

# Leica SP8 STED alignment

## Alignment

To be performed before each STED experiment

- Mount a gold beads slide, and set up focus using the 93x glycerol objective
- Load the pre-set acquisition settings for the alignment of one STED depletion laser. They are aligned separately, using separate settings for each depletion laser. Pre-sets are in the list - but do not use the Leica "Expert" ones, but the custom ones further down the list (source: [Hänsch, Sebastian](#) )
- This will use the PMT detectors in reflection mode, with the notch-filters removed, using the White Light Laser source as channel 1, and the STED depletion laser as channel 2.
- Use XZ scanning to find the coverslip reflection at the interface where the gold beads are resting
- Adjust motor collar correction of the objective until coverslip reflection has the brightest centre line, and doesn't skew to one side (up or down).
- Use XY scanning to find a gold bead with a nicely shaped and visible white-light laser and STED-laser PSF, and zoom into it maximally.
- Adjust the alignment (unlock "expert mode" in the laser configuration screen) by moving the STED depletion ring to be centered around the white-light laser PSF as best as possible.
- Evaluate in the Z-direction (confirm using XZ scanning). Double check alignment with other beads on the slide.
- Mount a reference slide with known structures (eg. nuclear pores), and use it to confirm that the alignment was successful.
- The system is now ready for use in STED experiments.