ABSORPTION AND REFLECTANCE SPECTROSCOPIC CHARACTERIZATION OF CANCEROUS AND PRE-CANCEROUS CERVICAL TISSUE

CARACTERIZACIÓN DE TEJIDO CERVICAL CANCEROSO Y PRE-CANCEROSO MEDIANTE ESPECTROSCOPIA DE ABSORCIÓN Y REFLECTIVIDAD

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Cancer diagnosis by non-invasive techniques is subject of cutting-edge research in biomedical field. This paper presents experimental results of absorption and reflectance spectroscopy for ex-vivo assessment of cancerous and pre-cancerous cervical tissue. Results show promising and provide a methodology to be integrated with the standard papsmear or screening and tests aimed at preventing deaths due to cervical cancer.

El diagnóstico del cáncer mediante técnicas no invasivas es objeto de investigaciones de punta en el campo biomédico. En este trabajo se presentan resultados experimentales de la aplicación de la espectroscopia de absorción y reflectancia para la evaluación ex-vivo de tejido cervical pre-canceroso y canceroso. Los resultados obtenidos pueden interpretarse como promisorios y develan una metodología que podría integrarse a la lectura convencional de papanicolaos o a campañas de tamizaje y prevención de muertes por cáncer cervicouterino.

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I. INTRODUCTION

Cancer diagnosis is among the worldwide established priorities recognized by the World Health Organization (WHO). Nearly ten millions of people die every year as a consequence of cancer and its incidence continues to grow at accelerated rates, with estimations to be doubled by 2035 [1, 2]. This makes extremely important the implementation of efficient and cost-effective diagnosis procedures to better assess in early stages the tissue characteristics and prevent cancer from developing. The WHO foresees an increase in the diagnosed cases worldwide in the next decades with more than 80 % of new cases found in less-developed countries [3]. In a comprehensive study by Murillo *et al.*, cervical cancer is recognized as leading mortality cause in Latin American countries [4]. It is one of the most frequent cancers worldwide, with estimations of 266,000 deaths only in 2012 [5, 6].

There exist several techniques to address the study of cervical tissue in terms of morphology, composition, and other elements. The standard screening method is cervical cytology, also called "Pap test". This method helps detect abnormal cells in the cervix, which can develop into cancerous cells. In this way, screening reduces the risk of developing cervical cancer. If cancer is already present, early detection with screening improves the chances of recovery. However, cytologies are not always completely accurate [7]. Sometimes, test results can appear normal even if there is a cancer or abnormal cells in the lining of the cervix. Some women who receive cytology results may therefore be wrongly diagnosed. Optical techniques are pushing the development of non-invasive methods for cancer diagnosis and characterization as only light interacts with the tissue. These methods can be classified into two main groups: those which use light to excite or illuminate the biological sample and collect a response from the media (fluorescence microscopy [8], laser speckle imaging [9], spectroscopy-based methods [10], among others.) and those which aim at studying directly the internal structure of tissues (optical coherence tomography, photoacoustics [11], just to mention a few techniques.).

A potential implementation of optical techniques in cervical cancer diagnosis is found in the analysis of optical properties of cervical cells and tissue. By registering absorption and reflectance spectra from these samples, features of abnormal tissues can be identified as they are compared with model healthy tissue. Thereby, early diagnosis of cervical cancer can be performed by analyzing cells extracted from the cervix, thus avoiding other more invasive established methods such as biopsy. To the best of our knowledge, this intermediary protocol has not been exploited so far.

In this paper, we present a set of experimental analysis of cervical tissue using absorption and reflectance spectroscopy. In this context, the optical parameters of tissues are interrogated to study the main features leading to a clinical criteria. We aim thereby to describe the spectroscopic features of healthy, pre-cancerous and cancerous cervical tissue with

clinical perspective.

II. MATERIALS AND METHODS

In the experiments performed pre-cancerous and cancerous cervical tissues are studied by absorption and reflectance spectroscopy. Samples are collected during conventional cytological analysis of salvadoran female patients.

