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DESIGN, ASSEMBLY AND START-UP OF AN EXPERIMENTAL SET-UP FOR OPTICAL CHARACTERIZATION IN BIOLOGICAL TISSUES

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Abstract: Spectroscopy and irradiation in the visible Ultraviolet (UVvis) to Near Infrared (NIR) ranges are important tools in the most recent research on biological tissues (Banavath, H.N., et al., 2018, pp. 35-40). On the one hand, the optical properties of tissues depend on the energy of the photons and, according to the spectral range and the irradiation dose, the application of light energy on biological tissues can lead to different applications both in the biological and health sciences (Hopkins, S.L., et al., 2016, pp. 644-653). To carry out experiments that allow to study the light-matter interaction, a flexible experimental set-up was carried out to characterize the usable light sources and also to perform Reflectance measurements (collimated) as well as Fluorescence studies in biological tissues using halogen light sources, LASER (405nm, 532nm and 650nm) and LED light emitting diodes (380nm, 395nm, 518nm, 590nm). m and 640nm). The experimental set-up uses easily available lasers and an optical system that allows preparing the light beam and focusing it on the sample under study. The phenomenological response is measured with a Silicon cell photometer and the spectral measurements are made with mini spectrometers in fiber optic assemblies.
Keywords: Biophotonics, LASER, LED, Spectroscopy, Biological tissues.

INTRODUCTION

Spectroscopy is a branch of optics that allows us to study the interaction between radiation and matter. Among the most widely used spectroscopic methods is electromagnetic radiation, which is part of the visible spectrum in the range from 380nm to 780nm. (Wallace, M.B., et al., 2009, pp. 233–242).

These spectra make it possible to determine the atomic and molecular composition of a material and, in this case, of a biological tissue and, depending on the characteristics of the light used, we can generate therapeutic applications on irradiated abnormal cells. (Dominguez, A., et al., 2009, pp. 21-28).

The biological and physiological effects are studied by controlling the irradiation on the growth of biological tissues using standard methods of reflectance, fluorescence and by microscopic observation of the morphological changes of the cells. (Gheewala, T., Skwor, T., & Munirathinam, G. 2018, pp. 130–137).

The light reflected by a material is a type of interaction of radiation with matter that is defined as the return of electromagnetic radiation by the material on which it falls, occurs in the same medium as the incident beam and depends on the ratio between the intensity of the reflected and the incident light. (Abebe T., 2010, pp. 8-9).

Fluorescence is a type of electromagnetic radiation emission that occurs when excited particles (atoms, ions, molecules) relax and go to lower energy levels, releasing excess energy in the form of photons. This emitted radiation has a longer wavelength than the radiation that caused the fluorescence. (Pérez, A., et.al. (2006). pp. 487–508).

The optical characterization in biological tissues is part of the previous studies for the application in this case of optical therapy that promises a new revolution in the research field, according to the properties of the sources used with good spatiotemporal resolution, selectivity according to its wavelength, specificity and directionality. (Glicksman, M.A. (2018), 1060–1065).

The optical effects of reflectance and fluorescence produced or induced by LASER irradiation are measured from the spectra of the irradiated samples (Darío, H., 2008, pp. 2-5). For example, the study of the effects of optical radiation of different wavelengths, and of almost monochromatic light produced by LASER on butterfly wings of the species *Heliconius Sara*, was carried out to verify

the design, assembly and start-up of the experimental set-up built for measurement in biological tissue. (Sedano, RE., Calero, H., 2021, pp. 374-384).

This experimental setup, constructed, assembled and characterized for spectroscopic measurement in biological tissues, will be used on leukemic cell cultures.

METHOD

The methodology implemented for the design, optical characterization and implementation of the experimental setup, controls certain factors such as optical power, the wavelength of the LASER and LED light sources used, the light intensity that the tissue receives and the exposure time to which it will be exposed. That is, the dose of light that will affect the biological tissue (Neira, R., et al., 2006, pp. 117–127).

To experimentally study the light-matter interaction, a setup was designed as can be seen in Diagram 1, characterizing the sources of light emission, measuring Reflectance and Fluorescence in biological tissues. (Solarte, E., et al. 2006, pp. 29-37).

