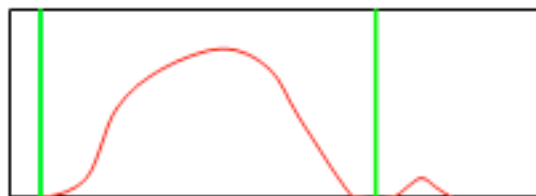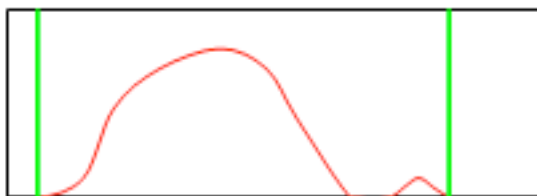
Grayscale Manipulation

TEM images usually require some grayscale adjustment. Traditionally most user adjustments are done after the image has been saved, in a program like PhotoImpact. But there are a few utilities that work very well (even 'best') in AMT, and can give a nice image in the shortest time. The AutoGain function in the AMT program is constantly adjusting grayscales in your image, in order to put the camera's contrast range to best use in displaying your sample. There are things you can to to effect the way that adjustment is made, and adapt it for special sample characteristics.

Contents

The Autogain Function
White and Black Level Adjustments
Gamma Adjustment
Sigma Adjustment
Image Sharpening
Image Processors

****

The AutoGain Function

AutoGain
In the histogram display shown here the area between the two vertical green lines represents the camera's output grayscale range, 0 to 255. The sample's grayscale range (the camera's input) is represented by the entire width of the display window. As you can see, the camera's output capacity is restricted to, or focused on, those sample grayscales which are occupied by your sample. This ensures that the available image contrast is put to best use displaying features, not analyzing the blacks and the whites. The camera actually does see all the sample's grayscales (the entire window), but pixels outside the green lines are seen as pure black or white, thus taking a minimum of the camera's analytical capacity.
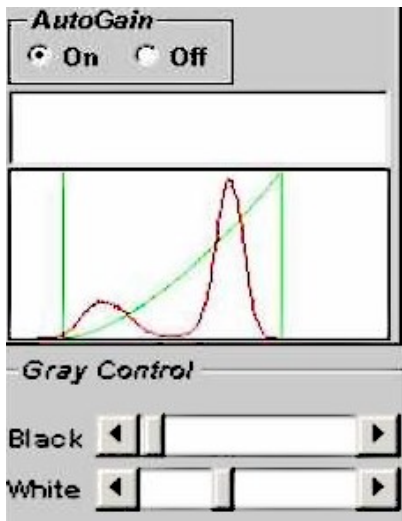
Thresholds and Tails
The *Thresholds* and *Tails* control where the green lines go (see "*Camera Settings and Control Window*"). If the thresholds are set to zero, the green lines stay at the edges of the display window, so there is no enhancement of contrast in the occupied pixels. Higher thresholds move the green lines closer together, enhancing contrast. The default for thresholds is 10 but some microscopists prefer thresholds of 50 or more. The tails move the green lines away from the center, by a percentage of the distance between them. Their main function is to control "blotchiness" when samples have a lot of extremely dark or light features.

White and Black Level Adjustments

A gridbar or other extreme black or white items often give your histogram extra sharp peaks on the left or right. This forces the system to divert the dynamic range from the main collection of pixels and into the far black or white regions, making the main features look "washed out" (See "*The Autogain Function*"). Manual "*Black*" and "*White*" level adjustments are often the easiest way to correct the contrast. By manually moving the green lines with the sliders you can keep those extremes from hogging the contrast. Essentially you're telling the camera "don't analyze what shades of black that gridbar has, just call it "black, and analyze my sample instead".

In order to change levels in live images you first have to click the AutoGain's *"Off"* button. You can leave it off while you collect a final image. <u>Be sure to turn it back on before you change magnification or illumination</u>.
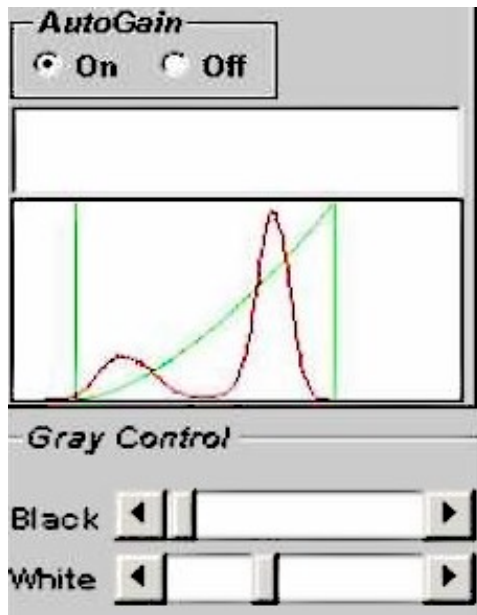
Adjusting Gamma

Adjustments to **Gamma** and **Sigma** can be done in a live or a final image, and does not require that the AutoaGain be turned off. Adjustments done in live imaging will stay in place for the session.

Changing the Gamma changes the shape of the gain distribution line (the diagonal green line between the vertical green lines). Adjust by sliding the *"Gamma"* button right or left while viewing either a Live or Final image. The same button is used for both the *"Gamma"* and *"Sigma"* functions. If it currently says Sigma, click it and it will go to Gamma. Clicking the *"Neutralize Gray Map"* button sets both Gamma and Sigma back to their default (neutral) value, which is one.

The histogram's (red lines) in the following two examples have two peaks. The larger one represents a concentration of pixels in the image's dark areas and the smaller one represents pixels in lighter areas. In the first example Gamma has been raised by sliding the *"Gamma"* button to the right. The gain line is now an up opening arch. This makes the <u>slope</u> of the gain line small in the part of the grayscale occupied by the large (dark) peak and large where the image's lighter regions have their peak.
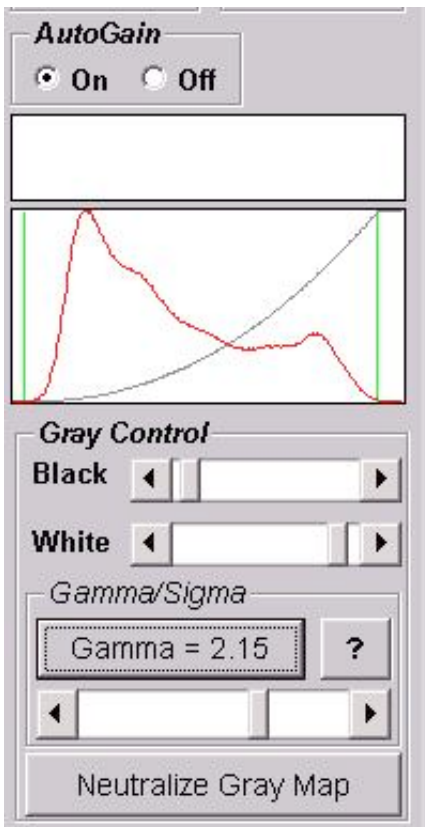
Gain line slope represents contrast. Therefore a raised Gamma means more of the available contrast is assigned to the lighter regions. This makes it easy to see the difference between light and very light features, but difficult to see the difference between black and almost black ones. On the right side of the raised Gamma image below, one can easily make out the differences in the thickness of the substrate. On the darker, left side of the image there's not much contrast available for distinguishing features. Dark and very dark are compressed together into a dark blob.

Gamma greater than one - light regions more contrasted

24
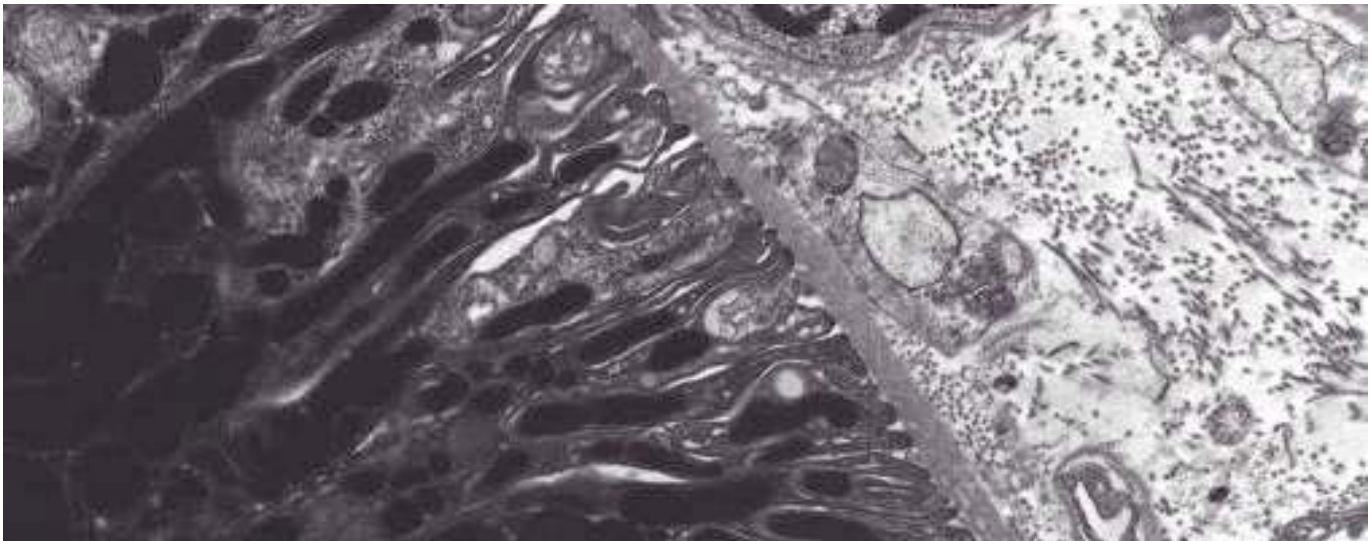
AutoGain

○ On   ○ Off

Gray Control

Black ◄ ║ ►

White ◄ ║ ►

Gamma/Sigma

Gamma = 0.55   ?

◄ ║ ►

Neutralize Gray Map

In the second example the Gamma has been lowered. Now the gain line is a downward opening arch. Its slope, and the fraction of the available contrast used, is much larger for the dark area peak than for the light area peak. This means that the differences displayed between light and very light will be diminished, and features in the light area are less distinguishable. Difference in the dark areas however are much more distinguishable. One can see what might be deposits in the dark tissue.

Gamma lower than one - dark areas more contrasted.

Changes made to Gamma in live imaging are preserved in a recorded image even though the displayed gain line will straighten out. The adjusted Gamma will still be in place when you go back to live imaging.
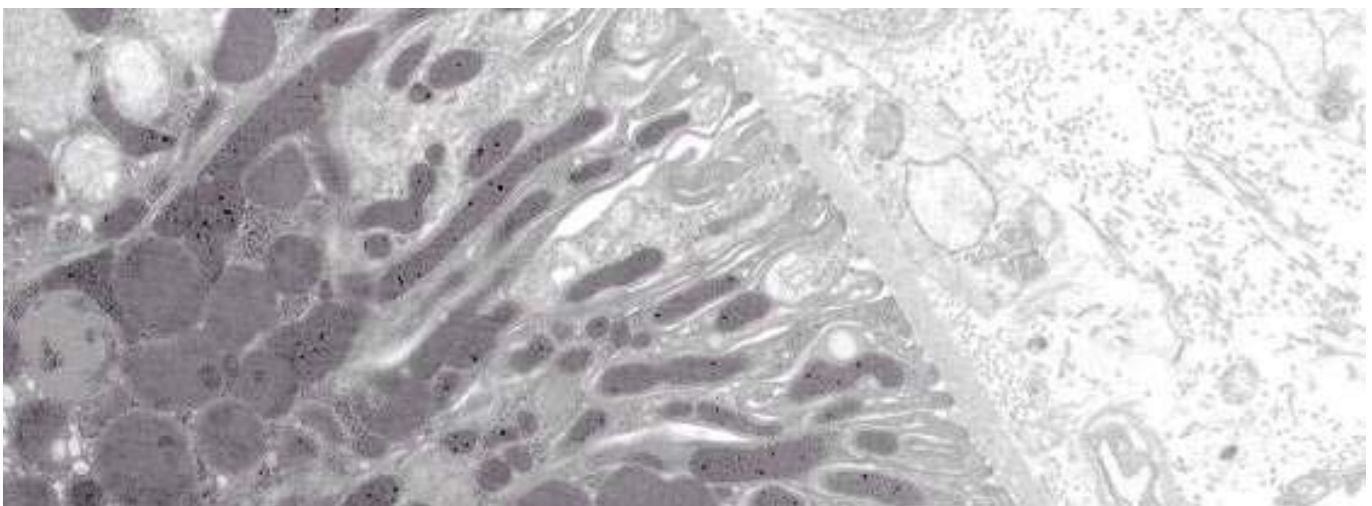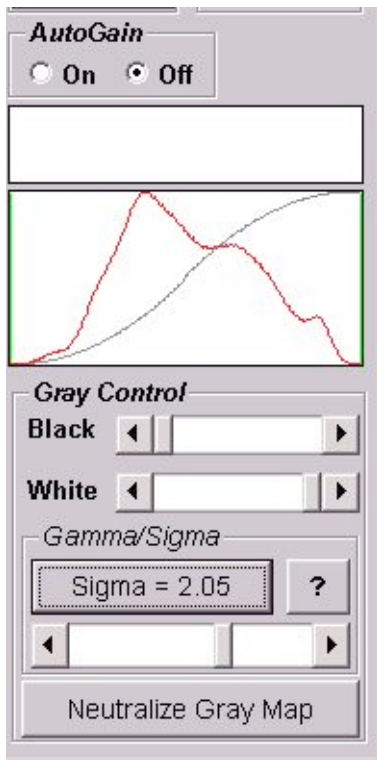
Adjusting Sigma

If you click the **"Gamma"** button it becomes the **"Sigma"** button, and the slider bar now adjusts *Sigma*. Like *Gamma*, Sigma changes that shape of the normally diagonal gain line, reapportioning the way contrast is distributed over the grayscales. Raising Sigma makes the gain line an "S", so there is less slope on the black and white ends and higher slope over the middle grayscales.

This puts more of the available contrast in the center of gravity of your histogram. The extra contrast in the middle is gained by sacrificing a little contrast on the dark and light extremes. In biology the middle gray scales are often where most of your important edges are, transitions from middle gray+ to middle gray-. So raising Sigma will often give a prettier picture, especially at low mag, and especially for users who print out their pictures.
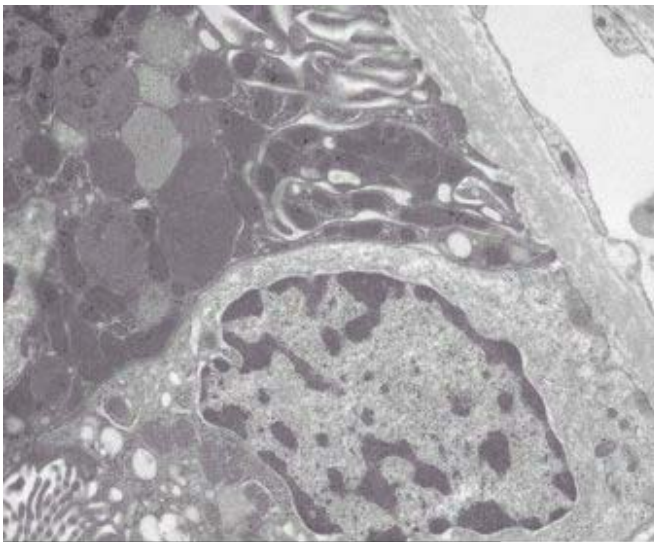
Sigma = 1   Sigma = 2
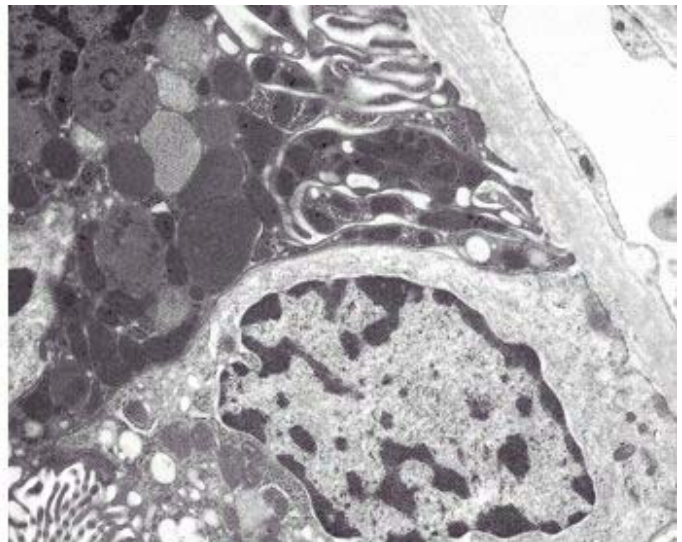
Note: If the features you're interested in are light features surrounded by a light background, or dark features surrounded by a dark background, this is not the best adjusting tool (See "*Gamma Adjustments*").
Clicking **"Neutralize Gray Map"** returns Sigma to its default of 1.

Image Sharpening

Not Sharpened
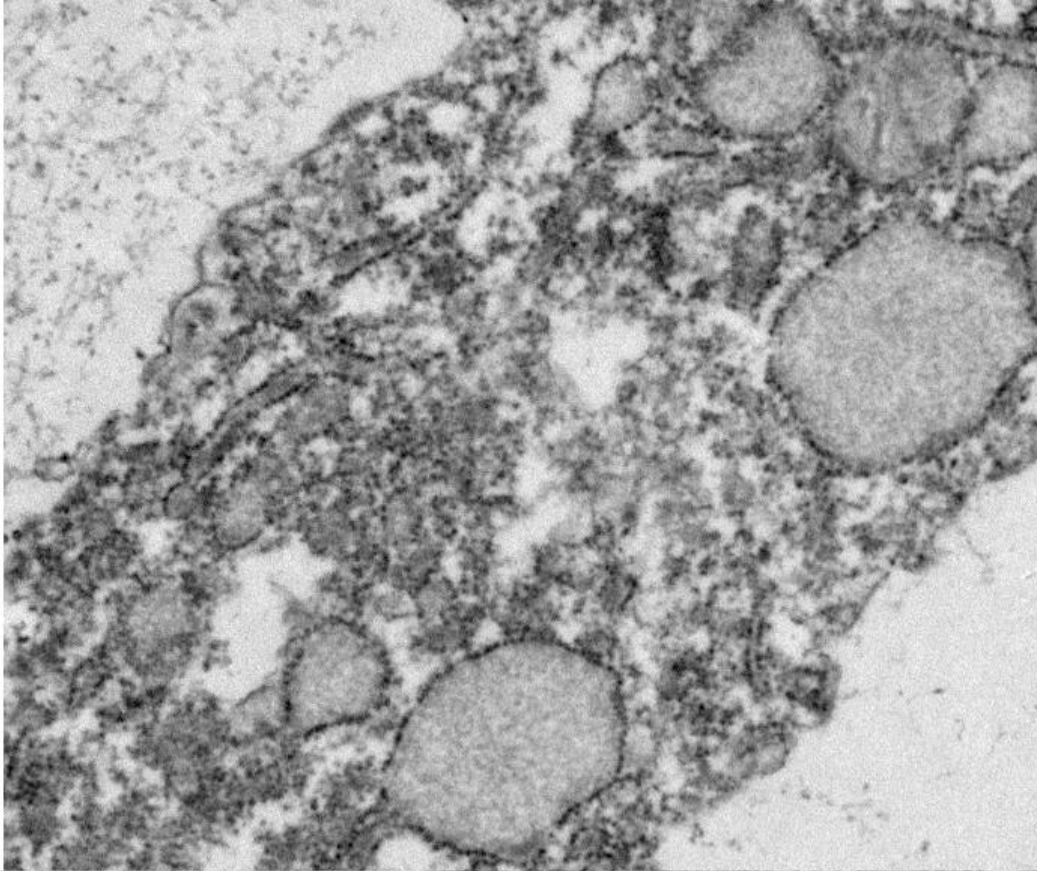
Compare Images
Sharpening can make a picture look prettier. It is most likely to be useful at lower mag, and with a sample that had some difficulty in preparation. It does involve a tradeoff. Sharpening can make a picture look gaudy and grainy and can interfere with the finest details. If your finest details are coming through O.K. you probably don't need sharpening. Deciding that requires experimentation, comparing image next to each other in a processor.
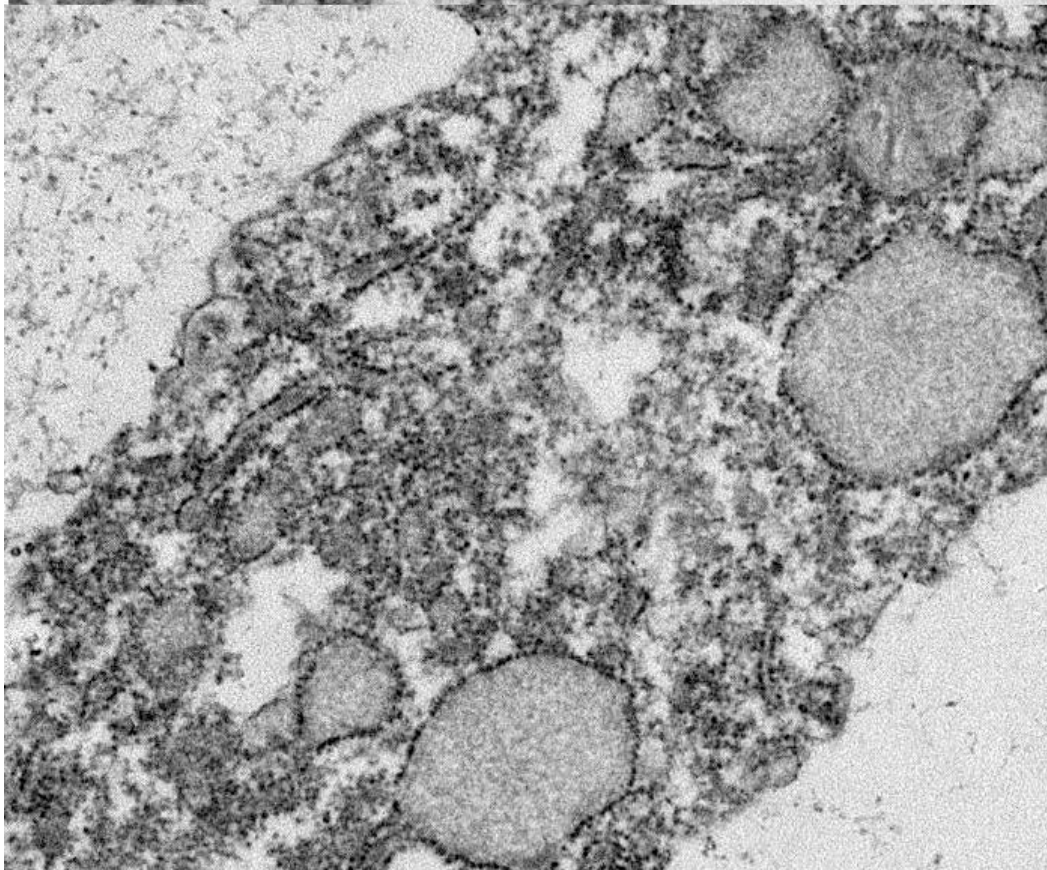
Sharpening Level 1

AMT Sharpening
If you do sharpen image, AMT sharpening is probably the best way to do it, because it is native to the image collection. There are two way to sharpen in AMT: "*Enhance current Image Only*" (on a collected final image) and "*Auto-Sharpen*" (on all final images).
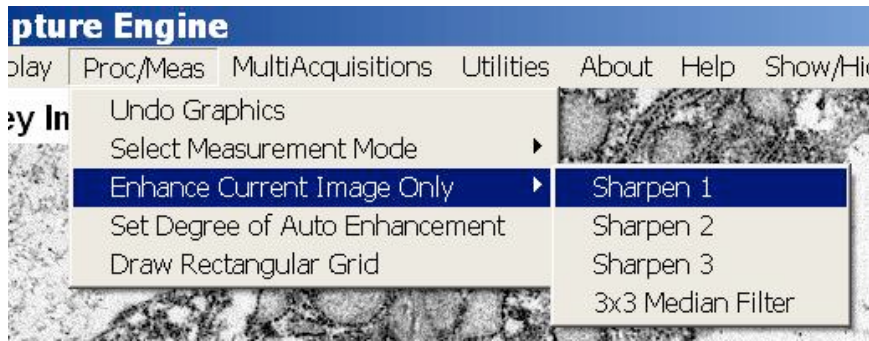
To enhance the current final image, click on "**Proc/Meas**" on the top menu and choose the Current Image enhancement option.
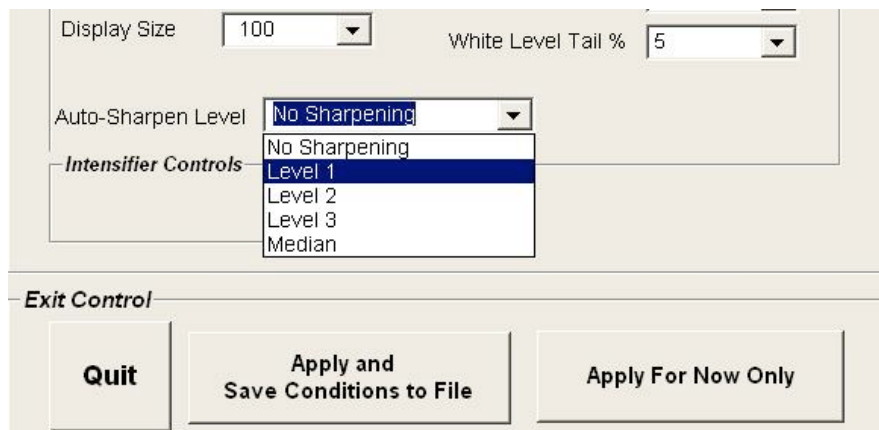
To set the **Auto-Sharpen** level click "**Set/Save"** to open the **Camera Settings Control** Window.

With either way of sharpening you can choose Level 1 to 3. Level 3 is the most extreme sharpening. ("**3x3 Median Filte**r" is a specialized function. It would be bad for normal imaging). Most sharpening jobs only require Level 1.
The severity of the sharpening is set in the camera's configuration folder in the file "Sharpen.txt".
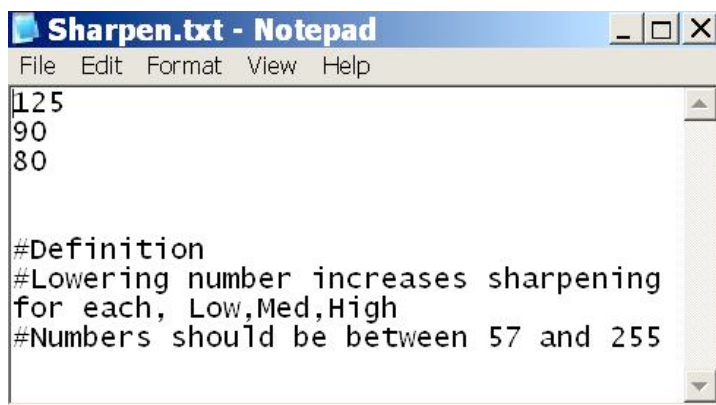
The top number sets the level for Sharpen 1, the bottom number for Sharpen 3. Higher numbers in this file mean softer sharpening. In some labs those three numbers are changed to 180, 125 and 90, so that Sharpen 1 is more like Sharpen 1/2 etc.

Image Processors

PhotoShop, PhotoImpact and ImageJ are examples of *image processors*. They have a variety of tools for manipulating grayscales and other image subtleties, sharpening, measuring, counting and analyzing. They also can be used to convert images into .jpeg images and for sending images to a printer. The two Image features you'll adjust most are Contrast and Brightness. In order to print out nicely, images often need their contrast increased until they almost look gaudy on the computer screen. When you adjust images in a processor, you probably want to save them with a different name, so you keep the originals.

Other basic processor adjustments include Levels, Gamma and Curves, all of which are used to divide up the available contrast, so it gets used most efficiently to display the important features in your image. See the PhotoImpact manual for directions on using its features. ImageJ a subchapter here and a lot of free documentation online. It has subroutines for counting particles, measuring area and doing fourier analysis. See "*In Depth Measurement and analysis using ImageJ*".

The AMT Capture Engine display typically has shortcuts to one or more image processors on it
The top two icons on the left toolbar of this AMT display are shortcuts for opening *PhotoImpact* and *ImageJ.*

****
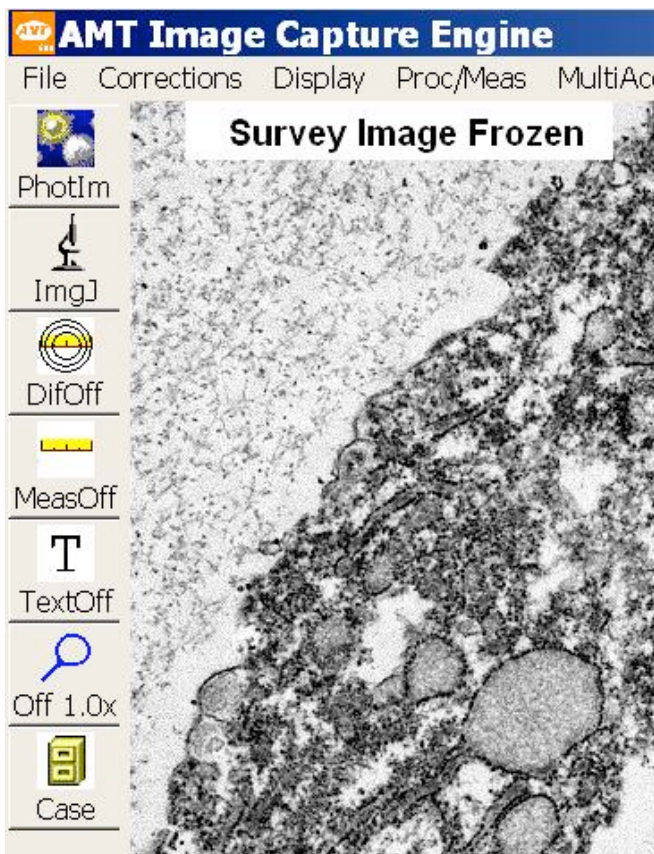
Image Properties and Corrections

The pages in this chapter are about how information for each pixel is collected, manipulated and stored. It is intended for users that need technical specifications for things like flat field correction, frame averaging and bit depths. Those printing these files as a general manual for new users can skip this chapter.

Contents

Bit Depth Of Images
Frame Averaging and Summation
Physically Based Flat Field Correction
Mag Factor In TEM Digital Imaging

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Bit Depth of Images

This page describes the collection and storage of information in binary registers. The information can be important for those who want to electronically analyze images.
The data is collected as follows

For both live and recorded data
   12 bit image data is generated by the camera and sent to the computer with two "8-bit bytes" per pixel.
   The pixels values are forced to be justified "high" so that the most significant data is in the high byte.
      This means that the first 4 bits of the low byte are zero.
   This does not make the data "perfect" in the sense that the maximum value is 65535. That comes later.
For live data only
   Only the most significant byte is used throughout the video chain. That is: the live data is 8 bits. This
   matches the SNR requirements of the TEM and display and allows faster data handling.
   The raw data is dark field and background corrected for each frame, and the histogram of the corrected
      data is shown in the graphics window. This is unaffected by gain and offset. (So the "red" line does
      not change with White/Black/Gamma.)
   The corrected live data is routed through an 8 bit video (Look Up Table) LUT for display. Since the data is
      not changed by the LUT all grayscale mapping is reversible.
   Maximum data values of 255 are possible but usually neither achieved nor desired.
For recorded data
   All recorded data is summed in an array S of 32 bit deep unsigned integers. After integration S is divided
      by the number of integrations and transferred to a 16 bit data array. At this point the data is justified
      "high" but the maximum of 65535 is still not necessarily attained in the image. It is simply the 16
      most significant bits of the "raw data."   Ideally, if the 12 A/D (analog to digital converter) were
      perfect, this array would lose some information for integrations of greater than 16 frames (16 x 12bits
      = 16 bits). In practice the A/Ds are not noise-free and the process can tolerate ~64 integrations
      before this becomes a concern (and then only for a near saturated image).
   The data is then dark field and background corrected with 32 bit arithmetic. The maximum of 65535 is
      still not necessarily attained in the image.
   If there AutoGain (and the thresholds are > 0) then the data are rescaled so that the maximum is 65535
      and the minimum is zero.
   The rescaling procedure was initially developed in conjunction with exposure   balancing. Ideally we
      "balance" the camera exposure times and gains so that setting the exposure in the live conditions
      automatically produces the the proper recording exposure levels. Remapping the data using the
      AutoGain criteria gives a matching contrast level. In fact the use of "tails" makes this procedure more
      forgiving and will produce matching contrast even if the exposures are not balanced.
   This procedure (coincidentally) made our images compatible with early versions of Photoshop and some
      other software, which could not "see" the lower byte of a 16 bit TIFF image. Our competitor who
      justified the data "low" had big problems with this.
   Setting Gamma to 1 and setting the thresholds to zero (with AutoGain ON) will disable rescaling but still
      give you the corrections. (Note that zeroing thresholds disables tails as well.)   This should give you
      quantitative data in the sense the camera becomes a light meter - as long as the dark and
      background images are constant. Absolute intensities become meaningful and can be calibrated.   It
      is also possible to disable background and dark field corrections, but I do not recommend this.

Histogram "Smoothness"

In principle dark field and background corrections add to the total uncertainty of the measurement and reduce the dynamic range of the data. Corrected Image = <mean of Back-Dark> [(Image + e1) - (Dark + e2)]/[(Back + e3) - (Dark + e2)] (error/Corrected Image)2 = [(e1 + e2)/(Image - Dark)]2 + [(e3 + e2)/(Back-Dark)]2 However, we integrate both the background and dark images during acquisition to keep e2 << e1 and e3 << e1 and Image ~ (Back-Dark) , so that this loss should be insignificant.

Any integration will increase the dynamic range by the SQRT of the number of integrations.

So we expect that the histogram will look relatively smooth at > 10 bits for a reasonable well illuminated image at the unity gain. We would expect gaps to appear for high gains and poor illumination.

Specific Suggestions for Diffraction

The bit depth information of images suggests using a separate set of settings for diffraction imaging. See the AMTHelpfiles chapter on *Diffraction Imaging.*

Frame Averaging and Summation

Typically TEM image quality is limited by beam statistics, where the signal-to-noise (SNR) per pixel is equal to the square root of the electron count assigned to the pixel. The electron counts per pixel are limited in the CCD by the well depth of the sensor. SNR can be increased by mathematically summing or averaging pixel values frame by frame.

Live Modes: In live imaging the camera system offers a Quality Mode, which frame averages the live image using a recursive filter:

$$R(x, y)_I = \left[\frac{255}{4095} \right] * \left[\frac{r(x, y)_I + (n-1) R(x, y)_{I-1}}{n} \right]$$

where
$r(x,y)_I$ is the incoming frame **I**
$R(x ,y)_I$ is the frame averaged value of the **I**th frame, and
n is the frame averaging constant – as specified in the "frame average" in the Quality Setting in the "***Camera Settings Control***" form.

Note that the result is normalized to 8 bit (256 levels) to accommodate the speed requirements of live display and processing.

Recording Mode: In the recording modes the camera system frame integrates multiple frames so that

$$R(x, y) = \left[\frac{65535}{(n*4095)} \right] \sum r(x, y)_I$$

for **I** = 1 to n, where
$r(x,y)_I$ is the incoming frame **I**
$R(x,y)_I$ is the frame averaged value of the **I**th frame, and
n is the number of frames in the sum-as specified in the "Record Integrations" in the "***Camera Settings Control***" form.

Camera Settings Control
ChangeCameraFormat (Factory Password)

Exposure/Gain/Integration Matrix for Camera

Modes	Exposures	Gains
Survey	NA	NA
Focus	NA	NA
Alternate	NA	NA
Record	NA	NA

Matrix Calculation
Balance Camera Exposures

Record Integrations 16

Display and Contrast Settings

Quality Setting (Frame Average) 2

Sample Region 95

Display Size 100

Black Threshold 10

Black Level Tail % 5

White Threshold 10

White Level Tail % 5

Auto-Sharpen Level No Sharpening

Intensifier Controls

Exit Control

Quit

Apply and Save Conditions to File

Apply For Now Only

This result is normalized to 16 bits (65536 levels), which allows internal calculations to be done at high precision.

The effect of multiple integrations is to increase the number of counts per pixel, which decreases the statistical noise. This is a physical effect that can be modeled. To first order the signal-to-noise ratio will be proportional to the square root of the integrations. You get a 40% improvement from 2 integrations, 100% from 4, 180% from 8 and 300% from 16. The most improvement per time unit is from 2 integrations. Integrations greater than 8 are rarely useful, because the noise becomes less than the detectability of the display and printer. Mathematically, improvements will occur up to 64 frames. These images need to be recorded in 16 bit TIFF format.

The improvement in signal-to-noise ratio is physically limited by the instability and drift in the specimen. It is also limited by the patience of the user.

Physically Based Flat Field Correction

The flat field correction normally used in AMT images is a combination of dark-field subtraction and gain normalization that accounts for multiple non-uniformities in the image.

Dark Image: Images are usually "flat-field" corrected. In the first step of this correction the dark image is subtracted from the incoming, raw data. In versions before 5.4.2.200 the dark image is collected by the menu operation.

This function collects a base image with no light or electron signal. The program collects dark images appropriate for Survey, Focus, FFT, Super and Record modes. They are stored in c:\AMTcommon\config\Settings\DefaultSettings or the other specifically named settings folders, as appropriate. Dark images are frame integrated 8 to 16 times and then normalized to 16 bit format. The dark image has both systematic and random components.

The random component is almost always much less than the statistical noise of the electron beam. The "DC" part of the systematic component can be significant for very low signal images where the raw signal is not 10 or more times larger than the DC component. For well illuminated specimens the dark image correction is not significant.

Dark images vary to a small extent with exposure time and are proportional to camera gain; so it is best to acquire them for each set exposure conditions. Dark images are very stable; so that they are valid for at least a year under a given set of gain and exposure conditions – unless some physical change is made to the camera electronics.

The dark image is always subtracted from the raw data (unless the dark image file is deleted from the particular AMT common folder). So the first step in the flat field correction is:

$R'(x,y) = R(x,y) - D(x,y),$

where

R(x,y) is the raw camera data,
D(x,y) is the dark image and
R'(x,y) is the dark corrected image.

Background Image: The second part of the flat-field correction is background normalization. The operation adjusts for the illumination variations in the lens and phosphor. Background correction assumes a linear model for the modulation of the raw image. That is the raw image is the product of the intensity of the ideal image and the modulation function.

$R'(x,y) = I(x,y)*M(x, y),$

where I(x,y) is the ideal image, and
M(x,y) is the modulation of the image due to imperfections the optics, phosphor and TEM.

Background correction requires the imaging a uniformly **illuminated phosphor with a spread beam**. The collection of the Background Image is accomplished by a menu item:

Background images are frame integrated 8 to 16 times and then normalized to 16 bit format.

The modulation function is computed from the background image as follows:

$B'(x,y) = B(x,y) - D(x,y)$

$$M(x, y) = \frac{B'(x, y)}{<B'>}$$

Where
B(x,y) is the Background value at each point,
B'(x,y>) is the dark corrected background at each point, and
<B'> is the mean of B(x,y).

Finally the ideal image is:

$$I(x, y) = \frac{<B'> * R'(x, y)}{B'(x, y)}$$

Background images vary mostly with mechanical drift of the system; so it is best to acquire them every day. The linearity assumption can also break down under extreme illumination conditions and where illumination profile in the TEM varies with magnification. This generally is not good operation, so spot size and apertures should be varied to avoid it.

Magnification Factor in Digital Imaging

There is much confusion about the "mag factor" which is often specified by the TEM beam projection alone. From this perspective a bottom-mount camera has only ~115% times the magnification of a negative (mag factor = 1.15!). Also by this standard a side-mount camera would have a mag factor of 0.3. Both are misleadingly low and do not correspond to user experience, because they do not account for the size of the detector (capture size) or field-of-view, which is generally much smaller than a negative.

All digital output devices are about the same size as photographic prints. But the smaller size of digital camera detectors results in more enlargement than needed for a photographic print. A meaningful "mag factor" must include this enlargement value.

To compare magnification factors you should use a common observable reference: negative, viewing screen, monitor, or print.

Magnification is the TEM magnification at the detector position multiplied by the *enlargement* of the the print. The enlargement is the ratio of the print size (~175mm) divided by the *capture size*, which is either the size of the phosphor or the size of negative.

TEM Mag x Print Size/Capture Size = Final Mag

TEM Mag		Print Size/Capture Size		Final Mag	
3000	x	175/24	=	21,875	Advantage HR
8000	x	Not Printed	=	8000	Viewing Screen
10000	x	175/75	=	23,333	Negative
11000	x	175/24	=	72,500	Advantage ERB
11000	x	175/30	=	58,333	XR60B/XR40B
11000	x	175/60	=	29,167	XR60C/XR40C

Bottom Mount Camera Choices

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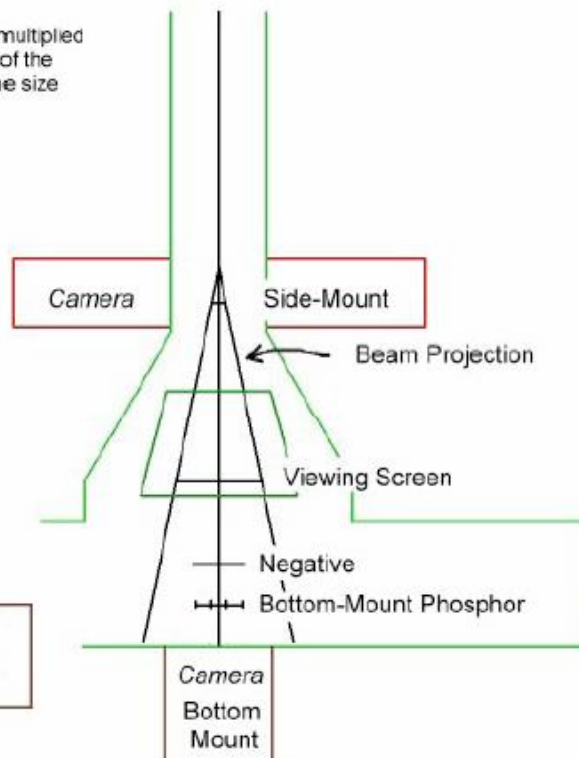
Measurements and Calibration

There are a variety of tools available to help you measure, count and otherwise analyze the images you collect. We'll discuss using and calibrating the ones that are part of the AMT Image Capture Engine, as well as some that are in other image processors.
There is also a section on Lattice Imaging with bottom mount digital cameras.

