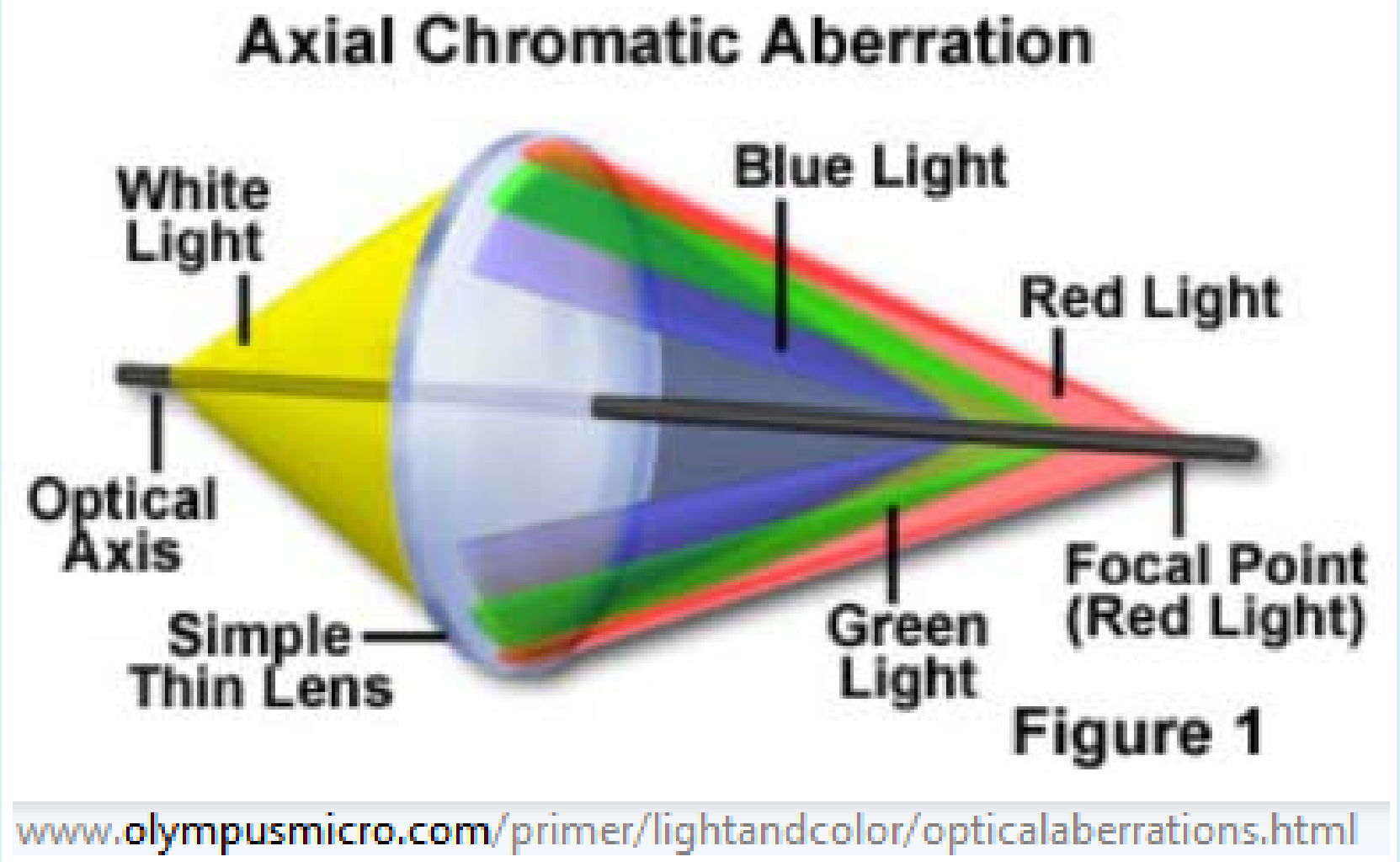
Chromatic Aberration and What It Could Mean To You.

Often we want to image (and measure) the location of different color proteins inside cells. XY registration is easy, but it is not so simple in Z.

