RG Raman microscope

User Manual

Version 202301

Lightnovo

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Safety

General Information

Read the following safety instructions carefully before operating RG Raman microscope and keep this manual for future reference available at any time. Always observe the instructions described in this manual to ensure user safety and to avoid property damage. Improper use or failure to follow these safety instructions can result in serious injuries and/or property damage. Any non-observance of the precautions will infringe the intended use (i.e., performing measurements by Raman spectroscopy) of RG Raman microscope. In this case Lightnovo ApS will not assume any liability. It is the operator's duty to plan and implement all necessary safety measures and to supervise their observance. Moreover, the operator must ensure that RG Raman microscope is in proper functioning condition. A safe and faultless operation can only be guaranteed if RG Raman microscope is transported, stored, installed, operated and maintained properly according to the procedures described in this manual. Never remove or deactivate any supporting safety systems during RG Raman microscope operation. Ensure that objects and/or material not required for the measurement is out of the instrument operating area.

Qualified Personnel
Primary installation and all maintenance and repair works not described in this manual should only be performed by Lightnovo service personnel. Only authorized operating personnel that have been briefed about the instrument operation and all relevant safety aspects should operate and maintain (i.e. only maintenance works that are described in this manual) the instrument. All repairs, adjustments and alignments on any RG Raman microscope component must be performed in accordance with the safety regulations and standards applied in the country in which the instrument is installed.

Correct Usage
RG Raman microscope and its components should only be used according to the instructions described in the manual or advised by a Lightnovo engineer. In case of accessories or components made by other manufacturers and used in connection with the microscope, Lightnovo does not assume any liability for safe operation and proper functioning.

Warning labels

This warning symbol indicates the existence of laser radiation.
Class 3B lasers **are hazardous for eye exposure**. They can heat skin and materials but are not considered a burn hazard. For visible-light lasers, Class 3B lasers' output power is between 5 and 499 milliwatts. Class 3B lasers are **normally hazardous under direct beam viewing conditions**, but are normally safe when viewing diffuse reflections.

Safety instructions

The following chapters describe all relevant safety aspects of the instrument operation. Depending on the degree of hazard the safety instructions are classified as follows:

Danger
indicates that death, severe personal injury or substantial property damage WILL result if proper precautions are not taken.

Warning
indicates that death, severe personal injury or substantial property damage CAN result if proper precautions are not taken.

Caution
indicates that minor personal injury or property damage CAN result if proper precautions are not taken. Important draws your attention to a particularly important information.

Note
draws your attention to an useful information on the product, e.g. product operation or to a special part of the manual.

The safety instructions Danger, Warning and Caution are marked by the corresponding warning labels.

Laser safety

General Information
The analysis system of RG Raman microscope uses the light of lasers that are installed in RG Raman spectrometer (532 or 785 nm) or miniRaman spectrometer (660 and 785 nm). The used laser diodes emit visible and partially visible laser radiation in the near infrared region. According to the standard EN 60825-1/10.2003, RG Raman microscope is a Class 3B laser product. Therefore, it is **normally hazardous under direct beam viewing conditions**, but is normally safe when viewing diffuse reflections.

Safety Features
The laser protective enclosure shields the area around the sample stage. The enclosure is opened and closed manually. For safety reasons, the operator has to close the laser protective enclosure before starting the measurement. This safety precaution protects the operator against accidental exposure to hazardous laser radiation exiting the objective.

Figure 1.
a) Open Laser Protective Enclosure b) Closed Laser Protective Enclosure

Safety Instructions

In addition to the safety instructions given below, also comply with all local regulations concerning laser safety.

The analysis system is specified as a laser class 3B product (depending on the model, see *Overview* section), i.e. it considered safe if handled carefully, with restricted beam viewing. Nonetheless, observe the following safety instructions:

Warning:

Avoid eye and skin exposure to direct or scattered laser radiation under all circumstances! Failure to do so can cause permanent and irreversible eye damage and/or serious skin injuries!

Do NOT remove the laser protective enclosure!

Installation

General Information

Unpacking and initial installation including hardware setup and cable connection is done by qualified Lightnovo service personnel. The operating company has to provide the required utilities and an installation site that meets the site requirements described in this chapter.

Delivery Scope

Standard Components

- RG Raman microscope (including user manual and quality test report)
- Several cables: power cord, USB cable. (See section *Cable Connections* below in this chapter.)
- Accessories (includes spares, adaptors, objective lenses, sample preparation tools etc.)

Inspecting the Packaging

After having received RG Raman microscope, inspect the packaging for damages. If there are any signs of damage, contact your local shipping representative before opening the shipping box.

Warning:

Do not put RG Raman microscope into operation if there are signs of damage. Failure to do so may result in severe personal injuries and/or property damage.

Transportation

When transporting the microscope, use the original shipping box to avoid damages.

Site requirements

Space Requirements

RG Raman microscope requires a space of 34 x 43 cm in lateral dimensions and 48 cm in height. (For the exact instrument dimensions refer to Specification.) At the rear instrument side, take a clearance of at least 10 cm into account.

When defining the installation site, take into consideration that the power supply connections are easily accessible at any time.

Note:

A power outlet box to which RG Raman microscope connected provides a suitable solution.

RG Raman microscope requires a stable and horizontal base which can carry the instrument (ca. 20 kg) and possible accessories. This is critical for applications that require high spatial resolution.

Environmental Requirements
To ensure optimum instrument performance and long-term reliability the following environmental conditions are essential:

- Ambient temperature range:18 - 35°C (64 - 95°F)
- Humidity (non-condensing): ≤ 70% (relative humidity)

Temperature variations can impair the results of long-term measurements. Therefore, the temperature variations should be less than 1°C an hour and not more than 2°C per day for high spatial resolution type of measurements.

Note:
For some kinds of application, the opportunity to black out the room is advantageous.

Vibration
Ideally, the instrument is not installed near vibration sources (e.g. ventilation hoods, air conditioners, motors, elevator).

Power Supply
The instrument power supply unit automatically adapts to the most common power sources. Valid voltage range:

- 100V microscope system: 100V to 120V AC 10%
- 200V microscope system: 220V to 240V AC 10%
- Valid frequency range: 50/60Hz

RG Raman microscope is an instrument of protection class I (electric safety).

Caution:
To avoid personal injury and spectrometer damage, connect RG Raman microscope only to a socket outlet with earthing contact.

To provide good data quality and a long RG Raman microscope service life, ensure that the following site requirements are met:

- Do not install RG Raman microscope near sources of potential inductive electrical interference (e.g. pumps, switching motors, microwave ovens etc.), sources of high energy pulses, and sources that might cause magnetic or radio frequency interference.
- Do not place devices such as large electric motors, heaters, welding equipment, radio transmitting equipment, units emitting pulsed electromagnetic radiation (e.g. NMR systems), or high powered lasers in close vicinity to RG Raman microscope. These devices can interfere with the instrument and cause instrument malfunction. Ensure that these types of devices are not connected to the same electrical circuit as RG Raman microscope.
- If there are any problems concerning mains power supply (e.g. brownouts, power surges, frequent thunderstorms), take precautions to ensure an uninterruptible power supply.

Cable Connections

Please unpack RG Raman microscope and remove it from the shipment box.

Please remove power adaptor and connect it to the right connector on the rear panel of the RG Raman microscope (Figure 2). Then connect USB cable to the lower connector on the rear panel and connect it to PC from the other side.

Figure 2. Rear side – overview of the connector sockets (bottom connector is USB 2.0 for connection with PC via USB cable, top connector is for power supply 36 V).

Software Installation

1. Download RG Raman microscope software package from Lightnovo website: https://lightnovo.com/lightnovo-software/
   Please select version 32 or 64 bit depending on your operation system.
2. Install all drivers from the corresponding software folder.
3. Run Miraspec.exe file to start the data acquisition software.
4. Software is ready for operation.

Removal and installation of RG Raman spectrometer

RG Raman microscope has an in-built product inside which is RG Raman spectrometer. It can be inserted and removed by the user without the need of realignment. Removal steps are shown below.