Contents

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AMT Measurements and Calibration

These are the utilities for measuring features on image in the AMT Image Capture Engine. The basic ones are Linear (point to point) measurement and Diffraction pattern measurement. Calibration is similar in the two utilities.

Linear Measurements
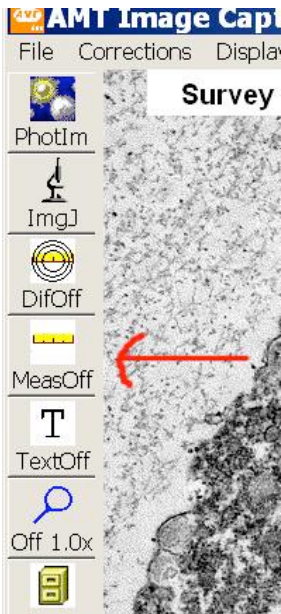
1. To make a linear measurement you first have to collect a final image.

2. Next click on the ruler icon on the left tool bar (or on menu item **Proc/Meas** ->**Point to Point Measurement**). This will open the "*Measurements*" window.
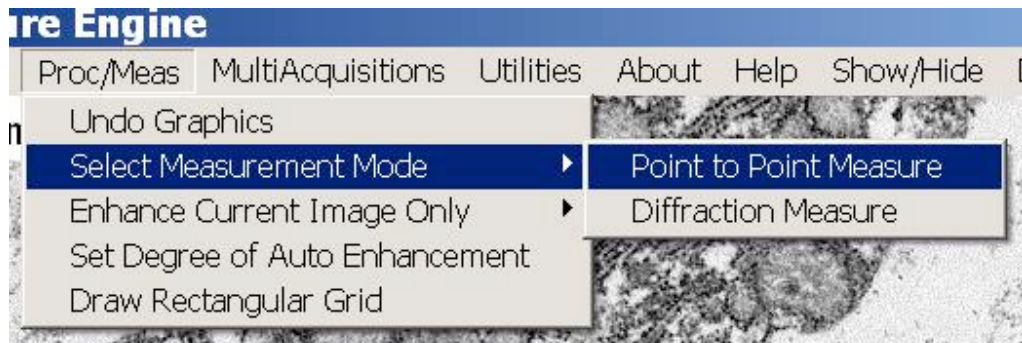
3. The correct voltage and mag need to be entered in the measurement window. If your system has communications, or if you've saved the image with the correct voltage and mag, the measurement window will already have the correct values.
4. Select the units on the measurement form before making a measurement.
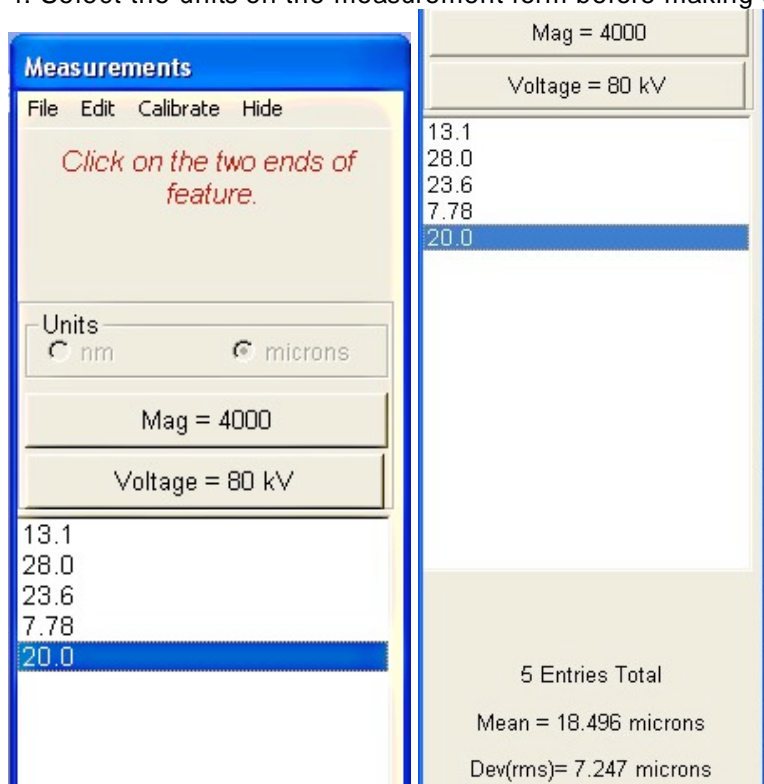
5. Make a length measurement by clicking once at each end of the desired feature to be measured. (Do not "drag".)   A line connecting the two points will be drawn and a measurement label added. The measurement will also be entered into the list on the measurement form. Any of the measurement in the list can be given a label or comment, or be deleted, using the Measurement form's "Edit" menu items. The number of entries, and the calculated mean and standard deviation of the measurements are shown on the bottom of the form. The measurements shown on the page will be preserved when the image is saved. To remove them click "**Proc/Meas -> Undo Graphics**".
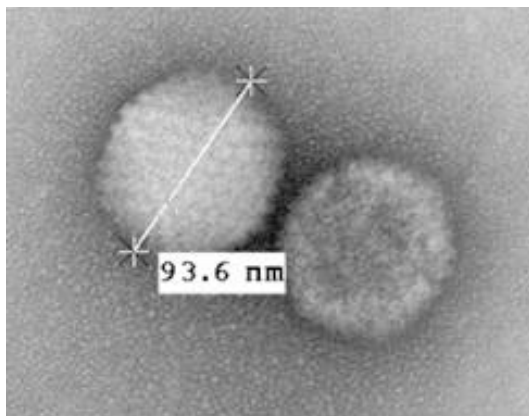
6. The list of measurements can be saved as a text file, with the labels you have added being a separate tab delineated column. To do this use "**File -> Save and Exit**" on the Measurement form's menu. The Mean, Standard Deviation and units of measurement will be saved with the list.

Diffraction Measurements

1. Be sure to use the proper precautions for diffraction imaging (See *"Diffraction Imaging"*).
2. After collecting a final image and going to Camera Out, click on the diffraction measurement button on the left tool bar. Alternately, click "***Proc/Meas-Select Measurement Mode->Diffraction Measurement***" from the upper menu.
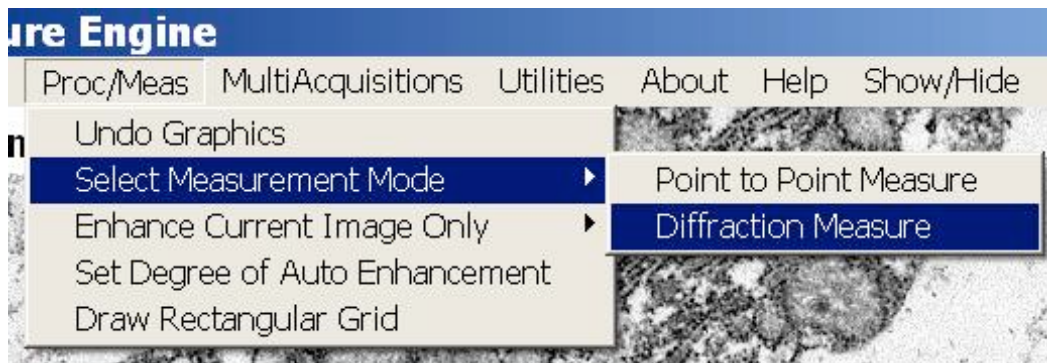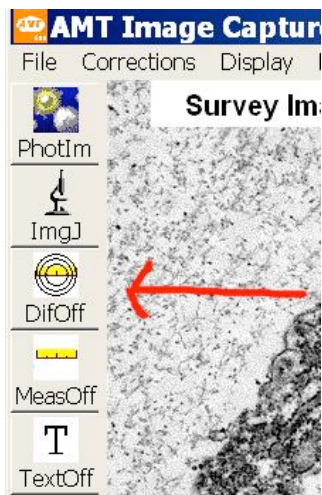
3. Select either diffraction spot measurement or diffraction ring measurement from the form that is displayed

4. Diffraction Spot Measurement. The distance between diffraction spots is measured in the same way that point to point image distances are measured. Click once on each spot to measure the line between them. The measured distance will also be entered into the measurement form list and a line and label will be drawn on the image.

5. Diffraction Ring Measurement Select ring measurement instead of spot measureme nt. Measure diffraction rings by clicking on three widely spaced points on the ring. A circle with the appropriate radius and center will be drawn and labeled. The radius of the ring is determined and entered into the measurement list.
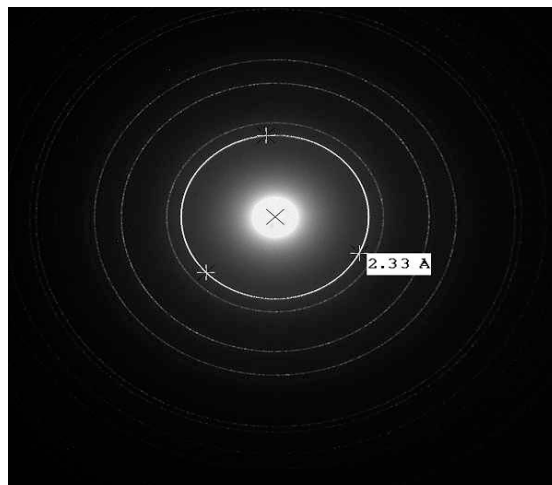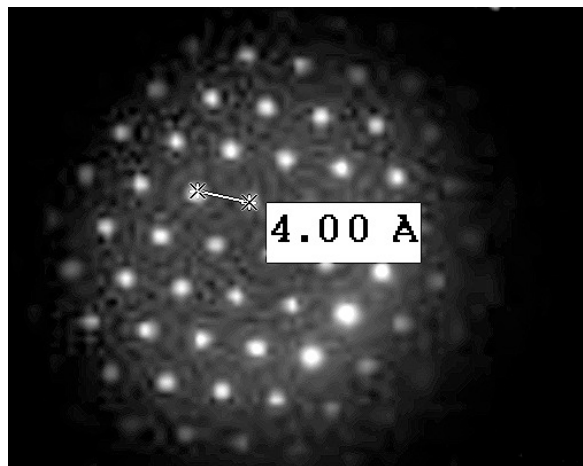
6. To calibrate diffraction measurement use the same steps as those in linear measurement calibration (See "*Measurement and Calibration->AMT Measurement->Calibration*"). The diffraction default calibration is kept in ***difCal.txt*** and the diffraction calibration Table Entrees are kept in ***camConst.txt.***

Calibration

Calibration of point to point measurement uses the same principles as calibration of diffraction measurement. Both types are password protected.

Default vs Table Entry

There are two kind of calibrations for point to point measurement - **Default** or **Normal** calibration and for more precision **Table Entry** calibration. For many labs default calibration is all that is needed. This single calibration is used for measurements at all mags and voltages, and it is extrapolated linearly (if the magnification you work at doubles the calibration factor is doubled to compensate). For doing the default calibration choose a mag near the middle choice of the choices in the range of mags you commonly measure at. So if you usual range is say 4KX to 40KX, and that range has 11 choices, pick something near the middle choice - on most scopes that will be 12KX.

As you get farther from the mag where the default calibration was done, the approximation will probably be less precise. Therefore it is possible to do a **Table Entry** calibration, for any specific combination of mag and voltage. The table entry calibration will automatically be used instead of the default whenever you measure at that mag and voltage. The steps for doing table entrees is basically the same as for doing a default calibration, except you choose **Table Entry** under **Calibration**. AMT can store as many table entrees as you like, up to one for each combination of mag and voltage.
The Table Entrees are kept in a file named "Mags4.txt" in the camera's configuration folder. You can bring that file up for review by clicking **Calibrate** on the Measurements Window's upper menu, and then clicking **Show Mag/KV Table** on the drop down list. The file has three columns: KV, Mag and Calibration Factor. The calibration factors may or may not be in scientific notation. There is one line for each of the table entrees you have done. If any line has a calibration factor that's very different from the rest, you may want to check the calibration at that voltage and mag again. Doing the table entry process over at a voltage and mag will overwrite that line on the table. The Default calibration is stored in the file "Calib2.txt" in the configuration folder. It gets overwritten when you redo default calibration.

Units of the Point to Point Measurement Calibration Factor

The calibration factors in Mags4.txt and Calib2.txt have units of "pixels per nanometer at 1000 X". You can compare that to the calibration that is displayed on a saved final image if, while saving, in the **Microscope Information** window, you choose **"Pixel Size"** under **Customize Magnification Display**. After saving, find the **Cal:** display on the final image. Convert it from microns/pixel to nanometers/pixel by multiplying by 1000 (unless it is already in nm/pix). Take 1/**Cal:** to get units of pix/nm. Since lower mag would obviously give more (image pixels)/(sample nm) divide the standard mag (1000X) by your picture's mag to get the calibration factor. So for instance if you take and save a picture at 3000X, and it displays Cal: = .004187 um/pix:

1/((**Cal:**) x 1000nm/um) times 1000X/3000X =

1/(4.187nm/pix) times 1/3 = .0796 nm/pix

.0796 is equal (within a ten thousandth) to the calibration factor for 3000X in Mags4.txt (or in Calib2.txt if there is not a table entry).

Calibration Stability

The phosphor placement, lens spacings and phosphor to CCD chip distance are all static. Distances and dimensions are fixed so this calibration also stays fixed over time. Uncertainties in the magnetic fields generated by the TEM lenses (magnetic hysterisis), uncertainties in positioning the sample at eucentric height, and uncertainty in determining the boundaries of a calibration object (Grating replicas do not have sharp features.) are many times larger and are the precision limiting factors in overall magnification calibration. A periodic check of calibration is still a good idea, mainly to correct for human error.

Calibration Steps

1. The first step in calibrating your measurements is to collect a final image of something you know the size of, like a calibration grid. Calibrating at high mag requires a different kind of standard (see High Mag Calibration Issues).
2. Next take some measurements. If using a repetitive grid measure a number of squares to reduce error. Measure in both directions. Delete the measurements that are obviously wrong.   If calibrating diffraction measurement, use spots which are a known distance apart, or rings of known size.
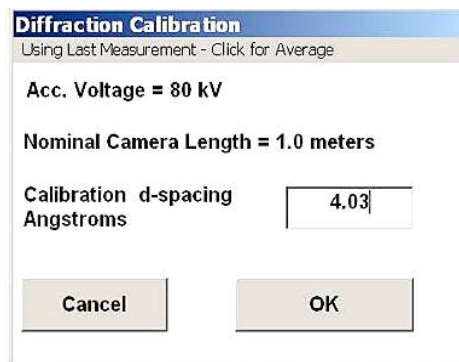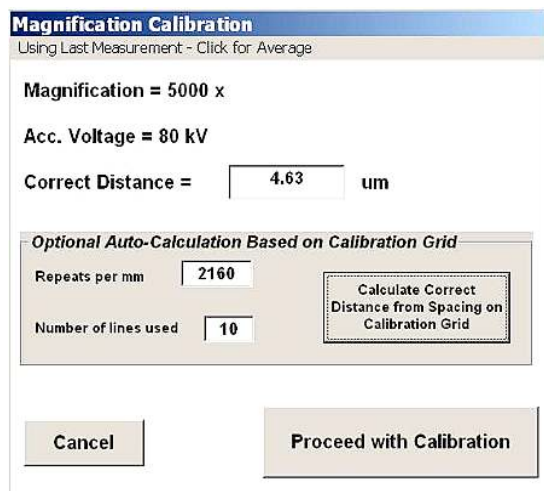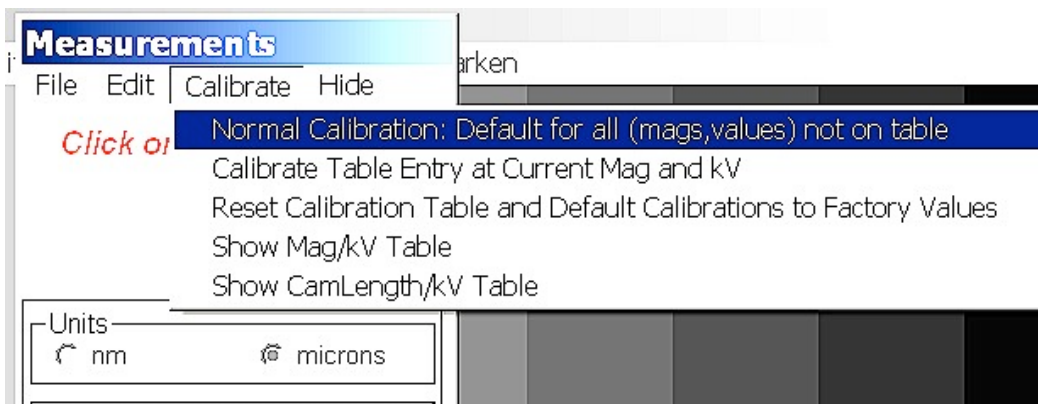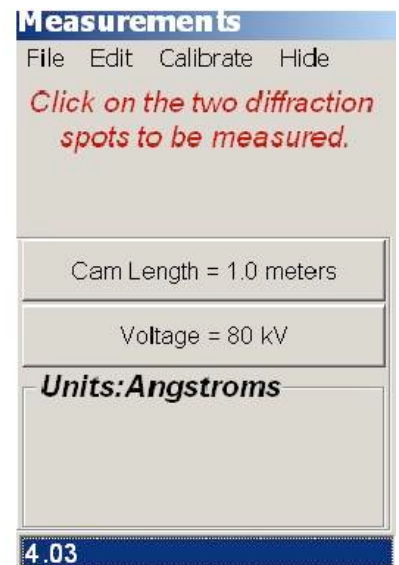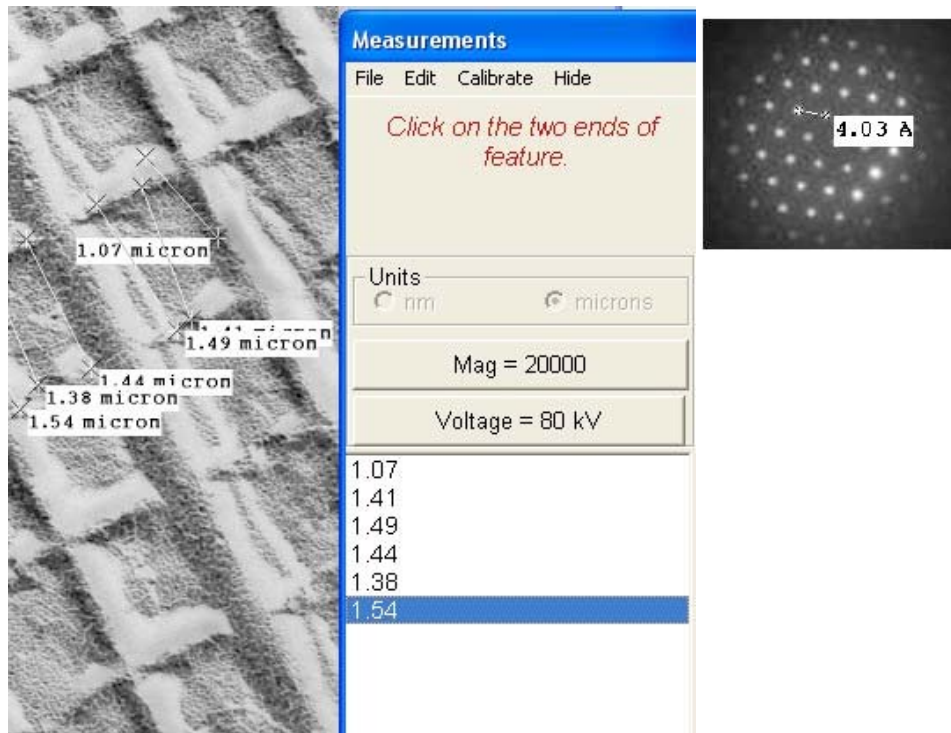
3. When you've got your target measurements click the **"Calibrate"** button. Besides options for the Normal and Table Entry calibrations, there are options to display the calibration tables for point to point measurement (Mag/KV Table) and for diffraction measurement (CamLength/KV Table). There's also a "Reset" option, for deleting all your table entrees and starting over. Resetting is not necessary for redoing a table entry or the default calibration. New values will overwrite the old ones.

4. After you choose the type of calibration and enter the password the Magnification Calibration window will appear (or, for diffraction, the Diffraction Calibration Window).

5. At the top of the Calibration window you choose whether to use the last (or selected) measurement or the Mean of the measurements as a standard. If you feel that one of your measurements is very representative use that as a standard. If you're measurements give kind of a spread, say, over different directions, use the Average as a standard.

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6. Enter the length of the known objects measured (Correct Distance). The window contains an optional use calculator to help calculate the distance of repeating patterns.
7. Clicking **"Proceed with Calibration"** finishes that calibration and adds the factor to the appropriate file(s).
8. Use the same steps for calibration of diffraction measurement. The diffraction default calibration is kept in **difCal.txt** and the diffraction calibration Table Entrees are kept in **camConst.txt.**

Measurement Labels and Properties

Editing Measurements: A measurement may be selected from the measurement list and a label added. Click **Edit** on the Measurement form menu.

Saving Measurements and Labels: If you choose to save a measurement list, measurements and labels are saved as two tab delineated colums in a text file.

Deleting Measurements: A highlighted measurement on the list deleted with the **Delete** key. If no measurement is highlighted the last value on the list is deleted. Only measurements currently showing on the image can be deleted.

Measurements in Images: An image saved with measurements displayed will retain those displays. To remove them before saving click **proc/meas -> Undo Graphics**.

Image Zoom

Image Zoom is a measurement and survey tool. The "*Zoom"* icon is near the bottom of the left toolbar. To use it one first needs a final image, either a new one or a reopened one.
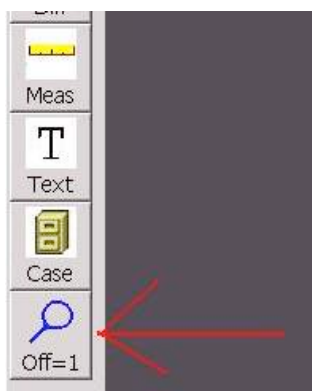
At the bottom of the Zoom icon it displays the current zoom factor. "Off = 1" is the unzoomed image.

Use the Zoom function by clicking once on the Zoom icon and then clicking on the image. The point where you click will become the center of the zoomed image. Zooming always increases displayed mag by a factor of 2.
After you've zoomed in, right clicking the image zooms out (unzooms) by one step.

Below is a final image of a biological specimen. Suppose we want to measure the width of the widest part of one of the cilia in the lower part of the image.
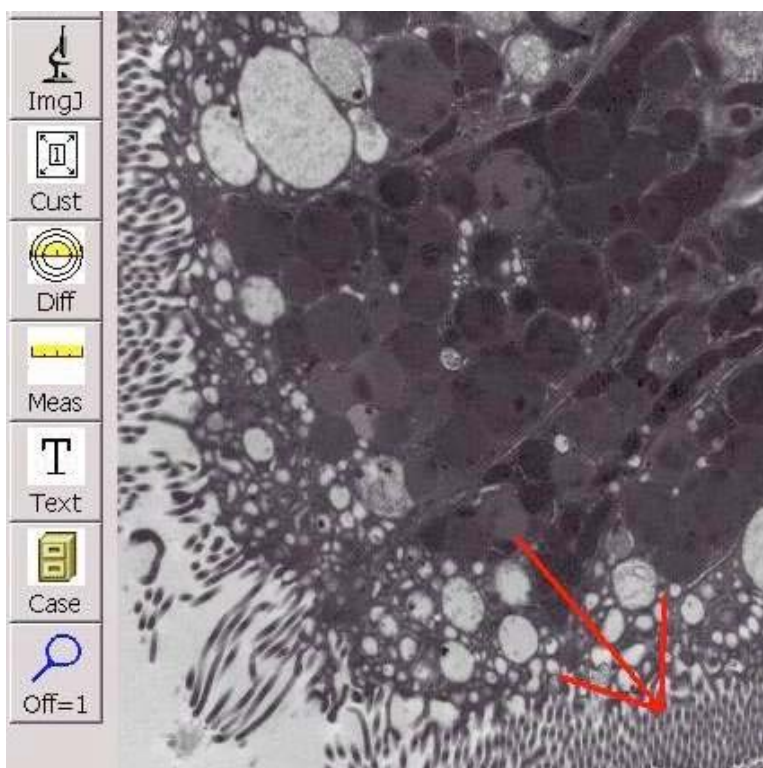
After we click once on the Zoom icon and once at the tip of the red arrow the picture below is displayed. The sample is displayed twice as large, and the Zoom icon button says "2X". The point where we clicked is now in the center of the image. If we had clicked the wrong place, we could undo our mistake by right clicking, to unzoom, and then zooming again.

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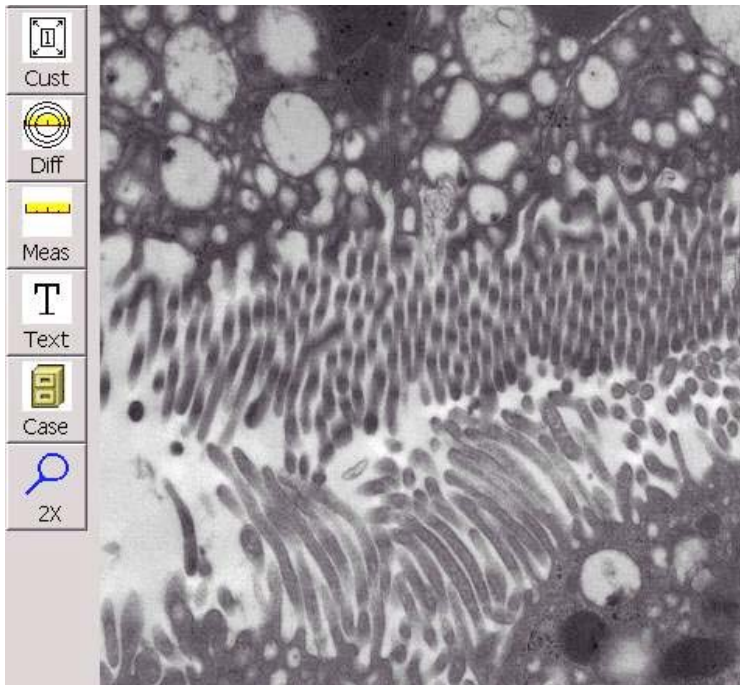
Here we've zoomed in a second time, so the displayed mag is 4 times the original. This is a good place to get the measurement. The measurement calibration is automatically adjusted to measure in the zoomed state. Click the measurement icon (the ruler) to open point to point measurement. Make sure the mag and voltage displayed on the measurement form are correct.
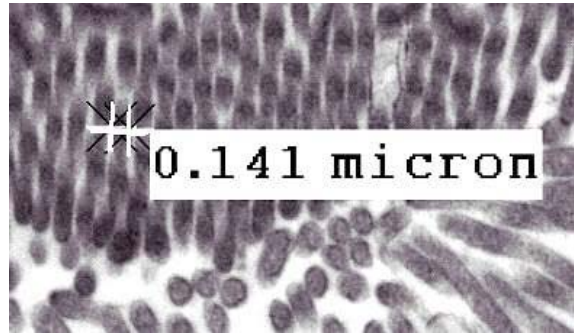
The same procedure can be used for diffraction measurement.

Calibration at High Magnifications

To calibrate in the higher magnification ranges it is a good idea to use a material standard. Available materials are:

    Catalase, which has two fairly large lattice spacings 87.5 A and 68.5 A.
    For higher mags Crocodilite has 9A and 4.5A.
    Copper Phthalocyanine is another material that is sometimes used but it is beam sensitive.

Lattice images of gold or graphitized carbon can also be used. The carbon layers tend to bend and finding any place with more than a few planes visible is not easy. There are also (very expensive) superlattice material that is made especially for TEM calibration.

Most suppliers have these materials available. You can check them out (for example) at:

SPI's website: http://www.2spi.com/catalog/index/
Crocodilite is SPI catalog # 02908 – AB
Catalase is SPI catalog # 02905 – AB
The MagiCal superlattice standard is SPI catalog # 02218 – AB.

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Measurement and Analysis Using ImageJ

ImageJ is an image processing application written by [Wayne Rasband](). It is a work of the United States Government, is in the public domain and is open source. It is available from the National Institutes of Health ([NIH Web Site]()). All modifications, source code, applications and additions to this program by AMT are also freely available from AMT.

This is a supplement to the existing documentation for *ImageJ* . Its main purpose is to highlight and introduce operations typically used with CCD images obtained by transmission electron microscopy. This orientation also describes customizations made by AMT for TEM imaging.

AMT Automatically Embeds Scaling Information

Spatial calibration information is incorporated into the tiff file header when AMT's Capture Engine saves a file. The customized version of ImageJ will read the calibration parameters for an image when it opens an AMT tiff file. If the calibration information is not present, an alerted box will remind you to manually calibrate the image (Use ImageJ's menu **Analyze/SetScale** to do this).

Calibration information may be lost if you open an image in another image processor and then overwrite the original image.

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Length, Area and Angle Measurement
Particle Counting
Intensity Measurement
Image Drift Measurement
FFT
Viewing Serial Images
ImageJ macros

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Measuring Length Using ImageJ

Open a Tiff Image in *ImageJ* and select the line tool from the *ImageJ* toolbar. The **last image saved** by the AMT Capture Engine may be opened by pressing the **F5** key.

Se lect the line tool from the *ImageJ* tool bar
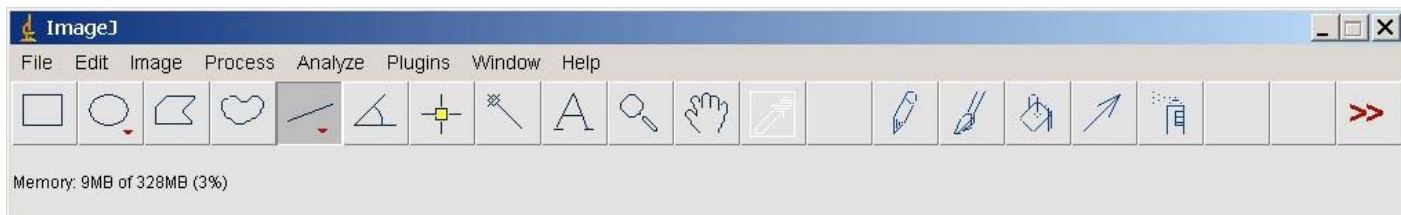
Position the cursor at one end of the object to be measured. Click and drag to the other end of the object. Type **F1** to enter the length of the line drawn into the measurement list. A line between the start and end points and a label will also be drawn on the image. Repeat the process for other objects that you wish to measure. For difficult or small objects you can use the zoom tool and then make measurements. More accurate measurements, of course, are obtained by collecting a higher magnification image.
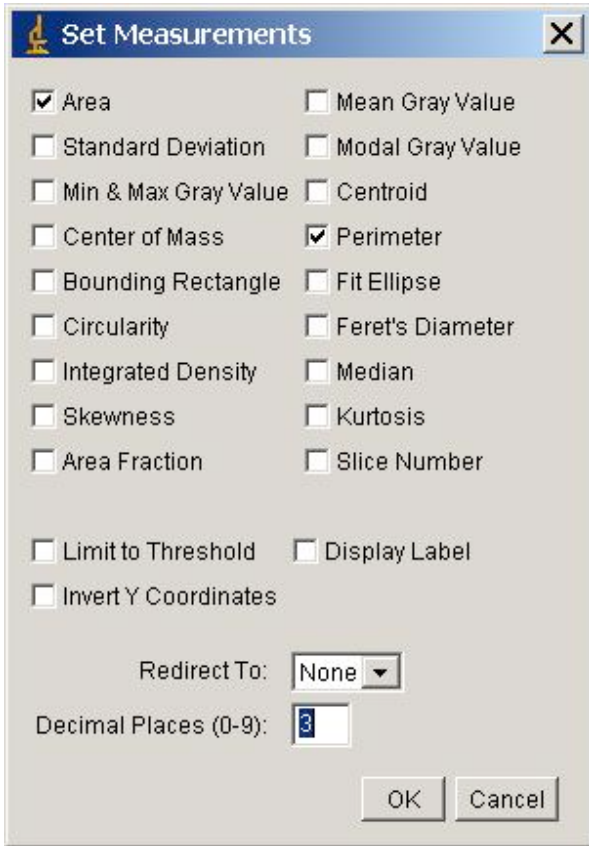
Measuring Perimeter and Area Using ImageJ

Distance along an irregular path or a perimeter can also be measured by selecting the Segmented Line Selection or the Freehand Line Selection tools in the *ImageJ* toolbar. You will need to select the measurement (area, perimeter, etc.) to be displayed in the menu **Analyze\Set Measurements** Menu item. After you have drawn the curve type **ctrl-m**. The measurements selected will be displayed in a Results box. Units are the length units of the image. In this case nm and nm2.
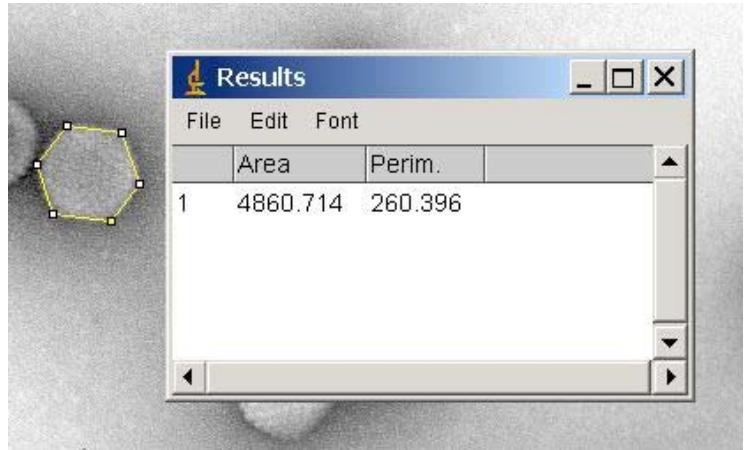
Measuring Angle Using ImageJ

Angles can be measured by selecting the angle measurement tool.

On an open image double click on the first point, single clicking on the vertex, and click once more at the terminus of the final ray. After you have drawn the angle type **ctrl-m**. The angle measurement is displayed in a Results Box.

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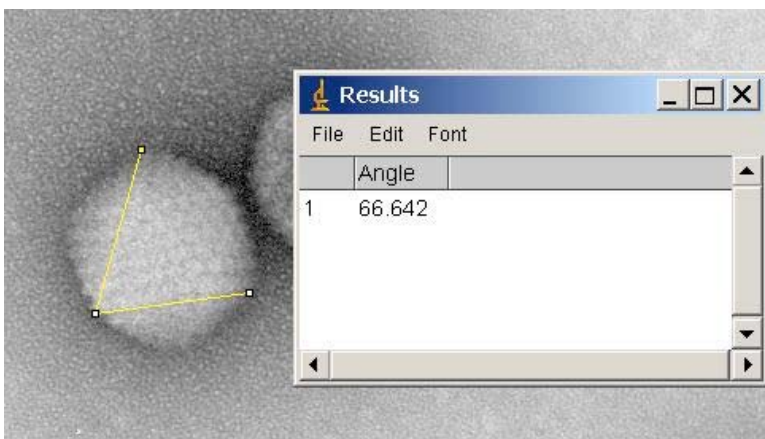
Particle Counting Using ImageJ

ImageJ can automatically count the particles in a whole image or a selected part of the image. You determine what gets counted as a particle by thresholding the image's grayscale values to suppress background features and accentuate the desired features, and also by setting limits to the size of a particle. It's also possible to require a set degree of "roundness" to be a particle. The steps below are a tutorial for particle measuring. For more information on this operation see the website: http://rsb.info.nih.gov/ij/docs/ .

