HOW TO USE THE HILO ZEBRAFISH MICROSCOPE

GUIDE - DCC LAB

GITHUB AND GROUPE PAGE

by

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Introduction

The following guide as been written to help new users apprehend the HiLo microscope possesed by the DCC Lab located at the institut CERVO in Québec City, Québec, Canada. This guide should contain all the needed information to prepare the softwares needed to control the microscope, start the microscope and finally take HiLo imaging. The reader and future user should be able to proceed to all of those steps with no difficulty, even if he never used a microscope in his life. While reading this guide, if there are sections or steps that are unclear or unprecise, feel free to message any of the authors so that the guide can be updated and upgraded. If you are more interested to the data processing after taking HiLo images, I suggest the lecture of this guide, explaining how to motion correct images and proceed to image registration, and also this guide, explaining how to use the HiLo algorithm. Finally, I also suggest reading the master's thesis of Valérie Pineau Noël [1], the builder of the HiLo microscope we possess, explaining in detail everything there is to know about the microscope and his caracterisation. In this guide it will be assumed that this lecture as been made.

1 The microscope and it's components

1.1 The setup

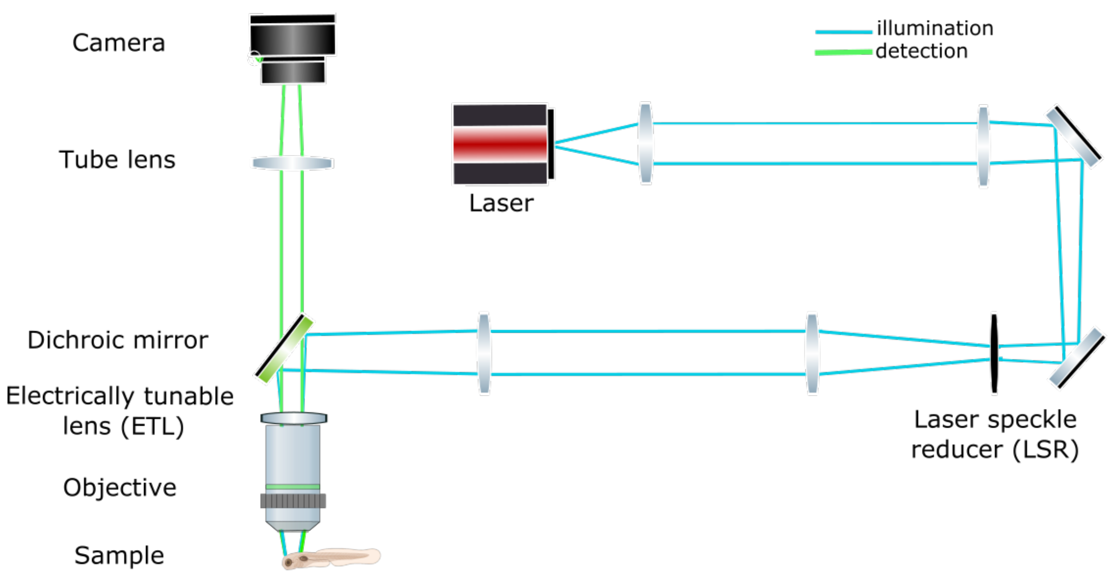


Figure 1 – Scheme of the way the HiLo microscope we use at the DCC Lab is designed

First, let us introduce the montage of the HiLo microscope and what optical components were used to build it. As we can see on the figure 1, the montage is fairly simple, and requires a few key parts: a camera, an objective, an electrically tunable lens (ETL), a laser speckle reducer (LSR) and finally a laser. In the following section (1.2), the choice of these parts and more will be detailed and listed. In reality, du to physical constraints, the montage looks like figure 2, where each location of each parts are shown.

