Basic guide for the use of analytical spectroscopy techniques applied to emerald crystals

Introduction

The Institute for Geosciences at the Johannes Gutenberg University Mainz has a sophisticated research infrastructure and laboratories with analytical spectroscopy techniques. These instruments are mainly used by the faculty and by master's and doctoral students. The principle of spectroscopy is the interaction between electromagnetic radiation and matter (González Cantellano and Montaño Zetina, 2015). However, depending on the type of interaction and analysis required, different spectroscopic techniques are used which, although they follow the same principle, require the understanding of specific concepts and methodologies for their application. Therefore, in order to improve and facilitate the understanding of these analytical techniques to inexperienced students or beginners, this basic guide will illustrate and explain in a simple way the fundamental principles, uses and possible obstacles when using the instruments, will be applied in the field of geology for the identification of minerals, in this case, emeralds.

Objectives

General objective: To develop a basic guide on Micro Raman confocal spectroscopy and ultraviolet-visible spectroscopy by collecting information and empirical tests to facilitate the understanding and handling of the instruments for beginners.

Specific objectives:

1. To compile information on the theoretical foundations of spectroscopic techniques and their main uses.
2. To illustrate the methodology necessary to make measurements on beryl crystals using the above-mentioned techniques.
3. To mention the most common errors when performing mineral measurements in each spectroscopic technique.
4. To synthesize common results in the analysis of emeralds with the mentioned techniques and their possible interpretation.

Theoretical framework

What is spectroscopy?

Spectroscopy is an analytical technique based on the detection of the absorption or emission of electromagnetic radiation by a substance. It consists of 3 basic elements: 1) A light source, 2) The sample to be analyzed and 3) A detector.

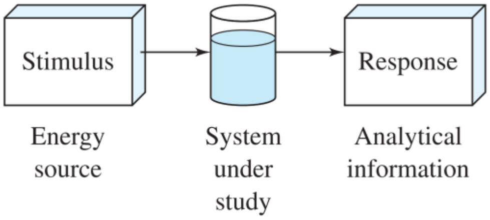


Figure 1. "Block diagram showing the overall process of an instrumental measurement" (Skoog et al., 2007).

To obtain the desired information from an analytical instrument, it is necessary to have a stimulus, in this case, a source of electromagnetic light that irradiates the sample and interacts with the substances that form it. The components of the sample modify or absorb a part of the light, so the detector receives the resulting light after passing through the sample.

The differences between the light that is directed at the sample and the resulting light make up the electromagnetic spectrum, in which the types of radiation are differentiated by their wavelength. The emission spectrum represents the light emitted by the sample when excited with energy. The absorption spectrum represents the light resulting from the absorption of certain wavelengths by the sample.

When comparing both spectra of the same element we will find that the radiations emitted in the emission spectrum are the same as those missing in its absorption spectrum. For this reason, the spectrum becomes a "fingerprint" of each element we analyze in the sample.

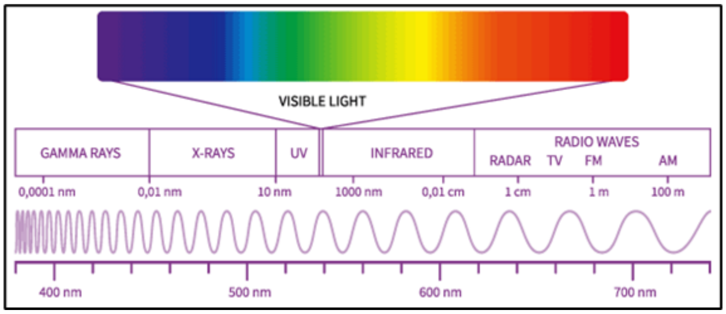


Figure 2. The electromagnetic Spectrum.

What is confocal Micro Raman spectroscopy?

Light scattering occurs when some photons collide with molecules and bounce off in all directions; light with short wavelengths is scattered more frequently than light with long wavelengths. This type of scattering is known as "Rayleigh scattering" or elastic scattering since the energy of the photons remains the same after scattering.

On the other hand, the wavelength of the scattered light, with a probability of 1 in 100 million scattered photons, can change, as can its energy. This type of scattering is known as "Raman scattering" or inelastic scattering since the scattered photon loses energy in the molecule and changes its wavelength. This energy change is known as a "Raman shift" and can be considered a fingerprint of matter.

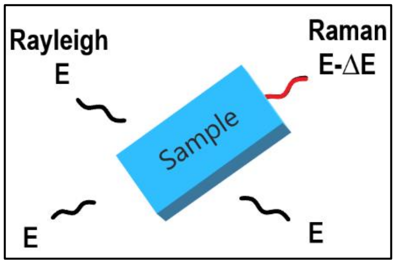


Figure 3. Raman Scattering.

Where does the energy go during Raman scattering?

In the Raman effect, the scattered photons undergo a change in their energy, as it is transferred in vibrational form to the molecules. This vibrational energy can only have separate states, i.e. there are no intermediate steps. When a photon collides with a molecule and transmits energy to it, the molecule will pass from a fundamental or base state to a higher vibrational state or, if it is already in a higher vibrational state, it can pass to an even higher state. In turn, the photon will scatter with lower energy and longer wavelength (Fig. 4). This process is known as Stokes scattering.

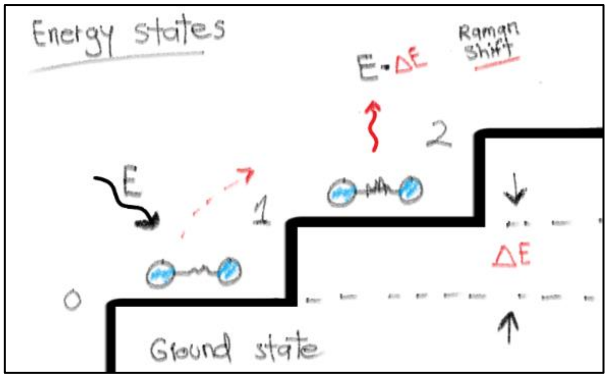


Figure 4. Vibrational energy states and Raman scattering.

Conversely, if the molecule loses energy by falling to a lower vibrational level than it had, the photon colliding with the molecule gains energy and scatters with a shorter wavelength. This process is known as anti-Stokes scattering. However, Stokes scattering is more common and, having higher intensity, is the one measured in Raman instruments. (Smith and Dent, 2005)

The energy difference between the ground state and higher states depends on the natural frequency of vibration. When our molecule has heavy atoms, the system vibrates at lower frequencies, whereas, if the atoms are lighter, the system will vibrate at higher frequencies. In the end, we will obtain a specific pattern for each molecule and, therefore, we will be able to use it as a "fingerprint of the molecule".

What are the main components of a Micro Raman instrument and how do they work?

A Raman instrument is mainly composed of 1) a laser, 2) an optical filter, 3) a spectrograph and 4) a detector (Fig. 5).

The sample to be analyzed is placed on the stage of the confocal Raman microscope, the laser light is reflected by the beam splitter and focused by the objective lenses on a specific point of the sample. Since Rayleigh and Raman scattering occurs, the photons pass through an optical filter to discard the Rayleigh scattering. The remaining Raman scattering is focused through a confocal hole and passes through a diffraction grating to the spectrograph, where the photons are separated by wavelength, i.e., they fall at different positions when they reach the CCD detector.

