J Cancer Res Clin Oncol (2013) 139:1083–1104
DOI 10.1007/s00432-013-1423-3

REVIEW

Optical techniques for the noninvasive diagnosis of skin cancer

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Received: 4 March 2013 / Accepted: 21 March 2013 / Published online: 4 April 2013

Abstract
Purpose The aim of this paper is to provide an overview of the most investigated optical diagnostic techniques: optical coherence tomography, fluorescence spectrometry, reflectance spectrometry, Raman spectroscopy, and confocal microscopy.
Methods A search of three databases was conducted using specific keywords and explicit inclusion and exclusion criteria for the analysis of the performances of these techniques in the pre- and postoperative diagnosis of skin cancers.
Results Optical coherence tomography has shown promising results in the assessment of deep margins of skin tumors and inflammatory skin diseases, but differentiating premalignant from malignant lesions proved to be less effective. Fluorescence spectroscopy proved to be effective in revealing the biochemical composition of tissue; early detection of malignant melanoma was reliable only with stepwise two-photon excitation of melanin, while tumoral margin assessment and differential diagnosis between malignant and non-malignant lesions showed some conflicting results. Characterization of the structural properties of tissue can be made using diffuse reflectance spectrometry, and the values of the specificity and sensitivity of this method are ranging between 72–92 % and 64–92 %, respectively. Raman spectroscopy proved to have better results both in carcinoma and melanoma diagnosis with sensitivities and specificities above 90 % and high above 50 %, respectively. Confocal microscopy is the closest technique to pathological examination and has gained the most clinical acceptance, despite the need for a standardization of the interpretation algorithm.
Conclusions In conclusion, these optical techniques proved to be effective in the diagnosis of skin cancer, but further studies are needed in finding the appropriate method or combination of methods that can have wide clinical applications.

Keywords Optical coherence tomography · Fluorescence spectrometry · Reflectance spectrometry · Raman spectroscopy · Confocal microscopy · Sensitivity and specificity indices

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Introduction

The need for more objective and quantitative methods to support the diagnosis is a priority for physicians, biologists, physicists, and engineers, and new optical imaging and spectroscopic techniques have been developed in order to answer to this demand. Optical techniques can provide

noninvasive, low-cost methods for a variety of applications. Techniques like optical coherence tomography (OCT) and fluorescence imaging have been widely evaluated for imaging of the retina and cancer diagnosis, respectively (Framme et al. 2005; Drexler and Fujimoto 2008; He et al. 2010; Alex et al. 2011). Reflectance spectroscopy is a well-known method in getting information on optical properties of tissue that can be used in skin cancer evaluation (Morales and Montiel 2012). Raman spectroscopy (RS) and confocal microscopy have gained some clinical acceptance after multiple trials (de Paula and Sathaiah 2005; Amjadi et al. 2011). All these methods offer, with minimal costs, new ways of accurate differential diagnosis between benign and malignant skin lesions.

This article aims to present five of all these techniques used for the diagnosis of skin cancer (OCT, fluorescence spectrometry, reflectance spectrometry, Raman spectroscopy, and confocal microscopy) and to analyze their value, based on a review of the available literature. Several other promising optical techniques such as near-infrared spectroscopy, differential pathlength spectroscopy, and coherent backscattering spectroscopy are not included in this study because they are relatively less studied in clinical diagnosis of skin diseases.

This study will try to answer the following questions: is there a place for clinical use of these methods in skin cancer evaluation? What devices/parameters can be used in optical diagnostics? What is the specificity and selectivity of optical methods for diagnosis of skin malignancies?

Methods

A review of the literature published between 2002 and 2012 on the optical methods for the diagnosis of skin cancers was performed. A rigorous search of major databases (including MEDLINE, CANCERLIT, and PubMed) was performed using specific keywords. "optical diagnosis" was associated with: "skin," "cancer," "cancerous," "malignant," "precancerous," "premalignant," "dermatology," and the following phrases used as text words: "skin cancer," "benign lesions," "skin imaging," "clinical diagnosis of skin disease," "spectroscopic method," "imaging method," "optical coherence tomography," "OCT," "fluorescence spectrometry," "reflectance spectrometry," "Raman spectrometry," "confocal microscopy," "sensitivity and specificity of diagnostic."

The relevant articles and abstracts that met the above criteria were selected for inclusion: (a) reviews, guidelines, and clinical trials, and (b) the device type, parameters, and sensitivity and specificity indices of the method were reported. We have excluded articles that dealt with laboratory experiments, although some of them were the bases for clinical trials included in our research. The papers published in a language other than English were also excluded.

Results

Optical coherence tomography (OCT)

Advances in optics, fiber, and laser technology have enabled the development of a novel noninvasive optical biomedical imaging technique, OCT. The first in vitro tomogram of the human eye was presented in 1991. In dermatology, OCT was introduced in 1995 (Schmitt et al. 1995) and is now increasingly used in clinical skin research (Gambichler et al. 2005).

