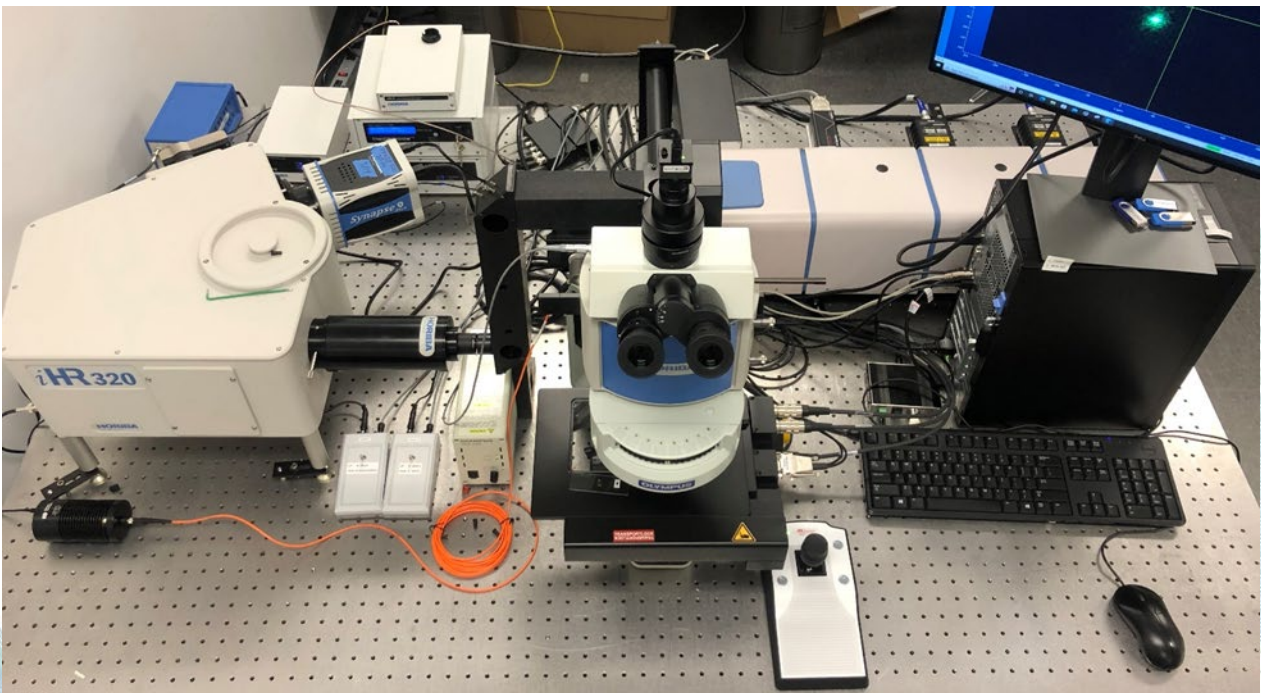


Custom Spectroscopy Systems Standard Microscopy System (SMS)



User Guide



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Safety information

- Read and follow these instructions carefully.
- Inform yourself in detail about:
 - dangers originating from the complete device;
 - dangers originating from the individual components installed in the device;
- Check frequently whether all means for safety and protection are followed and whether they are working properly;
- The operation of the device is only allowed for personnel with general expertise and experience in the operation of electrical equipment and who is in particular trained with the operation of the device and all of its components;
- The device must not be modified without proper technical advice and assistance from the manufacturer.

General safety advice

This system should only be operated by trained personnel. Detailed safety advice has to be given to all personnel involved.



Safety advice for operation

- Do not operate the laser without ensuring that adequate ventilation of the device is provided.
- During operation, the system must be kept between 15°C and 25°C;
- During operation and storage the system must be kept in a clean environment.
- During storage, the system must be kept between 5°C and 50°C;
- The system cannot be modified without the written agreement and the technical assistance of the manufacturer.

Improper use will cause all claims for liability and guarantees to be forfeited.

System Overview

The main components of the system are:

- Free space coupled 532nm laser
- Ocean Insight Halogen light source
- Double fiber-free space coupler
- Laser Filter Wheel
- Laser Periscope
- Laser Power Controller
- iHR-320 Spectrometer
- Sincerity Detector
- Symphony II
- Marzhauser XY stage controller
- Marzhauser XY stage
- Marzhauser XY stage joystick
- Personal computer running a) LabSpec software to control the spectrometer and acquire data from the Synapse detector.

