

# miniRaman microscope

## User Manual

**English**

version 1.0



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# Safety

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## 1 General Information

Read the following safety instructions carefully before operating miniRaman 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 miniRaman 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 miniRaman microscope is in proper functioning condition. A safe and faultless operation can only be guaranteed if miniRaman 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 miniRaman 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 miniRaman 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**

miniRaman 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.

# Safety

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## 2 Warning labels



This warning symbol indicates the existence of laser radiation. A Class 3R laser is considered safe if handled carefully, with restricted beam viewing. With a class 3R laser, the maximum permissible exposure (MPE) can be exceeded, but with a low risk of injury. Visible continuous lasers in Class 3R are limited to 5 milliwatts.



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.

## 3 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.

# Safety

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## 4 Laser Safety

### General Information

The analysis system miniRaman microscope uses the light of a lasers (660 and 785nm) that are installed in miniRaman spectrometer. 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, model MiniRaman microscope SERS is laser class 3R product and it is considered safe if handled carefully, with restricted beam viewing.

According to the standard EN 60825-1/10.2003, miniRaman microscope models: MiniRaman microscope Standard, MiniRaman microscope Standard Dual, MiniRaman microscope Power Dual, MiniRaman microscope Power are Class 3B lasers products. Therefore, they are normally hazardous under direct beam viewing conditions, but are 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 measurement. This safety precaution protects the operator against accidental exposure to hazardous laser radiation exiting the objective.



Figure 1. Open Laser Protective Enclosure



Closed Laser Protective Enclosure

# Safety

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## **Safety Instructions**

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

The analysis system is specified as a laser class 3R/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

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## 1 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.

## 2 Delivery Scope

### Standard Components

miniRaman 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 miniRaman 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 miniRaman 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 case to avoid damages.

Figure 2. Transportation case for miniRaman microscope

# Installation

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## 3 Site requirements

### Space Requirements

miniRaman microscope requires a space of 17cm in diameter and 28cm in height. (For the exact instrument dimensions refer to Specification.) At the rear instrument side, take a clearance of at least 5cm 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 miniRaman microscope connected provides a suitable solution. miniRaman microscope requires a stable and horizontal base which can carry the instrument (ca. 7kg) 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

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

# Installation

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**Caution:**

*To avoid personal injury and spectrometer damage, connect miniRaman microscope only to a socket outlet with earthing contact.*

## 4 Cable Connections



Please unpack miniRaman microscope and remove it from the transportation case (see Figure 3).

Figure 3. miniRaman microscope with transportation case.



Please remove power adaptor (Figure 4) and connect it to the right connector on the rear panel of the miniRaman microscope (Figure 5). Then connect USB cable to the left connector on the rear panel and connect it to PC from the other side.

Figure 4. Power adaptor.

# Installation

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Figure 5. Rear side – overview of the connector sockets (left connector is USB 2.0 for connection with PC via USB cable, right connector is for power supply 12V).

## 5 Software Installation

1. Download miniRaman 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.

# 6

## Removal and installation of miniRaman spectrometer

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

- 

remove top cover
- 

unscrew four side bolts of rear panel
- 

remove rear slide cover
- 

unscrew three bolts on the rear panel
- 

unscrew three bolts that hold miniRaman spectrometer
- 

remove miniRaman spectrometer
- 

remove small magnet
- 

assembly laser safety kit
- 

miniRaman spectrometer is ready to use

figure 6. Removal steps of the miniRaman spectrometer from the miniRaman microscope

# Overview

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## 1 General information

MiniRaman microscope has a modular design with reflection and off-axis white light illumination microscopy unit combined with motorized 3D stage and miniRaman 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.

MiniRaman microscope is also suitable for samples that tend to fluoresce when exposed to laser radiation. Due to the usage of a 785nm laser, the excitation energy is low enough for these samples not to fluoresces or only to a minor degree.

### **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.*

# Overview

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## 2 Specification

### Lasers

Depending on the selection of the miniRaman spectrometer model miniRaman microscope could be configured in the following models with different laser parameters:

- MiniRaman microscope Standard (includes one laser with optical isolation: 785 nm, power range on a sample 5-50 mW)
- MiniRaman microscope Power (includes one laser with optical isolation: 785 nm, power range on a sample 15-150 mW)
- MiniRaman microscope SERS (includes one laser with optical isolation: 785 nm, power range on a sample 0.5-5 mW)
- MiniRaman microscope Power Dual (includes two lasers with optical isolation: 785 nm, power range on a sample 15-150 mW; 660 nm, power range on a sample 1-32 mW)
- MiniRaman microscope Standard Dual (includes two lasers with optical isolation: 785 nm, power range on a sample 5-50 mW; 660 nm, power range on a sample 1-32 mW)

### Spectral range

- 400-2700  $\text{cm}^{-1}$  (at 785 nm laser excitation),
- 2750-4500  $\text{cm}^{-1}$  (at 660 nm laser excitation)

### Spectral resolution

- 10-15  $\text{cm}^{-1}$  (slit size dependent; slit size can be customized)

**Sensitivity in point mode at laser wavelength 785 nm** (determined as SNR of polystyrene spectrum)

- SNR 350:1
- spectral range 400-2700  $\text{cm}^{-1}$
- laser wavelength: 785 nm
- laser power: 100 mW
- integration time: 0.1s
- number of repetitions: 1

**Sensitivity in point mode from laser wavelength 660 nm** (determined as SNR of polystyrene spectrum).

- SNR 150:1
- spectral range 2750-4500  $\text{cm}^{-1}$
- laser wavelength: 660 nm
- laser power: 30 mW
- integration time: 0.5 s
- number of repetitions: 1

# Overview

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## **Spatial resolution**

- Diffraction limited (microscope objective dependent)
- 600 nm (lateral) at 660 nm excitation at NA=0.95 (100x)
- 750 nm (lateral) at 785 nm excitation at NA=0.95 (100x)

## **White light illumination**

- Reflected light microscopy with simultaneous laser spot visualization and Raman spectra registration
- Transmittance light microscopy with simultaneous laser spot visualization and Raman spectra registration
- Off-axis illumination microscopy with simultaneous laser spot visualization and Raman spectra registration

## **Microscopy configuration mode**

- up-right
- inverted

## **Motorized stage**

- lateral step size 50nm, axial step size 50nm
- bi-directional reproducibility 0.5um
- closed loop
- travel range in XYZ is 26mm

## **Weight**

7kg

## **Dimensions**

12x13x28 cm

# Overview

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## 2 Instrument design

MiniRaman microscope consists of three key units (see Figure 7):

1. miniRaman spectrometer
2. white light microscopy unit
3. motorized 3D stage

MiniRaman spectrometer can be installed and taken out by the user if necessary as shown in Figure 6.

