miniRaman spectrometer







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Safety

General Information

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

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 miniRaman spectrometer uses the light of a lasers (660 and 785nm). 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 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 spectrometer models: MiniRaman Standard, MiniRaman Standard Dual, MiniRaman Power Dual, MiniRaman 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 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!

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

- miniRaman spectrometer (including user manual and quality test report)
- USB-C 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 spectrometer, 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 spectrometer 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 spectrometer, use the original case to avoid damages.



Figure 1. Transportation case for miniRaman spectrometer

Site requirements

Space Requirements

miniRaman spectrometer requires a space of 12cm in diameter and 4cm in height. (For the exact instrument dimensions refer to Specification.) At the rear instrument side, take a clearance of at least 3cm into account.

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)

Note:

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

Cable Connections

Please unpack miniRaman spectrometer and remove it from the transportation case (see Figure 2).



Figure 2. miniRaman spectrometer with transportation case.

Accessories

One will receive in the package a set of accessories. Depending on the order each accessory can be used for a particular type of measurement and test.

The probes for contact and distance measurements should be oriented by the upper side as shown in the Table 1.

| Accessory | Description | Type of measurement and test | |
|-----------|---|--|--|
| | Long Working Distance Probe, f = 30 mm | Liquids in glass bottles, vials | |
| e | Middle Working Distance Probe, f = 15 mm | Liquids in glass bottles, vials | |
| | Short Working distance Probe, f = 6 mm | Powders, mostly solids | |
| | Contact probe for tissue and scattering media (0-20µm depth of focus) | | |
| | Contact probe for tissue and scattering media (40-60µm depth of focus) | Powders in plastic bags, solids | |
| | Contact probe for tissue and scattering media (80-100µm depth of focus) | | |
| | Calibration tool with polystyrene | Checking laser performance | |
| | Axial focusing accessory | Axial adjustment of a calibration tool with polystyrene | |
| | Light Protection Sample Cover | For liquid measurements in vials without ambient light influence | |
| | Adapter for Olympus objectives (10x; 50x) | Integration with microscopes | |
| 53 | Distance probe extender for Limited access measurements | Measuring liquids in plastic bottles (ethanol, acetone) | |

 Table 1. Accessories for miniRaman spectrometer.

Please connect USB-C cable to the connector on the rear panel (Figure 3) and connect it to PC from the other side.



Figure 3. Rear side - overview of the USB connector socket

Software Installation

- Download miniRaman microscope software package from Lightnovo website: <u>https://lightnovo.com/lightnovo-software/</u> Please select version 32 or 64 bit depending on your operation system.
- Install all drivers from the corresponding software folder.
- Run Miraspec.exe file to start the data acquisition software.
- 3. Run Miraspec.exe file to start the data acquisition sof
- 4. Software is ready for operation.
- 5. License .txt file 'Licence MiniRaman.txt' is needed for correct software operation.

Overview

General information

MiniRaman spectrometer has no moving parts (see section *Instrument design* below in this chapter.)

This instrumental setup allows for acquiring Raman spectra from 785 and 660nm lasers. Raman spectra reveal information about the molecular structure and chemical composition of a sample.

This instrumental setup is designed for industry and 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, identification of powders and liquids. MiniRaman spectrometer 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.

Specification

Lasers

Depending on the selection of the miniRaman spectrometer model device could have 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 10-90 mW)
- MiniRaman microscope SERS (includes one laser with optical isolation: 785 nm, power range on a sample 0.5-15 mW)
- MiniRaman microscope Power Dual (includes two lasers with optical isolation: 785 nm, power range on a sample 10-90 mW; 660 nm, power range on a sample 5-40 mW, 660 nm, power range on a sample 5-75 mW)
- MiniRaman microscope Standard Dual (includes two lasers with optical isolation: 785 nm, power range on a sample 10-90 mW; 660 nm, power range on a sample 5-50 mW)

Spectral range

- 400-2500 cm⁻¹ (at 785 nm laser excitation),
- 2500-4500 cm⁻¹ (at 675 nm excitation)
- 2750-4500 cm⁻¹ (at 660 nm laser excitation)

Spectral resolution

• 10-15 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 800:1
- spectral range 400-2700 cm⁻¹
- laser wavelength: 785 nm
- laser power: 80 mW
- integration time: 0.3 s
- number of repetitions: 10

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

- SNR 600:1
- spectral range 2750-4500 cm⁻¹
- laser wavelength: 660 nm
- laser power: 40 mW
- integration time: 0.3 s
- number of repetitions: 10

Sensitivity in point mode from laser wavelength 675 nm (determined as SNR of polystyrene spectrum)

- SNR 800:1
- spectral range 2500-4500 cm⁻¹
- laser wavelength: 660 nm
- laser power: 80 mW
- integration time: 0.3 s
- number of repetitions: 10

Weight

400g

Dimensions

112 mm x 39 mm x 34 mm

Instrument design

Key system components are shown on Figure 4.



