

CCD Montage Manual

Rev 2.1 – 28June04

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Installation

A. Enabling Montage:

The montage application needs to be turned on and files need to be created for controlling the montage process.

1) Use Notepad to open the file
c:\AMTcommon\config\initial540.txt.

Find the heading [SpecialAvailable] or [MontageAvailable] replace the word NO with the word MONT. Then Save the file and Exit Notepad.

2) Open the file
c:\AMTCommon\config\MagIndicesXXXX.txt.

The XXXX here indicates the TEM type. *For example*, if you have a JEM 1230 then the file is:
c:\AMTCommon\config\MagIndicesJEM1230.txt.

Use SaveAs to resave this file as follows:
c:\AMTcommon\config\magindices.txt.

If there is no file for your TEM model, create a file in Notepad called:
c:\AMTcommon\config\magindices.txt

Enter the available magnifications for your TEM from lowest to highest. Each magnification should be put on a separate line. See C:\AMTCommon\config\MagIndicesJEM1230.txt as an example of the file format. Call AMT (978)774-5550 for assistance.

B. Calibrating the TEM and Camera:

Careful stage and camera calibration are needed for automated montage. This section can be ignored for Manual Operation.

(I) Magnification Calibration:

Calibrate the TEM at all magnification and kV values combinations required for viewing and recording the montage image. This typically from 1000 to 50,000x. Use the standard magnification calibration routine.

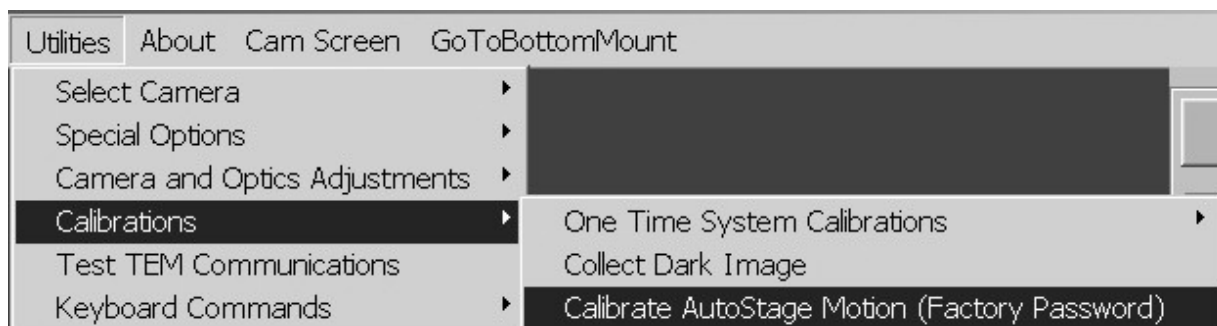
(I) Stage Calibration:

This procedure is a little complex and requires patience. It may need to be repeated if incorrectly done. But it needs to be done only once.

1) Set the TEM to 5000x (or the closest value to 5000x) on a sidemount camera or 2000x for a bottom mount camera.

This is a "first guess" of the appropriate magnifications. You may need to lower or raise it based on the results seen in steps 4 through 7.

2) Run the camera using AMT software (AMT Image Acquisition Engine). Find the menu item: **Utilities/Calibrations/Calibrate AutoStage Motion**



3) You will be asked to:
a) Enter a

Password. Use _____.

b) Enter the number of measurements, n , to be averaged ($n = 1$ to 10). The system will measure stage motion n times in the x direction followed by n times in the y direction. Since stages generally have greater than 0.5 micron random error, the precision of the calculation increases with multiple measurements.

Live imaging will begin and a message will appear:

"Starting X Calibration"

4) Another message will appear:

“Place recognizable point near center of image after backlash correction”

The point of this message is that you must be able to see this feature after it moves 10 microns.

5) After “OK” the stage will do a backlash correction. Another message will appear:

“Click mouse on the recognizable Point”

This will initiate a 10 micron stage move in the x direction (x as defined by the TEM manufacturer).

6) When the stage stops moving another message appears:

“AGAIN, Click mouse on the recognizable point after motion.”

