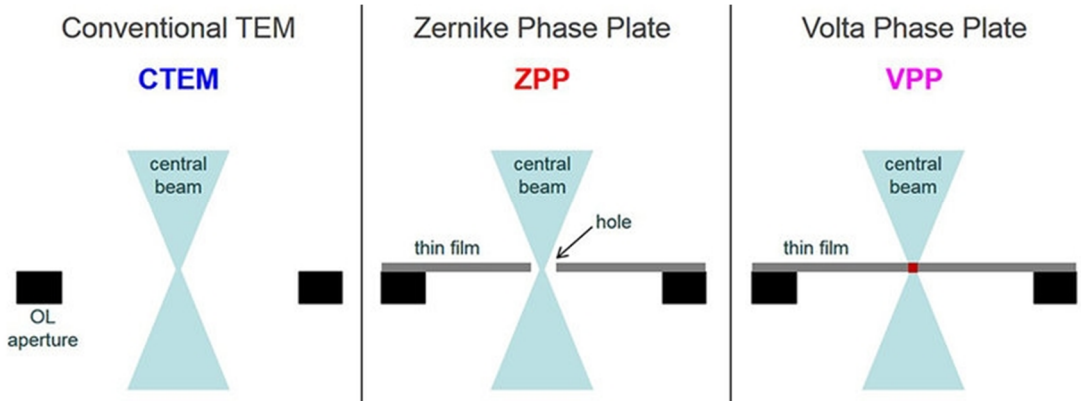
Voltage Phase Plate

Updated at 7/31/2019

This document shows how to align voltage phase plate (VPP) and set up data collection with VPP in Leginon at FSU BSIR.

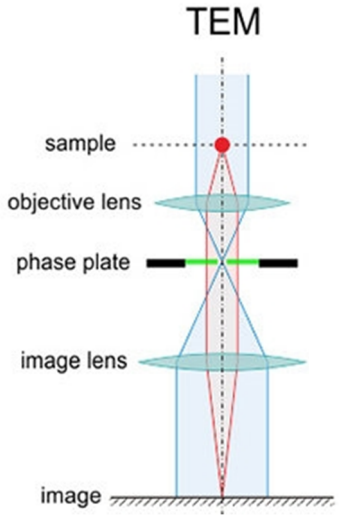
Please pay attention to the highlighted part (yellow) which is very very important.

1 Introduction of VPP



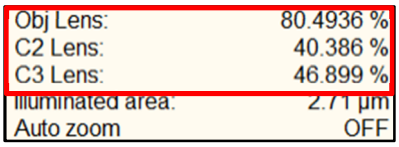
As a successor to the Zernike phase plate the Volta phase plate (VPP) has similar but simplified design. It comprises a thin (~ 10 nm) continuous amorphous carbon film constantly heated to ~ 250°C. Unlike the Zernike phase plate the VPP does not have a central hole. The phase shift is created "on-the-fly" by the interaction of the central diffraction beam with the film. (Max Planck Institute of Biochemistry)

2 Align VPP: on-plane phase plate alignment



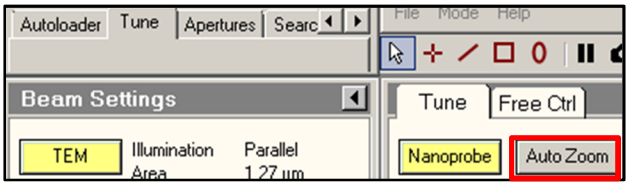
- VPP is located at the same position as the Objective aperture at the back focal plane below the sample and objective lens. Usually the microscopy has been calibrated and aligned that a parallel beam will be focused on the back focal plane where VPP is, but to best use VPP the beam has to be perfectly focused on it so here 'align VPP' indeed is to slightly change C2 and C3 lens to let the beam perfectly focus on the VPP, instead to change the VPP height. The reason that you have to align VPP whenever you change your exposure beam intensity (C2 lens) is because C2 change will in turn change the focus position. So it is necessary to align VPP every time when you change exposure beam (spot size C1, beam diameter C2), or switch phase plates, and the best would be to align VPP after your exposure beam is determined (ex or ec preset in Leginon).
- We have 6 phase plates on Titan, and each has 75 patches to use.
- Go to an empty hole

- Send exposure preset ex to microscopy. Make sure it is your final beam for data collection. Record the Obj, C2 and C3 Lens values at the right bottom of EM GUI.