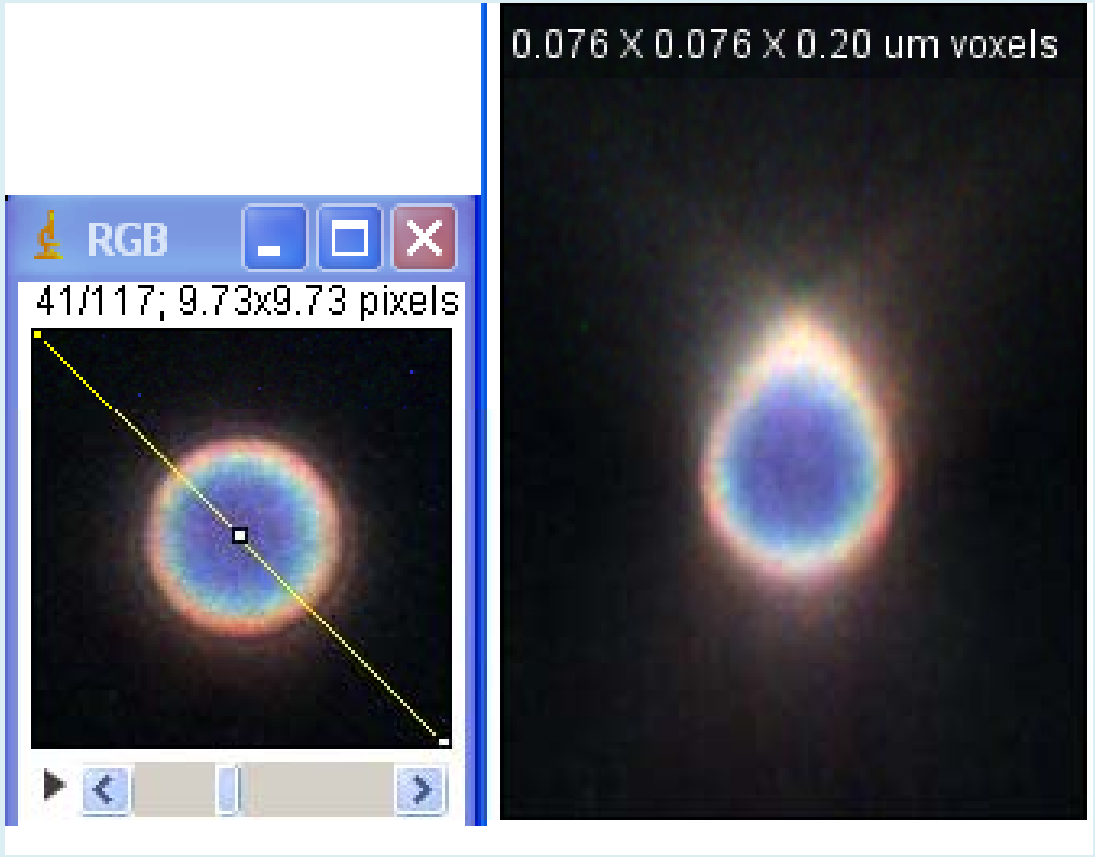
Michael Cammer Dec. 2011

Different λ of light focus at different Z axis positions



