

# Fringe Free Imaging

Please note that after the Fringe-Free Alignment (FFI) upgrade, there will be some changes visible in the behavior of the microscope:

- The shape of the spot in nanoprobe mode is different than before and not perfectly round anymore.
  - This effect does not influence the application performance of the system and, if desired, can be reduced by selecting a smaller C2 aperture.
  - In practice, condenser astigmatism is now best optimized by adjusting the stigmators to create a round beam in parallel mode, instead of optimizing the spot shape by condensing the beam.
- Similarly, in STEM mode, the spot cannot be made perfectly round anymore.
  - Also note that the sensitivity of the condenser stigmators has been reduced, and so the stigmators might report higher values after stigmation than before.
  - This is normal, and the Krios STEM specification will still be met. The probe shape can also be improved by selecting a smaller C2 aperture.
- In combination with a Phase Plate, the beam tilt pivot points cannot be adjusted perfectly anymore, since the perpendicular correction alignment for the beam tilt pivot points has been disabled to prevent the inadvertent misalignment of the beam shift pivot points.
  - This will not limit system performance. If the beam tilt pivot points are very far off, the advice is to iterate between beam tilt pivot point and rotation center or to request that a staff member re-align the beam shift pivot points in the phase plate alignments. Users are NOT permitted to do any alignments outside of the "Direct Alignments" panel in TEMUI.
  - On systems with such a configuration, the recommended way to adjust beam tilt pivot points for TEM experiments is to work with a parallel beam and change the magnification to allow displaying the full beam on the flucam viewer. Pivot points are then adjusted by minimizing the beam movement during the alignment.
- On Krios G3i systems, the improved cryo box might cause a beam cut-off at some magnifications below 5 kX. Again, this cut-off does not hamper the application performance of the microscope.

These side effects of FFI might require some getting used to. The main advice is to take the points above into account and set up and use the Krios exactly the same as before the upgrade. Also note that all Krios G4 systems shipped from the factory include FFI by default and show exactly the same behavior.

The full ThermoFisher Application Note is available here:

<https://stanford.box.com/s/ajjwy80a9ol6gc8bwcelxwsgkhtpmcwq>