Design for laser assembly in confocal Raman spectroscopy for *in-vivo* sample study

Diseño para ensamble láser en espectroscopía Raman confocal para estudio de muestras *in-vivo*

Urrieta-Almeida, Edgar*[a], Flores, Gil Aarón[b], Benavides, Olena[c] and Bandala-Garces, Magdalena [d]

[a] Universidad Autónoma del Carmen • 0000-0003-1668-1966 • 785442
[b] Universidad Autónoma del Carmen • 0000-0002-2302-2056 • 121166
[c] Universidad Autónoma del Carmen • 0000-0002-8124-0326 • 339830
[d] Universidad Autónoma del Carmen • 0000-0002-3102-3630

CONAHCYT classification:

DOI: https://doi.org/10.35429/H.2024.13.48.56

Area: Physics-Mathematics and Earth Sciences
Field: Physics
Discipline: Physical Chemistry
Subdiscipline: Molecular Spectroscopy

Key Handbooks

By visualising that the Raman spectroscopy technique has the versatility to be used to study in-vivo samples, such as, for example, the skin and fingernail of the human index finger. Another important fact is that humans have the same molecular conformation, but at different concentrations. This defines that a Raman spectrum is intrinsic to each individual, like a fingerprint. With these facts in mind, a laser assembly was implemented, which allows quality Raman spectra readings to be taken and thus a data bank to be created. The proposed assembly has the characteristics of allowing a sample to be taken in 10 minutes, without causing pain and without causing adverse effects to the skin. The aim is to identify biomarkers that will allow in the future a preventive or conclusive diagnosis in people with or without a diagnosis of chronic diseases through the processing of the database generated for each individual.

Citation: Urrieta-Almeida, Edgar, Flores, Gil Aarón, Benavides, Olena and Bandala-Garces, Magdalena. 2024. Design for laser assembly in confocal Raman spectroscopy for *in-vivo* sample study. 48-56. ECORFAN.

* ✉ [990615@mail.unacar.mx]

Handbook shelf URL: https://www.ecorfan.org/handbooks.php

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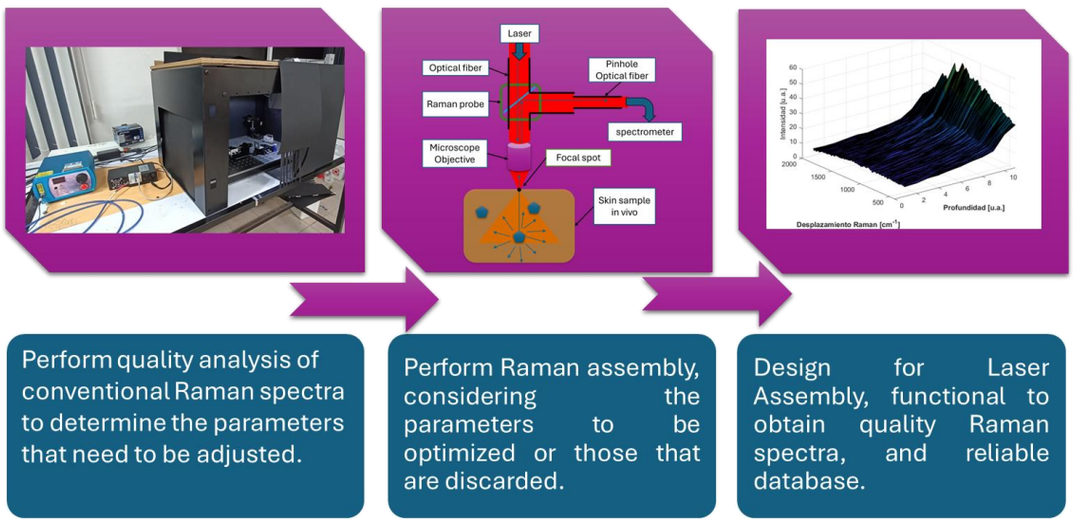
Abstract