Key system components are shown on Figure 7.

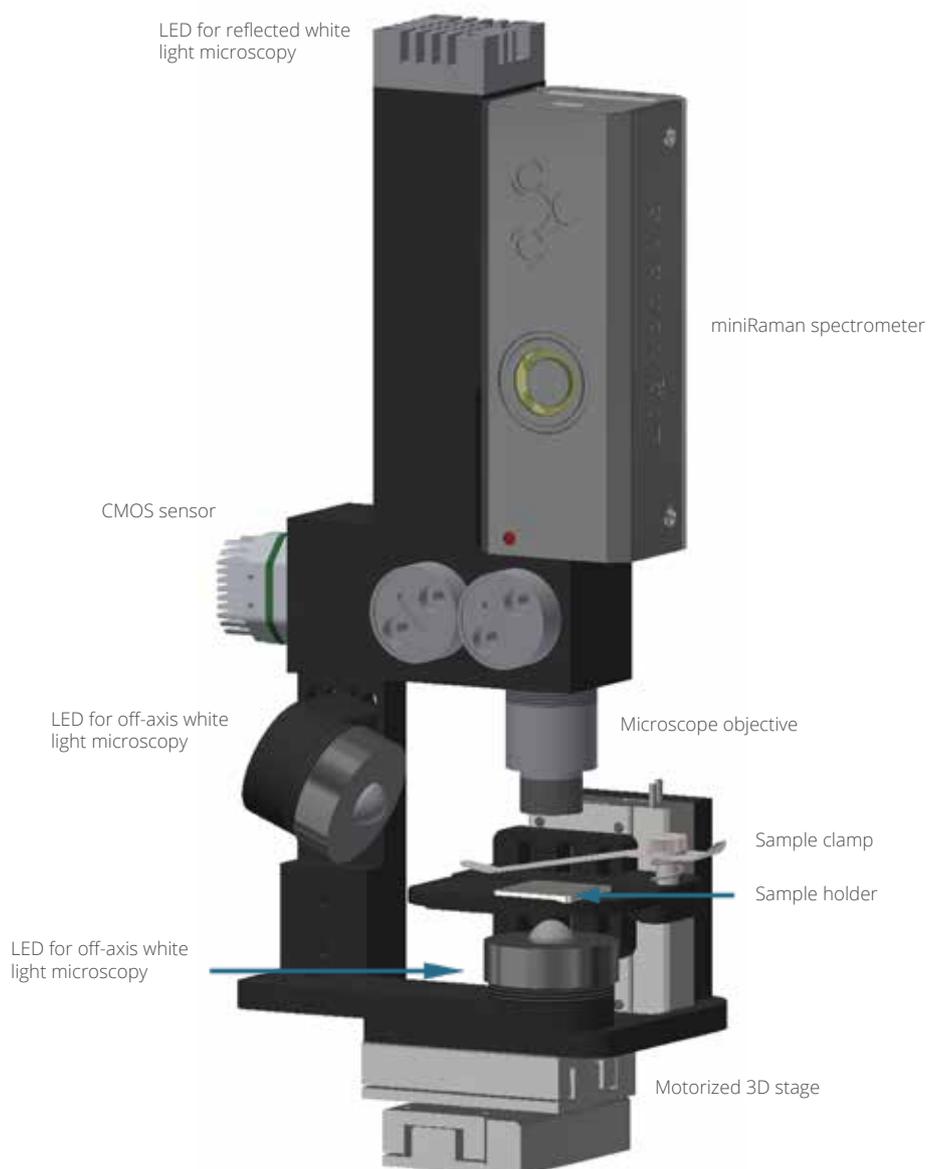


Figure 7. Opto-mechanical design of the miniRaman microscope.

## **3** General overview of control elements and components

MiniRaman microscope has 6 control elements:

1. Laser with wavelength 785nm (ON/OFF and power control)
2. Laser with wavelength 660nm (ON/OFF and power control)
3. Spectroscopic sensor for Raman spectra acquisition (exposure, gain, row selection and binning control)
4. LED for reflected light microscopy (power control)
5. LED for off-axis light microscopy (power control)
6. LED for transmittance light microscopy (power control)
7. CMOS sensor for white light microscopy (exposure, gain and color balance control)
8. 3D stage for sample movement and automated Raman mapping (sample scanning control)

# Operation

## 1 Hardware connection

MiniRaman microscope has two wire connections:

1. USB cable for system control and data transfer
2. Power cable (12V)

Both cables could be connected (order does not matter). User should wait for LED 1 shining "Blue" (constant) and miniRaman spectrometer bottom blinking "Blue". Then software is ready for connection ("Connect" bottom 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](mailto:service@lightnovo.com)).

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

During Raman mapping LED 3 will be blinking "Yellow".



Figure 8. LED indicators of miniRaman microscope.

# Operation

## 2 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 (785nm and/or 660nm). 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" bottom in Connection window (see Figure 9). This will initialize hardware accessories. Connection window can be found in Menu/Tools/Connection (Figure 11).