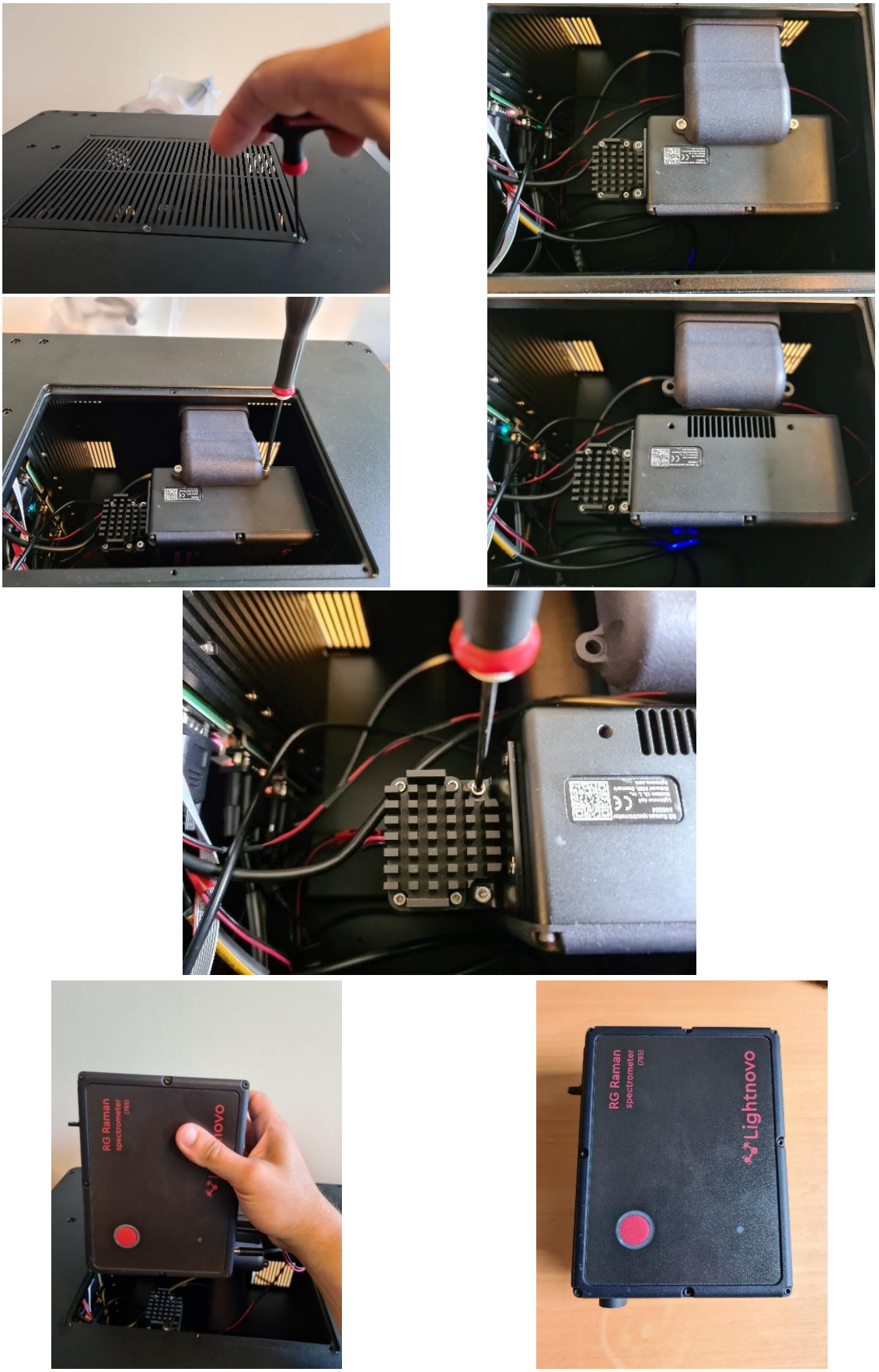

Figure 3. Removal steps of the RG Raman spectrometer from the RG Raman microscope.

Overview

General information

RG Raman microscope has a modular design with reflection white light illumination microscopy unit combined with motorized 3D stage and RG Raman spectrometer. See section *Instrument design* below in this chapter.
This instrumental setup allows for both sample viewing using the optical microscopy capabilities and performing measurements by Raman spectroscopy simultaneously. The visual observation reveals morphological details of a sample (e.g. color, size, shape), whereas the spectroscopic measurement reveals information about the molecular structure and chemical composition of a sample.

This instrumental setup is designed for demanding R&D application in materials science, pharmacy, life science or forensics, for example. Possible fields of application are analyses of SERS signals on plasmonic substrates, thin sample films, nano particles and surfaces.
RG Raman microscope is also suitable for samples that tend to fluoresce when exposed to laser radiation.

Note:
In Raman spectroscopy, sample fluorescence can yield a much more intense signal than the Raman scatter of the sample, masking any Raman bands in the spectrum. Therefore, Raman spectroscopy is normally not a suitable analysis technique for fluorescent samples.

Specifications

Table 1. RG Raman microscope when equipped with RG Raman spectrometer

Feature versus model*	405 / 405 HR•	532 / 532 HR•	633 / 633 HR•	785 / 785 HR•
Laser wavelength	405 nm	532 nm	633 nm	785 nm
Power range on a sample**	from 0.1 to 30 mW	from 0.1 to 75 mW	from 0.1 to 50 mW	from 0.1 to 65 mW
Spectral Range	80-3750 cm^{-1} (405) 80-1900 cm^{-1} (405 HR)	60-3750 cm^{-1} (532) 60-1900 cm^{-1} (532 HR)	80-3750 cm^{-1} (633) 450-1800 cm^{-1} (633 HR)	90-2500 cm^{-1} (785) 450-1800 cm^{-1} (785 HR)
Spectral Resolution	4-6 cm^{-1} (405) 2-4 cm^{-1} (405 HR)	4-6 cm^{-1} (532) 2-4 cm^{-1} (532 HR)	4-6 cm^{-1} (633) 2-4 cm^{-1} (633 HR)	3-5 cm^{-1} (785) 1.5-3 cm^{-1} (785 HR)
Signal-to-noise ratio at*:**	1000:1	1200:1	900:1	900:1
Lateral resolution****	280 nm	320 nm	500 nm	600 nm
Axial resolution or confocality****	600 nm	750 nm	1000 nm	1500 nm
White light microscopy	Reflected with simultaneous visualization of laser spot and Raman acquisition			
Microscopy configuration	up-right and inverted			
Mapping travel range in XYZ	102 x 102 x 25 mm			
Lateral step size	100 nm			
Axial step size	100 nm			
Physical dimensions	430 mm x 340 mm x 480 mm (LxWxH)			
Weight	20 kg			

• *HR – high resolution model*

* *Each model is based on the same microscope body; only the RG Raman / miniRaman spectrometer is different. RG Raman / miniRaman spectrometer can be replaced by the user if necessary.*

** *Actual laser power range can differ ± 2% per device. Please contact us if you need specific laser power range values.*

*** *Determined as peak signal-to-noise ratio of polystyrene spectrum at maximal laser power, integration time 0.3s, number of repetitions 10.*

**** *Determined at microscope objective NA=0.95 (magnification 100x)*

Table 2. RG Raman microscope when equipped with miniRaman spectrometer

Feature versus model*	Standard	Power	SERS	Power Dual	Standard Dual
Laser wavelength	785 nm			660/675 nm and 785 nm	
Power range on a sample**	5-50 mW	10-90 mW	0,5-15 mW	5-40 mW (660) 5-75 mW (675) 10-90 mW (785)	5-40 mW (660) 5-75 mW (675) 5-50 mW (785)
Spectral Range	400-2700 cm^{-1}			2700-4000 cm^{-1} (660) 2550-4000 cm^{-1} (675) 400-2700 cm^{-1} (785)	
Spectral Resolution	7-15 cm^{-1} (slit size dependent; slit size can be customized)				
Signal-to-noise ratio at*:**	500:1	1000:1	100:1	600:1 (660) 800:1 (675) 800:1(785)	600:1 (660) 800:1 (675) 440:1(785)
Lateral resolution****	900 nm			800 nm (660) 900 nm (785)	
Axial resolution or confocality****	3 µm			2.5 µm (660) 3 µm (785)	
White light microscopy	Reflected with simultaneous visualization of laser spot and Raman acquisition				
Microscopy configuration	up-right and inverted				
Mapping travel range in XYZ	102 x 102 x 25 mm				
Lateral step size	100nm				
Axial step size	100nm				
Physical dimensions	430 mm x 340 mm x 480 mm (LxWxH)				
Weight	20 kg				

* *Each model is based on the same microscope body; only the RG Raman / miniRaman spectrometer is different. RG Raman / miniRaman spectrometer can be replaced by the user if necessary.*

** *Actual laser power range can differ ± 2% per device. Please contact us if you need specific laser power range values.*

*** *Determined as peak signal-to-noise ratio of polystyrene spectrum at maximal laser power, integration time 0.3s, number of repetitions 10.*

**** *Determined at microscope objective NA=0.95 (magnification 100x)*

White light illumination
Reflected light microscopy with simultaneous laser spot visualization and Raman spectra registration.

