OPTOELECTRONICS AND ADVANCED MATERIALS – RAPID COMMUNICATIONS Vol. 4, No. 12, December 2010, p. 1960 – 1963

Combined optical techniques for skin lesion diagnosis: short communication

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Recent optoelectronic developments have helped scientists and dermatologists to design and improve equipments for the diagnosis of skin lesions. Among the numerous techniques which can be used in dermatology, optical coherence tomography (OCT) represents one of the best options, in terms of skin penetration depth and resolution, for retrieving morphological data of the skin. However, OCT has its own limitations and cannot offer the biochemical information of the skin. These data can be obtained with fluorescence spectroscopy, a non invasive, sensitive and real time technique. The present study aims to present the advantages of combining OCT with fluorescence spectroscopy for dermatological analysis. Examples of healthy skin and lupus erythematosus recorded with these techniques are given in this paper.

(Received November 22, 2010; accepted November 29, 2010)

Keywords: Optical coherence tomography, Fluorescence spectroscopy, Skin lesions

1. Introduction

Optoelectronic innovations have reached the dermatology domain with the development of new equipments which help physicians in the diagnosis of skin lesions. Skin diseases are generally diagnosed through visual examination by a trained dermatologist, procedure that usually implies some degree of objectivity [1, 2]. So, especially in the case of patients suspected of skin cancer, for a confirmation of the diagnosis excision biopsy and histology are used, which represent the gold standards for morphological investigations [3]. Due to the fact that biopsies are invasive and sometimes cause side effects, and that tissue sampling errors are often made, there is an increasing need for non-invasive, real-time and accurate methods for skin disease detection.

During the last decades, different techniques have been developed for dermatology, namely electrical impedance, ultrasound, confocal laser scanning microscopy, multispectral imaging, dermoscopy, digital photography, optical coherence tomography (OCT), fluorescence spectroscopy [4 – 8]. While some techniques, like electrical impedance or multispectral imaging, have not been sufficiently tested in order to give relevant data, other methods deal with issues such as limited penetration depth or resolution. For instance, confocal laser scanning microscopy is capable to provide very good resolution, but because of optical scattering the penetration depth is ~ 0.25 mm. On the opposite side, ultrasound gives very good penetration depth, up to 10 cm, but low resolution as depth increases [4, 9]. OCT fills the gap between microscopy and ultrasound with penetration depth of up to 2 mm, depending on the source wavelength and tissue, and resolution of 1 μm or less [6]. Also, according to Fercher [10], OCT is the only technique that offers cross-sectional imaging of the epidermis and upper dermis, *in vivo*. However, some OCT systems cannot properly analyse highly elevated lesions and have stability issues [11]. Hence, until these problems are addressed, recent studies concentrated on combining OCT with other techniques, like fluorescence spectroscopy, on colon cancer [12], ovarian cancer [13] or gastrointestinal tract diseases [14]. Fluorescence spectroscopy is non invasive and offers real time data about the biochemical composition of the tissue [15]. Despite the methods potential to characterise skin lesions, little attention has been given, so far, to the possibility of combining these two methods on the diagnosis of skin lesions. This paper aims to present, shortly, the principles of operation for OCT and fluorescence spectroscopy, with examples of normal skin and lesions, as a prospective approach in dermatology.

2. OCT principle

OCT is an optical technique that generates cross-sectional 2D and 3D images of backscattered or back reflected light from the tissue [10]. It is similar to ultrasound method, but it uses light instead of sound [6, 16]. OCT is based on Michelson interferometry, a more then 100 years old principle, and since its first realisation in 1991, it has been successfully applied to ophthalmology, cardiology, gynaecology or dermatology [17]. The low coherence light is split in two paths, by a beamsplitter. One path goes to the sample and the other to the reference mirror. The reflected light from the sample and reference mirror is then recombined and when the light path from the tissue is equal with the light path to the reference mirror and back, the constructed interference

occurs. So, the depth within the tissue is determined by the position of the mirror when constructed interference is detected [16]. This is a simplistic presentation of the method through which OCT distinguishes signals from different depths of the tissue [6]. The depth scan recording gives the A-scan. B-scans are generated by collecting numerous A-scans, in the x, y plane, forming a 2D image (Fig. 1) [16, 18]. By recording many B-scans on a certain surface a 3D image can be formed [6]. 3D images can be obtained by the use of an *en-face* OCT, which records T-scans and C-scans on a perpendicular plane to A-scans and B-scans. More details can be found in the studies of Podoleanu [18] and Bradu et al. [19].

