Collecting images in semi-automated fashion in SerialEM

1. Degauss omega filter, turn off MDS (2200FS)
2. Collect dark- and gain references on DE-20 camera using DE-IM program (without holder) (2200FS)
3. Start Digital Micrograph on Gatan PC
4. Transfer a grid, start SerialEM (on DE PC for 2200), open Navigator window; collect full grid montage at **100x** using US 4000 camera; switch back to **M** mode
5. Wait 15 – 20 min to warm up objective lens
6. Find an easily recognizable feature in the montage, mark it with permanent marker ("**add a marker**" in Navigator menu)
7. Switch to **4000x** and push "**go to marker**" in Navigator window
8. The feature found in the montage should be close to center of the screen. Collect "**view**" image in SerialEM, mark it with temporary marker and say "**shift to marker**" in Navigator menu. That should bring low mag montage and **4000x** image in register with low mag montage
9. Align the microscope as usual for high-res imaging, save alignment
10. Switch to low-dose in SerialEM; lower the screen. Check "**Continuous updates**" radiobox
11. Check beam centering etc. in **view** and **focus** modes; recenter beam, adjust beam shift/tilt with "**set**" radio box checked. Once completed for each mode, uncheck that box and switch back to record mode. Go back and forth until converged
12. Find a feature in record mode, center it with stage movement, acquire record image and make sure the feature is centered in the image. Make sure that image shift in SerialEM is zero
13. Acquire view image; uncheck "**move stage for large mouse shifts**" in "**alignment**" menu
14. Center the feature in view image using right mouse button; push "**set**" button (next to "**shift**"). Image shift value in SeiralEM should go to zero, another view image would be acquired and the feature should stay centered; if not, recenter it and push "**set**" again
15. Check "**move stage for large mouse shifts**" in "**alignment**" menu again

16. If you are using filter, make sure to insert the slit and adjust its width/position in the **Record** mode

17. Uncheck "**Continuous updates**" radiobox

18. If full grid montage is in register with **4000x**, setup a polygon montage in Navigator for a good grid square, if that is proved to be difficult go with "corner montage" using corners of that grid square. Use US 4000 for taking the montage. Close montage file in "**file**" menu

19. Switch to DE-20 camera (2200FS) using either "**setup**" button in camera and image menu or "**parameters**" in camera menu tab. Make sure that in **Record** mode it will save raw frames (and FinalImage if needed) on DE PC

20. Run macro #7 (**Init**) to initialize variables used in other macros. If need you could edit those.

21. Select points in the latter montage marking intended holes in the map. SerialEM allows to either mark all points manually or to make a grid of them filling a rectangle, circle etc.

22. Go to corner of the latter montage and run macro #9 (**InFoc8kx**) to adjust z-height. Push "**update z**" for that group of points. That helps focusing at individual holes when collecting datat.

23. Acquire view image, center a hole in it, open "**setup**" menu; select "**view**" and set the area to half of the camera size or a little larger (10 – 20%) for it; acquire an image where the hole is centered and save that to buffer "**P**". Switch view mode back to full camera size.

24. Run "**drift**" macro from macro menu, make sure that drift is getting below selected threshold. If not, play with holder to make it stable.

25. Check radio box "**acquire**" in Navigator for that group of points and check "**new file at group**" radio box as well. That will open a file setup dialogue.

26. Once the file is setup go to Navigator menu and push "**Acquire at points**" menu. That brings up a dialogue with initial action and main task for collecting data at each point. Switch to "run macro #10 (**LD**) as the main task, tell the program that you want to collect single images, not montages and setup a file for that.

27. Once everything is setup the program will start **LD** macro (main macro for auto-collecting data) and will run it at every point in the group

28. In the Init macro (opened with macro editor) you can set drift rate criterion and defocus range for data collection

29. Check nitrogen level in the specimen dewar once in a while