1. Open ImageJ, either by clicking on the ImageJ icon on the left side of AMT's display, or by clicking on ImageJ in *style=""">"Start -> Programs"*.
2. Open a saved image in ImageJ. For this tutorial, open "
    C:\AMTCommon\AMTSampleImages\ImgId1cal.tiff".
3. Use ImageJ's crop tool to separate an AMT image from caption area. One click with the tool will effect the separation. After the separation reselect the square tool, and close the caption window.
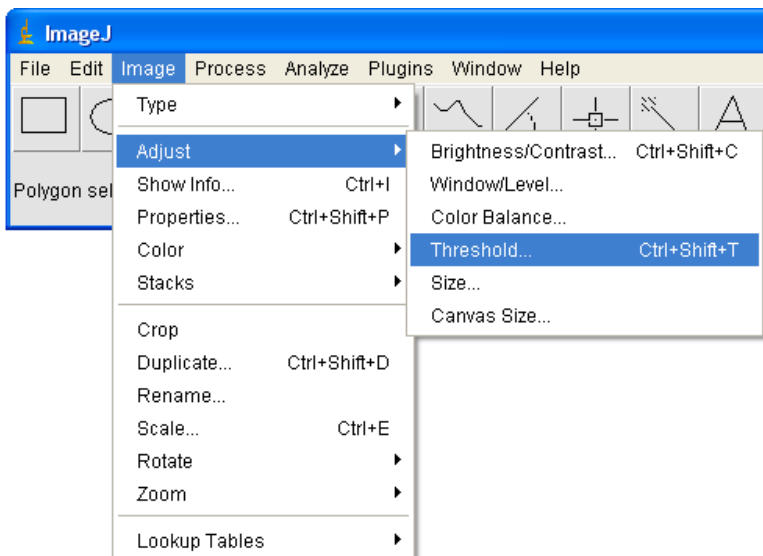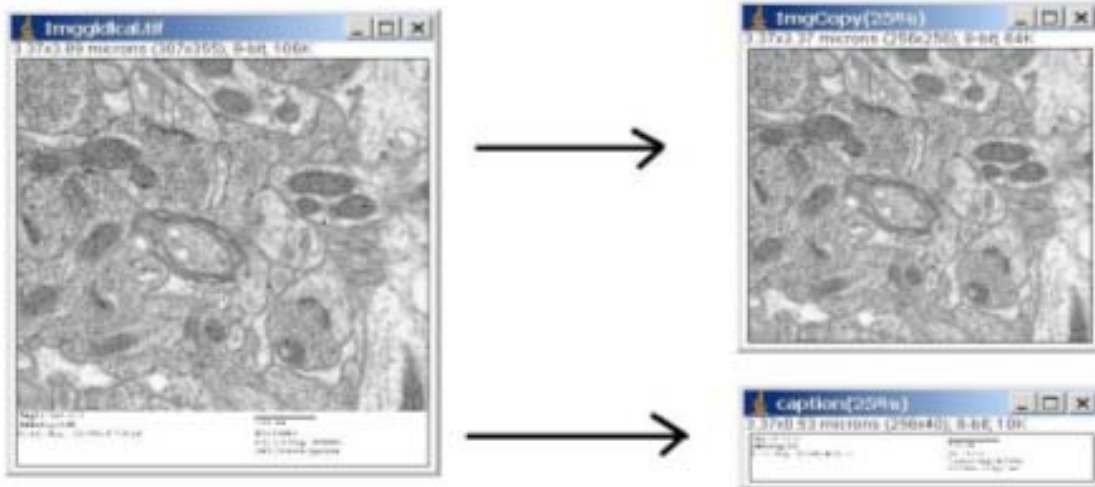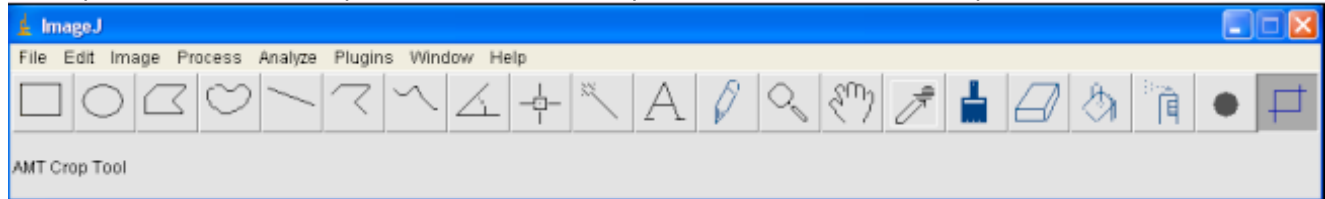
4. Next we separate the particles from rest of the image by setting the black/white thresholds around a narrow set of gray values. Make sure the image to be measured is selected (clicked). On the ImageJ menu click *"Image -> Adjust -> Threshold"*. Under the histogram that comes up you can change the thresholds by moving the bars or click *"Set"* to type values into a window. A lower value of 17 and an upper value of 70 worked well for our immunoGold particles at 10KX. You may want to experiment with these settings, but when you find values that work, be consistent will all your samples. After setting the thresholds click *"Apply"*, and click all three boxes in the dialog box setting threshold pixels to black and other pixels to white. This makes the image "binary" having all black particles on a white background.
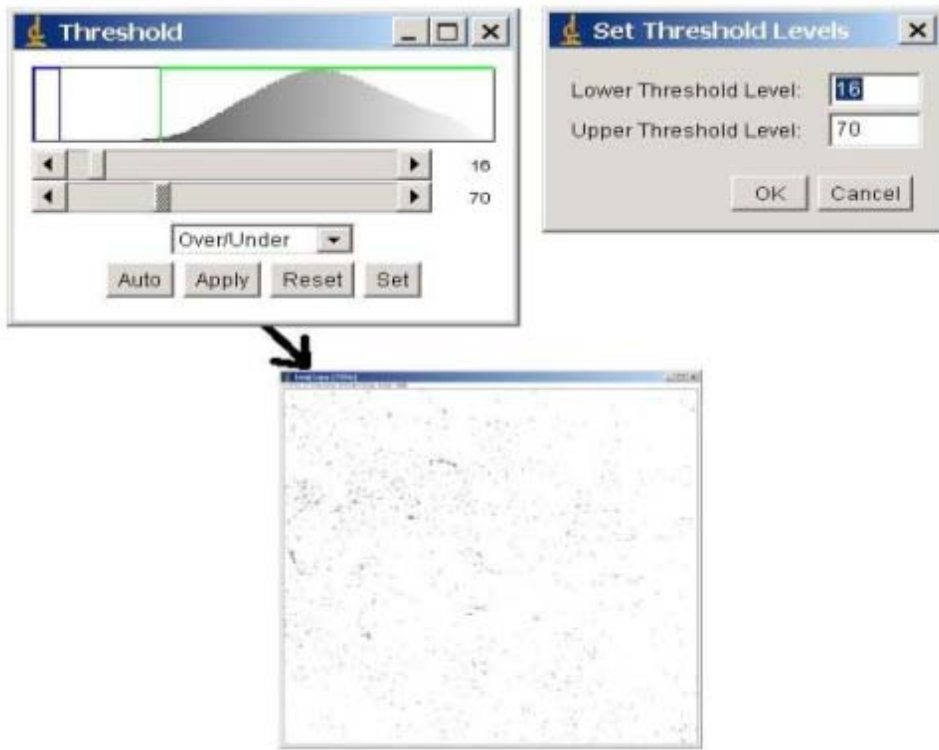
5. Tiff files saved from the AMT program have tags that will set the measurement scale for ImageJ. If other kinds of images are used you'll need to set the scale, using a feature of known size, in **"Analyze – Set Scale"**.
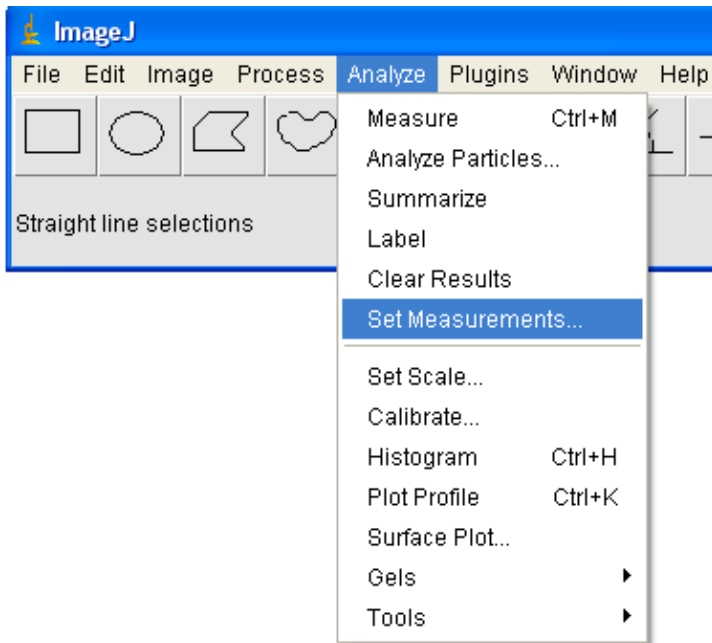
6. In **"Analyze – Set Measurements"** select which measurements you'd like saved for each particle, and the number of decimal places to be displayed in the results. Counting small particles in a 10KX image requires six decimal places. For other mags or for large particles you may have to experiment with decimal places. Grayscale measurements will not be terribly meaningful, since we've made the image binary (each pixel black or white) when we set thresholds.
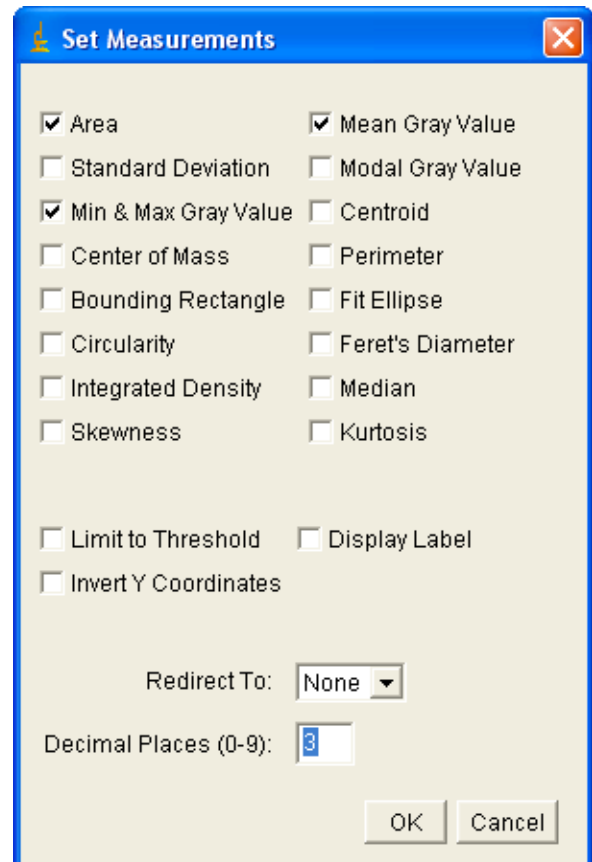
7. The "Result" window (upper right below) displays a number and the selected measurements for each particle. The "Summary" window gives the Count number, Average Size, Total area of the particles and the Area Fraction covered by the particles. The "Particle Size Distribution" window show a histogram with the particles grouped into bins according to their area. The statistical values displayed below are for particle area, not grayscale values.
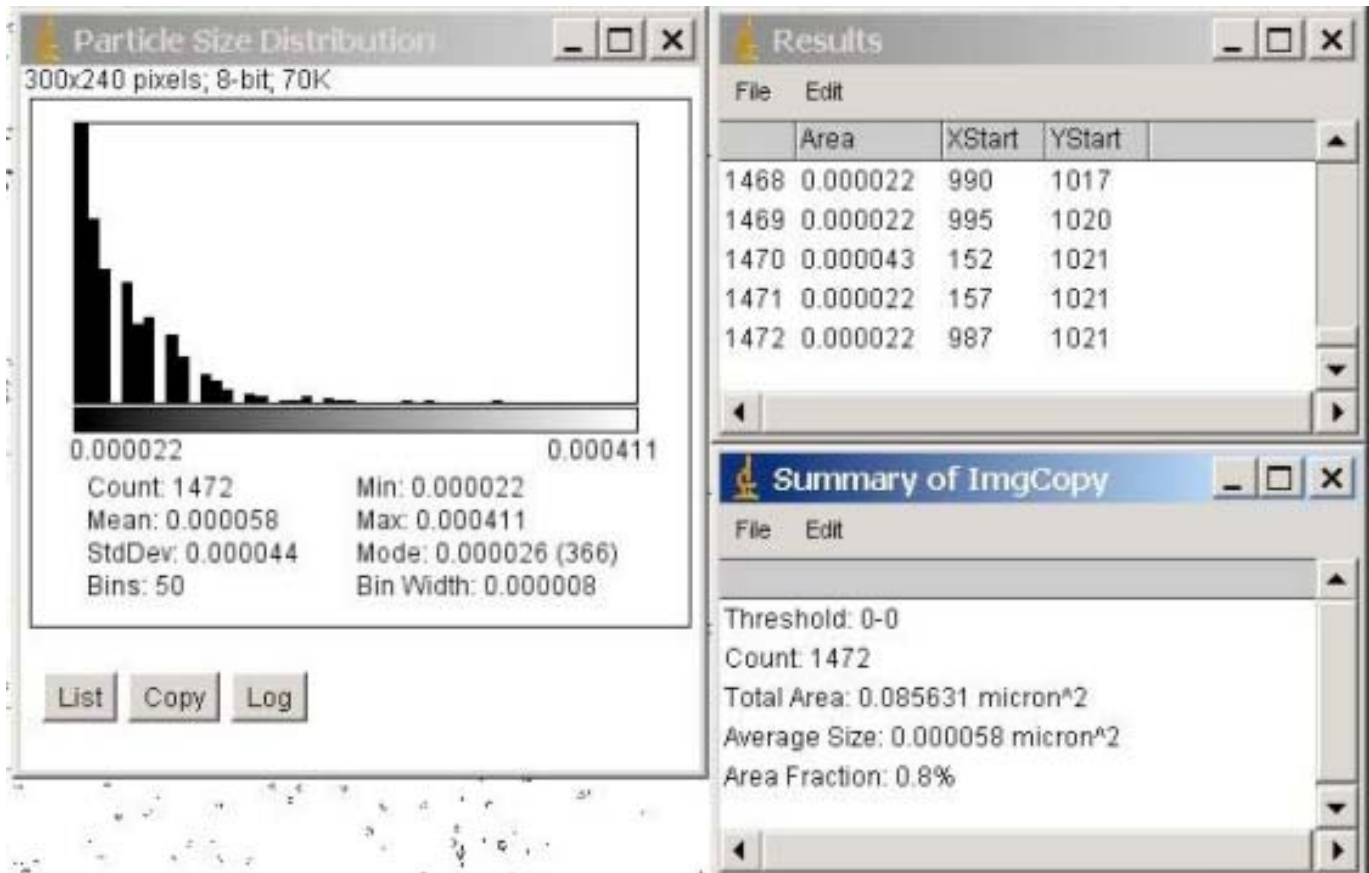
For more information about analysis with ImageJ see the website *http://rsb.info.nih.gov/ij/docs/*.

Measuring Intensity Using ImageJ

Intensity Measurement Cautionary Note

Measurement of image intensities and gray scales can also be done. However, it is important here to remember that the Capture Engine normally applies a contrast enhancement to images as they are aquired and displayed. This improves the appearance but modifies grayscale information. If quantitative measures are to be applied to image intensities, the contrast enhancement of the Capture Engine should be turned off and images should be saved as 16 bit tiff files.

To measure the gray scale value at a point simply position the cursor at a point of interest and the gray scale intensity is displayed in the status bar just below the *ImageJ* tools. Alternatively, the Point Selection tool can be used.

Magnifying glass (or use "+" and "-" keys)

Select a pixel with the tool and then type ctrl-M to display the intensity in the Results window. The values in the Results window can be saved.

Line profiles can be generated by drawing a line through the features and then selecting **Analyze\Plot Profile** or pressing the ctrl-K key. To improve the statistics of the plot one can select a rectangle in which case a column average profile is generated.
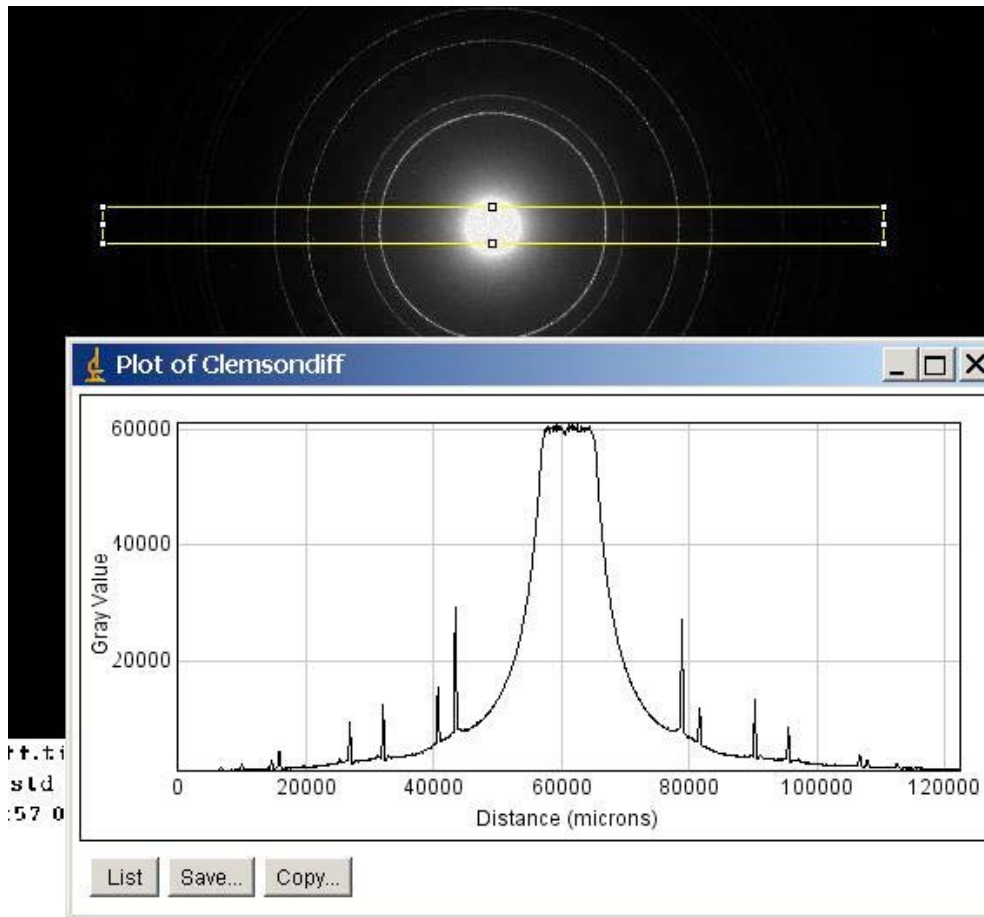
Image Math

Extraction of interesting features from a complex image can be a challenging aspect of image analysis. It is very useful to be able to perform simple mathematical functions on whole images and between two images. Once a sequence of operations is found to be useful a macro can be created to automate that sequence. Embedded in the particle counting macro discussed below, for example, is an algorithm to extract small dense particles from a complex image that includes both tissue and gold particles. This algorithm relies on some simple math. The image is first blurred and then a portion of it is subtracted from the original image. This tends to enhance small particles and suppress the background. The Image Calculator in the Process menu permits one to add, subtract, multiply, divide, and apply several other operators to pairs of images.

Image Drift Measurement
Installation
Use the ImageJ installer **ImageJ138g_V306_20070404** or later installer. Run Setup.exe and accept defaults. The installer will install the required startup macro, and the TurboReg plugin that it calls.

Setup
The input tiff images should be placed in a single folder and have file names that end in **_xxxx**. Where **xxxx** is the time of acquisition. E.G. test_0000.tif was acquired at time = 0000. The time units are seconds. All images should be taken at the same magnification and written by AMT's Capture Engine which embeds scaling information into the tiff image header. This information is used by the macro to calculate a real drift rate.
For testing purposes the installer placed a canned set of test images in the folder **C:\AmtCommon\AmtSampleImages\CannedImages**. These images will be used to demonstrate use of the drift measurement macro/plugin.

Running the Macro
The macro is activated by selecting the menu item **Plugins\Macros\AMT Drift Measurement** or equivalently by selecting the **F3** key. This will display an introductory message with acknowledgements to the TurboReg plugin authors and instructions.
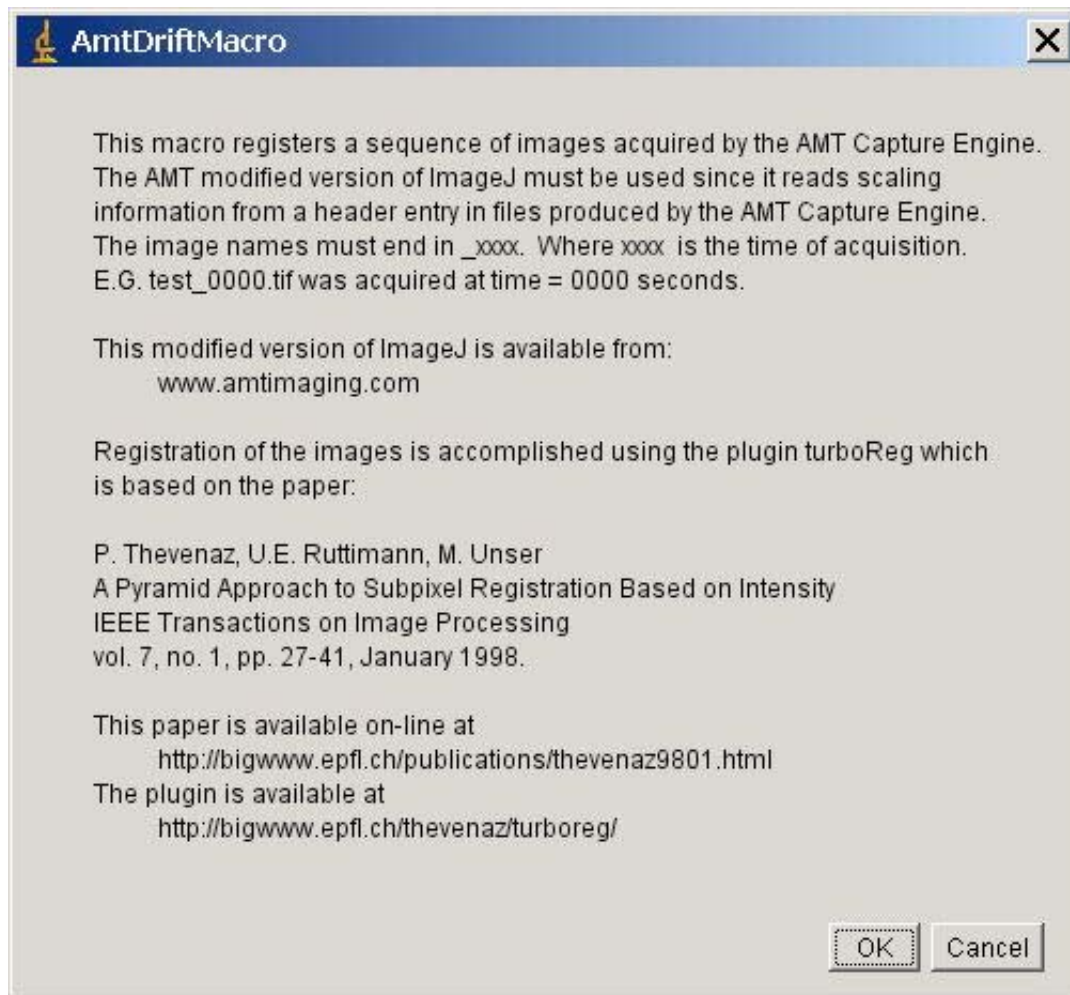
Click OK. Use the **"Choose Directory"** dialog that is displayed next to locate the images whose drift you wish to measure.
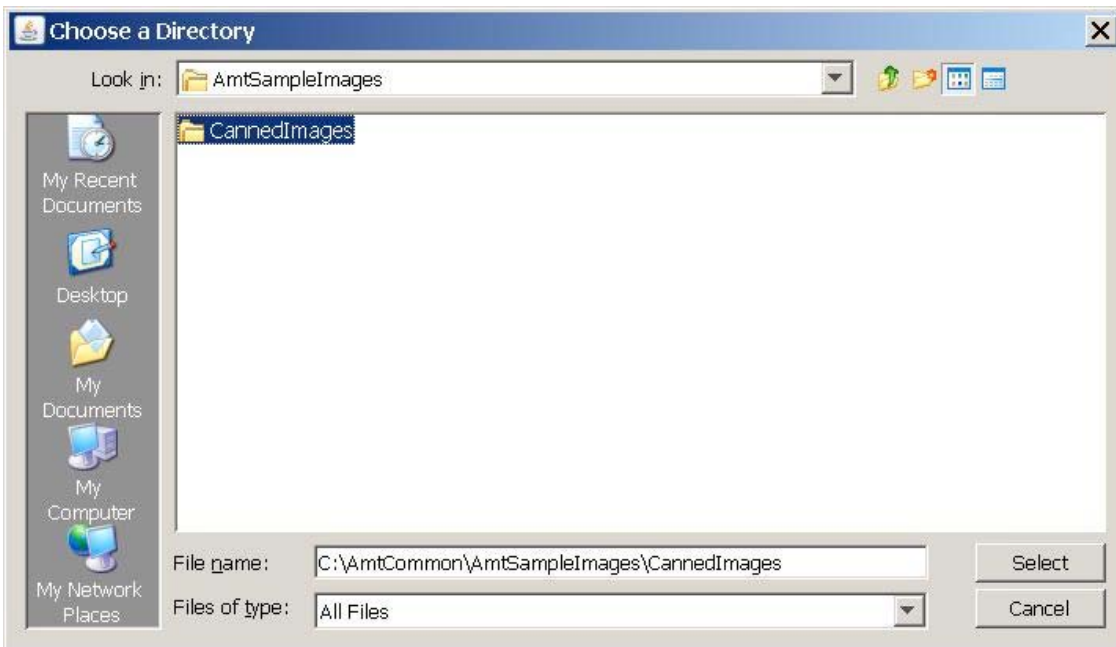
The macro will display the first image and request a choice of length units.

Select the unit and click **OK**.

The macro will now sequentially open the images measure the drift relative to the previous image and enter the results into a Log table.

ref Image	test Image	DeltaX[nm]	DeltaY[nm]	Vector Magnitude	Angle	Rate [nm/sec]
x_0000.tif	x_0010.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0010.tif	x_0020.tif	55.0821	0.0000	55.0821	0.00	5.50821
x_0020.tif	x_0030.tif	55.0821	0.0000	55.0821	0.00	5.50821
x_0030.tif	x_0040.tif	55.0821	0.0000	55.0821	0.00	5.50821
x_0040.tif	x_0050.tif	55.0821	0.0000	55.0821	0.00	5.50821
x_0050.tif	x_0060.tif	55.0821	0.0000	55.0821	0.00	5.50821
x_0060.tif	x_0070.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0070.tif	x_0080.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0080.tif	x_0090.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0090.tif	x_0100.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0100.tif	x_0110.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0110.tif	x_0120.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0120.tif	x_0130.tif	55.0821	0.0000	55.0821	0.00	5.50821
x_0130.tif	x_0140.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0140.tif	x_0150.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0150.tif	x_0160.tif	55.0821	0.0000	55.0821	0.00	5.50821
x_0160.tif	x_0170.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0170.tif	x_0180.tif	55.0822	0.0000	55.0822	0.00	5.50822
x_0180.tif	x_0190.tif	55.0822	0.0000	55.0822	0.00	5.50822

These results may be saved to a file by selecting **"File/Save As"** on the Log form if desired.

Fast Fourier Transform Using *ImageJ*

Backgound

The Fourier transform expresses an image in terms of the spatial frequencies in the image. A sharply focused image will be rich in high spatial frequencies. Large uniform areas are represented by low frequencies. Images with repeated features will have Fourier Transforms with repeated strong peaks. This can be used to select various parts of an image and can be very useful in removing artifacts, or improving image sharpness. *ImageJ* has a built in FFT and in addition there are several plugins posted on the NIH web site (NIH Web Site Plugins) that implement their own specialized versions of the algorithm. See for example: Band pass filter Arbitrary size 2D and 3D transforms

There are also plugins that rely on the FFT as an integral part of the algorithm they implement. These include convolution, filtering, and deconvolution plugins - these algorithms are more efficient when performed in fourier space. Many of these plugins are provided with references to the literature.

Doing the FFT

Doing the FFT of an image is straightforward. open an image. Select the region of interest on an open image and then in menu **Process menu select FFT**. Since the FFT is usually implemented for regions that are even powers of 2, the nearest power of two sized region is automatically used in the FFT.
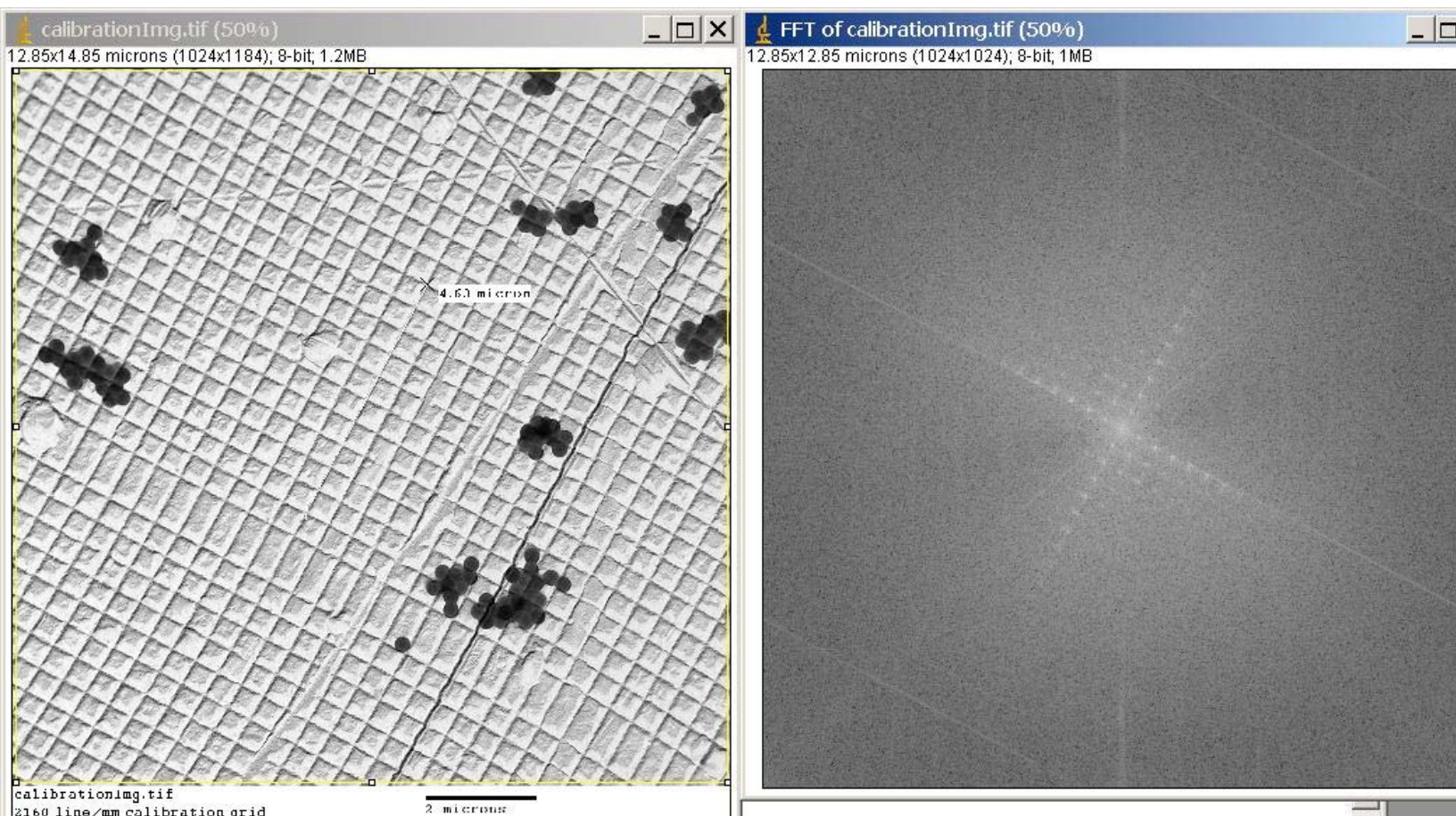
Viewing Serial Sections Using ImageJ Stacks

A sequence of images obtained from a serially sectioned sample can be used to create an ImageJ "Stack". A "Stack" is way to animate and visualize a sequence of images and ImageJ has routines that use stacks to display 3 dimensional information. In addition to the intrinsic capabilities of ImageJ plugins have been written to augment the 3D viewing capabilities of ImageJ.

The first step is to obtain a sequence of serial images through an object of interest. In a real experiment this is done by sectioning the object and then carefully collecting the sections in the order that they were sectioned. To experiment with and become familiar with the ImageJ stacks we include a simple macro to generate synthetic images which can be assembled into a stack. The macro is "**AmtDrawStack.txt**" and is located in the **C:\Program Files\ImageJ\macros** folder.

1)   The "AmtDrawStack" macro is a very simple macro that creates 50 images (01.jpg to 50.jpg) and stores them in the folder "**C:\images\ImageJ \SampleStack.** " You must create the folder C:\images\ImageJ\SampleStack before running the macro. Install the macro "AmtDrawStack.txt by copying it into the folder: C:\Program Files\ImageJ\macros. Another useful macro for this exercise is AmtOpenSet.txt. This macro opens the 50 images generated by AmtDrawStack. Install the macro AmtOpenSet.txt by copying it into the folder: C:\Program Files\ImageJ\macros

2)   The Volume Viewer plugin "Volume_Viewer.jar" was obtained (10/23/2006) from the ImageJ web site:
      http://rsb.info.nih.gov/ij/plugins/index.html#analysis
The web page "Volume Viewer.htm" has installation information, references to other possibly useful plugins, and author contact information.

3)   Install the "Volume_Viewer.jar" file by copying it into the
 C:\Program Files\ImageJ\plugins folder.

4)   Obtain serial images through an object of interest. To experiment with the plugin using synthetic (generated) images run "AmtDrawStack.txt" by going to the ImageJ menu Plugins\macros\Run and then locating the macro in the folder C:\Program Files\ImageJ\macros. The images generated by this macro will be found in C:\images \SampleStack

5)   Open the images in order. Use the macro AmtOpenSet or you can open the images directly from ImageJ using the keyboard shortcut Ctrl-O and then double click the image icon. These shortcuts may be helpful since Image J does not have a group open command.
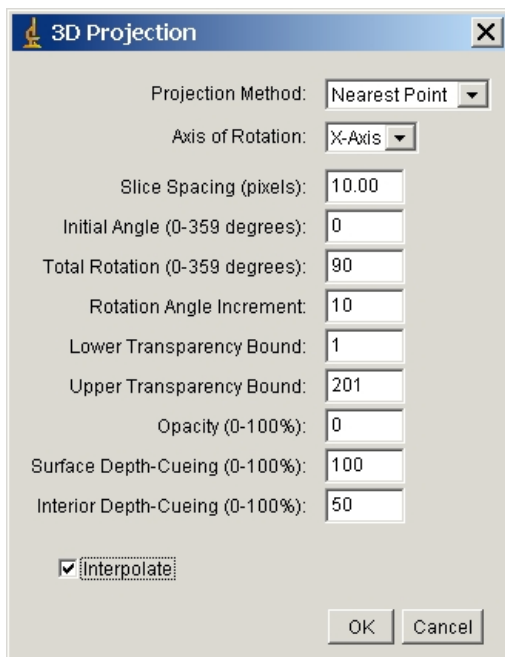
6)   Create a Stack from the images. Go to the menu "Image\Stacks\Convert Images to Stack.

7)   You can animate the stack by going to the menu: Image\Stacks\Start Animation. This will cycle through the images. You can also save the stack by using the File\Save or the File\SaveAs menu. You must save the stack in the tiff format.

8)   Once you have a stack go to the Plugins\VolumeViewer menu item. This plug in will allow you to observe the images from different orientations. ImageJ also has some viewing capabilities in addition to the simple animation in the Z dimension (See the other options in Image\Stacks). In particular, "Image\Stacks\3D Project" allows you to create an animation of the images rotating. Pixels of certain values can be set transparent so that embedded objects (like the cone) can be visualized. Try "Image\Stacks\3D Project" with the following parameters:

 The Upper and Lower Transparency Bounds allow one to specify pixel densities that are ignored. Since the cylinder and cone have different gray scale values setting the upper bound between the two values (eg. 170) makes it possible to visualize only one of the structures.

3D Projection

Projection Method:	Nearest Point
Axis of Rotation:	X-Axis
Slice Spacing (pixels):	10.00
Initial Angle (0-359 degrees):	0
Total Rotation (0-359 degrees):	90
Rotation Angle Increment:	10
Lower Transparency Bound:	1
Upper Transparency Bound:	201
Opacity (0-100%):	0
Surface Depth-Cueing (0-100%):	100
Interior Depth-Cueing (0-100%):	50

☑ Interpolate

OK   Cancel

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ImageJ Macros

Macros are sequences of commands, which can be executed as a single operation. A few examples of AMT generated macros have been mentioned and used above. Particle counting, drift measurement, and measurement and labeling make use of both intrinsic ImageJ operations, plugins (to be discussed below) and macos. An existing macro can be run by selecting the menu **Plugins\|Macros\Run** and then navigating to the location of the macro. Macros are usually kept in: C:\Program Files\ImageJ\macros   Macros can also be installed as a menu item or assigned a keyboard shortcut.

ImageJ has over ninety standard macros that are supplied as part of the initial installation. These macros range from functions that alter every image pixel, to measurement of image parameters and dimensions to drawing tools. More information is available on the NIH web site. ImageJ Plugins

The macro **StartupMacros.txt** is a special macro that runs when ImageJ is launched. It can be used to define shortcuts to macros and place macros in menus and tools. AMT has set this up to install a useful sample set of macros as menu items and shortcuts. It can be modified by the user as desired.

The AMT shortcuts are:

AMT Shortcuts		
Shortcut	Name	Description
F1	Draw and Measure	Draws a line between points defined by the line selection tool. The distance between the points is calculated, entered into the results table and drawn on the image.
F2	Measure	The same as ctrl M just a single key needs to be depressed.
F3	Drift Measure	Runs the drift measurement macro.
F5	Open Last AMT Image	Opens the last Image Saved by the AMT Capture Engine.

 In addition to these there are several ImageJ standard shortcuts.
See http://rsb.info.nih.gov/ij/docs/shortcuts.html

ImageJ Recording Macros
The easiest way to create a macro is to open the command recorder under the menu item **Plugins/Macros/Record** Then record a series of commands, and click Create. In record mode, each menu command you use generates a call to ImageJ's run() method. This method has one or two string arguments. The first is the command name. The optional second argument contains dialog box parameters.

ImageJ Plugins
Plugins are another form of extension available to the user of ImageJ. Plugins are very powerful and execute much faster than macros. More information as well as over 100 plugins are available on the ImageJ web site: http://rsb.info.nih.gov/ij/docs/menus/plugins.html   ImageJ has a large body of users and many plugins and macros have been created and contributed to the NIH site. In addition there are links there to other sites with additional macros, plugins, and documentation.

AMT Tools and Macros
AMT has generated some tools and macros that you may find useful. Macros in the **C:\Program Files\ImageJ\macros** folder whose name starts with AMT may be useful or provide useful examples for functions you may wish to build into your own macros. Macros are written as text files and these files begin with comments that document their function.

The AMT Crop function strips away the AMT image label so that image processing can be done on just the image.

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Diffraction Imaging

This chapter describes procedures and precautions for acquiring diffraction images.

Contents
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Acquiring a Diffraction Image

Precautions: Certain precautions are necessary for taking diffraction images. Diffraction produces bright concentrated spots or rings which can damage the phosphor of your camera. Lower illumination and shorter exposures are preferred. Please follow the following four precautions:

Use a smaller spot size (controlled by condenser 1). If your largest spot size is 1, try 5 or 6.

Put in the smallest condenser aperture. If your diffraction images are dim you can experiment with a larger aperture later.

Find and focus diffraction patterns with the traditional TEM viewing screen (side mount - phospor retracted, bottom mount - viewing screen down).

Use a "central beam blocker" if available. Many electron microscopes have one built in. It eliminates the bright central spot of a diffraction image.

Minimize "camera in" time. Using the first three precautions you should be able to get a nice pattern on the TEM viewing screen, without damaging the phosphor. Now insert the phosphor (for bottom mounts lift screen) for a short time to collect the image. Collect a final image and then retract the phosphor (for bottom mounts lower screen) right away.

For best diffraction image quality, if your camera has it, click "Display->Low Light Mode on/Click to Change to Diffraction Mode." This prevents overloaded pixels from "blooming" onto neighboring pixels. Don't forget to change back for normal imaging. Reopening the program will also bring back normal (Low Light) mode.

Diffraction Settings

Those who do diffraction often and analyze images offline can increase efficiency by creating a separate set of camera settings for diffraction using the Camera Settings And Control Window. The settings described below are designed for uniform quantitative calibration. (You can get "prettier" pictures using Gamma and non-zero thresholds.)

For information relevant to quantitative analysis of diffraction images, see the AMTHelpFiles chapter *Data Analysis - Bit Depth of Images*.

Set recording gain to 1. Use record exposure time to adjust brightness.

Set white and black thresholds to zero and run with AutoGain ON. This disables the Autogain's rescaling, but leaves corrections in place. (Note that zeroing Thresholds disables Tails as well.)

Set gamma to 1. These steps should give you quantitative data in the sense the camera becomes a light meter - as long as the dark and background images are constant. Absolute intensities become meaningful and can be calibrated.

Use as many final integrations as possible, keeping total exposure time below 3 seconds.

Save these conditions for diffraction - or some name that you like. The saved conditions will contain the zero thresholds. Do not use this condition as your default unless you do only diffraction.

Acquire a dark field (no beam) and background with very uniformly spread beam. I believe that the background should be taken once and only once to normalize the radial falloff of the lens. (Phosphor graininess should not be important in diffraction). If you are measuring absolute intensities picture-to-picture, do not repeat the background acquisition, unless you can measure the beam current independently and take new backgrounds under the same beam intensities (within your error tolerance). But note relative intensities within a picture will not be degraded by a new background.

Note that your saved backgrounds and dark images are valid only after new "diffraction" conditions are saved or recalled. Check your display to make sure you are saving to the right place.

As a precaution, save a copy the entire folder containing your settings and correction images. These can be found in c:\amtcommon\config\Settings\name of settings file There will be one .txt file and eight .bin image files in this folder. In fact it would be good idea to backup the entire Config folder to keep all your settings.

Save your diffraction images in 16 bit format.

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Lattice Imaging with Bottom MountDigital Camera

1) The phosphor pixel size determines the minimum magnification needed to see lattice. The minimum case has a bright region centered on one pixel and the dark region centered on its neighbor. So an absolute limit of 2 pixels for each "period" of the lattice oscillation.

2) The ERB camera has a ~23 micron pixels. So, a 3A lattice period would need to project to 46 microns on the phosphor. This corresponds to a minimum magnification of 46×10^{-6} m/3×10^{-10} m or 150,000x, <u>if the lines are horizontal or vertical.</u>
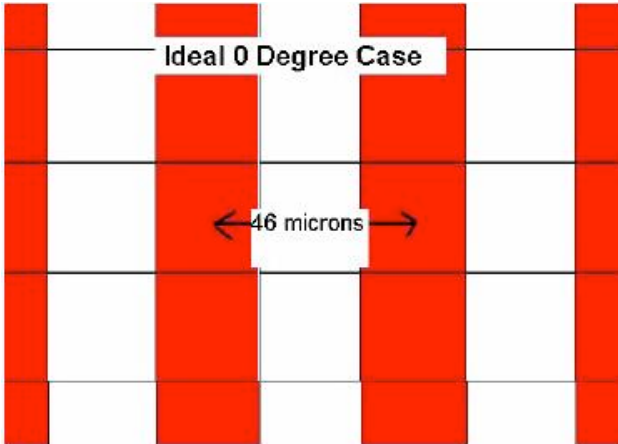
3) Slanted lines need higher magnification. The worst case is 45 degrees, where the lattice period corresponds to 65 microns. To see lines at this angle the minimum magnification is 220,000x.

4) The camera is below the film plane so that the magnification at the camera is 10 to 20 % higher than the "dial" or "film" magnification. Thus, 200,000x should be able to resolve 3A lattice in the ideal case.

5) But the world is not perfect.

a) This model assumes that the lattice sampling is perfectly synchronized with the CCD array, which is nearly impossible to achieve. There will always be sharing between adjacent pixels. There is also the possibility of Moire patterns, if the minimum magnification is used and there is only a small difference in lattice and CCD periods.

b) The model assumes high contrast in the image and nearly perfect response of the camera. Neither is true.
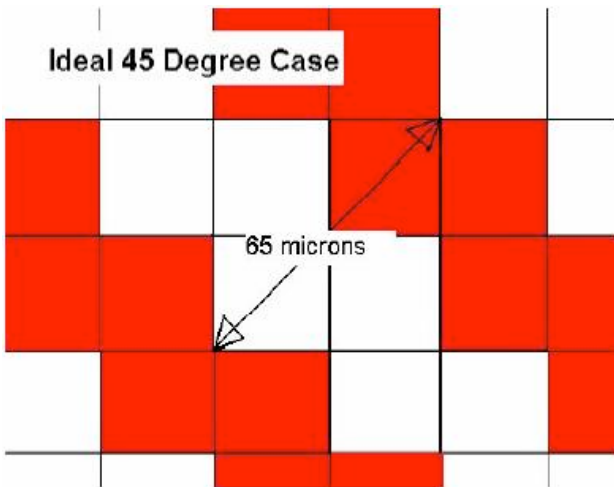
6) The general "rule of thumb" is to use ~2 to 3 times higher magnification than the ideal. This means that you should be more comfortable shooting 3A lattice at ~500,000x with a bottom mount camera. [Note: These values will be good for most bottom mount 1k cameras made by us or out competitors. Some 2k cameras have smaller pixels (i.e. Gatan's UltraScan = 15 microns, AMTXR60B = 12 microns); so a lower magnification can be used. Our large field, 7 mpixel bottom mount XR100 has 24 micron pixels; so the upper resolution limit is the same as the 1k but the field is 2.6x larger (Mag factor is 2.6 smaller).]

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Display Options

The AMT Image Capture Engine has lots of tools to change the way things are displayed during live and final imaging and while measuring.