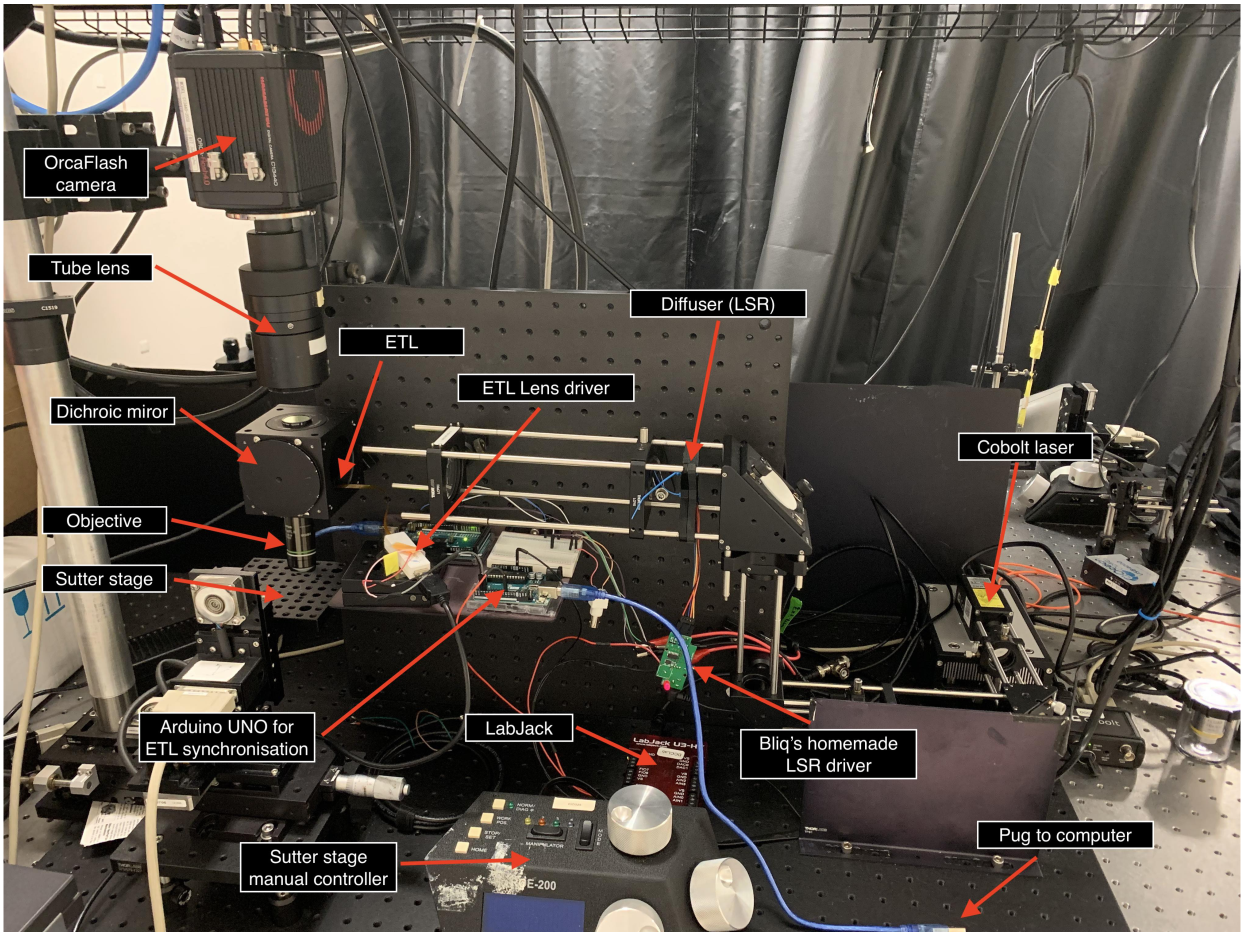


Figure 2 – Real montage of the HiLo microscope that is used at the DCC Lab

1.2 The components

In this section, we will display a list of all the important components composing the HiLo microscope. These components can also be visualized directly on the montage with the help of the figure 2. The one that is used in the DCC Lab has these specific components:

— The camera used to collect the images is the *ORCAFlash4.0 V3 - C13440-20CU*, a digital camera from *Hamamatsu*. The technical notes from the manufacturer are available through this link as well as the instruction manual, available here.

— The electrically tunable lens (ETL) used to modify the focal distance of the microscope is the *EL-16-40-TC-VIS-5D* from *Optotune* with the datasheet available here.

- To control the ETL via a Arduino or a computer, a driver was needed and was obtained from *Optotune*: the *Electrical Lens Driver 4*. It's documentation can be found at this location and the software to control the ETL can be downloaded on a Windows machine and is available here.

- The objective used is the *XLUMPLFLN20XW* water immersion objective from *Olympus*, and it's datasheet is available at this adress.

- The translationnal used is the Sutter stage *MPC-200* and it's documentation is found here.

- The Arduino used for the synchronisation of the ETL with the camera is the *Arduino Uno R3* and it's documentation can be found here.

- The laser speckle reducer (LSR) used in the microscope is the *LSR-5-17-24D-VIS-E* from *Optotune*, and it's description sheet can be found here.

- The blue laser that can excite the zebrafish permitting fluorescence used is the *Cobolt 06-MLD 488nm* de *HÜBNER Photonics*, with the datasheet available here.

- In order to synchronize the camera and the diffuser, we need a labjack in the system. The one we are using is the *LabJack U3-HV* and it's documentation is available here.

2 Prerequisites

2.1 Softwares to install and their setup

In this sub-section, we will list the softwares needed in order to use the HiLo microscope. Normally, they are all already present on the computer located here at the HiLo microscope setup. We will also explain how to set them up in order to acquisition a volumetric stack of HiLo images.