The experimental setup was assembled with light sources such as: 405nm (blue), 532nm (green) and 650nm (red) LASER, and a set of 380nm, 395nm, 518nm, 590nm and 640nm LEDs. (Solarte, E., et al., pp. 5–10).

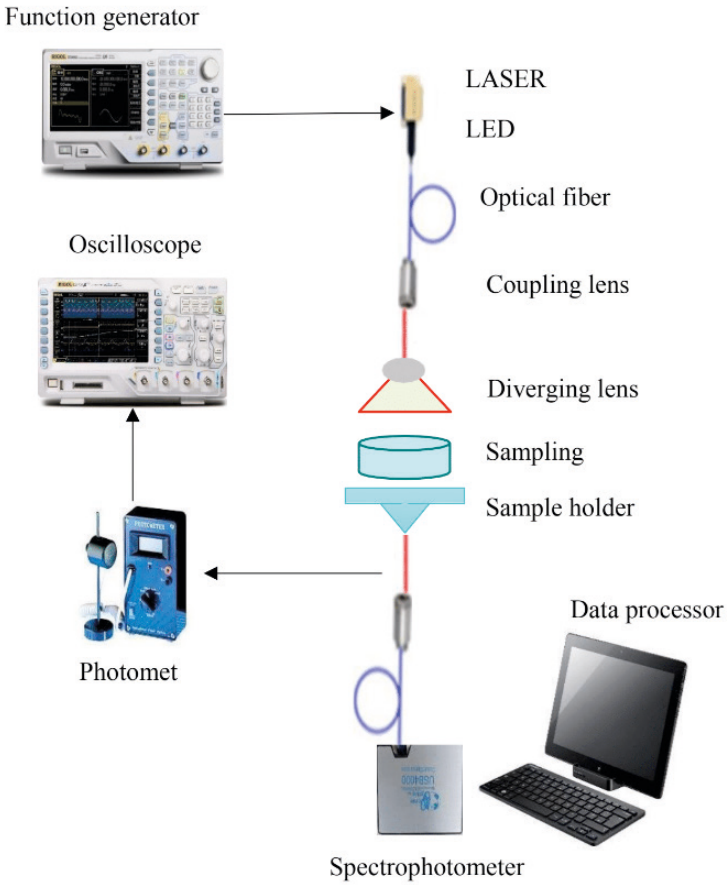
In addition, a function generator was included that sends a wave signal to the light source to activate it during certain periods of time, as well as an optical system comprised of a coupling lens between the source and the reflectance fiber or probe, depending on the study; which made it possible to guide the light beam, to diverge this beam with the help of a lens and to interact with the sample. (Solarte, E., et al., 2010, pp. 1-6)

The response was measured with a Si cell photometer, which allowed the optical response signal to be translated into an electrical signal, with which the energy absorbed by the tissue could be quantified. (Solarte E., et al., 2009, pp. 138-141). The signal in Volts of the photometer as a function of time was observed through the oscilloscope and the time in which the pulse was on and off was recorded. (Wallace, M.B., et al., 2009, pp. 233–242).

To verify the implementation of the experimental setup, the light emission sources of the system were characterized and the optical characterization of the *Heliconius Sara* biological tissue model was performed (Sedano, RE., Calero, H., 2021, pp. 374-384). For which, a reflectance probe was used that, by means of a bifurcated optical fiber, conducted the light beam from the source and collected the reflectance/fluorescence of the sample, which was analyzed by a spectrophotometer.

The application is in the preliminary phase of a research work entitled "Optical characterization of Insular and Continental populations of the diurnal Lepidoptera *Heliconius Sara* to examine the power of evolutionary fractionation", which includes the union of two research groups: the Eco-physiology, Biogeography and Evolution Group, and the Quantum Optics Group of the Universidad del Valle, with the aim of verifying the nature of color and the potential for evolutionary fractionation of wings of H. Sara populations. from optical spectroscopy under the direction of the professors: Raúl Sedano and Efraín Solarte and the participation of two undergraduate students Isabella Aguilera and Laura Gil. Therefore, copyrights are reserved. (Sedano, R., et al., 2023).