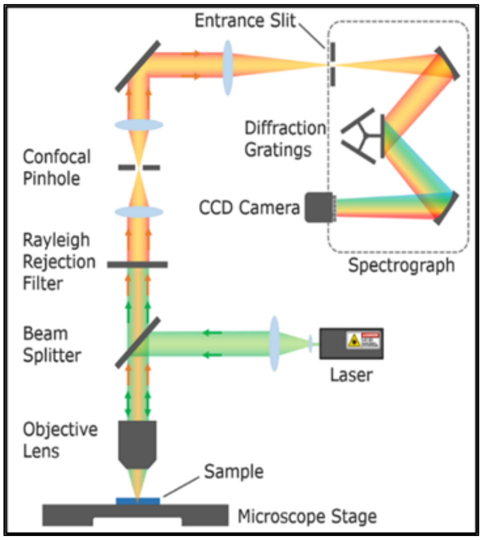


Figure 5. Components of a confocal Raman microscope.

What are the advantages of confocal Micro Raman spectroscopy and what applications does it have in geology?

Raman microscopy offers the possibility to chemically examine small objects with high spatial resolution (<1 µm) without destroying them during analysis. This tool provides information on chemical identification and characterization of molecular structures of geological materials, as well as to analyze fluid inclusions even below the sample surface. Samples do not require prior preparation and in-situ measurements are possible. Being a non-destructive method, it is ideal in multiple disciplines of geology such as: Gemology, mineralogy, paleontology, among others. (HORIBA, 2019).

What is ultraviolet-visible spectroscopy or spectrophotometry?

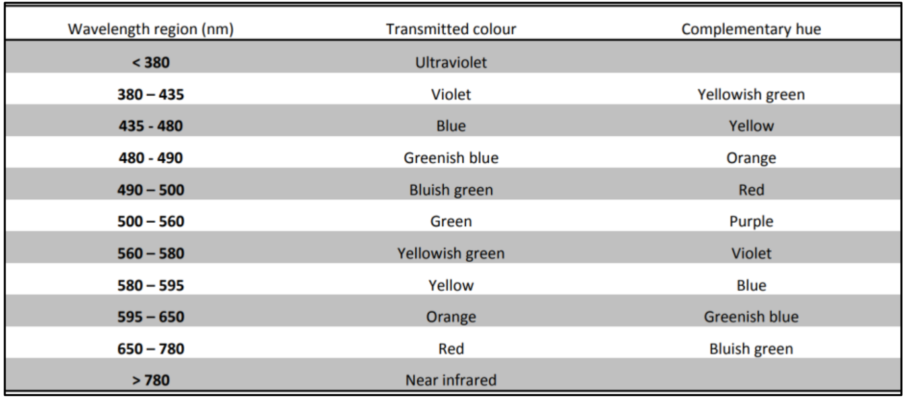
Spectra are produced when electrons in a molecule or atom move from a lower to a higher electronic energy level. This occurs when matter absorbs incident radiation, which eventually excites the electrons to reach higher energy levels. (Picollo et al., 2020).

The absorbed energy is equal to the separation between the two energy levels. UV-Vis spectroscopy measures the amount of ultraviolet (100-400 nm) or visible (400-700 nm) wavelength light that is absorbed or transmitted through a sample.

When light passes through the sample, the absorbed radiation is the difference between the incident (Io) and transmitted (I) radiation. The amount of absorbed radiation is called absorbance (A) and transmittance (T), which is the ratio (I/ Io) indicating the amount of light that has passed through the sample.

The Beer-Lambert law states that the absorbance of a sample is proportional to the concentration of the absorbing species and the distance light travels through the sample. This law can be expressed mathematically as $A = \varepsilon bc$, where A is the absorbance, ε is the molar absorption constant, b is the optical path length and c is the concentration of the absorbing species (Picollo et al., 2020). Absorption occurs at different wavelengths depending on the composition of the sample. Components that absorb visible light will be colored according to the wavelengths that the material allows to transmit, while those that absorb ultraviolet light are colorless (Royal Society of Chemistry, 2008).

Table 1. Relationship between light absorption and transmitted color (van Meerbeeck, 2010).



Wavelength region (nm)	Transmitted colour	Complementary hue
< 380	Ultraviolet	
380 – 435	Violet	Yellowish green
435 - 480	Blue	Yellow
480 - 490	Greenish blue	Orange
490 – 500	Bluish green	Red
500 – 560	Green	Purple
560 – 580	Yellowish green	Violet
580 – 595	Yellow	Blue
595 – 650	Orange	Greenish blue
650 – 780	Red	Bluish green
> 780	Near infrared	

What are the main components of a UV-Vis spectrograph and how do they work?

UV-Vis spectrographs attached to a microscope focus light on a specific point on the sample and collect the light that is scattered or emitted by the sample. In this case, the sample is illuminated by transmission, where the light is focused on the sample by the condenser and the part collected by the microscope objective has been transmitted through the sample (CRAIC Technologies, 2022).

The microscope objective lenses collect the light from the sample and focus it onto the diaphragm of the spectrophotometer. The digital imaging system presents an image of what the spectrophotometer diaphragm measures (CRAIC Technologies, 2022).

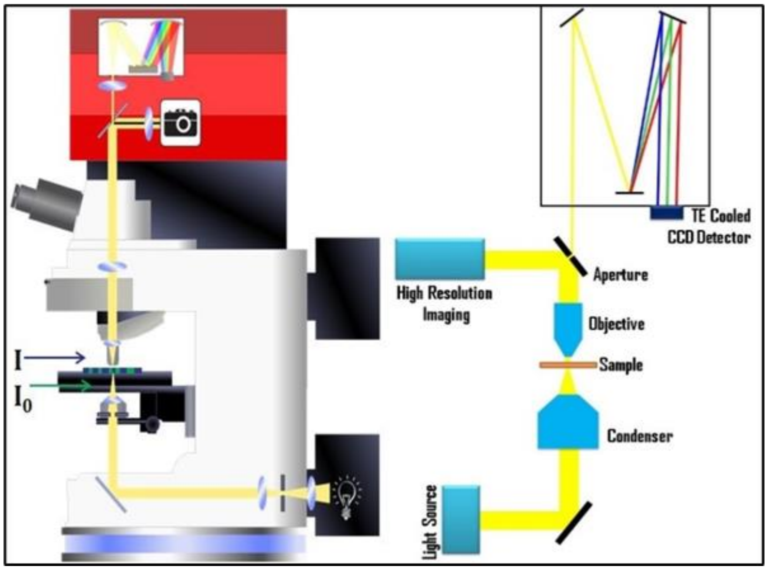


Figure 6. General schematic of a transmission microspectrophotometer (CRAIC Technologies, 2022).

The spectrograph consists of a light source emitting in the UV-Vis wavelength range (200-800 nm). In this case a mercury vapor lamp is used for the UV-Vis range and a xenon lamp covering the NIR.

A monochromator is also needed, consisting of an inlet, a diffraction grating or prism and an outlet. Its purpose is to scatter the light and let the desired wavelength pass to the output. The detector measures the intensity of the light that has passed through the sample. The detector signal is converted into an electrical signal which is processed and displayed on the screen (Tom, 2021).

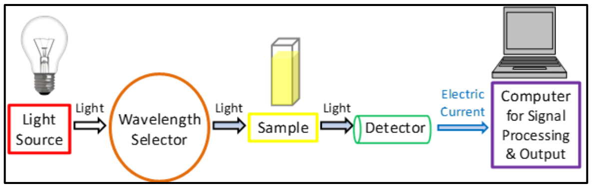


Figure 7. Simplified schematic of the main components of a UV-Vis spectrophotometer (Tom, 2021).

What are the advantages of UV-Vis spectroscopy and what applications does it have in geology?