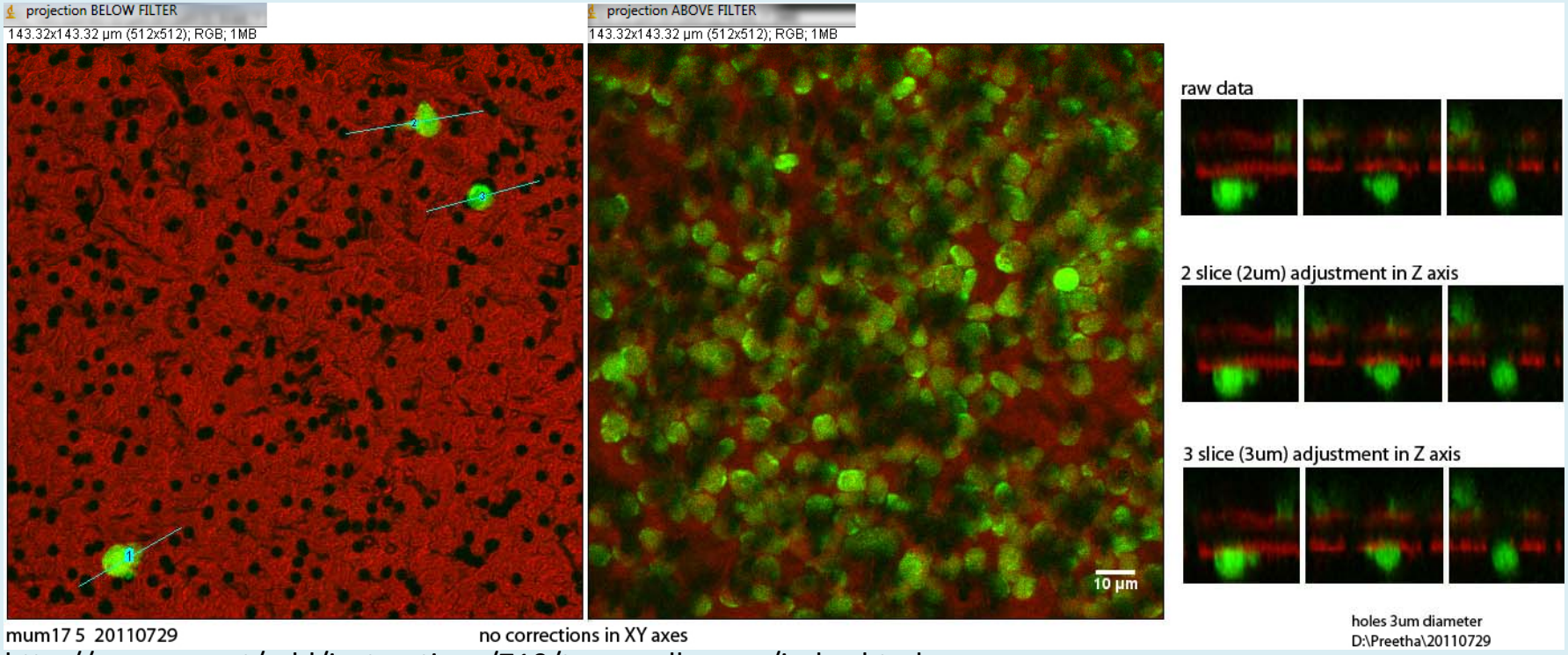
150X Olympus objective well corrected

Emissions from 500 to 670 nm.