2.1.1 Virtual machine

Install Parallel Pro to have Windows running at the same time as MacOS. This requires a license that you can buy at : `https://www.parallels.com/ca/products/desktop/pro/`. We have personally tried many other virtual machines and this one is the only one that I found that works with the laser we are using. It is essential and allows the user to change the power of the laser manually.

2.1.2 Laser control

To be able to manually control the laser, install the software for the Cobolt laser on Parallel Pro using this link: `https://hubner-photonics.com/downloads/`. Download the last version of the software as well as the driver for USB communication. If you are using a RS-232 to USB adaptor, **download the right RS-232 to USB adaptor driver instead**. Visit the website of the adaptor manufacturer to download their driver if this does not install automatically. Unfortunately, the software has no support on MacOS and is the reason why we need Parallel Pro.

Once this is all done, you simply need to open the Cobolt laser software on Parallel Pro and make sure that the laser is dectected by the computer.

2.1.3 ETL control

Install the Optotune software for the electrically tunable lens driver (*Lens driver 4 / 4i* on Parallel Pro using this link : `https://www.optotune.com/software-center`. If this link does not work, you need to send a request to the company. When dowloaded, follow these steps:

1. In the unzipped folder you can find 4 folders. In the folder *Optotune Lens Driver Controller 1.10.6108*, run *setup.exe* and follow the steps.

2. At some point, the steps a window will appear in one of the steps (figure 3). Follow the steps of this window to ensure proper communication.

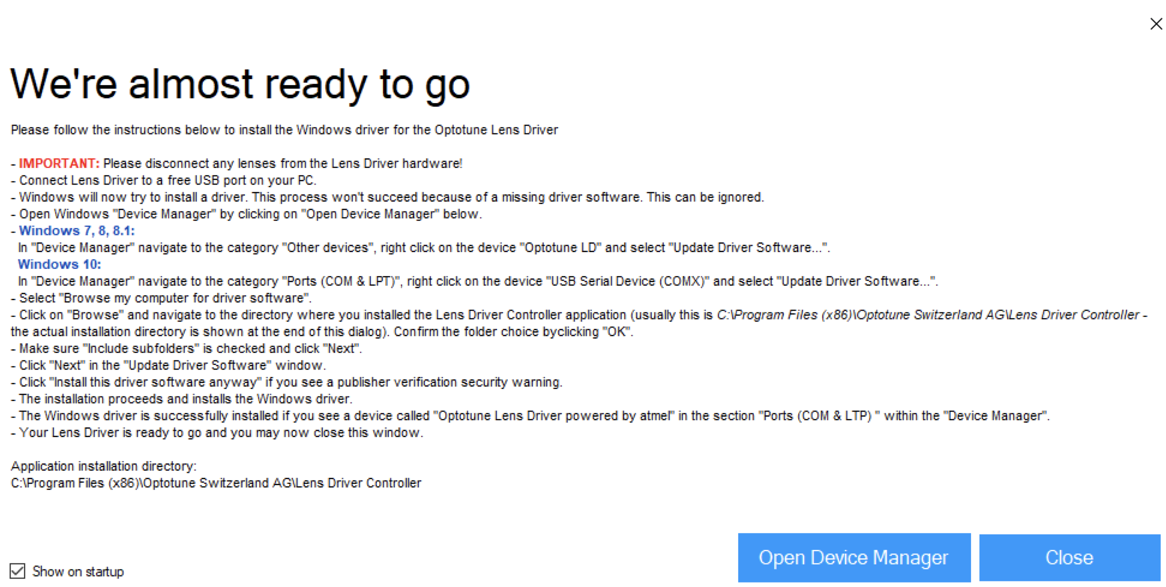


Figure 3 – Follow the steps showed in this window.

3. The ETL should be recognized now by the Lens Driver Controller.

2.1.4 Arduino

In order to synchronize images and focal changes of the ETL, you need to download the Arduino code for ETL on a personal computer. The code is available on this github repository under ArduinoCodeForETL, LoopCommandInHiLoSetup and the file you need locally on your computer is *LoopCommandInHiLoSparq.ino*. In order to run the code, you need the Arduino software that you can download here: `https://www.arduino.cc/en/software`.

Once these are downloaded, these are the steps to follow to use the Arduino:

1. Connect the Arduino to you computer using a **USB-B** cable.

2. Open the Arduino software you installed, and specify which Arduino you are using (see figure 4).

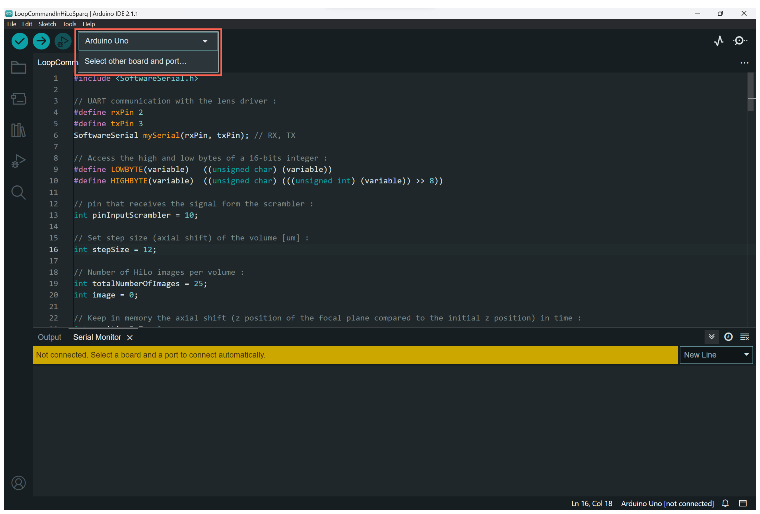


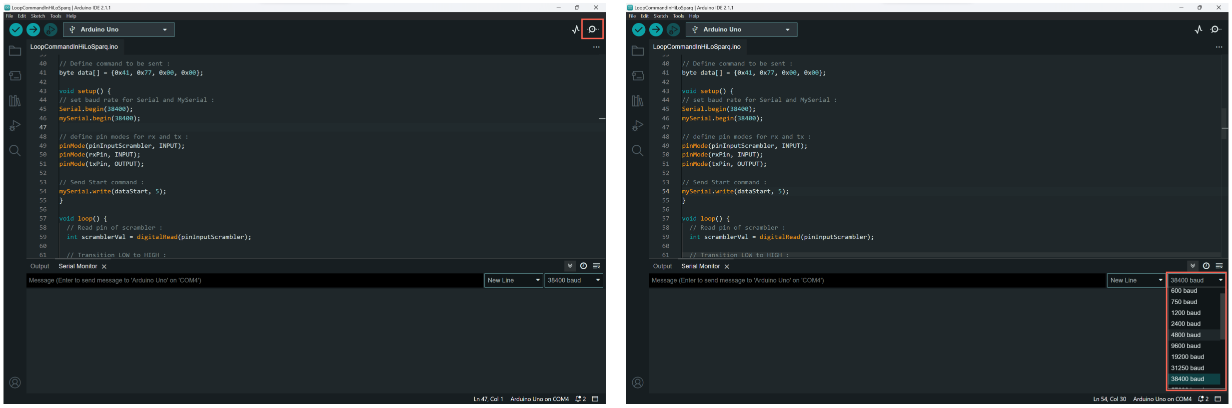
Figure 4

3. Once the computer recognised it, open you serial monitor and select the correct baud rate (see figure 5a and figure 5b). In the code, we are using 38400.

4. Finally, simply upload the code to the Arduino (see figure 6). If everything worked, you should see the message *Done compiling* appear in the bottom right of the screen.

2.1.5 Nirvana

In order to control the diffuser and/or the camera, you need the Nirvana software developped by *Bliq*. To install it or get their latest version (we use *NirvanaSv2.9.6*), you must contact them since it is not available on their website. Now that you have the software, you need to follow these simple steps to setup a volumetric acquisition with the sparq module:



(a) (b)

Figure 5

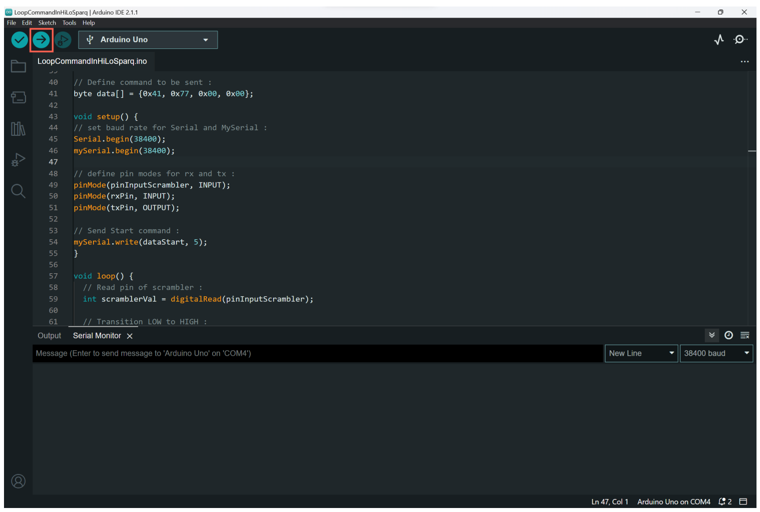


Figure 6

1. Open the Nirvana software on the main computer. Without any modules, the window appearing should look like figure 7.

2. Set the module of the software. To do so, click first on *Configuration* on the top-left of the screen, then *Edit available modules* (figure 9). This opens another window (figure 8b). Click Yes.