Optical coherence tomography is an interferometric, noninvasive tomographic imaging technique that generates cross-sectional 2D and 3D images of backscattered or back-reflected light from the tissue, ideal for real-time clinical applications such as in vivo surgical monitoring. OCT represents one of the best options, in terms of depth of skin penetration and resolution, for collecting morphological data of the skin. In its basic configuration, OCT systems employ Michelson interferometer. The light is split into two arms: a sample arm and a reference arm. An interference pattern is obtained only when the length of the reference arm of interferometer corresponds to the length of the sample arm within the coherence length of the light source.

Optical coherence tomography has been used until now as high-resolution imaging technique in the diagnosis of various diseases of: eyes (Bijlsma and Stilma 2005; Figurska et al. 2010), gastrointestinal tract (Sivak et al. 2000; Chen et al. 2007), vascular tissue (Farooq et al. 2009), dental tissue (Fried et al. 2002; Otis et al. 2003), and skin. The utility and the accuracy of OCT technique in diagnosing and monitoring skin cancerous and non-cancerous tumors are presented in Table 1.

We can see in Table 1 that the most clinical studies of the OCT have focused on basal cell carcinoma (BCC). Studies performed by Jorgensen et al. (2008) and Mogensen et al. (2009), on approximately 130 patients with 200 BCC lesions, have indicated that OCT images of BCC exhibit characteristic dark circular structures as compared with normal skin that has a layered structure. Some changes in the epidermal architecture and flattening the upper layer of dermis can also be observed. Analysis of these key indicators that fit well with histopathological test suggest that OCT can be used in the diagnosis of BCC. The main limitation of OCT in diagnosing BCC is its inability to identify subtypes of BCC (Mogensen et al. 2009). Assessment of the deep margins of the BCC is very

Table 1 Application of OCT technique for the diagnosis of skin cancer

Disease	Device/parameters	Results	Reference
Basal cell carcinoma (BCC)	M2-OCT system (LightLab Imaging Inc., Westford, MA, USA)	Twenty patients with biopsy-confirmed BCC were diagnosed by OCT. Depth of the neoplasm was determined through a computer-generated depth scale and direct measurement on analogous tissue specimens using a microscope micrometer. The results have demonstrated a good correlation between tissue thickness determined by OCT and routine histologic tests	Olmedo et al. (2007)
Basal cell carcinoma	–	Twenty-three patients with 49 lesions were selected for the study. Basal cell carcinoma was identified in 20 patients. The results demonstrated that there is excellent correlation between OCT images and histopathologic features of superficial, nodular, micronodular, and infiltrative basal cell carcinomas. The predictive value of OCT has not been evaluated due to the small number of patients	Olmedo et al. (2006)
Basal cell carcinoma	Swept-Source-OCT-System (OCS1300SS, Thorlabs, Dachau/Munich) $\lambda = 1{,}325$ nm Bandwidth = 100 nm Axial resolution = 12 μm Lateral resolution = 15 μm	Ten BCCs from 10 patients were included in this study. The accuracy of OCT and high-frequency ultrasound (HFUS) measurements in vivo was compared. The results demonstrated that OCT was superior to HFUS in terms of tumor thickness assessment	Hinz et al. (2011)
Basal cell carcinoma	–	In this study, 22 BCCs were examined by new high-definition OCT (HD-OCT) using the en-face and slice imaging mode. The characteristic morphologic features of BCC were evaluated in comparison with the histopathological test results. Results obtained in this study have shown that in the en-face mode, the lobulated structure of the BCC was more distinct than in the slice mode compared to histology. These results demonstrate that HD-OCT is better than conventional OCT giving additional information in the diagnosis of BCC	Maier et al. (2012)
Basal cell carcinoma (BCC) actinic keratosis (AK)	OCT scanner (Risoe National Laboratory, Roskilde, Denmark) $\lambda = 1{,}310$ nm Bandwidth = 60 nm $P = 20$ mW Axial resolution = 10 mm Lateral resolution = 20 mm	Forty-one BCC and 37 AK lesions from 34 patients were enrolled in this study. The lesions were located in different areas of the body: scalp, face, neck, lower back and upper extremities. The results demonstrated that OCT images of BCCs exhibit dark globules corresponding to basaloid islands. For AK lesions, white dots and streaks corresponding to hyperkeratosis were identified in OCT images. Classification accuracies of 73 % (AK) and 81 % (BCC) have been achieved using machine-learning analysis	Jorgensen et al. (2008)
Basal cell carcinoma actinic keratosis (AK) benign lesions	OCT system (Technical University of Denmark) $\lambda = 1{,}318$ nm Bandwidth = 66 nm Axial resolution = 8 μm Lateral resolution = 24 μm	One hundred four patients with 176 lesions were recruited. Sensitivity was 79–94 % and specificity was 85–96 % in differentiating normal skin from lesions. Location of basal cell carcinoma was in areola mammae	Mogensen et al. (2009)

