**CARS microscope**

1. **Turning on the setup**
   1. Turn on the acquisition box on the upper shelf.
   2. Turn on the 3D translation stage on the upper shelf (Sutter Instruments, MPC-200).
   3. Turn on Nirvana 2.*x* on the computer
      1. Connect BLIQ VSM (top right of screen). The polygon should start rotating.
   4. PUT ON OPTICAL GLASSES (Thorlabs LG9, at the entrance of the lab).
   5. Open the two shutters at the back of the picoTRAIN laser. The green laser beam that pumps the OPO will appear and the second beam is the 1064 nm output (only visible with a detection card).

With a detection card, make sure that you can see both beams (1064 nm and 816.8 nm) at the entrance of the polygon by opening the shutters one at a time.

* 1. Verify with the detection card that there is a rectangle beam shape after the top right mirror on the vertical part of the optical setup (after both the polygon and the galvo).
  2. With the Thorlabs power meter, verify that the power at the sample is 25 mW for the 1064 nm (picoTRAIN laser) and 40 mW for 816.8 nm (OPO laser). For both lasers, if needed, adjust the power at the sample with the polarisers which are right after the beam splitter (at the output of both lasers).

1. **Imaging**
   1. Place the sample under the microscope (do not scratch the tip of the objective with the slide or the coverslip).
   2. Put a drop of water on top of the coverslip.
   3. Lower the objective as low as possible near the sample (without touching the coverslip).
   4. Turn off the lights in the lab.
   5. Open the PMT with the slider on the right panel in Nirvana.
   6. Click on the “Live on” button in Nirvana. A uniform black image will be displayed.
   7. Rise the gain of the PMT in Nirvana to approx. 90-95%. A little bit of noise should appear in the image.
   8. Move the objectif up until the signal of the sample appears in Nirvana.

IMPORTANT:

Never open the lights in the lab while the PMT is ON. Also, DO NOT use any light source near the PMT/sample while the PMT is ON.

EMERGENCY:  
Turn off both lasers by shutting both shutters on the picoTRAIN laser.