The direct objective, for which the design, assembly and start-up of an experimental set-up that would allow spectroscopic characterization on biological tissue was carried out, is the direct application for which the experimental set-up was designed; with which the optical irradiation on cell cultures



Scheme 1. Experimental setup for optical characterization in biological tissues

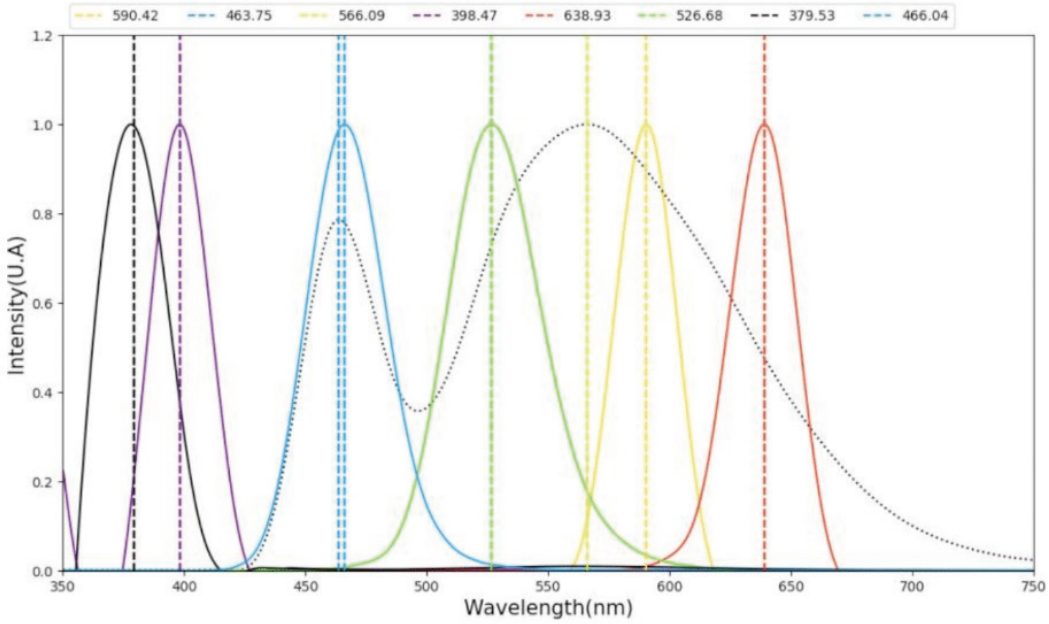


Figure 1. LED emission spectra at different wavelengths.

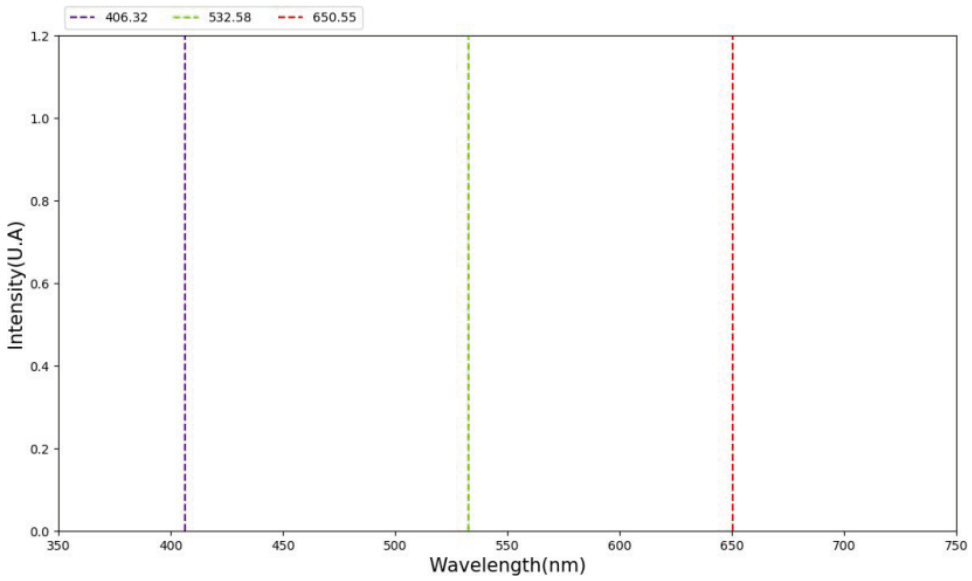


Figure 2. LASER light emission spectrum at different wavelengths.