This click determines the actual distance that the stage travels when asked to move 10 microns. If the recognizable point moves completely out of the field of view, then decrease the magnification and start over.

7) A message box indicating the current measurement appears:

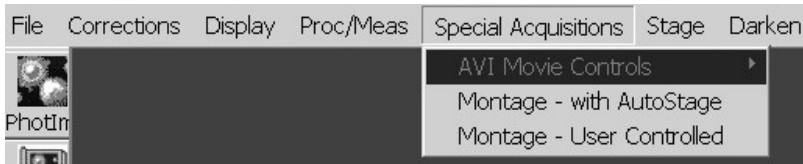
“Distance = xx.xx microns” where xx.xx should be within 15% of 10 microns.

8) Operations 4 through 6 will repeat *n* times for the x motion, followed by a similar *n* measurements for the y motion.

9) After y is completed the new calibration factors for x, y and stage orientation are recorded and then shown in a message box.

Collecting Montages

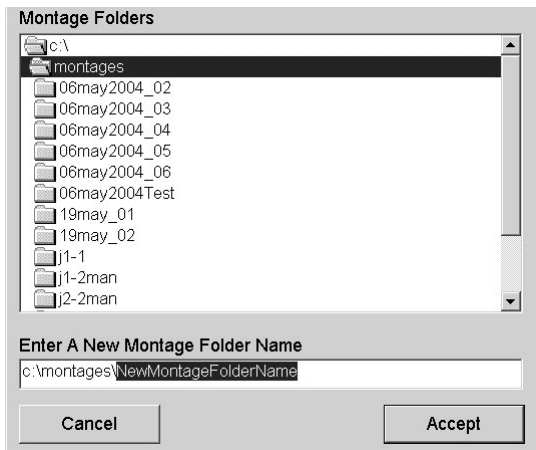
1) Select the appropriate Montage Acquisition Mode from the *Special Acquisitions* menu



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.

When AMT's application can control the stage, both options are enabled.



2) Provide a name for the folder where montage images will be saved.

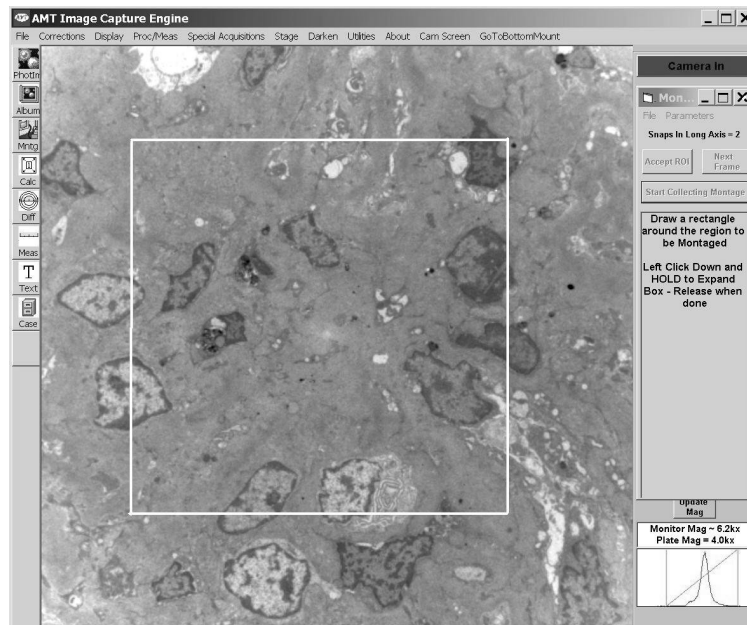
3) Once a folder name is selected, the montage control panel will appear on the right side of the screen.



4) The image will go “live”. Position the sample and use the mouse to draw a rectangle around the region from which you would like to collect montage images. To do this left **click the mouse** at the upper left hand corner of the desired region and **hold then release the mouse** at the lower right hand region.