Table 1 continued

Disease	Device/parameters	Results	Reference
Sun-damage skin actinic keratosis (AK)	BBS1310 JDS Uniphase (Ontario, Canada) $\lambda = 1{,}310$ nm Bandwidth = 50 nm $P = 11.5$ mW Axial resolution = 12 µm Lateral resolution = 12 µm	OCT images were acquired of 112 patients, with a subsequent biopsy. OCT image analysis revealed a statistically significant difference ($P < 0.0001$) between the average attenuation values of skin with minimal and severe solar elastosis and between undiseased skin and AK. The results of this study also demonstrated that AK could be distinguished from undiseased skin with 86 % sensitivity and 83 % specificity	Korde et al. (2007)
Sun-damaged skin actinic keratosis	BBS1310, JDS Uniphase (Ontario, Canada) $\lambda = 1{,}310$ nm Bandwidth = 50 nm $P = 11.5$ mW Axial resolution = 12 µm	In this study 20 patients with sun-damaged skin and actinic keratoses were investigated. OCT image analysis revealed that: Sun-damaged skin is characterized by increased signal in the epidermis and rapid attenuation of light; Actinic keratosis is characterized by high surface reflection, the presence of a low-signal band in the stratum corneum, and heterogeneous appearance in the epidermis/dermis; OCT method presents a sensitivity of 79 % and specificity 100 % for AK	Barton et al. (2003)

important in orienting surgical treatment, and OCT proved to have a good correlation with pathological examination (Olmedo et al. 2006, 2007; Hinz et al. 2011) and was superior to ultrasound imaging (Hinz et al. 2011).

A number of studies brought evidence of the performance of OCT technique in actinic keratosis (AK) diagnosis (Barton et al. 2003; Korde et al. 2007; Jorgensen et al. 2008; Mogensen et al. 2009). These studies indicate that AK, characterized by dysplasia and architectural disorder of the epidermis, appears in OCT images as white dots and streaks. The OCT accuracy in the diagnosis of AK has been assessed only in two studies (Korde et al. 2007; Jorgensen et al. 2008). In distinguishing AK from normal skin, a sensitivity of 79–86 % and a specificity of 83–100 % were found for OCT technique. It proved difficult to distinguish between AK and BCC using OCT. However, Jorgensen et al. (2008) using machine-learning analysis have developed classification models able to differentiate AK from BCC with a success rate of 73 % and BCC from AK with a success rate of 81 %.

In parallel with the development of medical applications of OCT, new OCT systems for dermatological purposes have been developed, some of which are already commercially available (e.g., LightLab Imaging Inc., Westford, MA, USA; OCS1300SS, Thorlabs, Dachau/Munich; BBS1310, JDS Uniphase, Ontario, Canada). Recent progress in the field of optoelectronics has led to more efficient OCT systems such as high-definition OCT (HD-OCT) system. The use of such OCT systems in dermatology may increase the diagnostic accuracy (Maier et al. 2012).

Finally, we can conclude that OCT can assess deep limits of soft tissue lesions with an axial resolution of 3–15 µm, which proves to be an advantage in evaluating certain lesions. As a disadvantage, OCT cannot offer biochemical information. This kind of data can be obtained with noninvasive and sensitive fluorescence techniques or diffuse reflectance spectrometry.

Fluorescence techniques

Fluorescence techniques are currently used in clinical practice for both noninvasive diagnosing and monitoring of the medical treatment. The principle of fluorescence diagnosis is based on the interaction between light with a specific wavelength (usually from UV–Vis range) and endogenous or exogenous fluorophores present in biological tissue. The fluorescence phenomenon has been described accurately by physicists in the first half of the twentieth century. Briefly, when a molecule (fluorophore) absorbs energy in the form of photons of visible/ultraviolet light, it passes from its ground electronic state to one of the various vibrational states in the higher-energy excited

electronic state. From this excited state, the molecule may return to the basic state by radiative (fluorescence and phosphorescence) or non-radiative processes (internal conversion, non-radiation relaxation, and intersystem crossing). The fluorescence emission appears when the molecule in excited state loses energy gained by the absorption of the photon initially before return to lower energy states. This means that the emitted fluorescence has a lower energy than the absorbed light, so the wavelength of emitted fluorescence radiation is higher than that of the light absorbed since the photon energy is inversely proportional with its wavelength. Consequently, the fluorescence spectrum of a molecule will be shifted to higher wavelengths in comparison with the absorption spectrum. The intensity of radiation emitted by fluorescence is proportional to that of incident radiation. This is understandable because the number of molecules excited by the absorption of photons is proportional to the intensity of the incident radiation. Therefore, in order to obtain intense fluorescence radiation, the sample must be exposed to intense light radiation fluxes using high-intensity radiation sources and/or focusing radiation on the sample to be analyzed. As light sources, both incoherent (lamps, LED) and coherent light sources (lasers) can be used if they emit radiation that can be absorbed by the fluorophores present in the investigated sample.