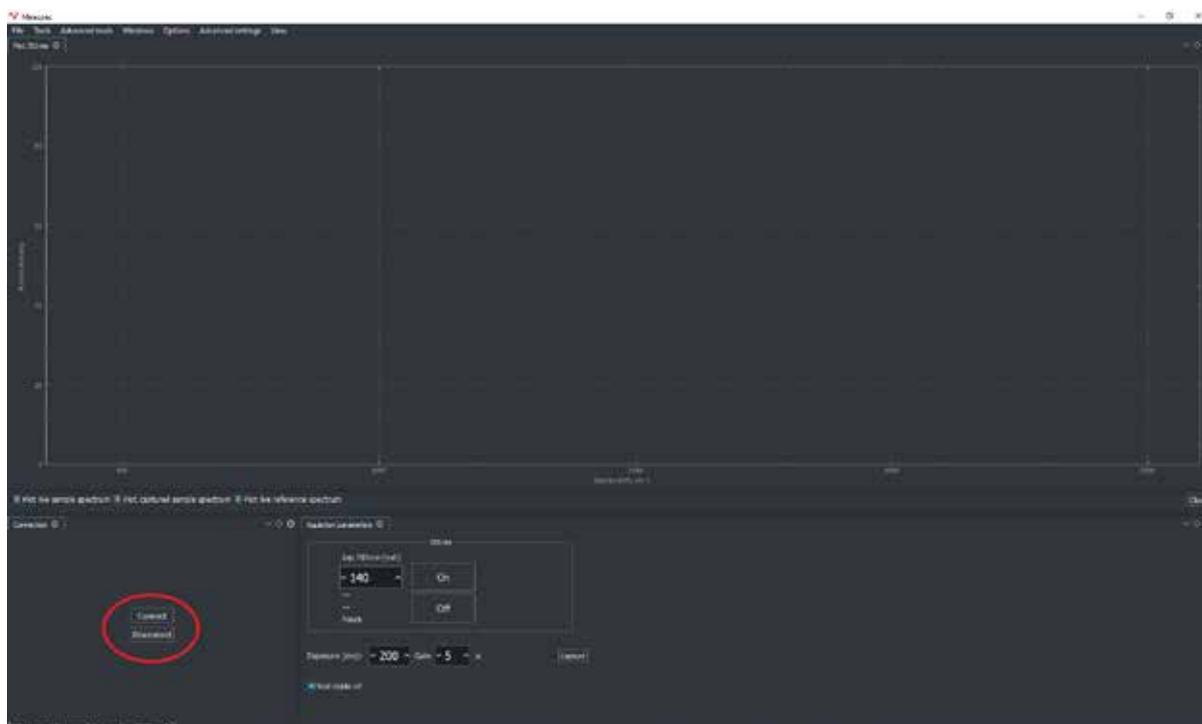


Figure 9. miniRaman microscope software interface; connection of accessories

# Operation

## 2. Turn ON/OFF lasers

Press "On" or "Off" bottoms, see below.

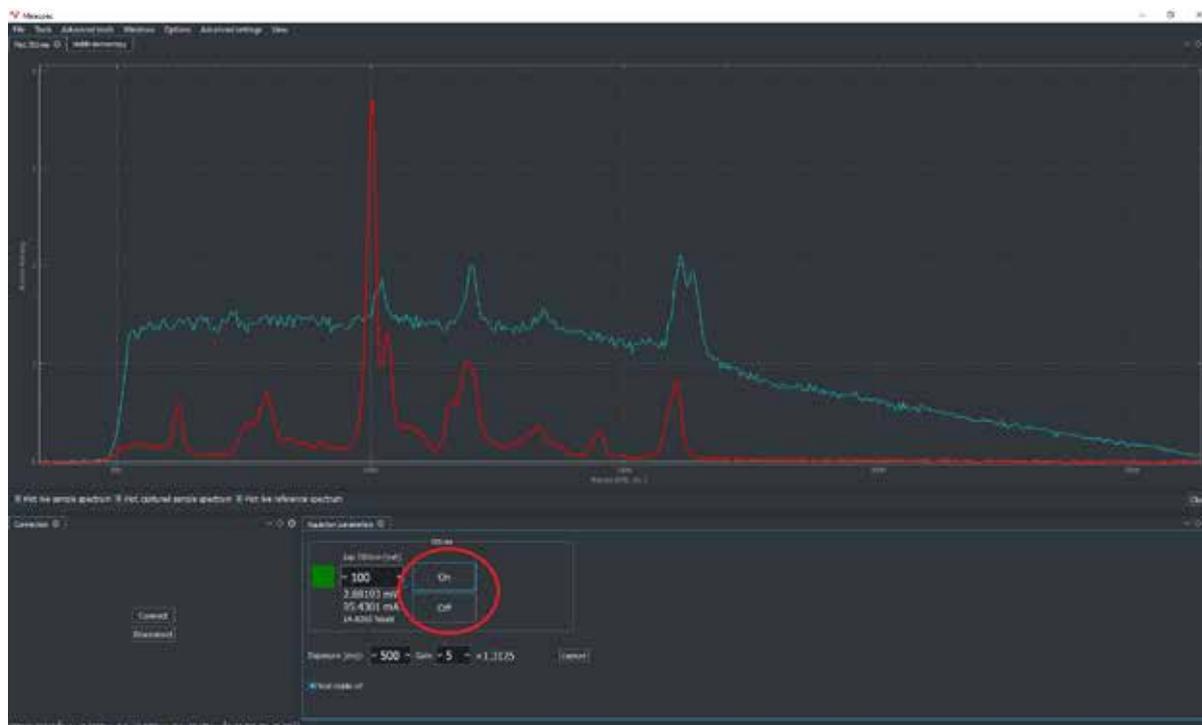


Figure 10. Switch on/off laser

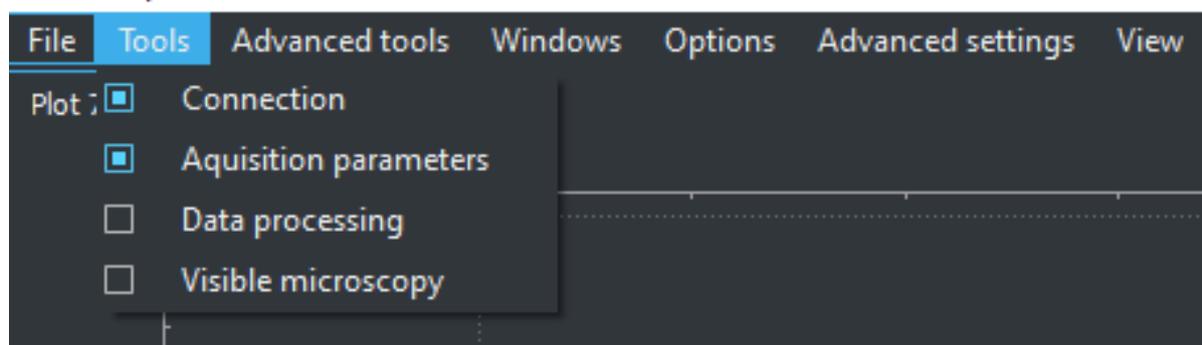


Figure 11. Tools menu

# Operation

## 3. Calibration of the motorized stage

Open Menu/Tools/Visible microscopy and press Home bottom (see Figure 12).