II.1. Sample collection procedures

Samples were collected using two different methods. In our investigation, we proceeded to the optical analysis of the collected cells which were previously classified with microscopy as healthy, pre-cancerous cervical intraepithelial neoplasia (CIN II) and cancerous carcinoma in-situ (CIN III). A smear test is performed on the patients following the standard procedure for this analysis [12]. In this method, a speculum is used to provide access to the cervix and a spatula is employed to smear the outer surface. This enables cells to get attached to the spatula surface, which are deposited in a glass plate for further lab treatment. In this procedure, the papsmear are colored with different dyes, which enable the identification of different cells and possible anomalies by microscopic observation. Cells are then subject to spectroscopic analysis to obtain their absorption and reflectance spectra.

For patients with clear identification of cells with cancerous features, a second sample collection using a colposcopy procedure is applied. In this method, a small portion of tissue is extracted from the cervical surface using a biospy Tischler punch. This tissue is also examined by using absorption and reflectance spectroscopic techniques.

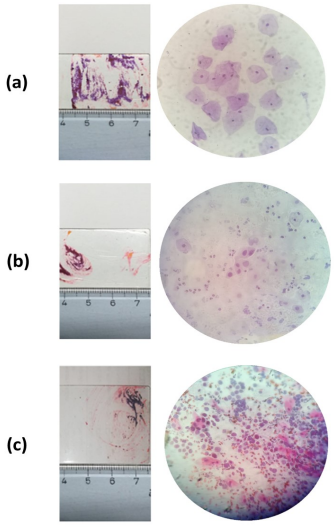

Figure 1. Optical imaging of the cell samples in between two plates and with x40 magnification. a corresponds to normal cells, b presents CIN II cells and c shows carcinoma *in-situ* CIN III cells.

The samples are represented in Fig. 1, where the studied cells are imaged with x40 magnification. The sample holders are also presented and scaled to illustrate how the cells are held in between two glass plates.

II.2. Optical set-up

In order to study our samples with the absorption and reflectance spectroscopies, an optical system projects light on the biological tissue to be evaluated. The light source is varied depending on the technique. For absorption spectroscopic measurements, a deuterium lamp is used and for reflectance measurements, a tungsten lamp is employed. Schemes of the optical setups employed are presented in Fig. 2 and Fig. 3.

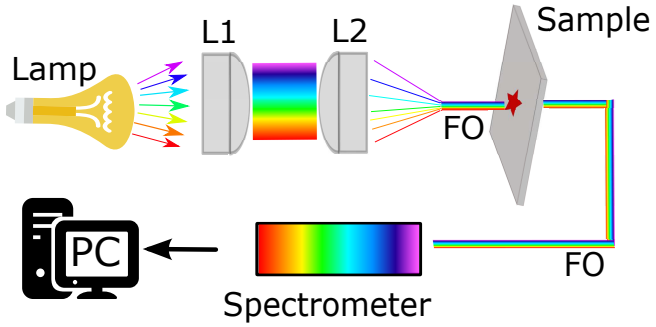

Figure 2. Optical setup employed for absorption spectroscopic measurement. L1 and L2 represent the set of lenses for beam shaping and FO is the optical fiber.

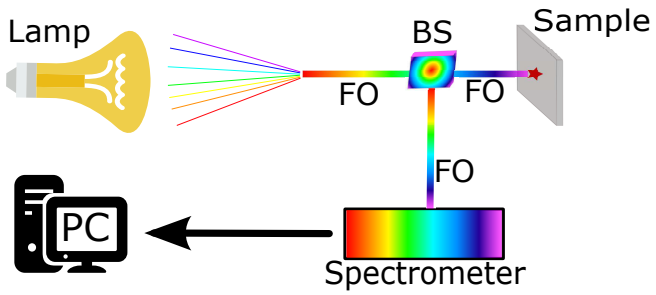

Figure 3. Optical setup employed for reflectance spectroscopic measurement. A beam splitter (BS) is used to derive the light towards the samples and the spectrometer simultaneously using the fiber optic (FO).

The spectrometer employed is an Ocean Optics model USB400. It is Czerny-Tuner configured with a focal distance of 42 mm at the input and 68 mm at the output. It has a plane diffraction grating with higher efficiencies between 300 and 400 nm and a spectral resolution of 1.5 nm in between 250 and 859 nm. It incorporates a cylindrical convex plane lens to reduce the image to the aperture of the detector. The detector operates in ambient temperature and integrates a Toshiba TCD130AP silicon charge-coupled device.