UV-Vis spectroscopy offers advantages such as its high sensitivity, since it can detect very small amounts of compounds in a sample, as well as being a non-destructive, fast, and easy to use technique. In geology it can be used in mineral identification, soil analysis and the investigation of contamination of water bodies (Tom, 2021).

What is a beryl and what are its characteristics?

Beryl $Be_3Al_2(Si_6O_{18})$ is a hexagonal cyclosilicate, formed by hexagonal rings of $(Si_4)^{-4}$ tetrahedra connected by Be ions in tetrahedral position and Al ions in octahedral position. The rings are aligned to form channels parallel to the crystallographic axis c of the crystal, where Na, K, Cs or OH, H_2O, CO_2, CH_4 ions can be accommodated.

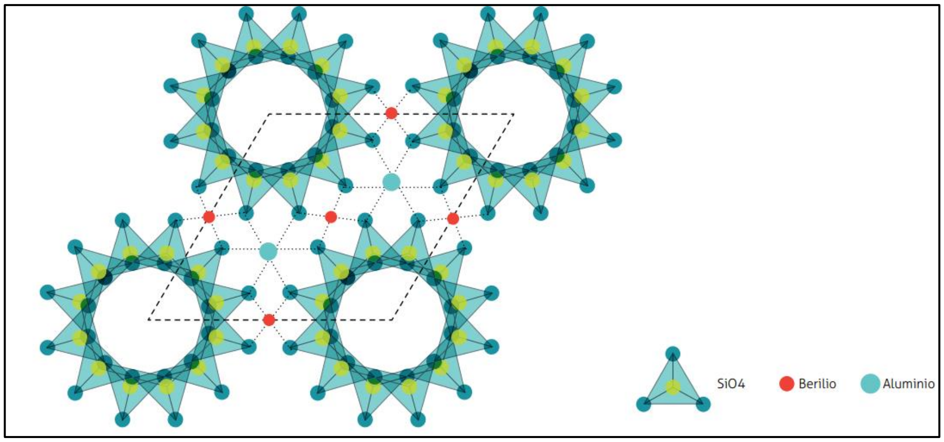


Figure 8. Crystal structure of beryl (Montoya and Moreno, 2019).

According to Hurlbut and Kammerling (1991) emerald is defined as "yellowish green, green or bluish green beryl revealing distinct Cr and/or V absorption bands in the red and blue-violet ranges of their absorption spectra". The study of the water molecules in the structural channels of the crystal has made it possible to classify them into H_2O *type I* and *type II.*

Type I water molecules are characterized by being solitary within the channels and with the H-H vector parallel to the crystallographic axis c of the emeraldine crystal and are associated with low alkali contents. In contrast, *type II* water molecules are associated with nearby alkali ions, so the H-H vectors are perpendicular to the crystallographic axis c of the crystal (Kolesov, 2008).

Raman spectroscopy makes it possible to see and differentiate the types of water present in the molecular structure of the emerald, since, in the spectrum, Raman bands below $3600\ \mathrm{cm}^{-1}$ are attributed to H_2O *type I* and Raman bands above $3600\ \mathbf{cm}^{-1}$ are attributed to H_2O t*ype II*. Hydrothermal synthetic emeralds show only the band above $3600\ \mathrm{cm}^{-1}$ and flow-grown emeralds show no band (Le Huong et al., 2010).

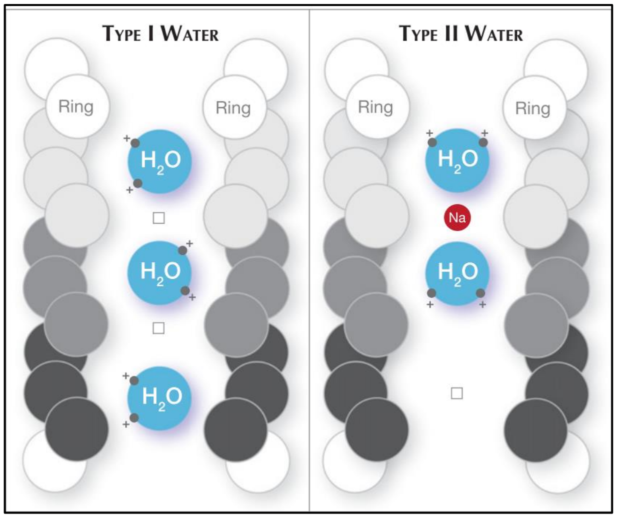


Figure 9. Water molecules of type I and type II crystalline channels according to their orientation with respect to the beryl structure (Le Huong et al., 2010).

Methodology for Micro-Raman measurements applied to beryl crystals.

To perform measurements on the Micro-Raman instrument, a laser with the appropriate wavelength must be chosen as the excitation source. There is no one wavelength that fits all situations. The laser will influence the spectral resolution, the signal intensity and mainly the fluorescence. The most used wavelength is 785 nm, as it offers low fluorescence while retaining a good Raman signal.

A laser with wavelengths below 300 nm will offer high Raman scattering intensity and low fluorescence. However, due to the high energies of the short wavelengths, holes may be generated or burn/degrade the sample. Lasers with wavelengths of 532 nm are good choices for analyzing metal oxides and inorganic material. However, in the study of some minerals it can trigger fluorescence that interferes with the measurements.

In the case of beryl, with a 532 nm laser, the results present a very high noise rate that masks the measurements. Therefore, an ideal laser for measuring beryl may be the 488 nm laser. In this way, an optimal signal-to-noise ratio is obtained.

1. **Sample preparation:** Although no prior preparation is necessary for Raman analysis, grinding and polishing the samples allows to obtain better results because it increases their transparency and the passage of light will be easier. In addition, if the crystal has an irregular or rough surface, its texture can influence the optical path of the light. Therefore, preparing the sample allows to obtain a uniform and clean surface, which improves the quality of the Raman signal.

Figure 10. Ground and polished beryl.

2. **Laser selection:** Choose the appropriate laser for the measurement by inserting it into the slot at the top of the instrument.

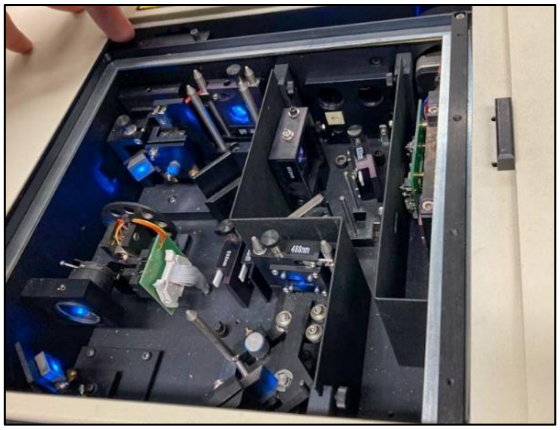


Figure 11. 488 nm laser in the top slot of the spectrograph.

3. **System calibration:** To calibrate the system, a standard silicon sample is used, which has Raman bands typically in the 520.7 cm^{-1}.

Figure 12. Calibration standard silicon.

Next, in the LabSpec6 software, the "AC" (autocalibration) option is selected, the diffraction grating presents 2 options: A 300 lines/mm diffraction grating and an 1800 lines/mm diffraction grating, the latter being the better option for small areas as it offers higher resolution. When measuring a larger area with an 1800 lines/mm diffraction grating the process will take longer. For this reason, for measuring larger areas, the 300 lines/mm diffraction grating is recommended.

The diffraction grating is placed in a position where all white light is reflected in the center of the CCD. If this is not the case, it is adjusted a little and then the diffraction grating is moved to the position where the Raman band of the standard silicon reaches the center of the CCD. On the screen the Si band will be observed, and all wavelengths will be reflected and not scattered (0.00).