Microscopy configuration mode

- up-right
- inverted

Motorized stage

- lateral step size 50 nm, axial step size 50 nm
- bi-directional reproducibility 0.5um
- closed loop
- travel range in XYZ is 102 x 102 x 25 mm

Weight

20 kg

Dimensions

430 mm x 340 mm x 480 mm

Instrument design

RG Raman microscope consists of three key units (see Figure 4):

1. RG Raman (miniRaman) spectrometer
2. white light microscopy unit
3. motorized 3D stage

RG Raman (miniRaman) spectrometer can be installed and taken out by the user if necessary as shown in Figure 3. Key system components are shown in Figure 4.

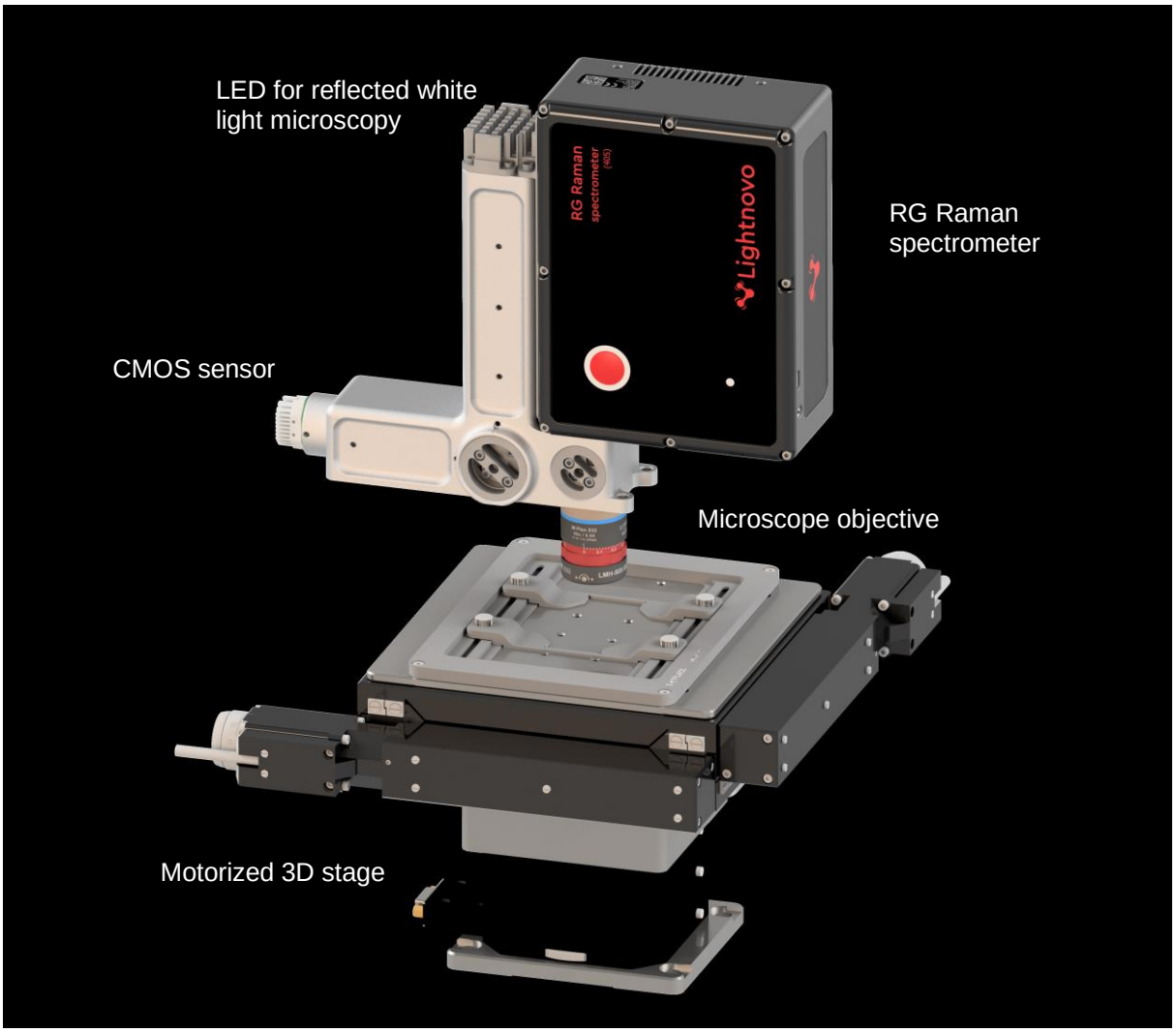

Figure 4. Opto-mechanical design of the miniRaman microscope.

General overview of control elements and components

RG Raman microscope has 5 control elements:

1. Laser (ON/OFF and power control)
2. Spectroscopic sensor for Raman spectra acquisition (exposure, gain, row selection and binning control)
3. LED for reflective light microscopy (power control)
4. CMOS sensor for white light microscopy (exposure, gain and color balance control)
5. 3D stage for sample movement and automated Raman mapping (sample scanning control)

Operation

Hardware connection

RG Raman microscope has two wire connections:

1. USB cable for system control and data transfer
2. Power cable (36 V)

Both cables should be connected (order does not matter). User should wait for LED 1 shining “Blue” (constant). Then software is ready for connection (“Connect” button in Miraspec). After connection LED 2 should be “Green”. It means that device is properly connected. If LED 2 is “Red” – device has connection problems. Please try to reconnect USB cable and power cable. If it does not help – contact service (service@lightnovo.com).

If connection with PC is lost, LEDs 2 and 3 will not shine.

During Raman mapping LED 3 will be blinking “Yellow”.

Figure 5. LED indicators of miniRaman microscope.

Operation procedure

Switching the system ON/OFF

It is highly recommended to switch off the miniRaman microscope LEDs for white light microscopy when they are not used for a longer period of time (several hours) and overnight. This action will prolong the service lifetime of the microscope LED.

When the analysis system is not used for a longer period of time, it is also highly recommended to switch off also the Raman excitation laser (785 nm and/or 660 nm). This action will prolong the service lifetime of the laser.

Switching off the PC is facultative. Take into account that each PC reboot requires a recalibration of the motorized stage.

Starting the Miraspec software

1. **Connection**

   Press “Connect” button in Connection window (see Figure 6). This will initialize hardware accessories. Connection window can be found in Menu/Tools/Connection (Figure 8).

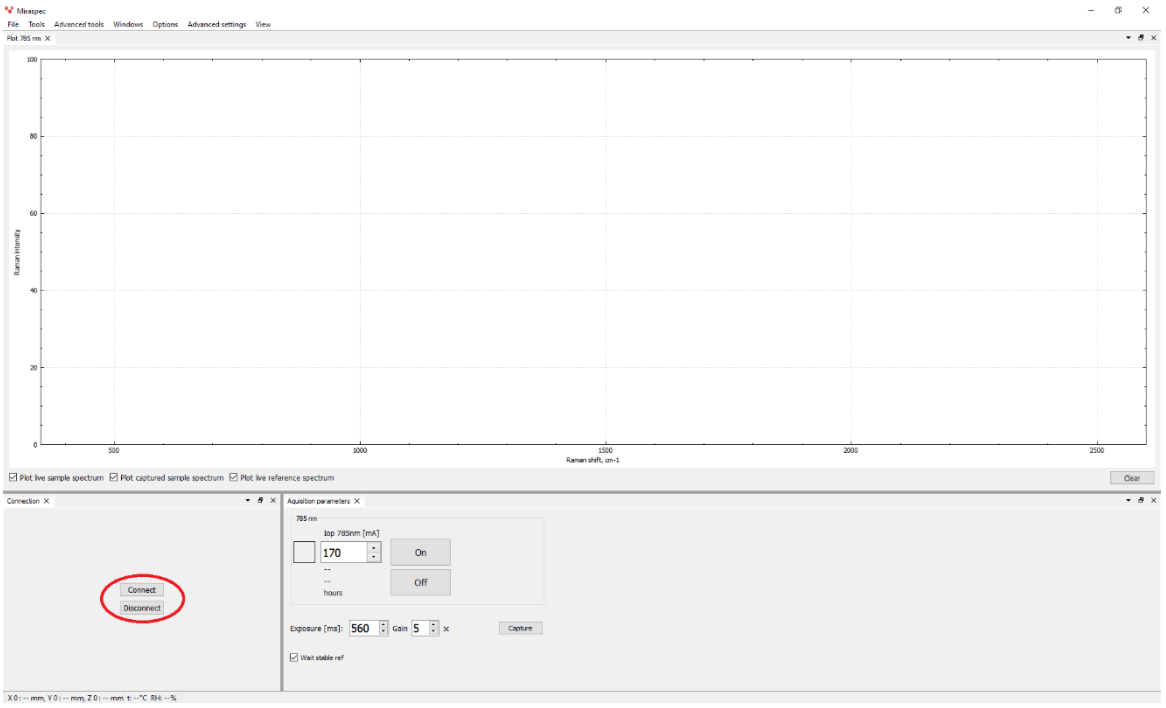

Figure 6. RG Raman microscope software interface; connection of accessories

2. **Turn ON/OFF lasers**

Press “On” or “Off” buttons, see below.