Using the microscope

Please refer to the instructions manual for the Olympus microscope and familiarize yourself with its operation.

1) *Trinocular*

The trinocular is used to send the image to the live camera or to the eyepiece. The laser will shut off when the trinocular is set to allow the image to the eyepiece. Pull on the knob to send the image to the eyepiece. Push in the knob to send the image to the camera. Refer to the section on laser operation to restart the laser.

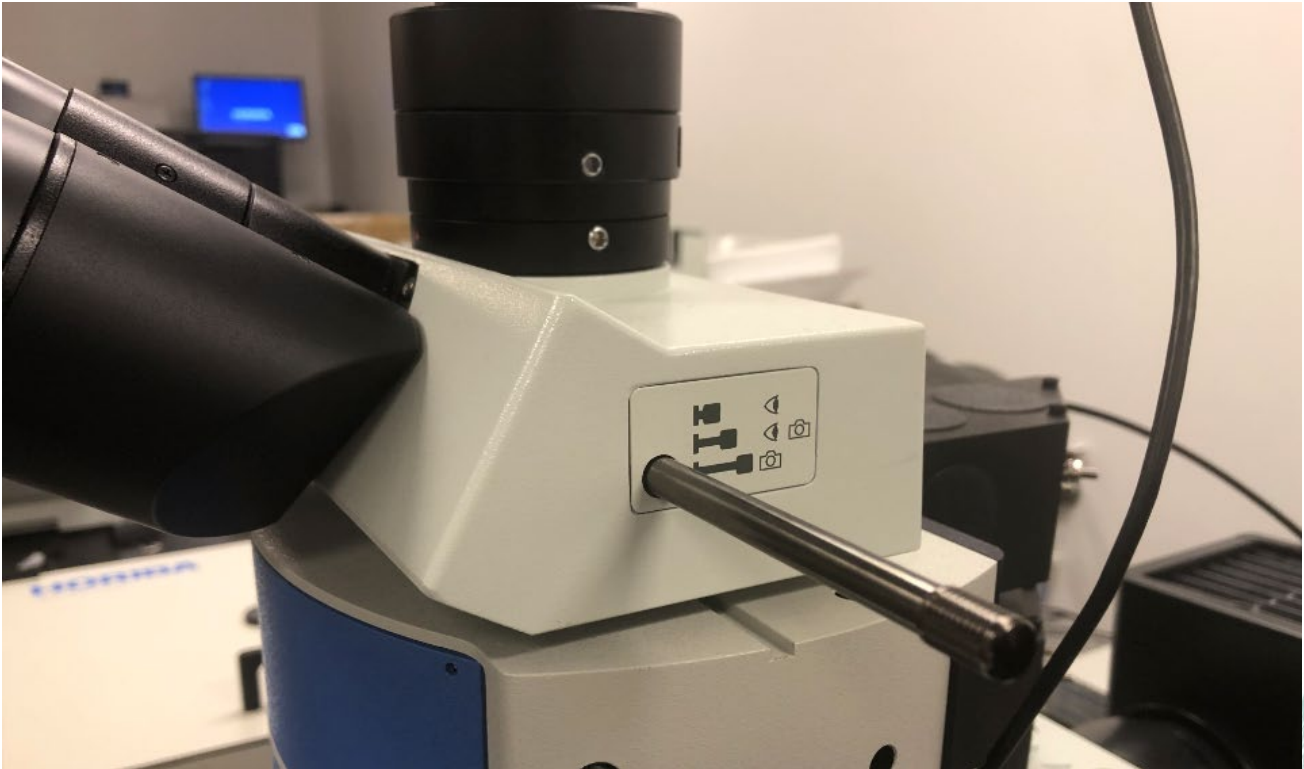


Figure 1: Camera system for microscope. The trinocular system consists of the eyepieces, the camera adapter, and the uEye camera.

2) *The UMA*

The UMA has been added to the microscope to facilitate easy integration and manipulation of the camera, the incident light path, and the emission light path. The UMA has two knobs: the upper knob controls the direction of the emission light path; the lower knob controls which filter module is in use. Upper knob position:

- I. **Bright field imaging:** this setting is used to send the bright field image of the microscope sample directly to the camera. The laser spot does not appear in the image of the sample as the filter module filters it before the camera.
- II. **Laser viewing:** this setting is used to send the image of the microscope sample to the camera, including the laser spot. A system of mirrors inside of the UMA bypasses the filter module so that the bright field image and the laser spot appear together.
- III. **Spectroscopy:** this setting is used during data collection. The bright field light source is turned off and only the laser should be on. Light is directed to the emission periscope of the system which takes it to the spectrometer.