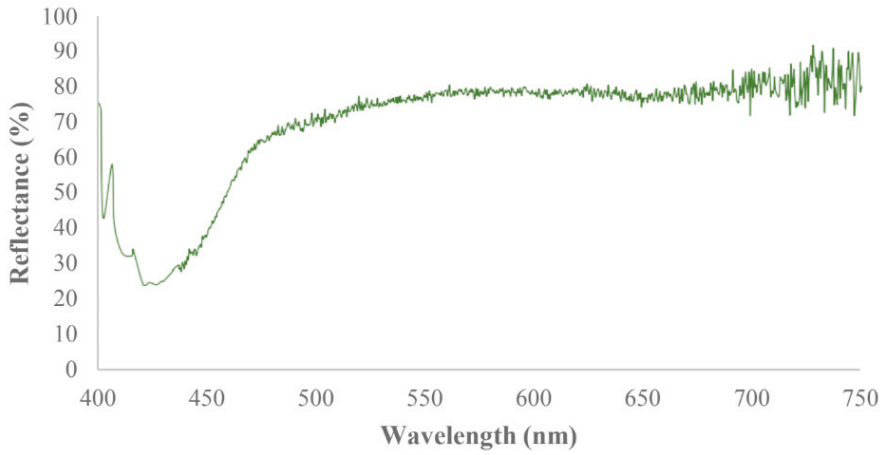


Figure 3. Reflectance spectrum with halogen light source on biological model of butterfly wings of the species *Heliconius Sara.*

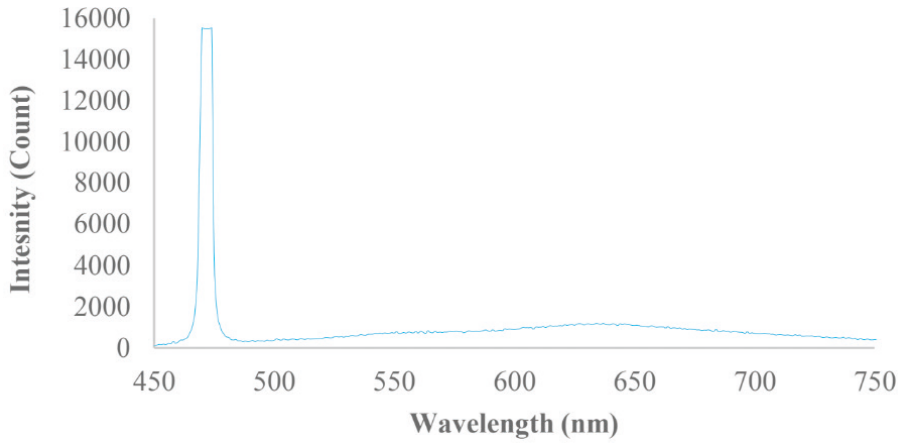


Figure 4. Fluorescence spectrum with a LASER light source on a biological model Butterfly wings of the species *Heliconius Sara*

will be carried out. *(The application of the experimental assembly is part of the doctoral project "Effects of optical irradiation with laser and led light sources in leukemia cell cultures"), for which copyright is reserved. (Ochoa, D.V, et al., 2023).*

RESULTS

To characterize the experimental setup; the emission spectra of the LED and LASER light sources were measured, as can be seen in figures 1 and 2.

Figure 1 shows the wavelength spectrum of the LEDs: white light represented by a black curve that covers the visible spectrum, wavelength at 380nm for the violet color, 395nm corresponding to purple, 405nm to the blue curve, 518nm to green, 590nm to yellow and 640nm to the red LED; with a representative bandwidth of the same.

Likewise, in figure 2 the characteristic emission spectra of lasers at 405nm for blue, 532nm for green and 650nm for red LASER are presented.

With the above, it was evidenced that the experimental setup allowed the measurement of the emission spectra of the different LED and LASER light sources used.

On the other hand, to verify the implementation of the experimental assembly built for measurement in biological tissue, the optical characterization was carried out on the biological model Alas de mariposa of the species *Heliconius Sara*.

In figure 3 resulting from reflectance in the UV - NIR range, it allows observing the absorption bands of the wings of the species: *Heliconius Sara.*

Figure 4 shows the spectrum of absorption between 400-500nm and emission between 600-700nm.

