

Everyday Procedures for CCD Imaging

***Version 5 rev.3
8Nov03***

Introduction

AMT's CCD based camera system for the transmission electron microscope is designed to provide an efficient means of producing high quality digital images. The purpose of this manual is to provide a standard scenario for routine operation.

The procedures shown are one set of many possible variations and more operations are available to users who wish to learn more intricacies of the application.

“Everyday” Procedure Summary

Setup:

- 1) Align TEM and center beam.
- 2) Acquire a Background Image
- 3) Set up a “Case” folder for saving images

Take Images

- 4) “Click for Live Image” to view specimen
- 5) “Click for Final Image” to acquire a quality, full resolution image.
- 6) Click on “Case” to Annotate and Save Images to the Folder
- 7) Modifying Exposure and Display Settings

Finish Up and Save to CD-R

- 8) Set up another Case or Close AMT Software
- 9) Write to CD-R
- 10) Printing Images
- 11) Measurements and Calibration

Contact Information and Help

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Setup

1) Align TEM and center beam:

Users will probably be more comfortable aligning the TEM using the standard TEM viewing screen and local alignment procedures. On side mounted systems be sure that the phosphor is retracted, so that the beam is visible!

In Manual Systems: Turn the wheel clockwise to the end position.

In Pneumatic Systems: Flip the switch away from column on retracting mechanism.

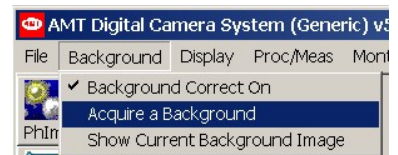
For H7500/H7600: If the AMT Camera Controller is running click on the “**Screen Out**” button on the top of the control panel on the right.

- 2) Camera operation is easiest when the “current axis” is aligned and the intensity remains centered as magnification changes.
- 3) If the TEM supports “Intensity Zoom,” use it.
- 4) The camera operates best at intensities equivalent to about _____ sec of film exposure.

Background Collection

Background correction is a very important software function that compensates for various phosphor inhomogeneities and optical imperfections. Background images need to be collected for both side-mounted and bottom mounted systems each day or whenever there is a change in the gun high tension. [Background images may need to be collected more often for retracting systems as mechanical drift can occasionally occur.]

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 between 2000x and 5000x and spread the beam with the condenser control so that the current is about _____ **sec** on the film exposure meter.

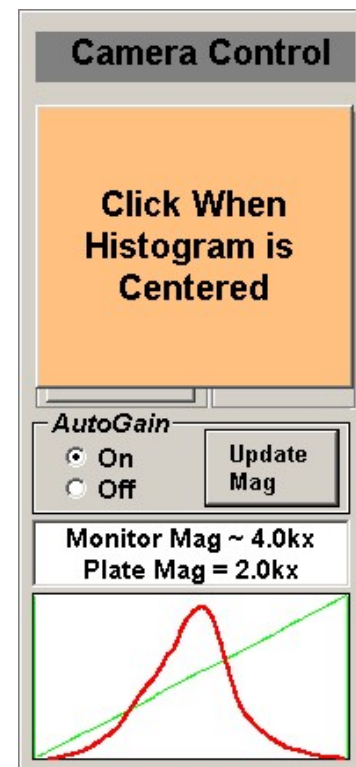
5) For side mounts insert the phosphor. (For bottom mounts lift the viewing screen.)

6) Select the Menu Item **Background - Acquire Background**

7) Adjust the second condenser (i.e. the TEM Intensity or Brightness knob) so that the histogram [red line in box] is **approximately centered** in the "box". When this is correct, click on the orange command button.

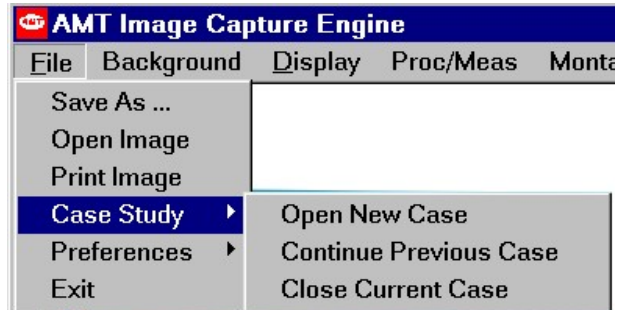
8) You will get a chance to cancel. If you proceed, it will take a minute or so for the backgrounds for the various modes to be acquired. WAIT until the "Click Live Imaging" button is re-enabled (gray letters return to black).


