Aim	Parameters	Sample	Protocol	Output	Action	Comments	
Objectives	**clean**	-	clean all objectives carefully and examine				
system	**microscope warm-up time**	beads	multi-point time lapse	shifts in position with time			
lasers	**laser warm up time**	power meter	check laser stability upon engaging				
	Laser Stability	power meter/mirror slide	Obj: 10X. Adjust AOTF to mid gray values. Detector on counting mode. Maximal zoom. run time lapse for 2h, 20sec intervals. Sequential scan- 1 laser at a time	plot Average Int±SD with time		acceptable range<10% for long times. 3% for 5 min	
measure alingment of excitation light path	**laser Intensity**	power meter	Obj: 10X . Keep all parameters fixed (low gain, avoid saturation) and recored each laser line at varied intensities. Pinhole open	overlay to extract intensity fluctuations	compare intensity fluctuations to signal fluctuations in samples	focus on a slide first then apply the same for meter	
	pinhole	power meter/ tetraspec bead	chanhe pinhole from 1AU to 2AU and measure intensity (<x3)		adjust pinhole position		
	detector	mirror slide	with fixed laser intensity- compare the gain required for saturation				
lasers	**axial resolution**	mirror slide	20nm wide detection windows centered around each laser line. Focus to the slide plan, change to XZ line scan. **Pinhole at 1AU and 0.5AU**.	line profile		preparation: glue the mirror to a glass slide and cover with #1.5, elvanol	
	spectral seperation	mirror slide	Obj: 10X ; lasers: 488, 561, 633. focus on surface, open pinhole and increase zoom to 8X. Adjust AOTF to avoid saturation at all scans. sampling: 256*256 . Run Xyλ 500-670, 5nm intervals	plot average image intensity as a function of wavelength			
	Z galvo Stability	mirror slide	Excitation:488nm. Obj:40X-63X. Pinhole 1AU and 0.5AU. Focus on slide, switch to XZT mode . Acquire Z stack at 30nm intervals, 256*256, 2 sec interval for 10min-1h.	plot peak position and FWHM over time			
objectives, laser alignment, scanner	**Field Illumination**	chroma slide (pink)	**Obj: All.** Main laser lines. Identify slide surface and penetrate : **10X:75µm ; 20X:50µm ; 40X:40µm ; 63X:30µm**. minimal zoom, pinhole=1AU detector on counting mode	apply smooth images, contrast enhancement and check intensity distribution. Divide by a homogen		preparation: cover with #1.5, elvanol	
Scanners	**Image Distorsion**	Grid	image a square at high zoom with good sampling	check for any deviation from square shape	create an edge map		
Co-localization	**chromatic shift**	1 µm tetraspec eads	pinhole 1AU , maximal zoom (apply over sampling), low laser power and low gain. Acquire Z stack (10X: 1µm ; 63X: 0.15µm) at sequential scanning for several laser lines	check shift between channels (X-Y-Z) and apply a correction factor for each direction. Project this correction to next images acquired and check shifts			
Stage	**stage stability**	1µm tetraspec beads	20X air objectives, exc 633 (avoid bleaching). Pinhole 1AU, XY pix=100nm. **1. multi point large distances**; **2. place 1 bead at different locations** (known distances) along FOV. Time lapse for 30 min, 20sec intervals.	filter to reduce noise, binary mask. Compare centers with time			
objective chromatic abb	**PSF**	100nm beads directly on #1.5, elvanol	488 laser line (intensity to give 75%image intensity from max bit range, use LUT), low noise (reduce gain). acquire Z stack (2X size of PSF)of **an isolated bead closest to the coverslip plane** with different objectives. **Pixel size: (NA=0.75/138*138*716)(na-0.95/87*87*208)(NA=1.1/75*75*246)(na=1.4/59*59*152). Pinhole:5AU, 1AU and 0.5AU**	generate PSF from image.	Check homogenoity. Make sure the entire P	*check if correction collar is adjusted properly. Allow the slide to sit for 30min before acq. Take several PSFs for each objective	
transmitted alignement	**condensor-objective**	tissue section	10x, kohler alignment. Center	even		more critical for wide-field	