Non-invasive spectroscopic techniques in the diagnosis of non-melanoma skin cancer

E Drakaki¹, IA Sianoudis³, EN Zois², M Makropoulou³, AA Serafetinides³, CDessinioti⁴, E Stefanaki⁴, AJ Stratigos⁴, CAntoniou¹, AKatsambas⁴, EC Christofidou⁴

¹Dept. of Optics & Optometry, Technological Educational Institute of Athens, Greece
²Laboratory of Telecommunications and Signal Processing, Dept. of Electronic Engineering, Technological Educational Institute of Athens, Greece
³School of Applied Mathematical and Physical Sciences, National Technical University of Athens, Greece
⁴Dept. of Dermatology, Medical School University of Athens, Hospital A. Syggros, Athens, Greece

E-mail: edrakaki@gmail.com

Abstract. The number of non-melanoma skin cancers is increasing worldwide and has become an important health and economic issue. Early detection and treatment of skin cancer can significantly improve patient outcome. Therefore there is an increase in the demand for proper management and effective non-invasive diagnostic modalities in order to avoid relapses or unnecessary treatments. Although the gold standard of diagnosis for non-melanoma skin cancers is biopsy followed by histopathology evaluation, optical non-invasive diagnostic tools have obtained increased attention. Emerging non-invasive or minimal invasive techniques with possible application in the diagnosis of non-melanoma skin cancers include high-definition optical coherence tomography, fluorescence spectroscopy, oblique incidence diffuse reflectance spectrometry among others spectroscopic techniques. Our findings establish how those spectrometric techniques can be used to more rapidly and easily diagnose skin cancer in an accurate and automated manner in the clinic.

Keywords: non-melanoma skin cancer, non-invasive diagnostic modalities, optical coherence tomography and fluorescence spectroscopy

1. Introduction

Skin cancers, both melanoma and non-melanoma skin cancers (NMSC), such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are still the most common of all human cancers and a growing health problem in most countries [1]. Although ionizing radiation based medical imaging continues to play an essential role in the diagnosis of cancer in several medical disciplines, in dermatology the discrimination of cancerous from healthy skin tissue is rather difficult with the conventional CT, PET or MRI techniques. The common routine diagnostic procedure for BCC and SCC is still the visual inspection, followed by skin biopsy and subsequent histopathology examination that takes days or weeks to produce a result. However, as early diagnosis of cancer is associated with improved prognosis, several non-ionizing radiation based, optical diagnostic, methods were developed to enable earlier detection of skin cancer, as laser induced fluorescence spectroscopy, diffuse reflectance spectroscopy, confocal microscopy, and optical coherence tomography [2]. These techniques have the potential to provide diagnosis of malignant and precancerous skin tissue, reducing...
the need for biopsy and the potential risk of tumor growth and/or metastasis, in some cases, because of time-extended procedures for histopathology based diagnosis. Our previous investigations showed that the laser induced fluorescence spectroscopy (LIF) is a useful tool to differentiate healthy from malignant (e.g. basal cell carcinoma - BCC, squamous cell carcinoma - SCC) skin tissue, eliminating the invasiveness of the biopsy and the cost and delay from histopathology procedures [3, 4]. Furthermore, our related research prospects are benchmarking fluorescence spectroscopy with other biophotonic techniques, e.g. optical coherence tomography (OCT). OCT has been previously used in ophthalmology and cardiology among other disciplines. In dermatology, OCT has been used in various pre-clinical studies and, in the latest years, it implements in medical practice [5]. OCT is able to visualize the epidermis, the upper dermis of skin, and blood vessels. OCT images have been shown to be useful in non-invasive monitoring and classification of non-melanoma skin tissues. The optical coherence tomography also provides an important tool for optical, noninvasive, delineation of the pathological area borders in surgical interventions and photodynamic therapy of skin lesions [6]. For these reasons this noninvasive diagnostic method also contribute to the minimization of the esthetic and psychological problems of malformation or scarring that may occurred by the biopsy and unnecessary or inappropriate skin excision of skin areas.

In this work, comparative studies of LIF and OCT results for non-melanoma skin cancer ex vivo diagnosis will be presented, while the possibilities of a relatively portable system are considered. The use of OCT in monitoring any change in the upper dermis and in deeper layers of skin tissue and the potential relation between the skin tissue optical properties in the relevant wavelengths for the LIF and OCT signals will also be discussed.

2. Materials and Methods

2.1. Tissue samples

All 25 skin tissue samples used in this study were obtained from 15 patients, which were clinically suspected for BCCs and SCCs and were already scheduled for Mohs micrographic surgery, at “A. Syggros” University Hospital. The skin samples were kept in a formalin solution, prior and after biophotonic measurements, while during the experiments they were held in constant temperature and humidity. All the samples were recorded with a classification number. After LIF spectroscopy or OCT imaging, a histopathology examination of the samples was performed and each sample was assigned as either normal skin or as diseased skin. The biopsy findings were used as the gold standard to compare the individual spectra for evaluation of the reliability of this diagnostic technique. Some OCT preliminary experiments were conducted on healthy human forearm skin of a volunteer and normally were not correlated with any microscopic skin image.

