



## Aligning Filters for Tucam Operation (Spinning Disk)

### To start. . .

1. Choose the 10x objective.
2. Set grid slide on sample stage and focus on center of grid.
3. Loosen cover thumb screw and remove cover.
4. Slide filter pack in and tighten the Allen-keyed screw that locks it into place.
5. Use c488-561 (or cBF) and 'Acquire' image.
6. Adjust exposure time and EM gain until signal is visible in both channels
  - EM gain 1* on left side of image (green channel)
  - EM gain 2* on right side of image (red channel)
  - Ultra 1 camera: longer wavelength (right); Ultra 2 camera – shorter wavelength (left)
7. Merge 2 channels:
  - a. Display > Split View > Align
  - b. Check *Show Alignment Image*
  - c. A split view align image is displayed
8. Adjust the Allen-keyed alignment knobs for x- (top right) and y- (bottom left) to align the two channels.
9. When channels are aligned, stop 'Live' acquisition
10. Keep cover off, and proceed to imaging (you can switch to a different objective).

### When done imaging...

1. Put cover back on and pull out filter pack.
2. Return grid slide (drawer).