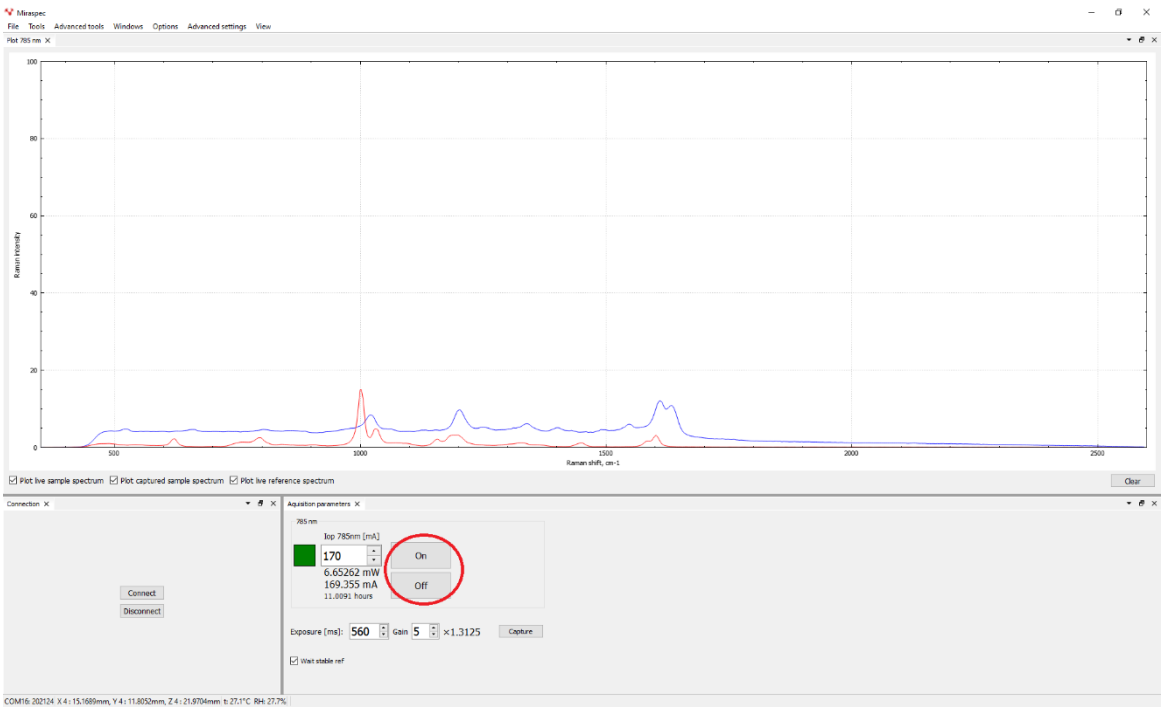

Figure 7. Switch on/off laser

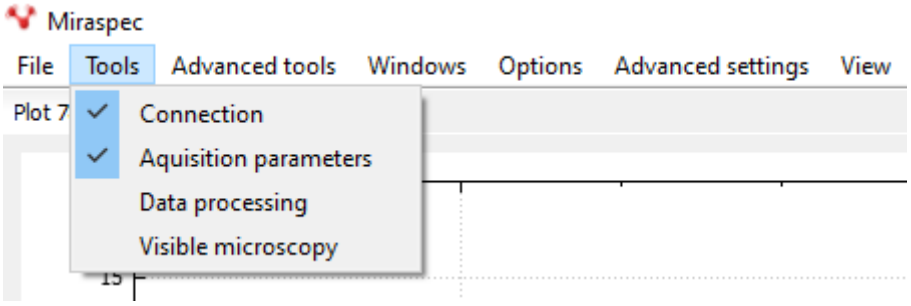

Figure 8. Tools menu

3. Calibration of the motorized stage

Open Menu/Tools/Visible microscopy and press Home button (see Figure 9).

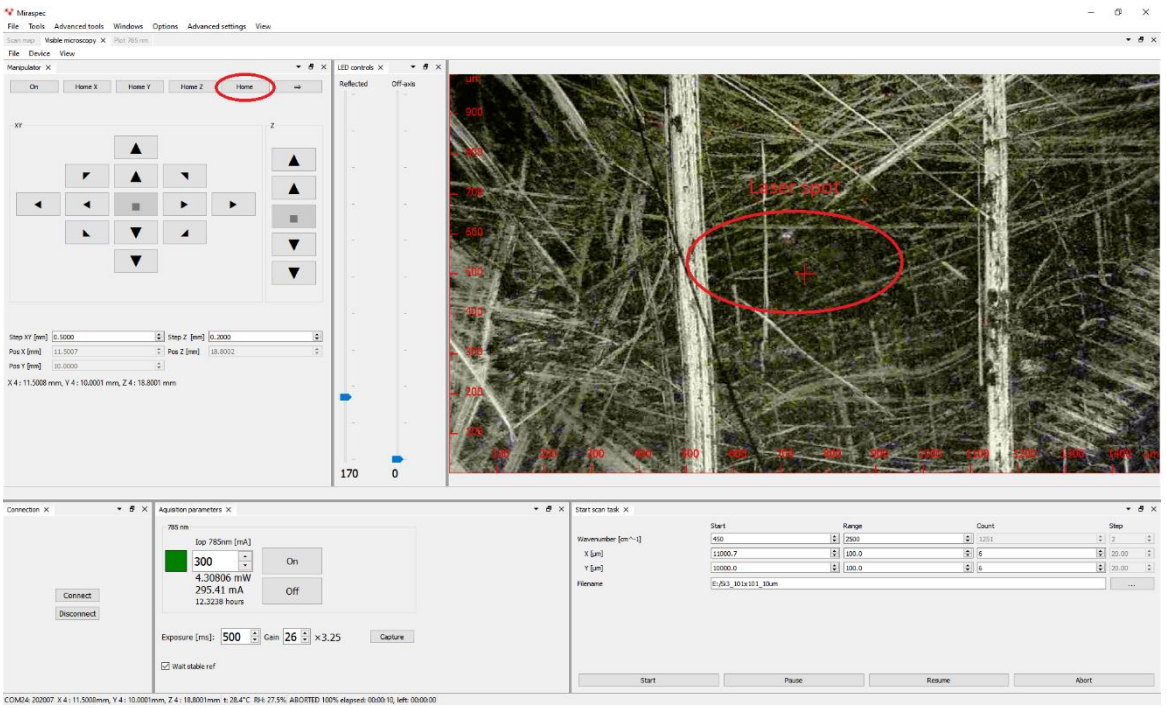

Figure 9. miniRaman microscope software interface; calibration of 3D stage

4. **Correcting the measuring position** in case of a slight offset of the point of incidence of the laser beam, i.e. offset with regard to the cross-hairs center (If this is not the case, skip this step.), see Figure 9.

5. **Positioning the sample on the microscope stage and visualizing the sample** (focusing, adjusting LED brightness, contrast etc.), see Figure 9.

6. **Measuring the Raman spectrum of the sample**

Raman spectra shown in the “Plot” window, see below.