The wavelength range for OCT is within 600 nm and 2000 nm, generated usually by superluminescent light diodes or swept laser sources. This spectral domain is optimum for biological tissues as their main constituents, water, pigments etc. are less absorbent in this region [18].

Depending on the wavelength, OCT can record two layers of the skin: epidermis and dermis, more precisely the upper (superficial) dermis. Stratum corneum (a sublayer of the epidermis) is generally visible at palmoplantar skin as a well-defined, thick, low scattering band containing sweat gland ducts [3]. Fig. 1b presents an OCT image of fingertip, where stratum corneum can be easily noticed with spiral, strong scattering sweat gland ducts [17]. The next layer is the epidermis, which at other skin sites is the first visible layer, since the stratum corneum is not thick enough [3]. Dermis, the last layer, has more intense signal compared with epidermis and therefore it shows more morphological information. Inside the dermis, blood vessels and hair follicles can be observed as darker areas, usually of round shape, and lower reflectivity lines, respectively [17]. Fig. 1 presents an example of venectasia where blood vessels are more pronounced.

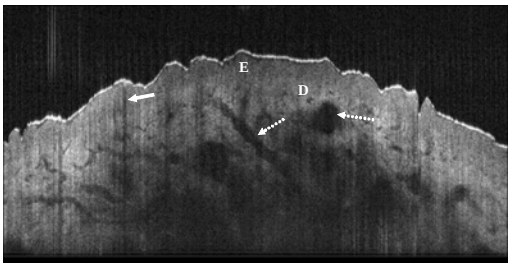


Fig. 1. OCT image for venectasia. E – epidermis, D – dermis, dotted arrow – blood vessels, simple arrow – hair follicle.

In the case of skin lesions, the uniformity of the layers is lost and low reflectance zones, with a particular form for each lesion, are found. More details on OCT analysis for various skin diseases can be found in Gamblicher et al. [3], Mogensen et al. [6] and Welzel [17].

3. Fluorescence spectroscopy principle

Fluorescence spectroscopy is a non-invasive, rapid and sensitive optical technique, successfully applied in numerous research fields. In the recent years, fluorescence spectroscopy is gaining popularity in medical studies, specifically as a potential method for *in vivo* disease diagnose [15].

Fluorescence is a special type of luminescence that describes the emission of light from molecules, named fluorophores, in electronically excited states. The fluorophores absorb energy in the form of light, at a specific wavelength, and release it in the form of emission of light, at a specific higher wavelength (i.e., with lower energy). The general principles of light absorption and emission can be illustrated by a Jablonski diagram, as seen in Fig. 2. When a molecule in its ground singlet state, S_0, absorbs light, the light energy is transferred to the electronically excited states: singlet states, S_1 or S_2. Afterwards, the molecule is subjected to internal conversion or vibrational relaxation, which implies the transition from an upper electronically excited state to a lower one, lasting from 10^{-14} to 10^{-11} s. In the final stage, emission occurs when the molecule returns to the ground state, S_0, in 10^{-9} to 10^{-7} s, emitting light at a greater wavelength, according to the difference in energy between the two electronic states. This process is known as *fluorescence* [20]. When excitation source is a laser, the process is called *laser induced fluorescence*.

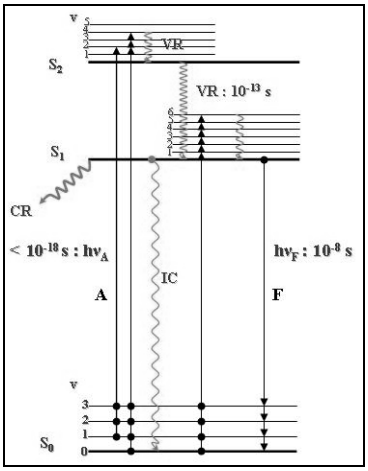


Fig. 2. Jablonski diagram presenting the processes of absorption (A) and fluorescence (F); VR – vibrational relaxation, CR – chemical reaction, IC – internal conversion.