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Changing Image Displays
    Refresh Display
    Contrast Inversion
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    Show Captions
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    Show/Hide Live Scale Bar
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Changing Image Display

The AMT Image Capture Engine has lots of tools to change the way things are displayed during live and final imaging and while measuring. Most of these options are accessed either the "Display" or "Proc/Meas" menu items.
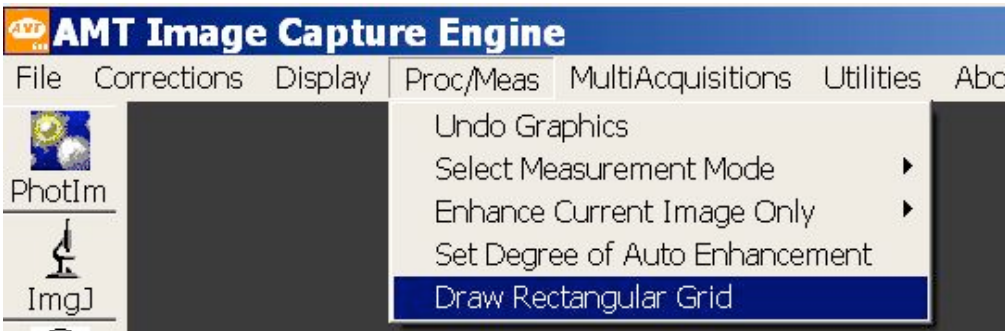
Refresh Display

This button is not needed often. It allows the user to refresh the display of a window.
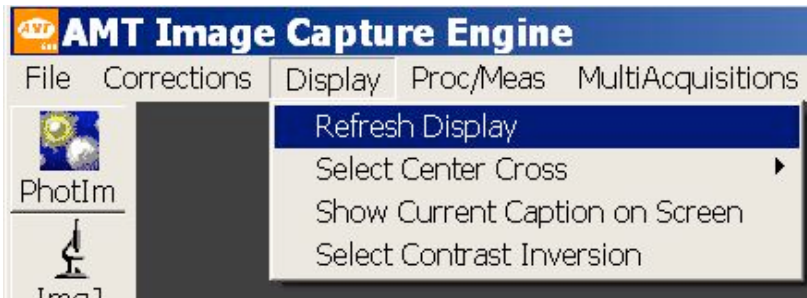
Contrast Inversion

Sometimes a user wants an image to be displayed with black pixels appearing as white and white pixels appearing as black, like a negative. This kind of display can be helpful when counting gold particles, for instance. The Display menu provides buttons for inverting the display, either just in the live image (with final images being normal), or on both the live and final images.
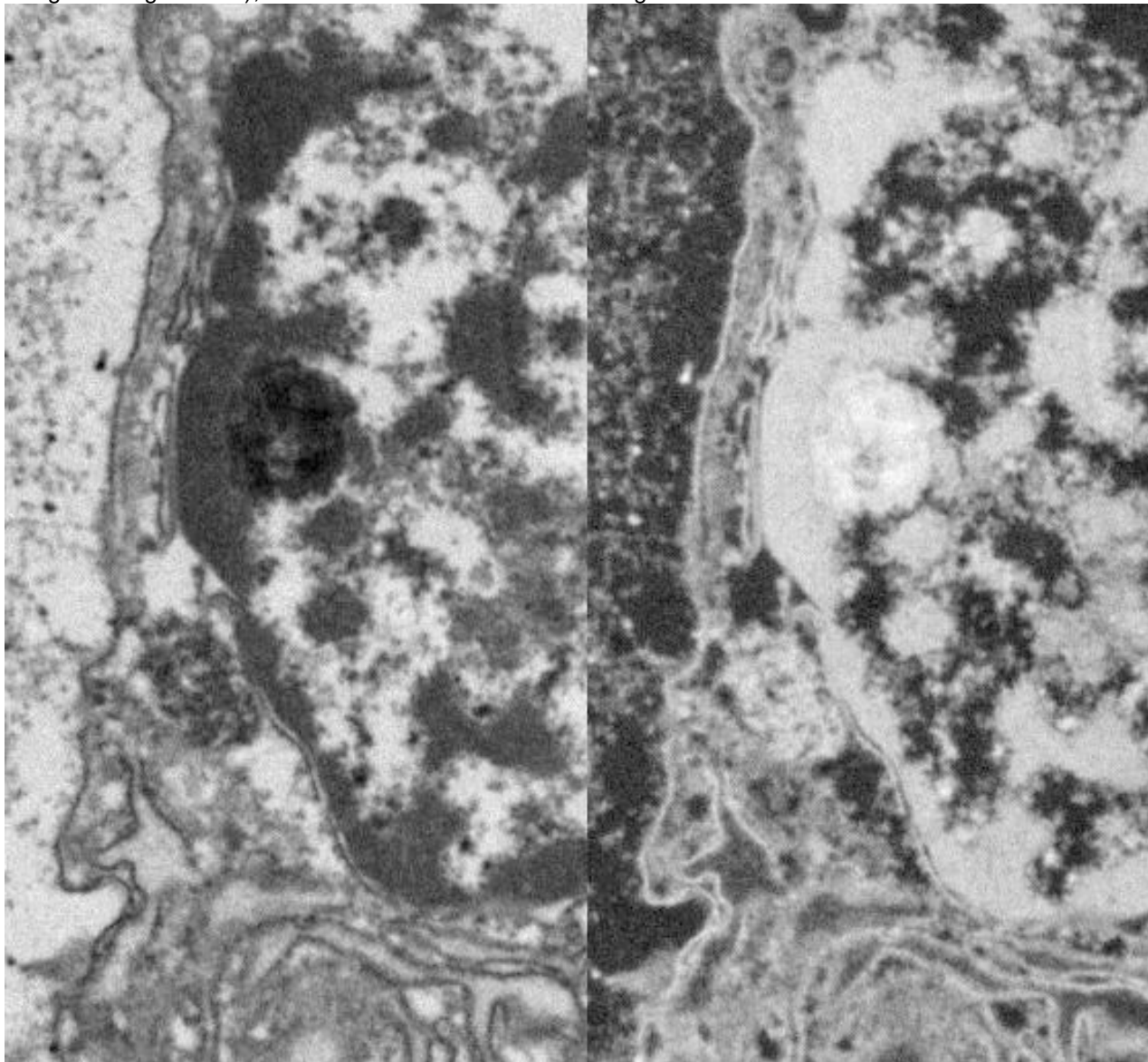
To invert contrast click the "*Display*" button on the upper menu and, on the drop down menu, click "*Select Contrast Inversion*".
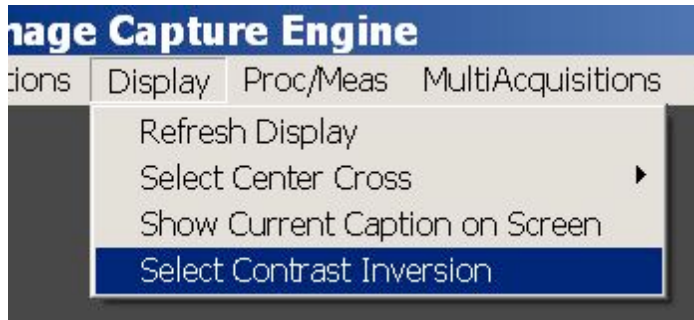
The contrast options window allows inverting contrast on live image only, live and final images, or neither.

After contrast is changed the buttons become buttons for returning to normal contrast.
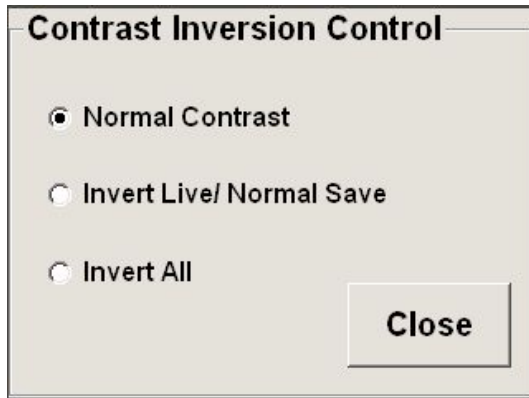
Center Cross

The Display menu provides a number of alternatives for showing a white cross over the center of the image. One can show the cross on the Live image only, on all final images or on just this final image.
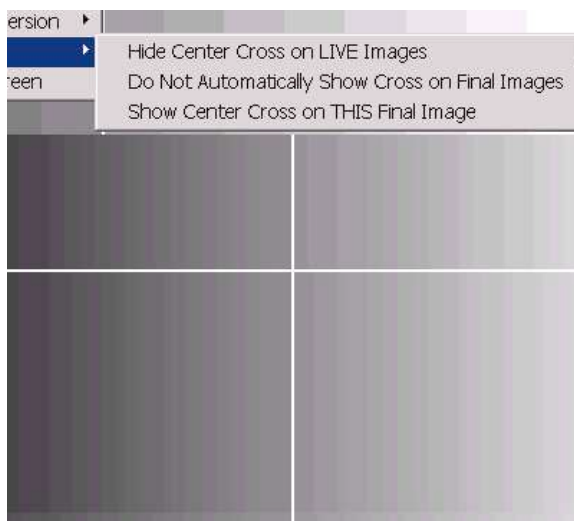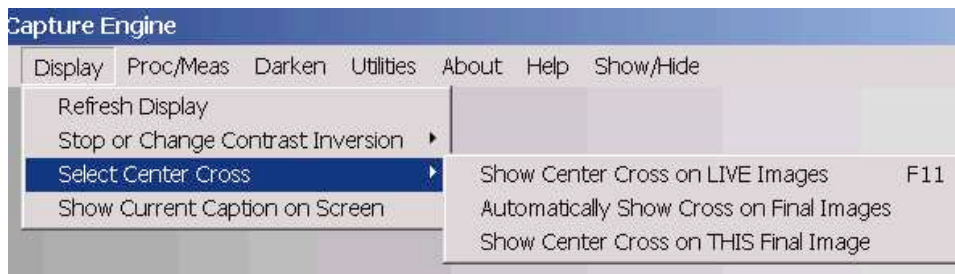
After a center cross option is activated, the clicked button becomes a switch for removing the cross.

Show Captions

  The last option in the Display menu give the user the option of showing, in the AMT display, the captions, Mag, HV, scale bar, and other information that is saved with an image, . This is the readout that appears at the bottom of an image when it is viewed in PhotoImpact or another image processor. To use this options one first needs to be displaying an image that has been saved. When an image is opened in a different image processor, such as PhotoImpact, this information appears underneath the image.

Undo Graphics

This option is under the "*Proc/Meas*" menu item. After you add graphics to a final image by measuring, drawing grid lines or creating a text box, clicking "*Undo Graphics*" will remove them to restore the original image.
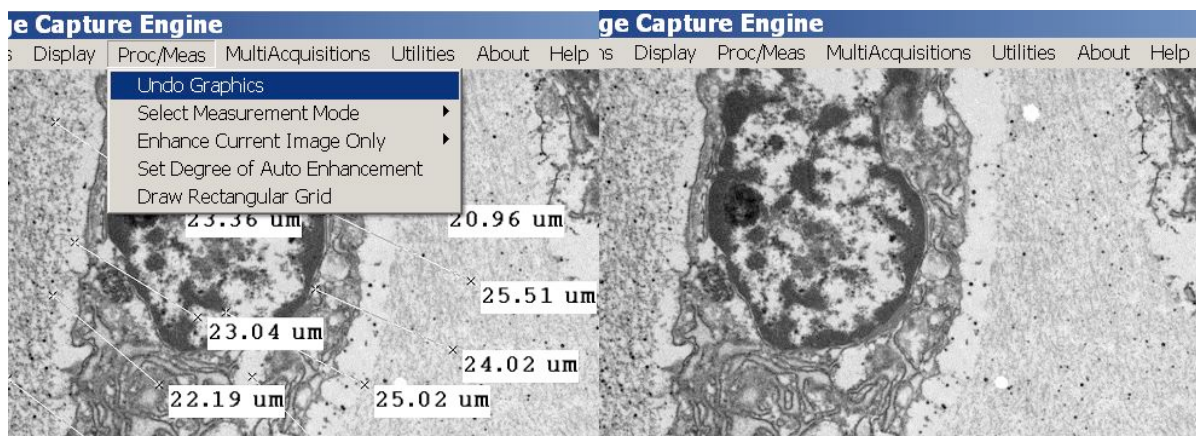
Draw Rectangular Grid

Having a regular grid on your image can facilitate counting or identifying features. To draw one click "*Proc/Meas*" and select *"Draw Rectangular Grid"*.
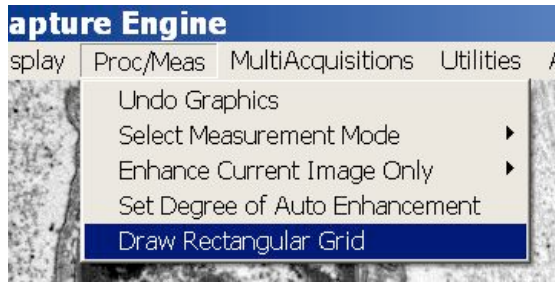
A window opens where you select the number of horizontal division for the grid.
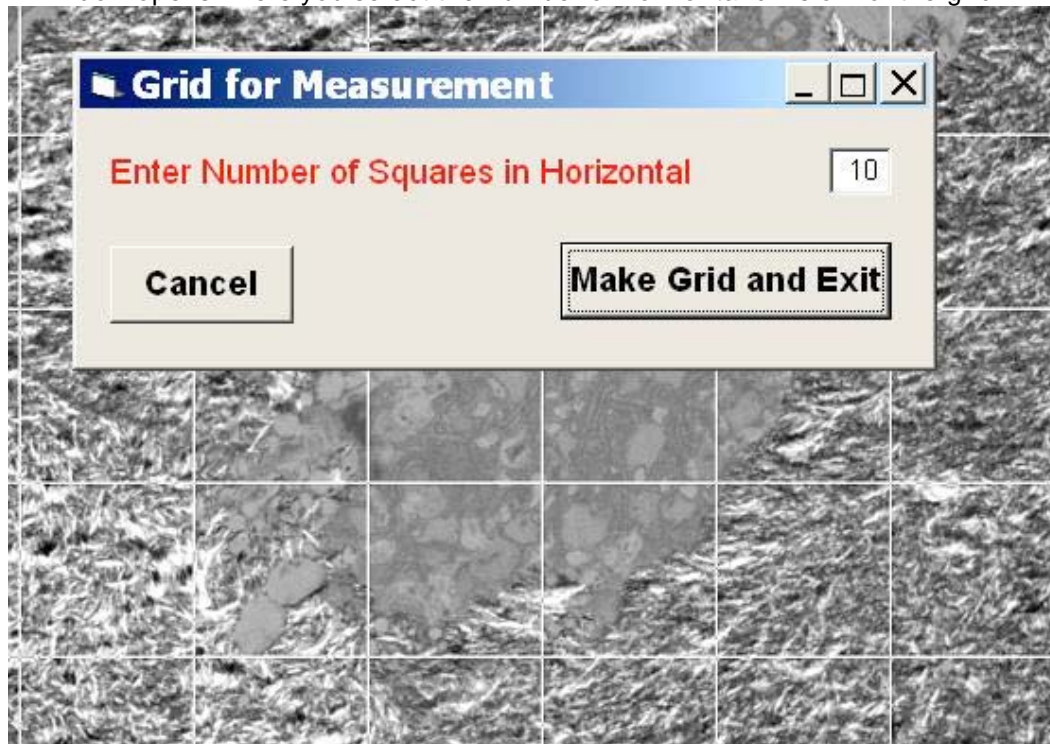
Darken

The "*Darken*" menu item temporarily darkens the entire monitor. It might be used while collecting a dark image, for instance. To bring back the display just click anywhere on the monitor.

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Show/Hide Options

Near the right of the menu bar is the button for the "**Show/Hide**" options. With these options you can make visible or invisible the Live Scale Bar and Control Panel, or change the size of the Mag Display.
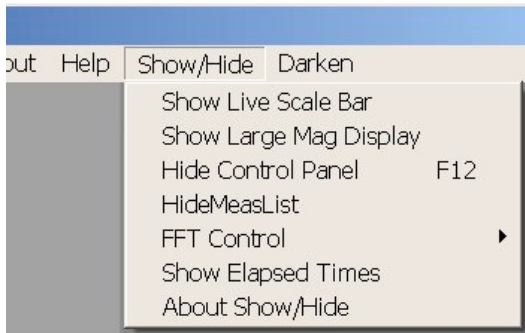
Contents

    Show/Hide Control Panel
    Show/Hide Large Mag Display
    Show/Hide Live Scale Bar
    Other Show/Hide Options

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Show/Hide Control Panel

One option under "**Show/Hide"** is for the Control Panel on the right and left side of the image. Hiding it might give you a more uncluttered view of the image.
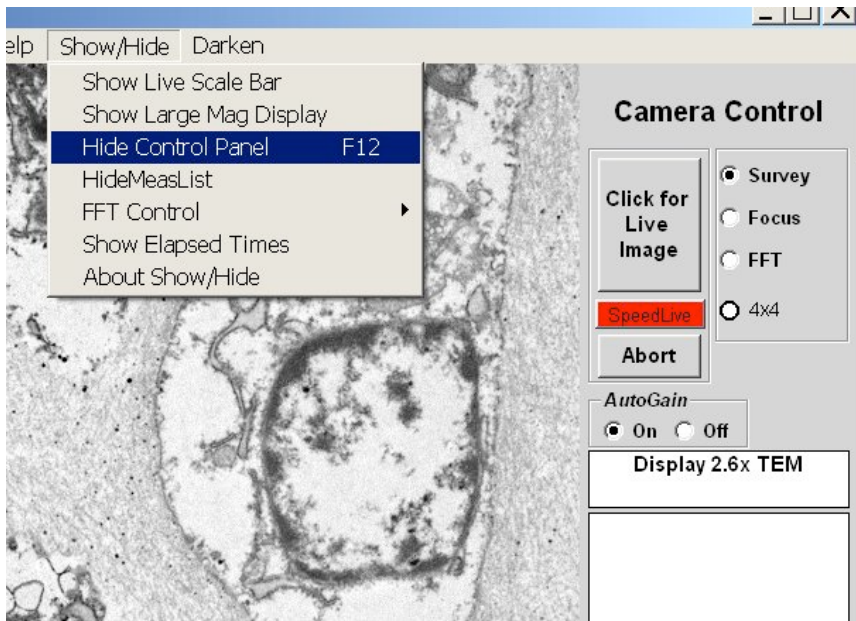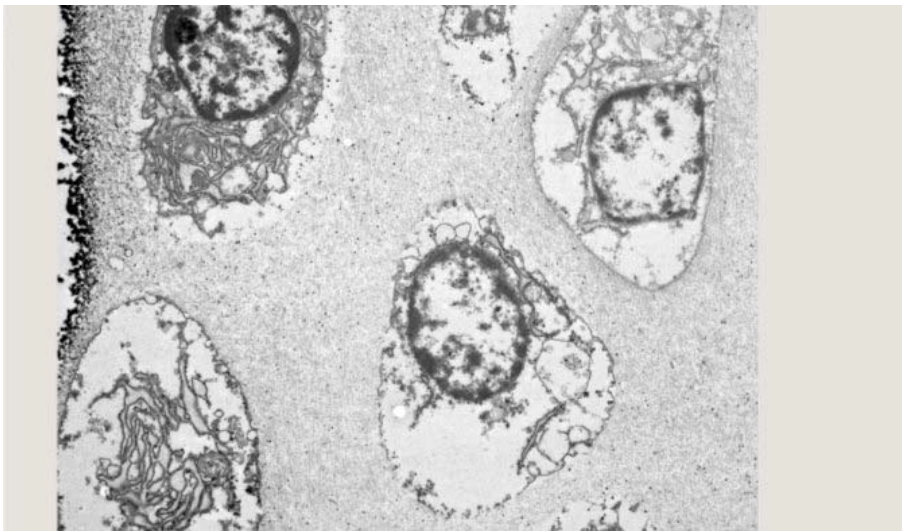
Image with the Control Panels gone

Show/Hide Large Mag Display
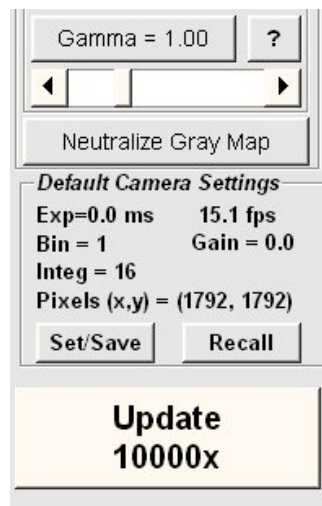
By clicking this item one can show or hide the large mag readout at the lower right part of the display.

Show/Hide Live Scale Bar

A scale bar can be displayed in live images. This scale bar is not saved with the image. It is for use in live or final images, but only in the AMT display. If you have TEM communications and continuous live mag updating, the scale bar will automatically change size or dimension when you change mag on the TEM. Otherwise it will change size after you manually enter the mag with a saved image.

The Scale bar normally appears in the lower left corner of the image, but it can be moved anywhere by right clicking a location. When the Live Scale Bar is showing, the menu item become "Hide Live Scale Bar".

Other Show/Hide Options

"**(Show/)HideMeasList**" controls the Measurements window, which can get hidden behind the AMT display during measurement. "**FFT Control**" has options for Show/Hide and size of the FFT window. There are also options to show image collection and saving times, and information about the Show/Hide feature.

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Preferences Display Items

   In File -> Preferences there are a number of choices to be made for the default display. These include:

Setting the (cursor) crosshair size
Making the default for live imaging "Speed Live" or "Quality Live".
For systems with communications, choosing to update the mag reading continuously or just with final images collection.
Setting a default path to use when images are save using "File -> SaveAs".
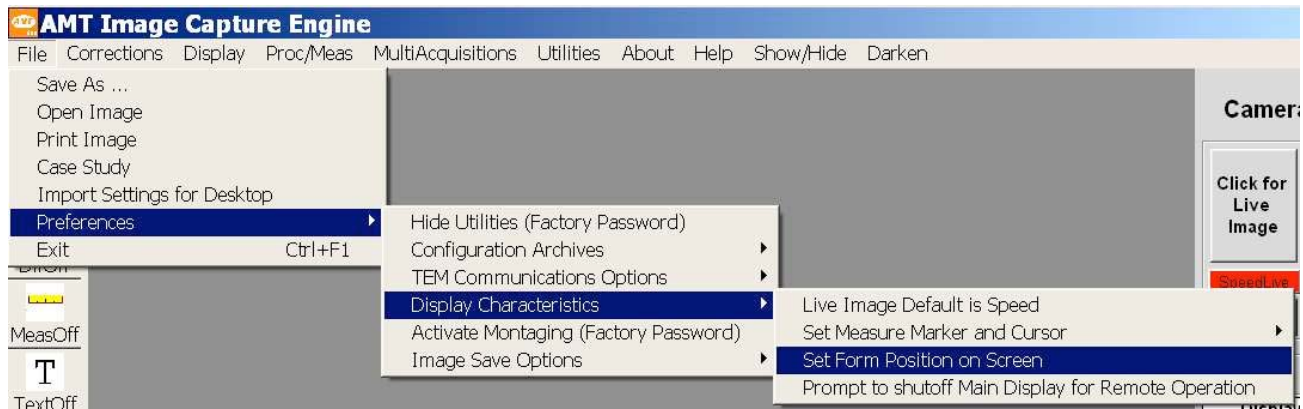
Contents

   Speed Live or Quality Live as Default
   Set Measurement Marker and Cursor
   Continuous Mag Read

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Speed Live Or Quality Live As Default

   When you open and use AMT it will automatically start imaging with either "**Speed Live"** and "**Quality Live**". Speed Live is faster, but Quality live averages together several frames in live mode to average out noise. Quality Live often gives a prettier live image, but the tradeoff is that imaging runs a little slower. You can toggle between them with the "**Speed/Quality Live**" button The choice you make in Preferences is which one the program open with.
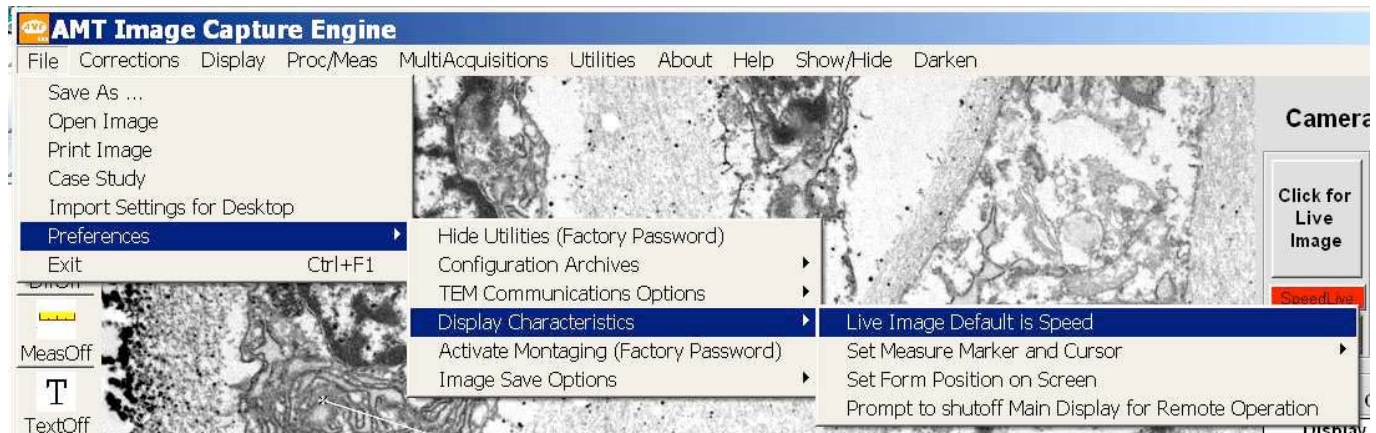
Here's where you would change the speed during live imaging:

Set Measurement Marker And Cursor

The **Measurement Cursor** surrounds the point which the mouse moves to points to measure the distance between them. The point can be surrounded by a circle or by crossed by crosshairs.
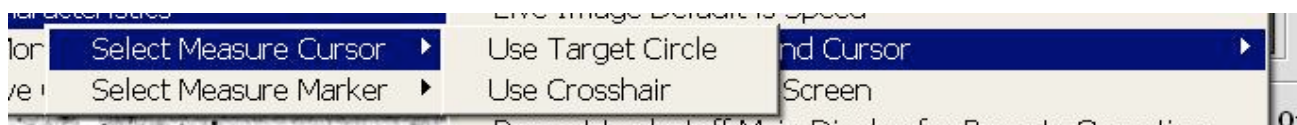
The **Measurement Marker** marks the endpoints of a line segment after it has been measured. The Marker can be Small, Medium or Large.
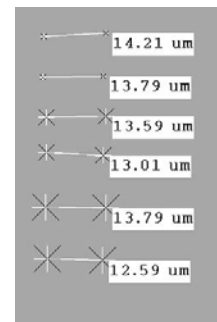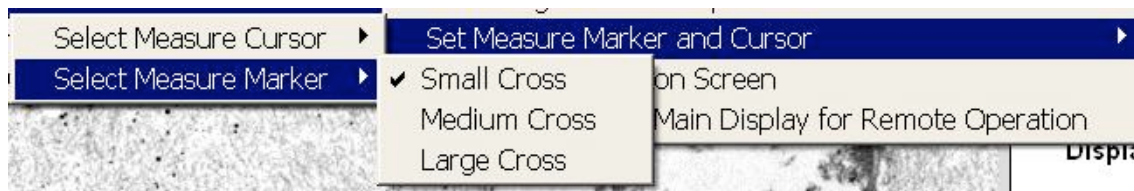
Continuous Mag Read

"*Preferences -> TEM Communications Options -> Enable/Disable Continuous Mag Read*" turns on and off the continuous live update of the mag display during live imaging. If it is off, in scopes with communications, mag will be updated when a final image is collected. The reason for providing the choice is that on some scopes the live imaging display is smoother without continuous mag updates.
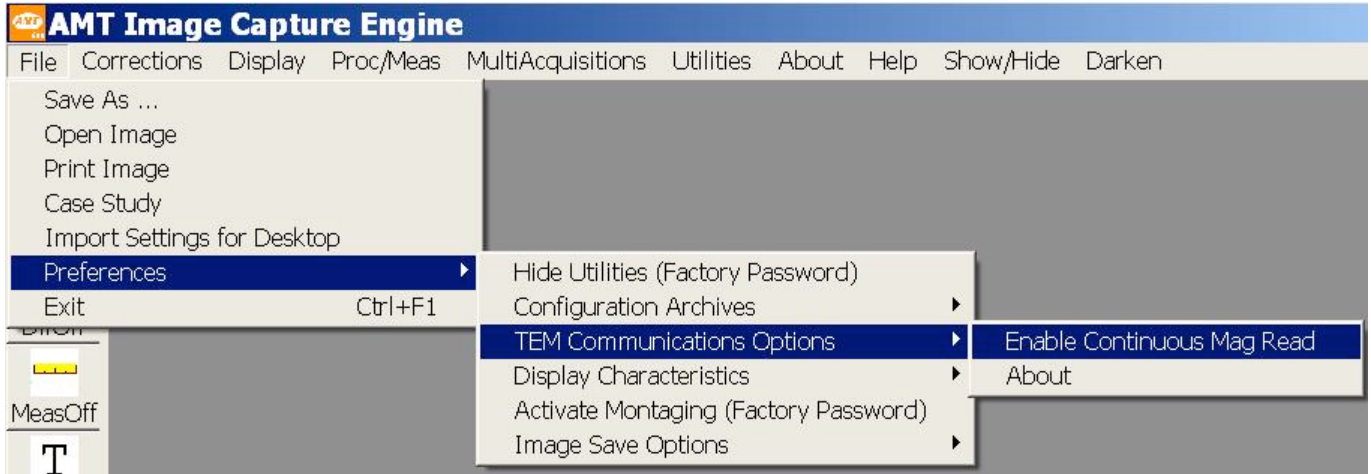
Below is the mag display, which gets updated.

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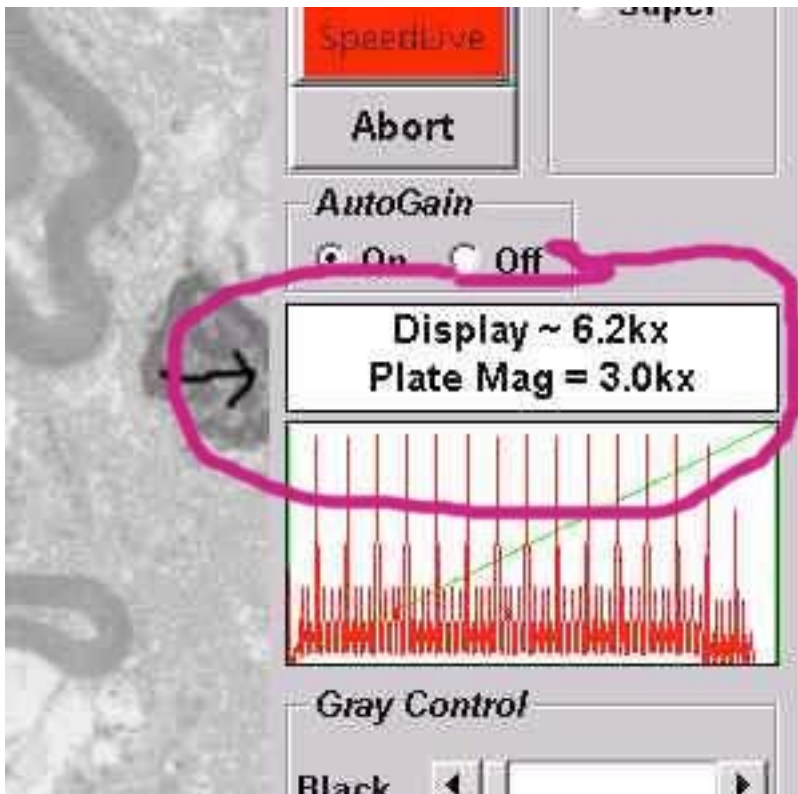
Authenticating Images

When the "Authenticate" mode is activated the camera software takes extra steps to make sure you can verify that your saved images are original and haven't been tampered with. This feature was designed to help labs comply with federal regulations (Code of Federal Regulations (CFR) Vol. 21 Part 11), which mandate that pathology images used for data generation are raw data and must be authenticated and archived.

When in Authentication mode certain features are activated and some features are deactivated.

Features Activated

- Images are Saved as "Read Only"
- An Authentication Marker is added to images. This marker is not visible and is lost completely if the image is saved in an image processor. In addition, this marker is a function of the image. Changes in the image will modify the marker thus flaging the alteration.
- A password is required to activate the application.

Features De-Activated

- Saved As is deactivated. Images are saved only through Case Study. This way images are sequentially numbered which aids in archival accounting of images.
- Saving images in the jpg format (a lossy compression based format) is disabled. Only tiff format is supported.
- Saving measurement lists in text format is disabled.

Ask your system administrator to activate the Authentication mode and assign user names and passwords.

Verifying the Authenticity Of Images

You can check the authenticity of stored images in the AMT program. You'll find the **"Verify Authentication"** button under "File" on the top menu.
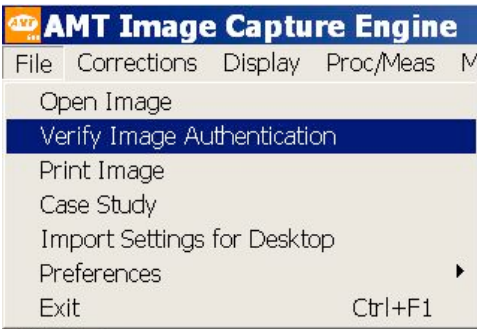
When you click the item the **AMT Authentication Checker** window will open. In the left pane of the window, locate the folder containing the images you want to verify. Double click the folder and the filenames of its images will be displayed in the second ("All Tiff Images") pane.

To verify the images click the "**Check Images**" button on the lower left of the form. The images will be sorted into Original Images and Other Images (those that no longer have the originality marker). In this example image #3 doesn't pass the test.

AMT Authentication Checker

c: [Local Disk] ▾	All Tiff Image Files	Original Images	Other Images
C:\ Images **BRAIN#587398**	BRAIN#587398_001 .tif BRAIN#587398_002 .tif BRAIN#587398_003 .tif BRAIN#587398_004 .tif	BRAIN#587398_001 .tif BRAIN#587398_002 .tif BRAIN#587398_004 .tif	BRAIN#587398_003 .tif

Check Images	Exit	Print Original/Other	Save Original/Other

To View An Image Double Click on the File Name

Double clicking an image filename in either the Original Images or Other Images pane will open that image in AMT. The two buttons at the lower right of the form are for saving the sort, either by printing it out or as a text file on your computer. This is what the saved text file looks like.

Brain#587398.txt - Notepad

File   Edit   Format   Help

```
Folder = C:\Images\BRAIN#587398
Original Images
BRAIN#587398_001 .tif
BRAIN#587398_002 .tif
BRAIN#587398_004 .tif

Folder = C:\Images\BRAIN#587398
Non-Original Images
BRAIN#587398_003 .tif
```

****

****
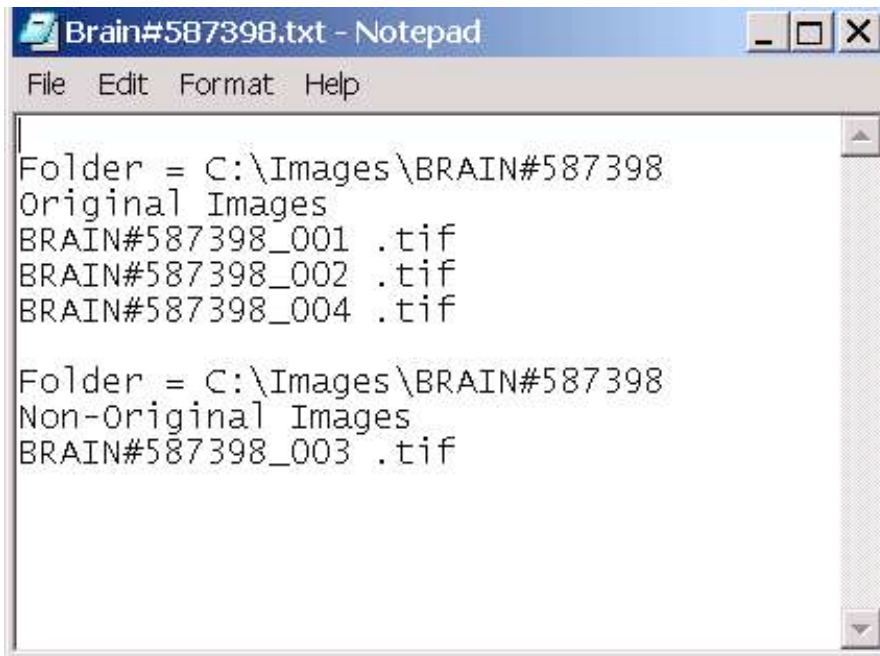
Database

<u>AMT Database and Meta-data</u> AMT maintains a database of the meta-information associated with images that are aquired using the Capture Engine. This is information such as the magnification of the image, TEM mode, the accelerating voltage of the TEM, the microscopist, the time and date the image was acquired, scaling information, the laboratory name, as well as two user supplied caption lines and up to 6 keywords.

This information is stored in several ways to provide flexability for the user and permit recovery of the information if it is inadvertently deleted. The information is written into an **rtf**(Windows WordPad) file, a predefined Windows Access database, the tiff header, and optionally into a logo band directly on the image.

<u>Contents</u>

AMT History .rtf File
Access Database File
Tiff Header
Logo Band

****

The AMTHistory RTF File

Metadata is written into the file C:\AMTHistory\AMTImagingDataBase1.rtf each time an image is saved. This is simply a text file which saves each field of information on the TEM Info Form that requests/displays the metadata just before an image is saved.

On systems with TEMs that support communication with external applications the Capture Engine is able to obtain much of the information automatically (Grey background). On TEMs that do not support communication the user will need to enter (if desired) the Magnification, accelerating voltage, TEM mode, xy position, and tilt. Some fields - caption lines and keywords always depend on user input(White Background).

Items that are checked on the information form will be written into the image caption band. However, all the information is written into the rtf database. This is simply a tab delimited table with the following fields:

FileName
Microscopist
Date_And_Time
TEM_Magnification
CaptionLine1
CaptionLine2
Pixel_Calibration
X_Position
Y_Position
Tilt
Accel._Voltage(kV)
TEM_Mode
TEM_Facility
Account
KeyWord1
Keyword2
KeyWord3
Keyword4
KeyWord5
Keyword6

If the field is empty it is left blank in the rtf file.

Importing the RTF into a Spread Sheet

An rtf file can be opened by Windows word Pad and a tab delimited table can easily be imported into a spreadsheet program. As an example, to import into Microsoft Excel:

Select a cell where you want to place the data. Go to the Data Menu and select Import:
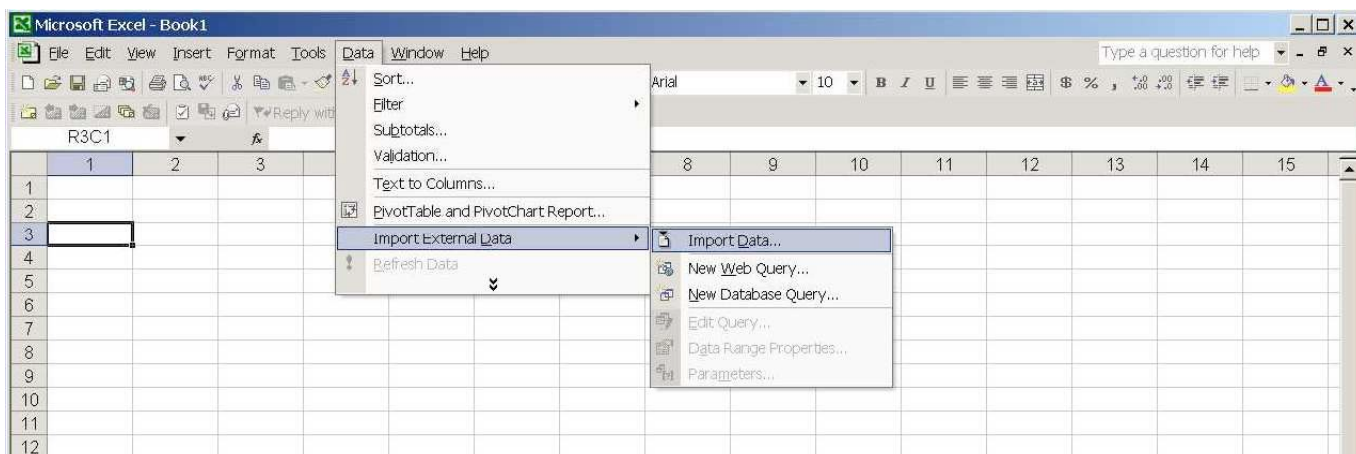
Confirm the information that the import wizard presents.
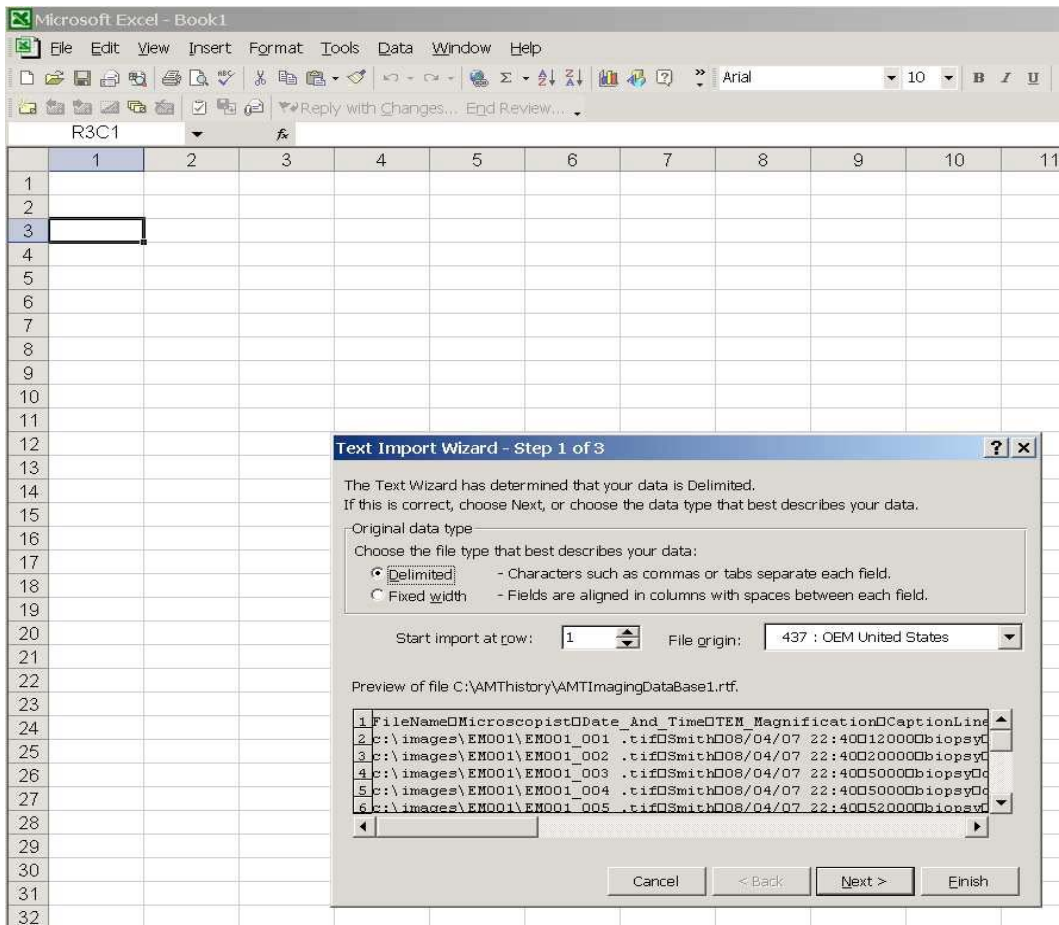
The table is **tab** delimited
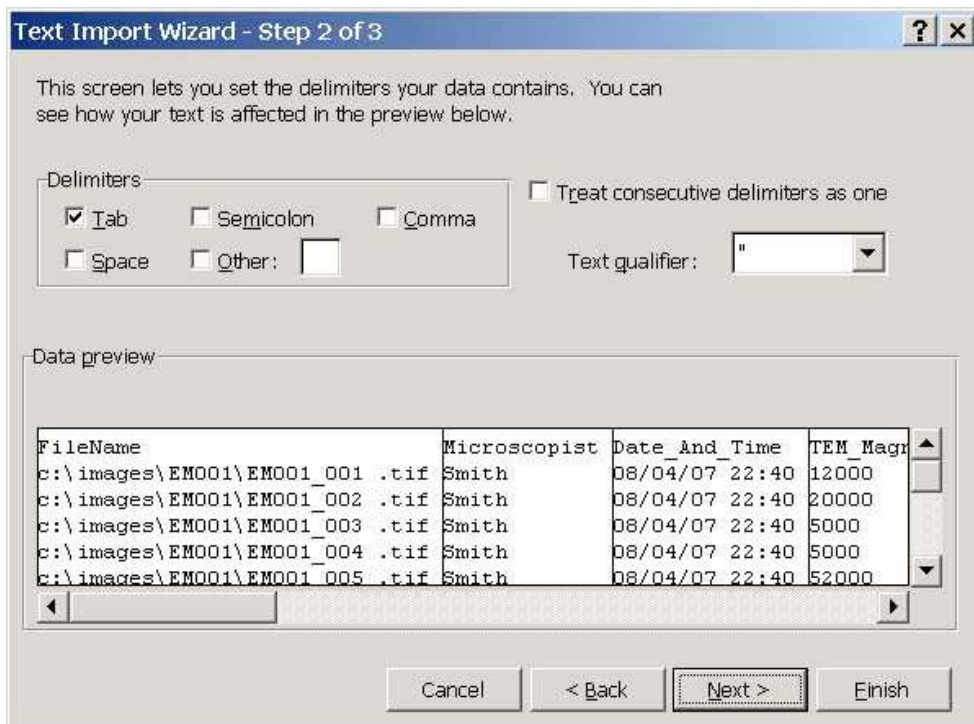
Chose the data format for the spread sheet
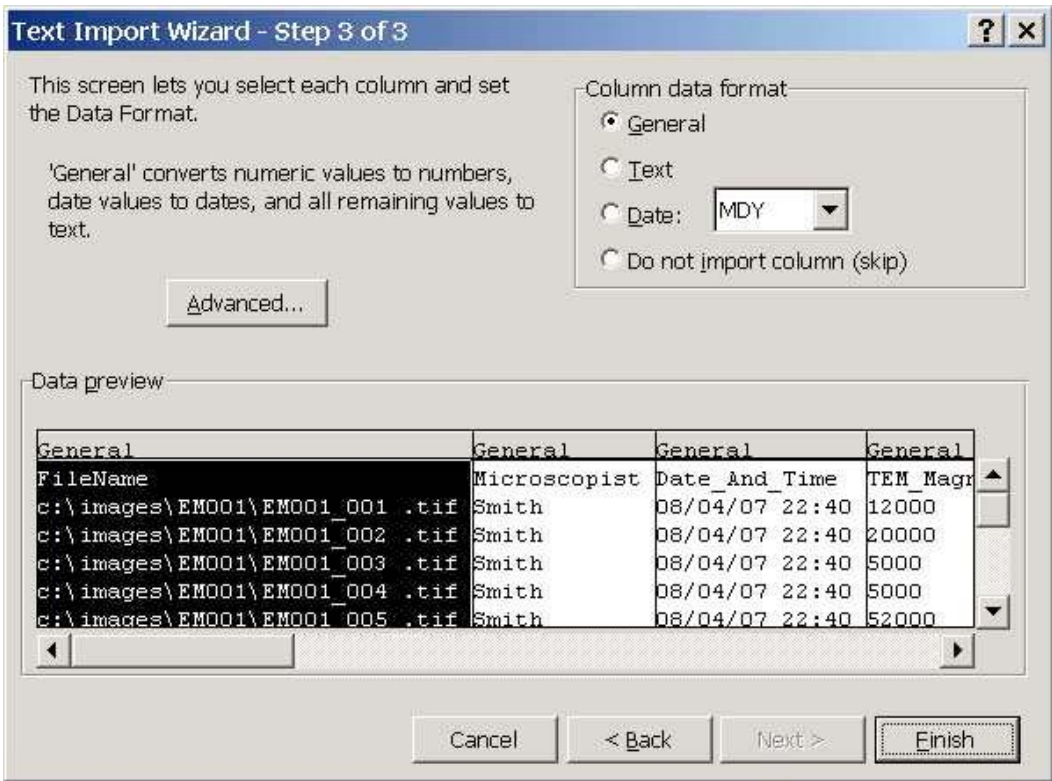
Confirm the location you selected above.
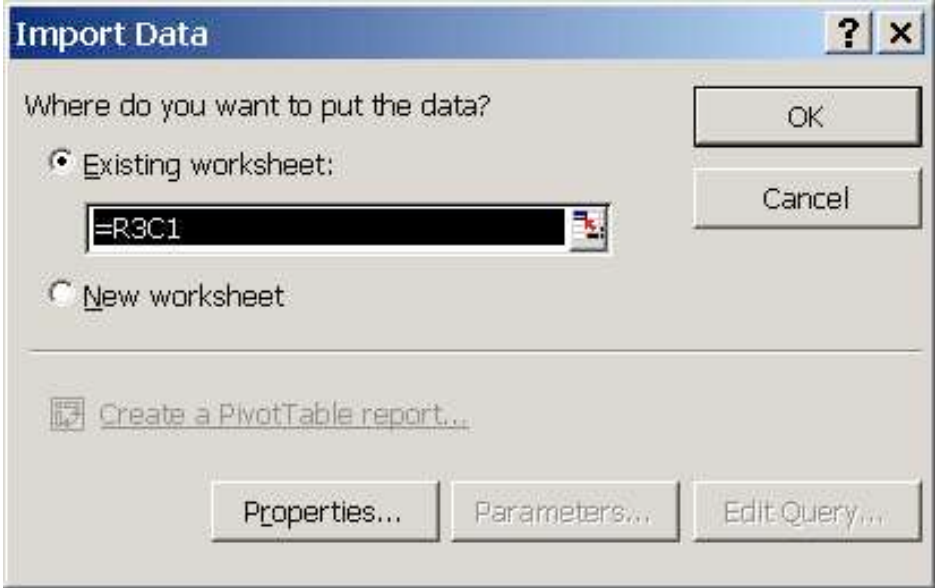
Other speadsheet programs are likely very similar.