Human skin contains several native fluorophores such as NADH, collagen, elastin, tryptophan, tyrosine, and porphyrins. (Richards-Kortum and Sevick-Muraca 1996) The fluorescent properties of these endogenous fluorophores have been studied widely until now, in correlation with the alterations in biochemical composition and tissue architecture induced by different pathologies. These studies have demonstrated that fluorescence technique [known as autofluorescence technique (AFT)] is a valuable tool for in vivo detection of various skin diseases and pathologies, especially skin cancer. The AFT has some advantages and disadvantages. The main advantages are its noninvasiveness, the real-time response, and low cost (it is not necessary to administer a drug to detect tissue fluorescence). The disadvantages of AFT are related to: low intensity of fluorescence radiation emitted by endogenous fluorophores, difficulty to distinguish among different fluorophores (because their emission and excitation spectral bands are wide enough so that often appears a spectral overlapping), and the complexity of interactions among different endogenous fluorochromes.

Tissue fluorescence can be improved by the administration of exogenous fluorophores (photosensitizers) with specific absorption and fluorescence properties which preferentially accumulate in diseased cells (especially cancer cells) and which, under the action of light radiation with specific wavelength, emit characteristic fluorescence radiation highlighting the injurious area. Such a technique is known as drug-induced fluorescence technique (DFT). The development of DFT for tissue diagnostics has come from parallel developments in photodynamic therapy of malignant lesions with fluorescent photosensitizers.

Clinical studies have demonstrated that DFT has a significant diagnostic advantage over AFT due to the increase in the fluorescence intensity by using exogenous fluorophores, but rises some problems related to: the choice of photosensitizer type, time interval between the photosensitizer administration and its exposure to light (which increases response time), side effects induced by photosensitizer administration, costs, and of course, necessary regulatory approvals.

Detection of fluorescence radiation (either autofluorescence or drug-induced fluorescence) may be achieved by two distinct ways: fluorescence spectroscopy and fluorescence imaging. Both techniques have become important modalities of investigation in clinical practice particularly in identification and localization of pre- and early cancerous lesions (Table 2).

We can notice from Table 2 that most of the clinical dermatology research on fluorescence diagnosis has focused, over the past decade, on the detection or diagnosis of melanoma and non-melanoma skin cancer as well as in demarcation of various skin cancers. Studies on early melanoma detection show that only the stepwise two-photon excitation of melanin autofluorescence gave some encouraging results in differential diagnosis between benign and malignant pigmented lesions (due to a low melanin autofluorescence) (Eichhorn et al. 2009; Leupold et al. 2011), but fluorescence was not compared with other noninvasive methods (like dermoscopy). Photobleaching analysis seems to generate some specific patterns, but the study takes into account many different lesions (Lihachev et al. 2011), while NIR fluorescence gives a different pattern for many lesions compared to normal skin, but does not show much clinical interest in terms of differential diagnosis (Huang et al. 2006).

Studies trying to assess the effectiveness of DFT in determining the BCC's lateral tumoral margins showed mostly discouraging results (Gambichler et al. 2008; Wetzig et al. 2010; Kamrava et al. 2012), the method is being less accurate than clinical examination. MAL-induced PpIX fluorescence imaging using fluorescence image analysis showed better results (Neus et al. 2008), but further studies are needed before the method becomes applicable. Early detection of skin malignancy (non-melanoma) was also a subject of investigation, DFT being evaluated mostly in comparison with histological examination. The results are conflicting from article to article, some of them showing good results in both sensibility and sensitivity of the method (Neus et al. 2008; Kamrava et al.