2.2. Experimental Setup.

For the LIF measurements, a Q-switched Nd:YAG laser operating at $\lambda=355$ nm, pulse width 6 ns, pulse repetition rate 1 Hz, energy fluence at 0.20 J/cm$^2$ was used for excitation. A PC plug-in HR2000 and in some cases a S2000 (slit width 25μm) micro spectrometer (Ocean Optics Inc, USA), controlled by a signal processing electronics and trigger system, was used for the laser induced fluorescence signals acquisition.

![Figure 1. Experimental set up of Optical Coherence Tomography.](image-url)
For the OCT measurements, all the experiments were conducted with the Thorlabs TELESTO SD-OCT Imaging System, a commercially available OCT scanner, non-specific for dermatologic imaging (Figure 1). With OCT imaging we obtained real-time two-dimensional cross-sectional and three dimensional volumetric images with micron-level resolution. The dimensions of the volumetric images span up to 10x10x2.5 mm, while the axial resolution was less than 7.5 µm in air. The OCT system is fully programmable with the relevant software.

3. Results and Discussion
Some representative results are shown in figures 2 and 3. The ex vivo samples, as that of figures 2a and 3a, were analysed histopathologically, after LIF spectral signal or OCT image acquisition, classified as normal, BCC or other skin lesion type.

![Figure 2](image)

(a) An excised human skin tissue sample, with basal cell carcinoma (BCC), and (b) the corresponding laser induced fluorescence spectra of the sample (excitation at λ=355 nm).

In figure 2a it is shown the photo of an excised human skin tissue sample (numbered 1627), suspected as basal cell carcinoma (BCC). The figure 2b exhibits the Laser Induced Fluorescence spectra of the skin sample 1627, where we can notice that the principal spectral areas are located at approximately 443 nm and 490-499 nm for the normal dermis with specific lesioned areas and malignant tissues with BCC only. The fluorescence of BCC showed significantly lower intensity compared to that of the normal dermis with specific lesioned areas. The fluorescence, excited at λ=355 nm, exhibits a broadband line shape with the peak spanning from 490 to 499 nm, attributed to the contribution of NADH, collagen and elastin. Spectral shape and intensity changes are noticed due to a decrease in collagen, elastin and NADH levels. We observed that the intensity in the blue region of the autofluorescence spectra is significantly reduced in BCC tissue area compared to dermis tissues, which shows that the concentration of NADH and NADPH in BCC tissues is lower than that in normal tissues.

![Figure 3](image)

(a) The digital image of a representative skin sample suspected for BCC malignancy, numbered 631. (b) The image of the top view of the tissue sample 631 recorded by the camera of the OCT device. (c) OCT image in B Scan mode of the tissue sample 631. (d) Histopathology analysis image of the previously examined by OCT skin sample 631.
The histopathology analysis of the skin sample 631 (figure 3d) reveals the following tissue details: i) macroscopic description: Fusiform skin patches 4.5 cm x 2 cm, with grayish lesion (mean diameter of 1.5 cm), ii) microscopic description: Texture of ulcerated BCC carcinoma, compact nodular type / total excision. As the gold standard of malignancy diagnosis, the histopathology analysis, shows that the examined by OCT area of the excised skin sample corresponds to BCC lesion, similar OCT images could be characterized accordingly.

Our preliminary results from the entire number of samples are in agreement with reported data in literature of OCT – skin diagnosis specialty, where BCC in OCT intensity imaging shows altered intensity signals when compared with surrounding dermis. From reported in vivo applications, OCT images show epidermis of lesioned skin significantly thinner and more signal-intense than the epidermis of non-lesioned skin [7], where basal cell nests show a characteristic hypo reflective border [8].

4. Concluding remarks and perspectives

Imaging with OCT could clearly differentiate the epidermis from the upper dermis, so that selected skin areas could be accurately measured and suspected skin areas are able to be revealed. LIF signals exhibited low intensity signals and a characteristic spectral shift in malignant skin tissues, while there were diagnostic difficulties in abnormalities below epidermis skin layer in wavelengths with low skin penetration (e.g. for 3rd harmonic Nd:YAG laser excitation at λ=355 nm). Specificity and sensitivity of LIF and OCT methods are compared with conventional histology and/or visual inspection analysis, with 83% of LIF spectra from NMSC were classified correctly as not normal, according to the histology examination results. OCT images distinguished lesion borders from diseased skin with 70% sensitivity and 60% specificity, which motivate us for further investigations in the future.

The object of this comparative study was to establish the possibilities of a relatively portable system that could combine a laser induced skin autofluorescence measurements device and an OCT to differentiate malignant from non-malignant skin lesions. However, as the sensitivity and specificity values are not yet optimized, we consider that more experimental and theoretical work is needed in this relative new field of dual diagnosis procedure. The optimization of the data analysis is essential for a potential use of these biophotonic emerging imaging techniques for clinical applications. For that, special care will be addressed on considering the safe levels of non-coherent and coherent (laser) radiation used for skin cancer diagnostic purposes, by consulting the relevant European and International guidelines on optical radiation exposure limits for both patients and personnel.

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