Lower knob position:

- I. Bright field imaging: this setting is equivalent to no filter set installed. In this configuration, light from the bright field setting of the sample passes undisturbed to the camera.
- II. 785nm filter set: this setting is used when the user wishes to send the 785nm laser to the sample.
- III. 647nm filter set: this setting is used when the user wishes to send the 647nm laser to the sample.

- IV. 50/50 beam splitter: this setting is used when the user wishes to see the visible light and laser at the same time or generally when user wants to send the laser light to the spectrometer for IRF measurements.

Notation:

Throughout this manual, whenever referring to the UMA knobs the notation is (upper knob position, lower knob position). For example, if the system is in spectroscopic mode using the 785nm laser filter set, the UMA is in (III, II) mode.

3) *Beam Splitter Selection*

The SMS system allows the possibility of using both bright field viewing and spectroscopy. Inside of the filter cube wheel is a 50/50 beam splitter at position 2. In position 1 there is nothing, and the emission light from the objective passes undisturbed to the UMA. During spectroscopy mode, the filter cube wheel should be set to position 1, otherwise the signal will be cut in half and will not be reproducible.

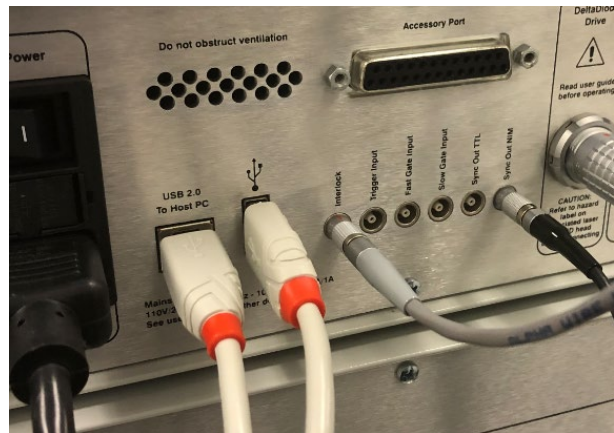
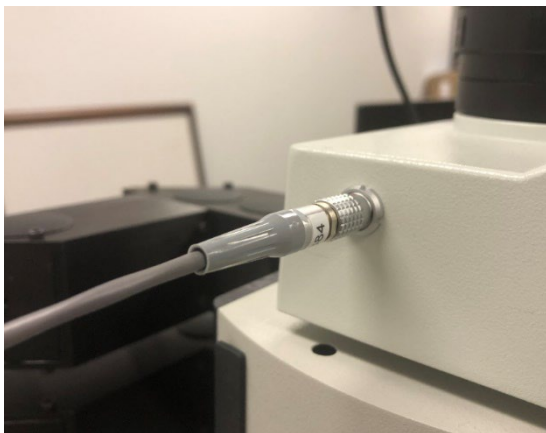
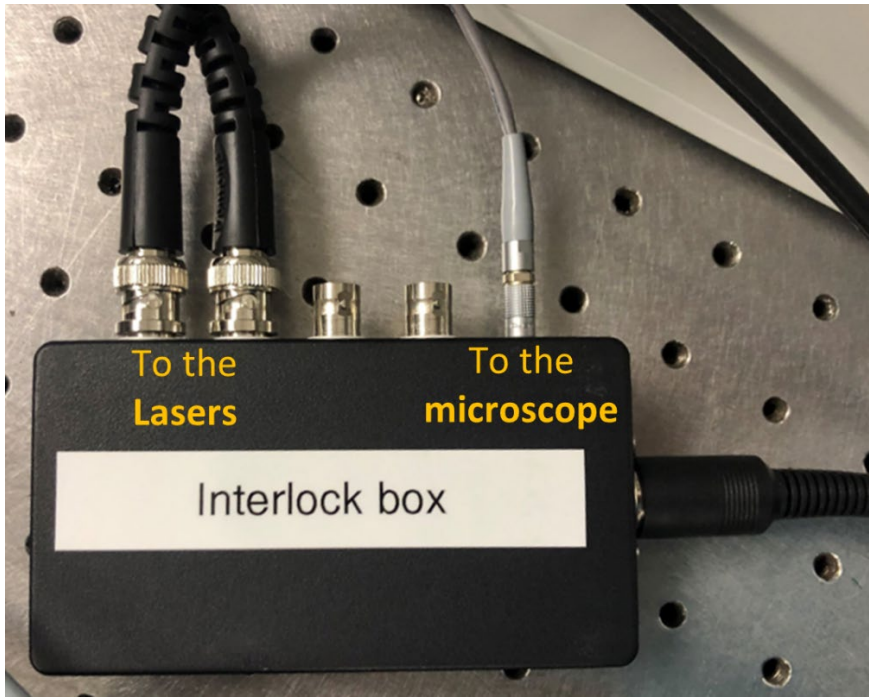