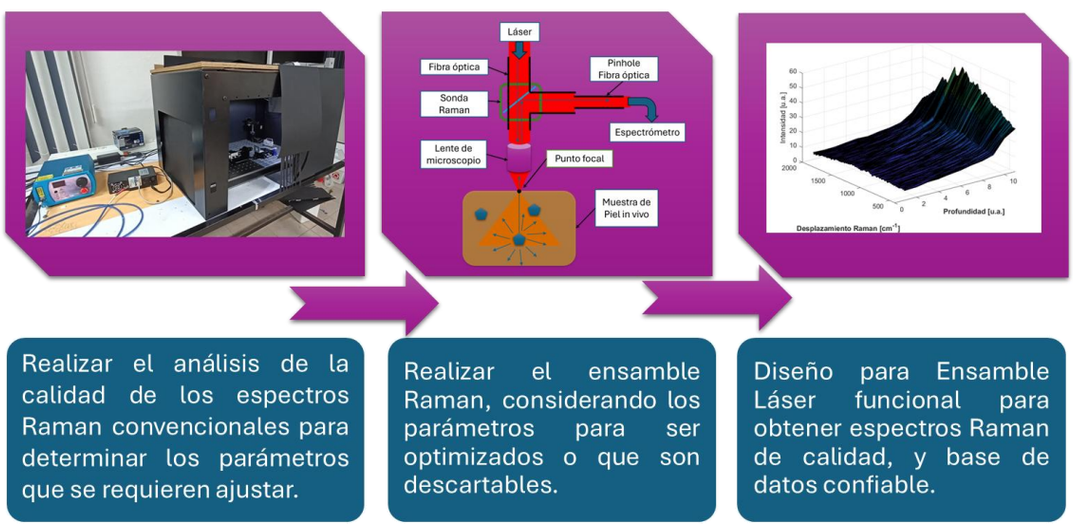
The study of biomarkers is a line of study that allows the early detection of abnormal biochemical processes, focusing on chronic human diseases such as diabetes, obesity, cancer, among others. Raman spectroscopy (RS) technology allows obtaining intrinsic data from a sample at the molecular level. RS allows studying biomarkers in biological samples in vivo, without contact, without pain, and in real time. This design for assembling a Confocal RS is presented, with a Raman laser equipment at 785 nm, a spectrometer QE65000 from Ocean-Optics, a microscope-type mount, and an optical fiber as a pin-hole. To validate the design, Raman bands of melanin, lipids and proteins are identified in spectral sampling at different depths, 1000 µm, in the fingerprint and index fingernail of a human volunteer. The design promises to obtain deconvolution spectra directly from the measurement in real time, without the need to apply numerical fitting methods in data processing.



Raman Spectroscopy, biomarcadores, Dedo índice, Muestras *in-vivo*

Resumen

Los biomarcadores son una línea de investigación, sobre la detección temprana de procesos bioquímicos anormales, enfocado a las enfermedades humanas crónicas, como la diabetes, la obesidad, el cáncer, entre otras. La Tecnología de espectroscopía Raman (ER) permite obtener datos intrínsecos de una muestra, al nivel molecular. La ER permite estudiar biomarcadores en muestras biológicas in-vivo, sin contacto, sin dolor, y en tiempo real. Se presenta un diseño para ensamble de un ER Confocal, con un equipo láser Raman a 785 nm, un espectrómetro QE65000 de Ocean-Optics, una montura tipo microscopio, y una fibra óptica como pin-hole. Para validar el diseño, se identifican las bandas Raman de melanina, lípidos y proteínas en el muestreo espectral a diferentes profundidades, hasta 1000 µm, en la huella y uña del dedo índice de un voluntario humano. El diseño promete obtener espectros en deconvolución directamente de la medición en tiempo real, sin aplicar métodos de ajuste numérico en el procesamiento de los datos.