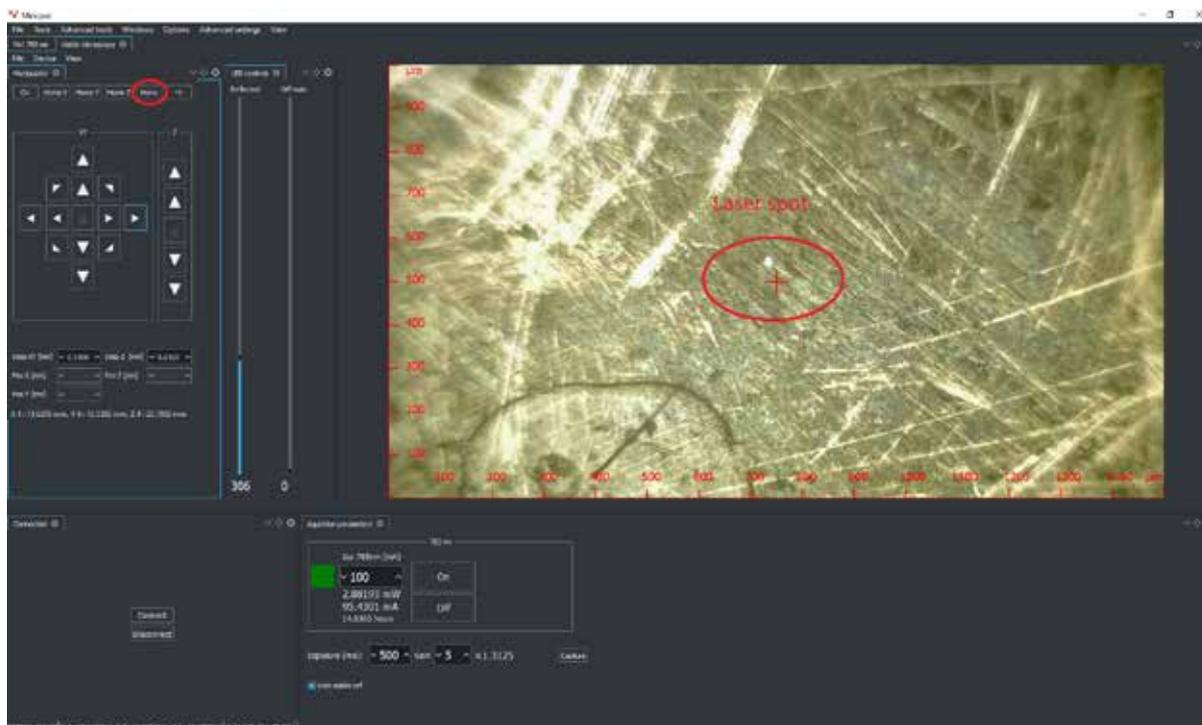


Figure 12. miniRaman microscope software interface; calibration of 3D stage

**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 12.

**5. Positioning the sample on the microscope stage and visualizing the sample** (focusing, adjusting LED brightness, contrast etc.), see Figure 12.

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

Raman spectra shown in the "Plot" window, see below.



Figure 13. Curve context menu

# Operation

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” bottom.

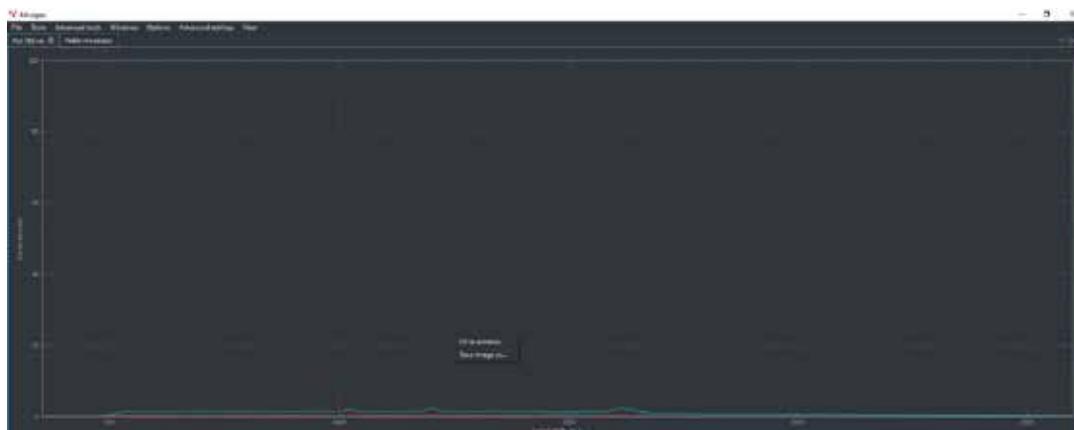


Figure 14. Fit spectra to window

Spectrum will be acquired by pressing “Capture” bottom. 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.

