SerialEM Note: Setup LD with Mix of uP and nP Modes

Adapted from Chen Xu's original file https://sphinx-emdocs.readthedocs.io/en/latest/serialEM-note-LD-mPnP.html Edited by Tong Wang (twangl@gc.cuny.edu)

Abstract

There are cases and situations that people want to use nanoprobe (nP) mode, but nP is not comfortable for lower mag range such as first a few magnifications just above LM, because the beam doesn't spread wide enough to cover entire camera area. This forces us to use microprobe (mP) for View and nP for rest of LD areas, namely F, T and R etc..

However, most people find it hard to setup LD conditions with the mix of nP and mP modes. I had frustrated time doing so too. This is, I think, mainly because nP and mP don't share the same origins for beam shift and defocus - they have their one origins. In SerialEM, all the LD conditions are linked together. Therefore, the seprate origins of focus and beam shift for mP and nP modes give extra hard time setting up LD in this mix use of nP and mP.

SerialEM already has a way to deal with this problem. I hope this doc makes it clearer to easier to follow practically.

Procedure Setting Up LD with mP and nP

In my case, I use mP for View area with -200 microns focus offset. All other areas F, T and R are with nP.

- 1. Before LD is turned on, make sure beam is centered for both *mP* and *nP* beam. I usually use Direct Alignments to do this with *mP* and *nP* beam. That is, turn *mP* on, Directly Alignments Beam Shift (multi-function to center) done. Repeat with *nP* mode.
- 2. Turn on SerialEM LD.
- 3. Lower down fluoroscreen.
- 4. From Task Specialized Options, make sure the "Adjust Focus on Probe Mode Change" is NOT checked.
- 5. Set View Defocus Offset to 0 using dial Up-Down button on SerialEM LD Control Panel.
- 6. Select R area (radio button) on LD control panel.
- 7. On microscope right panel, press "Eucentric Focus".
- 8. Reset Defocus (L2 button on our current setup for soft buttons on control pads), this makes defocus display 0.
- 9. Select V area (radio button) on LD control panel.
- 10. wait 6-7 seconds to allow scope to switch to this mag and mP mode.
- 11. On microscope right panel, press "Eucentric Focus".
- 12. Reset Defocus (L2 button on our current setup for soft buttons on control pads), this makes defocus display 0.
- 13. Set View Defocus Offset to target value (-200 in my case) using dial Up-Down button on SerialEM LD Control Panel.
- 14. From Task Specialized Options, make sure the "Adjust Focus on Probe Mode Change" is **NOW** checked.

That's it.