3) Use the Menu item **Parameters** on the Montage Control Panel to

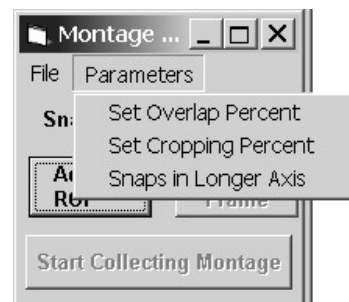
- Set image overlap
- Set image cropping.
- Set number of images in the montage



Overlap:

About 15% total overlap (~150 pixels in a 1024x1024 image) is needed to provide the stitching application with enough data to properly position constituent images. Use **Set Overlap Percent** to set this parameter.

Note also that mechanical stage drives have an inherent uncertainty or repeatability error associated with them. This variation depends on the model microscope as well as the age of the system.



Since larger montages require the stage to be repositioned more times the chances that the error in one of these movements is large is increased. In this case you should specify a larger pixel overlap.

Cropping:

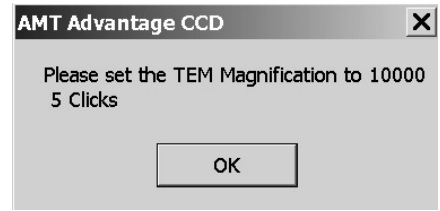
All TEMs have some distortion at the edges of the field-of-view. For bottom mount cameras with small angular field-of-view this effect is minimal. But for sidemount cameras this effect can cause difficulty in stitching. Use **Set Cropping Percent** to eliminate or reduce the distorted edges.

Snaps:

Snaps in the Longer Axis determines the number of images in the montage. This control sets the number of images in the geometrically longer axis. The application will automatically set the number of images in the other axis.

5) Click **Accept Region** if the region is correct. Then the system creates an overview image at the current magnification showing the region of interest from which the montage is collected and saved. The overview image is stored in the folder named in step 2 above and given the name Overview.tif

6) A message box will ask you to set the microscope to the magnification needed to produce the montage requested in step 5. (On some TEMs the magnification is automatically changed.)



7) Set the TEM to the requested magnification, focus the TEM, and adjust the intensity.

8) Click **Start Collecting Montage**. Now, the AutoStage and User Controlled options work differently.

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 "vst" 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 given large instruction area in the montage control panel on the right.



a) The first operation will be to move the lower right region of the intended montage to the center of the screen. (I.e. move image up and left.) Then click on **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 "frame" the non-overlap region. Initially the overlap region will duplicate on one edge of the live image. This region will flash occasionally to show the live image behind it. Move the image in the direction of the duplicate.

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.

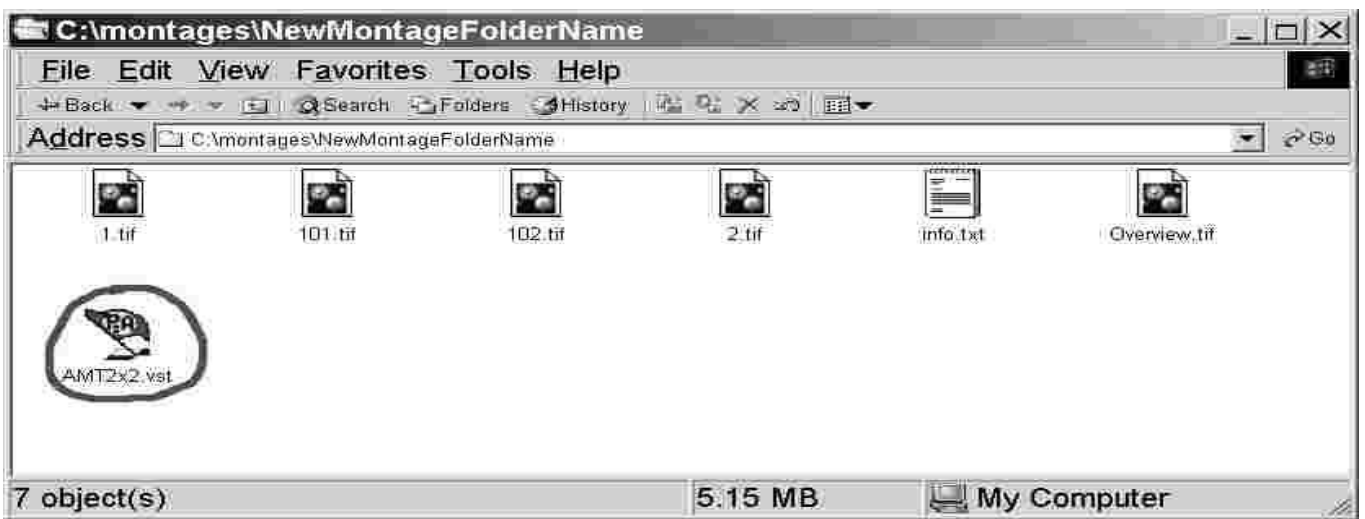
d) When done a message box will announce that acquisition is complete. The images and a "vst" file will be stored in the folder specified in Step 2.