Figure 2: Filter cube selection wheel. Setting 1 corresponds to spectroscopy mode. Setting 2 corresponds to bright field viewing mode.

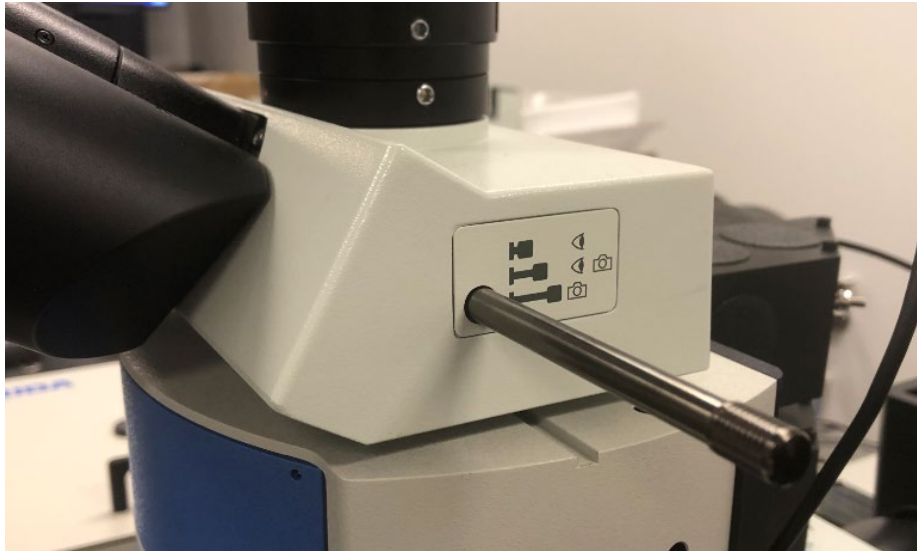
Laser

Interlock

Both of the 785nm steady state laser and DeltaDiode controller unit comes with an interlock protective cable that is used to prevent eye damage while the user uses the eyepieces and the laser at the same time.



The interlock cable for delta diode connects to the interlock box module and to the “interlock” port on the DDC.
The interlock cable for steady state laser connects to the interlock box module and to the “interlock” port on the back of the laser controller.
The interlock box is connected to the trinocular at the end.
The interlock system is triggered if the trinocular module moves into any view position other than the “camera only” position.



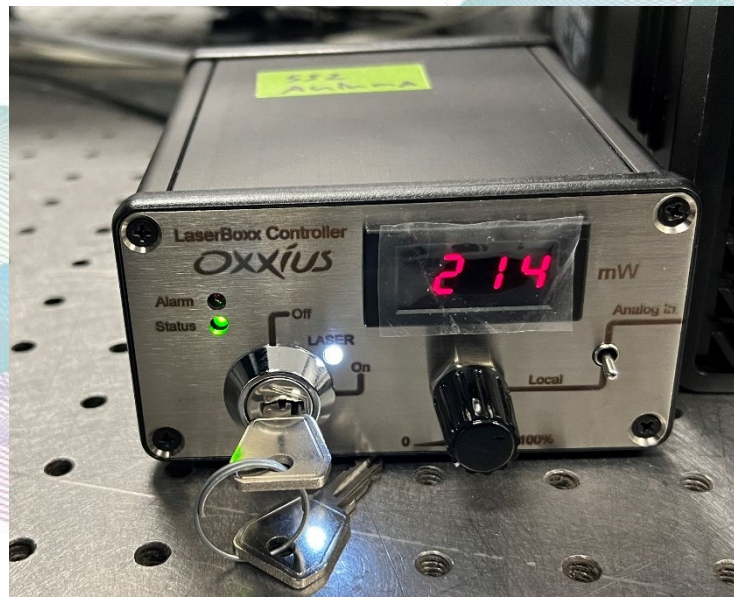
If the interlock system is triggered the following procedure must be performed before the laser can be used again.