DISCUSSION

Once the experimental set-up was characterized spectroscopically from the measurement of the reflectance spectra of the halogen light sources, the LED (figure 1) and the LASER (figure 2), it was possible to perform spectroscopic measurements on biological tissue (Teixeira, A. F., et al., 2018, 250–255).

As a direct application of the construction of the experimental setup, measurements were made with continuous visible light sources, LEDs and LASERS in biological tissues for the measurement of reflectance spectra (figure 3) and, under some modification of the experimental setup, to measure fluorescence spectra (figure 4) in the biological model - wings of *Heliconius Sara* specimens - (Wilts et al., 2017, pp. 1-12), (Sedano, R., Calero, H., 2021, pp. 374-384).

Figure 3 shows the reflectance spectrum with a halogen light source on the butterfly wing of the *Heliconius Sara* species, as a biomodel. From the reflectance maxima of the spectrum, the wavelength that the biological model absorbs the most is chosen and the fluorescence spectrum is measured using the blue laser (figure 4).

Figure 4 shows the absorbance of the biomodel between 400-500nm and at a longer wavelength, the emission of radiation between 600nm-700nm. Accordingly, the absorbance in the blue-violet and the emission of the radiation in the orange-red wavelength range are observed. What evidences the phenomenon of fluorescence on the biological model.

Biological tissues, based on their molecular, chemical and physical complexity, have a specific spectroscopic response depending on the wavelength with which they interact. Having this spectroscopic information in detail, clinical applications could be specified.

Finally, with the experimental setup

designed and assembled, it was possible to spectroscopically characterize, based on reflectance and fluorescence measurements, butterfly wings as a model biological tissue as evidence of the functionality of the experimental setup for optical characterization in biological tissues.

CONCLUSIONS

The experimental set-up that allowed spectroscopic measurements on biological tissue was designed, assembled, characterized and started up.

The emission spectra of the halogen, LED and LASER sources used were measured, coinciding with those expected according to their optical properties.

The resulting spectrum of reflectance in the UV-NIR range of the biological model was obtained, which allowed observing the absorption bands and from whose maximum it was found that the tissue under certain optical parameters has fluorescence characteristics.

The functionality of the experimental setup was verified by making reflectance and fluorescence measurements on the biological model - wings of the diurnal lepidopteran of the species *Heliconius sara* - with a halogen light source and LASER.

As perspectives, the respective optical tests will be carried out to determine the effect of the incidence of optical irradiation on cell cultures for a possible clinical application.

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REFERENCES

Banavath, H. N., et.al. (2018). Femtosecond laser pulse assisted photoporation for drug delivery in Chronic myelogenous leukemia cells. Journal of Photochemistry and Photobiology, B: Biology 187. 35-40. doi: https://doi.org/10.1016/j.jphotobiol.2018.07.031

Hopkins, S. L., et.al. (2016). An in vitro cell irradiation protocol for testing photopharmaceuticals and the effect of blue, green, and red light on human cancer cell lines. Photochem. Photobiol. Sci. 15, 644-653. doi: 10.1039/c5pp00424a

Gastrointest Endosc Clin N Am. 2009 April; 19(2): 233–242. doi: 10.1016/j.giec.2009.02.008.

Dominguez, A., Castro, P., & Morales, M. (2009). An In Vitro Study of the Reaction of Osteoblasts to Low-level Laser Irradiation. J Oral Laser Applications, 9, 21–28.

Gheewala, T., Skwor, T., & Munirathinam, G. (2018). Photodynamic therapy using pheophorbide and 670 nm LEDs exhibits anti-cancer effects in-vitro in androgen dependent prostate cancer. Photodiagnosis and Photodynamic Therapy, 21. 130–137. https://doi.org/10.1016/j.pdpdt.2017.10.026

Abebe T. (2010). Modelling of scattering and absorption by phantom tissue. 8-9. [Master's thesis, Addis Ababa University].

Pérez, A., et.al. (2006). Espectroscopía de fluorescencia inducida por láser en células, in Memoria de El Colegio Nacional. pp. 487–508.