Espectroscopia Raman, Biomarcadores, Dedo índice, Muestras *in-vivo*

Introduction

The study of public health problems is important for the Mexican society, especially for people who have problems in their quality of life. Considering the number of sick people and the number of chronic diseases registered, problems are generated in the socio-cultural and socio-economic development of the country. For example, Diabetes (NOM-015-SSA2-2010), Obesity, Cancer, which are known to have a great impact and generate social welfare problems (INEGI Communicated 2023). Chronic diseases such as cardiovascular diseases, hypertension, cancer, diabetes and also new conditions generated by the pandemic such as COVID-19. Each of these conditions represents a field of study for health science researchers in terms of prevention, diagnosis and treatment (INSP. 2023).

Raman spectroscopy (RS) has been used as a non-invasive method to study skin diseases and glucose between the Raman bands 1060 $^{cm-1}$ and 1125 $^{cm-1}$ (Flores A. et al. 2015), as well as haemoglobin 1549 $^{cm-1}$ (Gonzales-Viveros et al. 2022). The methodology may be feasible for the study of blood glucose in vivo, for in vitro cases (N. Gonzales-Viveros et al. 2021). Likewise, with Raman equipment systems coupled to confocal microscopes (Shao. J et al. 2012), frequencies between 1168, 1531, 1463, 1021 $^{cm-1}$ are also reported for diabetic blood samples (Firdous s. et al 2012) and studies in type 2 diabetes in blood samples (Gonzales-Solís et al 2018), under the condition of the American standard (ANSI) for type II lasers, which indicates that lasers with power lower than 100 mW on skin in humans should be used in experiments (American National Standards Institute 1993).

Raman spectroscopy (RS) is a technique that allows molecular information to be obtained from a material, in solid, liquid or gas state, whether organic or inorganic. Performing an ER analysis means shining a monochromatic beam of light on a sample and collecting the scattered light with a spectrograph. The light beam interacts with the molecular structure constituting the sample, causing changes in the vibrational state leading to changes in the frequency of the scattered light. The section of the scattered beam that contains the Raman information is known as inelastic scattering. The importance for analysing materials or substances is that the small frequency changes are specific and individual to each material (Smith, E. et al. 2005). With the proposed system and methodology it is expected to obtain detailed spectra on the molecular structures found on the skin of the fingerprint and the nail of the index finger.

Raman spectroscopy

The monochromatic light beam is incident on the sample, depending on the state of the sample there can be reflection, transmission, absorption and scattering. The scattering can be elastic or inelastic. It is the inelastic scattering that contains the information for ER. See Figure 1.

Box 1

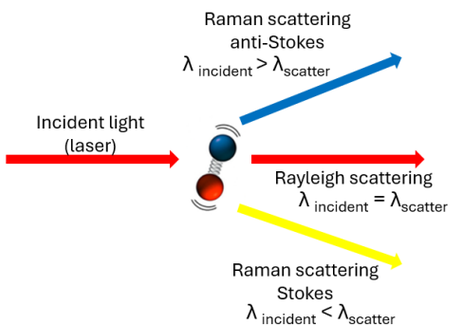


Figure 1

Incidence of a monochromatic light beam on a sample

Source: [*Own elaboration*]

A beam of light is a collection of photons, photons have energy according to the value of their frequency (where h is Plank's constant), photons interact with the molecular structure of the sample causing vibrational changes, the energy of an absorbed photon supplies that energy to move to a higher energy level which is called excitation. Upon loss of excitation, the molecule gives off a photon with a relatively different frequency from the incident beam (Drake G. et al. 2006).

While a molecule is in its ground state, a photon strikes and is absorbed, which inelastically excites the molecule to a higher virtual energy level. It then recoils to an energy level between the virtual level and the ground state, known as Raman Stokes, releasing a photon with a slightly different frequency and energy from the incident beam. If it returns from the virtual level to the ground state, this is called Rayleigh scattering. On the other hand, if the molecule is not initially in the ground state, when it moves to the excited state and returns to the ground state, it is called Anti-stokes scattering (Toporski, J. et al, 2018).

Raman changes depend on the natural frequency of vibration. The frequency depends on the mass of the molecule. For heavy molecule, it has long wavelength, low frequency and energy. For a light molecule, it has short wavelength, high frequency and energy. Complex molecules have a greater number of vibrational modes. Each molecule has its own natural frequency. The Raman spectrum is a fingerprint of molecules (Smith, E. et al. 2005).

