TEM Center for JEM-1400 window

(see following page)
Decided to close and open the TEM Center for JEM-1400 window each day. To open the small TEM Center Domain Controller window in desktop, click the TEM Center Domain icon. Next, click the Start TEM Center button. This opens the TEM Center for JEM-1400 window. The Screen window in the left-hand panel will require opening. The TEMCON icon in desktop opens the Controller for JEM-1400 window. This is the panel for engineers/service, but it can be used to verify information in the TEM Center for JEM-1400 window. For example, use it verify if the HT is off (blue) before changing filament. If it is on, HT is highlighted in green. Also, use this window to re-select the full range of magnifications if the TEM Center for JEM-1400 window crashes ..... the only mag that will be available is 10K. From the menu > Options > Mag Select > recheck the full range of magnifications in both low and high mag. Go back to the TEM Center for JEM-1400 window to verify ..... Image Forming System window in left-hand panel.
To open double-click GATAN DigitalMicrograph icon (see previous page). Select (✓) Camera Inserted.
Each day de-select Camera Inserted, close DigitalMicrograph window, open again, and select (√) Camera Inserted. This refreshes program so that it will not malfunction during the capture.

Check the HT: reading at bottom of window. It should be the same as high tension (80KV) set for scope. If it reads (0KV), simply close and open the window again.
To acquire a gain reference, be sure that the beam is uniform over the whole field of the camera and that no specimen is in the way.

A relatively bright beam, spread considerably larger than the field of the camera, is recommended.

- Target number of counts: 7500
- Number of frames to average: 10

Reference Binning:
- 1 (can be used with all camera binnings)
- 2 (can be used only with even binnings - odd binnings will use the existing unbinned reference)

This new reference will replace an existing reference:
- Average dark reference: 8 times
- Calibrate dose with last image

ARE YOU READY TO PROCEED?

- YES
- NO

If specimen/rod is inserted for capture the night before, pull out rod to the first hold position (pull out until it stops and turn counter clockwise until resistance is met). Turn on beam (1 and 1/2 minutes), center it, and spread a couple of partial turns outside the edge of the screen. All values in adjacent window are default. Click YES to proceed.
Click OK and you are ready to proceed.
After refreshing Digital Micrograph and performing Prepare Gain Reference, remove specimen rod and make exchange to next section to be captured.* Once in scope, turn on beam and set up at 150X (low mag). Select spot size 1 for strongest/brightest beam and cook (4 to 5 minutes) the region of section to be captured. Then switch to 60X and note that the image rotates 90 degrees. Spread beam and click Record from left-side menu of SerialEM to capture image. Open Navigator window (Navigator>Open).

*It is important to position the grid so that the long axis of the sample to be captured is parallel to the long axis of the specimen rod. Positioned as such, the long axis of the sample is in the vertical plane at 150X, the magnification chosen for pre-exposure to the beam. When the magnification is changed to 60X (for capture) the sample will rotate 90 degrees counterclockwise, and place the long axis of the sample in the horizontal plane. As such, the vertical columns of capture in SerialEM will be at their minimum. This produces less stress on the software used to montage individual image tiles.
Select Add Polygon
Select first point for drawing polygon around area to be captured.
Continue to click points to construct polygon around area to be captured.
Click Stop Adding to draw the final line. If you need to outline an additional area of capture, select Add Polygon again.
The next step is to select Acquire (A) in Navigator window. Once clicked, New file at item is activated.

Note the appearance of the letter A in the dialogue line for item 1 in the dialogue box.
The next step is to select New file at item. This will bring up Properties of File to O... window.
Select Montage Images.
Select Fit montage to polygon.

Click OK.
Upon clicking OK in Properties of File to O... window, the Montage Setup window is opened. In this version of the window increase the mag to 5000 (Magnification: 60) using the adjacent up button. As you do so the bottom of the window will expand downward (see next page).
Montage Setup

FITTING TO NAVIGATOR AREA: Change mag to adjust number of pieces. Changing mag, binning, overlap, or "Move stage" will refit to area.

Magnification: 60

Binning: 1

Pixel size: 187 nm

Number of pieces in X: 1

Y: 1

Piece size in X: 1360

Y: 1008

Overlap in X: 490

Y: 490

Reset

Minimum overlap: 12% and 0.5 micron

Total Area: 1360 x 1008 pixels

254.2 x 188.4 microns

- Move stage instead of shifting image
- Skip pieces outside Navigator item
- Do full rectangle; ignore list of pieces to skip
- Ask about making map after each montage
- Use settings for high-quality stage montage

OK

Cancel
In the expanded Montage Setup window the only item that needs to be corrected is Pixel size in X: Y: ....... the number of pixels should be 4080 by 4080. All the other settings are default such that once selected they appear so in subsequent captures. Click OK to proceed.
**Montage Setup**

**FITTING TO NAVIGATOR AREA:** Change mag to adjust number of pieces. Changing mag, binning, overlap, or "Move stage" will refit to area.