9) You are now ready to collect images.



Set Up a “Case” Folder for Saving Images

- 1) Set up case by selecting menu items:
- 2) To select existing folder select Continue Previous Case (See below.)
- 3) To start a new case select Open New Case. You can also start a new case from the menu of the Continue Case form.



- 4) The Hot Button  has various meanings based on the whether a case is open.
 - If no case is open, it will activate the Continue Case form (or you can start a new one using its menu.)
 - If a case is open and Final Image has been selected then clicking on the button initiates a save.
 - If a case is open and the image is Live or Aborted Image then clicking on the button creates a Final Image (integration) and initiated a save.
- 5) To exit a case or start a new one find the Menu item

“Click for Live Image” to View Specimen

1) Insert the phosphor screen:

Manual Systems: By turning the wheel counter-clockwise to the end position.

Pneumatic Systems: By flipping switch toward the column on retracting mechanism.

H7500/H7600: Clicking for Live Image controls phosphor screen. There is an additional control, Camera Control, which also controls the screen.

Starts Camera then changes to "Click for Final Image".

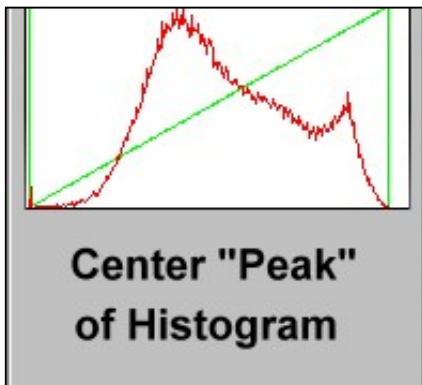
Speed (red) is fast display
Quality (blue) is frame averaged live.

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



3) The **Survey** (*round*) button should be selected.

4) Click on command button “**Click for Live Image**”.



5) Adjust the brightness of the electron beam to position the histogram approximately centered in the box. The green line on the right hand side should be visible and slightly away from the right side of the white box.

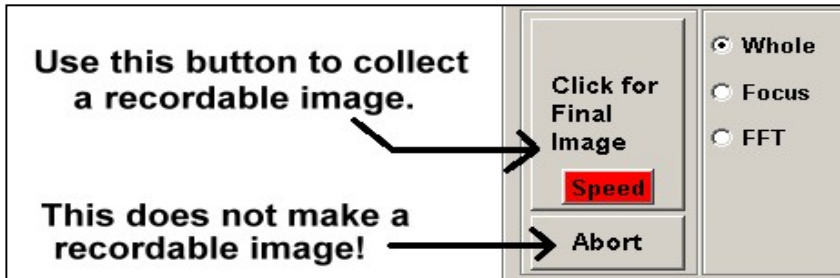
6) The **Speed/Quality** button switches between frame-averaging, “**Quality**”, and the faster, no frame-averaging “**Speed**” mode. Use this to make viewing more effective. This button has no effect on the final image.

7) You can fine focus using the Focus button, which enlarges the center region of the image.

8) Note: Starting in version 5.4 there is a SuperPix option for most cameras. This setting runs at near TV rates with a smaller display area.

“Click for Final Image” to Acquire a Quality, Full Resolution Image

1) When the “Live” image is focused and illuminated properly then “Click for Final Image” for an image that can be saved (Final Image).



2) This will take approximately 10 seconds.

3) This image is ready for digital storage.

Alternatively clicking the Case Save Icon will produce a Final Image from the Live or Aborted state when a case is open.

Saving Images

1) Most individual save operations will activate the Annotation form shown below and the saving process.

Use either the SaveAs option using the File menu to save individual images or the Case Hot Button to save using the Case Study protocol.

Microscope Information

Keyboard Commands

Customize Magnification Display

Print Mag Pixel Size None Print Size in mm Show Micron Bar?