Figure 4. Optical schema of MiniRaman spectrometer. See details for patent # US20210072158 here: <u>https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2019145005</u>.

- 1 laser
- 143 collimation lens
- 141 Wollaston unit
- 145 laser line filter
- 147 focusing lens
- 155 mirror
- 3 slit and Raman filter
- 157 polystyrene (reference sample)
- 109 collimation lens
- 11 Raman probe lens
- 5 sample
- 149, 6 Raman filters
- 103 collimation lens
- 12 grating
- 13 focusing lens for spectroscopic sensor

General overview of control elements and components

MiniRaman spectrometer has multiple 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)

Operation

Hardware connection

MiniRaman spectrometer has two connection options:

- 1. Via USB-C cable for system control from PC
- 2. Via Bluetooth for system control from Android based smartphone or tablet

Connection via USB-C cable

miniRaman spectrometer starts loading process when USB cable connected. It takes between 30sec to 60sec. When bottom is blinking "Blue" device is ready for connection from PC.

Connection via Bluetooth

miniRaman spectrometer starts loading process when USB cable connected. It takes between 30sec to 60sec. When bottom is blinking "Blue" device is ready for connection from Android based smartphone or Tablet.

Operation procedure

Switching the system ON/OFF

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

Warning:

Do not work with miniRaman spectrometer at laser power that is high than specified for particular model. This could lead to the decreased life time of the laser diode or laser damage.

Starting the Miraspec software

1. Connection

Press "Connect" bottom in Connection window, left bottom (see Figure 5). This will initialize hardware accessories. Connection window can be found in File/Connection (Figure 6).

| Miraspec | - | σ× |
|--|---|-----------------------|
| File Spectroscopy Visible microscopy Raman mapping View Help | | |
| B 🖸 🧱 🔺 | | |
| Raman from 660 nm (0) | | $\sim \diamond \odot$ |
| 20 | | |
| 130 130 | | |
| | | |
| 1010 1250 1500 1750 2000 Raman shift, cm-1 | 2250 500 1000 1500 2000 Raman shift, cm-1 | 2500 |
| Plot live sample spectrum Plot captured sample spectrum Plot live reference spectrum | Clear Plot live sample spectrum Plot captured sample spectrum Plot live reference spectrum | Clear |
| Concertion (2) | | ~~~ |
| Connect 600 million (mkl) Wat stable rd* 0ff Begoing (mkl) 100 million Wat stable rd* Smoothing Besoine cont | iop 205 mm (mW) ↓ 49.97 ^ On 1.17316 hours Off wort 1 ^ Capture Tecton Capture and add to Data analyzer | |

Figure 5. miniRaman spectrometer software interface; connection of accessories.

| ₩ М | iraspec | | | | | |
|------|--------------|--------------------|---------------|------|------|--|
| File | Spectroscopy | Visible microscopy | Raman mapping | View | Help | |
| R | Save | | | | | |
| | Connection | | | | | |
| | Exit | | | | | |
| | 100 | | | | | |

Figure 6. Connection window



Figure 7. Connection of accessories takes a certain time.



Figure 8. The status of connection.

Once accessories are connected, one can operate the lasers.

2. Turn ON/OFF lasers

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

| Raman mapping settings Laser parameters 🕄 | |
|---|--------------------------------|
| 660 nm | 785 nm |
| Iop 660 nm [mW] | Iop 785 nm [mW] |
| ~ 8,95 ~ _{On} | ⊻ 49,97 ^ On |
| | 215.918 mA |
| 0.824153 hours Off | 1.17316 hours Off |
| Exposure [ms]: v 100 ^ Gain v 10 ^ × 1 | 1.625 Rep. count v 1 ^ Capture |
| Wait stable ref Smoothing | Baseline correction |
| | |
| | |
| | |
| | |

Figure 9. Switching on/off laser

3. Measuring the Raman spectrum of the sample in real time. Raman spectra can be visualized via Spectroscopy/Plots, see below.

| 💎 Mii | raspec | : | | | | | | |
|-------|---------|-----------------|--------------------|-----|-----------------|-----------------|---------------|------|
| File | Spec | troscopy | Visible microscopy | | | ору | Raman mapping | View |
| | | Aquisitio | nParamet | ek | 1 | | | |
| | Plots > | | | Ran | nan from 660 nm | | | |
| Rama | | Preprocessing > | | | Ran | nan from 785 nm | | |
| | | Data ana | lysis | | | Way | velength | |

Figure 10. Visualization of Raman spectra obtained via excitation at 785 nm.