Stitching Images in Panavue

The montage procedure has been developed for use in Panavue. Images are named so that they will automatically be placed in the proper order for Panavue's stitching routines.

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.

Of course, there are other programs available for stitching. The images are all in TIFF format so using them in other packages should be straightforward.



1) Use **Explorer** to select the montage files you specified before recording the component images. Open the file **AMT n x m .vst** the blanks will be filled in with the dimensions of the montage that you have just collected.

2) This will launch the stitching package **Panavue**, which launches a control screen.



3) Use the **File** menu and select **Open All Images** or press the key combination **Alt O**. This will bring all the images to the screen.

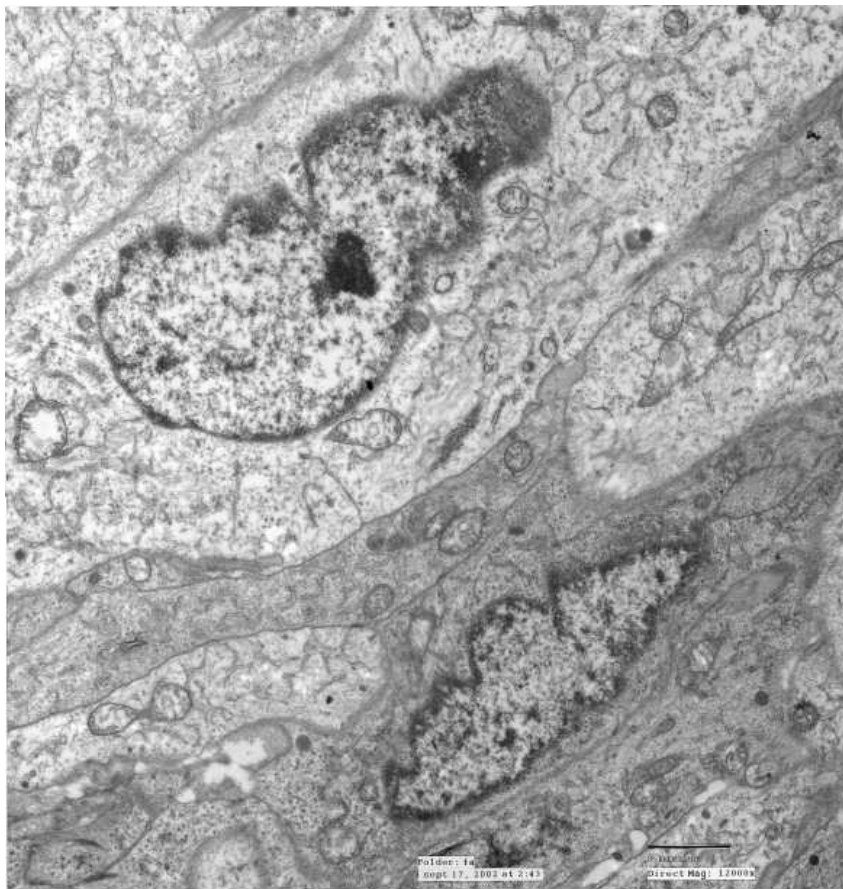


4) Use the **Action** menu and select **Execute** or type **Ctrl E**.

5) The resulting image will be displayed when stitching is completed. Note: The lower right hand image has the micron bar.

You can then chose to save the image (We recommend an 8 bit gray scale TIFF format.).

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.



6) This image can be compared with the low resolution Overview.tif image that was created when the region was defined.