3. This open a window where you can select the modules you need for the HiLo. Please select the modules according to figure 9a and click *Save*. This last step allows the software to access certain modules, but they will not appear automatically in the software. To use the modules, you need to select them one at a time when clicking on *Add module* at the bottom-right and bottom-left of the software (figure 9b).

4. If your Nirvana window looks like figure 10, your Nirvana is now ready to go!

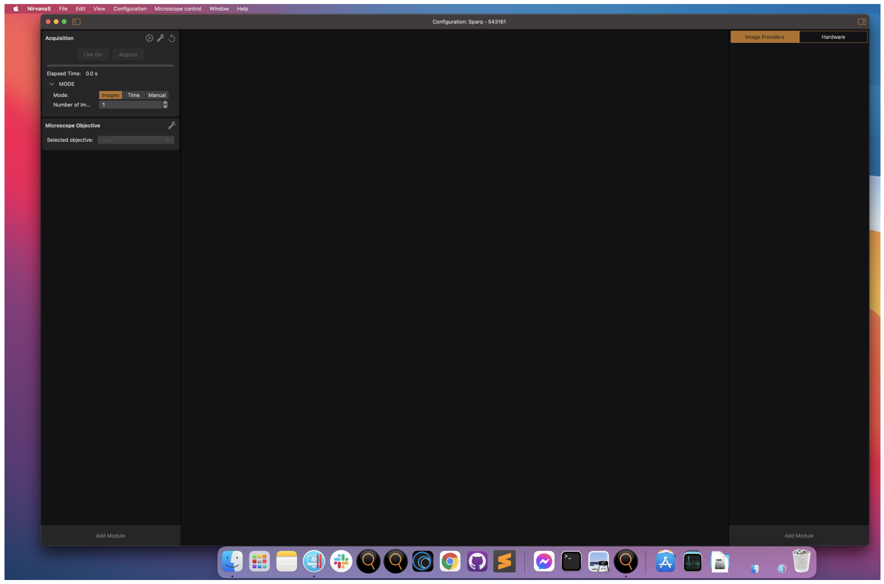
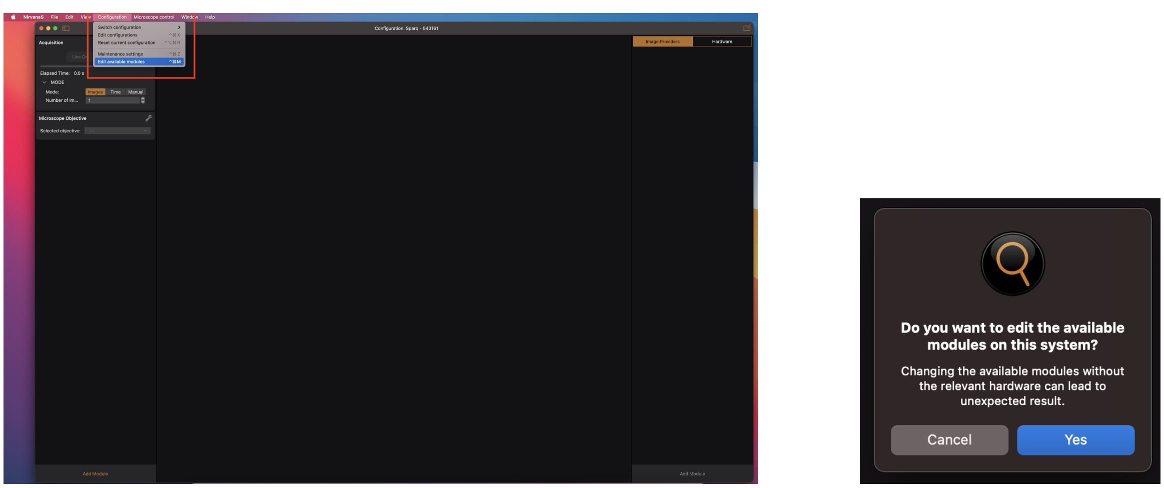
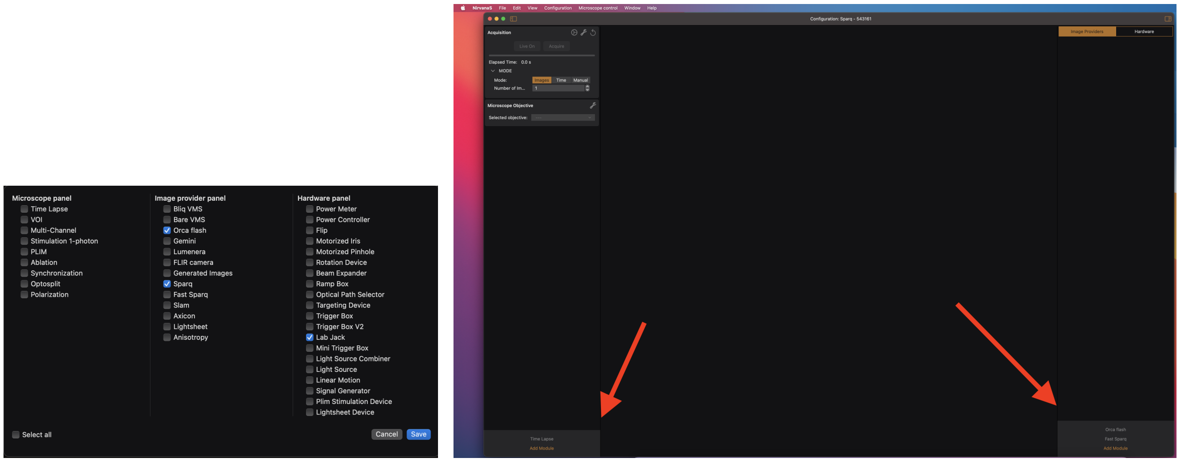