The information provided by the CCD device incorporated in the spectrometer is interfaced with the computer using an analog-to-digital converter and spectra are digitally registered using a USB port. Data acquistions are configured using the software SpectraSuite.

III. RESULTS

The absorption spectra for healthy (normal), pre-cancerous (cervical intraepithelial neoplasia, CIN II) and cancerous (carcinoma in-situ, CIN III) cells extracted from the cervical tissue are presented in Figure 4. They have two clearly formed

bands with maxima at 532 nm and 600 nm, which correspond to the absorption bands of hemoglobine [13]. The maximum located at 532 nm corresponds to the oxihemoglobin [14]. The spectral characteristics of this tissues are highlighted from the absorption measurements and the differences between a normal tissue and tissues with dysplasia are evident. Healthy tissues absorb much less of excitation light. The maximums remain at the same wavelengths and the spectral shape is the same in each type of cells. Moreover, as can be easily appreciated, absorption is higher in the pre-cancerous tissue with more advanced dysplasia associated (CIN III > CIN II) as compared to a normal tissue.

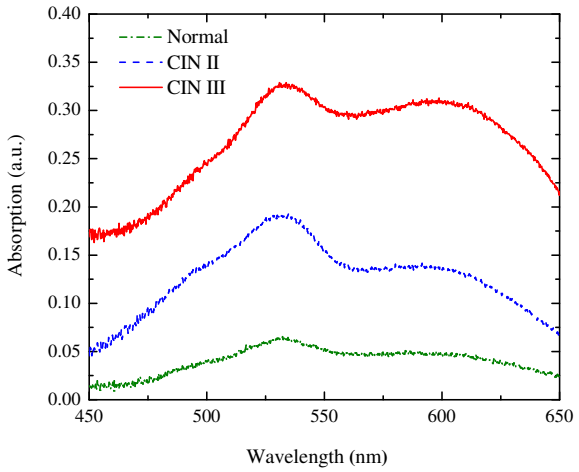

Figure 4. Absorption spectra of normal (healthy), CIN II and CIN III tissues. For better distinction among the plots, the reader is referred to the online version of the article where the plots appear in color.

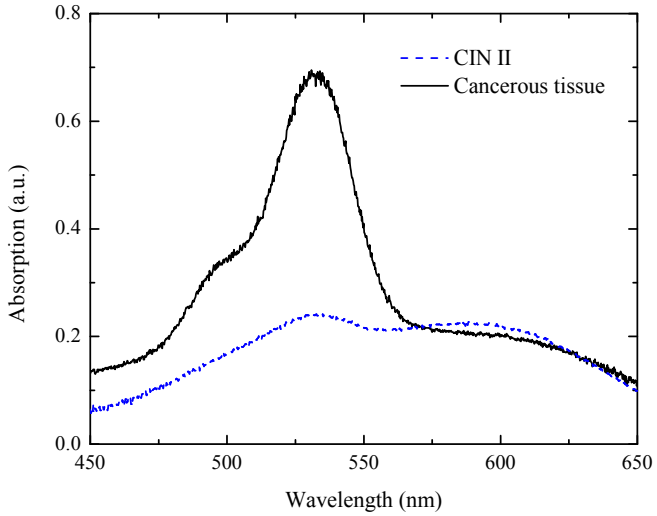

Figure 5. Comparison of the absorption spectra for cancerous cervical tissue and CIN II cells. For better distinction among the plots, the reader is referred to the online version of the article where the plots appear in color.

A comparison of a cancerous cervical tissue with a cervical intraepithelial neoplasia (CIN II) is presented in Figure 5. It can be appreciated the localization of the absorption maxima around 532 nm. It is clear the correlation existing between the abnormal tissue and its absorption features.

The absorption features shown in Figures 4 and 5 demonstrate that early assessment of the cervix using only the extracted cells is possible, thus avoiding the invasive nature of biopsies as much as possible and providing spectroscopic results on the degree of the cancer manifestation without extracting small portions of tissue, thus making the procedure less painful and still reliable. In addition, these cells samples can be directly examined in a microscope for further analysis in search for pathologies.