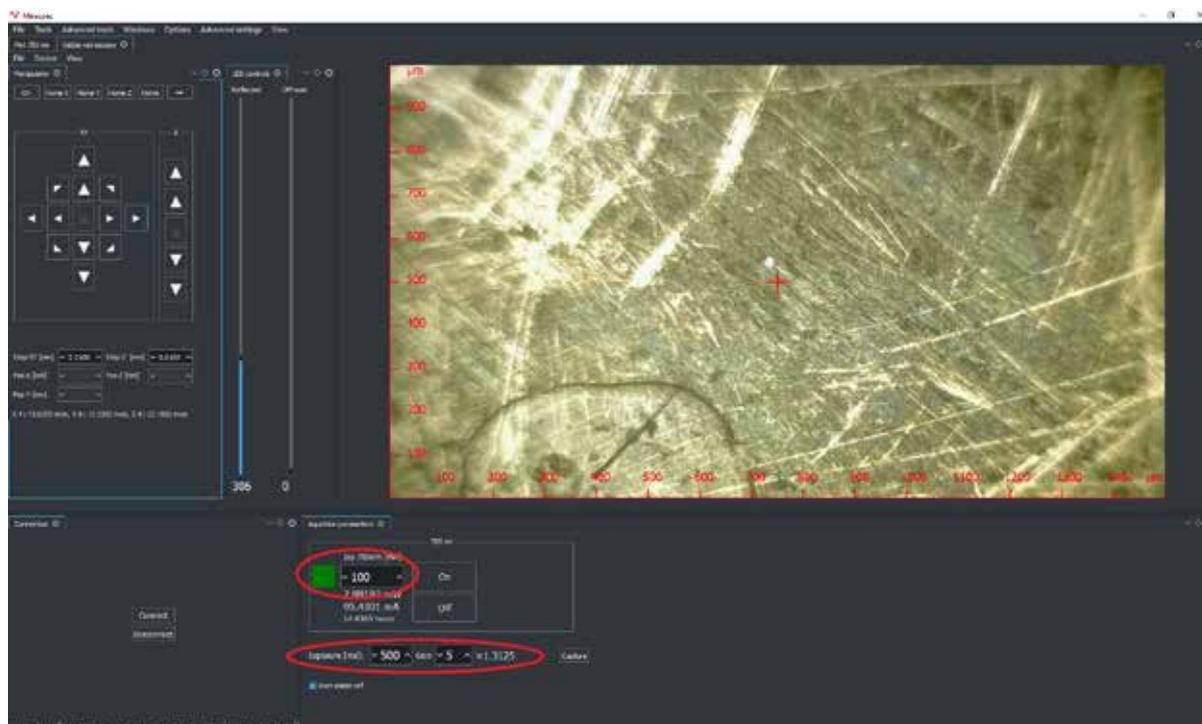


Figure 15. miniRaman microscope software interface; laser settings

# Operation

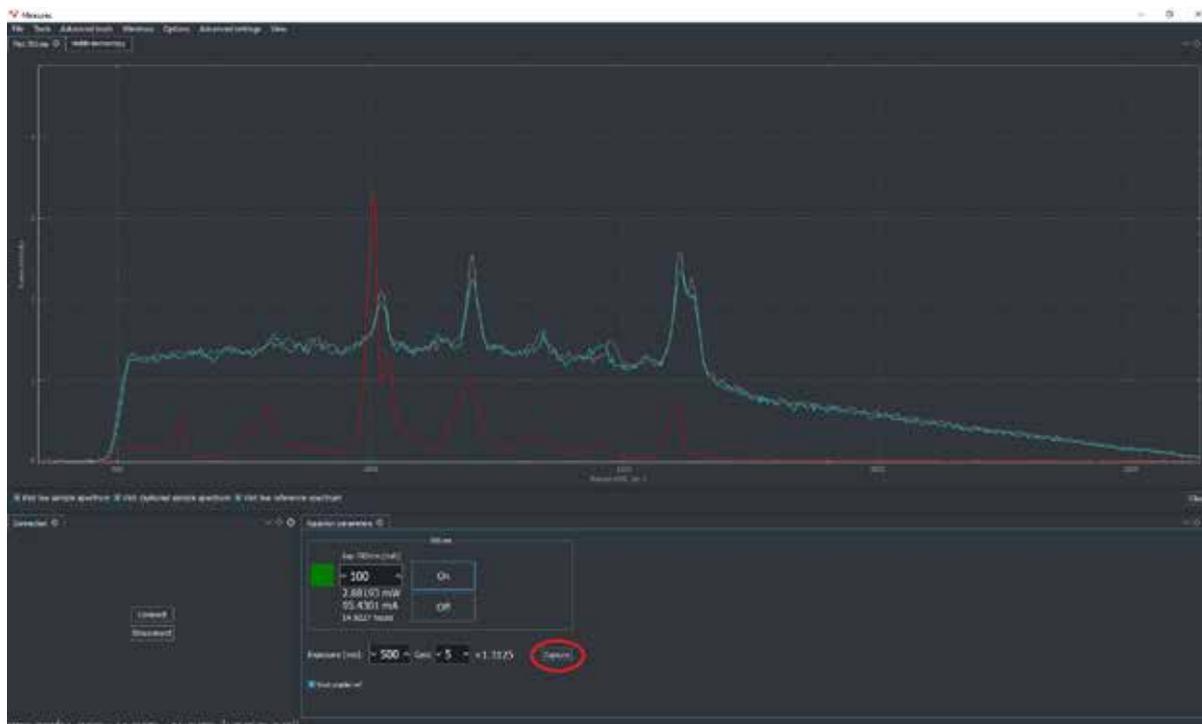


Figure 16. Spectrum capture

## 9. Defining Raman mapping parameters, see Figure 17.

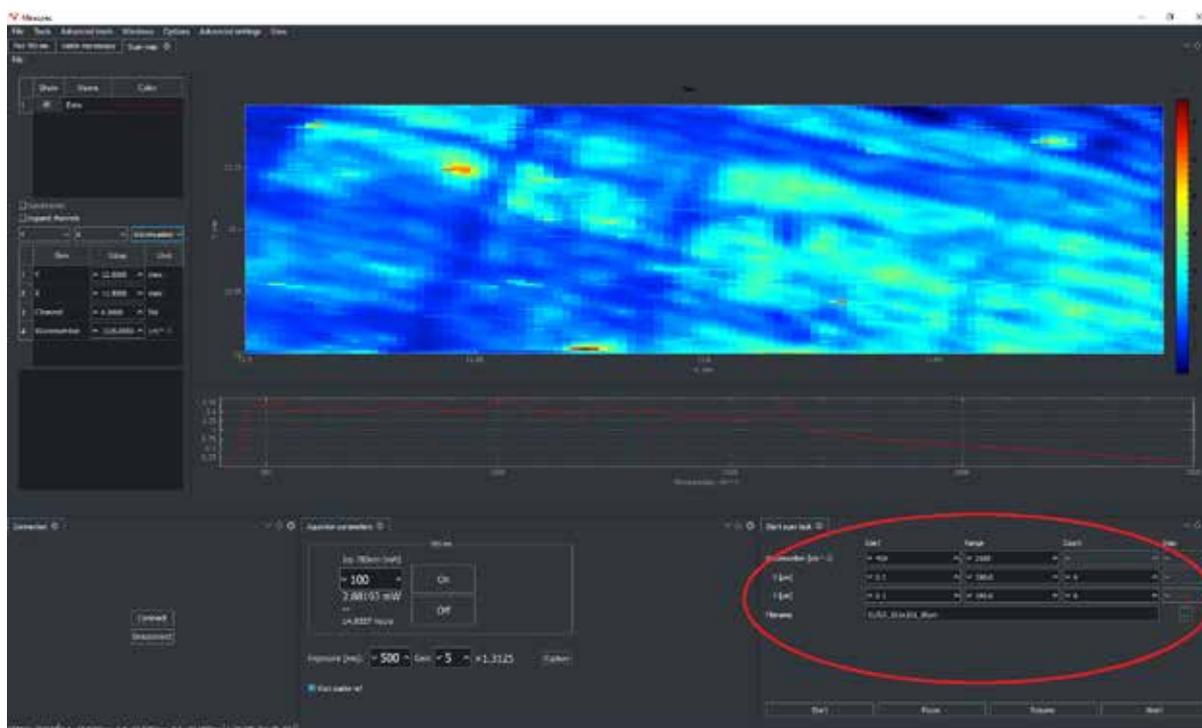


Figure 17. miniRaman microscope software interface; Raman mapping settings

# Operation

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

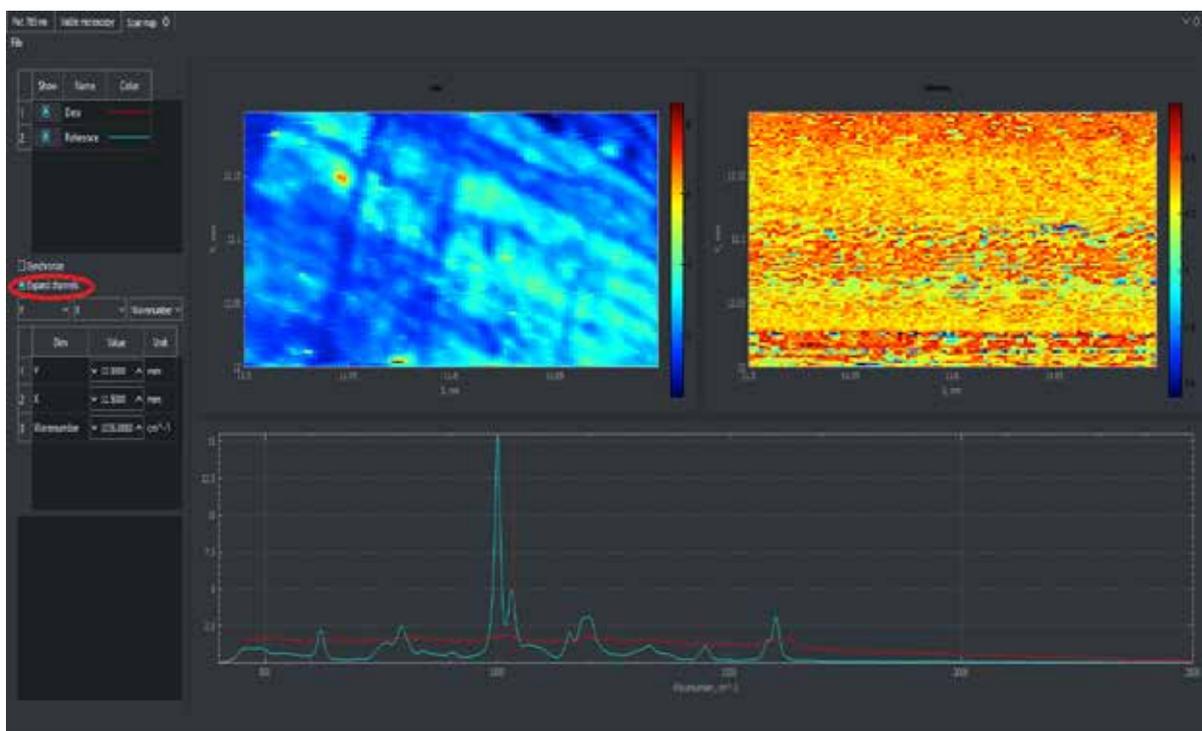