Table 2 Applications of fluorescence techniques for the diagnosis of skin cancer

Disease	Fluorescence technique	Device/parameters	Results	Reference
Melanoma	AFT	Nanosecond laser pulses $\lambda_{excitation} = 810$ nm	On the basis of a newly developed method to selectively excite melanin fluorescence of skin tissue by stepwise two-photon excitation, the authors of this study investigated information from this melanin fluorescence with respect to the differentiation of pigmented lesions. The results revealed a distinct difference between the melanin fluorescence spectrum of malignant melanoma (including melanoma in situ) and fluorescence spectrum of benign melanocytic lesions (i.e., common nevi) for freshly excised samples as well as for histopathological samples. A specific fluorescence was also recorded for dysplastic nevi. These results prove that early detection of malignant melanoma can be achieved by AFT	Eichhorn et al. (2009)
Melanocytic nevi malignant pigmented melanoma	AFT	–	In this study, using a new mode of stepwise two-photon excitation, melanin-dominated fluorescence spectra of pigmented skin lesions are reported. The results of this study revealed that the pure melanin fluorescence spectra of normal pigmented skin, melanocytic nevi, and malignant pigmented melanoma show distinctly different spectral shapes. Melanoma gave a characteristic fingerprint with a fluorescence band peaking at 640 nm, independent of the melanoma subtype. The melanin fluorescence spectra peaked at 590 nm for all types of common melanocytic nevi. In a series of 167 cases with melanocytic nevi and melanomas, the sensitivity of this new method to diagnose melanoma was 93.5 %, the specificity 80.0 %, and the diagnostic accuracy 82.6 %	Leupold et al. (2011)
Pigmented and vascular lesions	AFT	The fluorescence experimental setup: cw laser, optical fiber bundle and AvaSpec-2048-2 spectrometer $\lambda_{excitation} = 532$ nm DP = 65 mW/cm^2	In this study, 141 pigmented and vascular lesions were investigated by laser-induced skin autofluorescence photobleaching analysis. The results of this study revealed that: each of skin pathologies has a specific autofluorescence photobleaching characteristic; autofluorescence intensity of healthy skin decrease exponentially; autofluorescence intensity of pigmented nevi varies around the initial value; pigmented cellular nevus and cherry angioma have different dynamic features. Results of the present study show considerable sensitivity of skin pathologies of the autofluorescence photobleaching analysis method	Lihachev et al. (2011)
Vitiligo compound nevus nevus of Ota melanoma post-inflammatory hyperpigmentation	AFT	Fiber-optic NIR spectrometer $\lambda_{excitation} = 785$ nm	Twelve patients with: vitiligo, compound nevus, nevus of Ota, superficial spreading melanoma, and post-inflammatory hyperpigmentation were evaluated by NIR-AFT. The results of this study showed that all these conditions exhibited significantly greater NIR fluorescence than the surrounding normal skin, except vitiligo which presented a lower autofluorescence. Based on these results, the authors concluded that NIR fluorescence techniques could be used to evaluate the skin disorders involving melanin	Huang et al. (2006)

Table 2 continued

Disease	Fluorescence technique	Device/parameters	Results	Reference
Basal cell carcinoma	DFT Photosensitizer: Methyl aminolevulinate (c = 160 mg/g)	Digital fluorescence imaging system (DyaDerm; Biocam GmbH, Regensburg, Germany) $\lambda_{excitation}$ = 405 nm DP = 0.8 mW/cm^2 $\lambda_{emission}$ − red (PpIX fluorescence) $\lambda_{emission}$ − green (autofluorescence)	In this study, the authors evaluated the clinical performance of a preoperative definition of the lateral borders of BCC by fluorescence detection in comparison with its definition by clinical diagnosis. The main results obtained in this study are as follows: the mean tumor area as determined by fluorescence detection was significantly smaller than the tumor area as determined by clinical diagnosis; the sensitivity of fluorescence detection was 38.5 %; the specificity of fluorescence detection was calculated as 88.4 %. Based on these results, the authors conclude that preoperative fluorescence detection combined with clinical diagnosis of nodular BCC localized in the high-risk H-zone has no additional clinical benefit compared with simple clinical diagnosis alone	Wetzig et al. (2010)
Basal cell carcinoma	DFT Photosensitizer: 5-aminolevulinic acid (c = 20 %) $t_{incubation}$ = 3 h	Digital fluorescence imaging system (DyaDerm, Biocam GmbH, Regensburg, Germany)	Fluorescence diagnosis and clinical diagnosis were used in this study as methods for the noninvasive detection of tumor boundaries. The results of this study showed that the mean tumor area that was visualized by fluorescence diagnosis was significantly smaller than the tumor area determined by clinical diagnosis (97.9 ± 34.7 mm^2 vs. 124.5 ± 37.6 mm^2). These results lead to the conclusion that fluorescence technique is less sensitive than clinical diagnosis of the tumor boundaries	Gambichler et al. (2008)
Basal cell carcinoma	DFT Photosensitizer: Methyl aminolevulinate	–	In this study, the clinical efficacy of PpIX fluorescence images using fluorescence image analysis to define the lateral border between the tumor and tumor-free areas of facial BCC was evaluated. The rate of tumor detection from BCC lesions using PpIX fluorescence with the fluorescence image analysis tool showed a sensitivity of 94.1 % and specificity of 82.6 %. These results suggest that MAL-induced PpIX fluorescence imaging using fluorescence image analysis is quite sensitive and specific for detecting tumor and occult tumor in facial BCC lesions	Won et al. (2007)
Basal cell carcinoma	DFT Photosensitizer: 5-aminolevulinic acid (c = 20 %) $t_{incubation}$ = 3.5 h	Wood lamp	The fluorescence diagnosis and histopathological examination were used in this study as methods for the detection of tumor margins. The study findings showed that: In six BCCs, the DFT-defined BCC margin did not correlate with the histopathologically assessed tumor borders; The sensitivity and specificity rates of DFT were 79 % and 100 %, respectively; DFT is fairly sensitive and highly specific method for the demarcation of BCC margins; DFT does not seem to be substantially superior to simple clinical evaluation of tumor margins	Neus et al. (2008)