Darío, H., Criollo, W., Solarte, E., Cuántica, G. D. Ó., Física, D. De, Farmacología, G. De, & Ciencias, E. De. (2008). Efectos de la Radiación de Baja Potencia Sobre el Crecimiento Celular en Cultivos de Meningioma. [Tesis pregrado en física. Universidad del Valle].

Glicksman, M. A. (2018). Induced Pluripotent Stem Cells: The Most Versatile Source for Stem Cell Therapy. Clinical Therapeutics, 40(7), 1060–1065. https://doi.org/10.1016/j.clinthera.2018.06.004

Hussain, A., Eduardo, J., & Brown, D. (2010). Total body irradiation. In Quality and Safety in Radiotherapy (Fourth Edi, pp. 373–379). Elsevier Inc. https://doi.org/10.1201/b10448

Ochoa, D. V. (2018). Efecto de las reacciones químicas, en fase gaseosa, sobre la producción de monóxido de carbono, durante la combustión in situ. Cali, Colombia. [Tesis de maestría en ingeniería química, Universidad Nacional de Colombia]. Repositorio institucional de la Universidad Nacional de Colombia. https://repositorio.unal.edu.co/handle/unal/68705

Neira, R., Toledo, L., Arroyave, J., Solarte, E., Isaza, C., Gutierrez, O., … Ortiz-Neira, C.

L. (2006). Lowlevel laser-assisted liposuction: The Neira 4 L technique. Clinics in Plastic Surgery, 33(1), 117–127. https://doi.org/10.1016/j.cps.2005.09.005

Solarte, E., et al. (2006). Laser Light Propagation in Adipose Tissue and Laser Effects on Adipose Cell Membranes. SPIE Vol. 6026, pp. 29-37. ISBN 0819460575.

Solarte, E., Gutierrez, O., Neira, R., Arroyave, J., Isaza, C., Ramirez, H., … Ortiz, C. (2004). Laser induced lipolysis on adipose cells. Proceedings of SPIE, (October), 5–10. https://doi.org/10.1117/12.589307

Solarte, E., Urrea, H., Criollo, W., & Gutierrez, O. (2010). LED illumination effects on proliferation and survival of meningioma cellular cultures. Dynamics and Fluctuations in Biomedical Photonics VII, Vol. (7563, 756309), 1-6. https://doi.org/10.1117/12.843060

Solarte, E., Valdés, C. P., Banguero, Y. (2009). Estudio Experimental y Reconstrucción de la Propagación de un Haz Laser en un Modelo de Tejido Blando. Revista Colombiana de Física, ISSN 0120-2650. Ed. Sociedad Colombiana de Física. Vol. 41, No. 1, pp. 138-141.

Teixeira, A. F., Alves, J. R., de Souza da Fonseca, A., & Mencalha, A. L. (2018). Low power blue LED exposure increases effects of doxorubicin on MDA-MB-231 breast cancer cells. Photodiagnosis and Photodynamic Therapy, 24(May), 250–255. https://doi.org/10.1016/j.pdpdt.2018.07.016

Wilts et al. (2017). BMC Evolutionary Biology. 17:226, pp. 1-12. doi 10.1186/s12862- 017-1073-1

Valdés, C. P., & Solarte, E. (2010). Estudio experimental de la propagación de luz láser en medios con alto esparcimiento. Cali, Colombia. [Tesis de maestría en ciencias física, Universidad del Valle].

Sedano, R., Aguilera, I., Ochoa, D.V., Gil, L., Solarte, E. (2023). Caracterización óptica empleando fuentes de láser o LED, de poblaciones insulares y continentales del Lepidóptero diurno *Heliconius Sara* para examinar el potencial de fraccionamiento evolutivo. Universidad del Valle, Cali, Colombia.

Sedano, R., Calero, H. (2021). Genetic characterization of the Heliconius Sara (Nymphalidae) population in the Gorgona Island, Colombia. Acta Biológica Colombiana, 26(3), pp. 374-384. https://doi.org/10.15446/abc.v26n3.86205

Ochoa, D.V., Gutiérrez, J.O., Solarte, E. (2023). Efectos de la irradiación óptica con fuentes de luz láser y led en cultivos celulares de leucemia. Cali, Colombia. [Tesis para optar a grado de Doctora en Ciencias Biomédicas, Universidad del Valle]