Nail and skin anatomy

The spectra are sampled on the fingerprint and on the nail of the index finger. The epidermis is a compact cellular layer measuring 120-200 µm, with regional differences according to skin function. The dermis is 15-40 times thicker than the epidermis. The nail is 500-600 µm thick. In the present work, readings are being taken from the surface up to 10 internal steps of 127 µm, i.e. up to 1270 µm inside the footprint and nail (Conejo-Mir, J. et al. 2018). See figure 2 and 3.

Box 2

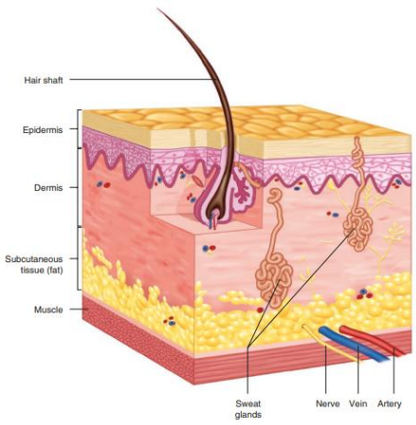


Figure 2

Anatomy of the skin

Source [*Manual of Dermatology, ISBN: 978-84-7885-627-5*]

Box 3

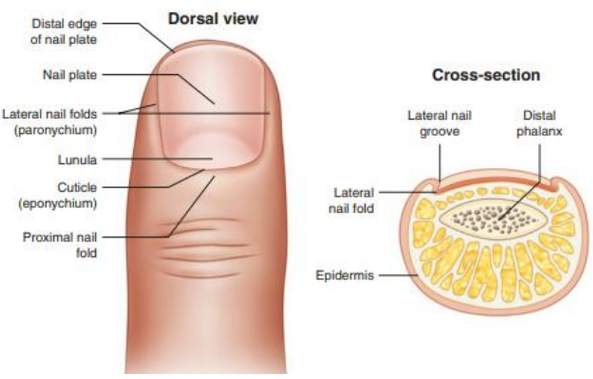


Figure 3

Anatomy of the nail

Source [*Manual of Dermatology, ISBN: 978-84-7885-627-5*]

Results

Confocal optical system development

In the present work, a unidirectional optical system was assembled to take Raman readings of the fingerprint of the index finger and the fingernail, in a first stage of healthy volunteers. Although it is worth commenting that this system could well be included in a CNC machine, such as the one used for industrial systems (Antonio, V. E., et. al 2023), as in the case of laser welding in the automotive industry (Yepes Mayoral, E. (2024), Thesis). But in our case, we need to take measurements at different depths inside the index finger, either at the front of the finger, or through the fingernail.

The optical system consists of the laser module at 785 nm, connected to a bifurcated optical fibre, with a 100-micron multimode fibre core, coupled to the laser, and a 200-micron multimode fibre coupled to the spectrometer, called a Raman probe whose tip has a focal length of 0.7 cm. The Raman signal is captured with the QE65000 spectrometer through the same probe. The 785 nm laser module is modulable in power, from 1 to 400 mW, and 50 mW was used for this project. The spectrometer allows Raman signals to be recorded in the spectral window of 400 - 2000 $^{cm-1}$, with a resolution of 4 $^{cm-1}$. The optical system also consists of an intermediate microscope lens between the probe and the sample, as well as a 50 micron fibre between the 200 µm fibre and the spectrometer. These two complements increase the resolution and reduce the laser spot, which reduces the power required. See Figures 4 and 5.

Principle of operation of the optical system

Figure 5: A schematic of how the focal plane shifts in a sample as it approaches the Raman probe is shown. An ER reading is taken for each displacement which is 127 µm.