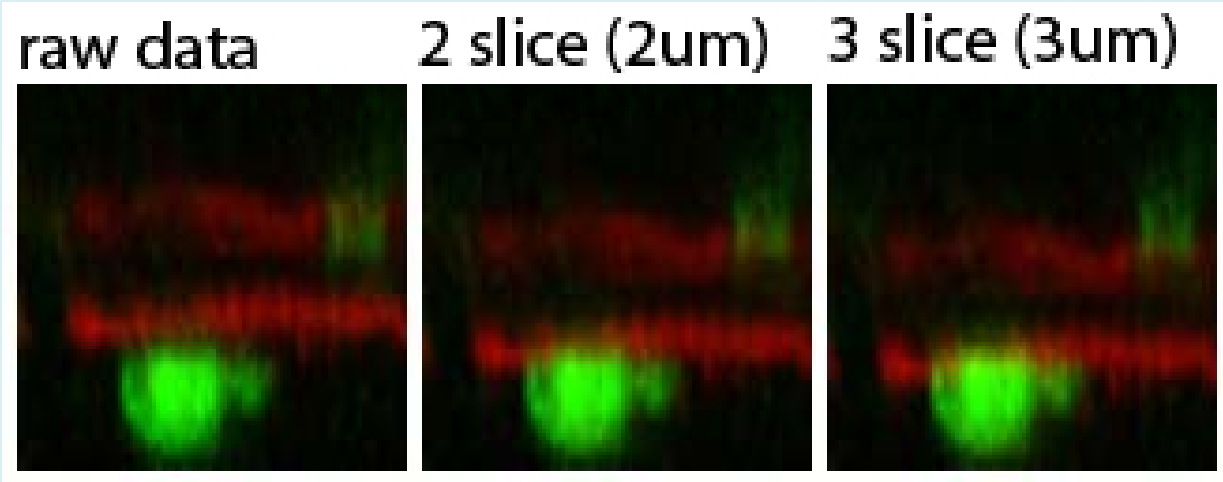


4umbeads_150X_Z020_XZ

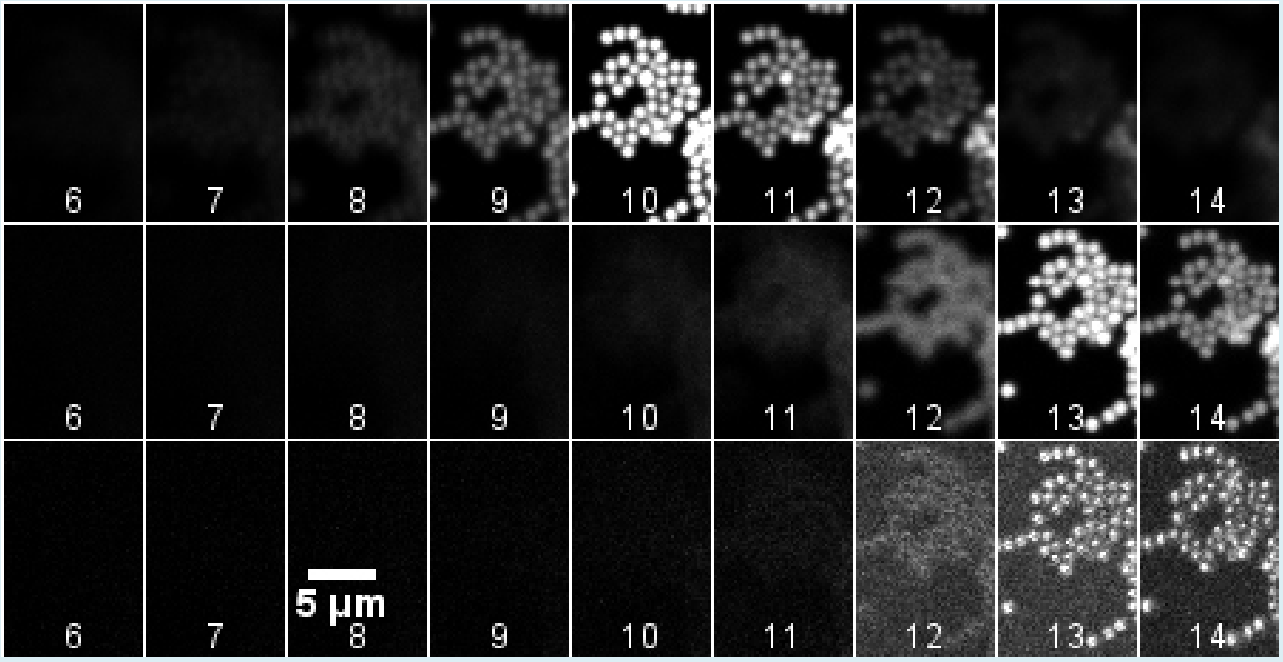
Example with transwell assay

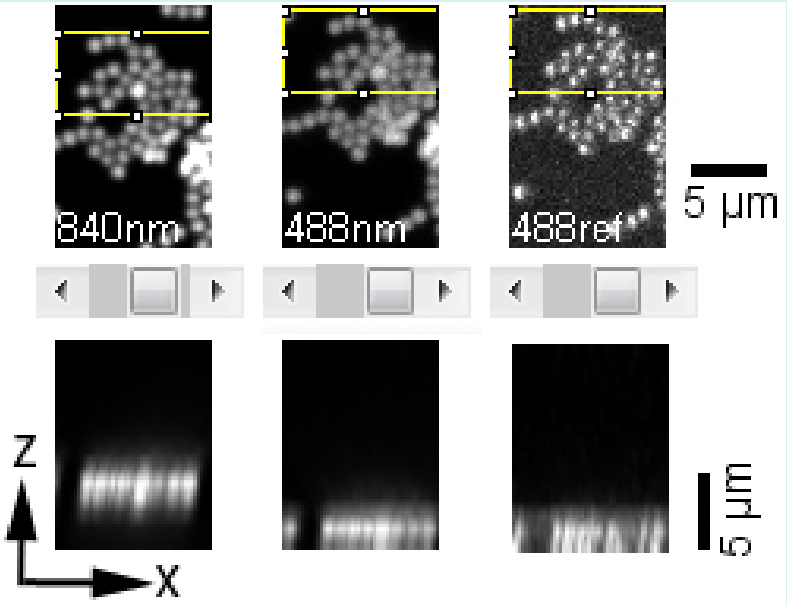


http://cammer.net/mld/instructions/710/transwellassays/index.html



How to calibrate

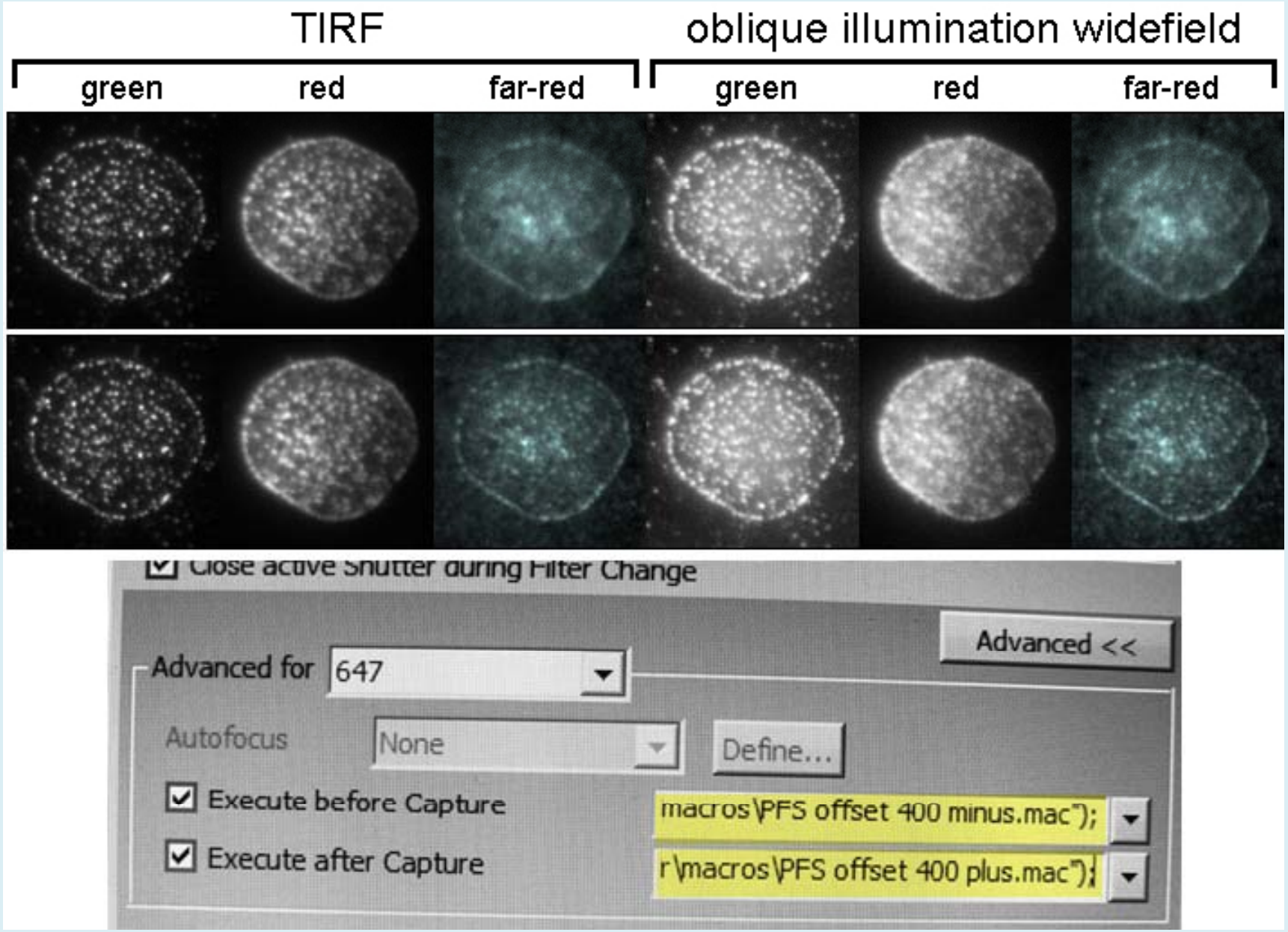