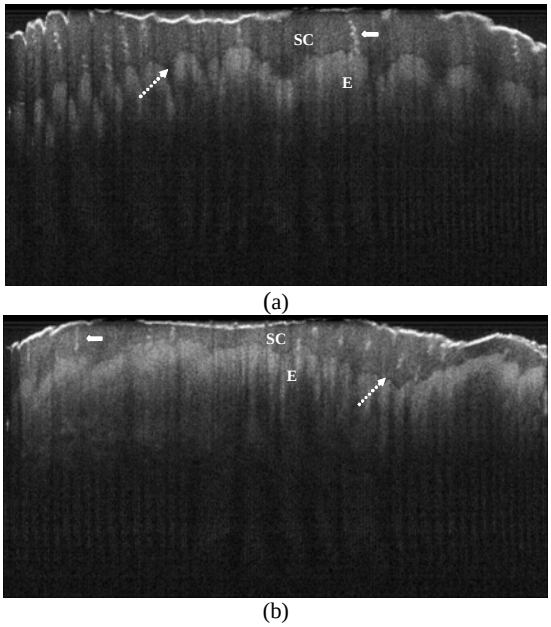
The fluorescence signal in biological tissues is given by extrinsic and intrinsic fluorophores. Although, extrinsic fluorophores are more fluorescent then intrinsic fluorophores and have known photophysical and pharmacokinetic properties, they present issues regarding the safety and toxicity of the drug being used. Therefore, it is much safer to analyse the intrinsic fluorophores. Typical intrinsic fluorophores detected in skin include tryptophan, tyrosine, collagen, elastins, porphyrins, flavins and lipids [21, 22]. According to Gillies et al. [21], the fluorescence at excitation wavelengths shorter then 315 nm comes from

the epidermis and at excitation wavelengths longer then 315 nm fluorescence is attributed to the dermis layer.

4. Combined OCT and fluorescence spectroscopy

As shown in previous sections, OCT and fluorescence spectroscopy are very effective in the diagnosis of skin lesions, but when used in combination, complementary data can be obtained, enabling higher sensitivity and specificity to the state of a certain disease [23]. Combined OCT and fluorescence spectroscopy technique has been applied on colon cancer [12], aorta [23] or uterine cervix [24, 25]. All studies have shown that the combination produced less false positive results then either method used separately and may be very effective in monitoring the effects of treatments [12]. To the authors knowledge, only one study [26] has taken into consideration the combination of these two techniques for skin lesion diagnosis, offering promising results in the diagnosis of basal cell carcinoma.

In order to evidence the effectiveness of combining OCT with fluorescence spectroscopy, for dermatology, an example of healthy skin and lupus erythematosus measured on the same patient is shown. Fig. 3a presents an OCT image of a healthy fingertip, where stratum corneum can be easily noticed with spiral, strong scattering sweat gland ducts. The next visible layer is the dermis, which is separated from stratum corneum by a highly absorbent layer, stratum granulosum (the second layer of the epidermis). As can be seen in Fig. 3b, lupus erythematosus produces hyperkeratosis of the stratum corneum and atrophy of the epidermis. Similar results have been obtained by Gamblicher et al. [27].

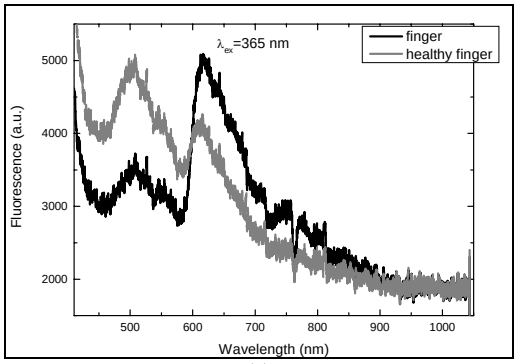


(a)

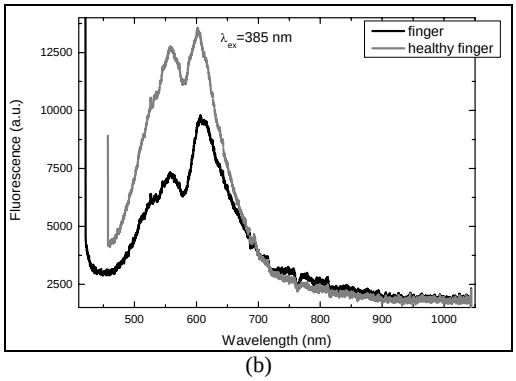
(b)

Fig. 3. OCT images for (a) healthy and (b) lupus erythematosus on fingertip. SC – stratum corneum, E – epidermis, dotted arrow – stratum granulosum, simple arrow – sweat gland ducts.