- Move the trinocular module to the “camera only” position.
- Turn the emission key from “On” to “Standby”.
- Turn the emission key from “Standby” to “On”.

It is not advised for the user to use the eyepieces and the laser at the same time for safety reasons. If the user desires this, then the interlock cable can be replaced by the manual interlock override key on the DDC.

1) Turning off the laser

- To turn off the laser, turn the emission key from “On” to “Standby”.



The XY Stage

1) Turning on the XY stage

- To turn on the Marzhauser stage, turn the “On/Off” switch at the back of the controller to “On” (see Fig. n).

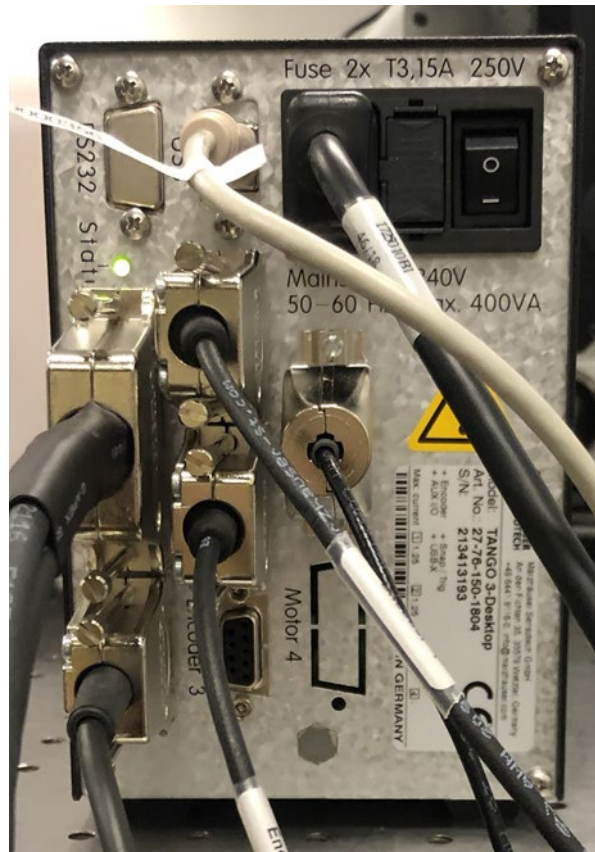


Figure n: Back view of Marzhauser controller.

2) Manually adjusting the stage

- The XY – directions of the joystick handle adjusts the XY stage.
- By turning the joystick handle clockwise or counterclockwise, the user can adjust the height of the XY stage.

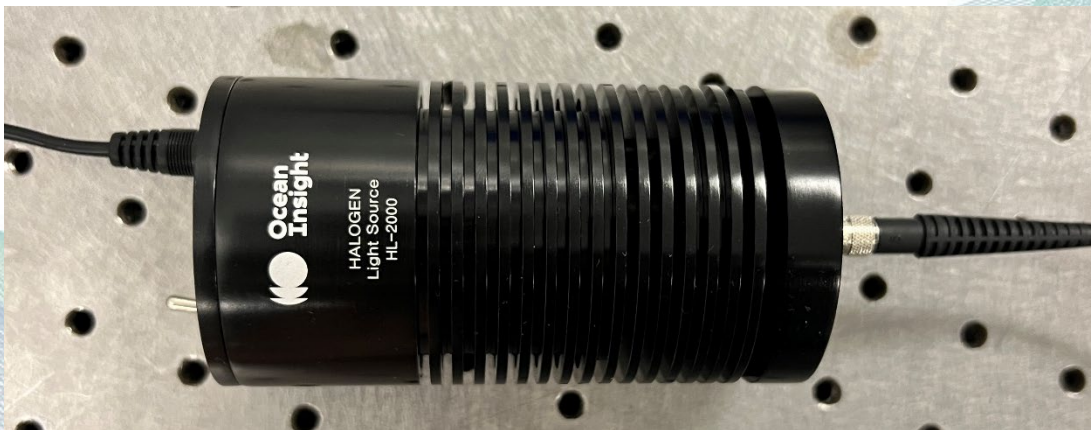
Operating the light sources

The system can operate with 532, 633nm, and 785nm free space coupled Laser source or with a Fiber coupled white light source. This capability has been added using a double coupler added prior to UMA.



