Stanford-SLAC Cryo-EM Center

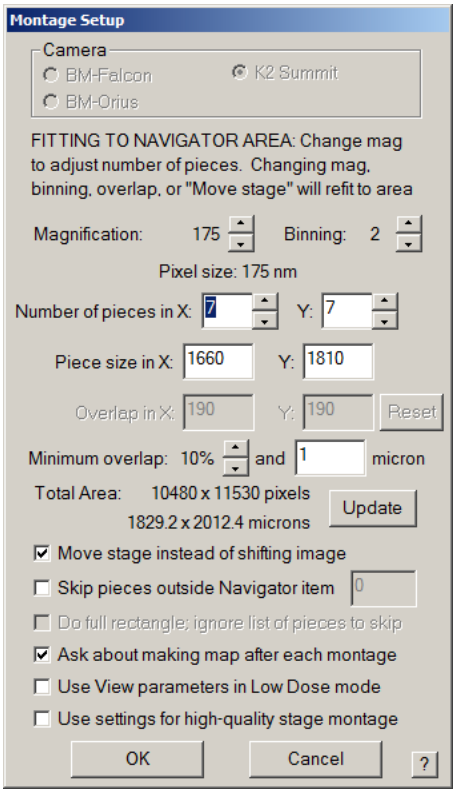
Author: Yan Liu

SOP: Collecting tilt series using SerialEM

- Open SerialEM server
- Open SerialEM
- Load settings

Creating a Low Magnification Montage

- Open **Navigator** menu and load "**Imaging States**"
- Double click on "LMM" mode (LM 145X, spot size 9, C2 100%)
- Check in TIA FluScreen viewer if the setting is ok (Obj. aperture is removed and C2 aperture is 150 um)
- Open **Camera** -> **Parameters**-> **Record**, make sure K3 is selected, **Record** is in Counting mode, mag is 145X, exposure time 1 s, Bin 2, no dose fractionations. Click **OK**
- Open **Navigator** menu, click **Montaging & Grids** and **Setup Full Montage**
- In **Montage Setup**, use "7X11" tiles, 20% overlap and make sure "Move stage instead of shifting image" and "Ask about making map after each montage" are selected



- Click **OK** and save .st file with mag and exposure dose. Save files as Atlas.mrc
- Load one grid and open column valves
- To start the montage, go to "**Montage Controls**" and click on **Start**

- Click “Yes” to make final overview of montage into a map
- Close the montage file

Image Shift LMM to View:

- Turn on low dose mode
- Change C2 aperture to 50 um
- Identify a bad grid square with a feature, add a point on the feature and **go to XY**
- Open **Tasks** menu -> **Eucentric Rough**
- Take a **View** image, find the feature
- Add a marker
- Open **Navigator** -> **Shift to marker**
- This process can be repeated until LMM and **View** are well correlated
- **If the shift is not correct, you can always undo this by clicking Navigator -> Undo last shift.**

Set up low dose imaging:

- Turn on low dose mode and move the stage to a broken square or a square can be sacrificed
- Make sure you are eucentric and in focus
- Go to **View**, check continuous updates. Open DM, adjust magnification so that you can find the samples. Adjust spot size and intensity so the dose rate of K3 is between 15-25.
- Open **Camera** -> **Parameters**, for **View** mode: 5600X, 0.5 s, bin by 2, full readout
- Uncheck continuous updates
- Go to **Record**, check continuous updates, adjust optics for data collection. Make sure beam is in parallel illumination (C2 value). Measure dose.
- Calibrate dose in SerialEM (1 s exposure and bin 1), and set up exposure time and dose fractionation in Record
- Open **Camera** -> **Parameters**, for **Record** mode: 45,000X, 2+ s, bin 1X (counting), full readout, dose fractionation on
- Copy **Record** settings to **Trial** and **Focus**.
- **Trial**: 45,000X, 0.2 s, bin by 4, full readout
- **Focus**: 45,000X, 0.2 s, bin by 4, full readout
- Not over vacuum, take a **View** and set the “define position” of area for **Focus** and **Trial**
- Take a **Preview/Record**, then **Trial/Focus** to verify the position, then **View**.