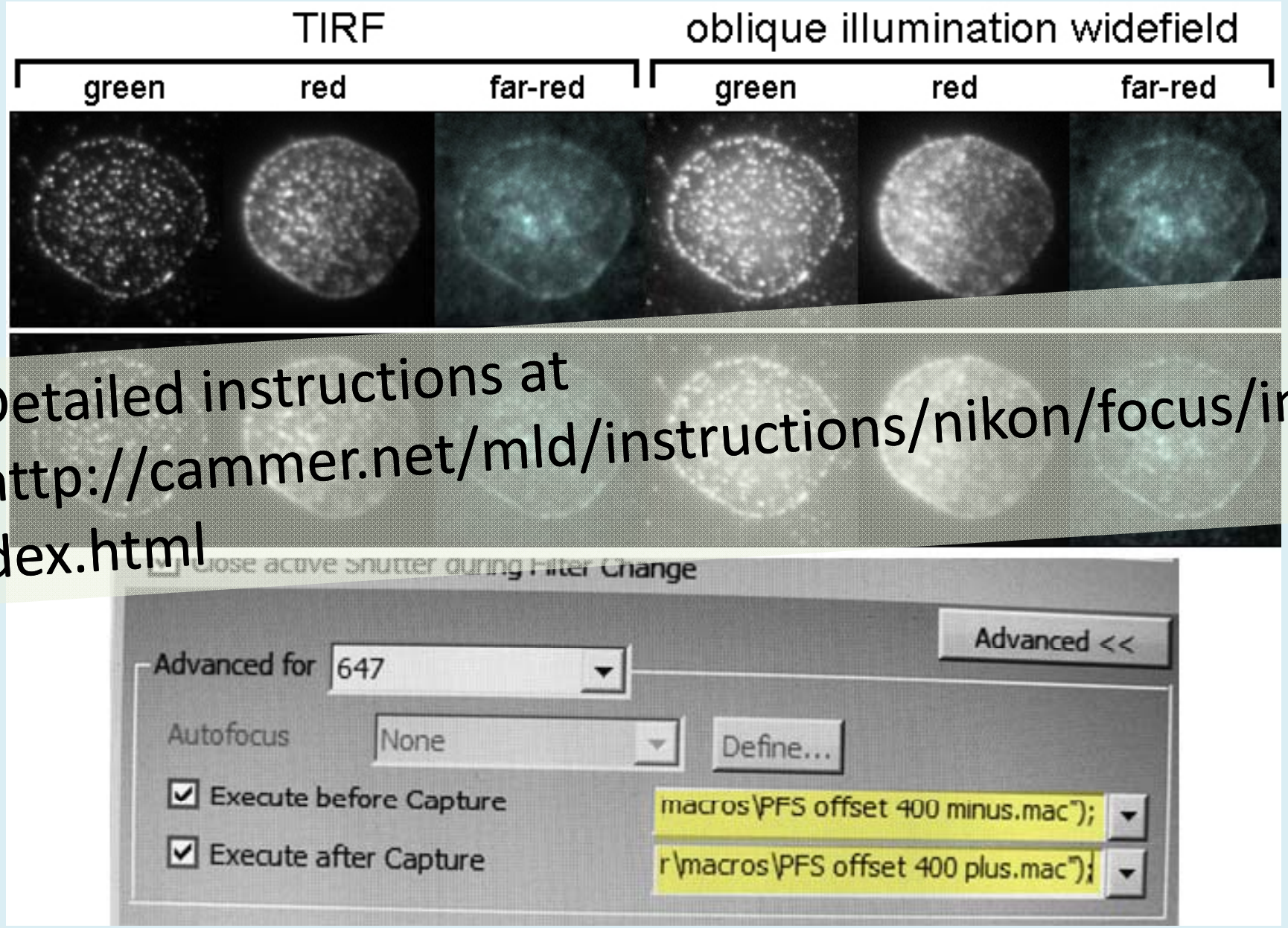
This is caused by chromatic aberration. The reflection is at 488 nm and the multiphoton excitation is at 840 nm.

Based on measurements with beads, the focal point from one to the other wavelength shifts by approximately 3 um.

Chromatic aberration a problem in TIRF too



Chromatic aberration a problem in TIRF too



Conclusion

When doing precise colocalization, need to check the offset of different wavelength probes along the optical axis.

When taking single optical section pictures of different probes, need to adjust focal planes appropriately.