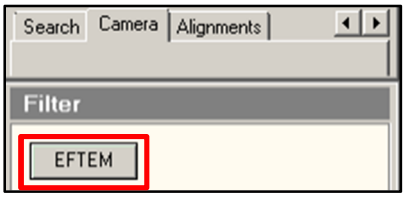


- Have Flu camera inserted, and switch from Natural to High Resolution (2X zoomed in) and Manual mode (at the bottom of the image)

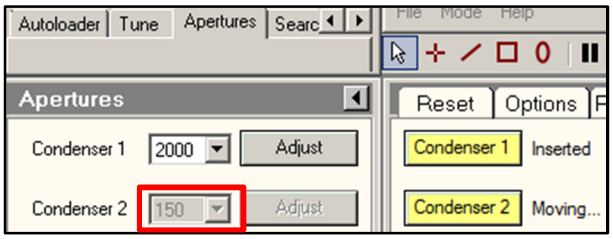
- EM GUI -> Tune -> Auto Zoom off



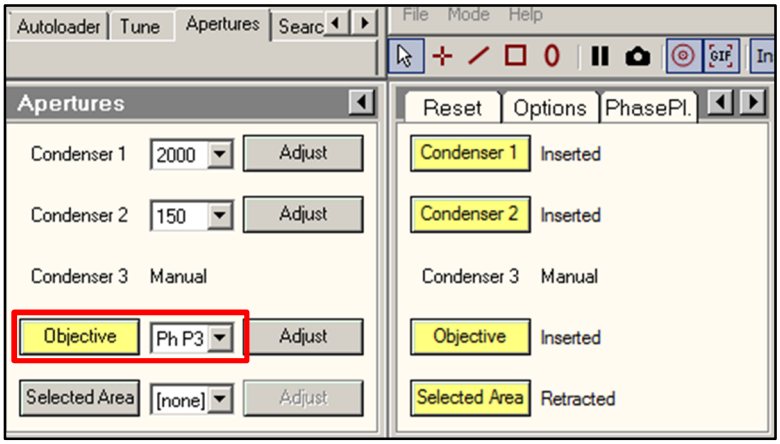
- EM GUI -> Camera -> EFTEM off



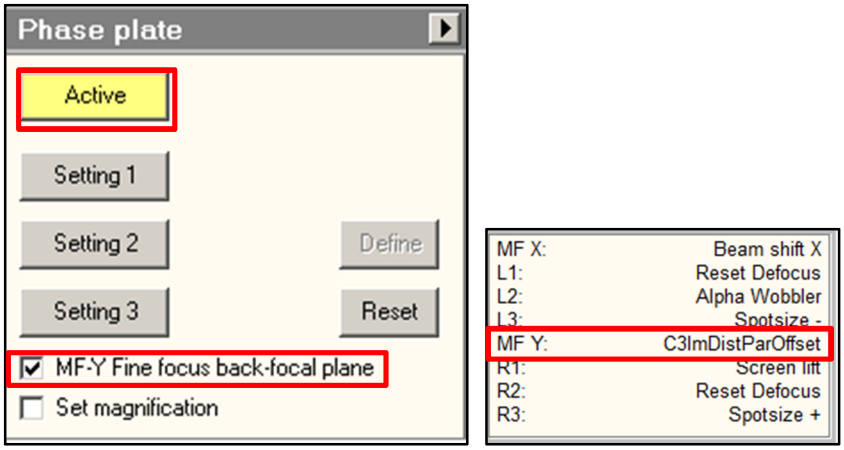
- EM GUI -> C2 aperture 150 (usually it is 70)



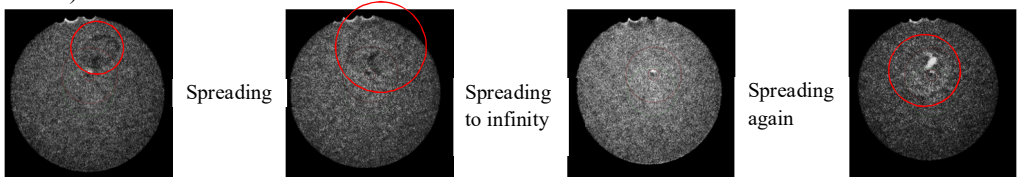
- EM GUI -> Aperture -> insert VPP (Ph P1-6 at the Objective aperture list)