Note that although all lasers can be turned on during normal operations, it is not ideal due to laser lifetime considerations.

Halogen insight lamp:



633nm/532nm laser:

The 633nm and 532nm lasers both are a delta diode laser. Since there is only one delta-Diode controller in the system, and two laser power cords supplied. These two lasers can not be on simultaneously. To turn on each delta diode laser, the related-power-cord must be connected to the “DeltaDiode drive” port in Delta-Diode controller. There are two power cords supplied for the

detladiode lasers. It is not suggested to remove the power cord from the lasers because this might move the laser box, and this will misalign the out-put laser light toward the sample.



785nm laser:

Flip the laser train switch to “785nm” if it is not already.

Turn the laser controller’s key switch to ON.

- The laser can take several minutes to stabilize.
- The status LED on the laser controller will blink during the startup procedure.
- The status LED will become solid green once the laser has locked onto its target power.
- The output power of the laser can be adjusted after it has locked into normal operation.
- The laser controller displays the output power of the laser head.



Oxxius 532nm laser system controller. (left) Front view. The key switch should always remain inside of the Oxxius box for safekeeping. (right) Back view. To turn on the controller, flip the “On/Off” switch to the “On” position.

1) Laser Aperture

The laser head has an aperture that (when closed) temporarily eliminates the laser spot from the live video. To close the aperture, move the aperture lever from the “Open” position to “Closed”.

2) Laser stability

It is recommended that the laser shutter be closed while the laser powers up; this will avoid back reflections which may destabilize the laser. It is also important to avoid excessively bending the fiber optic line or placing objects on the line, lest the inner core become damaged.



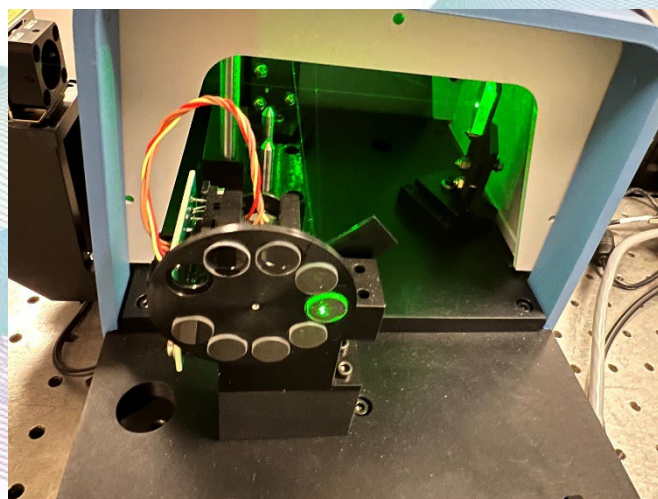
Oxxius 785nm laser Zoom in of the laser aperture switch.

1) Laser stability

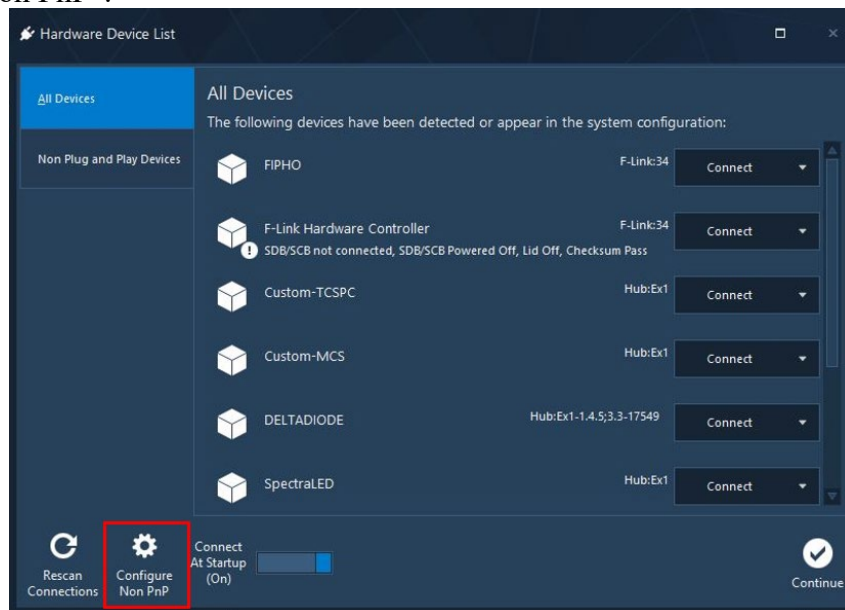
It is recommended that the laser shutter be closed while the laser powers up; this will avoid back reflections which may destabilize the laser. It is also important to avoid excessively bending the fiber optic line or placing objects on the line, lest the inner core become damaged.

