Objective correction for Glycerol and Water immersion objectives

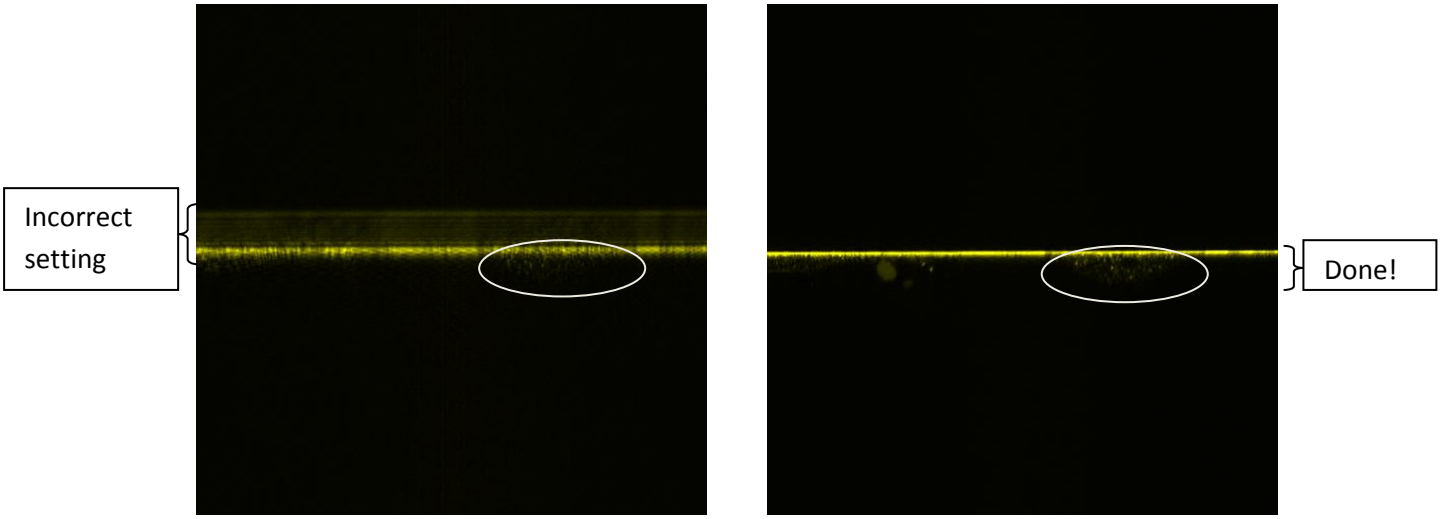
This protocol describes how to use the correction collars on the 63x glycerol and 63x water objectives in order to optimize the objective for different coverslip thickness and temperatures on the SP5 microscope.

- The 63x 1.2 NA Water and the 63x 1.3 NA glycerolobjectives can be corrected for differences in coverslip thickness and temperature.
- Since users use different coverslips and work at different T this correction should be performed at the start of each session.
- The 63x oil objective cannot be corrected; for optimum results it should only be used with the correct coverslips.

How to proceed step-by-step

- Select either the 63x glycerol or the 63x water objective
- Choose a single laser line; take one which you are going to use also for your experiment, 488 is eg. a good choice. Set it to 10 %.
- Activate one PMT and select range such that the laser line shines into your detector
- Set this PMT to rather low gain (500-600 V)
- In Scan mode change from **xyz (default) to xzy**
- Click onto the 'AOBS' change setting to 'Reflection'
- Visually select a sample using the fluorescence lamp in order to get the system focused
- Open the incubation box: lower right door; if using CO2 block CO2 supply with green button!
- Start scan in 'Live' Mode
- If not already there, place the bright line in the middle of the image **using the z-motor on the joystick control** (NOT on the panel!!) ,
- The line is the reflection of the laser on the coverslip; depending on your sample one or two lines can be visible; the **upper one is the important one** (you can see the specimen from the side.)
- Insert one finger between objectives and try to reach the correction collar
- Turn the collar in order to make the line as bright and as thin as possible (=focusing)
- Save your microscope settings for the next time under 'Objective Correction' or so
- Switch to experimental settings and proceed
- ***see example on next page for live cells expressing an EYFP-fusion protein with the 63x water objective***

Before… **After!**



Note that after optimization

1) blurr is completely gone

2) many more details are visible in the indicated cell

3) the coverslip appears as a single focused line

☺ ***With some practicing the whole process takes only few minutes…***