Figure 7



(a) (b)

Figure 8



(a) (b)

Figure 9

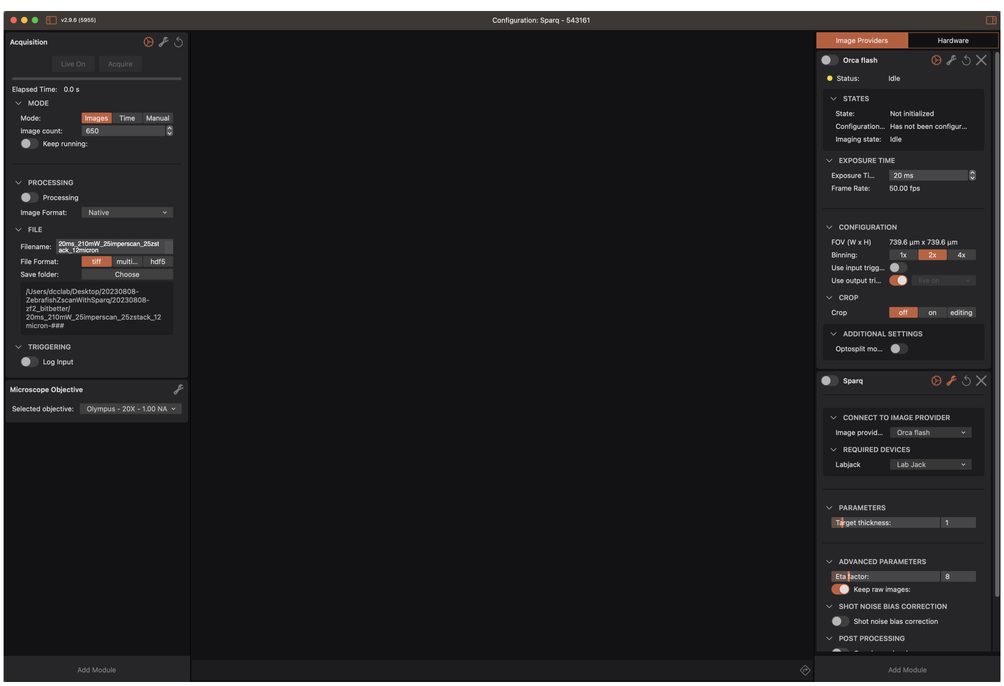


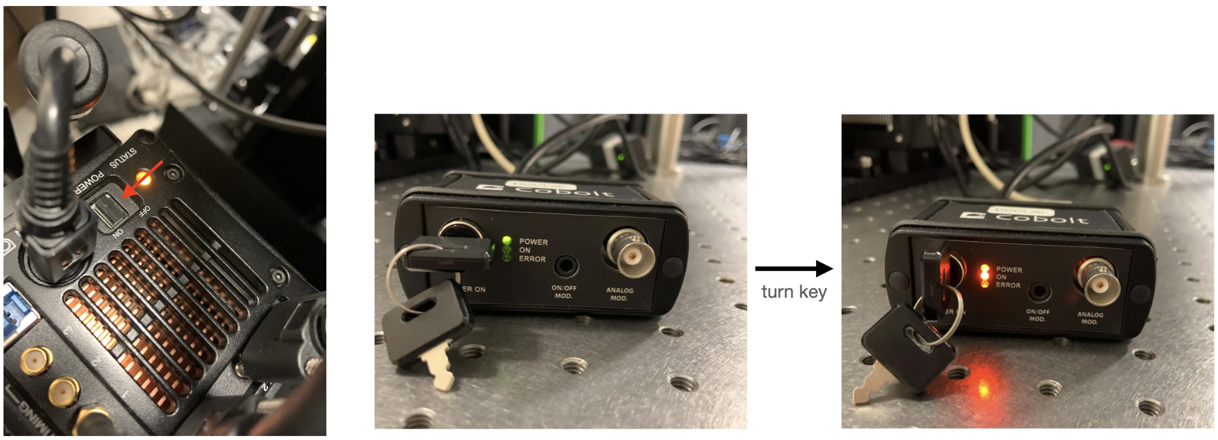
Figure 10

3 Getting ready for imaging

In this section, we list all the steps in order to prepare everything needed before taking the first images. The steps go like this:

1. Turn on the ORCA Flash camera (see figure 11a).

2. Turn on the laser (see figure 11b).



(a) (b)

Figure 11

3. Turn on the LSR driver switch. There should be a red light appearing (see figure 12a).

4. Connect the ETL's driver to the main computer. There should be a green light appearing (see figure 12b).

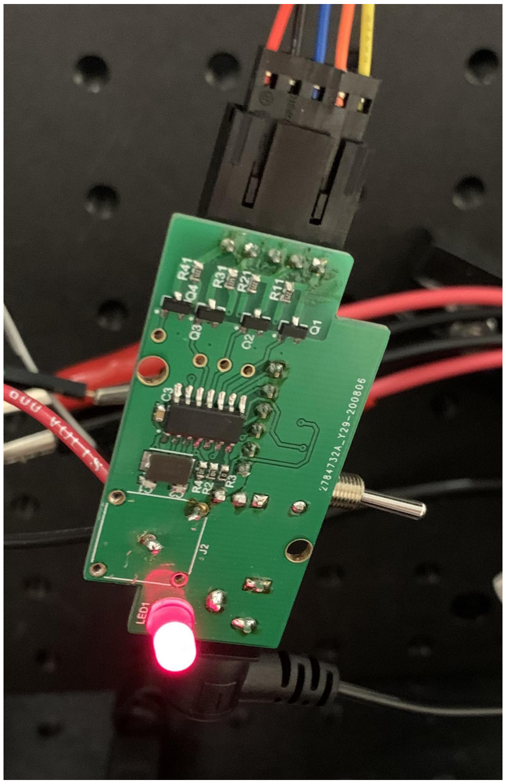
5. Make sure your Arduino is working and connected to your computer as explained in section 2.1.4.

6. Open the latest version of Nirvana.

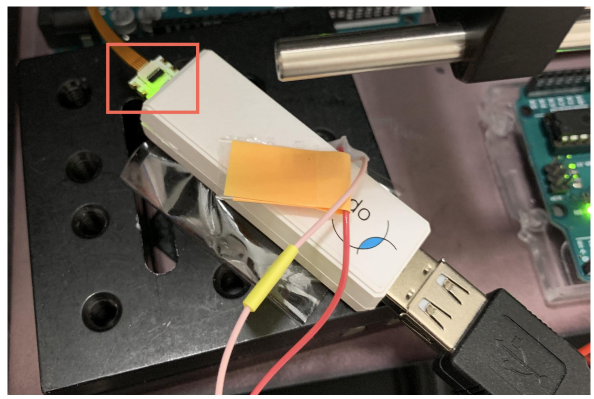
   (a) To make sure that the Orca Flash camera is detected by Nirvana:

      i. Turn on the Orca module in Nirvana by clicking on the top-left orange switch (see figure 13a).

      ii. You should see the status turning green with the state *Ready*, meaning that Nirvana detects the Orca Flash camera and the latter is ready to acquire images.



(a)



(b)

Figure 12

iii. Click on *Live on*.

iv. Click on the information button (little *i* in a circle on the top-left of the software (see figure 13b)). The frame rate should show the number of images per second the Orca Flash would acquire by clicking on *Acquire*.

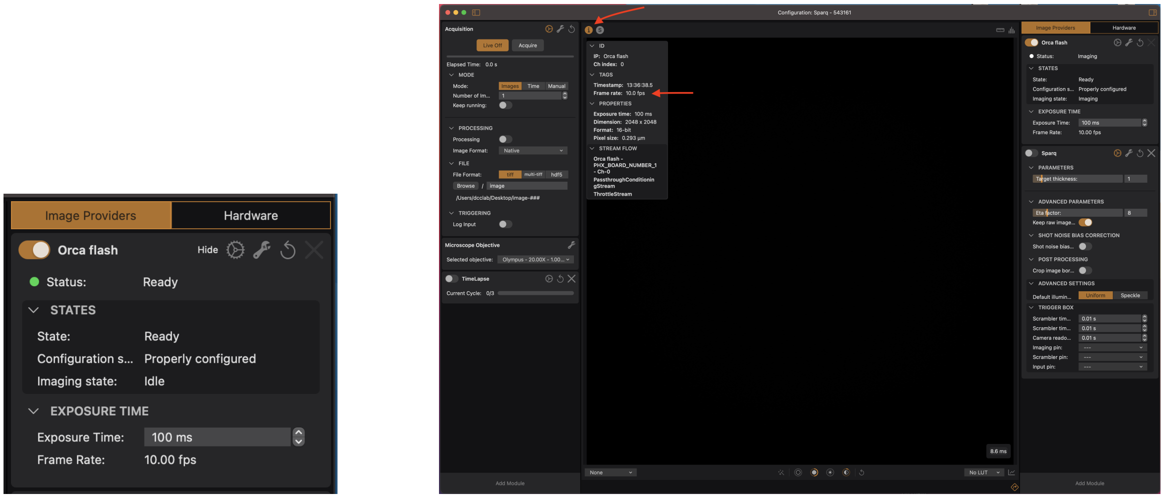
v. If all of this works, the Orca Flash is ready to acquire images with Nirvana. **If anything does not work the way it was just explained, talk to Bliq.**