Figure 13. Details of the calibration process. Green detector, AC (auto-calibration), 488 nm laser and a x50 microscope objective.

4. Parameters: Before starting the measurements on the sample, it is important to adjust some parameters in the system, the most important are:
    - <u>The measurement range:</u> It will depend on the sample, so it should be previously consulted in the bibliography in which range or approximate wave number the characteristic Raman bands appear for the material to be analyzed. In the case of emeralds, the OH range and the molecular vibrations of water usually appear in the bands (3700-3500 cm^{-1}). To identify the mineral beryl, a range spanning the wavenumbers (100-3700 cm^{-1}) is used.
    - <u>Spectrum accumulation:</u> Represents the number of times the Raman spectrum is acquired at the same point on the sample. It measures a point several times and averages the results to improve the quality and accuracy of the data. In the case of beryl, 2 accumulations will be sufficient.
    - <u>Acquisition time:</u> This is the time taken by the instrument to collect data at each point on the sample and should be long enough to obtain a good signal-to-noise ratio in the Raman spectrum, but also short enough not to damage the sample. In the case of beryl, a time of 120 s will be sufficient.

  - Microscope objective: This refers to the magnification used to obtain the Raman spectrum. The higher the magnification, the smaller the size of the analysis area. Lower magnification objectives (such as 5x or 10x) may be better for large or heterogeneous samples, while higher magnification objectives (such as 50x or 100x) are better for small samples or specific regions of interest. In the case of beryl, a 50x objective will be adequate.

5. **Mounting of the sample:** The orientation of the crystal is important; therefore, it should be evaluated if the crystal is anisotropic or isotropic. If it is isotropic the orientation will not be an important factor since its physical properties are the same in all directions. On the other hand, if the crystal is anisotropic, as in the case of beryl, the properties will vary depending on its orientation. In the case of emeralds, measurements will be made in the direction of the electric vector E parallel and perpendicular to the crystallographic axis c.

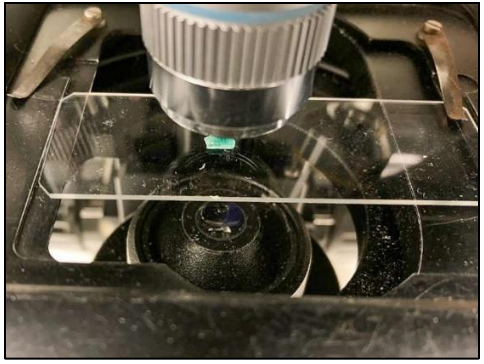


Figure 14. Emerald crystal with E perpendicular to the c-axis.

6. **Measurement:** After mounting the sample, it is important to focus to a point free of inclusions, usually a clean and transparent area of the glass for measurements. As an additional measure, focus slightly towards the inside of the sample to avoid surface effects. Then remove the polarizer, turn on the laser, cover the sample surroundings and turn off the lights. It is necessary to get rid of any ambient light, as it can affect the quality of the Raman signal. In the LabSpec6 software the circular option "Start acquisition" is chosen, then the generation of the Raman spectrum will start.

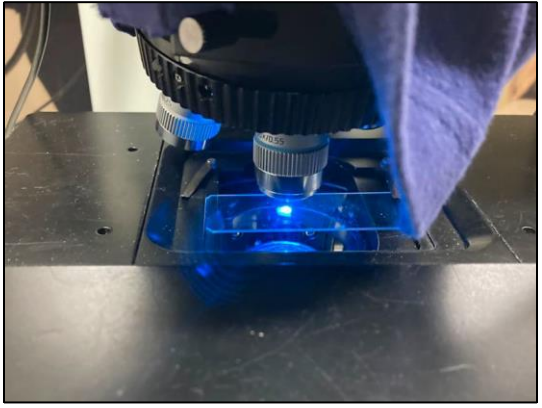


Figure 15. 488 nm laser on emerald, surrounding a "curtain" to avoid ambient light.

If there are doubts about the spectrum being visualized, it is possible to rely on the software library, in this way it will be known to which mineral the Raman bands that have been generated correspond. It can also be used to compare the results obtained with typical spectra of the analyzed material.

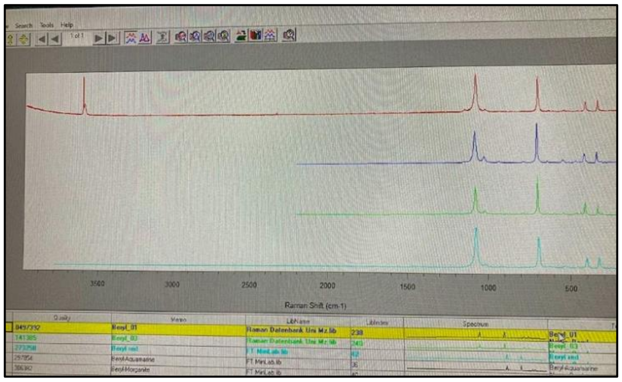
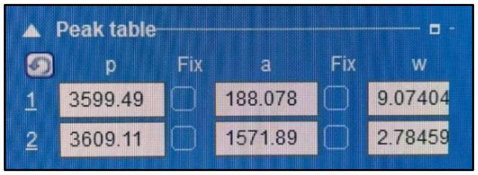


Figure 16. Raman library, typical spectra for beryl.

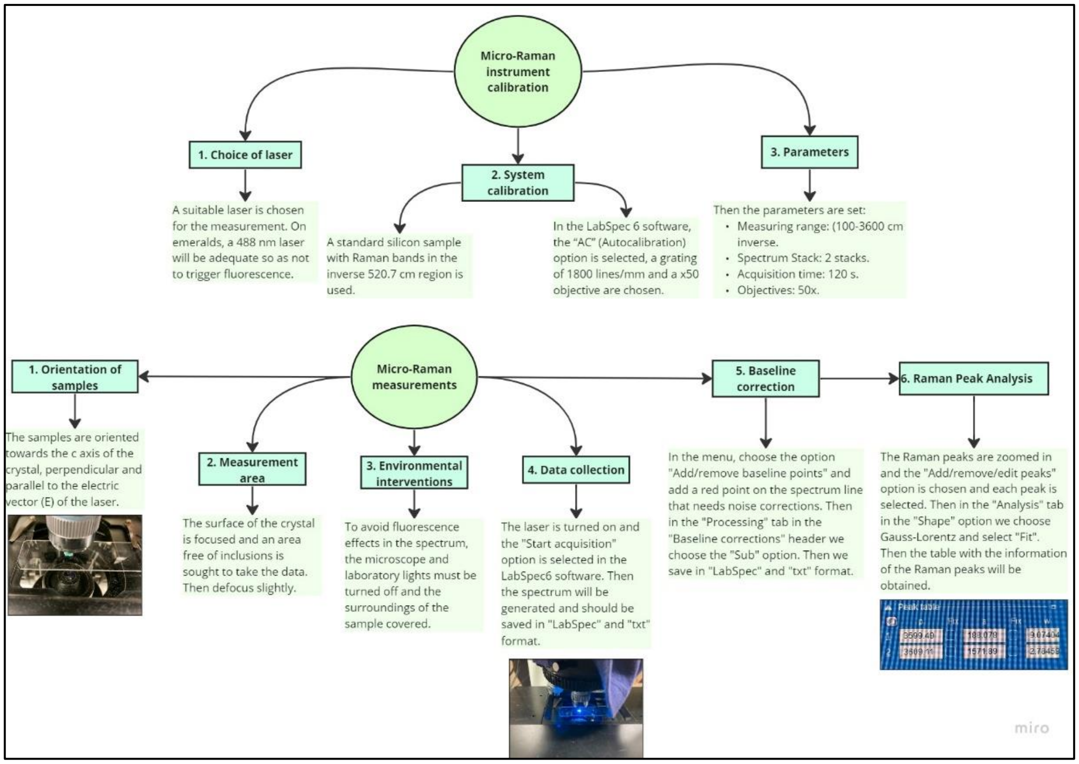
7. Baseline correction: Once the Raman spectrum is generated, the data is saved in the "Save as data" tab in LabSpec6 and in text (txt) format. A manual correction must then be performed. In the vertical menu, select the "Add/remove baseline points" option and click to add red points at each location in the spectrum that needs a noise correction. Then, in the "Processing" tab and under the heading "Baseline corrections", select the "Sub" option. In this way the background signal is removed and the Raman peaks corresponding to the molecular vibrations of the sample can be displayed more clearly and accurately. This correction should also be saved in both formats (LabSpec and txt).