Figure 18. miniRaman microscope software interface; Raman map of the in-built reference channel

## 10. Measuring the Raman map of the sample

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

## 11. Saving and/or exporting the data

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

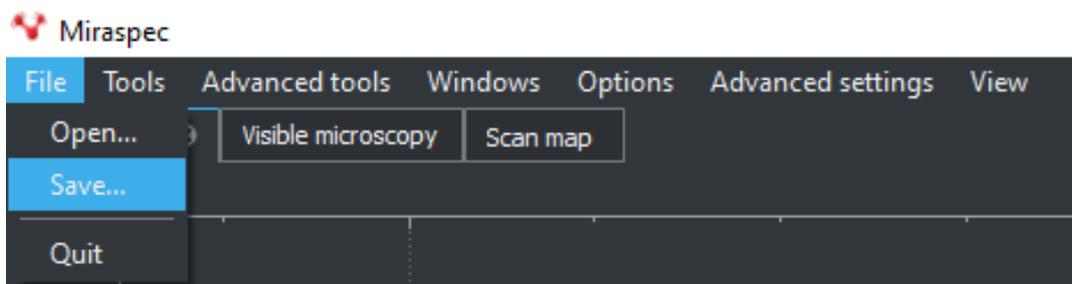


Figure 19. Saving captured spectrum

# Operation

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).

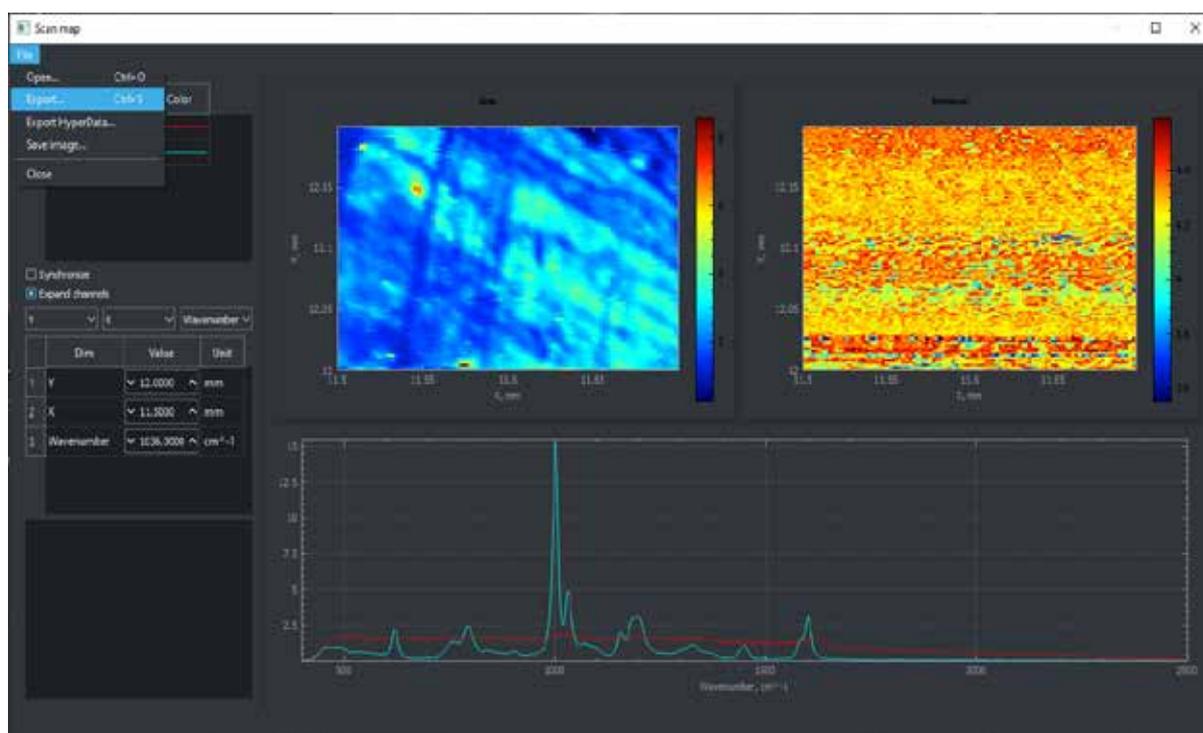


Figure 20. miniRaman microscope software interface; data export.

