

Normal Procedures for AMT CCD Imaging on Morgagni

The AMT (Advanced Microscopy Techniques Corp., Danvers, MA) CCD system has been installed on FEI Morgagni 268 electron microscope since March 03, 2004. It is a bottom mounted CCD camera. This document gives you a brief description of everyday operation of this AMT CCD camera. For details, please refer to the series of the original documents that comes with the equipment.

Here is some basic information about three major parts of this system:

The camera itself is a nominal 1k x 1k camera (the display size of the installed chip is set to 1280x1024, actual chip size is a little bigger than the above dimensions). The pixel size @ 1280x1024 is 24 microns. It is a lens-coupled optical system with a very thin layer of phosphor on the top facing electrons. This is a 12-bit camera.

The controller box has two cables: one is the controlling cable that connects to the camera, the other cable is a firewire (IEEE 1394) cable that transfers data to the computer.

The computer is an ATX PC computer with a P4 2.40 GHz processor. Installed software includes the AMT software and a photo editing software, PhotoImpact. Due to hardware dependencies, it is running the very latest version of the Windows 2000 Professional operating system.

1. Get Morgagni Ready

Before collecting any CCD images, the microscope must be properly started and aligned. Check "Beam --> Int. Zoom" is in action (button yellow). The CCD camera operates best at intensities equivalent to the conditions set for normal dose film imaging, i.e., about 1.0 seconds with small screen measurement.

Note:

Make sure the large screen is DOWN before going to next step.

2. Turn On the AMT CCD camera

Usually, the CCD controller box is left ON after use, if not, switch on the CCD controller box. Login (or unlock) AMT computer as emlab, then launch the AMT software, from "Start" --> "amtv600".

Note:

In case the you have to switch ON the controller, wait for about 10 seconds before starting the software.

3. Background Collection (optional)

Background correction is important to compensates for the phosphor inhomogeneities and optical imperfection. For optimal performance, background images should be updated every day or whenever there is a change in the gun high tension, however you can skip this step if just want a quick check of your sample without too much concern about the quality of your images. In that case, you can simply use the current acquired background.

To acquire a new background, pull out specimen from the column or find an empty position or hole on specimen grid and set the magnification to roughly what you will use to take image. Spread the beam with C2 control clockwise so that the current is about 1.0 second on the small screen film exposure meter. Lift the large screen and cover the viewing glass window and adjust Intensity and beam shift so that the beam is optimally bright and evenly spread. When this is correct, select the Menu Item Background - Acquire

Background and click on the orange command button. It will take a minute or so for the background collection for various modes to be acquired - WAIT until the "Click Live Imaging" button is re-enabled.

4. Viewing your Specimen

With a specimen in and a region of interest centered on the phosphor screen, lift the screen by pressing the square button on the left hand side of the viewing window and click on the "Click for Live Image" button to activate the feed from the CCD.

Notes:

The Speed/Quality button switches between frame-averaging. This button has no effect on the final image. The current computer is fast enough that there is no advantage to using the Speed mode.

The FFT mode (radio button) is very useful to adjust the objective stigmatism and defocus.

The "Click For Final Image" button will acquire a Full Resolution Image

5. Saving Images

With AMT software, there are four image format options: 8-bit TIFF, 16-bit TIFF, Lossless JPEG (100% compress) and Normal JPEG (50% compress).

There are two ways to save an image:

Directly save a recorded image from File -> Save As. You have to give filename each time. You can define one of the four image formats in "File Type".

Alternatively, one could utilize the hot button Case. As long as a Case is opened, each new file will be saved in "Case" folder with incremental index in the filename. One has an option to choose 8-bit or 16-bit TIFF as image file format. The default is 8-bit, which is sufficient for most screening purpose.

You may set up a "case" so that images saved will have filename reflecting the case name and of incrementing if multiple images are taken.

To start a new case, select File - Case Study - Open New Case.

To use an existing case, select File - Case Study - Continue Previous Case.

To exit a case, select File - Case Study - Close Current Case.

Once a case is opened, clicking on the Hot Button "CASE" on the left side of the main window will save a Final Image. In addition, if a case is open and the current image is Alive or Aborted Image, then click on the button creates a Final Image and initiates a save.

Note:

In either of the two ways above, one has choice to save the TIFF image with or without a caption. If "Save with Captions" is clicked, the information is not only stored in the image Tag, but also made into an extra TIFF image which is appended on the real electron image to form final image with captions.

6. Manage Your Image Files

All images should be directly saved in a user-specific folder located in "C:\Images". This folder can be accessed on various platforms as detailed below. There is no way to locally transfer your images to other storage media. Instead, use the Samba server to access your images from any on-campus computer.

LINUX

To start up the ftp-like smbclient and connect to the share from the command line, enter:

```
% smbclient //amt.rose2.brandeis.edu/Images -W EMLAB -U emlab
```

The user will be prompted for the password for the emlab account.

WINDOWS

To mount the share on windows, use the "Map network drive" function. The name of the drive to be mapped is \\amt.rose2.brandeis.edu/Images.

When prompted, the user should authenticate using User name emlab and the password for the emlab account. A window into the share should open on the desktop.

On Windows 7 computer, you can directly put the line \\amt.rose2.brandeis.edu/Images into "Search":field, it should also work.

MAC OS X

Use CMD-K to pull up the Connect to Server dialog box, then enter the following in the Server Address field:

```
smb://amt.rose2.brandeis.edu/Images
```

The user will be prompted for authentication information and should enter:

Workgroup or Domain: EMLAB [if not already filled in]

Name: emlab

Password: password for emlab account

An icon for the share should appear on the desktop.

Note:

Brandeis LTS policy restricts Samba access to on-campus computers only. You will not be able to access your data from off-campus.

The local hard drive is not for long-term storage purpose. Any user data on AMT CCD computer should be transferred/saved somewhere else. Any data left on the local drive will eventually be deleted.

Pixelsize and Magnification Calibration

The default factory magnification calibration might not be good enough if you need a accurate value for size measurements. Finally, I did a mag and pixel size calibration for each mag. The specimen used for this calibration are 463nm grating replica grid for mag of 8900x and lower, and homemade Tropomyosin paracrystals in negative stain for 8900x and higher.

Mag_ScreenDown(x)	Mag_ScreenUp(x)	Mag_OnCCD(x)	Pixelsize@1280x1024(nm)
880	1000	1,188.47	21.19
1100	1300	1,519.85	15.79
1400	1600	2,029.25	11.83
1800	2000	2,400.90	10.00
2200	2500	3,229.22	7.43
2800	3200	3,796.33	6.32
3500	4000	4,896.05	4.90
4400	5000	5,982.03	4.01
5600	6300	9,940.55	2.41
7100	8000	10,971.83	2.19
8900	10000	12,895.61	1.87
11000	13000	16,204.35	1.48
14000	16000	20,102.28	1.19
18000	20000	25,108.86	0.956
22000	25000	30,571.14	0.785
28000	32000	40,205.91	0.597
36000	40000	51,524.05	0.466
44000	50000	61,768.10	0.389
56000	63000	83,240.51	0.288
71000	80000	100,392.04	0.239
89000	100000	124,046.58	0.194
110000	125000	154,389.87	0.156
140000	160000	197,574.68	0.122
180000	200000	232,283.54	0.103