Access Database

If Microsoft Access is installed on the acquisition system the information is entered automatically into an Access Database file (mdb). The Access database is a Microsoft standard file and can be moved to an external work station and opened with Access 2007.

Information In The Database

Each image saved enters information into the fields shown in Table 1. The database has 6 keywords reserved for use by the microscopist. The principle investigator or lead microscopist for a project should define these categories and suggest possible entries. For example, keyword 1 can be designated as the sample type. In a biological setting this might tissue type, keyword 2 could be a common feature or organelle, keyword 3 could be a suspected pathology. In a materials setting the first keyword might be the sample material, project, or experiment. The second might be a description of features in the image. Another keyword might be a patient designator, or the source of the sample.   Again the keywords should be defined by the Principal Investigator to facilitate recalling information about important groups of images.

Using the Database

Database filtering aids in finding examples of previous work with a particular sample, feature, account, microscopist, date, or technique. For example a facility manager might be interested in how many images were taken for a particular project or account.

Filter by Account

Open the full database (See for example Figure 1).
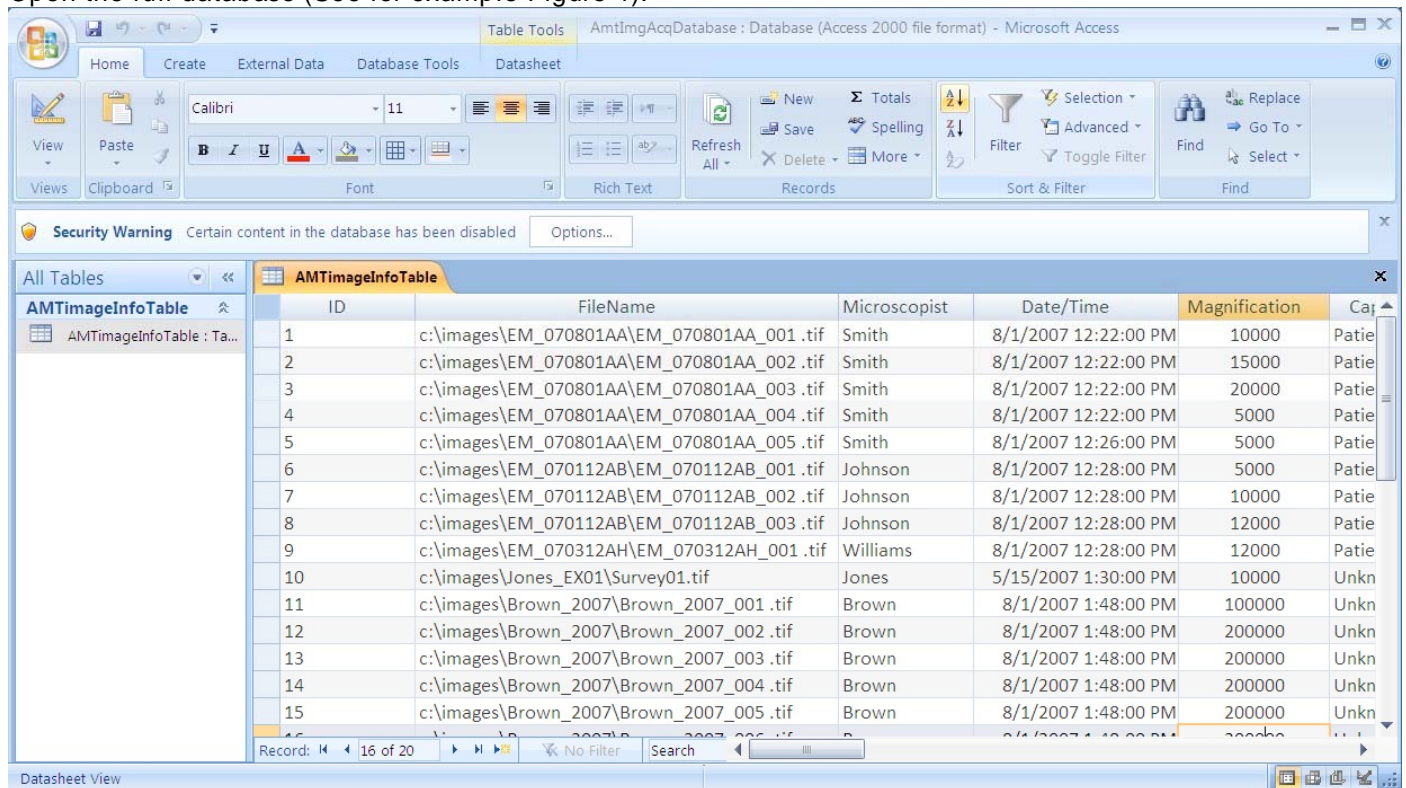
Figure1 Select the Home tab and the account column and then click on the filter icon (funnel) in the filter and sort section. This will display a panel listing all the accounts that have been entered (Figure 2).

Leave only the account of interest checked and then click OK. The display will collapse to show only those entries associated with the specified account "XYZ-001" (Figure 3).
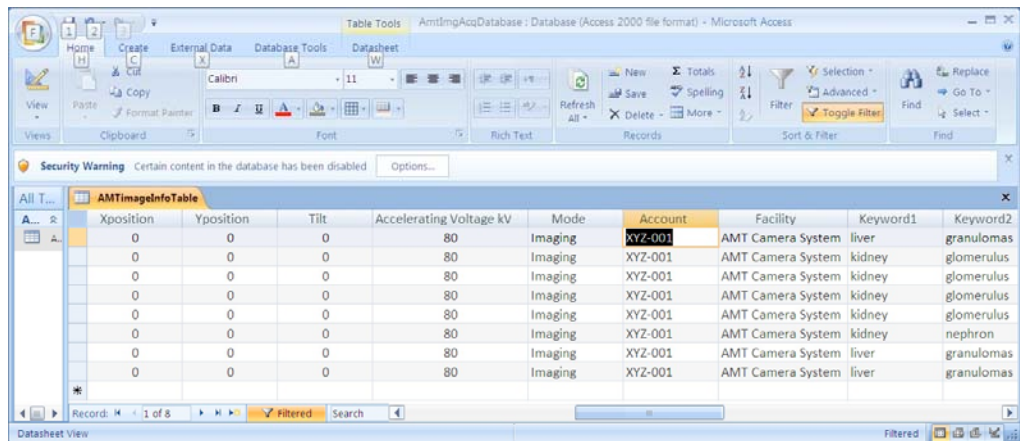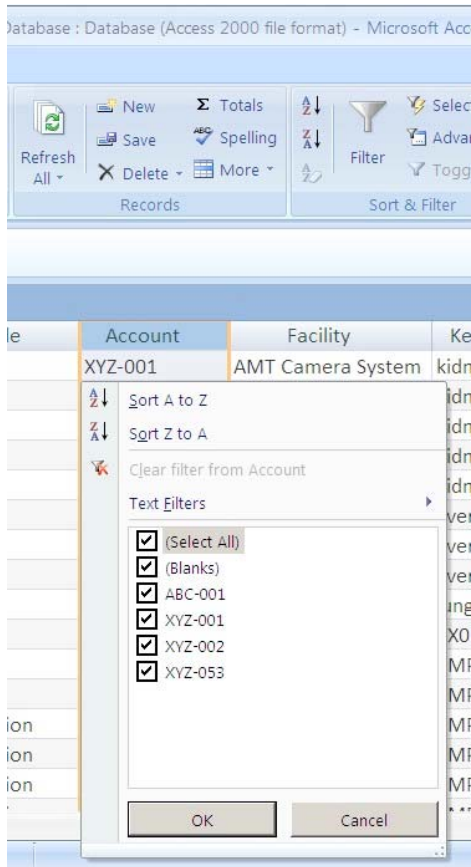
Scrolling left and right one can see the microscopists, who did the work, when the work was done, where images were stored, as well as captions that were entered on the images and keywords that characterize the sample and observations.

Filter by Keyword

The keywords allow one to extract from the database only those information records that relate to a particular field. In this case, for example, keyword 4 has been used to store a patient number. One can extract records related to only a particular patient.
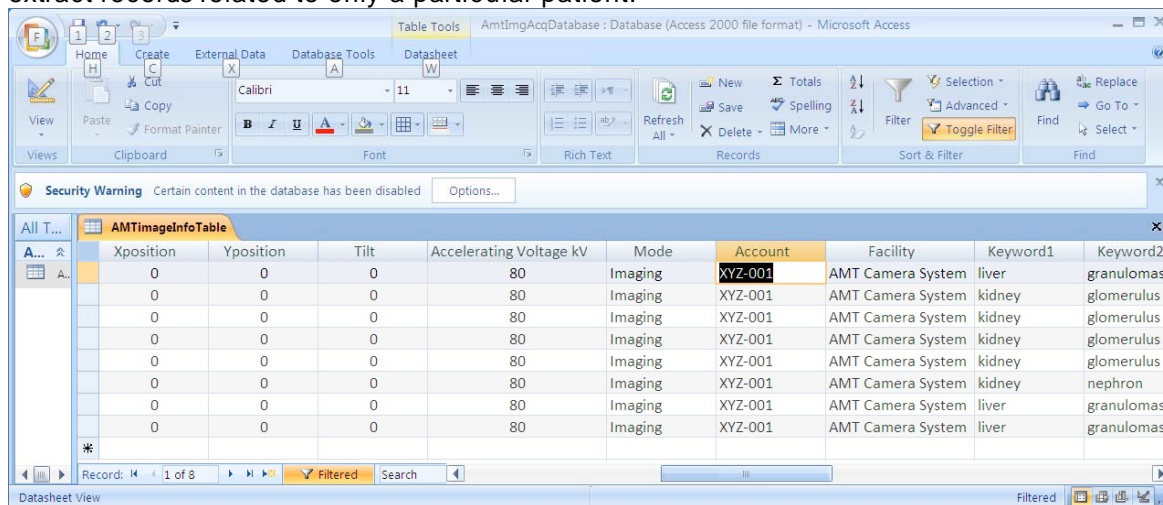
Report Generation

Tabular representation of the database can be very informative, but may be lacking esthetically. This is especially true when some of the fields are not critical (or are blank). Microsoft Access allows one to design a report form and display the information in the underlying database in a fashion that is more pleasing. These reports can be created and modified by the user to suit differing needs. Filtering operations are still available in this format.

A Very Brief Tutorial

This is necessarily a very brief tutorial. For more information there are some references at the end of this section.

Open the database, select the Create tab, and then select the Report Design icon. This will generate a blank report. Then click the Add Existing Fields Icon. This will display the fields in the AMTImgInfoTable in a panel to the right of the blank report form (Figure 5). Fields can now be selected and dragged from the panel on the right to the report form. These fields and their labels can be positioned and sized as desired. There are a variety of tools on the Design, Arrange, and Page Setup tabs that can be used to generate a report layout. View icon on the left allows you to switch between design, layout, report view, and print preview displays. To save the report layout click on the disk icon in the top left corner and supply an appropriate name. Figure 6 shows a simple report.

It is important to note that the filtering operations can still be applied from a report. Select the field of interest and then click on the filter icon in the tool bar. Access will display possible field entries (Figure 6) select those desired and click OK. Only records matching the filter requirements will be displayed. At this point additional filtering may be done or the report can be printed.
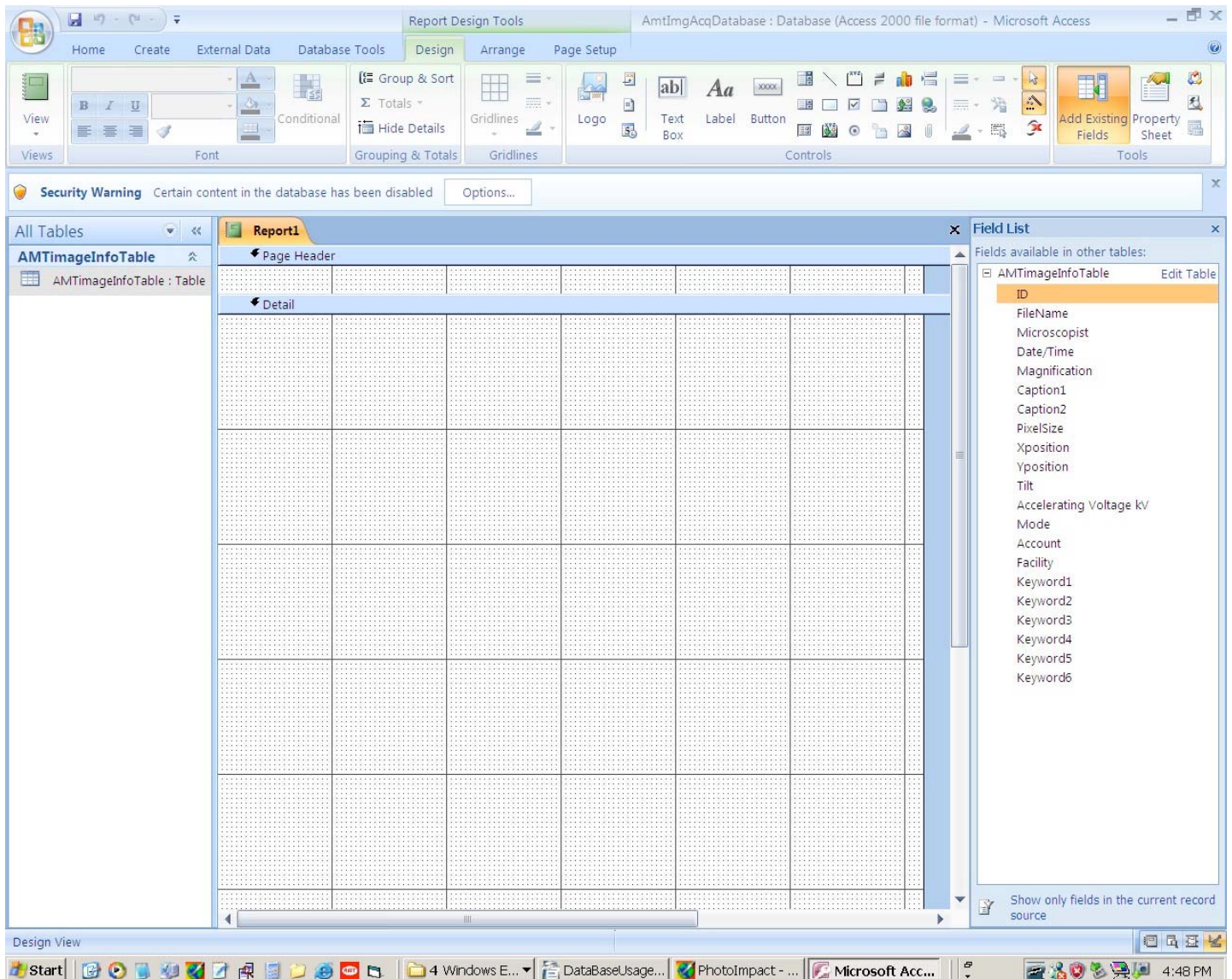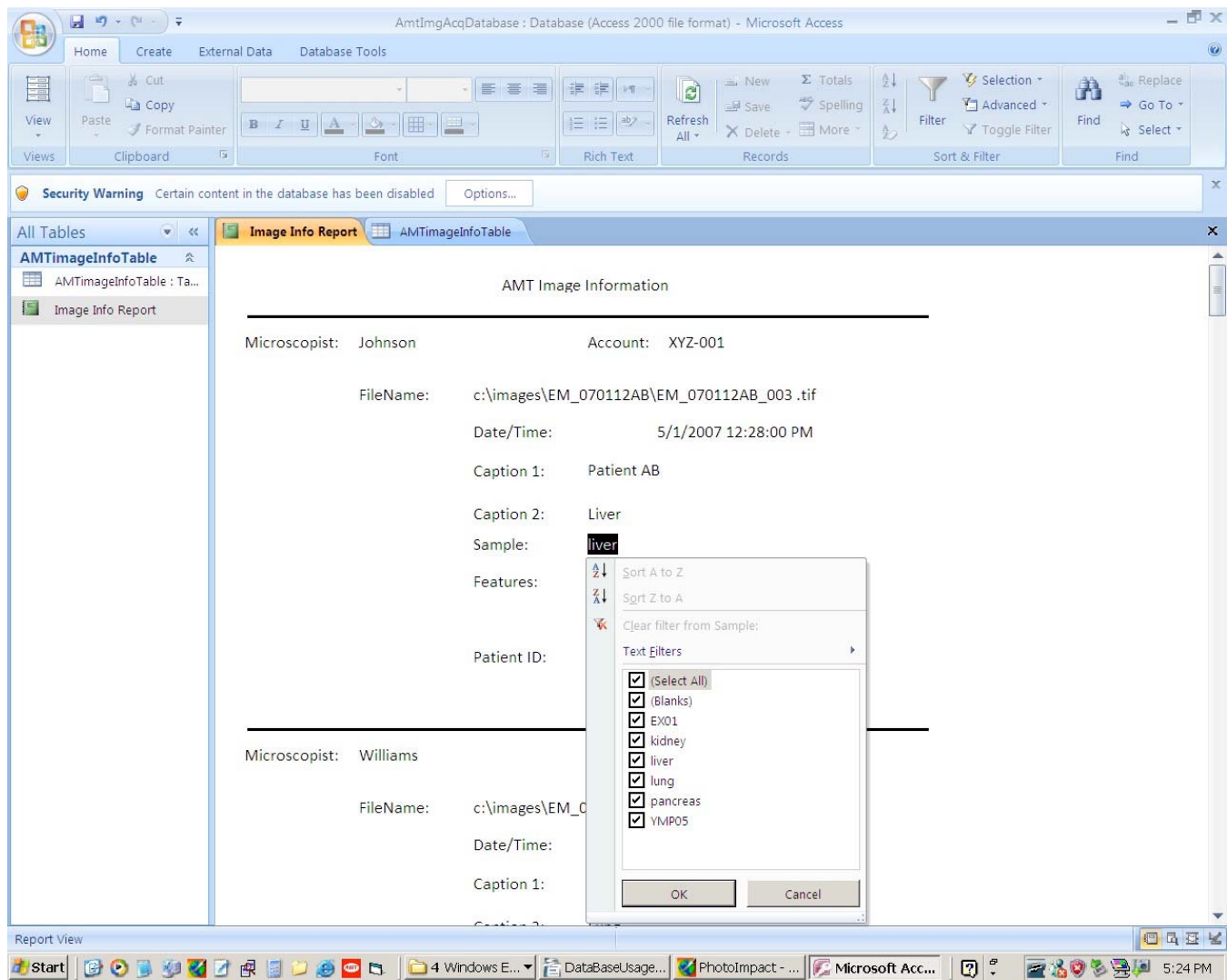
Figure 6

References

This is an extremely brief introduction meant to get you started. Many books have been written on the use of Microsoft Access. See for example: Microsoft Office Access 2003 Inside Out by John L. Viescas Microsoft Access 2003 Forms, Reports, and Queries (Business Solutions) by Paul McFedries Access 2003 Bible by Cary N. Prague, Michael R. Irwin, and Jennifer Reardon

Appendix

AMT Access Database Fields		
Field Name	**Description**	
ID	A sequential number identifying the image.	
File Name	The full path name where the image is originally saved.	
Microscopist	The name of the microscopist.	
Date/Time	The date and time the image is collected.	
Magnification	The TEM Magnification setting.	
Caption 1	A text string that the user can enter as a label on the image caption band.	
Caption 2	A second text string used as an image label.	

Pixel Size	The size of a pixel at the current magnification in microns/pixel or nm/pixel.	
X Position	The x-position of the sample as read from the microscope.	
Y Position	The corresponding y-position of the sample as read from the microscope.	
Tilt	The sample tilt.	
Accelerating Voltage (kV)	The accelerating voltage of the electron microscope in kV.	
Mode	The mode of the microscope. This is usually Imaging or diffraction.	
Account	The account under which the work is being done.	
Facility	The microscope facility name.	
Keyword 1	See main text.	
Keyword 2		
Keyword 3		
Keyword 4		
Keyword 5		
Keyword 6		

Meta-data In the Tiff Header

The meta-data is also stored in the tiff header. Information from this location is used to scale images automatically when opening them in *ImageJ*. In addition it may be possible in some image processors to display this information for examination.
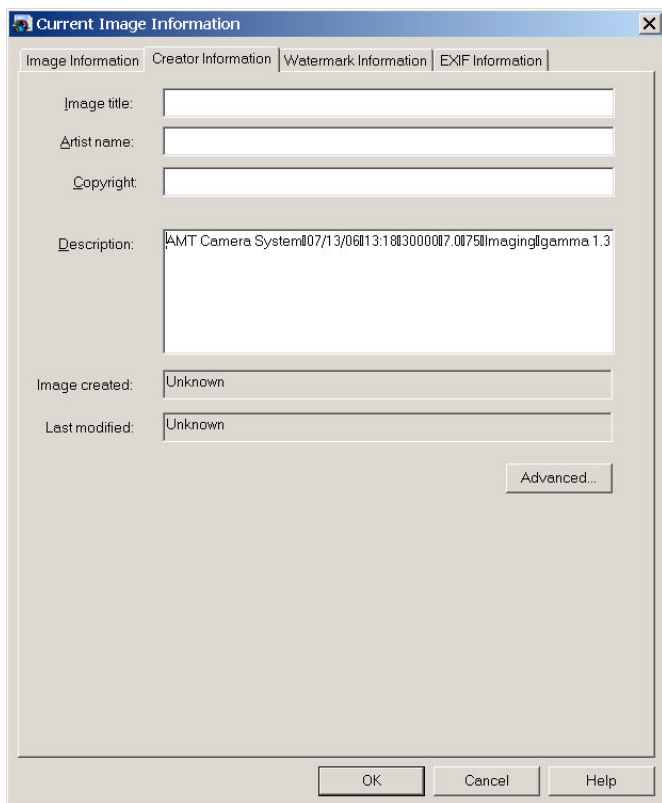
Corel Paintshop Pro Information Display

Some databases may also have the capability to extract this information for entry into database fields.

Fields in this tag are delimited by a Carriage Return and occur in the following order:
TEM_Facility
Date
Time
TEM_Magnification
Print Width
Accel._Voltage(kV)
TEM_Mode
CaptionLine1
CaptionLine2
Keyword1
Keyword2
X_Position
Y_Position
Tilt
Pixel_Calibration
Pixel_Calibration - this is a duplicate and will be phased out in a future version
Calibration Unit
AMT tag format version marker

AMT Logoband

Information in separate files may become separated from the image to which they refer. Not all image programs open and preserve the tiff information tag. If they do not then openning an image in the image processor and saving over the original image effectively strips away the metadata. To address these limitations we have introduced a new method for embedding this information directly into the image. The information is encoded into pixel intensity and then placed in a logo band at the bottom of the image.

To activate this method of storing the metadata be sure to check the **"Show Logo and Metadata"** checkbox on the TEMinfo Form.
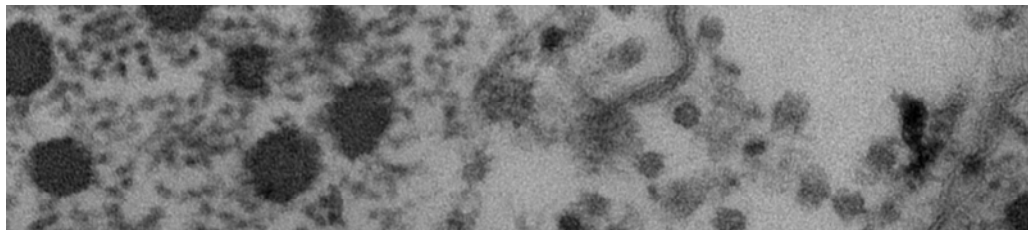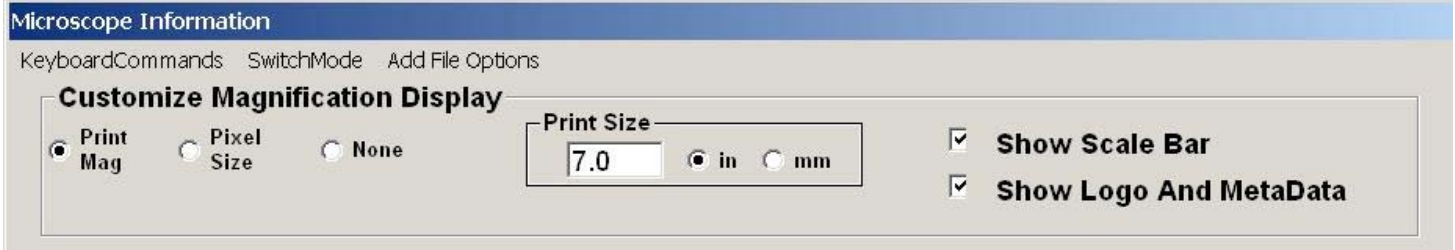
When the image is saved a band containing the AMT Logo and the metadata is added to the bottom of the image.

The information can be extracted from the image by selecting the Extract Metadata menu option.
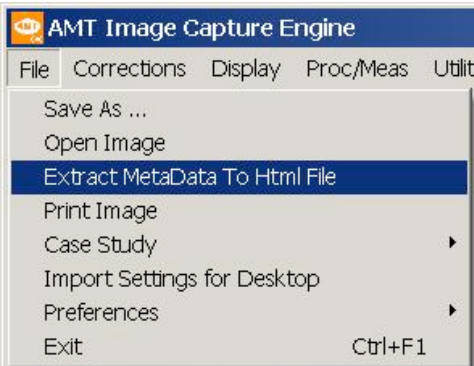
This reads the information and creates an html file in the same folder. This file can be opened by your browser to display the metadata.

****
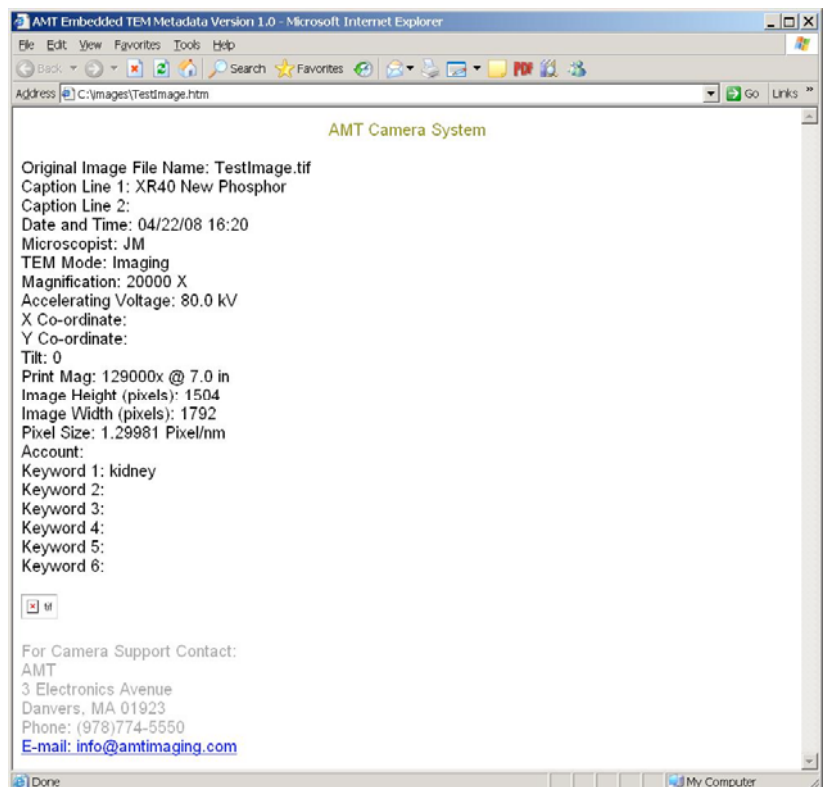
Montaging

AMT's montage package employs stage motion to scan an array of images. Thus it is capable of collecting low magnification montages. The program can be used with or without a motorized stage.

Contents

Collecting Images For A Montage
Collecting And Naming Conventions
Stitching With Panavue Image Assembler

****

Collecting Montages

1) Select the appropriate Montage Acquisition Mode from the Special Acquisitions menu.

In the **AutoStage** option, the computer controls the stage motion and image collection automatically after setup.
In the **User Controlled** option, the camera application guides the user through the montage process, but the user controls the stage.
If the TEM provides stage control, both options are enabled. If not, only User-Control is enabled.

2) Provide a name for the folder where montage images will be saved. You may select a previously existing folder in which case you will be asked if you want to delete the contents of that folder. Answer YES if you intend to overwrite everything otherwise NO.
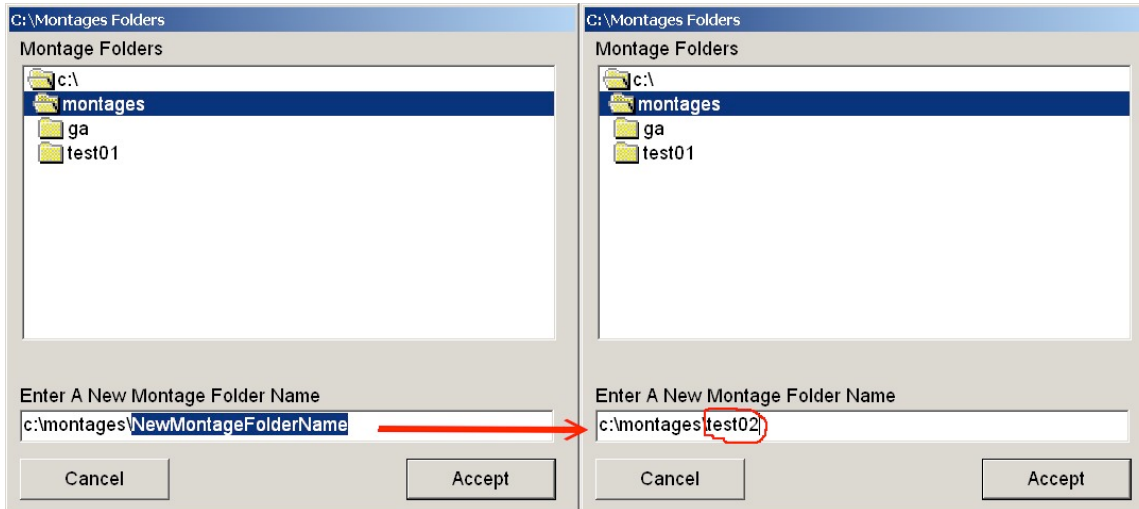
3) The montage control panel will appear.
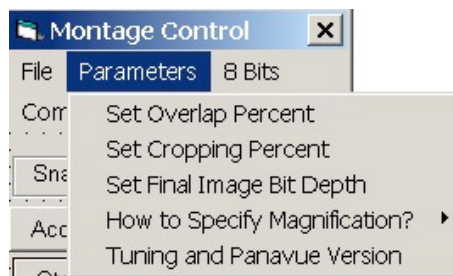
4) The image will go "live."   You should now set up the parameters for acquisition.

Typically **overlap** should be 20% for automatic stitching. Note also that

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mechanical stage drives have an inherent uncertainty or repeatability error associated with them. This variation depends on the microscope. At higher magnification this uncertainty is a greater fraction of the image field of view. Generally higher magnifications will require larger overlaps to compensate for stage variations.

15+% **cropping** is needed for side mounted cameras to reduce "S" distortion of the projector lens. But 0% cropping should work for bottom mounted cameras.

Set Final Image **Bit Depth** refers to the number of bits used to represent the intensity in a pixel. At present this value must be 8.
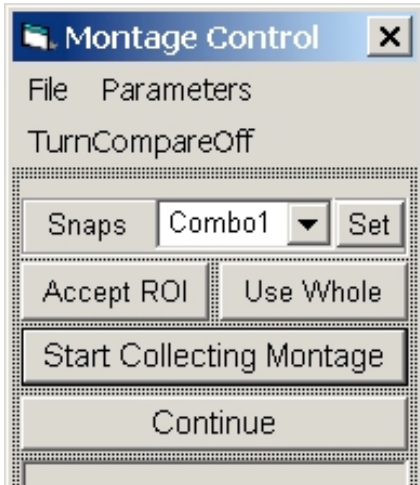
Choose the **mode** for setting the magnification. Select either **final magnification** OR the **number of snaps** (pictures) in the longer axis. The magnification or snap values are selected in the pull down menu immediately below.

Tuning and Panavue Version allows you to **enable/disable backlash** correction, specify a **Stage Drift Time** (the amount of time required for the stage to stop drifting after it has arrived at a programmed position.) and select the version of the Panavue Image Assembler that you are using.

5) Select the acquisition area for the montage by drawing a **ROI** (region of interest) and then clicking **Accept ROI** or alternatively click **Use Whole** to select the whole field of view.

Selecting a ROI.
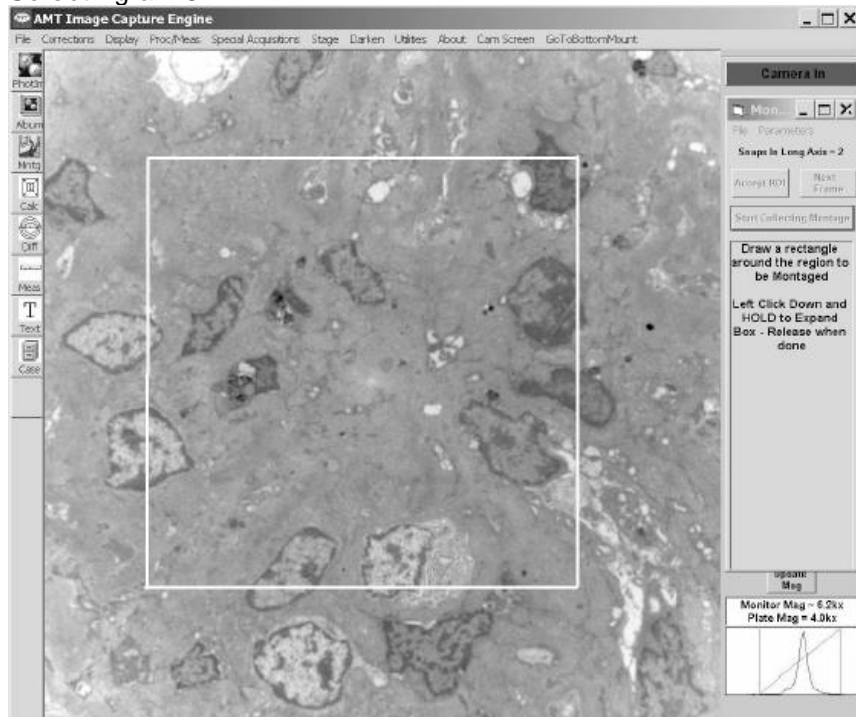
6) Once the area is selected the program will set the magnification (If the microscope remote command set supports external control of the magnification.) or request that you do so.
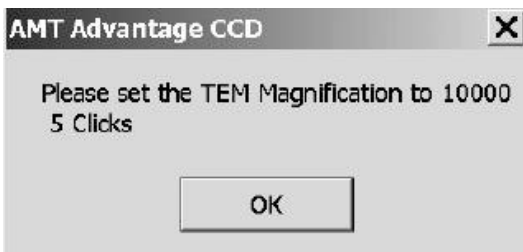
Set the TEM to the requested magnification, focus the TEM, and adjust the intensity. The intensity as observed on the histogram should be approximately centered.

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Click **Start Collecting Montage**. Status of the collection ia displayed in the panel on the right.

AutoStage Mode:
The program automatically moves the specimen to the appropriate positions and records images. Status messages will be displayed in the Montage Control Message window. When done a message box will announce that acquisition is complete. The images and a Panavue project file ("xia" file) will be stored in the folder specified in Step 2.

User Controlled Mode:
This is a computer assisted mode of operation, which prompts the user to move the specimen and indicate when to record a picture. Instructions are displayed on the montage control panel on the right.

Motion prompts refer to the direction of motion of the image - not the stage.
   A. Start by moving the lower right region of the intended montage to the center of the screen (i.e. move image up and left.) Then click **Next Frame**.
   B. After recording the image, the program will prompt you to move the image in a certain direction (left, right or down) and will freeze the expected overlap region. The overlap region is duplicated on one side of the live image. This region will flash occasionally to show the live image behind it or display in partial transparency the image behind it. Move the image in the direction of the duplicate and line them up.

There is also a movable guideline that can be used to assist following a specific point across the field. Clicking the mouse on the image moves this line.

C. When finished moving, click on **Next Frame** again. Then Step b) will repeat until finished.
D. When done a message box will announce that acquisition is complete. The images and a "xia" file will be stored in the folder specified in Step 2.

If you have Panavue Version 3.4 you will be asked if you wish to assemble the montage now (in the background) or save it for later assembly. If the montage is large you may wish to save and assemble later as stitching can take quuite a while. See **Stitching with Panavue Image Assembler** for an introduction to the use of a Panavue project.