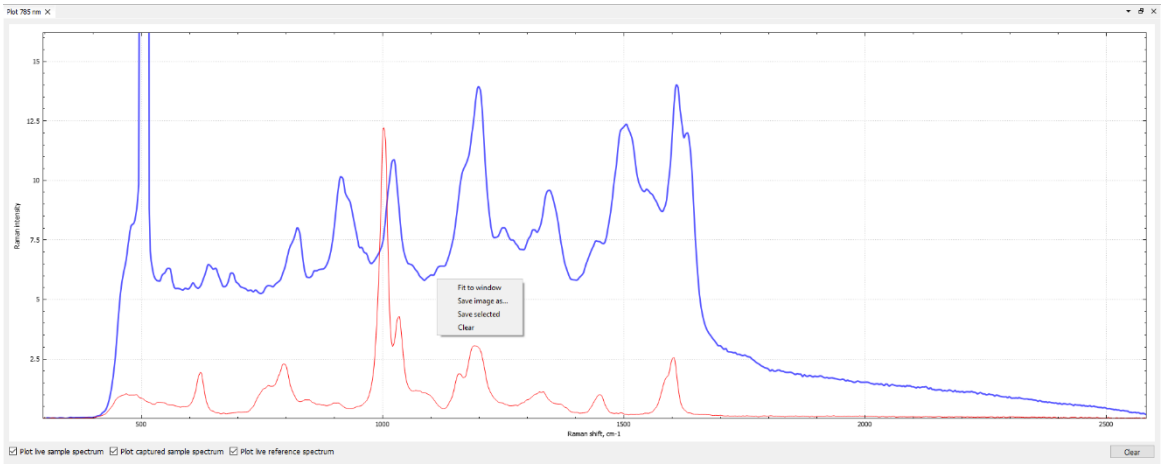

Figure 10. Curve context menu

If laser is ON system should show the “live” spectrum, see below. Spectrum scale can be adjusted by right click of the mouse and selection of “fit to window” button.

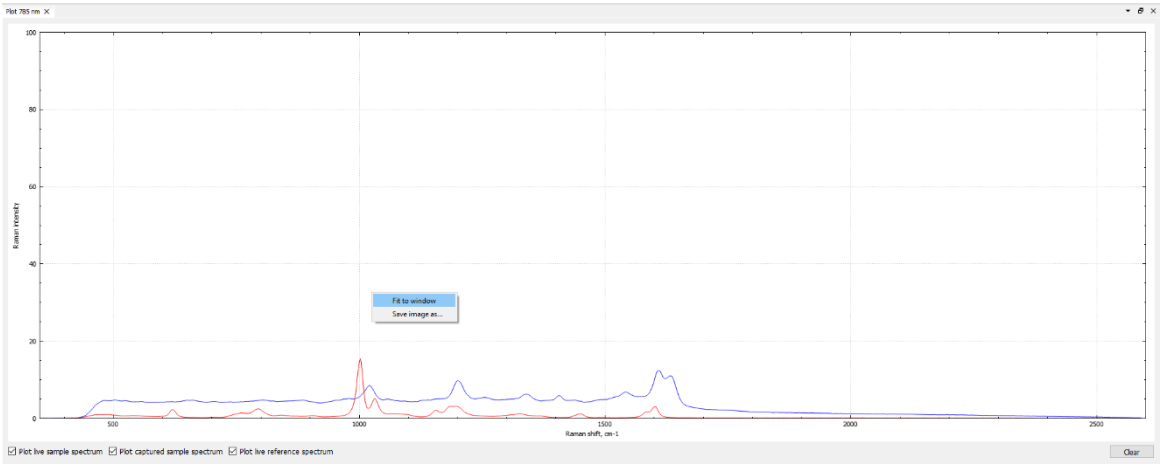

Figure 11. Fit spectra to window

Spectrum will be acquired by pressing “Capture” button. Acquisition parameters like laser power, gain of CMOS sensor and exposure time can be adjusted. Icon “Wait stable ref” should be applied if experiment require high resolution of Raman spectra.