8. Analysis of the Raman peaks: To visualize the information of the Raman peaks obtained, in this case, corresponding to water types I and II, in the vertical menu select the "Zoom" tab to have a better view of the peaks and then select the "Add/remove/edit peaks" tab and select each of the peaks. Next, in the "Analysis" tab and in the "Shape" option choose Gauss-Lorentz and the "Fit" option repeatedly until the peaks show no numerical changes. This mathematical function is used to adjust the shape of the peaks, improve the accuracy and resolution of the measurements. At the end you get a table with information of the peaks about their wave number (Peak position), area (a) and width (w).



	p	Fix	a	Fix	w
1	3599.49	☐	188.078	☐	9.07404
2	3609.11	☐	1571.89	☐	2.78459

Figure 17. Table of information on the peaks obtained from water types I and II with E perpendicular to the c-axis of an emerald.



A suitable laser is chosen for the measurement. On emeralds, a 488 nm laser will be adequate so as not to trigger fluorescence.

A standard silicon sample with Raman bands in the inverse 520.7 cm region is used.

In the LabSpec 6 software, the "AC" (Autocalibration) option is selected, a grating of 1800 lines/mm and a x50 objective are chosen.

Then the parameters are set:
- Measuring range: (100-3600 cm inverse.
- Spectrum Stack: 2 stacks.
- Acquisition time: 120 s.
- Objectives: 50x.

The samples are oriented towards the c axis of the crystal, perpendicular and parallel to the electric vector (E) of the laser.

The surface of the crystal is focused and an area free of inclusions is sought to take the data. Then defocus slightly.

To avoid fluorescence effects in the spectrum, the microscope and laboratory lights must be turned off and the surroundings of the sample covered.

The laser is turned on and the "Start acquisition" option is selected in the LabSpec6 software. Then the spectrum will be generated and should be saved in "LabSpec" and "txt" format.

In the menu, choose the option "Add/remove baseline points" and add a red point on the spectrum line that needs noise corrections. Then in the "Processing" tab in the "Baseline corrections" header we choose the "Sub" option. Then we save in "LabSpec" and "txt" format.

The Raman peaks are zoomed in and the "Add/remove/edit peaks" option is chosen and each peak is selected. Then in the "Analysis" tab in the "Shape" option we choose Gauss-Lorentz and select "Fit". Then the table with the information of the Raman peaks will be obtained.

Figure 18. Methodological map of Micro-Raman measurements.

Methodology for UV-Vis-NIR measurements applied to beryl crystals.

1. **Sample preparation:** If the sample to be analyzed were liquid, it would be necessary to place it in a quartz cuvette to analyze the ultraviolet light and in a plastic or transparent glass cuvette to analyze the visible region. However, in the case of crystals, a grinding and polishing process in which the sample has a transparency that easily allows the passage of light will suffice.
2. **Preparation of the instrument:** Start by turning on the spectrographs and the microscope, adjusting its illumination to a medium point, since an excess of illumination can cause interferences in the measurements. To view the microscope image on the computer, access the J&M TidasVISION software. The following settings are selected: View: "Always on top". There you will see an image of what is in focus on the microscope and the position of the condenser.

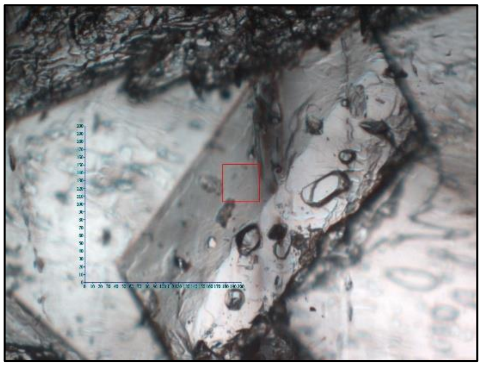


Figure 19. TidasVISION software, showing the surface of an emerald focused from the microscope.

3. **Calibration:** In the TIDASDAQ software (UV-NIR), parameters will be chosen to perform a non-sampling measurement to measure the luminous intensity. The duration of the measurements will be 240 ms for the visible range and 260 ms for the NIR.
   To perform the measurement, focus the surface of the sample holder on the microscope, then in the software choose the "Shutter" option, which will allow observing the microscope's condenser, which will appear as a bright square that can be focused with the condenser adjustment knobs (they have a diagonal movement). Once centered, choose "Scan type: Reference" in the software and make a measurement. Make sure that both intensities (UV-Vis and NIR) are above 80%. Next, under "N" enter the number of measurements to perform or average per spectrum, in general 20 measurements will be a suitable parameter and click on the symbol (✓).
   Additionally, a measurement is performed by choosing the "Dark Sweep" option, which consists of an unsampled measurement that evaluates the noise or background signal present in the system to improve the accuracy and precision of the measurements.
   Finally, a "Reference Scan" is performed without sampling that evaluates the luminous intensity and ensures that subsequent measurements are accurate and precise.

4. **Mounting of the sample:** The orientation of the crystal is important since beryl is an anisotropic mineral, therefore, measurements should be taken in the parallel and perpendicular directions of the electric vector E with respect to the c-axis of the crystal. In an area preferably free of inclusions, the stage is rotated until the extinction angle of the crystal is found.
   To perform this step, the polarizer must be inserted between the sample and the observer and, to simplify the process, it is possible to place the crystal with the crystallographic c-axis parallel to the direction of the second polarizer (located between the condenser and the sample). This way you will know that when the crystal is aligned to the direction of the second polarizer, it will be in parallel direction and you will be able to look for the extinction angle (Straight in beryl) from this position. From the extinction angle you will look for the next one and you will have the perpendicular position on the crystal.

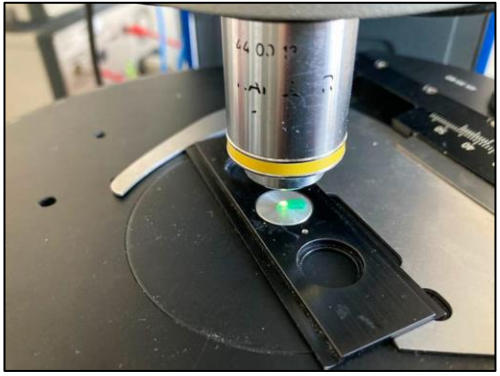


Figure 20. Beryl crystal in UV-Vis spectroscopy microscope oriented in E direction perpendicular to the c-axis.