(b) To make sure that the sparq module is detected by Nirvana:

i. Turn on the orange switch of the Sparq module.

ii. You should see the status turning green with the state *Ready*, meaning that Nirvana detects the LabJack needed for the sparq module and the latter is ready to go.

iii. This should also change the frame rate in the information window when Nirvana is in *Live on* mode (see figure 13).



(a) (b)

Figure 13

iv. If all of this works, the LabJack is ready to acquire images with Nirvana. **If anything does not work the way it was just explained, talk to Bliq.**

7. In Parallel Pro:

   (a) Open the Cobolt Monitor, the Lens Driver Controller and the Zaber Console. Figure 14 shows the software and what it should look like when well connected.

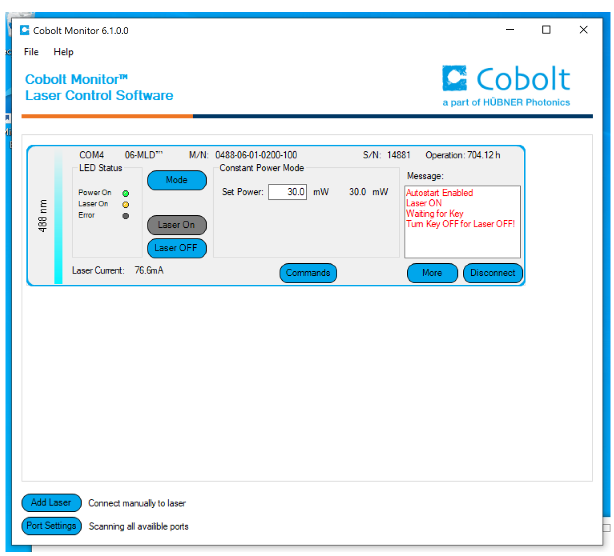


Figure 14

   (b) In the Cobolt Monitor, the laser power at the laser output is set only by changing the value in the box *Set Power*. The maximal output power is 210 mW.

4 Taking images

Now that you have proceeded through all the steps of downloading the prerequisited softwares and you turned on all the components of the HiLo microscope, you should be ready to take you first set of images. Here, we will go through the process of taking a single plane over time or a volumetric scan of a certain sample. We will cover the case where our sample is a GCaMP zebrafish because it is what we mostly work with here at CERVO. In reality, the process would be the exact same for any sample that is fluorescent with GFP.

4.1 Single plane over time data acquisition

Doing a single plane data acquisition is pretty simple since it does not require the need of the ETL. Therefore, you don't need to use the Arduino codes for these types of acquisitions. Here are the steps to be able to do such an acquisition:

1. First, chose the laser's power
2. Then, on Nirvana, there are a few parameters to determine:
   (a) Acquisition time
   (b) Total number of image during the acquisition
3. When all of these parameters are chosen, you can directly acquire the image and the LabJack will take care of taking uniform and speckle images for each time.

4.2 Volumetric scan over time data acquisition

For a volumetric scan, you need to specify a few more things. Here are the steps to be able to do such an acquisition:

1. First, chose the laser's power.
2. Then, on the Arduino program, there a few parameters to determine:
   (a) The total number of images per volume, listed as *totalNumberOfImages*.
   (b) The difference in microns between each images of the volume taken, listed as *stepSize*.

   (c) Once this is specified, upload the code to the Arduino.

3. Then, on Nirvana, there are a few parameters to determine:

   (a) Acquisition time.

   (b) Total number of image during the acquisition. For each volume taken, the first image is a trash image to account for the mechanical effect of the ETL. You must therefore add one more image per volume for all volumes. If you chose 25 images per volume and you want to do 20 volumes, then you must specify $25 \times 20 + 20$ images in Nirvana.

4. When all of these parameters are chosen, you can directly acquire the image and the LabJack will take care of taking uniform and speckle images for each plane and the ETL will take care of the volumetric scan.

References

[1] Valérie Pineau Noël. *La microscopie à illumination à tavelure laser de type HiLo pour l'imagerie volumétrique rapide de l'activité calcique du cerveau du poisson-zèbre*. Master's thesis, Université Laval, Québec City, Québec, Canada, 2022. 3