Laser Filter Wheel

The laser train is equipped with a filter wheel in the first compartment. The filter wheel can be controlled manually to set for a desired filter in the laser path.



- If new hardware is being installed, such as a camera or XY stage, click “Configure Non PnP”.



- Follow the steps needed to install the new hardware, then click “Ok”.
- Open Labspec.
 - This will give the user control of the spectrometer.

Operating the Laser Train

Please refer to the instructions manual for the laser train and familiarize yourself with its operation.

Note that although lasers can be turned on during normal operations, it is not ideal due to laser lifetime considerations.

There are two control motors to chose the desired laser illuminate to the sample.

The image of the tow motors are shown below. For detailed description, mirror number 1 is the most closer mirror to the vertical periscope. If mirror one is UP, it will block all other lasers other than 785nm laser which will be guided toward the sample. Moreover, if mirror 1 is down, depending on the position of the mirror #2, which is UP/DOWN, the 633nm or 532nm lasers can be guided toward the sample.



Acquiring a spectrum

Refer to the LabSpec reference manual for a detailed description of LabSpec's various functions. Pages 32-35 of the manual show the basics of acquiring a spectrum. Pages 42-46 provide a breakdown of the acquisition parameters. The basic procedure for acquiring a point spectrum is described below.

1) *Locating the sample*

If possible, use the video camera to locate and focus the sample. Remember that position 3 of the UMA will block the light from entering the camera if used. Using the camera will allow you to see the laser spot on the sample. Use a neutral density filter to reduce laser power and avoid saturating the camera.

2) *Illumination*

The full field of view will be visible using the camera software (uEye) or the LabSpec video software when using the microscope condenser to illuminate the sample with transmitted light. Use position 1 of the upper knob of the UMA to view the bright field image. The laser beam will be visible only when using the filter module setting of the UMA and position 2 of the upper knob of the UMA.

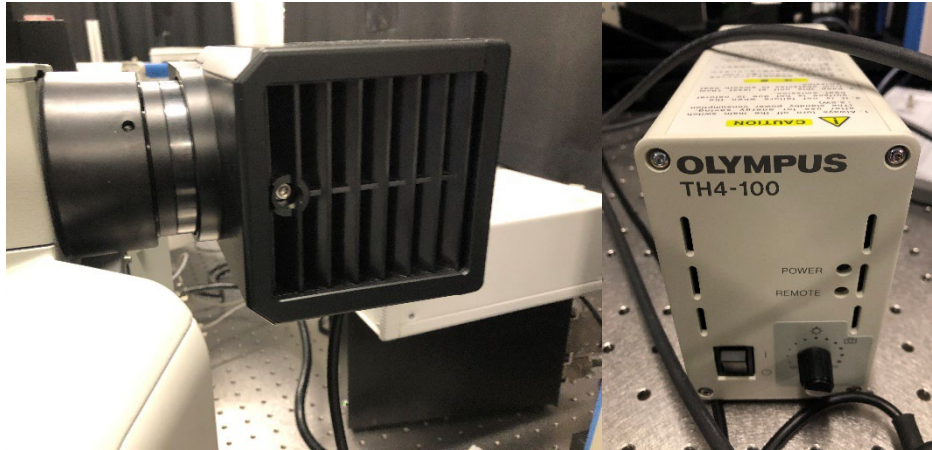


Figure 4: Bright Field Illumination System. (left) Microscope condenser. The lamp inside of the black box should illuminate when turned on. (right) Bright field controller.

3) *Image stitching*

The LabSpec image stitching feature, called montage, is useful for viewing larger samples and determining the appropriate area to map. This feature is found within the video options, under the acquisition tab. See page 39 of the LabSpec reference manual.

4) *Focusing*

There are many factors which can affect how focus differs from signal strength, such as: changing objectives, penetration depth of the laser, the nature of the sample, and a saturated view of the laser spot on the camera. Measurement repeatability can be accomplished by always focusing on the sample with the camera and then taking a spectrum.