Caption Line1

Caption Line2

Date and Time

Imaging Mode

Microscopist

Magnification

Acc.Voltage (kV)

X Coord Y Coord

Tilt

Facility

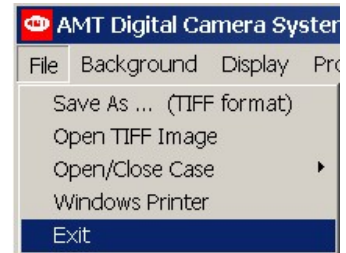
2) Fill in the appropriate fields (especially check magnification). Normally click on **“Write Full Caption”**. AMT discourages saving without a caption since this makes images difficult to trace.

3) The image is now saved. Proceed with next image.

Closing AMT Software

To close the AMT Software click on the menu items:

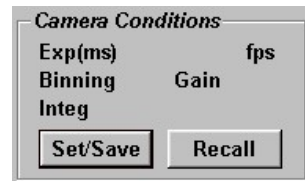
Alternatively click on the  button on the upper right hand side of the control form.



Modify Camera and Display Settings

1) Use Recall or Set/Save Control to Set Camera Parameters

This operation is needed when different users set the camera differently



To change camera conditions to a non-previously store state, click on the **Set/Save** button. To recall a saved set of parameters click on the **Recall** button.

The **Set/Save** button will bring up a form which controls camera exposure time and gain. It also controls record integrations, live frame averaging, histogram sampling, and contrast settings.

The image shows a larger dialog box titled "Camera Settings Control". It has several sections:

- Special Functions**:
 - Camera Setup Procedures**:
 - Exposure/Gain/Integration Matrix for Camera**: A table with columns for Modes, Exposures, and Gains.

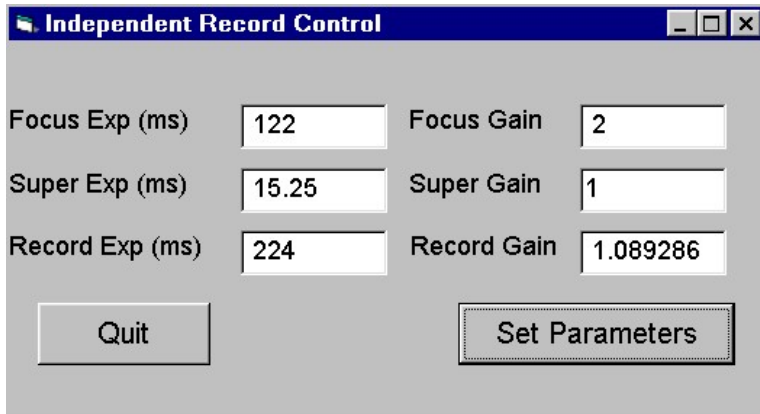
Modes	Exposures	Gains
Survey	61	1
Focus	122	2
Super Pix	15.25	1
Record	224	1.089286
 - Record Integrations**: 8
 - Matrix Calculation**: A button labeled "Balance Camera Exposures".
 - Display and Contrast Settings**:
 - Quality Setting (Frame Average): 4
 - Sample Region: 80
 - Display Size: 100
 - Black Threshold: 10
 - Black Level Tail %: 5
 - White Threshold: 10
 - White Level Tail %: 5
 - EBTV Intensifier Controls**: (Empty section)
 - Exit Control**: Two buttons: "Save and Apply" and "Apply For Now Only".

Exposure Control: The user first selects values for Survey Exposure and Survey Gain. Then the user clicks on **Balance Camera Exposures** to balance the exposure settings between the various exposure modes of the camera.

Limitations of **Balance Camera Exposures**:

Drifting Specimens: If specimen drift is a problem, try lessening the **Record Integrations** **AFTER** clicking on **Balance Camera Exposures**.

Large Variations in Exposure Time: Typically this control works for stable specimens and exposure conditions similar to those set during installation. For very short or very long



exposures the balancing algorithm may not produce a set of matching exposures. In these cases you may need to override the algorithm. To do select Special Functions-Change Exposures. This will bring up another form which can be used to independent all non-survey conditions.