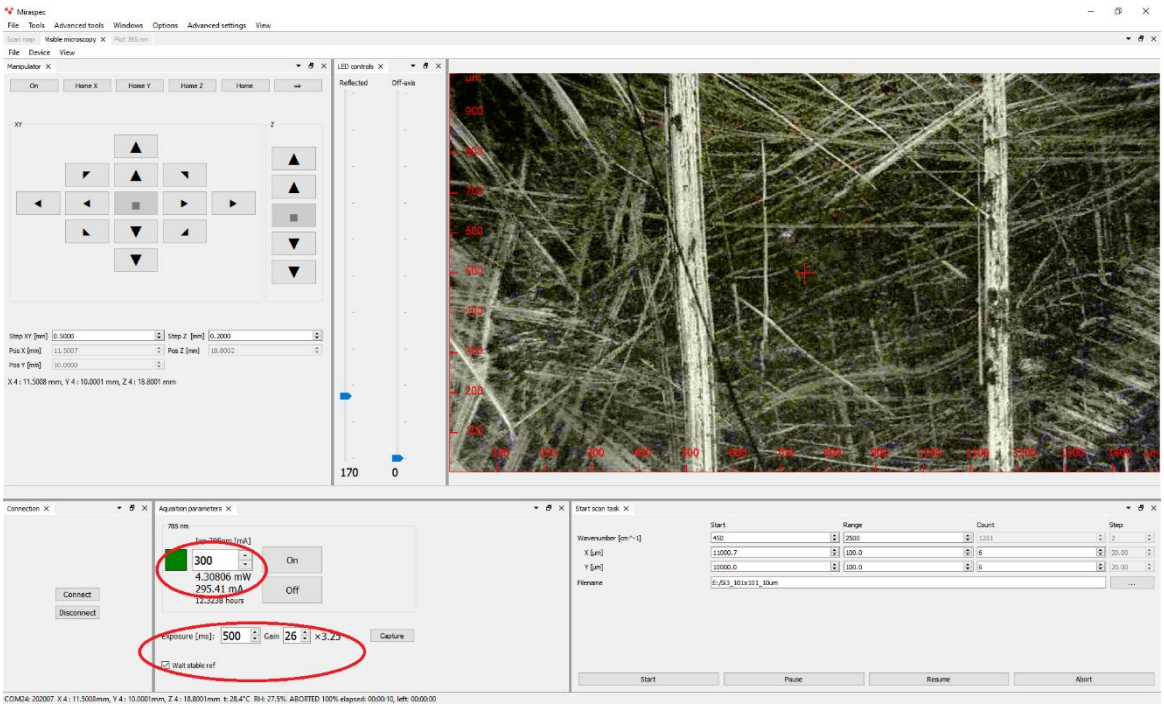

Figure 12. RG Raman microscope software interface; laser settings

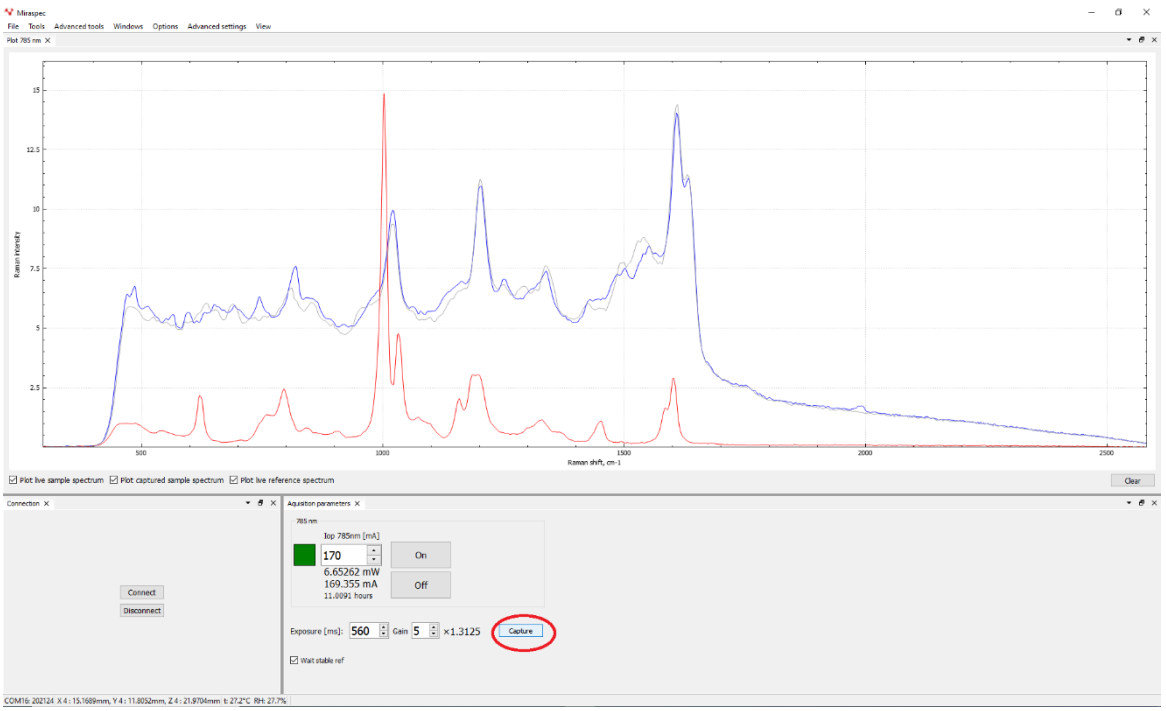

Figure 13. Spectrum capture

7. Defining Raman mapping parameters, see Figure 14.

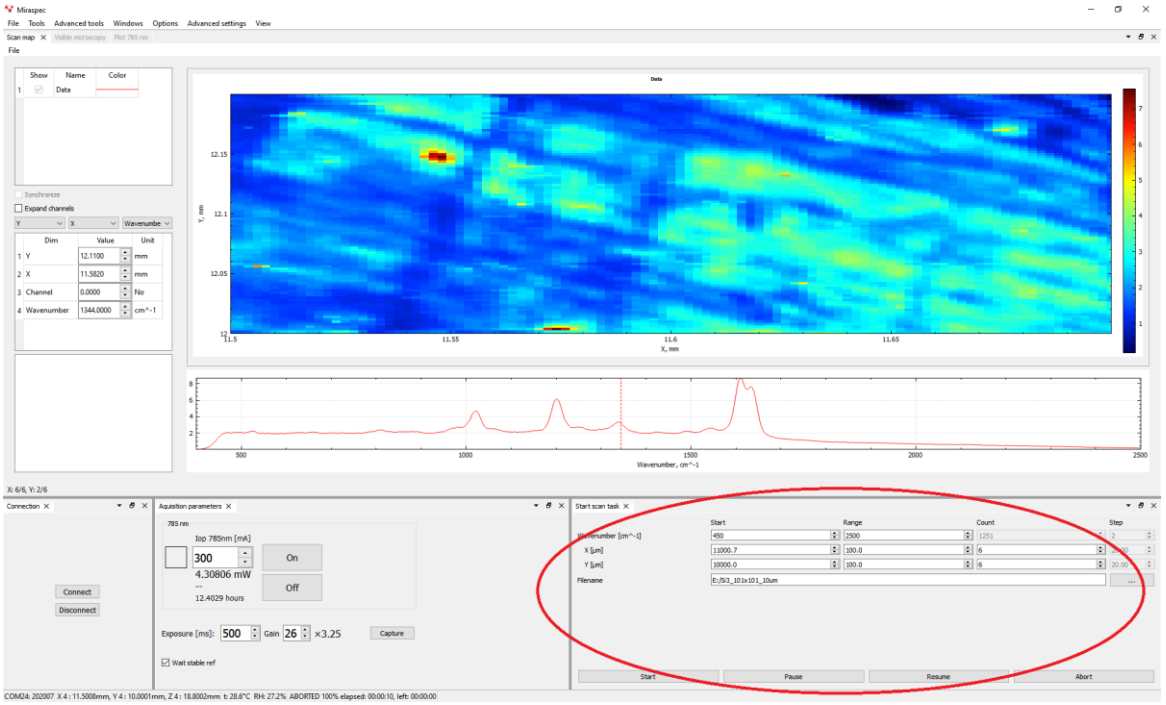

Figure 14. RG Raman microscope software interface; Raman mapping settings

Raman intensity fluctuations during mapping can be visualized by map of reference channel, see below.

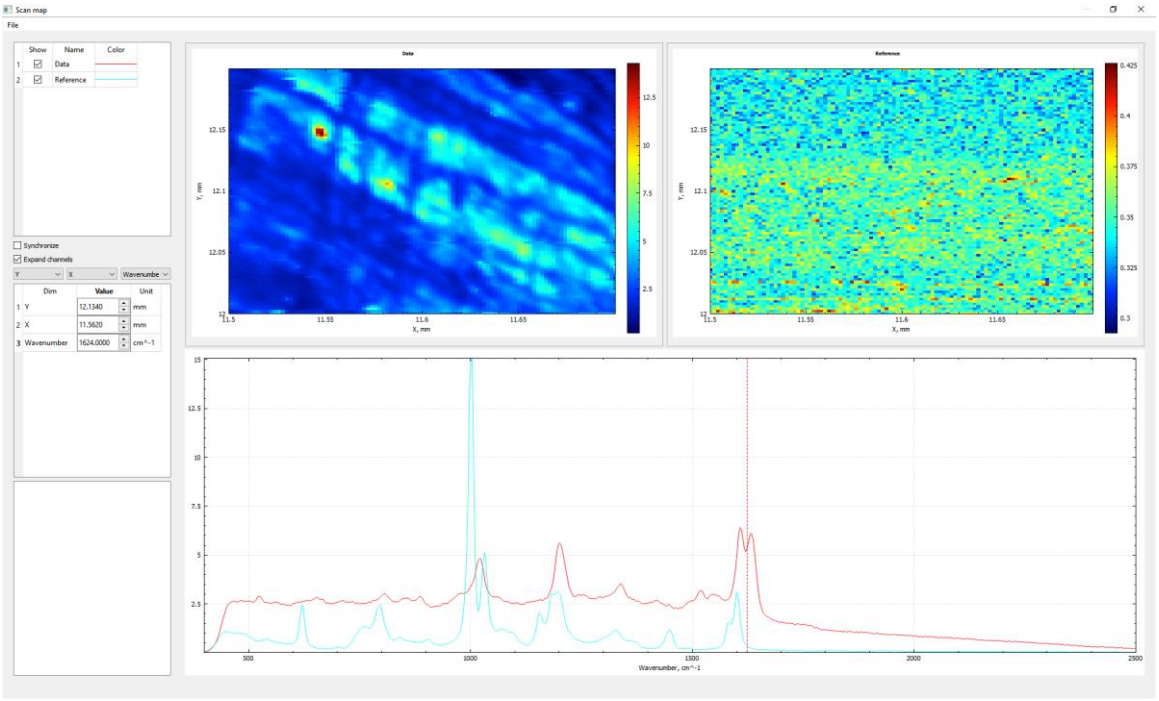

Figure 15. RG Raman microscope software interface; Raman map of the in-build reference channel

8. Measuring the Raman map of the sample

Press “Start” button in order to initiate the mapping process. If necessary, options of pause, resume and abort of the mapping process are also available.

9. Saving and/or exporting the data

Export spectrum in tsv: Menu/file/save (see Figure below).

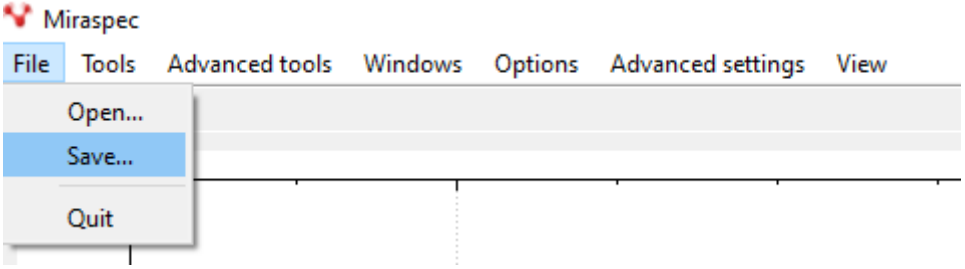

Figure 16. Saving captured spectrum

Map is saving during acquisition process in *.mrspectra. Map name and path must be selected in Raman mapping parameters before acquisition.
Export map in Matlab: Menu/advanced tools/scan map/export (see Figure below).