5. **Measurement:** After mounting the sample and having it at its extinction angle, choose in the TIDASDAQ (UV-NIR) software the "Shutter" option, which represents the activation of the shutter, to control the exposure of the sample to the light source. Once activated, you will observe on the microscope the appearance of a bright square on the sample, which is an image of the condenser being illuminated by the light source. This bright square must be focused and centered to optimize the signal captured by the spectrometer detector and to obtain accurate spectral measurements.

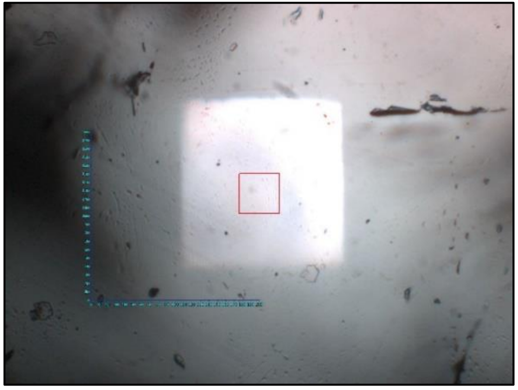
   

   Figure 21. Image of the condenser (bright square) on beryl crystal in UV-Vis spectroscopy.

   You can also view the condenser from the TIDASVision software, which provides a picture of what is seen through the microscope (when the polarizers are inactive).

   Finally, to perform the measurement, remove both polarizers from the microscope and in the TIDASDAQ software (UV-NIR) designate a name for the measurement and choose the "Start Scan" option, then you will see in the software the generation of the UV-Vis-NIR spectrum.

6. **Data:** At the end of the measurement save the data in format (.spec) and in text format (txt). It is also possible to take pictures of the focus on the microscope from the TIDASVision software, in the case of beryl, it is useful to record images of inclusions.

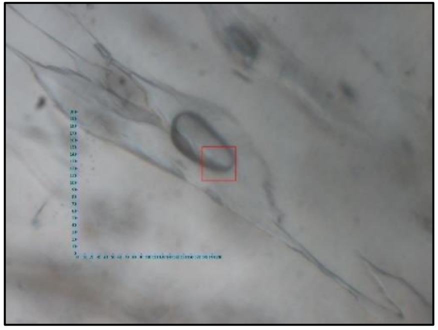
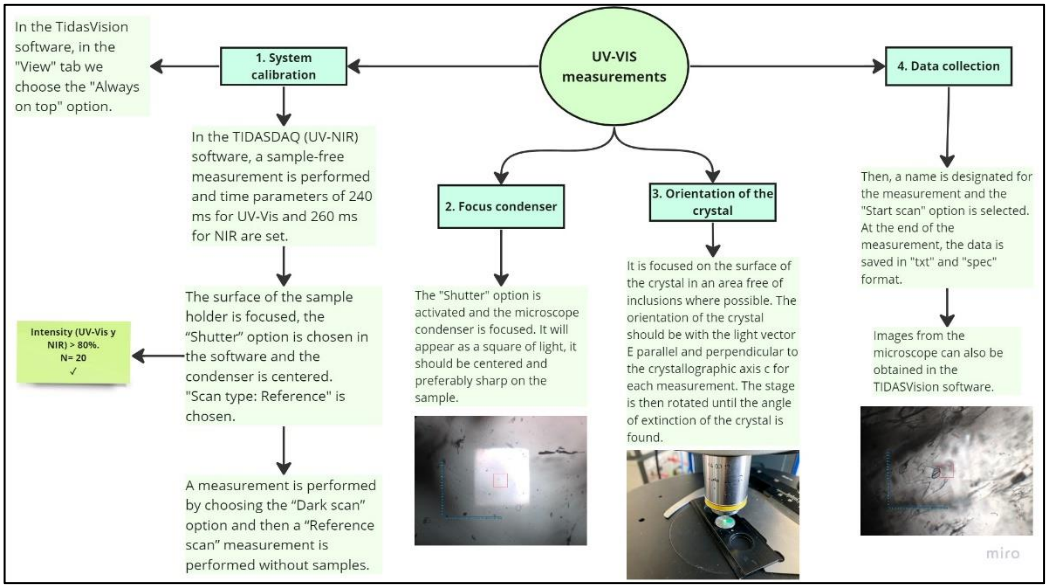
   

   Figure 22. Inclusions in emeralds taken from UV-Vis micro spectroscopy.



In the TidasVision software, in the "View" tab we choose the "Always on top" option.

In the TIDASDAQ (UV-NIR) software, a sample-free measurement is performed and time parameters of 240 ms for UV-Vis and 260 ms for NIR are set.

The surface of the sample holder is focused, the “Shutter” option is chosen in the software and the condenser is centered. "Scan type: Reference" is chosen.

A measurement is performed by choosing the “Dark scan” option and then a “Reference scan” measurement is performed without samples.

The "Shutter" option is activated and the microscope condenser is focused. It will appear as a square of light, it should be centered and preferably sharp on the sample.

It is focused on the surface of the crystal in an area free of inclusions where possible. The orientation of the crystal should be with the light vector E parallel and perpendicular to the crystallographic axis c for each measurement. The stage is then rotated until the angle of extinction of the crystal is found.

Then, a name is designated for the measurement and the "Start scan" option is selected. At the end of the measurement, the data is saved in "txt" and "spec" format.

Images from the microscope can also be obtained in the TIDASVision software.

Figure 23. Methodological map for UV-VIS-NIR measurements.

What are the most common mistakes when performing Micro-Raman spectroscopy?

Raman spectroscopy offers great results if a proper process is conducted in the use of the instruments, for this some common mistakes should be considered to avoid when performing the measurements and thus obtain adequate results. According to Ryabchykov et al. (2022) some of the most common mistakes are:

1. **Improper sample preparation:** Although Raman sample preparation is not critical, it may contain impurities that affect the quality of the results. Therefore, the sample should be clean and well prepared prior to measurement.
2. **Incorrect choice of laser:** Choosing the wrong wavelength can cause fluorescence problems and excessive noise in the spectrum. Short wavelengths provide greater sensitivity to Raman scattering; however, they can also present greater problems with respect to fluorescence. Long wavelengths present fewer interference problems but may heat the sample. The laser you choose will depend on the sample you are analyzing and the reaction of its components at each wavelength.
3. **Omitting calibration:** Omitting system calibration can result in inaccurate measurements, wavenumber, wavelength, and intensity calibration should be performed. In addition, it is important to select a suitable calibration standard, such as silicon (Si), as it exhibits well-defined Raman peaks that can be used to accurately calibrate the Raman spectrometer. The Raman spectrum of silicon is characterized by a sharp, intense peak around 520 cm^{-1}. You should also ensure that the reference material is stable and constant over time. The Raman peak of

Si is very stable, with minimal shifts over time, making it a reliable reference standard for calibration.

4. **Environmental interference:** Ambient light can interfere with the Raman signal, so it is important to perform measurements in a dark space to avoid background noise in the spectra and to cover the sample area around the microscope stage.
5. **Noise removal or baseline correction:** By baseline correction, the background signal of the Raman spectrum, caused by fluorescence or other non-Raman scattering processes, is removed. If baseline correction is not performed, the signal may be artificially elevated or shifted, leading to incorrect peak identification and inaccurate quantitative measurements.
6. **Incorrect identification of the sample or its components:** Correct identification of the sample or its components is important to ensure accurate analysis. To avoid this, the library of the software being used can be consulted for a correlation between the spectrum obtained and its possible classification.
7. **Incorrect interpretation of the data:** Correct interpretation of the data is important to avoid errors in the analysis of Raman spectra.

What are the most common mistakes when performing UV-Visible spectroscopy?