Figure 11. Different live spectra. Red curve corresponds to live sample spectrum, blue spectrum is polysterene reference.



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 12. Fit spectra to window for better spectra visualization.

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 requires high resolution of Raman spectra.

| Raman mapping settings Laser parameters 🕲 | |
|---|----------------------------|
| 660 nm | 785 nm |
| Iop 660 nm [mW] | Iop 785 nm [mW] |
| ~ 8,95 ^ On | ∽ 50,88 ^ On |
| 0.864906 hours | 1.34366 hours Off |
| Exposure [ms]: V 500 A Gain V 10 A × | 1.625 Rep. count 1 Capture |

Figure 13. miniRaman spectrometer software interface; laser settings.

| Ұ м | iraspec | | | | |
|------|----------------------|-------------------|----------------|---------------------|--|
| File | Spectroscopy Visible | microscopy | Raman mapping | View Help | |
| | AquisitionParame | te x Advar | cedSettings > | Binning line editor | |
| | Plots | > 🔳 🛛 Las | er parameters | RefData line limits | |
| Wave | Preprocessing | > 🔳 🛛 Way | venumber range | Laser time limits | |
| | | | | _ | |

Figure 14. miniRaman spectroscopy settings; advanced settings menu.

| Wavenumber rang | je | | | \times |
|-----------------|--------|---------------|---------------|----------|
| 6 | i60 nm | | – 785 nm –––– | |
| First [cm^-1] | | First [cm^-1] | | |
| ~ 2600 | ^ | ~ 400 | | ^ |
| Last [cm^-1] | | Last [cm^-1] | | |
| ~ 4000 | ^ | ~ 2600 | | ^ |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

Figure 15. Wavenumber range settings.



Figure 16. Stability threshold settings.



Figure 17. Illustration of spectrum capture which is done by averaging of fixed number of recorded spectra per time. Grey curve illustrates spectrum capture. Bottom menu allows to enable/disable showing necessary spectra.

4. Saving and/or exporting the data

Export spectrum in tsv format: File/save (see Figure below).

| 💎 м | iraspec | | | | |
|------|--------------|--------------------|---------------|------|------|
| File | Spectroscopy | Visible microscopy | Raman mapping | View | Help |
| B | Save | H M | | | |
| | Connection | | | | |
| | Exit | | | | |
| | 100 | | | | |

Figure 18. Saving captured spectrum

5. Spectra preprocessing

Once the spectra is saved, one has many options for data preprocessing. It is available via Spectroscopy/Preprocessing.

| 💎 Mira: | spec | | | | | |
|---------|---------------------|---------------------------|--------|---------------------|------|------|
| File S | pectroscopy | Visible micro | oscopy | Raman mapping | View | Help |
| | Aquisition Plots | nParamete) s > | A | | | |
| Rama | Preproce | ssing > | _ Smo | othing | | 1 |
| A | Data anal | lysis 🗌 | Base | line correction | | |
| | | | Bacl | ground correction | | |
| | | | _ Tran | smittance correctio | n | |
| | | | | | | |

Figure 19. Different options for spectra preprocessing.

Let's start with spectra smoothing and background correction.

| Smoothing & ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ | Smoothing O V Savitzky-Golay filter |
|---|-------------------------------------|
| G Smoothing | Smoothing Method |
| ○ Savitzky-Golay ◎ AsLS | © Savitzky-Golay O AsLS |

Figure 20. Smoothing methods. Left panel – AsLS algorithm, Right panel – Savitzky-Golay method.

| Background correction 🕲 | Background correction 3 |
|--|-----------------------------|
| Lambda v 10 v Tolerance v 0.0010 v Max iterations v 21 v | Rolling circle filter |
| Enable Method | Enable Method |
| ○ Rolling circle | ◎ Rolling circle ○ arPLS |

Figure 21. Background correction methods. Left panel – arPLS algorithm, right panel – Rolling circle.

| Background correction 🛛 | Laser parameters | | | \lor \diamond \otimes |
|-------------------------|------------------|--------|-------|-----------------------------|
| | | 660 nm | | |
| | | | | |
| | | 785 nm | | |
| Enable correction | | | Reset | Capture reference |
| | | | | |
| | | | | |
| | | | | |

Figure 22. Activation of background correction for different Raman spectra.