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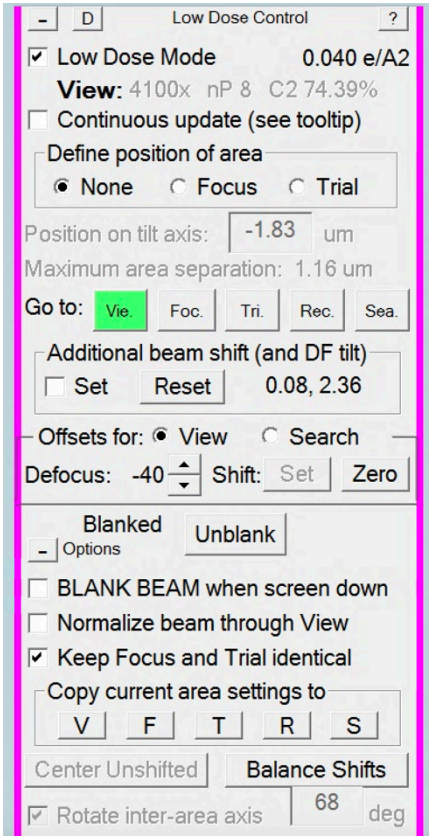

Setting K3 Camera (GIF Tune and Gain Reference)

- Move the stage to an empty square and set the microscope magnification as the same in **Data Acquisition** preset
- Set Spot Size 1, C2 aperture 150 μm
- In **Digital Micrograph**, select **Tune GIF.** This will take about 15 minutes.
- After GIF Tune, under **Camera** tab select **Prepare Gain Reference**
- Follow instructions: put down FluScreen, adjust **Spot Size** to change the counts first and use **Intensity** to fine tune it

Make a Medium Mag Montage:

- Add a Polygon of desirable squares (add all and then stop adding)
- **Navigator** -> **Acquire** and **New file at item**
- Set up the MMM.st file (define number of pieces) bin 2, 0.5s exposure, counting mode, deselect “ask about making map after each montage” box.
- Click **OK**
- Save all info in extended header
- **Navigator** -> **Acquire at items,** check **Rough Eucentricity**, select **Acquire map image or montage**
- Click **Go** and start Montage
- Save the **Navigator** file

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Fix the register between View and Record:

- Center feature in **Preview** image
- Take **View** image
- Check continuous update
- Uncheck “move stage for big mouse shifts” in the Image alignment and focus control panel.
- Right click and drag to center feature
- Under offsets for view click “set”
- Take preview to ensure feature is centered, repeat if necessary

Beam alignment, Coma and Stigmatism:

- Move the stage to a square can be sacrificed
- **Tasks** -> **Rough Eucentric**
- Under **Focus and Tune,** set defocus target to 0
- Start **Autofocus**, review **FFT**, should be at zero. If not, charge focus knob to make it to zero
- Perform direct alignment on the microscope on carbon
- **Autostigmate by CTF**
- **AutoComa by CTF**

Set up batch tilt series:

- Put a marker located in medium-mag maps.
- **Go to marker**
- Take **View** and **Preview** images
- **Save A,** to save all the points (positions); Open a file for maps of the areas to be acquired
- Make a **New map** and check **Tilt Series** in the **Navigator**
- Press the **TS Parameters** button
- Define file properties as needed then define tilt series properties (-50 to 50°, beam tilt 1.7 milirad, defocus, etc).
- To adjust the automatically generated filename, press the **Filename** button
- Change focus positions
- Select **Acquire at Items** from the **Navigator** menu. Tilt series acquisition will be selected as the action to perform.
- Check **Realign to Item** to align to the maps at each position; and **Fine Eucentricity** if this operation is not selected in the tilt series parameters for each series (OR run a script: Eucentric_tomo)
- Set **Close valves at end** and press **Go**