5) *Real time display*

Refer to pages 33 and 39 of the LabSpec reference manual for a brief overview of using real time display (RTD). This feature will show a spectrum which is continually updated.

6) *Acquiring a spectrum*

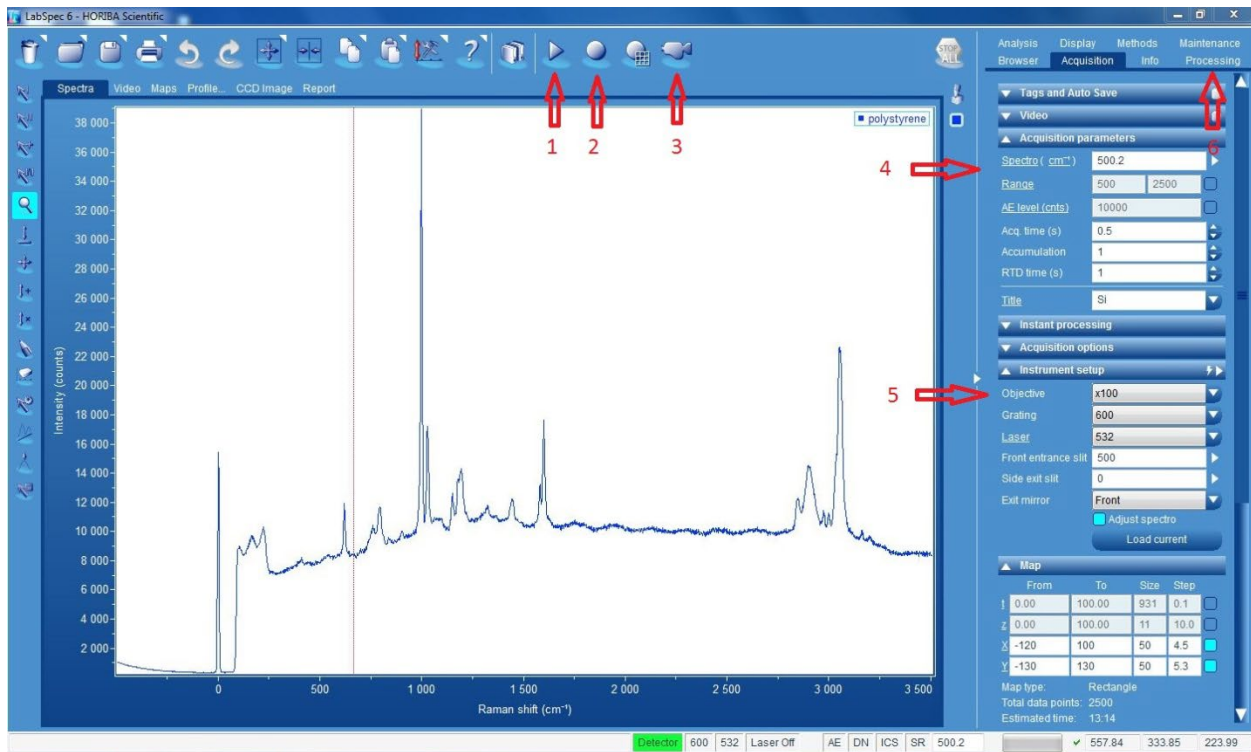


Figure 5: Polystyrene spectrum in LabSpec. The most common icons and data acquisition parameters are pointed to with red arrows.

Use RTD (1) to optimize the acquisition parameters including acquisition time, slit width, and focus. When using the video camera (3) to focus the sample, ensure that the correct objective (5) has been selected.

Acquire a single spectrum (2) with the spectrometer grating fixed, or over a specified range (4). Post processing (such as smoothing and baseline correction) is found under the processing tab (6).

7) Mapping a sample

Page 46 of the reference manual displays the map options available in the acquisition tab. Make note that 'size' refers to the number of points to sample along an axis and 'step' refers to the spacing (in μm) between sample points. The SWIFT mapping option decreases the time required for mapping by allowing the stage to trigger the detector while it moves.

8) Displaying a map

Many options are available for highlighting features in a map. See pages 66-68 of the LabSpec reference manual for some of these options. A useful feature is the ability to select up to 3 arbitrary wavelength ranges and have them displayed as 3 separate colors. This false color feature can be used to display a narrowband emission as green over a broadband background displayed as red.