1. **Inadequate sample preparation:** Samples must be free of contaminants to avoid the appearance of interfering signals and must also be of adequate thickness to allow light to pass through. If the glass is too thick the light will have difficulty passing through it and may have trouble focusing the condenser.
2. **Focusing problems:** Incorrect focusing can result in inaccurate measurements or weak signals. It is important to make sure that the focus is correctly adjusted both on the surface of the glass and for the condenser.
3. **Excessive illumination on the microscope:** Placing too much illumination on the microscope can decrease image resolution. Therefore, it is important to carefully adjust the illumination without exceeding the exposure limits of the specimen.
4. **Incorrect calibration:** It is important to ensure that the wavelength of the spectrometer is correctly set before measuring. If the wavelength is out of adjustment, the results may be inaccurate. Also, if the intensity of the UV and Vis light is too low, the signal may be weak and difficult to detect, which can affect the accuracy and reliability of the results.
5. **Leave polarizers active:** Polarizers can block the light used to measure the sample, which can result in a weak or undetectable signal. In other cases, the orientation of the polarizers may interfere with the measurement and affect the precision and accuracy of the results.
6. **Data analysis:** Incorrect data analysis may affect the accuracy of the results.

What are the expected results of Raman and UV-Vis spectra on emeralds?

Micro-Raman Spectroscopy

The analysis of emeralds with Micro-Raman spectroscopy allows obtaining information about their chemical identification, the molecular nature of the water in the channels of their crystallographic structure and their authenticity. Figure 24a illustrates the typical spectrum obtained for emerald in the region of (200-

1600 cm^{-1}) with typical bands according to Bersani et al. (2014) at 319 and 529 (ring vibrations), 682 (ring vibrations, Be-O stretching), 1009 (Si-O stretching), 1066 (Si-O or Be-O stretching) and 1242 cm^{-1} (internal ring vibrations).

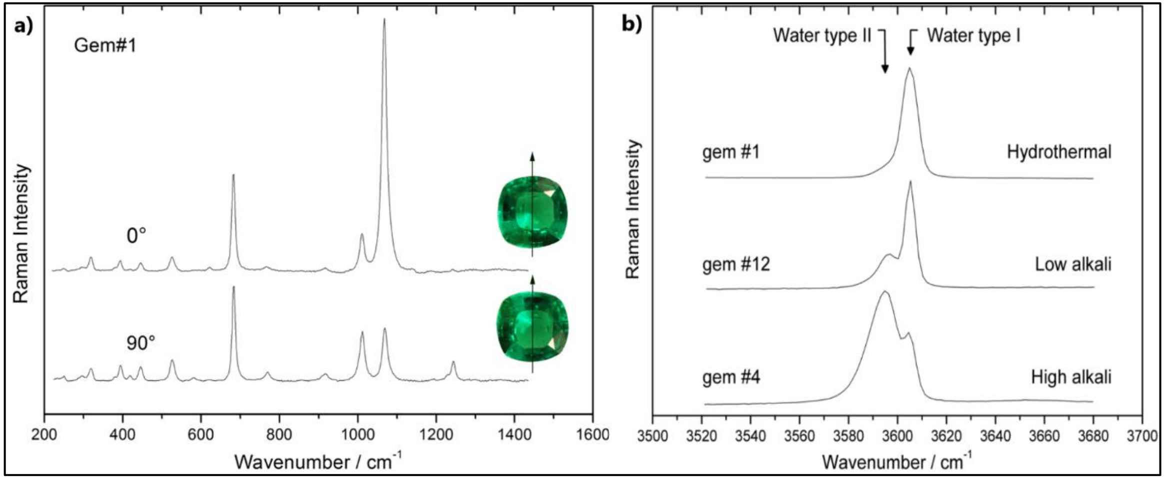


Figure 24. a) Raman spectra obtained parallel to the c-axis of the gems. The black arrows show the polarization of the laser beam. **b)** Raman spectra, in the OH region, of three gems of different origin (Bersani et al., 2014).

Figure 24b illustrates the region (3500-3700 cm^{-1}) that is characterized by containing the different types of water hosted in the channel cavities present in the beryl structure. Therefore, this technique also allows investigating the geographical origin of natural emeralds (Le Huong et al., 2010).

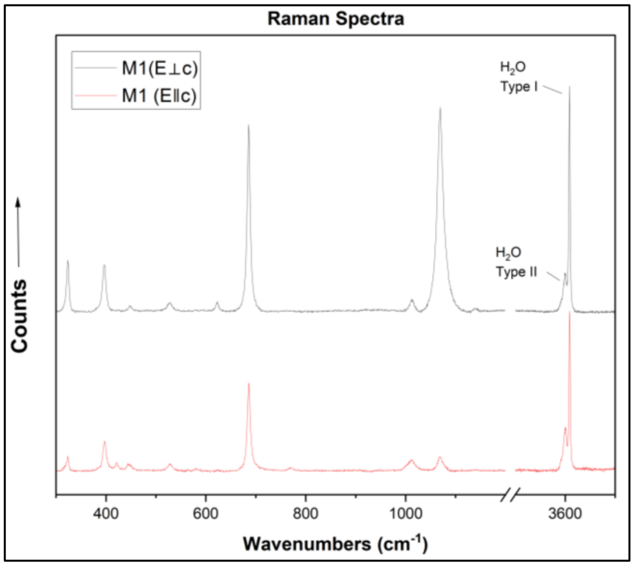
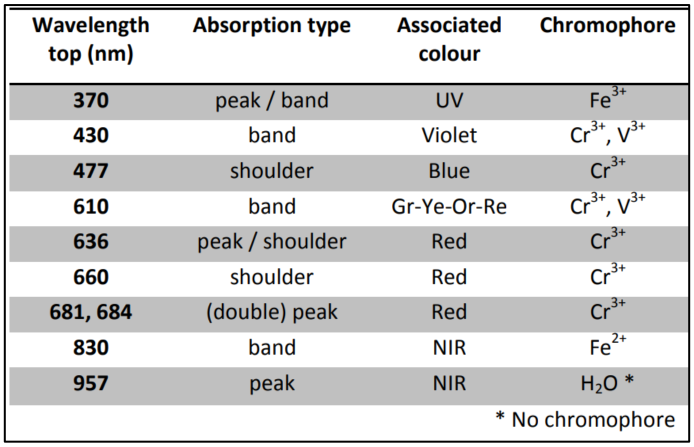


Figure 25. Raman spectrum with E in the direction parallel and perpendicular to the c-axis in a Colombian emerald (Muzo). This graph was obtained following the current guide, the typical spectrum for beryl and the characteristic Raman bands for water types I and II are observed.

UV-Vis-NIR spectroscopy

The analysis of emeralds with UV-Vis-NIR spectroscopy allows obtaining information about the color and origin of the crystals. According to Wood and Nassau (1968) the absorption in the UV-Vis-NIR regions are mainly caused by chromophore transition metals, in the case of emerald, the green color is produced due to the substitution of $\mathrm{Al}^{3+}$by Cr^{3+} or V^{3+} these chromophores selectively absorb violet, yellow, and transmit colors such as blue and green. Some emeralds contain Fe^{2+}, obtaining a yellowish-green color.



Wavelength top (nm)	Absorption type	Associated colour	Chromophore
370	peak / band	UV	Fe^{3+}
430	band	Violet	Cr^{3+}, V^{3+}
477	shoulder	Blue	Cr^{3+}
610	band	Gr-Ye-Or-Re	Cr^{3+}, V^{3+}
636	peak / shoulder	Red	Cr^{3+}
660	shoulder	Red	Cr^{3+}
681, 684	(double) peak	Red	Cr^{3+}
830	band	NIR	Fe^{2+}
957	peak	NIR	H_2O *

* No chromophore

Figure 26. Typical absorptions associated with chromophores (van Meerbeeck, 2010).