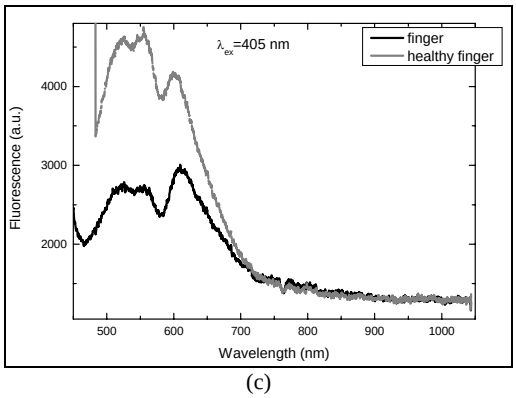
The example of fluorescence spectra on skin is shown in Fig. 4 for lupus erythematosus and normal skin, at three excitation wavelengths (365 nm, 385 nm and 405 nm). All spectra present three major fluorescence peaks, two of them belonging to lipids, ~ 510 nm and 550 nm, and one peak to porphyrin, ~610 nm. The fluorescence intensity of all peaks is lower for the lesional skin compared to healthy skin. These results are confirmed by other studies, like Lohmann et al. [28], Chwirot et al. [29] or Drakaki et al. [7]. Lohmann et al. [28] state that fluorescence intensity decreases linearly with the distance from the centre of the lesion to healthy skin boundaries. Therefore, fluorescence spectroscopy can be used in various surgical procedures by defining the margins of resection [15].



(a)



(b)



(c)

Fig. 4. Fluorescence spectra for healthy and lupus erythematosus fingertip, at three wavelengths: (a) 365 nm, (b) 385 nm and (c) 405 nm.

Based on the presentations above, it can be concluded that if OCT and fluorescence spectroscopy are combined, the biochemical and morphological data of the skin can be simultaneously obtained. Also, the disadvantages of both techniques can be surpassed. For example, the fluorescence signal is influenced by cosmetic creams [30] and some OCT systems cannot be properly used on elevated skin lesions. Therefore, it is recommended to utilize these two optical techniques, OCT in combination with fluorescence spectroscopy, for dermatology in order to obtain complementary data.

5. Conclusions

Due to recent optoelectronic advances, OCT and fluorescence spectroscopy may be applied, with success to dermatology, as non invasive, real time and accurate methods. However, the combination of OCT and fluorescence spectroscopy can be more effective because it offers complementary data, biochemical and morphological, with less false positive results. The examples of healthy skin and lupus erythematosus, shown in this study, highlighted the capabilities of OCT and fluorescence spectroscopy, used in combination.

OCT distinguished the surface layers of the epidermis, stratum corneum and stratum granulosum, from the fingertip. Also, the blood vessels in the dermis and hair follicles were seen in an OCT image for venectasia. Furthermore, OCT images showed structural changes in lupus erythematosus on fingertip skin, like atrophy of the epidermis and hyperkeratosis. Fluorescence spectroscopy generated emission spectra with higher intensity for healthy skin compared to lupus erythematosus, identifying three major peaks which correspond to lipids and porphyrin. As a consequence of this study, the combination of these two optical techniques may be useful in dermatology by providing both biochemical and structural data of the skin.

Acknowledgements

This work was financially supported by the Research Project TE 160, through the contract No. 39/28.07.2010 between UEFISCSU and Carol Davila University of Medicine and Pharmacy Bucharest, within the Human Resources Program- PN II.