Collection and Naming Conventions

Normally the AMT Capture Engine will generate a Panavue project file for the images it collects from a montage area. In some cases, for example a large irregular region, the user may wish to create a custom project to assemble the images. The following discussion explains the oder that images are collected and the naming convention used.

Image Collection Order

Odd Line Finish

Even Line Finish

Start Point
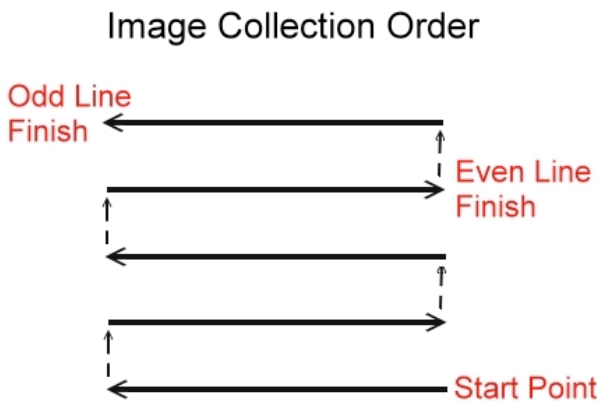
** reference final image

1) Montage images are collected in a serpentine path (boustrophedonic) from the bottom right corner of the image to the upper left corner.

2) The image names have two components as shown below.

The first component is a number that corresponds to the reverse of the collection order. The second corresponds to the row and column.

3) These image plus an overview and information file (with images positions) are stored in the folder created for the montage during the setup process..
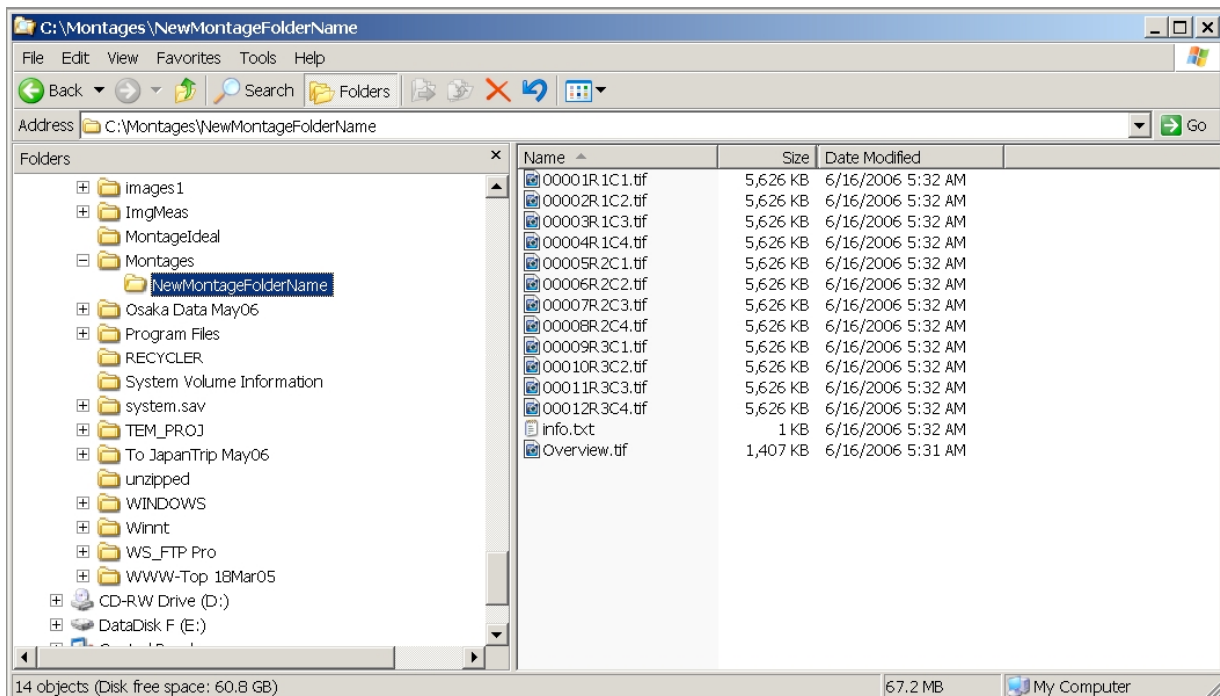
Image Naming Convention

	Number	Row/Col	
Last Image	00001	R1C1.tif	
	00002	R1C2.tif	
	00003	R1C3.tif	
	00004	R1C4.tif	
	00005	R2C1.tif	
	00006	R2C2.tif	
	00007	R2C3.tif	
	00008	R2C4.tif	
	00009	R3C1.tif	
	00010	R3C2.tif	
	00011	R3C3.tif	Lower Right
First Image	00012	R3C4.tif	Corner

Montaging with PanaVue Image Assember 3

The montage procedure has been developed with the Panavue image stitching program in mind. The montage routines in the AMT Capture Engine automatically names images in the Panavue project order. However, for Version 3.4 of Panavue the Capture Engine not only aquires the montage images but it also creates and runs the stitching project. Since the user may elect to assemble the montage at a later time the following discusses some aspects of running a Panavue project. You can also obtain more information from the Help menu in Panavue. It should not be necessary to create the project.

The user may delay assembly of images for a later time. In addition, the quality of the automatic stitching result will depend on the content of the image, distortion and amount of overlap. There are also option settings for Panavue that may need to be changed for a given environment. A manually assisted procedure is also available in Panavue.

1) Open the Panavue project created by the Capture Engine by double clicking on the xia file in the montage folder you defined during setup. This will open the Panavue project.

2) Go to the **file**menu and select **Open All Images**. This will open all the images in the project.

3) Use the **Action** menu and select **Full Run** or type **Ctrl R.**

4) The resulting image will be displayed when stitching is completed. Note: The lower right hand image has a micron bar and a label with the TEM Direct Magnification in it. You can then choose to save the image (We recommend an 8 bit gray scale TIFF format.).

5) You should evaluate this image and determine whether stitching has been successful. If there is a problem with the result you can change the options and/or reset the project for manual positioning and repeat the stitching. This might be necessary if, for example, the overlap is less than 30% and the resulting image has not been stitched properly. These options are found on the Set Options tab of the Panavue project.

6) This image can also be compared with the low resolution **Overview.tif** image in the project folder. This image was created when the region was defined at low magnification.

****

Web Broadcasting

All AMT cameras have an option to produce an NTSC signal that can be used for a live "webcam" signal. This capability is useful for remote collaboration, demonstration and instruction.
This section describe the equipment, software and procedures for routing this signal for display via the internet.

Contents

Web Broadcasting Manual

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AMT Web Broadcast Manual
Rev1.3
10Jan2006

This option requires:

Scion AG5 Video Output Board (or nVidia Dual Display Video Card with Video Output and cables)
Winnov Videum 1000 VO Plus Board
WebCam32 software
Scion Cable
Male BNC to male RCA adapter

1) Follow Scion's standard installation procedure to install the AG5 board
     OR
   Install the nVidia card with dual display, set the secondary display resolution to 640x480.
2) Follow Winnov's standard installation procedure to install the Videum 1000 board.
3) Connect the output of the Scion board to the input of the Videum board using the Scion cable and the BNC to RCA adapter.
4) Install the WebCam32 software.
5) Configure your network. If you have a fixed IP address users outside your local area network will be able to connect to your port and view the images.
Check with the network administrator at the customer location to see if this is allowable.

(You can find out what addresses are currently assigned to your system by running **Start|Programs|Accessories|Command Prompt** and then typing **ipconfig** in the black window that is displayed.)   If fixed addresses are permitted go to **Start|Settings|Network and Dial-up Connections**. Right click on Local Area Connection select **Internet Protocol(TCP/IP)** and click on the properties button. Click on the **Use the following IP address** and enter the IP address, Subnet mask, and Default Gateway.
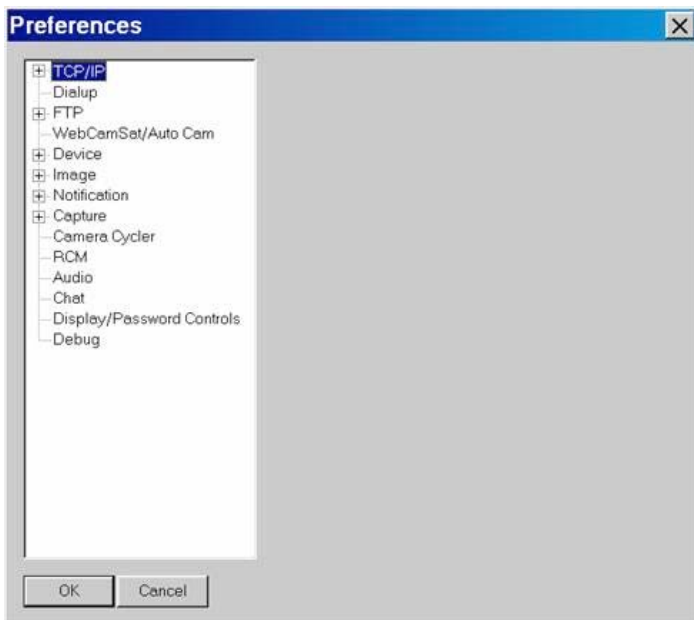
6) Configure WebCam32 –
   a) Launch WebCam32
   b) Select **File|Videum Video Capture**
   c) Select **File|Preferences**

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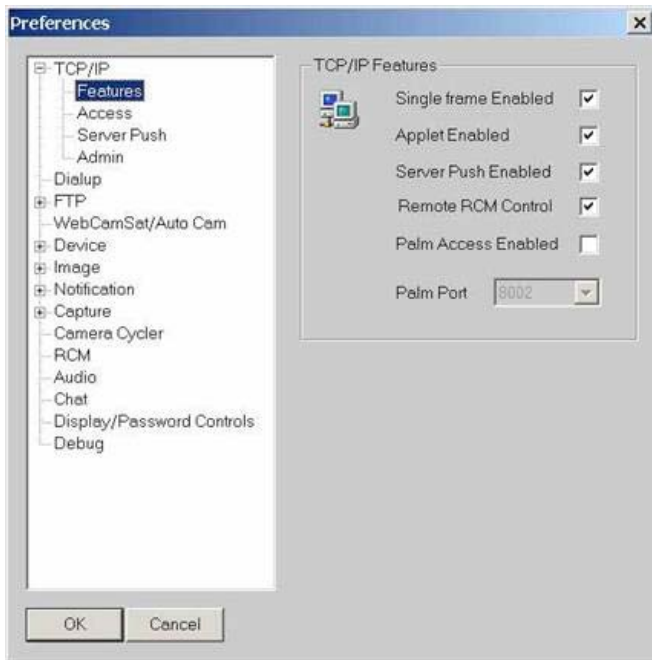
d) Expand **TCP/IP|Features** - check all option boxes except Palm Access Enabled.

e) **TCP/IP|Access** – Confirm that the IP Address shown is the same one returned by ipconfig above.   Take note of both the IP Address and the port.   They will be needed later.
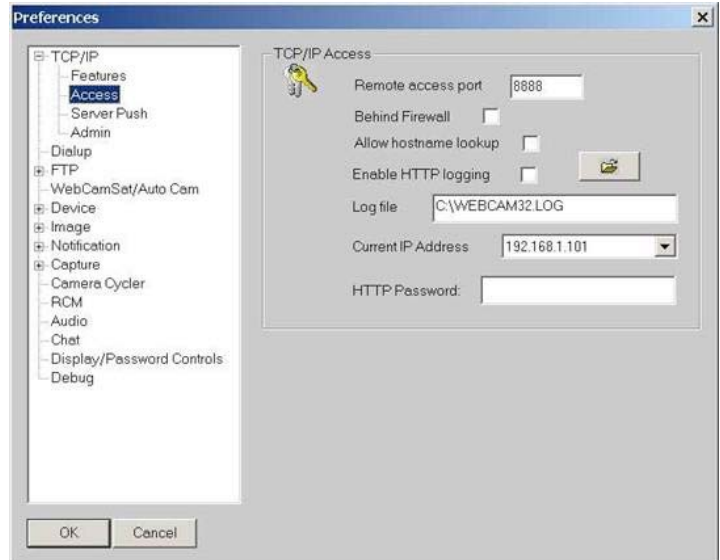
 f) Expand **Device|Format** Select a standard image size of 320x240 and check black and white. Set Format to RGBT: 24bit RGB.
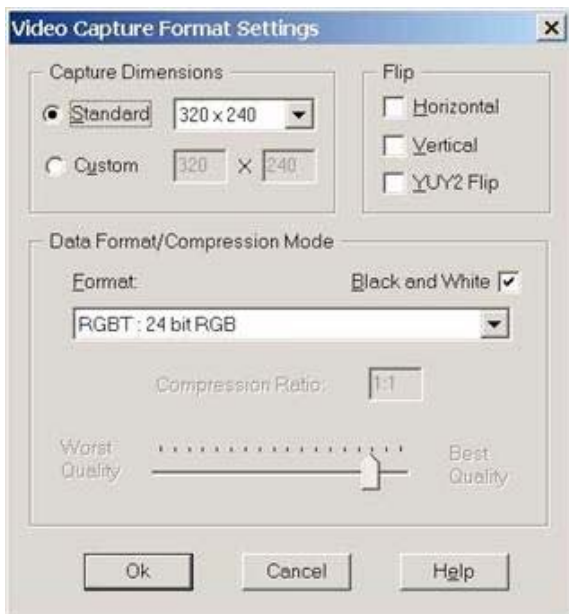
   g) Select **Device|Source**. This will display the Video Source Settings window below. Set the popups to Composite, NTSC, and Camera as shown. Picture adjustments of Brightness 50, Contrast 25, Saturation 25, and Hue 50 are reasonable places to start.

Be sure to click the Default command button so that the next time the program is run these settings will be used.

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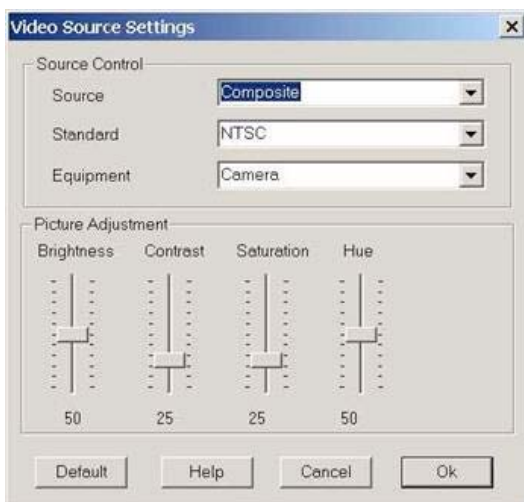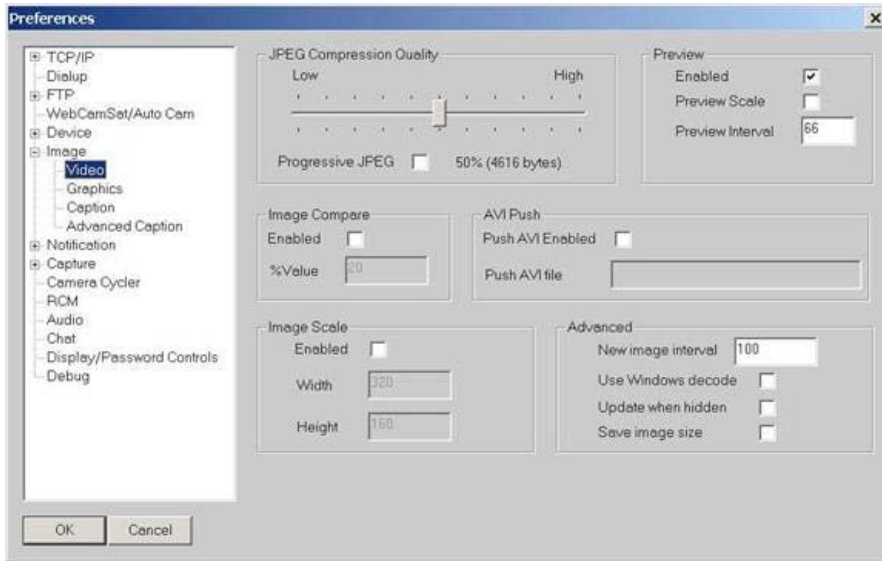
h) Expand **Image|Video** and set JPEG Compression Quality to around 50%. Check Preview Enabled to display the image being sent out.   This can be turned off later. A small preview window appears which allows you to see what is being made available to the remote observer.

i) Select **Camera Cycler** and make sure that only Videum Video Capture is checked.

7) Go to **Start|Programs|WebCam32**. Create a shortcut (right click) and drag this into the bottom task bar.
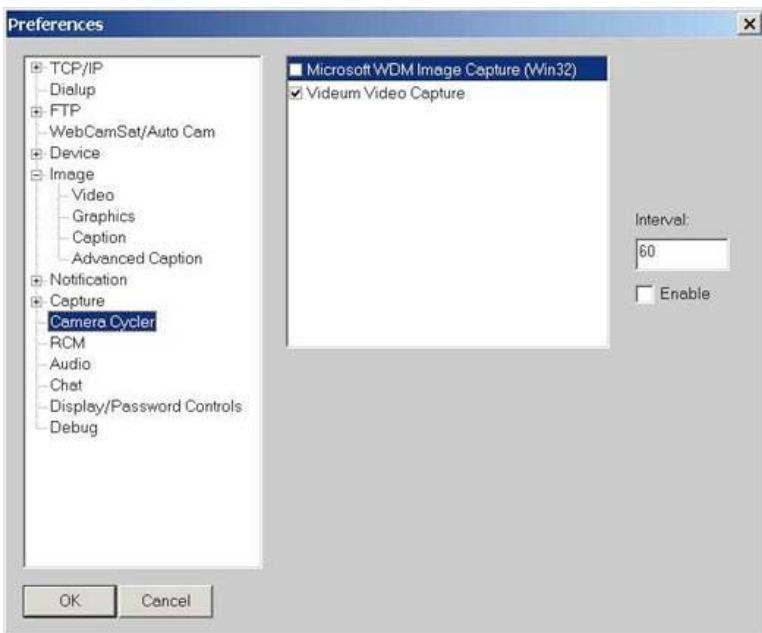
8) The remote user will need to run AMTWebCast.html to observe the video stream. A prototype of this is located in **C:\AMTCommon\config.** A complete listing is appended here with the required changes highlighted.
a) Open Notepad - edit **AMTWebCast.html** file to customize:
   1) The header string <H1 align=center> header string <\H>.
   2) The ip address and port number must be set to match 6e.
   3) If you wish to test other resolutions Width and Height specifications can be modified.   These values should match acquisition parameters set up in Cam32 (**Device Format** 6f). If the network connection between the Camera computer and the remote computer is fast enough it may be reasonable to increase the size of the transmitted image.
9) The **AMTWebCast.html** file can either be distributed to remote users or posted on central web site and the URL distributed to the remote users.

Appendix
1) Replace the following -shown in blue- with the name of the Microscope Facility:
Microscopy Center Image from AMT Camera on XYZ TEM

2) Replace the IP Address and port – shown in green in 4 places 256. 256. 256. 256:8888 - with the correct IP Address obtained from ipconfig and displayed in the TCP/IP Access Preferences page and the port from the Properties page.
3) To try a different resolution use the Preferences Device|Format to select another resolution and replace the values 320 and 240 below.   Shown in orange. It may also be necessary to adjust form sizes set here to 350.

AMTWebCast.html Listing

<!-- Sample Modified from NIH/Rocky Mountain Lab -->
<html lang="EN">
<head> <title>Image Pull</title> </head>
<body>
<H1 align=center> **Microscopy Center**

Image from AMT Camera on XYZ TEM </H1>
<TABLE align=center border=0 cellPadding=1 cellSpacing=1 width="75%">
<TR>
<TD align=center height=350 width=350 vAlign=middle style="BACKGROUND-COLOR: lightyellow">
</TD>
<TD align=center height=350 width=350 vAlign=middle style="BACKGROUND-COLOR: lightyellow";
HEIGHT: 350px; WIDTH: 350px>

```
<script LANGUAGE="JavaScript">
<!--
browserType = navigator.appName;
newImage = new Image();
document.onstop = document_onstop;

function startstop_onclick()
{
    if (startstop.value == "Stop")
    {
        stopLoad();

    else
    {
        startstop.value = "Stop";
        newImage.onload=loadNewImage;
        loadNewImage();

}

function document_onstop()
{
    stopLoad();
}

function stopLoad()
{
    newImage.onload="";
    startstop.value = "Start";
    window.status = "Live video stopped ...";
}

function loadNewImage()
{
    uniq = new Date();
    uniq = uniq.getTime();
    document.images.webcam32.src=newImage.src;
    newImage.src="http://256. 256. 256. 256:8888/video/pull?"+uniq;
    window.status = "Displaying live video ...";
}

function initialImage()
{
    uniq = new Date();
    uniq = uniq.getTime();
    newImage.onload=loadNewImage;
    newImage.src="http:// 256. 256. 256. 256:8888/video/pull?"+uniq;
    document.images.webcam32.onload="";
}

if (browserType == "Netscape")
{
    document.write('<IMG SRC="http:// 256. 256. 256. 256:8888/video/push" align=center width=320
height= 240>');
```

```
}
else
{
    document.write('<IMG SRC="http:// 256. 256. 256. 256:8888/video/pull" name=webcam32
onload="initialImage()" align=center width= 320 height=240>');
}

//-->
</script>

<P><INPUT id=startstop type=button value="Stop" LANGUAGE=javascript onclick="return
startstop_onclick()"></P></TD>
<TD align=center height=350 width=350 vAlign=middle style="BACKGROUND-COLOR: lightyellow">
</TD>
</TR>
</TABLE>
</body>
</html>
```

```
**************************************************************
```

Directions for Changing a Phosphor

Occasionally the microscope's technician or user is called on to replace the phosphor for an AMT camera. It's a simple procedure but requires a little caution. This chapter describes the change procedure for side mount and bottom mount systems.

Contents

Phosphor Evaluation
Phosphor Types
Changing Sidemount Phosphors
Changing Bottommount Phosphors

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Phosphor Evaluation

Phosphors may accumulate dust and occasionally it is possible to burn a phosphor by exposing it to a beam that is too intense for an extended period of time. If a dust particle is thin enough that the background correction is able to compensate for the lost intensity then cleaning the phosphor can be delayed until an opportune time. Unfortunately in some cases the dust particle is too thick or moves relative to the background image when charged by the beam or jarred by the motion of phosphor insertion. In these cases the phosphor should be cleaned immediately.

When evaluating the state of a phosphor it is sometimes the case that a background corrected image confuses the issues. It may be helpful to turn off all corrections for this purpose. This can be done by selecting the menu item: **Corrections/Diagnostic Tools/Suspend corrections and Zero Thresholds**

Phosphor Types

There are three main physical designs for phosphors used by AMT. For all three, in the scope, the aluminum side points up (towards the beam).

Film Type (IF)

One design is called Film Type. It has a shiny aluminum coating on the side facing the beam, and phosphorescent material on the bottom. Both sides are delicate and shouldn't be touched. The transport case protects both sides.

Round Glass Bottom (IB)

Another type type is round with glass on the bottom and a dull aluminum finish on the side that points toward

the beam. They can be placed, glass down, on a soft surface, or lifted by something soft on the glass side, but don't touch the aluminum side.

Rectangular Glass Bottom

Like the round glass ones, these have a glass bottom that can be touched by something soft, and a delicate gray side facing the beam. They are transported in a curved bottom dish with the delicate side down. As with the other types, grab it by the edges.

Glass side up in dish. Aluminum side points toward beam.

Sidemount Phosphors

Here are the three types of sidemount phosphors, each mounted in its respective holder for installation. They each have either the shiny aluminum side or the dull aluminum side up.

Phosphors for Sidemount Cameras
4 to 8 Megapixel Cameras XR40/XR60 — FB6, IB6
1 Megapixel Cameras HR/HRL/ER — GA8
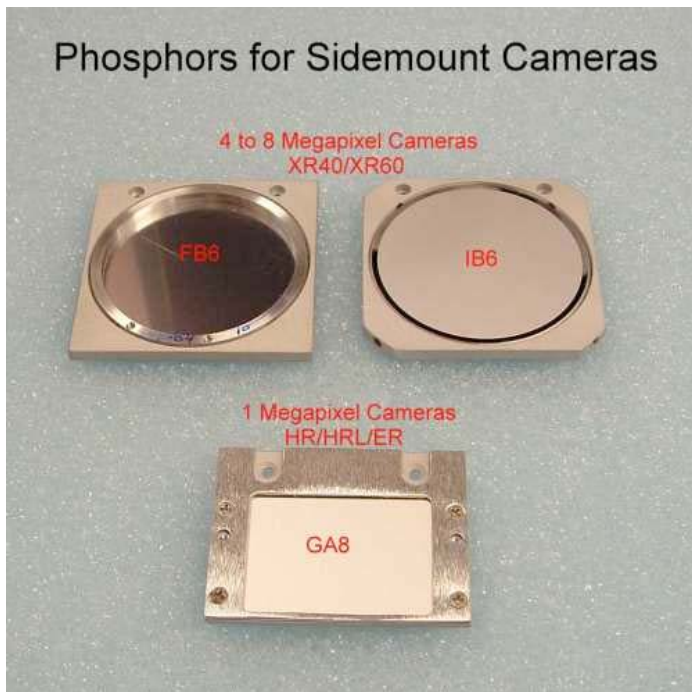
Procedure for Changing Round Phosphors

1) Vent the TEM camera (film) chamber only. Some old JEOL100/200C and Zeiss "orange column" microscopes need to vent the column as well. You never need to vent the gun.

2) Make sure the mirror is in the retracted (camera out) position. You may have to remove the pneumatic tube that powers the mechanics. Remove the 35 mm retracting flange from the TEM, using a hex wrench for the bolts. Side-mount systems normally install the retractor on the right [exceptions H7000/H7100 and some orange column Zeiss's].

3) Remove the phosphor. After removing the assembly put the mirror in the extended (camera in) position for working on it. Loosen the four set screws (one at each corner) that hold the phosphor in the holder. In film phosphors there are small holes in the rim of the phosphor. The small hex wrench you used for the set screws will fit into the holes, allowing you to pry up the phosphor so you can grab it by the sides and move it to an empty holder. Glass phosphors can be pushed up from the bottom by something soft. Be careful to not have anything touch the mirror. After removing the phospher, make sure the set screws are fully retracted so the new phosphor will drop into place.

Film Type Phosphor
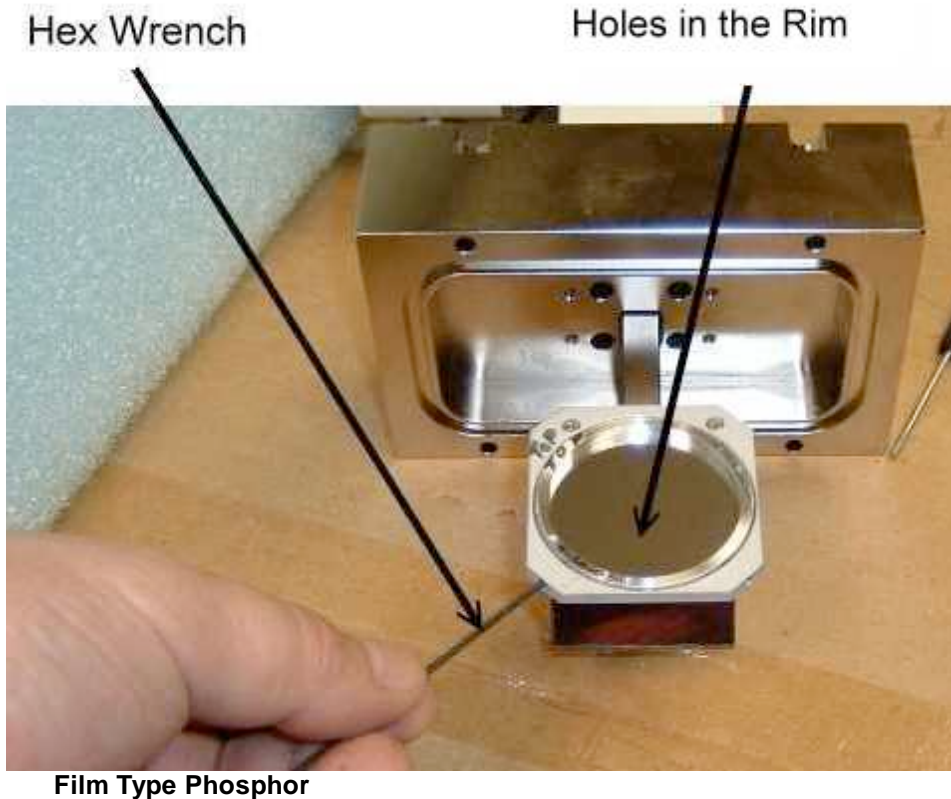
4) Mount the new phosphor. Grabbing it from the sides, lift the new phosphor into place. If it's a glass phospher you'll have to flip it after lifting out of the transport case to get the dull aluminum side up. The film ones are transported with aluminum up. After the phosphor is in place, tighten the corner set screws. They need some torque, but not much. If the phospher is a film type, carefully check the top to make sure there's no buckling of the surface.
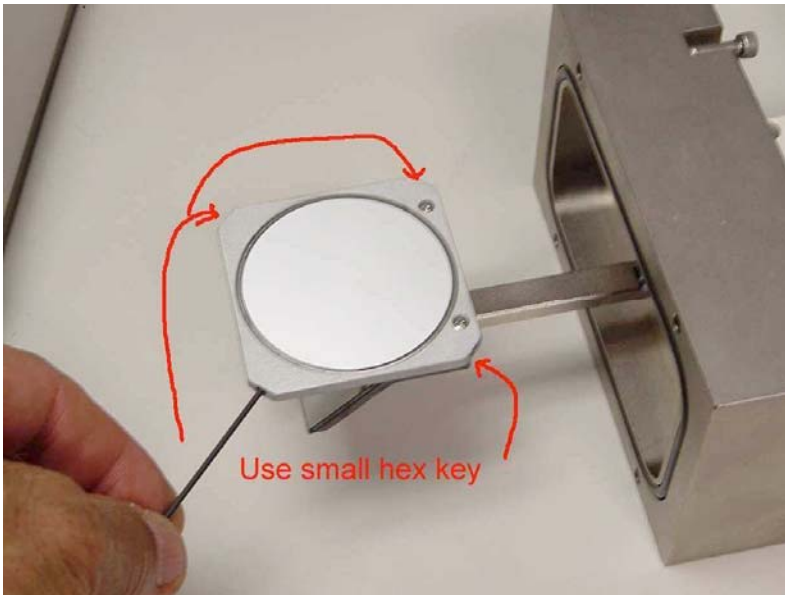
Glass Type Phosphor

5) Gently blow off any dust. Using a can of compressed air or other gas, blow off the top of the phosphor and the front of the mirror surface. This requires caution. Hold the can at least eight inches away from the target. Pull the trigger part way, to get a low pressure stream of air. Blow the stream at your hand first to set the stream and make sure it's all gas, no liquid. During the cleaning spray, keep the can in the same orientation - don't turn or shake it.

6) For putting the assembly back on the column, make sure the mirror is retracted (camera out). Be sitting for the insertion and put your elbows on the table top.

Rectangular Phosphor Replacement

If you need to replace a rectangular (GA) type phosphor, it will probably arrive already fastened into a mounting bracket, with a flat cover held onto the top with small screws. You change the bracket using the two screws at the rear. Make sure the glass side is down when mounting. After the bracked is attached remove the cover. Be careful not to let the screws or the screwdriver touch the surface of the phosphor.

Bottommount Hardware

Changing a bottom mount phosphor requires removing all the AMT hardware from underneath the column. The kick cover, camera and lens, and the lens mounting block can be removed without breaking vacuum. Remove the lens and camera together. Be sure not to tamper with the screws that hold the lens to the camera. The Flange to TEM contains the phosphor. It is fairly heavy, although it shouldn't require a floor jack. Use a rubber band to fasten something soft over the glass on the bottom of the Flange before you remove it.

Procedure for Changing BottomMount Phosphors

The phosphor is in the flange to TEM. When you have the flange on a table carefully remove parts until you get to the phosphor. Mark parts as to orientation and position for reassembly. Be careful not to drop screws onto the phosphor. Film type phosphors have holes in the rim which can be used for lifting. Glass phosphors can be lifted from the bottom by something soft. When you're done with replacement the phosphor's aluminum side, either dull aluminum or shiny aluminum, should be facing the beam.
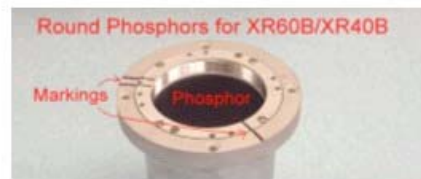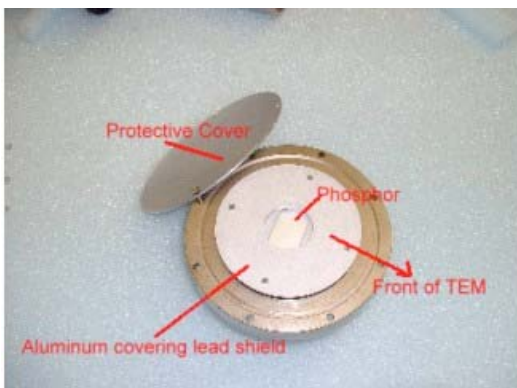
When the phosphor is in place gently it blow off any dust using a can of compressed air or other gas. This requires caution. Hold the can at least eight inches away from the target. Pull the trigger part way, to get a low pressure stream of air. Blow the stream at your hand first to set the stream and make sure it's all gas, no liquid. During the cleaning spray, keep the can in the same orientation - don't turn or shake it. Use the same procedure on the other hardware parts as you reinstall them.

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Computer Issues

This chapter discusses computer issues which are commonly handled by scope users or their I.T. Department personnel.

Contents

User Accounts
Maintenance Utilities
Kavoom! KM
Networking

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User Computer Accounts   AMT computers are usually shipped with two accounts for use: "Administrator" and "User1". Both accounts have administrative privileges in Windows. If you create separate accounts for camera users they will not be able to use AMT until the new user has full control of the folders listed below. The folders' "Security" tab might not be available until, in Windows Explorer, to go to **Tools->Folder Options** and on the "**View**" tab, unclick "**Use Simple File Sharing**".

Users need Full Control to the following folders (including sub folders): C:\AMTcommon C:\AMThistory C:\Images C:\ImgMeas C:\Montages NOTE: A network drive, or other designation for saving images, must be write enabled also. If your AMT software has these folders in the "All Users" folder, they should already be fully accessible.

Basic Microsoft Utilities

The TEM digital camera is a high speed data acquisition system that puts heavy real-time demands on its computer and generally produces many, very large files. Maintaining performance requires some routine maintenance procedures that are readily available in the Windows operating system. This sections describes some of the most basic operations that should be done monthly.

Checkdisk:
This is your most powerful tool for restoring efficiency. To turn Checkdisk on find the **C drive** in "**My Computer**" or "**Windows Explorer**", right click it and click "**Properties**". "**Tools**," where you will find "**Error Checking**". Click "Check now" and select the first cleanup option, "**Automatically fix file system errors.**" Then the system will tell you to reboot your computer.

Note: If you select "**Scan for and attempt recovery of bad sections**" this operation will take a long time. This operation should be used only when you suspect a physical problem with the hard drive or you have a few hours to spend on the operation (i.e. overnight).

Safe Mode:
This utility is a barebones version of Windows, which is used for diagnostic and repair purposes. It also rewrites registries and repairs files during the boot process, so just opening in Safe Mode can sometimes fix a system. To get into Safe Mode "**Restart**" your computer. Before Windows starts, push and hold down the F8 key on your keyboard. This should produce a screen with several options including the SafeMode Start option. The startup takes a few minutes during which file names are displayed. After the startup your Desktop Icons will be enlarged since the graphics card is not running. When the computer is done starting up tell it to restart again, this time without pushing F8.

Defrag:
This can be done after Checkdisk and may not be needed every time. See how many files are fragmented when the Defrag display starts. The Defrag actuator is on the C drive's **"Properties - Tools**" tab along with "Error Checking". This is another time consuming procedure for lunch or overnight break.

Disk Cleanup:
If you do not browse the internet you probably will not have a lot of temporary internet files plugging things

up. However, it still wouldn't hurt to do a cleanup occasionally, especially if you're on a network. To start the process click "***Start Programs/ Accessories/ System Tools/ Disk Cleanup***". Select the C drive and check the categories that contain unneeded files

Directions For Installing Kavoom! KM Kavoom! KM combines the keyboard and mouse for two computers. Kavoom! KVM would also combine the monitor.   **Caution** Be careful not to use or disturb the network that is doing communications between the scope and its computer. Use "***Start->Run->cmd***" to open a command line interface and then type "**ipconfig**" and [enter]. Use ipconfig together with "Network Connections" in the Control Panel to figure out which port is doing what. Any hub or switch you use will be outside the computer. Sometimes the scope's computer will have a row of Ethernet looking ports in the back that looks like it might be a hub, but it is not a hub in the normal sense, and plugging into it could cause problems.

 Create A Network The Kavoom! network will need an Ethernet port on each computer. A single port can be used for two networks if it is split with a hub or a switch. An Ethernet card can be added if needed, or a USB to Ethernet adapter (available from newegg.com or trendnet.com).
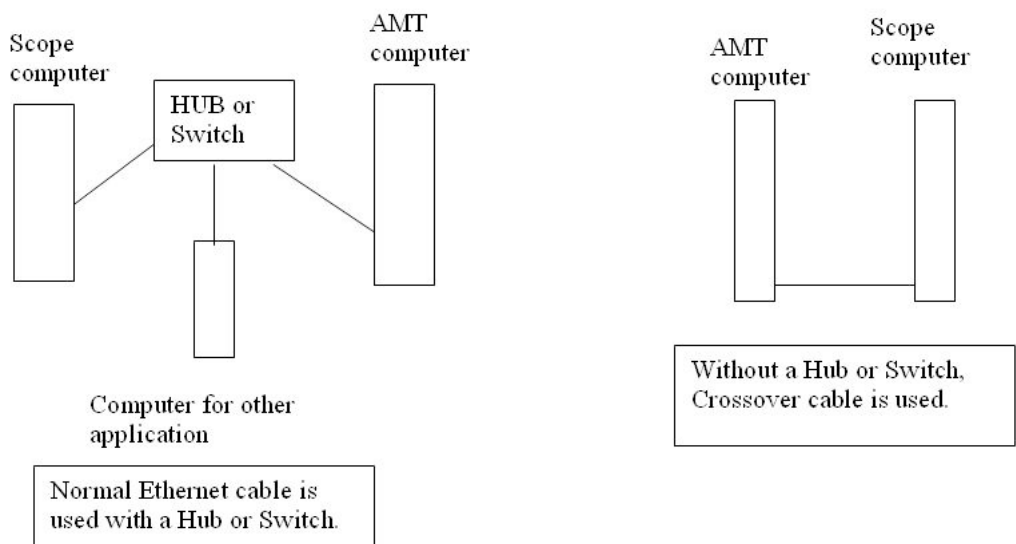
 Unless using a hub or switch the network cable will be a crossover cable (NOT straight Ethernet). The network we create will be minimum duty, without file sharing between the computers. This facilitates safety if either computer is connected to an outside network. The protocol for the network is TCP/IP, which will probably be there automatically when the network is created. Kavoom! works best with assigned I.P. addresses on each computer. A convenient pair of addresses is 172.17.41.1 (scope side) and 172.17.41.2 (AMT side). If the network includes another camera just increment the last digit for its address. Both should have a subnet mask of 255.255.0.0. To assign addresses right click the network's icon and click "Properties". Then highlight "Internet Protocol" and click "Properties". After you have set-up the cables and assigned I.P. addresses the computers should be able to "see" each other via Network Neighborhood. If you are not sure you have a good connection, try "pinging" over the new network. Get a command line interface by clicking ***"Start -> Run"*** and typing in "**cmd**", then type "**ping**" [space] and the I.P. address of the other computer and [Enter]. If the ping does not get positive results you may have to restart one or both of the computers. You may also have to turn off Windows Firewall on one or both computers. Access its on/off switch in the Windows ***Control Panel***.   **Install Kavoom!** If your AMT computer is on the internet, you can download the Kavoom! KM software from the Kavoom! Website www.kavoom.biz. Kavoom! gives a 30 day free trial, and you can the license the program via the Internet. If you do not have internet in the lab use the Kavoom! Corporate edition, which you can get from AMT or by downloading from http://www.kavoom.biz/k0000/KaVoomKM3.22Corporate_Setup.exe. If you use the corporate edition you will have to get the activation code from AMT, and the AMT computer should be named amt1 (case not important). Run the Kavoom! installer on both computers. Open the Kavoom! program on both. Click "Change" to start the configuration process. It will ask you how the monitors are arranged. Hopefully the computer's Kavoom! programs will see the other computer for configuring. If not, you may have to turn off Windows Firewall on one or both computers. If you want Windows Firewall on, because you will be

connecting to an outside network which may not have a good firewall, you may be able to make an exception in Windows Firewall to allow Kavoom!. **Other Notes** Usually both keyboards and mice are left installed in case either computer is used alone, but one set can be tucked away out of sight. When either computer is restarted Kavoom! should restart automatically. If it does not, open the program from **"Start->Programs**" and click "change" to get the connection started. A problem starting automatically often means Windows Firewall is blocking the program.

Networking

For many labs, the AMT computer is used without networking. Each user downloads their images onto a CD or a flash drive. Ideally, even a non-networked computer should have an antivirus utility if there's a lot of users. That decision depends on what priority you place on the data stored on the computer. Any computer that does get networked needs an antivirus utility, unless it's on a super secure network.

Bare Bones Networking

For those AMT computers that do get networked, for file sharing and/or for Internet, there are a few precautions. First, AMT is most compatible with "Bare Bones Networking". This kind of network give the I.T. Department enough control so they can, for instance, upgrade the virusware and run Windows updates from a central location, but less options for remote control than is common in some labs. "Zen Masters" and similar utilities for complete remote control are ruled out. When networking is done there should still be only one Display Adapter visible in the computer's Device Manager (right click **"My Computer**" and choose "**Properties**", then "**Hardware**" and "**Device Manager**".

Frames Per Second

There is convenient way to at least partially monitor the effect of networking software. Before the process of creating a network begins, do a check on the frames per second (**fps**) at which your camera runs. This number is given at the lower right part of your AMT display, above the buttons for "**Set/Save"** and "**Recall**". Check what the rate is for Survey mode and Focus mode imaging, and make a record of it. When networking is finished the fps should be the same as before networking. If networking slows down AMT it will affect image quality. If that happens a lighter network protocol needs to be adopted.