## 12. GUI settings

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

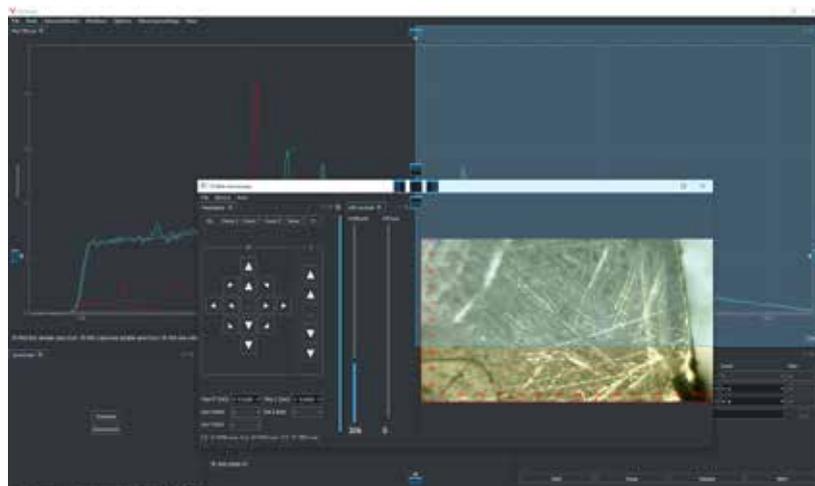


Figure 21. Docking widgets

# Operation

Each user interface can be saved, see below.

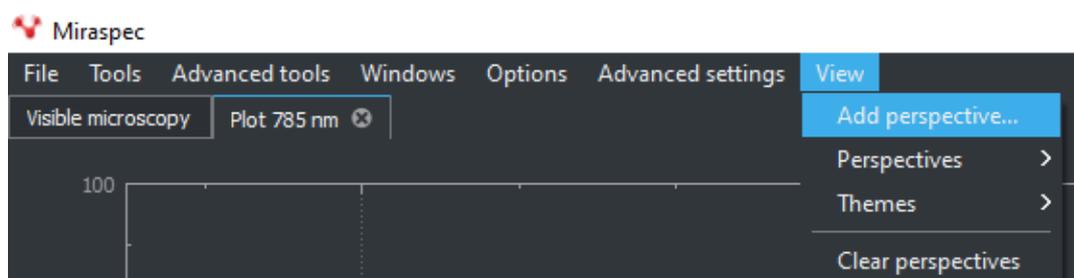
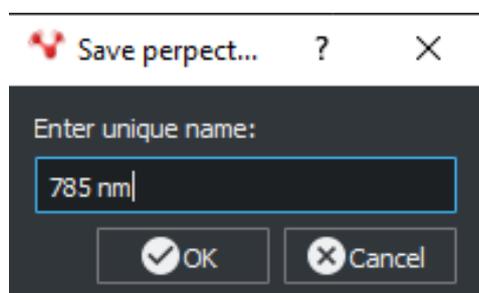


Figure 22. Adding perspective



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

Figure 23. Saving perspective

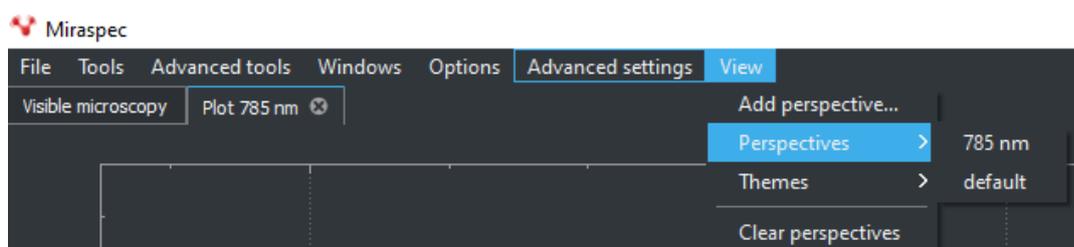


Figure 24. Saved perspective list

## 13. System indicators.

miniRaman microscope has in-built humidity and temperature sensor, see sensor position on Figure 8. Values are shown in connection status bar, see Figures below.