- **Magnification:** 5000
- **Binning:** 1
- **Pixel size:** 2.18 nm

- **Number of pieces in X:** 32
- **Y:** 23

- **Piece size in X:** 4036
- **Y:** 4080

- **Overlap in X:** 490
- **Y:** 490
- **Reset**

- **Minimum overlap:** 12% and 0.5 micron

- **Total Area:** 113962 x 83060 pixels
- **248.0 x 180.7 microns**

- **Move stage instead of shifting image**
- **Skip pieces outside Navigator item**
- **Do full rectangle; ignore list of pieces to skip**
- **Ask about making map after each montage**

- **Use settings for high-quality stage montage**

<table>
<thead>
<tr>
<th>High-quality stage montage parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autofocus at each piece</strong></td>
</tr>
<tr>
<td><strong>Repeat until drift is below 2.5 nm/sec</strong></td>
</tr>
<tr>
<td><strong>Autofocus in blocks of 3 x 3 pieces</strong></td>
</tr>
<tr>
<td><strong>Realign with image shift up to 10 microns</strong></td>
</tr>
<tr>
<td><strong>Go from center out; anchor at mag:</strong> 2000</td>
</tr>
<tr>
<td><strong>Realign at new column and after 8 pieces</strong></td>
</tr>
</tbody>
</table>

- **Maximum alignment shift as % of piece:** 60

- **Delay time after moving stage:** 5 sec

- **Skip correlations used to align pieces**

[OK] [Cancel]
4080 by 4080
FITTING TO NAVIGATOR AREA: Change mag to adjust number of pieces. Changing mag, binning, overlap, or "Move stage" will refit to area.

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High-quality stage montage parameters

- Autofocus at each piece  
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Maximum alignment shift as % of piece: 60

Delay time after moving stage: 5 sec

- Skip correlations used to align pieces

OK  Cancel  ?
Clicking OK in Montage Setup window opens File Properties window. All selections are default. Important one is Save images to: Series of TIFF files listed in an Autodoc file. Click OK to open Save As window.
Create new folder in which the images from the present capture will collect.
Create New Folder ------->
Name New Folder
Select file name. Have been routinely choosing 1 as file name for all captures.
Save As dialog box with the following options:

- **Save in**: 10131
- **File name**: 1
- **Save as type**: Image Autodoc files (*.idoc)

Options available:
- **Save**
- **Cancel**
Note the appearance of the letter F in the dialogue line for item 1 in the dialogue box. AF is the abbreviation for acquire file and indicates that the two previous steps (Acquire and New file at item) are completed.
Change the magnification to 5,000X before proceeding to the next step, image shift calibration. Make sure the objective aperture is inserted before increasing the magnification.

* If the magnification is not changed from 60X to 5000X before performing the image shift calibration, SerialEM is corrupted and will not function until the following correction is made:

Carl,

I think you are suffering from that bad initial image shift calibration at 60x - I'm glad you mentioned it. This gets remembered in the file SEMshortTermCal.txt in the C:\Program Files\SerialEM folder

Exit SerialEM and rename that file to something else so that I can see what is in there at some point. Restart SerialEM and it should behave OK.

We can add a property setting to have the program always ignore the short term cals, which would probably make sense in your case.

David
**Image Shift** calibration is found in the drop down menu under Calibration in the SerialEM menu.
Before clicking Yes and proceeding with the image shift calibration, again make sure the mag is set at 5,000X. Also, make sure the objective aperture is centered and the beam is both centered and condensed until the current density is in the range of 100 pA/cm2 with the small fluorescent focusing screen in place. The current density reading is found in the drop-down Screen panel located in the left-hand TEM Systems Task Bar of the TEM Center for JEOL-1400 window.

* 100 pA/cm2 is the current density routinely chosen for capture.
To calibrate, 9 images will be acquired using the Trial parameter set. Before starting, be sure that the beam brightness and exposure time are set so as to give an image with moderately high counts with Trial.

Are you ready to proceed?

[Yes] [No]
Camera 0, 5000x: the scale matrix is

7.5 -34.3 28.1 8.1

The maximum range between paired estimates is 0.0 (0.0% of maximum scale value).
The maximum range of drift estimates is 0.3 pixels.