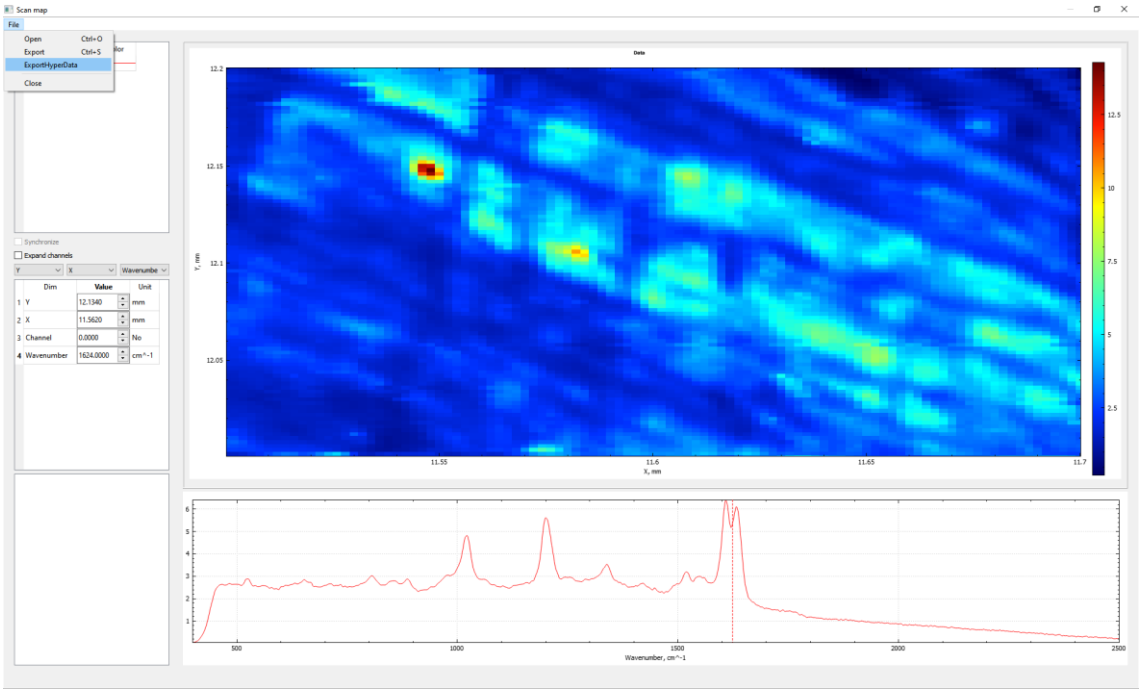

Figure 17. RG Raman microscope software interface; data export.

10. GUI settings

It is possible to optimize user interface based on customer preferences. Windows can be adjusted with docking widgets, see below.

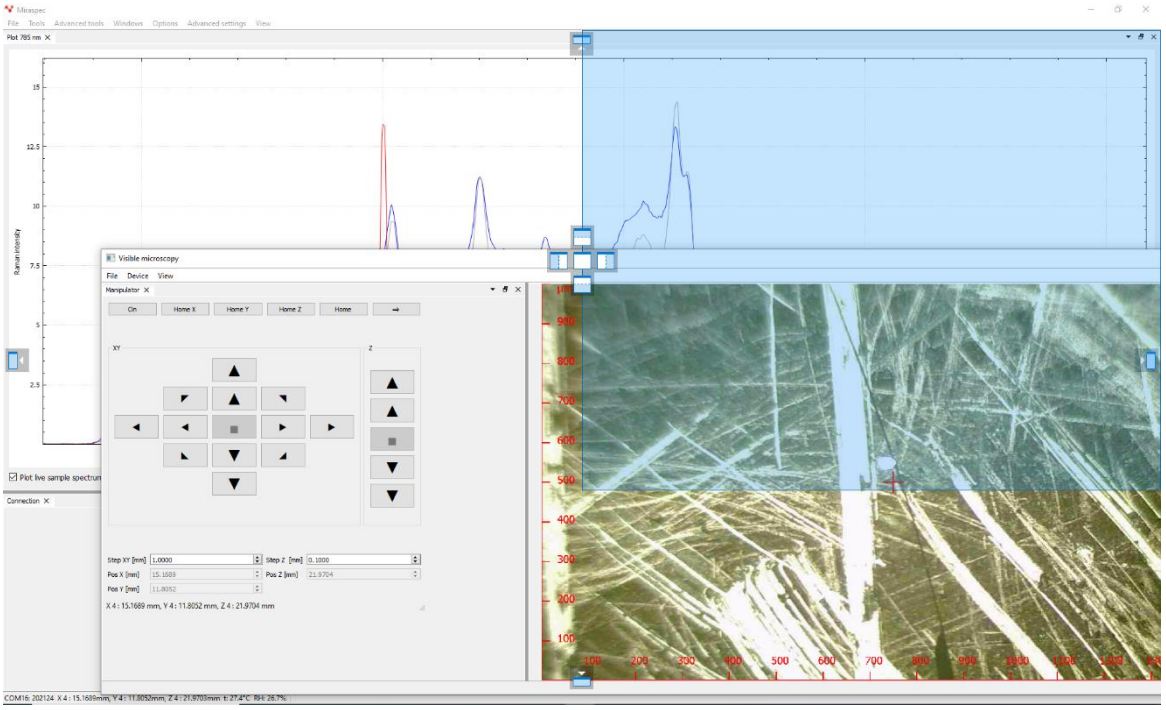

Figure 18. Docking widgets

Each user interface can be saved, see below.

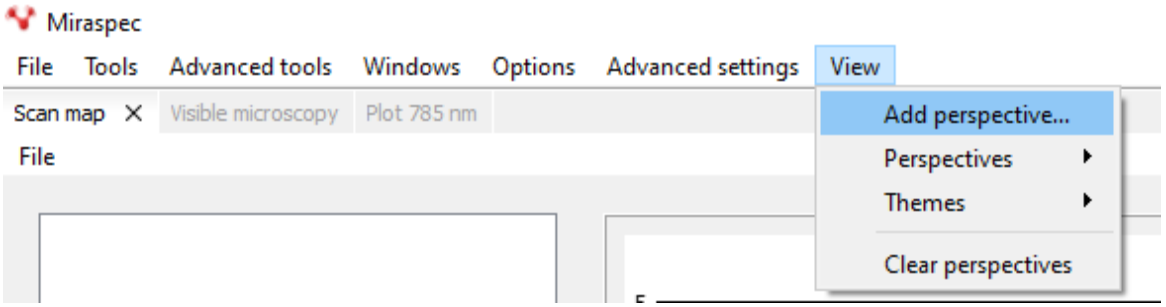

Figure 19. Adding perspective

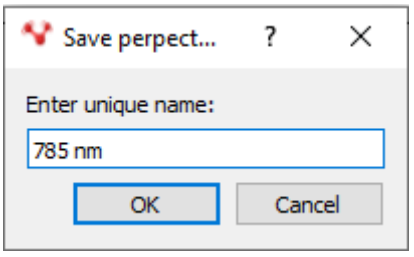

Figure 20. Saving perspective

Saved perspective can be opened at any time later, see below.

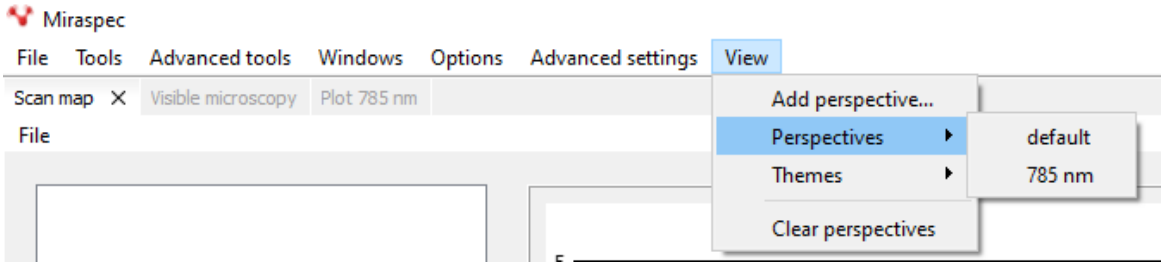

Figure 20. Saved perspective list

11. System indicators.

miniRaman microscope has in-build humidity and temperature sensor, see sensor position in Figure 21. Values are shown in connection status bar, see Figures below.

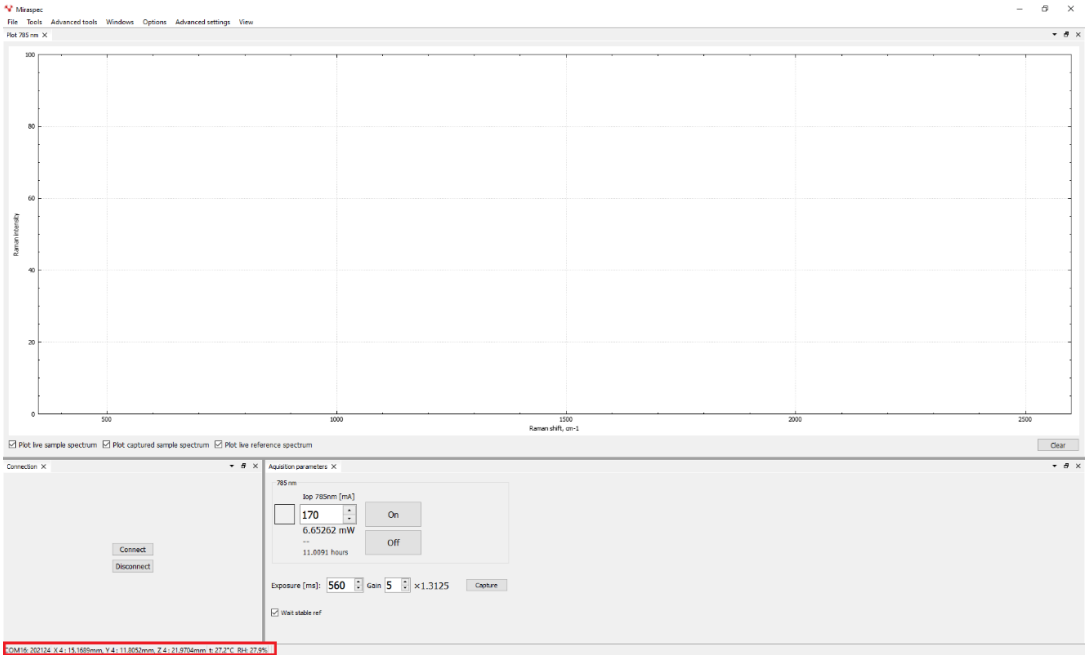

Figure 21. RG Raman microscope software interface; hardware connection status and in-build sensor data.