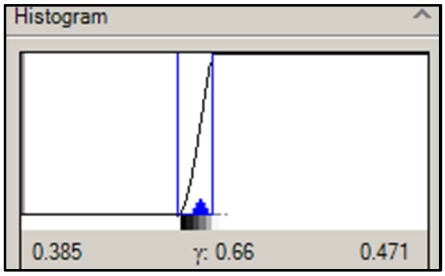
- Go to Mag 5K,
- Activate VPP, and have ‘MF-Y Fine focus back-focal plane’ checked. The MF-Y will be functional as ‘C3lmDistParOffset’



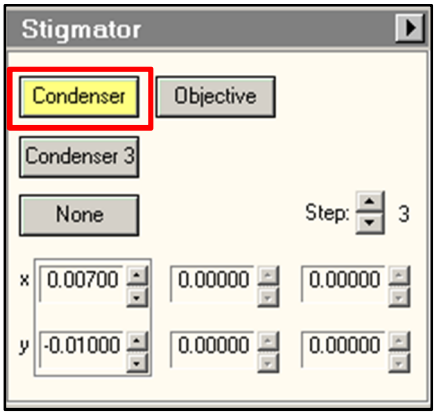
- Adjust MF-Y clockwise you should see the feature (an example feature in the red circle) spreading -> in-focus -> spreading again. Finally stay at in-focus which should be featureless (the 3rd image below).



Adjust the image histogram (right side of the image) simultaneously when you adjust MF-Y.



- Record the Obj, C2 and C3 lens values. The C2 and C3 will change slightly if MF-Y is adjusted.
- EM GUI -> Stigmator -> Condenser, Adjust MF-X and Y button to make it in-focus as featureless. Remember to click None after you are done.



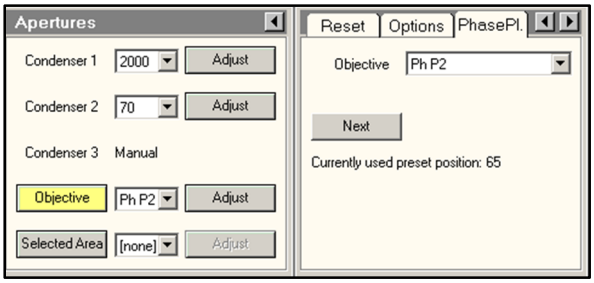
- EM GUI -> C2 aperture 70
- EM GUI -> Camera -> EFTEM on
- Switch back to Natural mode
- Send ex to microscope to check if C2 and C3 lens values are consistent as after VPP alignment.