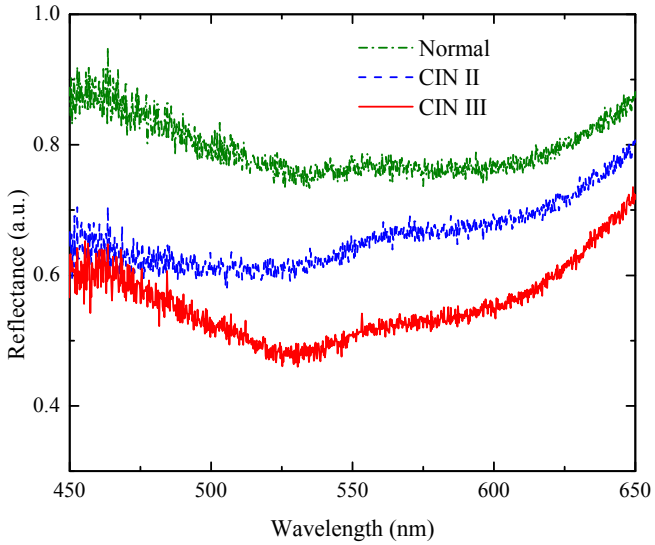

Figure 6. Reflectance spectra for cells with different classifications: healthy (normal), CIN II and CIN III. For better distinction among the plots, the reader is referred to the online version of the article where the plots appear in color.

For the absorption measurements the main contributions to the band are associated to the hemoglobin. This is explained by the fact that as cancer develops, other processes such as angiogenesis become relevant. Angiogenesis is a mechanisms ruling the formation of new blood vessels from existing vessels and is well related to the presence of cancer [15].

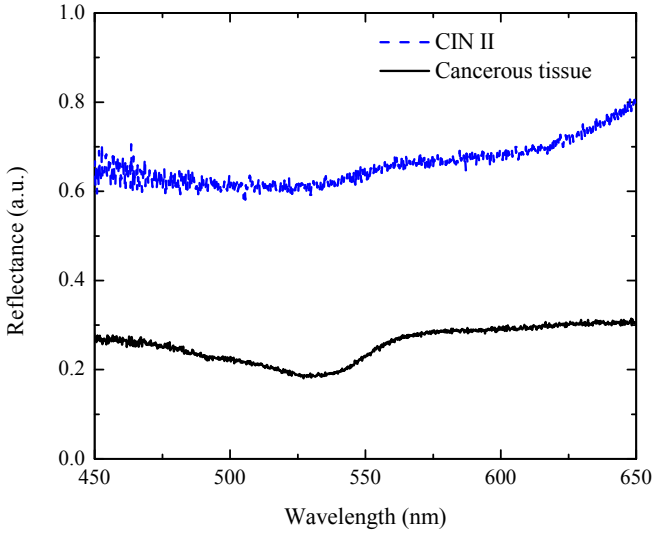

Figure 7. Comparison of reflectance spectra of cancerous tissue and cells (CIN II). For better distinction among the plots, the reader is referred to the online version of the article where the plots appear in color.

The reflectance spectra for cell samples extracted from cervical tissue are shown in Figure 6. As evidenced in these graphs, the results indicate an optical response aligned with the

absorption results presented in Figure 4. The more cancerous the cells are the less reflective and more absorptive they behave. Furthermore, the processes involved in the optical properties of these cells explain these results. As cells grow in size, their nuclei also grow, which turns to contribute significantly to the optical properties of the sample: it absorbs more light and reflects less [16].

The sample with cells issued from the smear test reflect more light than the extracted tissue as evidenced by the spectra from Figure 7, and maintain the spectral profile in the region 450-650 nm. Therefore, by using these reflectance features, early evaluation of the cervix can be performed, leading to quantification of the status of the cervical tissue. The threshold enabling the diagnosis is around 0.3 for the reflectance.

IV. CONCLUSIONS

Absorption and reflectance spectroscopies can potentially be employed to assess cervical cells and tissue. The spectral features provide information connected to degrees of development of cancer. The described methods enable this optical non-invasive technique to be considered in practical clinical assessment to enable an accurate diagnosis. Biopsies can be avoided using the methodology explained here and early detection of cervical cancer is enabled with non-invasive protocols.

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