Box 4

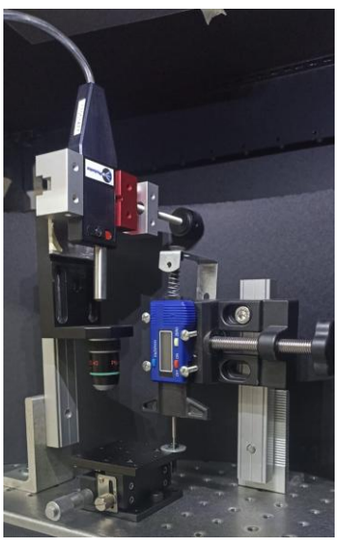


Figure 4

Raman spectroscopy system assembly

Source: [*Own elaboration*]

Sampling methodology

Activate the optical system, which are: Laser, spectrograph and computer equipment.

Set the software parameters, acquisition time in 5 s, 5 s average scanning time and level 3 filtering.

Clean the volunteer's index finger with alcohol to remove moisture and other impurities.

Place the area of the index finger, fingerprint or fingernail.

Locate the focal point on the surface of the area.

Collect the spectrum of the surface.

Reduce the distance of the moving base every 0.127 µm to collect spectra for each displacement, up to a total of 11 spectra.

Save the data obtained in SpectraSuit software.

Box 5

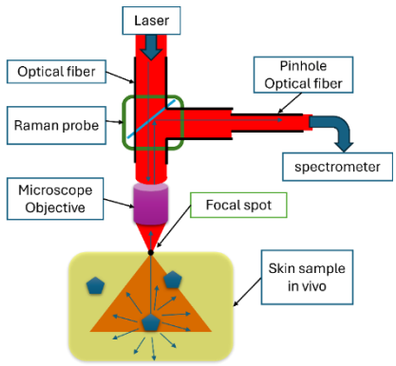


Figure 5

Raman spectroscopy system assembly

Source: [*Own elaboration*]

Processing of Raman spectra

The spectra obtained by the optical system are captured in digital form in a data file with the SpectraSuite software. The set of spectra is saved in a *.txt text file. The file name has the format date_time_name_region_notes. A comprehensive Matlab code was developed to automate the processing of the set of spectra to generate individual files and graphs in the format required for the comprehensive analysis. In general, the steps that the Matlab code executes are to trim the spectra in the range of 400 to 1800 cm-1. The next step is to generate the attenuation plots, for each band specified in the code, and to perform the flat fitting of the spectra. The output files are, attenuation plot sorted by volunteer name with respect to sampling dates, flat-fit plot of the bands specified in the code, to perform deconvolution band analysis. The qualitative analysis of the bands of the set of Raman spectra is to determine differences in intensity, width, areas, shifts and deconvolution, which are significant for correlation with the inner layers being studied, of the volunteer's footprint and fingernail, see figure 6.

Box 6

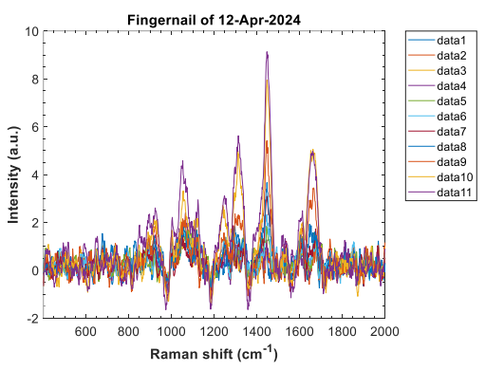


Figure 6

Flat-fit plot of a full spectral set of the nail.

Source: [*Own elaboration*]

The reliability of the spectral set obtained is qualitatively verified with the surface type graph, see figure 7. With which it is possible to visualise the exponential attenuation, as the focal point moves in the inner layers of the skin.

Box 7

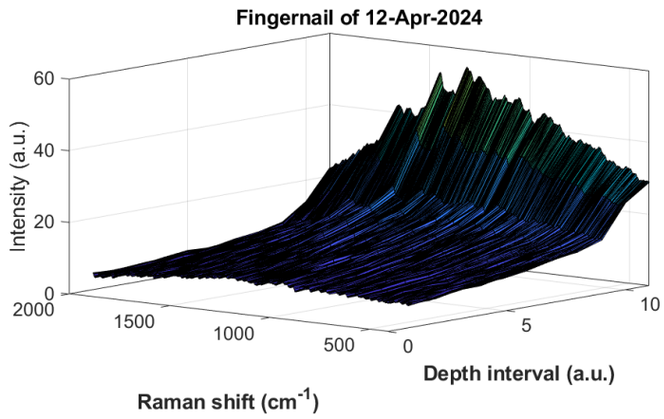


Figure 7

Surface type plot of the attenuation of the spectral array versus focal spot depth