To differentiate the origin of the emeralds, it is possible to analyze the type of crystalline inclusions and their shapes, however, UV-Vis spectra can also be useful to distinguish the origin.

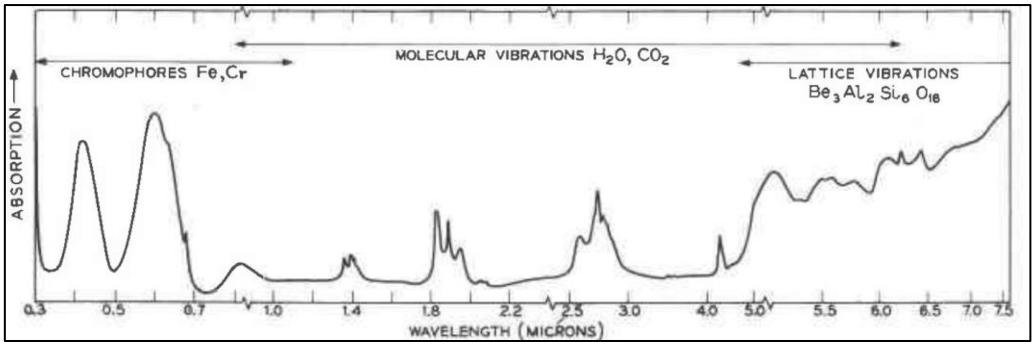


Figure 27. Composite spectrum of beryl showing lattice vibrations, molecular vibrations, and chromophore absorption.

According to Saeseaw et al. (2019) emeralds housed in schists have higher Fe contents than those present in hydrothermal/metamorphic emeralds. Figure 28a shows typical absorption spectra for emeralds of hydrothermal/metamorphic origin, whose most prominent absorption bands are Cr^{3+} around 430, 600, and 683 nm. Figure 28b shows typical spectra for shale-hosted emeralds, showing an Fe^{3+} absorption band around 372 nm and an intense Fe^{2+}/ Fe^{3+}band around 810 nm.

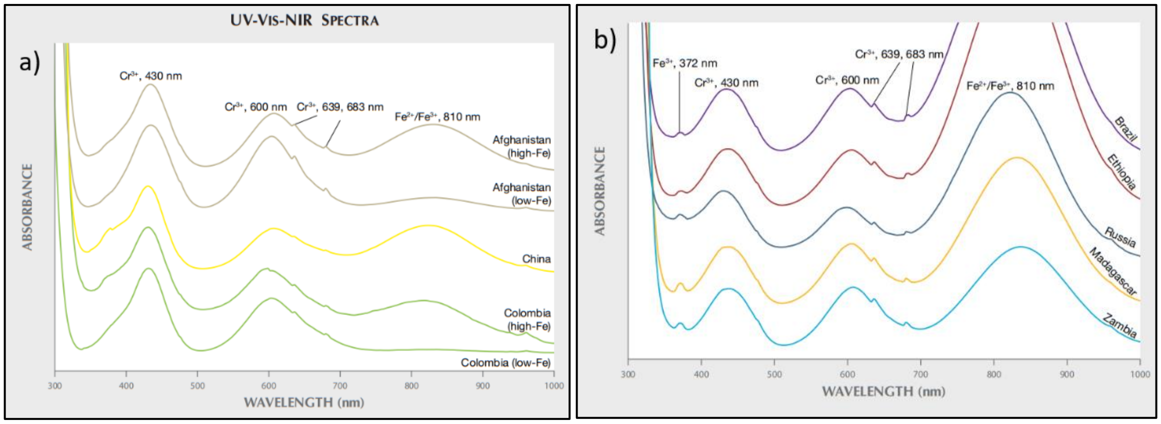
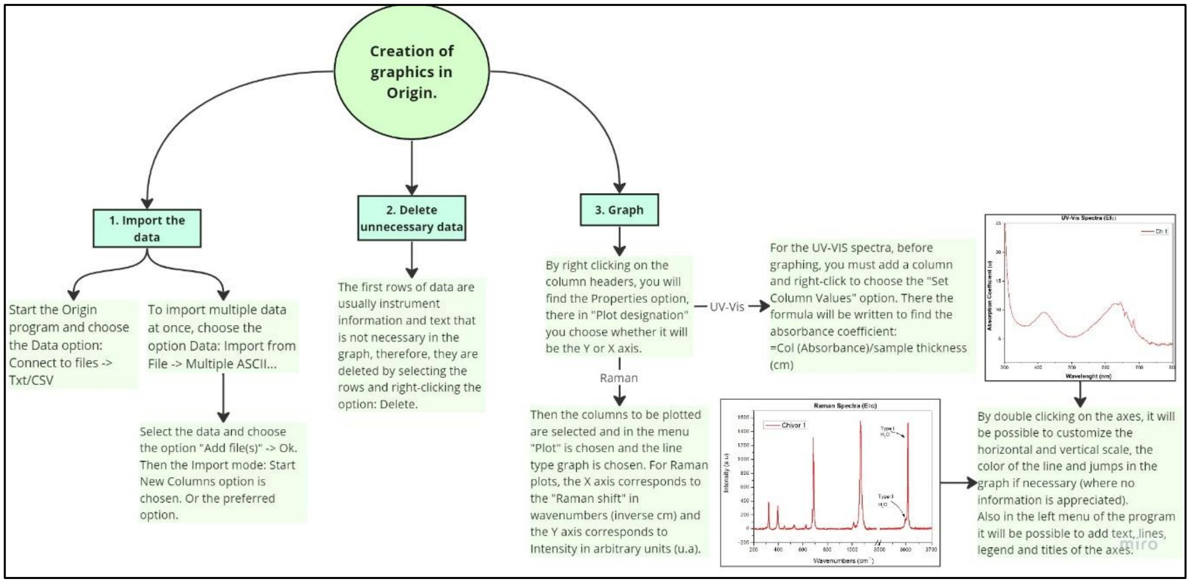


Figure 28. a) Representative UV-Vis-NIR spectra of metamorphic/hydrothermal emeralds. **b)** Representative UV-Vis-NIR spectra of emeralds hosted in schists (Saeseaw et al., 2019).



Start the Origin program and choose the Data option: Connect to files -> Txt/CSV

To import multiple data at once, choose the option Data: Import from File -> Multiple ASCII...

Select the data and choose the option "Add file(s)" -> Ok. Then the Import mode: Start New Columns option is chosen. Or the preferred option.

The first rows of data are usually instrument information and text that is not necessary in the graph, therefore, they are deleted by selecting the rows and right-clicking the option: Delete.

By right clicking on the column headers, you will find the Properties option, there in "Plot designation" you choose whether it will be the Y or X axis.

For the UV-VIS spectra, before graphing, you must add a column and right-click to choose the "Set Column Values" option. There the formula will be written to find the absorbance coefficient: =Col (Absorbance)/sample thickness (cm)

Then the columns to be plotted are selected and in the menu "Plot" is chosen and the line type graph is chosen. For Raman plots, the X axis corresponds to the "Raman shift" in wavenumbers (inverse cm) and the Y axis corresponds to Intensity in arbitrary units (u.a).

By double clicking on the axes, it will be possible to customize the horizontal and vertical scale, the color of the line and jumps in the graph if necessary (where no information is appreciated).
Also in the left menu of the program it will be possible to add text, lines, legend and titles of the axes.

Figure 29. Methodological map for the creation of graphics in Origin.

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