Saving To A Network

If you plan to save images to a network drive make sure the drive is write enabled. The account of any user that saves images must be set to have full access on the drive's "**Properties -> Security**" tab. If the Security tab isn't displayed deselect "Use Simple File Sharing" in Windows Explorer's "**Tools - Folder Options -> View**" tab.

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Software

This chapter is intended to help with first time installations as well as upgrades, moving and troubleshooting. Most of the software needed can be downloaded from AMT's web site for free. Please contact us before upgrading software for advice and for sign-on information. Our phone number is 978-774-5550 or email us at info@amtimaging.com.

You must have Administrative privileges to install software on your computer. If you do not think you are a "computer person" someone here may be able to "walk you through it" on the phone. Another possibility is to find an "IT" person or local computer guru to help you. Whoever does the upgrade, whether coached or alone, should contact AMT about the specifics first.

It is also possible to send us your computer for new software installation for a nominal labor charge.

Contents

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AMT Software

An upgrade of AMT software often requires an upgrade of camera driver to be in place first. Please contact us before upgrading software for advice and for sign-on information. Our phone number is 978-774-5550 or email us at info@amtimaging.com. You can see which release is available for you system in the page **"Which AMT Version"** . Software updates and camera drivers are posted on www.amtimaging.com.

You must have Administrative privileges to install software on your computer. If the upgrade you install is in the same release (the version numbers have the same first three digits) as your current version, upgrading should be very simple. If you are upgrading to a newer release it is a little more complicated.

It is also possible to send us your computer for new software installation for a nominal labor charge.

The AMT Image Capture Engine, and the other drivers we distribute, are designed to run on computers with Windows XP or Windows 2000 as an Operating System.

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Which AMT Version?

<u>**Version 600 or 5.4.2**</u>
The Table below describes the combinations of cameras and boards that are supported by AMT600. If your system uses one of the board/camera combinations that shows "N", your upgrade should be to AMT version 5.4.2.544q ("AMT's Final Release Advantage Plus"). It the camera is supported by AMT600 the download link is "Latest Release All XRs, AMT 16000s, AdvHRs, v600...". Both versions are available on the AMT web site **www.amtimaging.com**, after clicking on "*Support*" and then "*Click Here To Start Downloading*". Contact AMT for the password.

----------------------- **AMT600 Systems Table** ---------------------------

Camera	Frame Grabber Board					
	PCDIG	ICPCI	1394 FireWire	Active Silicon Phoenix	CamLink EDT	GigE
Advantage HR/B						
Hamamatsu NR/ER	AMT600	N	N	AMT600	N	N
Hamamatsu G04/ERG	N	N	AMT600	N	N	N
DVC 1312	N	N	N	N	N	N
DVC 1412	N	N	AMT600	N	N	N
Advantage HS						
DVC 1500	N	N	N	N	N	AMT600
XR40/40B						
Optronics MacoFire	N	N	AMT600	N	N	N
XR41/B/C/M/HTV						
DVC4000	N	N	N	N	AMT600	AMT600
XR60/B/C/M XR100/111						
Hamamatsu ORCAHR	AMT600	N	N	AMT600	N	N
AMT 16000S/B/M, TLD						
Redlake EC11000	N	N	N	N	AMT600	N
Redlake EC16000	N	N	N	N	AMT600	N
Advantage Plus/B/AM						
Kodak Megaplus 4.0	AMT600	N	N	N	N	N
Advantage R/10						
Kodak Megaplus 1.4/1.4i	N	N	N	N	N	N
Kodak Megaplus 1.6/1.6i	N	N	N	N	N	N

Identifying The Hardware

<u>**Identifying the Camera Head**</u>
The camera model is written on the camera head. Normally it is printed on the label with the serial number. If you cannot find, this call AMT for assistance.

<u>**Identifying the Frame Grabber Board**</u>
a) The following cameras have only one choice for frame grabber board, so further research is not needed.

Optronics MacroFire
DVC1412
Hamamatsu GO4
Hamamatsu ERG
Redlake EC11000
Redlake EC16000
Kodak Megaplus 4.0

b) These cameras have two or more board options:

DVC4000
Hamamatsu NR
Hamamatsu ER
Hamamatsu ORCAHR

If you have a working version of AMT software, you can identify the board using Windows' ***Device Manager***. For the Hamamatsu Cameras: PCDIG and ICPCI boards can be identified under "***Sound, Video and Game Controllers***", while the Active Silicon board shows up under "***Imaging Devices***".
For DVC4000: The EDT board will show up in the Device Manager under its own heading and the GigE board will show up under the PRO/1000 Grabber Devices heading.

In all cases you can identify the board by visually examining the board itself.

Backup Information First

<u>**Save AMTCommon**</u>
The data stored on your computer in the folder **C:\AMTCommon** contains the settings and configurations for your camera. Whether you are upgrading a system on the current computer or moving it to a new computer having the old **AMTCommon** can save hours of work. If the new software will go on the current computer back up **AMTCommon** by copying it and pasting it to the C drive, creating a **"Copy of AMTCommon"** folder. If moving to a different computer, download the **Copy of AMTCommon** to a flash drive or a CD. If you will be upgrading to the 600 release from and earlier version, renaming the copy to **"Copy of AMTCommon542"** for instance, will help keep things clear.

<u>**Writing Down Info**</u>

If you are upgrading to the 600 release from an earlier one, or if you are upgrading to release 542 from a version before that, you can save time by making a written record of some of your parameters before making a change.
  It is always a good idea to record the AMT version number. On the upper menu click ***"About"*** and then ***"Version"***. Write down all the information that gets displayed. This often will include X and Y pixel dimensions.
  Find the Align Offsets by clicking ***"Utilities"*** on the upper menu, then click ***"Camera and Optics Adjustments"*** and finally ***"Align Camera Center".*** When the horizontal and vertical centering controls appear, write down and record the numbers they contain (don't change anything, just click "**OK**").
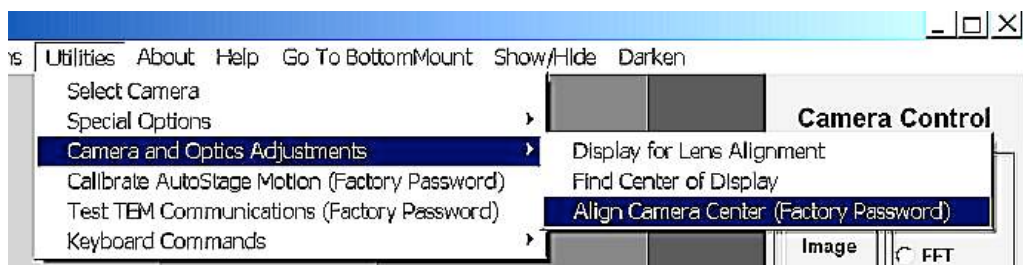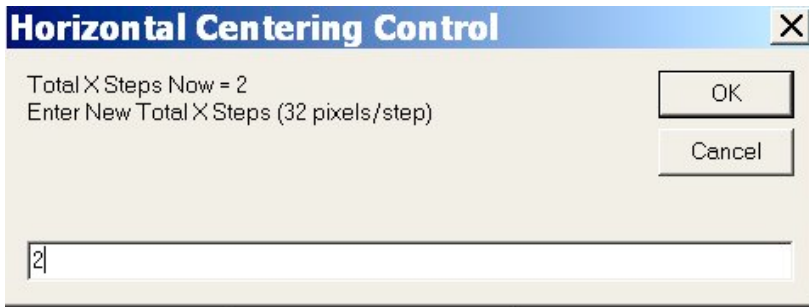
Write down the Exposure Times and Gains for the various sets of settings. Click "**Recall"** on the lower right part of the AMT display and view the various Settings that have been created. One by one click on each of them (including the **DefaultSettings**) and open them by clicking the "**Set/Save"** button to open the **Camera Settings Control** Window. For each set of Settings write down exposure times, gains and record integrations, to transcribe into the new install.
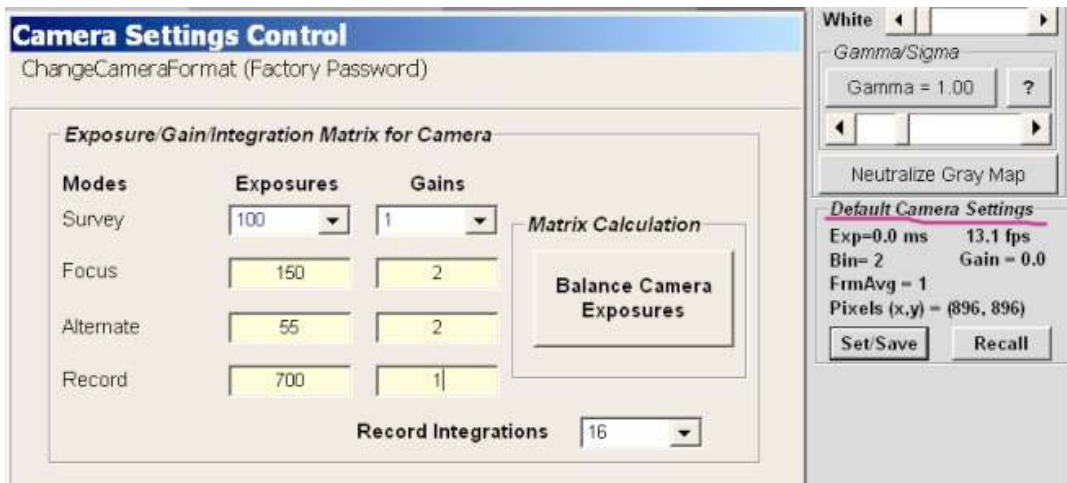
In systems with TEM communications it is often useful to have written down which communications protocol is selected. To find that, on the upper menu click "**Utilities"** and the click "**Special Options - TEM Communications".**
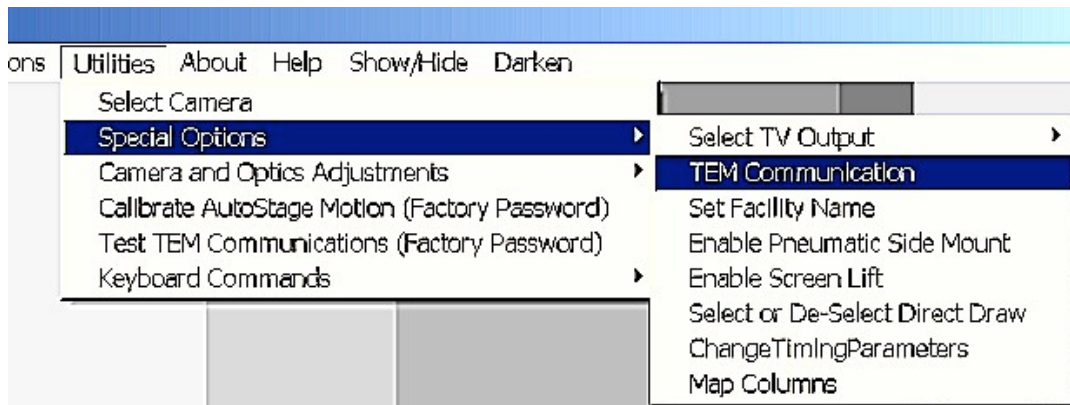
Broken Computer
If the computer with AMTCommon is not working, you may still be able to salvage AMTCommon. Ask the I.T. Department, or local computer guru to remove the hard drive and read it on a different computer.

Fresh Install
If you are installing AMT for the first time in this lab you obviously don't have anything to back up. The settings will have to be done from scratch.

Installation AMT And Start Camera

Download and Unzip Software

After a camera driver version and an AMT version are selected (call AMT for help with this), and after your configuration information is backed up, you are ready to download and install the AMT software. It is available on the website **www.amtimaging.com**. The links to downloads are *"Support"* on the top of the home page, and *"Click Here To Start Downloading"* on the support page. Contact AMT for sign-on information. The upgrade to the 600 release is labeled "**Latest Software Release All XRs, AMT16000s, AdvHRs, v600...**". The upgrade to the latest 542 version is labeled "**AMT's Final Release AdvantagePlus v542544.....**". Since the installation files have been zipped with **WinZip**, you should use WinZip to unzip them. **WinZip** has a free downloadable evaluation version available at **www.winzip.com.**

Camera's Configuration Folder

The Configuration folder is the specific folder, within **AMTCommon**, where cameras settings are stored. In AMT versions within the 600 release, the folder name is **ConfigCam1** (for the first or only camera) or **ConfigCam2** (for the second camera). In earlier releases the configuration folder name is **Config** (for the first camera) and **ConfigB** (for the second one).
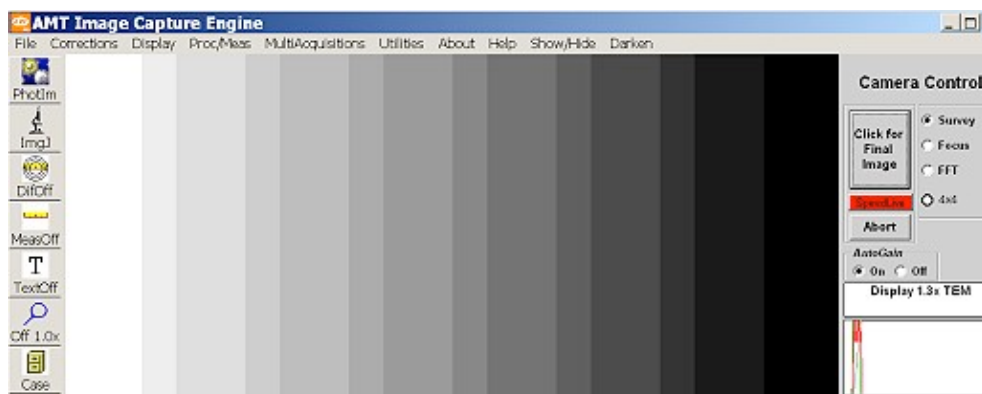
Run the Install

If you have a working version of AMT that starts with 542 or 600, leave it in place (don't uninstall). You might use the old version for diagnosis. When the new version is working just delete the links to the old one on the *Desktop* and in the *Programs* menu. If your version is older than the 542 release call AMT to see if you should uninstall it before proceeding. If you are upgrading from a 600 version to a more recent 600 version, look first in the configuration for the file **"Mags4.txt".** Make sure the lines in this file have comma separations instead of tabs. If it is delineated by tabs change them to commas and save. In the AMT installation folder double click on *"Setup.exe".* If the file suffixes are hidden, the executable is the *"Setup"* file with an icon that contains a computer monitor. Click *"OK"* or "**Run**" for any warnings about an **"Unknown Publisher".** Accept all the defaults during the installation, just click *"Next"* and *"Finish".* This will be a "*Typical"* setup.

Run AMT

If this is a fresh install, or an upgrade to version 600 you will be asked a couple of questions when you start AMT. On most modern computers you can say *"Yes"* to **Direct Draw** (**DirectX**). If there's problems with imaging you can then turn *DirectX* off for a test. The purpose of *DirectX* is to speed up the program. The second question will be **"Is this system sharing a computer with an H7650".** Unless AMT is installed on the same computer that is running that kind of scope, the answer is *"NO".* If you give the wrong answer to either of these questions, you can re-answer them by going to the primary configuration folder, **(Config** or **ConfigCam1**) and deleting the file **"DirectDraw.txt"** or **"UseLeadTools.txt"** respectively, and reopening AMT.

Click "*OK"* to warnings about Darkfield Corrections and Calibrations. Those comes later.

For fresh installs and upgrades to the 600 release, AMT is now ready to run in "**Null Camera**" mode. Having no camera selected, AMT will show a test pattern consisting of a series of vertical lines delineating gradations in grayscale, which change spacing and progress from dark to light. If you can not make AMT run in null mode there is a problem with the installation. Call AMT for support. If there is a conflict you may have to uninstall all versions of AMT and start over.

If this install is an upgrade within a release (first three digits of version number are the same), and if the install was done with the old **AMTCommon** in place on the **C** drive, then your old setting should have been incorporated, and for most systems your upgrade should be complete and ready to use. For some systems an upgrade of communications software may also be needed (See the **"Communications"** subchapter). In the meantime you can run your camera with communications turned off. Use the menu item "***Utilities -> Special Options - TEM Communications"*** to set communications to ***"None Of The Above"*** (after making a note of which communications had been selected).
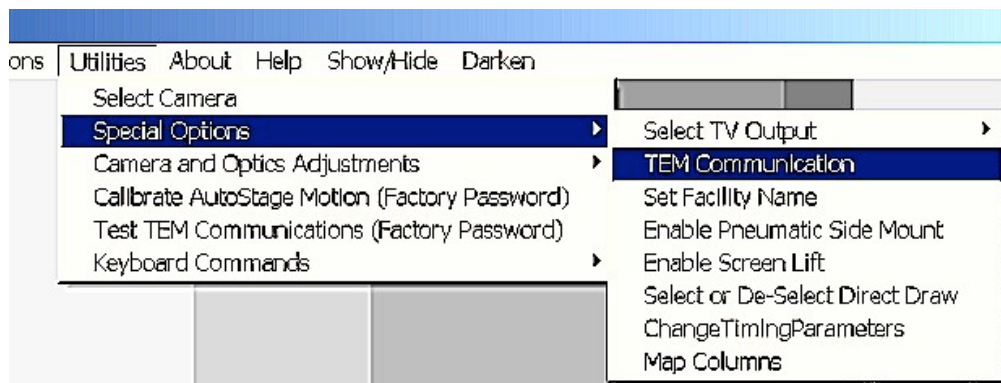
Select Camera
If your system has two cameras skip this paragraph and go to the page **"Two Cameras"**. When your new system is working in **NULL Camera** mode, you can select a camera by clicking on ***"Utilities"*** on the upper menu and then "***Select Camera"***. If you don't already know which camera and frame grabber board you have, look in the configuration folder inside the saved **Copy Of AMTCommon** . Camera and board information are in the file **InitialXXX.txt**, where **XXX** is the release number. Categories in that file are in brackets, and the option under each category that is not preceded by a pound sign is the one selected. After selecting a camera close AMT to implement the new settings, make sure that the camera power supply is turned on, and for GigE cameras make sure you have found the camera using **DVCco's** ***"GigE Camera Finder"*** program (See **"Camera Drivers"**). When you reopen AMT and go to live imaging the camera should work. If not try turning everything, including the camera's power supply, off for a minute and try again. When it works proceed to the **"Camera Settings"** page.

Two Cameras

If cameras are mounted at both the side port and underneath the column, AMT will need to be configured for two cameras. If you are doing an upgrade within a release (first three digits of the version number are the same) and if you run the upgrade with the old **AMTcommon** in place on the C drive, then the two camera configuration, and other settings, from the old software should be integrated into the new software without problems. If that doesn't happen, or if you are doing a fresh install, or an upgrade to the 600 release, you will need to configure the two cameras manually.

Camera Locations
The configuration folders are inside the folder **C:\AMTCommon**. For AMT software with version numbers that start with "600", the first opening camera's configuration is in **ConfigCam1**, and the second opening camera's configuration is in **ConfigCam2**. For all other versions, the first camera is in **Config** and the second one is in **ConfigB**.

### Get One Camera Working First
As a starting point for this discussion it is assumed that the camera drivers are in place, that AMT is installed, and that you are able to run AMT in *"Null Camera"* mode (no camera selected). It is also assumed that cameras' power suppliers are turned on, and that for **GigE** cameras, you have successfully run **DVCco's** *"GigE Camera Finder"*. Let's start by selecting one camera to run. This will be the camera that will open first when you run AMT (provided you aren't forced to switch the camera's positions during setup). Click *Utilities*" on the upper menu and then click *"Select Camera"*. The **Select Camera** window will open.
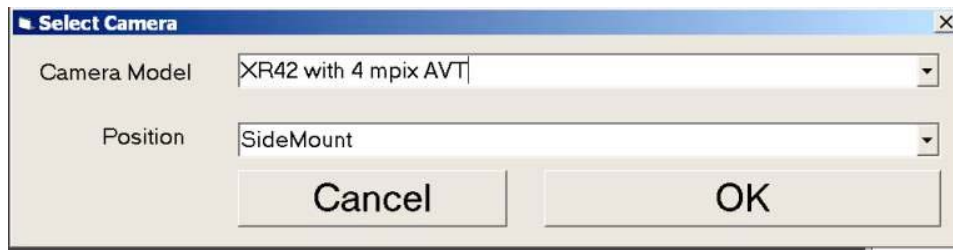
If you're not sure which camera you to select, check what the old system was set for in the saved "AMTCommon" (now renamed). In the folder **Copy Of AMTCommon\ConfigCam1** (or for release 542, "**Config**"), open the file **initialXXX.txt,** where **XXX** is the release number. That file should show the camera and board selections as the choices that are not preceded by a number sign in the list. Select your camera in the upper drop down box, and select its position in the lower box. Click *"OK"* and select *"Yes"* for "**Exit Program To Activate New Settings?**". When you reopen AMT and "*Click For Live Image"* the camera should work. You can usually see this without needing a beam on in the scope. If a problem shows up during this step call AMT for support.

Set For Two Cameras
After you have one camera working, open the folder **C:\AMTCommon** and then open the folder **ConfigCam1** (or **Config**, in AMT release 542). In there open the file _**NumberOfCameras.txt.** Change the uncommented number from "**1**" to "**2**", save the file and close it. Next open _Cam1Properties.txt just to look at it. This will be set to "**1**" on new software. Close the file without changing it, and open _**Cam2Properties.txt.** If your second camera will be the same kind of camera as the one you have working, set **Cam2Properties** to "**2**". This indicates that the other camera will be the second one of that kind. For all other camera types make Cam2Properties "**1**". Now close all files and run AMT. When the first camera opens It will be labeled as "**SideMount".** Don't worry if that is not correct. You can switch labels later. Hopefully the first camera is still working as before. If not you can try changing the _**cam2Properties** number, and then try switching the _**Cam1Properties** and _**Cam2Properties** numbers. If neither of those makes it work change _**Cam1** and _**Cam2** properties back where you originally set them, and try selecting the other camera as the first opening camera. Sometimes the program will only accept the cameras in its own desired order. When one camera is working, with _**NumberOfCameras** set to **two**, you can navigate to the second camera. Always navigate between cameras by selecting that menu item on the top menu. This makes for the least problems.
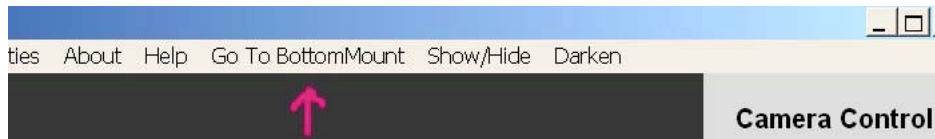
When the second AMT camera display (not necessarily the Bottom Mount) is opened, select the second camera in **"Utilities"**. Exit the second camera to activate settings and then open it again. Now the second camera should run. If not, try changing the **_Cam2Properties** number (from 1 to 2 or visa versa), or switching the **Cam1** and **Cam2** properties numbers. If it still won't work you might be forced to set both cameras to **NULL** (no camera) and begin again by selecting your second camera as the first camera to open. When you are done, if you have two of the same camera, **_Cam1Properties** and **_Cam2Properties** should be 1 and 2 or visa versa. And if you have two different cameras the numbers should be 1 and 1.

Switch Labels
If the first camera to open is the bottom mount, both cameras will have the wrong label. To switch labels open the file **C:\AMTCommon\(ConfigCam1 or Config)\SwitchCameraLabels.txt** and change the order of the labels.

Camera Settings

Now that you have your camera(s) working, they need settings for **X and Y Limits Align Offsets, Exposure Times and Gains**, besides **Calibrations** (which are discussed on the next page). If this has been an upgrade within a release (first 3 digits of the version number the same), and if you ran the install with the old **AMTCommon** in place on the C drive, then all your old settings have been incorporated into the new software and the new system is ready to use (In some cases the scope's computer also needs a communication driver update. See **"Communications"**.) For upgrades or fresh installs where you don't automatically incorporate the old settings, you need to transcribe into AMT the parameters you saved from the old software. These will be in the backup you made, **Copy of AMTCommon,** and/or in written notes you made before upgrading. If you don't have old settings to copy in you will have to set them up manually, which we discuss at later on this page.

Camera's Configuration Folder
The Configuration folder is the specific folder, within **AMTCommon**, where cameras settings are stored. In AMT versions within the 600 release, the folder name is **ConfigCam1** (for the first or only camera) or **ConfigCam2** (for the second camera). In earlier releases the configuration folder name is **Config** (for the first camera) and **ConfigB** (for the second one). Within the configuration folder will be the **Settings** folder, which contains separate folders for the various sets of settings, including **DefaultSettings**. These each contain a .txt file, which has information about Exposure Times and Gains. For the 600 release this .txt file also contains the X and Y Limits. The .bin files in this folder are background and darkfield corrections.

X and Y Limits
The X and Y Limits are the dimensions, in pixels, of your active CCD area. Your written notes from "*About -> Version"* from your old installation may contain these limit numbers. Otherwise look in the A**MTCommon** backup from the old software. If the former installation was release 600, look in the old configuration folder, in the **Settings** folder. Find the **DefaultSettings** folder and inside there, the file **DefaultSettings.txt**. The **Record Width** is the X-Limit and **Record Height** is the Y-Limit. If the earlier version was prior to release 600 (e.g. 5.4.2) look in the configuration folder for the file **InitialXXX.txt**, where **XXX** is the release number. In that text file the X and Y Limits are listed. (Ignore **InitialDefault...txt** files.)
Once you have the X and Y limit numbers, enter them into the new installation. If you do not have any record of the X and Y limits, see "**Creating Settings Manually**" below.

Entering Saved X and Y Limits Into Release 600
For release 600, open the AMT software and click the *"Set/Save"* button on the lower right of the window. At the top of the Camera Settings Control window click "*Change Camera Format*" to open **Camera Mode Configurator**.
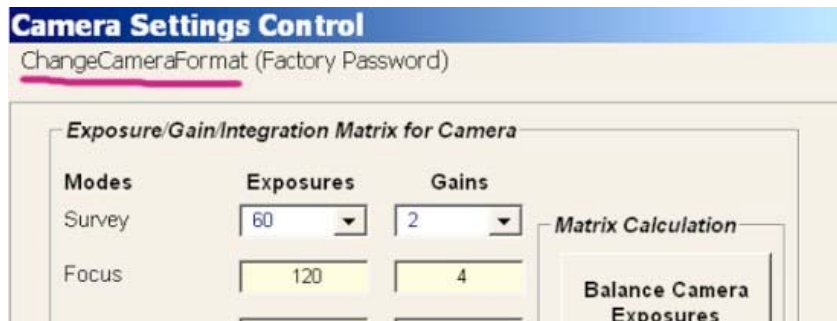
The top box in the Configurator, for the Record Mode, has the horizontal pixels (X Limit) and vertical pixels (Y Limit). Set those values to the ones you have recorded from the previous software. You can ignore the other numbers in this window.

When done click **"Accept"**, and then in the **Camera Settings Control** window click "**Save and Apply Conditions To File"** and save the changed settings as the **Default Settings**. Any new sets of settings you create, using the **DefaultSettings** as a starting point, will have the correct X and Y limits.

Entering X Saved and Y Limits Into AMT Release 542
In AMT 542 release, X and Y Limits are entered directly into the file **Initial540.txt** (in the configuration folder).

Enter the correct value under the delineator and save and close the file. The value will be used for all sets of settings.

Align Offsets

Align Offsets electronically move the selected area of the CCD chip, right or left, or up or down, on the available CCD pixels. If you are using the entire chip then there is no room to move, and changing the Align Offsets will do nothing. The purpose of Align Offsets is to help you perfectly center the camera, so that you can use the maximum possible selected CCD area, within the fixed aperture shadows of your scope. Adjustments to Align Offsets are made by clicking **"Utilities"** on the top AMT menu, then **"Camera and Optics Adjustments"** and **"Align Camera Center"**.
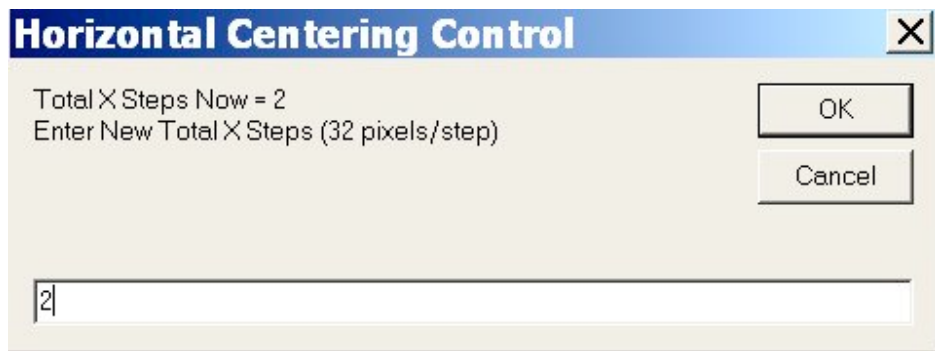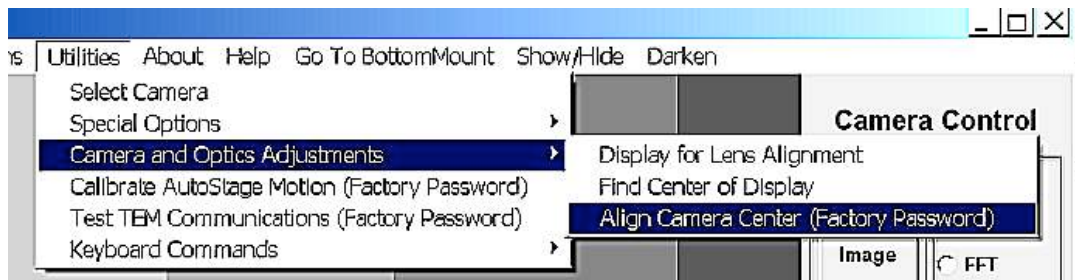
Entering Saved Align Offsets

Obtain the Align Offsets numbers from the notes you took before upgrade, or from the old **AMTCommon**. The numbers are stored in a camera's configuration folder in a file called **AlignOffsets.txt** (pre 600 releases) or **AlignOffsets2.txt** (600 release). Adjustments are done in units of 32 pixels, so a horizontal adjustment of **"2"**, and a vertical adjustment of **"-1"** in the Centering Control will be saved in the Align Offsets file as **"64, -32"**.
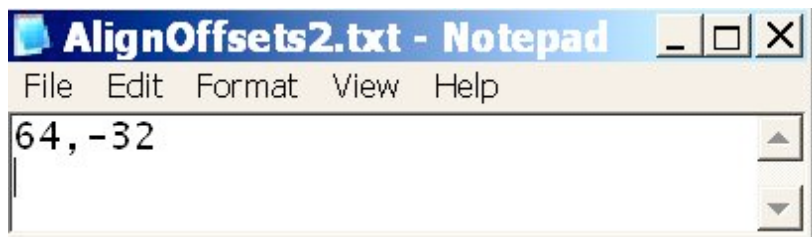
The best way to enter the numbers in the new software is to use the Center Control shown above (entering "1" for a 32 in the **AlignOffsets file**). Alternately the align offsets file can be copied into the new **AMTcommon** (making sure to rename it **"AlignOffsets2.txt"** first if the new software is in release 600).

Exposure Times and Gains

Exposure Times and **Gains** control the camera's speed and sensitivity. The **DefaultSettings** are what the program opens with, and in some labs it is all they every use. Other settings trade in speed for more sensitivity, for instance at high mags, or are used to store separate backgrounds. If you wrote down the exposure times and gains from the pre-upgrade system enter them in the new software. If you do not have a written record, get the exposure times and gains from your backup **Copy of AMTCommon**. In the camera's configuration folder, look in each folder that is inside the **Settings** folder. Each one will have a **.txt** file which has the exposure times and gains for each of the imaging modes, for that set of settings. Write down

the values for each set.

Entering Exposure Times and Gains
you can now copy the numbers into the appropriate windows in the DefaultSettings, after clicking the "Set/Save" button. When done click "Save And Apply Conditions To File" and save as "Default Settings". Click "Set/Save" again and enter the numbers for the next set of settings, which you can then save with that name. Repeat until all the sets of settings are copied in. After copying in settings from a previous installation it is usually a good idea to check them for balance, as described in this manual's chapter **"Camera Settings And Control Window -> Exposure Times And Gains".**

Doing Settings Manually
This section of for installers who do not have backup information for one or more of the settings parameters, either because it was lost, or because this is a fresh installation.

    X and Y Limits: To see how big you can set the limits in each direction set the scope mag to 12KX (or nearby). Turn the camera's background corrections off and retract the sample. Click the *"Set/Save"* button and manually set the **Black** and **White Thresholds** to **"0"**, and click *"Apply For Now Only"*. Now when you go to live imaging it will be easy to see the limits imposed on the corners by the fixed apertures. Remove the objective aperture so that doesn't confuse you. If you see a corner that might be the condenser aperture, tweak its centering to make sure that is not what the corner is. Now set the limits large, possibly use the whole CCD (see "Entering Saved X and Y Limits" above). You can see where the edges are and about how much you will have to crop. Figure it out by trial and error. Make reductions in the X and Y Limits in increments of multiples of 32. When you get all the constrained edges out of the picture save that as your Limits in the **Default Settings** (See above).

    Align Offsets: While Setting your X and Y Limits, you may be able to get a few more pixels out of the CCD by shifting things up or down or left or right with this centering tool (See Above). Alternately, you can also use align offsets to center the image by placing a sample feature at the crosshairs on the AMT image (use *"Display -> Select Center Cross"*), to compare the feature's position to that on the TEM viewing screen. After centering you may need to recheck the X and Y Limits.

    Exposure Times and Gains: To set these up use the directions in this manual's subchapter **"Camera Settings And Control Window -> Exposure Times And Gains"**. For a starting place for the **DefaultSettings** use Focus Mode. Set the exposure time for focus mode to 150 and gain to 3. (Set gain to 1 for 1K sidemounts.) Use the **"Exposure Times And Gains"** directions to balance the other modes with this focus mode standard. To create MediumMagSettings and/or HighMagSettings, start by bumping up the focus mode, and use the directions to balance the others. For SuperHighMagSettings, start with the HighMagSettings and bump up Survey and Record modes (you can ignore Focus, which won't be used there).

When you have finished transcribing in settings, or creating settings manually, recall each set of setting and collect a DarkField (*"Corrections -> Acquire A Dark Image"*).

Saving Calibrations

For calibrating measurement on a newly installed system, see this manual's subchapter **"Measurement And Calibration -> AMT Measurement -> Calibration".** For upgrades and moves, if your measurement calibration is not extensive (i.e. has only a Default calibration), if you are unsure of the calibrations' accuracy or if they are very old, you should probably do the calibration over from scratch. It is almost as easy, and they should be checked once in a while anyway. However if you have a large calibration table that you want to save, or don't have the time or equipment right now to redo calibration, you can transcribe the numbers from the previous installation into the new configuration folder. If upgrading or moving a system that uses Montaging with **AutoStage**, you will also need to copy in the stage motion calibration files (see below).

As with the other camera settings, if your upgrade is within the 542 release, and if you ran the installer with the old **AMTCommon** in the correct place on the hard drive, then your calibrations are already saved and integrated into the new software. If you already have the 600 release, and are upgrading to a more recent version of 600, you need to check the formatting of **Mags4.txt.** In the new versions **Mags4** is comma separated. If your current file has lines separated by tabs change the tabs to commas, as shown below.

Linear Measurement
In the 542 release the default calibration is in the file **Calib.txt** and the table entrees (voltage and mag specific calibrations) are in **Mags3.txt**. In the 600 release these files are replaced by **Calib2.txt** and **Mags4.txt** respectively. All these files are in the camera's configuration folder, which is described on the previous page ("**Camera Settings**"). If your upgrade is within release 542 you can copy these calibration files directly from your saved **"Copy of AMTCommon"** from the old software into the new **AMTCommon**. If the upgrade is from a 600 version to a more recent 600 version, the numbers can also be copied in without change, but make sure **Mags4.txt** is comma delineated as shown below. However if the change has been to release 600 from 542 or earlier, then the calibration files have to be renamed and refactored, besides making the file comma delineated, before being imported.

Transcribing Calib.txt to Calib2.txt
To import the default calibration from an earlier release into AMT600, first make a copy of the **Calib.txt** file (copy and paste it in the same folder). Change the name of the copy to **Calib2.txt**. The file contains one number in quotation marks. That number needs to be made smaller by five orders of magnitude (move the decimal point 5 spaces to the left) and then put in scientific notation. So, if the number you see in **Calib.txt** is " 4257.479", first move the decimal point five places to the left, so the new number is " .04257479". Then move the decimal point to the right side of the first non-zero digit, keeping track of how many place it moved to get there. The number in scientific notation will be " 4.257479E-2", which means "4.257479 times 10 to the minus 2". E and a minus number means you would have to move the decimal point to the left to get the number back to it's non for scientific notation status. E and a positive number means you would have to move it to the right. When you have the changed and reformatted number in **Calib2.txt**, you can copy that file into the camera's new configuration folder to be the default calibration.
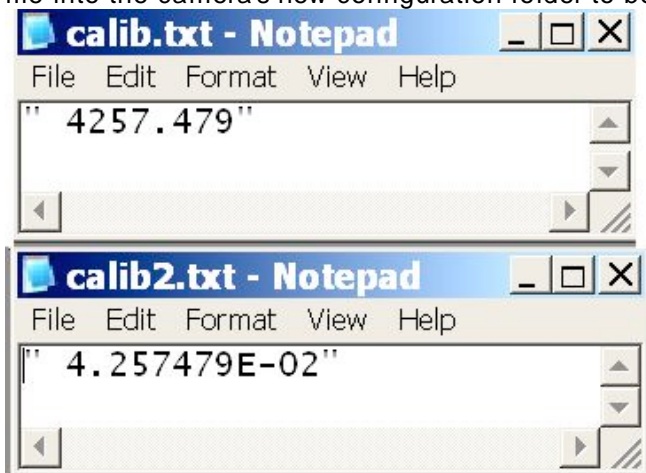
Transcribing Mags3.txt to Mags4.txt
For the table entry calibrations the process is very similar. First open your old **Mags3.txt** file to see if it looks reasonable. Each line is supposed to have a voltage, a magnification and a mag factor, with the numbers separated by commas or tabs. If there are many lines with all zeroes, the file is corrupted and cannot be

used. Make a copy of **Mags3.txt** in the same folder and rename it to **Mags4.txt.** Open **Mags4.txt** and start by making sure the separating units in the lines are commas, not tabs. On each line, wherever you see a tab separating numbers, delete it and replace it with a comma. On each line, the rightmost number is the mag factor. Reduce each of the mag factors by five orders of magnitude and put them in scientific notation as described for **Calib2.txt**. When **Mags4.txt** has the correct numbers and formatting it can be copied into the cameras new configuration folder.
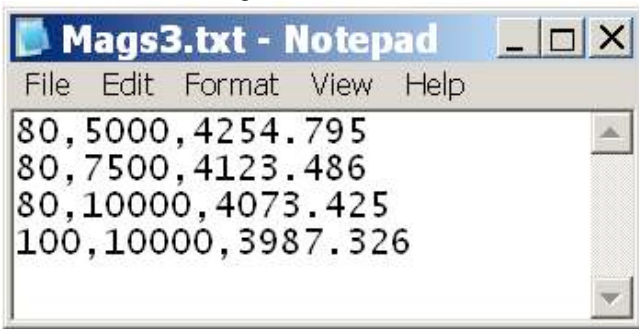
An Old, Tab Delineated Mags4
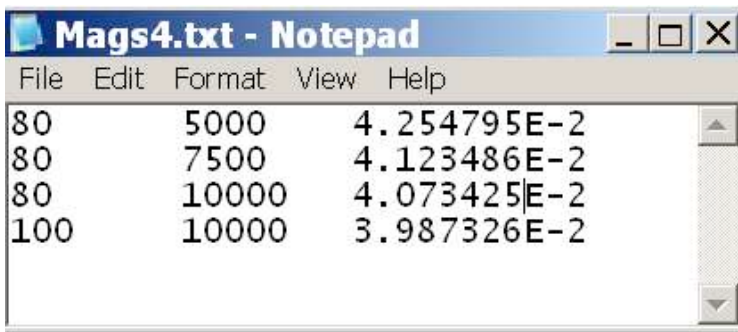
A Comma Delineated Mags4
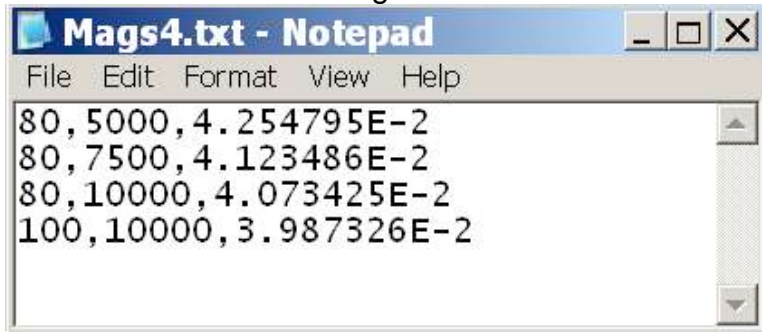
Diffraction Measurement
The files in the camera's old configuration folder that have the Default diffraction calibration and the Table Entry diffraction calibrations are **DiffCal.txt** and **CamConst.txt** respectively. The format for **Diffcal.txt** has not changed. Therefore that can be copied into the new configuration folder without change, whether the upgrade is to the 545 or 600 release. **CamConst.txt** also does not need any number changes, but make sure the file is comma delineated. Then it can also be saved and copied into the new configuration folder. Both file names will remain the same.

Stage Motion Calibration
The state motion calibration files are **Rangle0.txt**, **Rangle2.txt** and **StageXYcal.txt**. These can be copied into the new configuration file without change. However it might be helpful to save the new configuration folder's originals of these files, with a changed name, for comparison in case adjustments are needed.

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Camera Drivers

Camera drivers need to be in place before AMT can be used with a camera. An upgrade of AMT software often requires that camera drivers be updated also. Before upgrading, contact AMT about which drivers you need.
This subchapter is designed to assist with installing and upgrading the drivers. As with the AMT software, most camera drivers are available on the AMT website, www.amtimaging.com.

The drivers AMT distributes are intended to run on computers with Windows XP or Windows 2000 as an operating system. If you have W2000 rather than XP mention that when asking about drivers, since it could make a difference in the driver version.