Data analysis is available via the tab Spectroscopy where one can open the saved spectra in.tsv format and perform data treatment.

| 🜱 Mi | raspec | | | | | | | |
|---------|---------------------|--------------------|-------------------|--------|-------|---|--|---|
| File | Spectroscopy | Visible microscopy | Raman mapping | View | Help | | | |
| | Aquisitior Plots | nParameteis | | | | | | |
| Rama | Preproces | ising > | | | | | | |
| | Data analy | ysis 📃 Da | ta analysis | | | | | × |
| | | File | Preprocessing Ana | alysis | | | | |
| | | S | how Nam | e | Color | | | |
| | | | | | | | | |
| | 80 | | | | | | | |
| | | | | | | 4 | | |
| | | | | | | | | |
| | 60 | | | | | | | |
| tensitγ | | | | | | 3 | | |
| man inl | | | | | | | | |
| Ra | 40 | | | | | | | |
| | | | | | | 2 | | |
| | | | | | | | | |

Figure 23. Data analysis tab.

| 😵 Miraspec | |
|--------------------------------------|---|
| File Spectroscopy Visible microscopy | Raman mapping View Help |
| 🕂 🖸 🏢 🗰 🎤 | Data analysis - C X |
| Raman from 660 nm 🙁 | File Preprocessing Analysis Open Name Color |
| | |
| 80 - | |

Figure 24. Opening the spectra.

| 🖌 Miraspec | | | |
|--------------------------------------|---|---|---|
| File Spectroscopy Visible microscopy | Raman mapping View Help | | |
| 2 🗉 🏢 📠 🖉 | Data analysis | — | × |
| Raman from 660 nm 🔇 | File Preprocessing Analysis SmoothingBackgroundCorrection | | |
| 100 | Normalization | | |

Figure 25. Smoothing background correction and normalization features of data analysis.

| * 1 | Viraspec | | | | |
|------------|------------------|--------------------|---------------|------|--------|
| File | Spectroscopy | Visible microscopy | Raman mapping | View | Help |
| | 3 🖸 📕 | | | | |
| Dat | a analysis 😳 🛛 R | aman from 660 nm | | | |
| File | Preprocessing | Analysis | | | |
| | Show | Name | Color | | |
| 1 | | 12Data - | | | |
| 2 | | 12Ref | | 75 | m, , |
| | | | | | |
| | | | | | |
| | | | | 5 | |
| | | | | | |
| | | | | | |
| | | | | 2 = | |
| | | | | | |
| | | | | | |
| | | | | | multil |

Figure 26. Raw spectra (red) and reference spectrum (green). "Show" tab enables to visualize desired spectra.



Figure 27. Data analysis of complex sample is possible via different methods.



Data analysis / Analysis / Calibration

Figure 28. Possibilities for calibration data analysis.

6. GUI settings

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



Figure 29. Docking widgets

Each user interface can be saved, see below.

| 😵 Miraspec | | | | | | | | |
|-------------------------------|-----------------|--------------------|---------------|------|-----------------|-----|--|--|
| File | Spectroscopy | Visible microscopy | Raman mapping | View | Help | | | |
| | | | | | Add perspectiv | e | | |
| | | | | | Perspectives | > | | |
| Rama | n from 660 nm 🛽 | | | | Themes | > | | |
| | F | • <u>i</u> • • • | | | Clear perspecti | ves | | |
| | | | | >_ | Show Console | | | |
| Figure 30. Adding perspective | | | | | | | | |



Figure 31. Saving perspective

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

| 💙 Miraspec | | |
|--------------------------------------|---------------|------------------------|
| File Spectroscopy Visible microscopy | Raman mapping | View Help |
| | | Add perspective |
| | | Perspectives > default |
| Raman from 660 nm 🛞 | | Themes > |
| | . <u>.</u> | Clear perspectives |
| | | 🖂 Show Console |
| | | |

Figure 32. Saved perspective list

Technical drawings



Figure 33. miniRaman spectrometer drawing with dimensions

Lightnovo ApS

miniRaman spectrometer

User manual

Service addresses

Lightnovo ApS

Blokken 15, 1. Tv. 3460 Birkerod Denmark

Phone: +45 71370410 https://lightnovo.com/ service@lightnovo.com