Table 2 continued

Disease	Fluorescence technique	Device/parameters	Results	Reference
Squamous cell carcinoma	DFT Photosensitizer: Radachlorin $c = 1.0$ mg/kg $t_{incubation} = 4$–5 h	Fluorescence imaging system (Fluotest) $\lambda_{excitation} = 633$ nm $P = 100$ mW	The accuracy of DFT for the diagnosis of SCC was investigated in 40 patients. The results of fluorescence and histopathological studies similarly showed: malignant lesion (in 27 cases) and non-malignant lesion (in 8 cases). In 5 cases, the results of these two methods were different. The performances of DFT in diagnosing SCC were also evaluated: 90 %—sensitivity; 80 %—specificity; 87.5 %—accuracy; 93 %—positive predictive value (PPV); 72 %—negative predictive value (NPV); 4.5—positive likelihood ratio (PLR) and 0.125—negative likelihood ratio (NLR). These results prove both accuracy and reliability of DFT method for detecting SCC lesions	Kamrava et al. (2012)
Squamous cell carcinoma Basal cell carcinoma Adenocarcinoma Chondrosarcoma	DFT Photosensitizers: Hematoporphyrin derivative ($c = 2.5$–5 mg/kg) $t_{incubation} = 12$–24 h 5-aminolevulinic acid ($c = 20$ %) $t_{incubation} = 2$–8 h	System based on blue light–emitting diodes $\lambda_{excitation} = (378$–$426)$ nm $P = 1$–10 mW	Ninety-eight patients with malignant, premalignant, and benign skin were investigated by DFT. The study highlighted the following: The tumors margins can be clearly and precisely outlined under fluorescent vision; The most appropriate wavelength for DFT is 401 nm in order to achieve complete visualization of malignant lesions after the application of a tumor selective photosensitizer; In the blue light mode, there is background blue fluorescence in normal tissue and red fluorescence in malignant areas. The authors concluded that DFT is applicable for detecting early superficial tumors	Liutkeviciute-Navickiene et al. (2008)
Sebaceous Gland hyperplasia Actinic keratosis Basal cell carcinoma Squamous cell carcinoma	DFT Photosensitizers: 5-aminolevulinic acid ($c = 0.5$ %) Methyl aminolevulinate ($c = 16$ %) $t_{incubation} = 3$ h	DyaDerm fluorescence detection system Light source: LED $\lambda = 405$ nm $\tau = 5$ ms $v = 1$ Hz $P = 1.0$ W	Sixty-one patients with 287 lesions (212 benign lesions, 71 premalignant lesions, 3 BCC, and 1 SCC) were investigated in this study by DFT, using ALA or MAL as photosensitizer. The fluorescence intensities of lesions were evaluated in comparison with the histopathological examination. The fluorescence image of MAL-treated skin areas showed very high and homogeneous fluorescence intensity with low discrimination between normal and diseased skin. The ALA-treated areas showed low autofluorescence of the normal skin and moderate, but distinct fluorescence of actinic keratoses, resulting in a high discrimination between the normal and the diseased skin. The results of this study revealed that the specificity of combined method (DFT, clinical investigation, and dermatoscopy) is about 92 %	Leeuw et al. (2009)
Actinic keratosis, morbus Bowen, basal cell carcinoma Benign lesions	DFT Photosensitizer: 5-aminolevulinic acid ($c = 0.5$ %) $t_{incubation} = 2.5$ h	Dyaderm fluorescence detection system (formerly Biocam GmbH, Regensburg, FRG). $\lambda = 407$ nm	The study was conducted on 30 patients suspected of having one or more non-melanoma skin cancers (NMSC). A comparison between the accuracy of non-normalized and normalized fluorescence methods was done. The results of this study revealed that the specificity and sensitivity of non-normalized fluorescence method are substantially lower than those of normalized fluorescence detection method (27 and 39 % vs. 100 and 97 %)	van der Beek et al. (2012)