References

[1] J. Weissman, T. Hancewicz, P. Kaplan, Opt. Express **12** (23), 5760 (2004).
[2] J. Smolle, Color atlas of melanocytic lesions of the skin, Editors: H. P. Soyer, G. Argenziano, R. Hofmann-Wellenhof, R. H. Johr, Springer, 47 (2007).
[3] T. Gambichler, G. Moussa, M. Sand, D. Sand, P. Altmeyer, K. Hoffmann, J. Dermatol. Sci. **40**, 85 (2005).
[4] Y. A. Glickman, O. Filo, M. David, A. Yayon, M. Topaz, B. Zamir, A. Ginzburg, D. Rozenman, G. Kenan, Skin Res. Technol. **9** (3), 262 (2003).
[5] K. Nouri, Skin Cancer, McGraw-Hill Professional, 655 (2007).
[6] M. Mogensen, L. Thrane, T. M. Joergensen, P. E. Andersen, G. B. E. Jemec, Semin. Cutan. Med. Surg., **28**, 196 (2009).
[7] E. Drakaki, E. Kaselouris, M. Makropoulou, A. A. Serafetinides, A. Tsenga, A. J. Stratigos, A. D. Katsambas, C. Antoniou, Skin Pharmacol. Physiol. **22**, 158 (2009).
[8] N. Kollias, G. Zonios, G. N. Stamatas, Vib. Spectrosc. **28** (1), 17 (2002).
[9] J. Fujimoto, W. Drexler, Optical coherence tomography: technology and applications, Editors W. Drexler and J. G. Fujimoto, Springer, (2008).
[10] A. F. Fercher, Z. Med. Phys., in press (2010).
[11] A. M. Forsea, E. M. Carstea, L. Ghervase, C. Giurcaneanu, G. Pavelescu, J. Med. Life, in press (2010).
[12] L. P. Hariri, A. R. Tumlinson, D. G. Besselsen, U. Utzinger, E. W. Gerner, J. K. Barton, Laser. Surg. Med. **38,** 305 (2006).
[13] E. Kanter, R. Walker, S. Marion, P. Hoyer, J. K Barton, Prog. Biomed. Opt. Imaging **6** (30), 58610P.1 (2005).
[14] L. P. Hariri, A. R. Tumlinson, N. H. Wade, D. G. Besselsen, U. Utzinger, E. W. Gerner, J. K. Barton, Comp. Med. **57** (2), 175 (2007).
[15] N. Ramanujan, Neoplasia **2** (1 – 2), 89 (2000).
[16] P. J. Tadrous, J. Pathol. **191**, 115 (2000).
[17] J. Welzel, Skin Res. Technol. **7,** 1 (2001).
[18] A. Gh. Podoleanu, Br. J. Radiol. **78**, 976 (2005).
[19] A. Bradu, A. Gh. Podoleanu, B. Rosen, J. Optoelectron. Adv. Mater. **7** (6), 2913 (2005).
[20] J. R. Lakowicz, Principles of Fluorescence Spectroscopy, Third edition, Springer, New York (2006).
[21] R. Gillies, G. Zonios, R. Rox Anderson, N. Kollias, J. Invest. Dermatol. **115**, 704 (2000).
[22] A. Shahzad, M. Edetsberger, G. Koehler, Appl. Spectrosc. Rev. **45** (1), 1 (2010).
[23] J. K. Barton, F. Guzman, A. Tumlinson**,** J. Biomed. Opt. **9** (3), 618 (2004).
[24] R. V. Kuranov, V. V. Sapozhnikova, H. M. Shakhova, V. M. Gelikonov, E. V. Zagainova, S. A. Petrova, Quantum Electron. **32** (11), 993 (2002).
[25] N. M. Shakhova, V. V. Sapozhnikova, V. A. Kamensky, R. V. Kuranov, V. B. Loshenov, S. A. Petrova, A. V. Myakov, J. Appl. Res. **3** (2), (2003).
[26] E. Borisova, E. Carstea, L. Cristescu, E. Pavlova, N. Hadjiolov, P. Troyanova, L. Avramov, J. Innov. Opt. Health Sci. **2** (3), 1 (2009).
[27] T. Gambichler, J. Hyun, G. Moussa, N. S. Tomi, S. Boms, P. Altmeyer, K. Hoffmann, A. Kreuter, Lupus **16**, 35 (2007).
[28] W. Lohmann, Maturwissesschaften **75**, 201 (1988).
[29] B. W. Chwirot, S. Chwirot, J. Redzinski, Z. Michiniewicz, Eur. J. Cancer **34** (11), 1730 (1998).
[30] L. E. Rhodes, B. L. Diffey, Br. J. Dermatol. **136**, 12 (1997).

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