Contents
HamDCAM
DVC
ICPCI
IFC
Optronics
EDT
ActiveDCAM

****

HamDCAM Drivers

Download and Unzip
Almost all Hamamatsu cameras now use the **HamDCAM** Drivers, which are labeled as such on the AMT Downloads page. After downloading them, unzip the package with **WinZip**, which has a free trial version available on **www.WinZip.com**. If your operating system is Windows 2000, you may need an earlier version than what is posted.

Get A Baseline
If this is an upgrade to a working system, take a look at Windows' **Device Manager** to get some starting information. If you have *"Imaging Devices"* on the Manager's top level, look in there for either a camera or an Active Silicon board. PC-Dig boards and ICPCI boards show up under *"Sound Video and Game Controllers"*. A firewire board is also labeled "1394". Right click the device and click *"Properties".* See what you can find out about the drivers - Hamamatsu versus other, Driver Date and Driver Version. Write down what you find. If the system has two similar cameras, find out which one is which in the **Device Manager** by turning one off. This information will help you select the right modules during installation, and will tell you when the drivers have been updated. It can also be useful diagnostic information if there's trouble.

Uninstall Obsolete Software
In Windows' Control Panel, select *"Add Remove Programs"*. Uninstall any **Hamamatsu** programs and any **HamDCAM** or **DCAM** programs. Also uninstall any versions of **IFC** that you see there (*"IFC"* followed by two or three digits). When unistallation is done restart the computer. Do not uninstall hardware in the Device Manager. Having hardware installed there will facilitate updating the drivers.

Install HamDCAM
During installation, on the **"DCAM-API Setup"** page, take note of the date of the installer. Run the downloaded **HamDCAM** installer, taking care to select the correct module for your hardware, "*IEEE 1394"* for firewire, *"Active Silicon Phoenix"* for Phoenix boards and *"Coreco IFC Module"* for PC-Dig boards. When the installation is done restart the computer. Find your device in the **Device Manager** and check the driver. Usually the driver will now be updated and ready to go. If not, right click the device and click on *"Update Driver"*. When the **Hardware Update Wizard** appears, choose *"No, Not This Time"* for the question about contacting Windows. Click *"Next"* and then choose *"Install From A List Or Specific Location"*. Click *"Next"* and uncheck the box for searching *"Removable Media"*. Check the box that allows browsing. Browse to **C** drive and to the folder **"Windows"** or **"WINNT"**. Inside that folder go into the location **"System32\DCAMAPI\Setup"** and in there find the folder that matches your hardware. Complete the Wizard. Go next to the "**AMT Software**" subchapter.

DVC Drivers

The link to **DVC** drivers is labeled *"XR-41 and DVC Drivers"* on the AMT Download page. After downloading them, unzip the package with **WinZip**, which has a free trial version available on **www.WinZip.com**. **DVC** cameras use a range of framegrabber boards including Gig-E and Firewire (both covered on this page), EDT (see the EDT page) and PC-DIG (see IFC).

Firewire Cameras
If upgrading **DVC** first find your device in the **Device Manager** and take some notes about the driver, so you can see the change when it is updated. Uninstall the old **DVC** program first ("Control Panel -> Add Remove Programs"). Also delete the **DVCco** folders from the hard drive. There's one on the top level on the **C** drive and one in **C:\Program Files**. Leave your device installed in **Device Manager**. Restart the computer before installing the new **dvcInstall.exe** (the other installers in the package are for GigE only). To start the **Hardware Wizard** find your device in the **Device Manager**, right click, and click *"Update Driver".* In the **Wizard** choose *"Install From a list or specific location"* and on the next screen choose *"Don't search, I will choose driver"*. The camera should be clearly identifiable in the list. If you don't see it in the list, back up one screen and browse to the driver. It will be in the folder **C:\DVCco\Drivers**.

GigE Cameras
If your DVC camera uses an Ethernet connection to transfer image data then it is **GigE** ("Gigabyte Ethernet"). The data path is designed to bypass the operating system, to transfer data faster. Therefore when it is configured, that Ethernet card will <u>not</u> show us as a Network Adapter in the **Device Manager**. Rather it will appear under the category **"Pro 1000/Grabber Devices"**. All of AMT's GigE installations use the **Intel Pro1000** adapter, so that the fastest **iPORT** drivers can be used.

Upgrading An Existing GigE Driver
Find your Pro 1000 device in the **Device Manager** and take notes about the driver version and date, so you can tell when it is updated. Leave that device in place. In *"Add/Remove Programs"* uninstall the DVC software. Also uninstall the **iPORT** program. Delete the two folders named **"DVCco"**. One is on the top level on the **C** drive, and one is in the **Program Files** folder. Restart the computer and run the new **dvcInstall.exe**. Also run the **iPORT_IPEngineSDK** installer. During the installation of **iPORT** select the *"Runtime"* type. If the device becomes uninstalled in the **Device Manager** see the directions for Installing a new **GigE** device. Now proceed to "**GigE Driver Update"**.

Installing a new GigE Device
First look in the **Device Manager** at what Network Adapters are already in place, so you know which one is the new one. Power off and unplug the computer and install the Gigabit Ethernet card. Touch the computer chassis first to discharge static electricity. On restarting, if the **Hardware Installation Wizard** appears, allow it to automatically install the card's drivers. You will change them later. Run the new **dvcInstall.exe** and then the **iPORT_IPEngineSDK** installer. During the installation of **iPORT** select the *"Runtime"* type. If the new card does not appear in the **Device Manager**, run the installer **PRO2KXP.exe**, which is in the package you unzipped. This should install the card as a Network Adapter. In rare cases it has been necessary to temporarily disable the <u>onboard</u> Network Adapter during GigE installation (using the computer's BIOS), to get the new card installed. Now proceed to "**GigE Driver Update".**

GigE Driver Update
Once your card and your software are installed, click on *"Start -> All Programs"*. In the Programs menu find the category "*Pleora Technologies Inc"* and select *"eBUS Software -> Ethernet Bus Driver Tool"*.
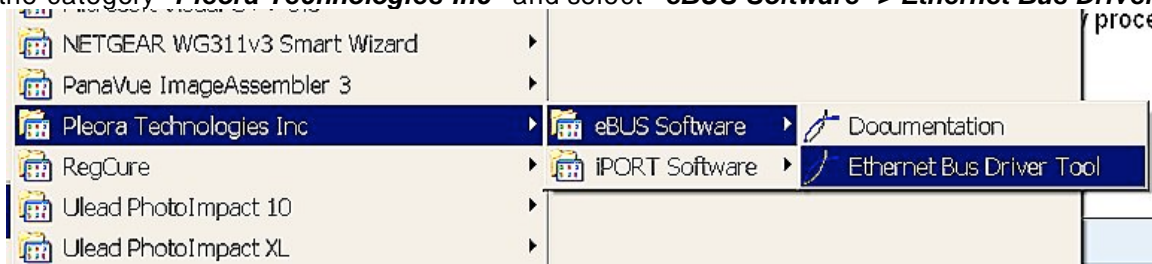
This will open the **Driver Installation Tool** window. In the upper window select the Network Adapter used for the camera. Then click *"Configure"*.
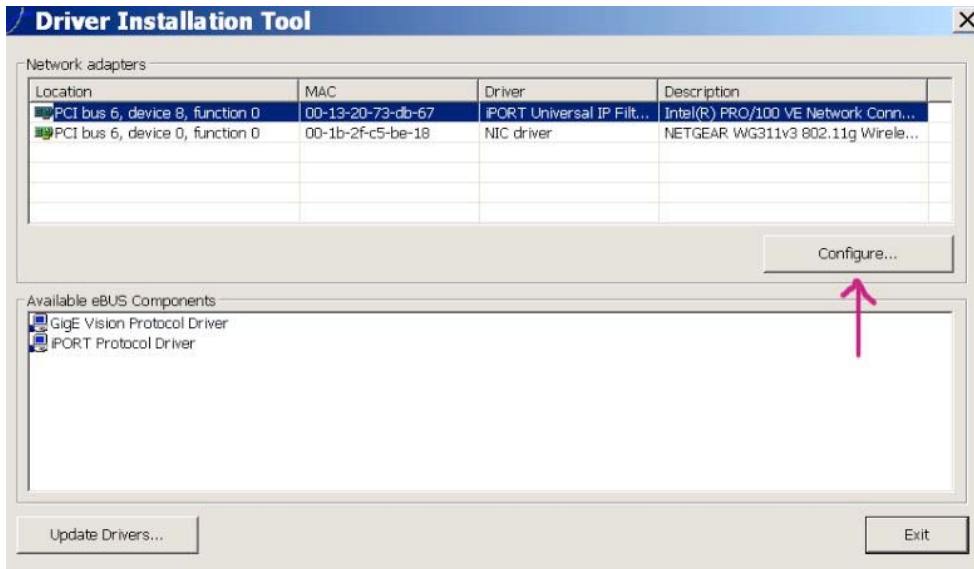
This will bring up the **Network Adapter Configuration Wizard**. Choose the **iPORT Family Of Drivers**. Then click **"Next".**
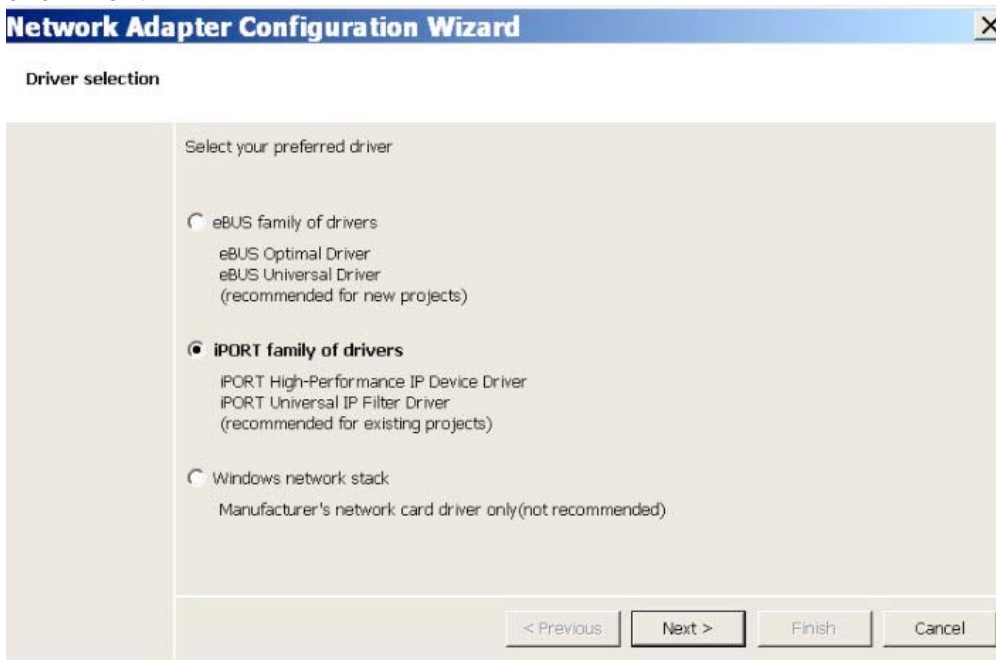
In the Wizard's next window choose **"iPORT High-Performance IP Device Driver".**

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Click **"Finish"** to continue.
In the **Driver Installation Tool** window click **"Update Drivers"**. This will open the **Update Drivers** window.
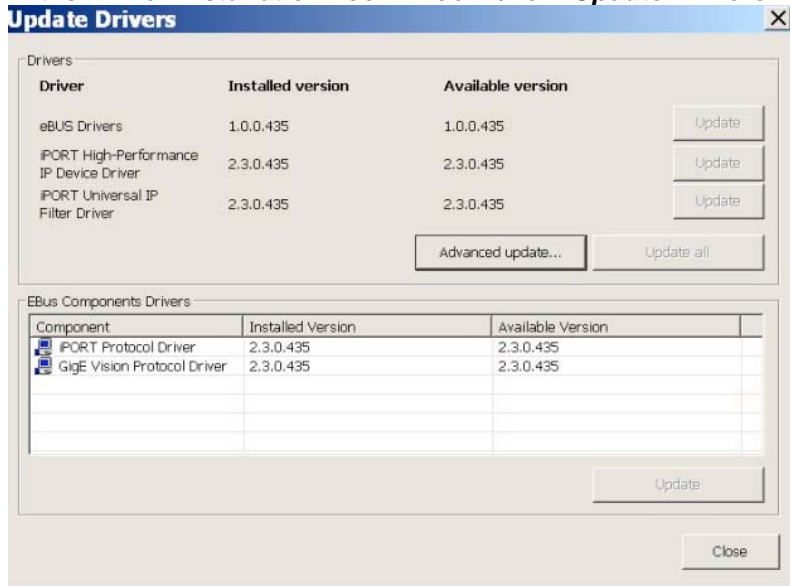
If the iPORT High-Performance Driver's "Installed version" does not match it's "Available Version", click that row's **"Update"** button. Close the window. The computer will now need to be restarted. After restart open the **Driver Installation Tool** again to confirm that the drivers are updated. Look in the **Device Manager** to make sure that the **Pro 1000 Grabber Devices** is installed and functional.

Cable Up and Power On Camera
After cabling up the camera switch on its power supply. After about a minute both LEDs on the camera should light up as green, and one should be flashing on and off about once a second. An amber color means the connection does not support a gigabit transfer rate.

"Find" The Camera, To Fix Its Address
To fix the camera's address run the program **"Start -> All Programs -> DVCco -> GigE Camera Finder"**. This only needs to be done the first time you run the camera. The address fix is stored in one of the **DVCco** folders on the hard drive. Check that the first three numbers of the Camera IP match those of the Adapter IP. If necessary change the Camera IP and click **"Reset IP"**.
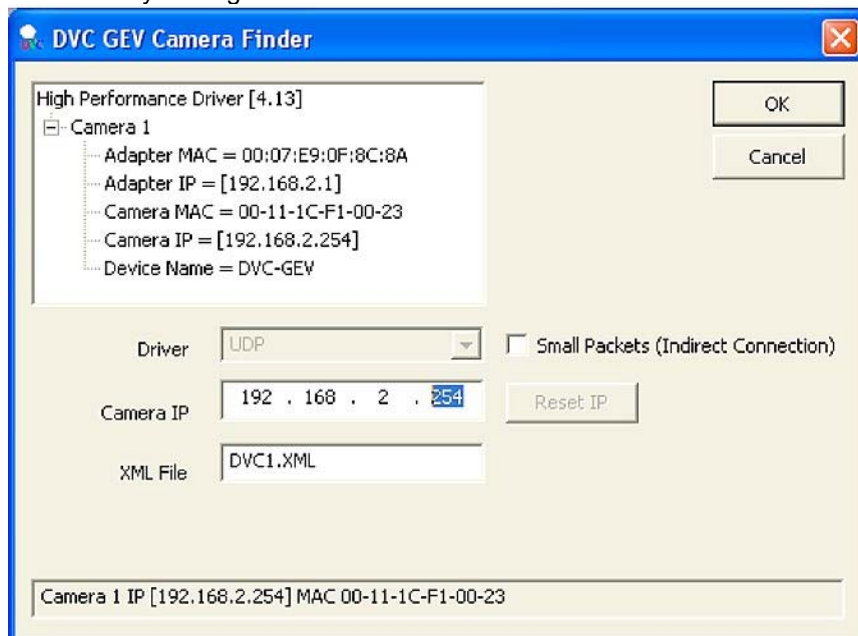
The camera is now ready to work with AMT. It will also work with **DVCView** (for diagnostic purposes).

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ICPCI Drivers (ITEX41)

The **ITEX41** package of drivers is for all cameras that use an **ICPCI** board as a frame grabber. These are the only drivers that those cameras need. The **ITEX41 Drivers** are posted on AMT's download page. After downloading them, unzip the package with **WinZip**, which has a free trial version available on **www.WinZip.com**.

Avoid Generic Drivers
If you find that your **ICPCI** board is installed in the **Device Manager**, automatically or otherwise, <u>before</u> the **ITEX** drivers or its patches are installed, then the board has the wrong drivers. Make a note of the driver's name and location, and then use the **Device Manager** uninstall the board. Find the generic drivers and rename them, so they don't interfere.

Install ITEX
Run the **ITEX** installer, and select *"ICPCI Board"*. Other than that selection, accept the defaults during installation.

Copy In Patches
For **ITEX** to work properly on **Windows 2000** and **Windows XP** there are two files that have to copied onto the computer's hard drive. There are separate versions for the two operating systems, so be sure you have the right one. They are both located in the package that you unzipped. You will copy these files into folders within the **Windows** folder. If these folders already contain files with the same name, rename them to keep them separate.

itipci2k.inf   Should be copied into the folder   **C:\Windows\Inf**
mvcntp.sys   Should be copied into the folder   **C:\Windows\System32\Drivers**
Install the board
In the **Hardware Installation Wizard** choose *"Install the Software Automatically"*.

IFC Drivers

Download and Unzip
A few of the cameras with PC-DIG boards cannot use the HamDCAM drivers and must use IFC instead. These include AdvantagePlus (KodakES4.0) and certain DVC cameras. Use the latest version, IFC5.10, which is labeled as such on the AMT Downloads page (**www.amtimaging.com -> Support -> Click Here To Start Downloading**). After downloading, unzip the package with **WinZip**, which has a free trial version available on **www.WinZip.com**.

Get A Baseline
If this is an upgrade to a working system, take a look at Windows' **Device Manager** to get some starting information. PC-DIG boards show up under *"Sound Video and Game Controllers"*. See what you can find out about the drivers - Driver Supplier, Driver Date and Driver Version. Write down what you find. If the system has two similar cameras, find out which one is which in the **Device Manager** by turning one off. This information will help you select the right modules during installation, and will tell you when the drivers have been updated. It can also be useful diagnostic information if there's trouble.

Switch Off Power to The Camera
Turn it back on after the new board drivers are in place.

Uninstall Obsolete Software
In Windows' Control Panel, select *"Add Remove Programs"*. Uninstall any old IFC programs (*"IFC"* followed by two or three digits). When unistallation is done restart the computer. Do not uninstall hardware in the Device Manager.

Install IFC5.10
Run Setup.exe in the IFC510 package. Click *"Run"* if given a warning about an "Unknown Publisher". The installer will present a page to "Select Components". PC-DIG is the only one that needs to be selected. Reboot the computer.

Install The Device Drivers
Find your device in the **Device Manager**, right click and select *"Update Driver"*. When the **Hardware Update Wizard** appears, choose *"No, Not This Time"* for the question about contacting Windows. Click

"Next" and then choose *"Install From A List Or Specific Location"*. Click *"Next"* and in the next window choose *"Don't search. I will choose the driver to install"*. Click *"Next".* In the next window choose the **PC-DIG** device and finish. The process may repeat for the two serial ports connected with the PC-DIG board. There is more detailed driver installation notes available in *"Programs -> IFC 5.10 Runtime -> Release Notes".* The notes file is "**IFC510_00.doc**", which is in **C:\IFCRuntime** and is also on the CD .

Optronics Drivers

The Optronics camera is also known as the "XR-40". The **XR-40 Drivers** program (named **"PictureFrame"**), is posted on the AMT download page. After downloading them, unzip the package with **WinZip**, which has a free trial version available on **www.WinZip.com**.

Check For Upgrade Version
If upgrading the **Image Capture Engine**, contact AMT to see if you also need an upgrade of **PictureFrame**.

Install PictureFrame
First go to *"Add/Remove Programs"* in the **Control Panel** and uninstall the old **PictureFram**e version. Leave the device installed in the **Device Manage**r. Restart the computer and install **PictureFrame**.

Install The Camera
Find your device the **Device Manager** and click *"Update Driver"*. In the **Wizard** choose *"Install From a list or specific location"* and on the next screen choose *"Don't search, I will choose driver"*. The camera should be clearly identifiable in the list. If you don't see it in the list, back up one screen and browse to the driver. The main part of its name will be **"MacroFire"** and it should be in the folder "S**ystem32"** in **"Windows"** or **"WINNT"**.

EDT Drivers

Use these drivers for cameras that use the EDT CamLink Board. Currently this includes AMT 16000 PIACON/Redlake Cameras, TR111, TR160, TLD111, TLD160 and some DVC cameras. These have gone through many changes, so be sure to check with AMT to make sure you have the correct one for your camera. For DVC cameras download the *"XR-41 and DVC Drivers"* from the AMT Download page. This installation then has a link to the EDT drivers which are also needed. For the other cameras use the EDT CD supplied by AMT.

Installation Procedure

Caution:
After installation the EDT cable MUST plug into **Port #1** on the EDT Framegrabber board.
Be sure that the cable in plugged in and then secured with the screws. Do not use the screws to pull the plugs into place - this often results in a poor connection. For bottom mounts, leave the cables wrapped around the camera head and held there with a cable tie.

1. Uninstall old versions of both DVC and EDT software. Delete the folder **"DVCco"** from both the **"C-drive"** and the **"Programs Files"** folder.
2. If the board is not installed yet, leave that out until the software is installed. If it is installed, find it in the **"Device Manager".** It will appear under **"System devices"**. Record what information you can about the driver (date, version, etc.) to help you know when you've updated it.
3. **For DVC Cameras:** Run the DVC software installer. Then in the folder **"C:\DVCco\Drivers\CameraLink"** find the link to the EDT driver installer and download it. If this PC is not networked you can paste this url in the browser on another computer - **"http://www.dvcco.com/software/drivers/cameralink/windows/EDTpdv.exe".** When you hit "Enter" a download screen will appear behind the browser. After EDTpdv.exe is downloaded, install it on the camera computer. Ignore the warning about "Unknown Publisher".
4. **For All Other Cameras:** Insert the EDT CD and run **Winsetup.bat**. Ignore the warning about "Unknown Publisher". Select *"PCI DV"* and accept the defaults.
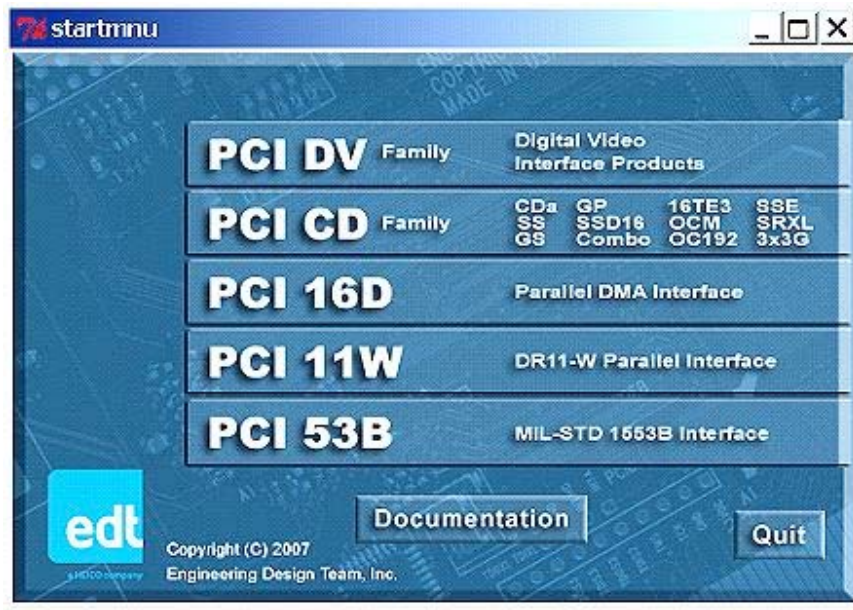
5. Now install the board and start the computer. If the board is already installed at this point, find the device in the **"Device Manager"** and uninstall it. Then restart the computer. The Hardware Installation Wizard will appear.
6. In the Wizard click **"No"** for contacting Windows. In the next screen choose **"Install from a list or specific location"** and in the next window choose **"Search"**. Browse to *"C:\edt\pdv"*. The Wizard is looking for the file **pdv2k.sys**. Finish the Wizard.
7. Check in the Device Manager to make sure it sees the EDT Pdv c-link board. Cable up the camera and turn on its power. On RedLake cameras, the power light will turn green, the status light will flash green and the data lights will turn green. On the DVC's with LEDs, both will turn solid green and then one will start to flash on and off.
8. You are now ready to configure the AMT software.

ActiveDCAM Drivers

USE
The XR-42 camera uses ActiveDCAM drivers and a FirewireB board.

Documentation
Detailed installation instructions are available from AMT.

Board Speed
In order for the camera to work properly, the FirewireB board must be set to run and 800 MBPS. For Windows XP systems with Service Pack 2 or 3, the Microsoft **"Hotfix"** mentioned in the instructions is Not required. However the "**Rollback**" of the drivers is necessary.

Resetting Camera
Since this camera does not have a separate power supply to recycle, resetting the camera is done by rebooting the computer.

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Communications

"Communications" is where the scope's computer tells the AMT computer its mag and KV. This sub chapter discusses preserving communications during upgrades and setting up communications for the first time.

Contents

AMT Side Configuration
HyperTerminal
Scope Side Configuration

****

AMT Side

Basic Configuration

These directions are for AMT camera's that have their own separate computer. For Integrated systems, or systems where the scope and camera share a computer, contact AMT for directions. The first thing to do on the AMT side for communications is to make sure the correct ComPort is selected in the software. That assignment is made in the camera's configuration folder in the file **"SerialTEMPort.txt"**. Once that is done select the communications protocol by clicking ***"Utilities -> Special Options -> TEM Communications"*** and select your microscope in the window that opens. Restart AMT to incorporate the change. If communications doesn't work at that point you can check the connection using **"HyperTerminal"** (See next page).

Select Microscope

Select Your Microscope

- ○ Hitachi External Control H-7500 H-7600 H-7650
- ○ Hitachi H-7600
- ○ Hitachi H-7100
- ○ Hitachi H-7650
- ○ Hitachi H-9500

- ○ JEM 2010
- ○ JEM 1230
- ○ JEM 1210
- ○ JEM 1010
- ○ JEOL 1011
- ○ JEM 1011UK
- ○ JEM 1400
- ○ JEM 2100/2200
- ○ JEOL 3010 Native
- ○ JSM 7001

- ○ External Tecnai
- ○ Internal Tecnai
- ○ Morgagni
- ○ CM Series With Stage Ctrl
- ○ CM Series With No Stage Ctrl
- ○ EM400/410

- ○ Leo/Zeiss

- ⊙ None of the above

Select and Close Form

H7650

If communications is with an H7650 scope, another text file <u>may</u> need to be created and saved to the camera's configurations folder. The file is **"TEMComSetup.txt"** and it specifies the baud rate, parity, data bits and stop bits. It sets those parameters on the AMT side to match those used by the scope computer's comport. The text that usually appears in the file is **"9600, N,8,1"** <u>including</u> the quotation marks. On the scope's computer, look at the comport setup in the menu **"Maintenance\TEM adjustment"** to see what parameter numbers are being used.

For almost all of the H7650s and the H7600s, the correct choice for communications is the triplet **"Hitachi External Control H-7500 H-7600 H-7650"**.

FEI Morgagni, 208 and CM

To communicate with FEI Morgagnis and 208s, the AMT computer uses another utility called **"RS232Remote"**. For CMs the extra utility is **"Remote Control Driver"**. If your AMT upgrade is for one of these scopes, contact AMT to see if the communications utility needs to be upgraded.

Incorporating Settings Automatically

If your upgrade is within a release (first three digits of the version number are the same), and if you run the install with **AMTCommon** in place on your computer's hard drive, then the communications settings are preserved and communications will usually resume working at this point. If it doesn't the scope's computer may also need a communications software update (See the page **"Scope Side"**). Check with AMT about needed communications package upgrades before upgrading AMT. If you get to this point and communications won't work, turn them off temporarily using the window pictured above. You can also use **"HyperTerminal"** to check the connection (See next page).

Transcribing In Settings

The information you need to configure the AMT software for communications are the TEM Communication <u>type</u> and the <u>number</u> of the serial port used for TEM communications. If this information is not in your written notes it is in the backup **"Copy Of AMTCommon"** that you made (and possibly renamed). The TEM Communications type usually just goes by the scope name, but there are occasionally exceptions. Look in the camera's old configuration folder at the file **"InitialXXX.txt"** (where **XXX** is the release number) and find the category **"[TEMcom]"**. The option under that heading, without a pound sign in front of it, is the selected one. In the same folder look for the file **"SerialTEMPort.txt"**. The number in that file is the number of the serial port that the old software used for communications. Transcribe that number into the **"SerialTEMPort.txt"** in the new configuration folder. If you don't have a backup of the serial port number, you can see which ports you have, in the **Device Manager** and try them in order. If you need to confirm a connection, you can use *"HyperTerminal"* (See next page). Cameras on some of the H7650s also have the file **"TEMComSetup.txt"** in their configuration folder. If you find one in the old configuration folder, copy it into the new folder.
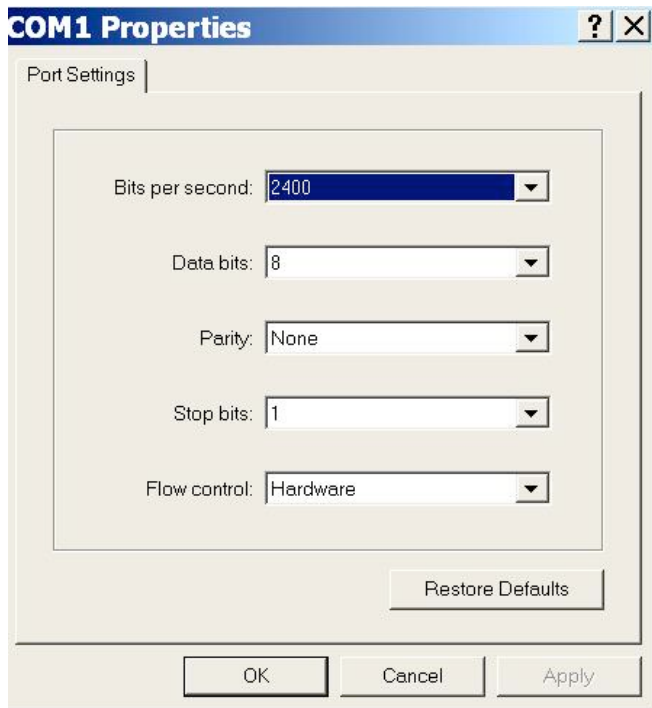
HyperTerminal

Running Windows' **"HyperTerminal"** accessory on both computers simultaneously allows you to check whether a connection is live, and find out which Comports are used.
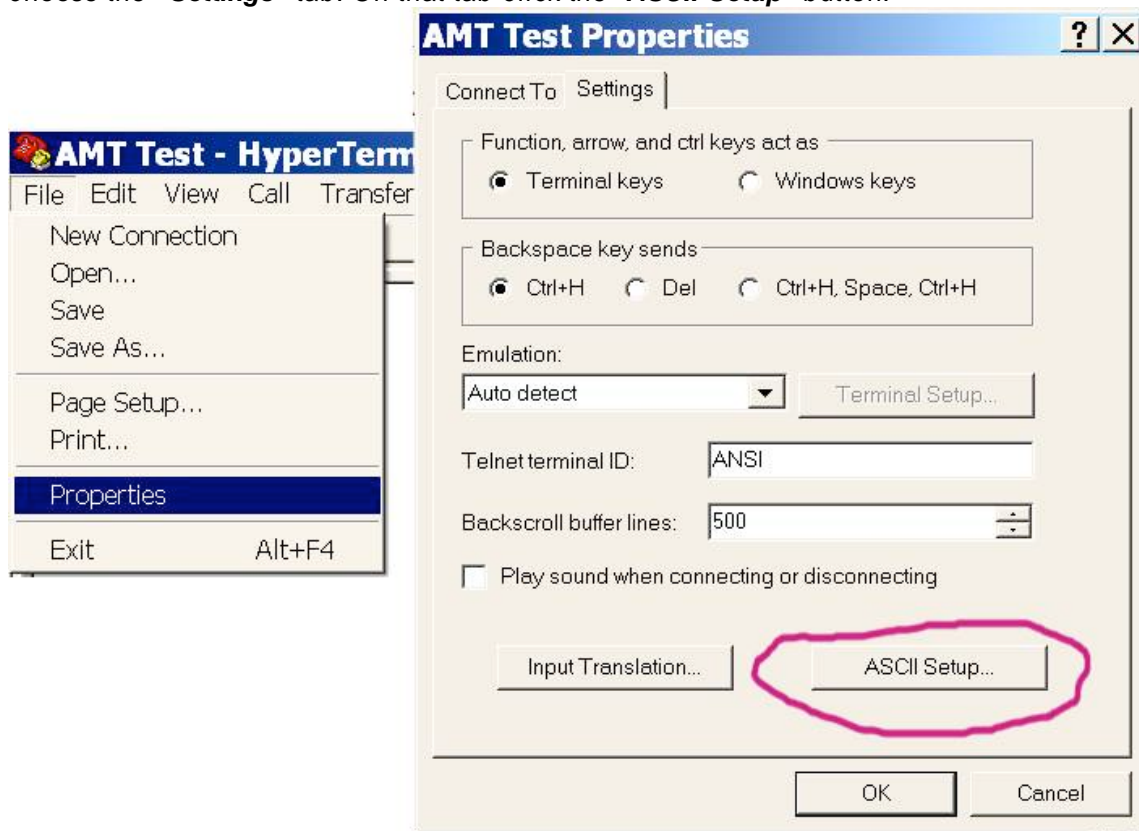
Setting Up HyperTerminal
This test requires that no other program is trying to use the specified Comport to communicate, so turn off AMT and on the scope side, turn off the user interface to the scope. The path to open the HyperTerminal program is **"Start -> All Programs -> Accessories -> Communications -> HyperTerminal"**.
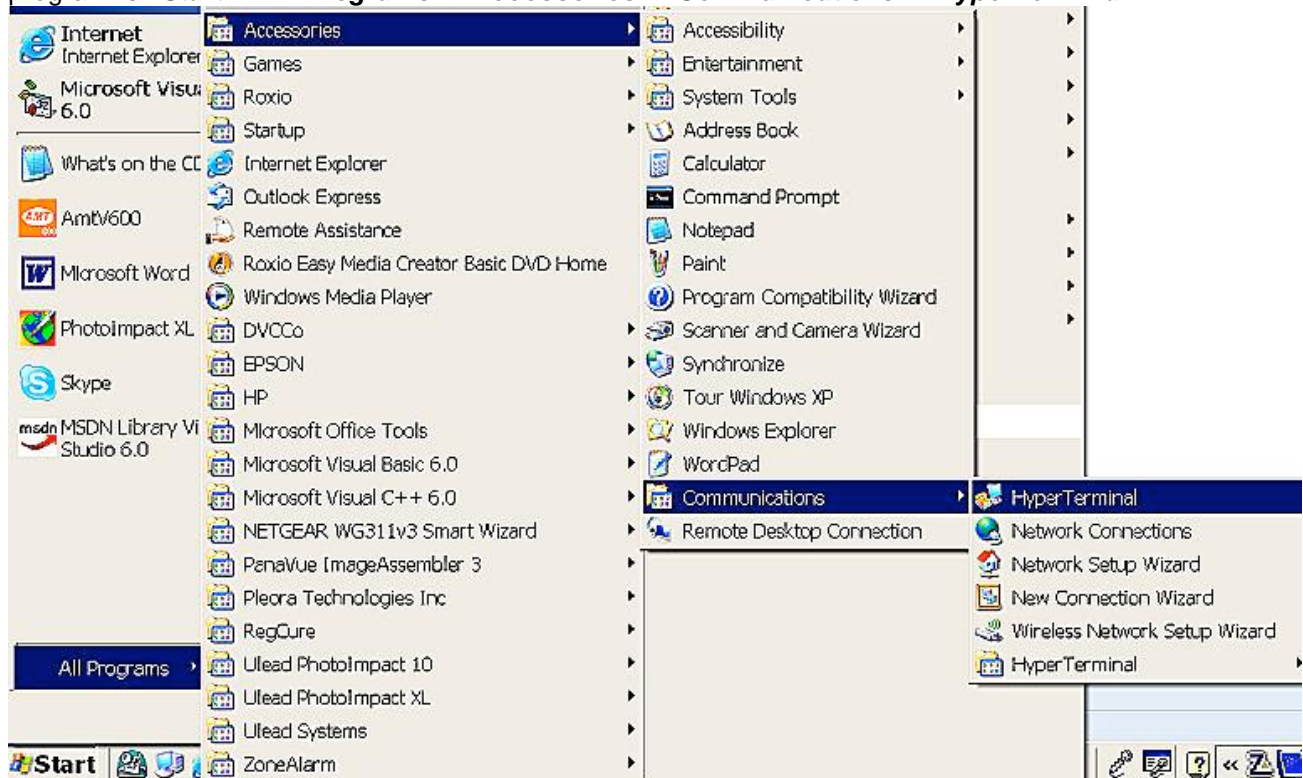
 When the program opens it will ask about making HyperTerminal a default program, to which you can say **"No"**. It will ask for a name for this connection. Anything works. Then you choose which comport to use. If it asks for an Area Code, just make one up. Click **"OK"** to continue. When the **"Port Settings"** tab comes up accept the default values ("2400", "8", "None", "1", and "Hardware"). Just make sure both computers' are using the same values. Click **"OK"** to continue.

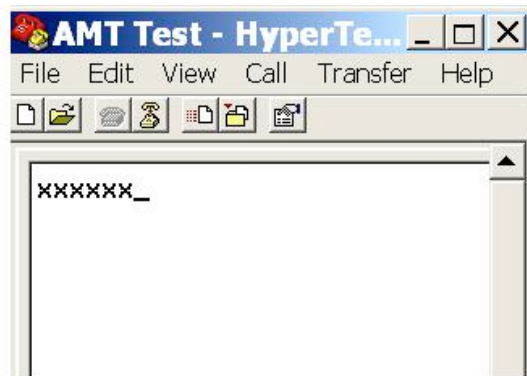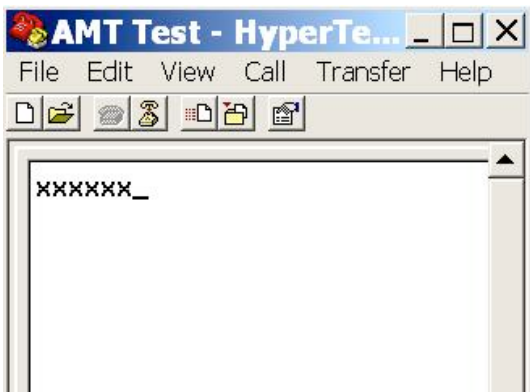On the top menu of the **HyperTerminal** window, click *"File -> Properties",* and in the window that comes up choose the *"Settings"* tab. On that tab click the "*ASCII Setup"* button.

Check all the rows on the **ASCII Setup** page. Click *"OK"* to continue.

ASCII Setup

ASCII Sending

☑ Send line ends with line feeds

☑ Echo typed characters locally

Line delay: 0 milliseconds.

Character delay: 0 milliseconds.

ASCII Receiving

☑ Append line feeds to incoming line ends

☑ Force incoming data to 7-bit ASCII

☑ Wrap lines that exceed terminal width

OK   Cancel
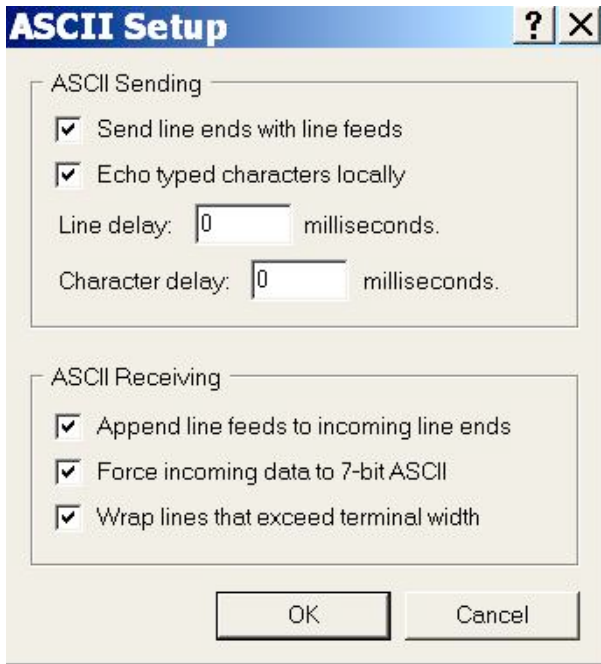
Now, if the computers are connected by the specified ports, text typed into one of the windows will show up in both.

AMT Test - HyperTe...

File Edit View Call Transfer Help

xxxxxx_

AMT Test - HyperTe...

File Edit View Call Transfer Help

xxxxxx_

Scope Side

Communications software on the scope's computer is usually turned on and configured by the scope engineer, and is not usually a user function. The two exceptions to that rule are described here.

EXT 1 and EXT 0
For some JEOL scopes communications are turned on and off by typing **"EXT 1"** **[Enter]** and **"EXT 0"** **[Enter]** (including the space) respectively, into the scope's input panel. The AMT computer may have been doing that for you, but if the sequence gets interrupted, say by a power outage, you will have to manually set it to zero. Whether it is done manually or by AMT you can see it being entered on the JEOL monitor.

FEI Tecnai
The Tecnai is the one scope where AMT regularly adds and does upgrades to a communication program on the scope's computer. If doing an upgrade of AMT, contact AMT to see if the scope's **"ExtTecnai"** program need to be upgraded also. As a diagnostic test, you can see if **ExtTecnaiG2** is running by looking in that computer's **"Task Manager"**. Open by pressing the keyboard's **"Ctrl"**, **"Alt"** and **"Delete"** keys. **ExtTecnaiG2** should be running whenever the computer is running.

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AMT Support

The first section has contact information for applications support and camera repair. The section briefly describes software upgrade via website downloads.

Contents

Applications Help, Repair and Problem Solving
Software Upgrades

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Applications Help, Repairs and Problem Solving

Please call at: Advanced Microscopy Techniques
                      3 Electronics Avenue
                      Danvers, MA 01923 USA

Or use our website: http://www.amtimaging.com for further information.

You can reach us in person at (978) 774-5550 or fax us at (978) 739-4313

Our e-mail address is: info@amtimaging.com

Upgrading AMT Software

Most of the time software updates can be downloaded AMT's website for free. Please contact us before upgrading software for advice and for sign-on information. Our phone number is 978-774-5550 or email us at info@amtimaging.com.

You must have Administrative privileges to install this software on your computer. If you are not familiar with this or WinZip or install procedure, please have your "IT" person or local computer guru contact us.

It is also possible to send us your computer for new software installation for a nominal labor charge.

For more information about upgrading software, see this manual's subchapter **"Software -> AMT Software".**