Table 2 continued

Disease	Fluorescence technique	Device/parameters	Results	Reference
Actinic keratosis squamous cell carcinoma (SCC)	DFT Photosensitizer: Methyl aminolevulinate $t_{incubation} = 3$ h	Digital fluorescence imaging system (Dyaderm, Biocam GmbH, Regensburg, Germany)	In the present study, the potential applicability of DFT in discriminating Aks from SCC was investigated. The lesional/non-lesional fluorescence ratio of Aks was compared with the ratio of SCC. 13 patients with 36 lesions suspected for AK or SCC were included in this study. All lesions were diagnosed by DFT and histopathological examination. No significant differences were found in the fluorescence ratio (lesional/non-lesional skin) between Aks and SCCs, although macroscopic fluorescence was significantly higher in Bowen's disease and microinvasive SCCs	Kleinpenning et al. (2010)

2012), while other researchers concluded that DFT had no value in early skin cancer detection in premalignant lesions (Kleinpenning et al. 2010; van der Beek et al. 2012). Some studies insisted only in comparison between photosensitizers (Liutkeviciute-Navickiene et al. 2008) or the best way to analyze data [normalized fluorescence detection showing better results (Leeuw et al. 2009)].

Nevertheless, the fluorescence spectroscopy offers the advantage that it can directly probe the biochemical composition of tissues by means of detecting specific biomolecules which emits characteristic fluorescence signals. Furthermore, fluorescence is very sensitive to factors such as pH and temperature in addition to other uncontrollable physiological factors that induce wide variability to the data. Having the huge advantage of being a noninvasive method, fluorescence, both natural and drug-induced, has not shown constant results in determining early malignancy, nor in tumor margin assessment, which calls for further studies concerning its clinical value.

Diffuse reflectance spectroscopy (DRS)

Diffuse reflectance spectroscopy is a potentially affordable technique that can be used for fast, noninvasive and accurate diagnosis of skin disease. This technique is sensitive to both scattering and absorption properties of the tissue, over a wide range of wavelengths, and consequently, it can provide spectra that contain valuable information about the morphology of the normal or abnormal tissue as well as the chromophore content (e.g., hemoglobin, melanin, bilirubin, and water). The use of DRS for tissue diagnosis is based on the fact that many tissue pathologies exhibit significant architectural changes at the cellular and subcellular levels which can be evidenced by spectral measurements of the diffuse reflected light.

The principle of diffuse reflectance spectrometry consists in sending a light beam toward a sample and detecting light reflected from its surface in many directions in the hemisphere surrounding the surface. The general mechanism by which the skin reflects light diffusely does not involve only the skin surface, but also the presence of scattering centers located below the skin surface. By measuring the changes in the diffuse reflectance spectrum, information about changes of scattering centers (and thus of the specific structures of the skin) can be obtained. Unlike the fluorescence techniques, DRS does not provide information about the chemical composition of tissue in a direct way, but using some analytical models of light transport in biological tissues (Zonios et al. 2001) or numerical methods (Wang and Jacques 1995; Yudovsky and Laurent Pilon 2010), some skin constituents can be determined (e.g., hemoglobin and melanin). Hence, DRS which provides important data about both structure and

chemical composition of tissue is now considered to be an appropriate technique for early diagnosis of some disease, especially premalignant or malignant skin lesions (Table 3), having the advantage of avoiding tissue biopsy and providing diagnostic signatures, noninvasively and in real time.

According to Table 3, the values of sensitivity and specificity of the DRS technique strongly depend on the specific features of the spectra obtained from normal, precancerous, and cancerous skin lesions taken into account in discriminant analysis used by different researchers. Such specific features of the skin spectra reported to date in differentiating between normal and pathological tissue are mean or integral value of absorption coefficient and reduced scattering coefficient (Zonios et al. 2008; Garcia-Uribe et al. 2011), spectral slope (Canpolat et al. 2012), and integral value of the diffuse reflectance for specific wavelength region (Canpolat et al. 2007; Jiao et al. 2009; Upile et al. 2012). Based on the comparison between these specific spectral features, the values of the specificity and sensitivity of DRS were established to be between 72–92 % and 64–92 %, respectively.

Recently, several research groups have demonstrated that these performances of DRS in differentiating disease from normal surrounding tissue, mainly in detection of cancerous and precancerous changes in human skin can be improved by combining this noninvasive diagnostic technique with AFT. Using this combined technique (DRS/AFT), some parameters related to the biochemical, architectural, and morphologic state of tissue can be simultaneously measured and used to diagnose various skin conditions. Thus, Thompson et al. (2012) using a compact steady-state diffuse reflectance/fluorescence spectrometer and a fiber-optic-coupled multispectral time-resolved spectrofluorometer have correctly diagnosed 87 % of the BCCs in 25 patients. Very good results were also reported by Rajaram et al. (2010) in a pilot clinical study performed on 40 patients with 48 lesions. They have demonstrated that, using a combined method (DRS/AFT), BCCs can be classified with a sensitivity and specificity of 94 and 89 %, respectively, while actinic keratoses and squamous cell carcinomas with a sensitivity of 100 % and specificity of 50 %. Troyanova et al. (2007) reported that the differentiation between normal skin and different cutaneous lesion types (hemangioma, angiokeratoma, and fibroma) and among lesion types themselves can be done with the sensitivities and specificities higher than 90 % by common use of laser-induced autofluorescence (LIAF) and reflectance spectroscopy (DRS). The same technique (LIAF/DRS) was also used by Borisova et al. (2012) for skin cancer diagnostic. They reported a sensitivity of 92 % and specificity of 78 % of combined LIAF/DRS technique in discrimination between malignant melanoma from dysplastic nevi. All these results demonstrate that the diagnostic accuracy can be improved by the use of combined technique (AFT/DRS) together the specific discriminant analysis.

