Polystyrene bead calibration of spinning disk confocal microscope's lasers

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I have done a sort of calibration test on the spinning disc scope that we can use as a baseline. I used 2.5 um InSpeck fluorescent polystyrene beads (Molecular Probes/Invitrogen) mounted in the media they provide. The general idea is, using specific imaging settings (which I copied from a typical imaging session of my own, and will describe in detail below), I adjusted the exposure time to the point at which one is starting to just barely see fully saturated pixels in each sphere within the frame. Using the protocol below, I hope to reproduce these results in the coming weeks to see if they are consistent. I still need to find a permanent spot in the lab for these beads to live, so everyone has access to them. My protocol follows.

objective: 100x oil, alpha plan apocromat, 1.46
laser line: 488/568/647
emission discrimination
filters for each line: 488 - Green; 568 - Blue/Red; 647 - Cyan/Far Red
laser control: 20%
power for each line: 50%

Beads used for testing:
488 line = InSpeck Green 0.3% 2.5 um diameter
568 line = InSpeck Orange 0.3% 2.5 um diameter
647 line = InSpeck Deep Red 0.3% 2.5 um diameter

Bead slurries are stored at 4 C in white plastic cylinders. Do not freeze them.

1) Vortex bead slurry. Pipette 1 uL onto a glass cover slip and let dry for ~10 minutes (I cover it with an opaque box of some sort.).

2) After beads have fully dried, pipette 10 uL mounting media and mount cover slip on a glass slide. Let this cure for a few hours, or overnight at room temp. Seal cover slip with nail polish.

3) Image beads using the microscope settings above. Start at 1 ms exposure time and increase in the appropriate channel until you start to see saturated pixels in each bead in the frame. Use the color scheme GreenGreyRed.tab to view this. Red pixels are fully saturated. Because these beads can not be fixed to the glass, some beads will move in the mounting media during imaging despite its high viscosity. I find that clusters of beads tend to remain still and are best for imaging.

My initial test results:
488 line: 30 ms
568 line: 150 ms
647 line: 3 ms

I will add these results in the log book, which is now by the spinning disc. Each time you perform this test, please record your results in the log book.