

# Using Macros, Camera Issues, and Setting Up SerialEM

# Macros

- A macro is a sequence of commands with some modest control logic
- Macros are good for simple repetitive tasks, for testing scope properties, and for troubleshooting problems
- It is possible to program a basic tilt series with macros
- There is a control dialog which will stop a macro on a number of conditions, one of which is a image too dim
- It is relatively easy to add a macro command to the program

# Example Macros

Macro to take unfiltered and filtered image pairs

```
SetSlitIn 0
R
S
R
S
SetSlitIn 1
SetEnergyLoss 0
R
S
R
S
SetEnergyLoss 30
R
S
R
S
```

Macro to take 2° series from 6° to -6°

```
TiltTo 10
TiltTo 6
R
S
Loop 6
TiltBy -2
R
S
EndLoop
TiltTo 0
```

Macro to measure drift every 10 seconds

```
T
AlignTo B
ReportAlignShift
Delay 10
Repeat
```

# Calibrating Camera Gain

- You must calibrate your screen current with a Faraday cup
  - Make sure the entire beam is on the screen; measure with Faraday cup and SerialEM current meter
  - ADJUST the ScreenCurrentFactor by the ratio of Faraday cup to SerialEM current and restart program
    - E.g., if existing factor = 0.7, SerialEM gives 4.2 na and cup gives 5.7 na, change factor to  $5.7 * 0.7 / 4.2 = 0.95$
  - Factor is not always 1 on Tecnai and  $< 0.1$  on JEOL (?!)
- Calibrate shutter dead time
- Set up beam and exposure so
  1. All of beam fits on camera but it nearly fills camera (to within 10% of edges)
  2. Use spot size that gives a current that can be measured accurately ( $> 1$  na, up to 5 na preferred)
  3. Use binning 1 and set exposure time to avoid saturating camera
- Take image and find the mean
  - $\text{Gain} = (\text{mean} * X\_size * Y\_size) / ((\text{exposure} - \text{dead\_time}) * \text{current} * 6.242 \times 10^{-9} \text{ e-/na-sec})$

## Options for Dealing with 16-bit Cameras

- Originally the MRC format defined only a *signed* 16-bit format (range -32768 to 32767)
  - If data from 16-bit camera do not range above 32767, they can safely be saved in this format
  - Two options to save data bigger than this: subtract 32768, or divide by 2
- Agard's lab defined a new mode for *unsigned* integers (range 0 to 65536) so I adopted this
  - 16-bit data can be saved in this mode: better than subtracting 32768
  - IMOD will process these data
- But when 16-bit data are gain-normalized and still stored as unsigned, negative values get truncated at zero
  - This could be a problem for very low dose images, especially if dark reference drifts
- These problems and choices go away if “Divide by 2” is selected as a *camera* option (not a file storage option)
  - Take dark reference of small area not crossing quadrant boundary and measure SD with Ctrl-I
  - If  $SD > 2$  you have at least one bit of noise and dividing by 2 is fine

## Using Settings Files

- SerialEM always starts by reading SerialEMsettings.txt in the “Start in” folder defined in the shortcut properties
- Each user should have a copy of the SerialEM shortcut that “Starts in” their own working directory
- All but a few user parameters are stored in the file and restored on restart
- Settings are automatically saved when you exit, unless you first close the settings file
- You can save settings to a file under a different name, which becomes the current file until you exit
- When you load settings, it replaces the current parameter values
- The most recently used file list allows rapid switching between settings

# Calibrating Specimen and Stage Geometry

- Image shift calibration is key to proper tracking, etc. and will take things a long way
- But other information is needed:
  - Angle of tilt axis on camera, to shift along axis in low dose
  - Pixel size and angle of tilt axis, for reliable stage moves
  - Relation between stage movement and position on specimen
- IF relation between image shift coil value and movement on specimen is fixed, the program can use image shift calibrations at two mags to “transfer” a pixel size and rotation from one mag to another
  - On Tecnai, this is true within LM and within regular mag ranges
  - On JEOL, this is approximately true within subranges separated by boundaries where the relationship changes by  $> 10\%$