# Operation

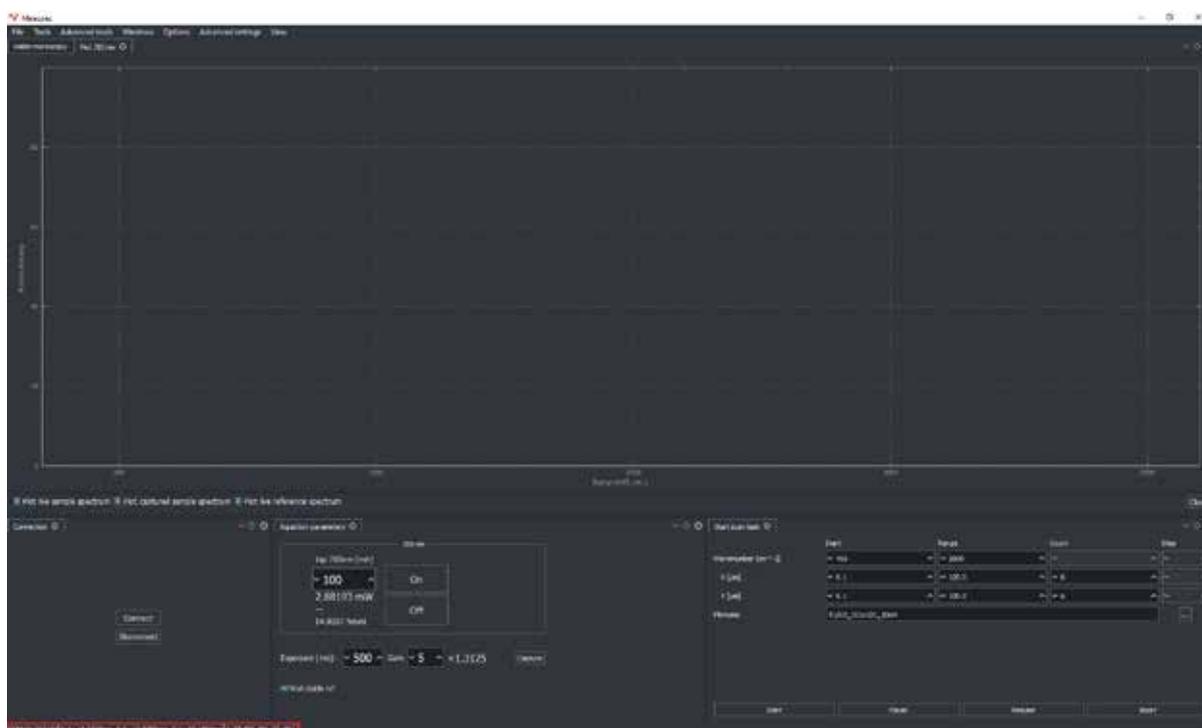


Figure 25. miniRaman microscope software interface; hardware connection status and in-built sensor data.

```
COM16: 202124 | X 4: 13.6207mm, Y 4: 12.5303mm, Z 4: 22.1803mm | t: 25.9°C RH: 35.3% |
```

Figure 26. 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.

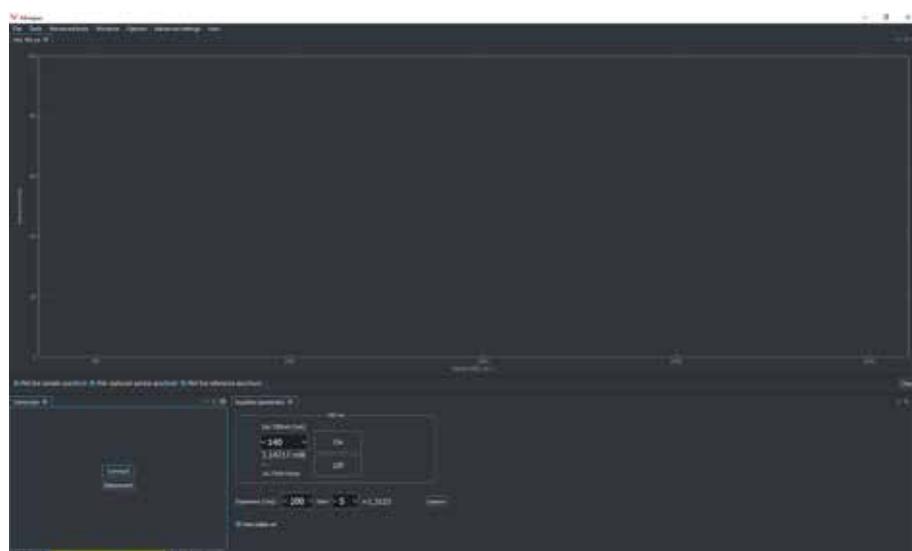


Figure 27. Connection status bar yellow when 3D stage requires calibration.

# Operation

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## 3 Upright and Inverted operation

miniRaman 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 on Figure 28.



Figure 28: a) Upright operation



b) Inverted operation

# Technical drawings

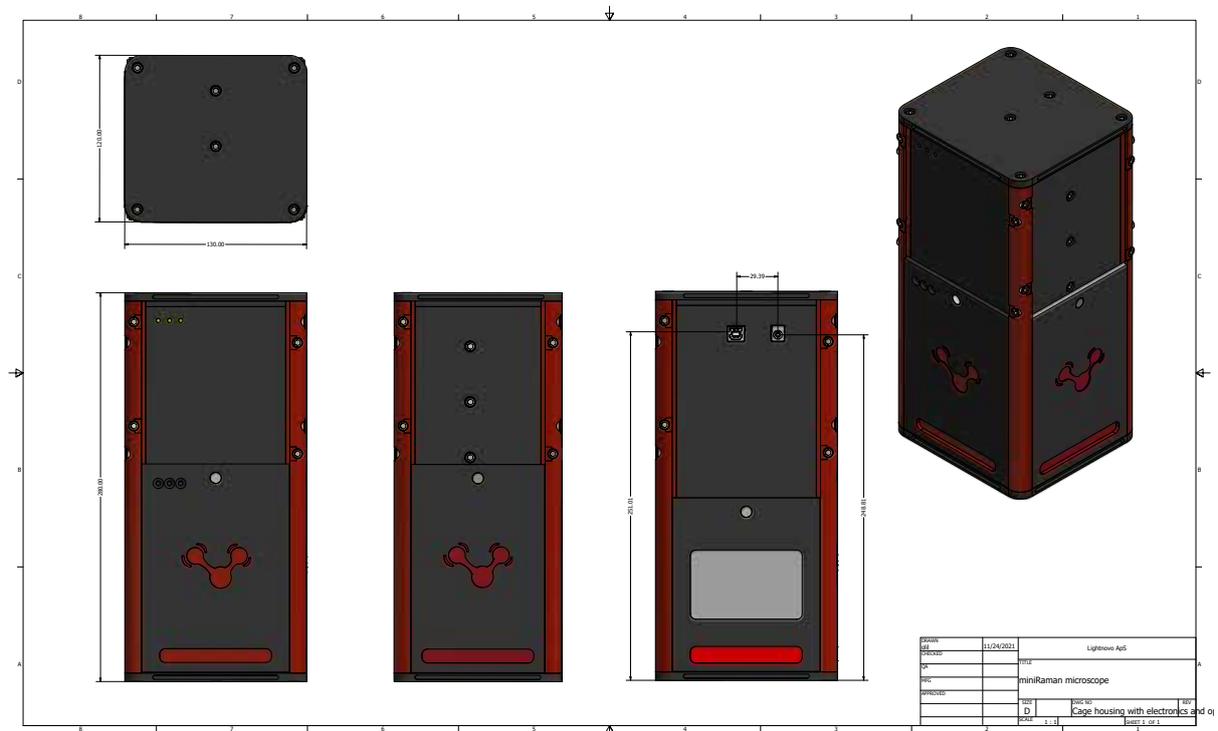


Figure 29. miniRaman microscope drawing with dimensions

# Service addresses

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