Record Integrations and Quality Settings: These are similar operations where multiple frames are added to improve signal quality. The *Quality* setting work on the live image and the *Record Integrations* settings apply to Final images only.

Contrast Control: The auto-gain control uses the Black and White Thresholds to determine the contrast window. Then Tails are added to each side of the windows. The Tails are a percentage of the contrast extending the respective Black or White levels.

Sample Region: The contrast is determined by sampling a rectangle within the image determined by **Sample Region**. Selecting a sampling region can eliminate effects of very dark or very light features on the tonality of an image. To use the whole image select 100%. To avoid effects of grid bars or holes at the edges of an image use an 80% rectangle. To ignore feature closer to the center select a smaller value. It is helpful to use a non-zero value of the Tails when the sample is less than 100%.

Apply and Exit: This applies the changes until the system is shutdown or reset. It does not permanently save the new parameters.

Save and Apply: This saves the exposure and display parameters to a file which can be loaded later. Rigorous microscopy requires that a separate background and dark-field be acquired for each saved set of conditions. **The file DefaultSettings is loaded on start up of the program. Saving Defaults requires a password. No other settings file needs a password.**

Write to CD-R

[Example using Roxio Software and CD-R (not RW) Disks.]

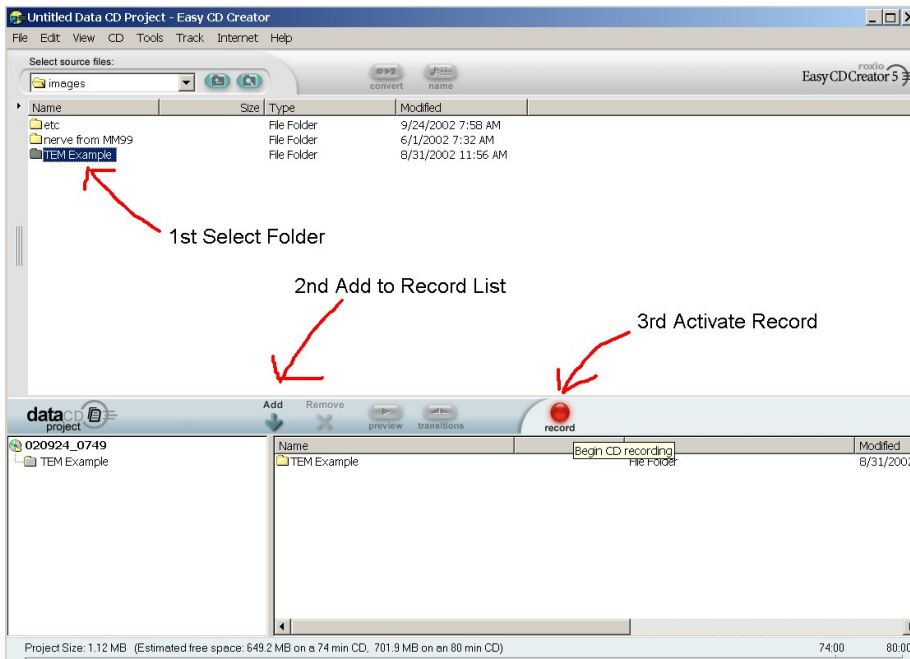
Open the CD Creator application. Select **Data** Disk



1) Select the folders that need to be archived.

2) Add folders to Record List.

3) Click on Record button.



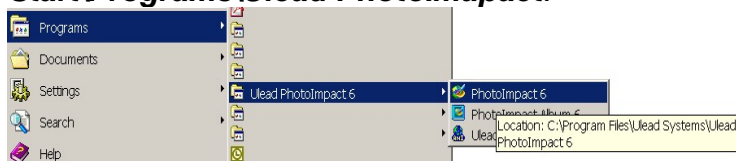
Printing

AMT Print function

AMT's internal print function uses a standard Windows print protocol under the File Menu. This will provide a simple print. More complex operations and batch printing should be down with PhotoImpact.

Printing from PhotoImpact

Open *PhotoImpact* by double clicking on either the desktop icon or by double clicking on **Start\Programs\Ulead PhotoImpact**.