THIS IS GOOD
Navigator Menu

(see next page)
With the scope ready for capture and the image shift calibration performed, proceed with the next and final step, starting the capture. Go to the drop-down menu for **Navigator** in the upper **Menu** bar. Select **Acquire at Points** to pull up the **Acquire at Items** window. The settings are default. **Make sure to select Turn of filament at end.**
Acquire at Items

Initial Actions after Moving Stage
- Rough eccentricity
- Autocenter beam
- Realign to item
- Cook specimen
- Fine eccentricity
- Autofocus
- Only at start of group
- Run macro # 1

Primary Task
- Acquire map image
- Just acquire and save image
- Run macro # 8
- SerialCapture
- Acquire tilt series
- Restore scope state after aligning to item
- Turn off filament at end
- Send email at end

GO  Cancel  ?
Initial image shift calibration capture at 2,000X.
The capture starts at a midpoint in the polygon drawn around the area to be captured. The initial segment of the first row is captured in a downward direction from this point. The remaining segment of the first row is completed by capturing in an upward direction from the midpoint. The capture moves one row to the left and proceeds in the same pattern as the first. After the left-hand side of the polygon has been captured, the right-hand side is captured in the same manner.

* Position the capture so that the long axis is in the horizontal plane.

Watch the capture of the first row in both the downward (south) and upward (north) direction to insure that the area of interest is within the capture polygon. This is necessary because of the shift from low mag (60X) in which the polygon is drawn to high mag (5,000X) in which the capture is made. This shift is primarily from south to north and varies from 10 to 20 um.
PANELS and SCREENS
CAMERA SETUP
WINDOWS
Parameter set
- View
- Focus
- Trial
- Record
- Preview

Parameters for View

Acquisition
- Continuous
- Single Frame

Processing
- Unprocessed
- Dark Subtracted
- Gain Normalized

Binning
- 1
- 2
- 3
- 4
- 6
- 8

Exposure time 0.05 sec
Drift settling 0 sec

Minimum 0.12 if not 0.0

Shutter mode
- Beam blanking only
- DM film shutter with beam blanking
- Dual shuttering - minimum exposure

Positioning
- Top 0
- Left 0
- Bottom 4096
- Right 4096

Recenter
Swap X & Y

Area size
- Quarter
- Half
- Full
- Wide Quarter
- Wide Half
- 10% Less
- 10% More
- A Bit Less

Force new dark reference next time only
Take new dark reference each time
Average dark references 4 times

Dose: Not calibrated
Update Dose

OK
Acquire
Cancel
Camera Parameters -- Ultrascan 895

Parameters for **Record**

**Acquisition**
- Continuous
- Single Frame

**Processing**
- Unprocessed
- Dark Subtracted
- Gain Normalized

**Binning**
- 1
- 2
- 3
- 4
- 6
- 8

**Exposure time** 2 sec

**Drift settling** 1 sec

Minimum 0.05 if not 0.0

**Shutter mode**
- Beam blanking only
- DM film shutter with beam blanking
- Dual shuttering - minimum exposure

**Positioning**
- Top 0
- Left 0
- Bottom 4096
- Right 4096

**Area size**
- Quarter
- Half
- Full
- Wide Quarter
- Wide Half
- 10% Less
- 10% More
- A Bit Less

**Force new dark reference next time only**

**Take new dark reference each time**

**Average dark references** 10 times

**Dose:** Not calibrated

**Update Dose**

OK

Acquire

Cancel

Swap X & Y

Recenter
Camera Parameters -- Ultrascan 895

Parameter set
- View
- Focus
- Trial
- Record
- Preview

Parameters for Focus

Acquisition
- Continuous
- Single Frame

Processing
- Unprocessed
- Dark Subtracted
- Gain Normalized

Binning
- 1
- 2
- 3
- 4
- 6
- 8

Positioning
- Top 1536
- Left 1024
- Bottom 2560
- Right 3072

Binned size: 1024 x 512
382.87 x 191.43 um @ 374 nm

Exposure time 0.3 sec
Drift settling 0.3 sec
Minimum 0.05 if not 0.0

Shutter mode
- Beam blanking only
- DM film shutter with beam blanking
- Dual shuttering - minimum exposure

Force new dark reference next time only
Take new dark reference each time
Average dark references 4 times

Area size
- Quarter
- Half
- Full
- Wide Quarter
- Wide Half
- 10% Less
- 10% More
- A Bit Less

Dose: Not calibrated
Update Dose

OK  Acquire  Cancel  ?