Source: [*Own elaboration*]

In the spectral range from 1550 to 1750 cm^{-1} it is possible to visualise deconvolution peaks that have been obtained using the proposed methodology. The highest intensity peak measured at shallow levels, which is in the range of 1630 to 1700 cm-1, is interpreted as the sum of the lower intensity peaks obtained at deep levels, which are the peaks at 1650 cm-1, 1675 cm^{-1}, and the peak at 1700 cm^{-1}. See figure 8. This interval corresponds to the vibrations associated with melanin, the molecule that provides skin colouring (Wu et al., 2023).

This is the most substantial advance of the project since we have identified molecular components, it is necessary to continue with the interpretations of the spectral ranges for other species of molecules that are present in human skin.

Box 7

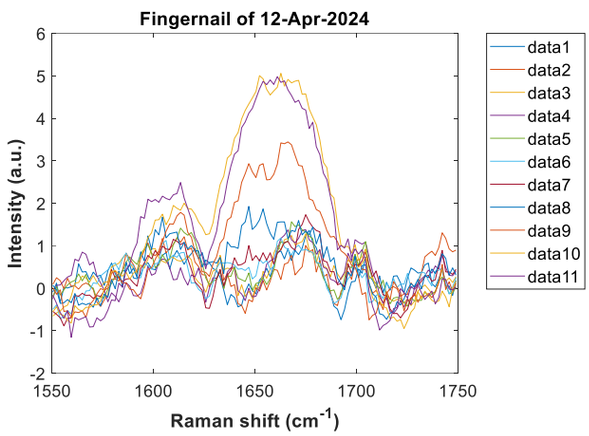


Figure 8

Flat-fit plot of a segment of the nail spectral array, from the range 1550-1750 cm^{-1}

Source: [*Own elaboration*]

Conclusions

The design of the confocal Raman system allows Raman spectra of the human index finger to be taken from different inner layers of the skin at different depths. This allows the following to be found:

- The integration time and power through the confocal system is reduced compared to standard Raman spectroscopy systems. This allows powers well below the ANSI international standard (100 mW). In our case, with a power of 25 mW and integration times of 25 seconds, spectra of good spectral quality are obtained.

- Systematic recording of Raman spectra of the skin, surface and internal, is possible. With displacements of 127 µm, reaching a depth of 1200 µm.

- The Raman spectra of fingerprint and fingernail show qualitative differences, i.e. there is no coincidence of Raman bands in both parts of the index finger, which implies studies of different molecular components (biomarkers) of the human.

- It was found that the intensity of the main Raman bands decay exponentially with depth, and that these bands actually deconvolute with sub-bands that may well be associated with biomarkers.

- The confocal system allows deconvolution of the spectra obtained for different depths.

- Deconvolution can allow the analysis of biomarkers that are not observable by standard Raman spectroscopy.

- The set of Raman spectra obtained are consistent, reproducible and intrinsic. Therefore, it is feasible to obtain correlations of the diversity of molecular components (biomarkers) with human physiological and pathological conditions.

Declarations

Conflict of interest

The author declares that he has no conflict of interest. He has no known competing financial interests or personal relationships that might have appeared to influence the article reported in this chapter.

Authors' contribution

Urrieta Almeida Edgar: Preparation of this paper.

Flores Gil Aarón, Benavides Olena, Bandala Garces Magdalena: Support in the development of the project and in the writing of the paper.

Availability of data and materials

The information contained in this document is available upon request from the lead author.

Funding

No funding was received.

Acknowledgements

Thanks are due to the PhD programme in engineering sciences of the Universidad Autónoma del Carmen and the CONAHCYT scholarship programme, and to the professors for their advice in the development of the project.

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