# What is the Minimal Calibration to Get from Specimen to Camera Coordinates?

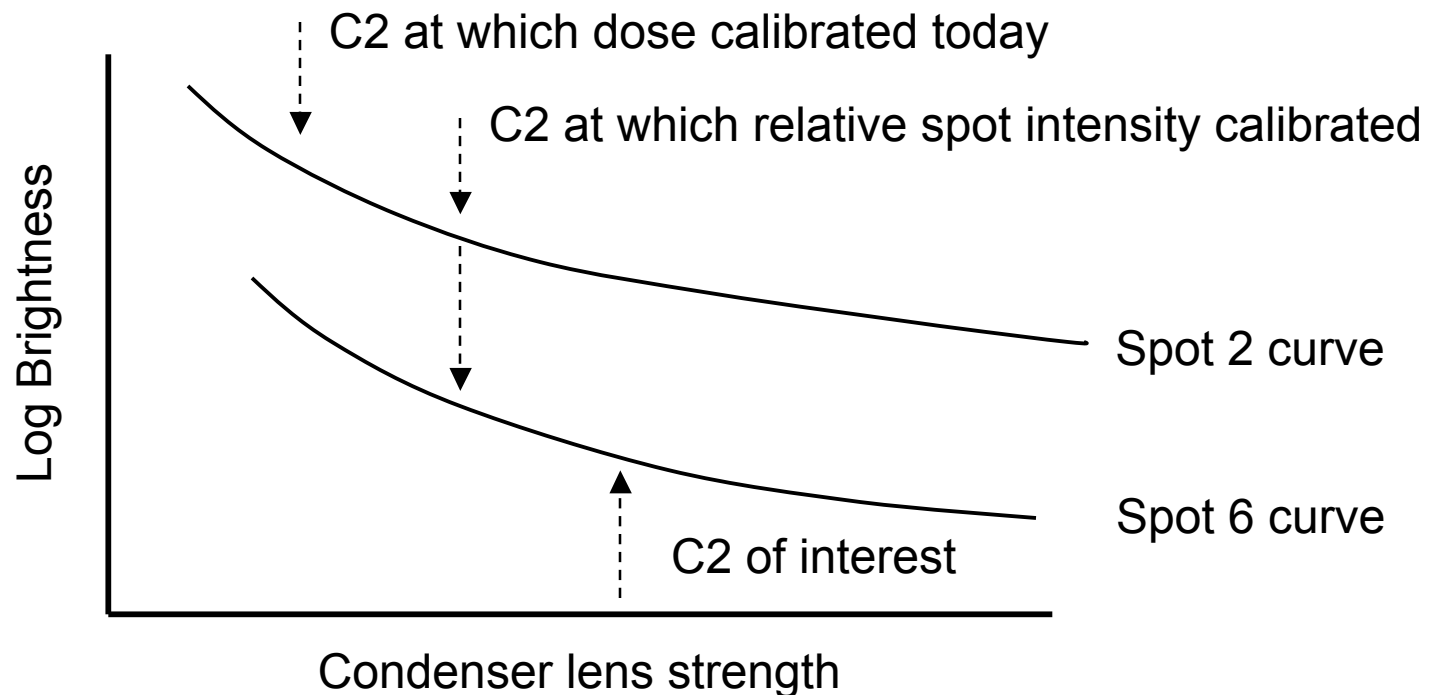
- In principle, sufficient information is provided by
  - Image shift calibration at each mag of interest (including LM)
  - One pixel size in each mag range
  - One calibrated tilt axis rotation angle (solved from fiducial tilt alignment)
  - Relative rotation between each mag range
- This strategy can be used on Tecnai
  - But you may want direct measurement of pixel size for all mags where data will be acquired, to protect against problems with image shift calibrations
- On JEOL, an alternate strategy is used because image shift is not a reliable enough basis
  - A complete set of grating images are taken and analyzed with alignment and modeling to get pixel size and relative rotation at all mags of interest (including LM)
  - Image shift is calibrated only in the higher mag range where it is used

## What about the Stage Calibration?

- The other needed piece is a stage calibration, because stage axes may not be perpendicular and movements can vary from true distance by 10%
  - Should be done at low enough mag(s) to average over non-uniformities
  - Calibrations at multiple mags are simply averaged because there is just one relationship between stage and specimen coordinates
- Relation between stage and camera at a mag is thus derived by combining this relationship with the relationship between specimen and camera
  - This could be done by direct calibration instead, but for now that is the way it is
- A practical point: waffle grating is a hazardous specimen to use for lower mag calibrations. But you can check whether cross-correlation found the right shift after doing calibration

# Beam and Dose Calibration

- The automatic dose calibration during gain reference allows dose to be computed at multiple spot sizes provided:
  - The spot intensity calibration is done
  - Beam intensity is calibrated for each relevant spot size
- Because computation involves following a chain of ratios, it is advisable to calibrate dose also at or near the spot and intensity of interest



## **Beam, Spot, and Crossover Calibrations**

- Column alignment on the Tecnai can change the beam crossover point, which will impair accuracy of beam intensity calibration
- This will be dealt with in next version – requires storing the crossover intensity calibration when beam intensity is calibrated
- After this change, you should always run crossover calibration before a beam or spot intensity calibration
- Thereafter, simply redoing a crossover calibration after column alignment should keep the intensity calibration accurate

## General Points on Program Setup

- Most Priority 2 items are needed to support some neat feature, so what can you skip?
  - The redundant calibration of image shift and pixel size/rotation can be pared back
  - “For the sake of completeness” can be ignored
  - Tietz binning offset hasn’t been an issue on F415, may just need a spot check on 2K cameras
  - You can live without the X-ray removal from dark references unless you see that they are a problem. But findhotpixels is in IMOD 3.9 so should be easier to run
- Doing the setup seems to require digesting a lot of words and following step-by-step instructions. It’s not obvious what else I can say to help this