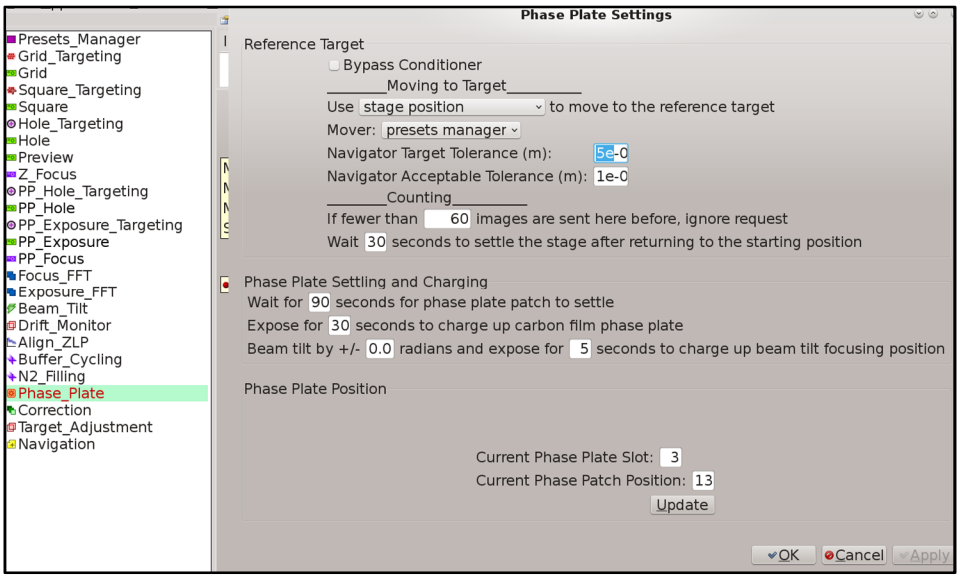
3 Align beam with VPP

- Perform direct alignment
- Insert the phase plate
- Align VPP: On-plane phase plate alignment
- Retract the phase plate
- Perform the coma-free alignment
- Insert the phase plate: wait for ~5min for the thermal drift to settle
- Stigmate the objective

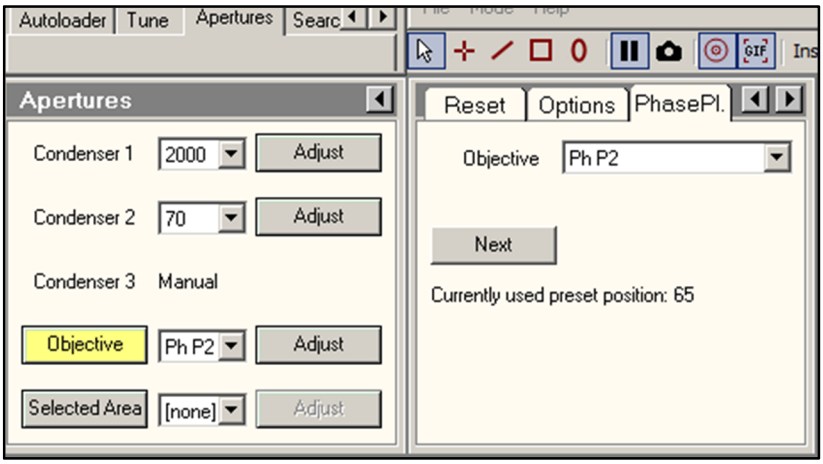
4 Set up VPP in Leginon

- Application: MSI-T2-PP
- Use 'stage position' for PP_Exposure node since no image shift is allowed for VPP use.
- Data collection defocus range: -0.5 ~ -0.8µm
- Best phase shift rage is 0.2~0.8π (36°~144°)
- Phase_Plate node settings
  - 'By pass conditioner' should be only checked for test, and should be unchecked when data collection starts.
  - Moving to Target: all default
    Use 'stage position' to move to the reference target
    Mover: Presets manager
    Navigator Target Tolerance (m): 5e-07
    Navigator Acceptable Tolerance (m): 1e-06
  - Counting
    If fewer than xx (60) images are sent here before, ignore request. This number is tested that after these many images collected, the phase shift reaches the max (0.8 π). It is exposure beam dependent.
    Wait 30 seconds to settle the stage after returning to the starting position
  - Phase Plate Settling and Charging
    Wait for 90 seconds for phase plate patch to settle.
    Expose for xx (30) seconds to charge up carbon film phase plate. This number needs to be tested that after xx seconds of fc (focus beam) the phase shift reaches the min (0.2π). It is focus beam dependent.
    Beam tilt by +/-0.0 radians and expose for 5 seconds to charge up beam tilt focusing position.
  - Phase Plate Position. These number have to match the EM GUI, manually fill in and click 'update' button.
    Current Phase Plate Slot: 2
    Current Phase Patch Position: 65





- Phase_Plate reference position: Every phase plate patch position needs to be charged up before use, and the charging needs to take place at an empty broken hole or bad ice area. This place is called reference position. Usually we save a position of empty broken square and sent it to microscope, then click  to acquire an image to let the program remember its coordinates.
- During phase plate data collection, the EM GUI has to stay at the page of Aperture -> PhasePlate, otherwise, Leginon will not be able to move to the next phase plate patch position.



5 Clean up

- Deactivate VPP and remove it
- VPP has to be removed before turning Turbo pump on.

6 Tips

- Someone said that the middle patches are better than those on the edge on each phase plate, but it is impossible to only choose them for imaging.
- Expand the search beam (hl?) beyond the parallel range by ~2µm in nano probe and ~5µm in micro probes to prevent the creation of a parasitic spot on the phase plate in search mode.