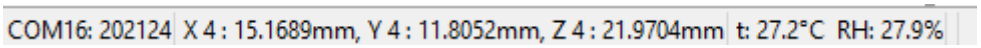

Figure 22. Connection status bar

Connection status also represents the COM port number and current position of 3D stage in XYZ axis.
If XYZ stage requires calibration, those values will be highlighted in yellow, see below.

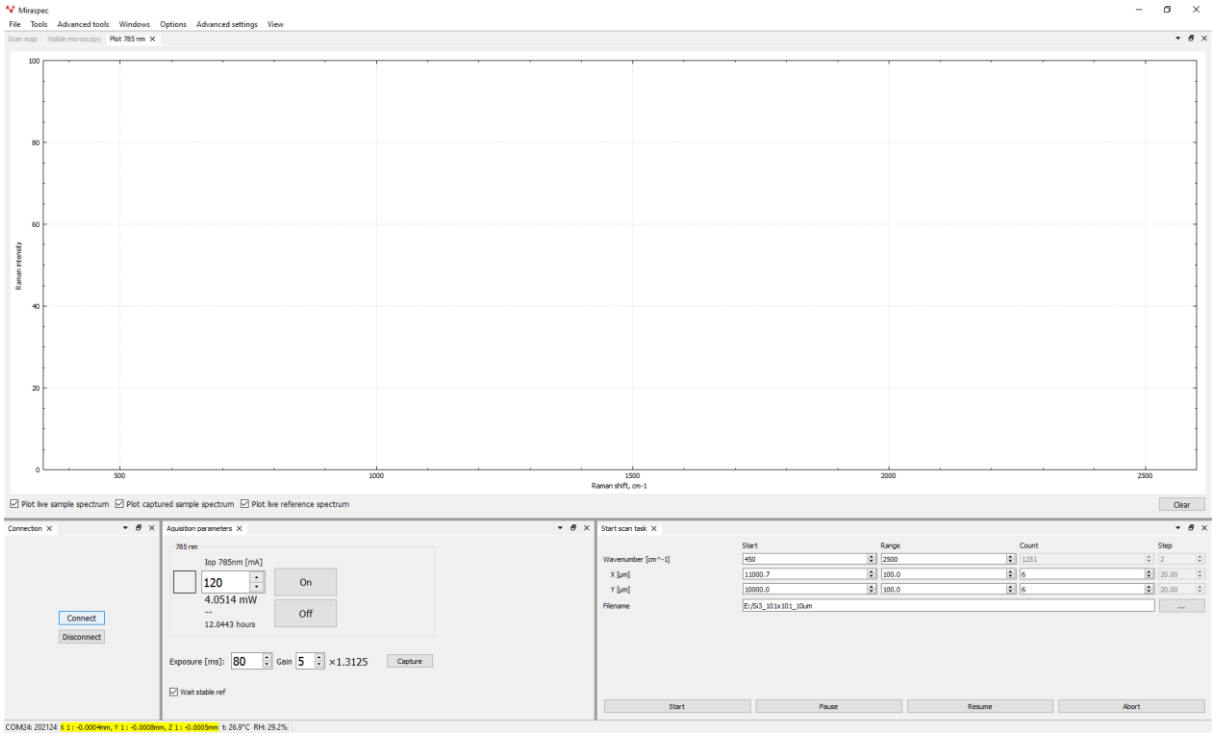

Figure 23. Connection status bar yellow when 3D stage requires calibration.

Upright and Inverted operation

RG Raman microscope can be used in upright and inverted configurations without any hardware modifications. In order to switch between different operation modes it needs to be rotated as shown in Figure 24.

Figure 24. a) Upright operation b) Inverted operation

Technical drawings

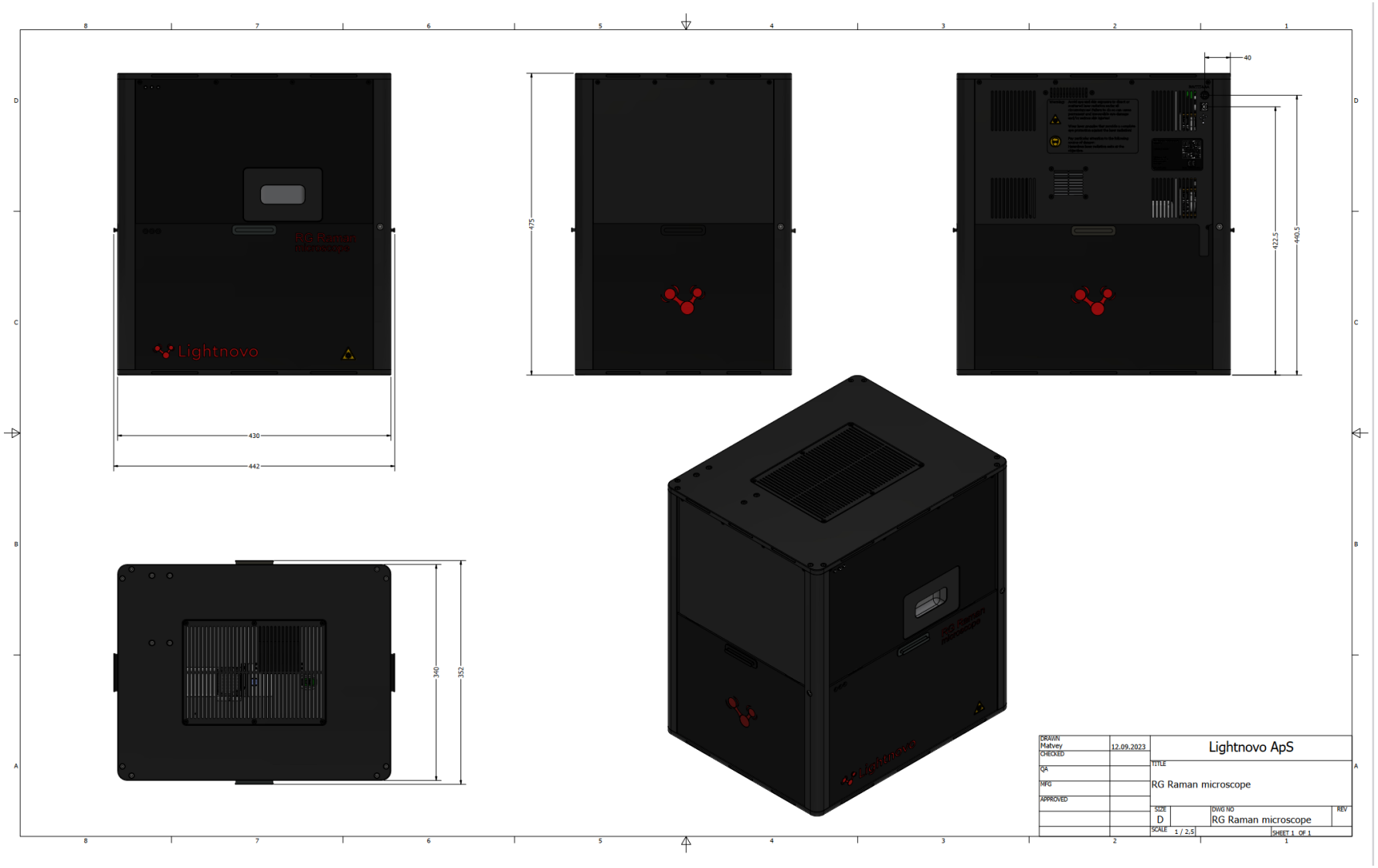

Figure 25. miniRaman microscope drawing with dimensions

Service addresses

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