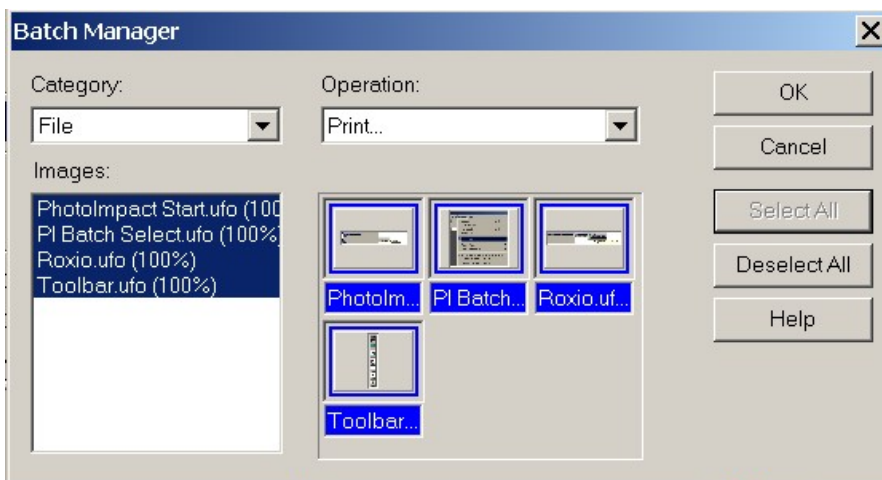
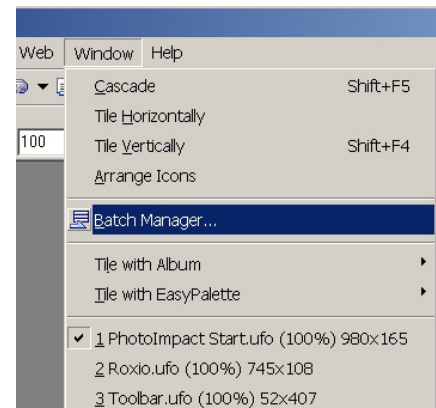


To Print a Single Image:

- 1) Go to the menu: **File\Open** and open the image you wish to print.
- 2) Select Print and check the options you wish to enable. [You may want to select center horizontally and vertically.]
Then click "OK".

To Batch Print Images:

- 1) Click the menu: **Window\Batch Manager**.
- 2) This opens a small form. Click the button "Select All" on that form.
From Category select **File** and
From Operation select **Print**.
Then click on OK.



Measurements and Calibrations

(See Measurement Manual for more details.)

1) Linear Measurement

- a) The operator selects units before making a measurement.
- b) Length measurements are made by clicking at each end of the desired feature to be measured. (It is no longer necessary to hold the mouse down.)
- c) Units are displayed on the image and entered once at the head of the data file saved.

2) Diffraction Spot Measurement

- a) The distance between diffraction spots is measured by clicking the mouse on each spot.

3) Diffraction Ring Measurement

- a) Diffraction rings are measured by clicking on three widely spaced points on the ring. The radius of the ring is determined and entered into the measurement list.

4) Calibration

- a) Calibration has been unified with measurement.
- b) Calibration is normally disabled and is password protected. Users can enable calibration from the Utilities/Calibrations menu.
- c) When calibration is done, measurements completed on the current image are rescaled using the newly calculated calibration constant.

5) Measurement Labels

- a) A measurement may be selected from the measurement list and a label added.
- b) If labels have been added and measurements are saved, labels appear in a tab delimited second column.

6) Undo Feature

- a) Individual labels on the image corresponding to a deleted measurement are erased from the image.
- b) If added labels appear in the saved txt files in a tab delimited second column.

7) Live Micron Bar

- a) A calibrated micron bar appears on the live image when point-to-point measurement is active.
- b) Use the mouse to change its position.
- c) The size of the bar is determined by the magnification entered in the Measurement Form.

Applications Help

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

or use our Web Site <http://www.amtimaging.com> for further information.

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

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

Other AMT Documents

- 1) Installation Manual
- 2) Montage Manual
- 3) Image Authorization Manual
- 4) Measurement Manual