Besides these spectroscopic techniques (DRS and fluorescence spectroscopy), Raman spectroscopy has also been used for the same medical applications.

Raman spectroscopy

Raman spectroscopy is an optical technique which uses the inelastic scattering of monochromatic light (usually with wavelength in the visible, near-infrared, or near-ultraviolet range) to analyze vibrational modes of molecules. The inelastic light scattering process occurs when the photons interact with the vibrating molecules or the excited electrons in the sample in such a way that molecules take up energy from or give up energy to the photons, so that the scattered photons are shifted in frequency up or down in comparison with the incident photons. The shift in the photon frequency is correlated with the difference between initial and final vibrational energy levels of the scattering molecule. The change in energy or shift in photon frequency indicates molecular information and its photon mode in the sample. These changes in energy and frequency are molecular-specific and they appear as a series of peaks in a Raman spectrum. The positions and relative magnitudes of these peaks correspond to the vibrational energies associated with specific chemical bonds in specific molecules. Many molecules have distinguishable spectra, so that one can determine the molecular composition of a sample from its Raman spectrum.

Raman spectroscopy has a wide range of uses in various biomedical issues such as early detection of neoplastic lesions (Qiang and Chang 2012), intraoperative tumor border determination (Haka et al. 2006; Keller et al. 2011), determination of atherosclerotic plaque composition (Motz et al. 2006; Rocha et al. 2007), assessment of the chemical substance toxicity (Pyrgiotakis et al. 2009), and identification of pathogenic microorganisms (Kalasinsky et al. 2007; de Siqueira et al. 2012). since it can provide details of the molecular and/or biochemical changes associated with the morphological changes that occur in tissue as a result of disease.

In dermatology, RS has beginning to be recognized as a potential technique for the diagnosis of skin cancer and characterization of neoplastic progression of tissues with a high degree of specificity at the molecular level (Table 4).

As we can see in Table 4, RS can be applied both in vivo and on tissue samples (Zhao et al. 2008; Zeng et al. 2011; Lui et al. 2012). Both skin carcinomas and melanomas have been taken into account during research, with fewer studies concentrating on only one type of skin malignancy (Nunes et al. 2003; Zhao et al. 2008; Larraona-Puy et al. 2009). The

Table 3 Clinical applications of diffuse reflectance spectrometry for the diagnosis of skin diseases

Disease	Device/parameters	Results	Reference
Melanoma Dysplastic nevi Common nevi	Oblique incidence diffuse reflectance spectroscopic (OIDRS) system: Light source: halogen lamp (455–765) nm; Fiber-optic sensor probe: one or more source fiber for a 45° oblique incidence and 2 linear array of 10 collection fibers	In this study, DRS was applied on 144 pigmented skin lesions (16 melanomas, 98 dysplastic nevi, and 30 common nevi). The optical absorption and scattering spectra of these skin lesions were estimated by the authors of this study from the measured diffuse reflectance data. The results revealed that the absorption spectra for the melanoma and dysplastic cases are similar and generally are higher than those for the benign ones. Also, it was found that the reduced scattering coefficient generally increases with the degree of dysplasia or malignancy of the skin lesions within the entire visible spectrum	Garcia-Uribe et al. (2011)
Melanomas dysplastic nevi common nevi Basal cell carcinoma squamous cell carcinoma	Oblique incidence diffuse reflectance spectroscopic (OIDRS) system	In this study, the OIDRS system was used for the diagnosis of melanoma and non-melanoma skin cancer. The results showed that pigmented melanoma was diagnosed with sensitivity and specificity of 90 % for a blinded test set. The sensitivity and specificity of DRS method have increased to 92 % in the case of differentiation of non-pigmented basal cell or squamous cell carcinomas from non-cancerous skin abnormalities (actinic keratoses and seborrheic keratoses)	Garcia-Uribe et al. (2012)
Melanoma Common nevus Dysplastic nevus	Fiber-optic probe: 400 μm core multimode fibers arranged in a 6 illumination around 1 collection geometry with a single fiber–fiber spacing of 470 μm $\lambda \in$ (550–1,000) nm	The study assessed discrimination of early melanoma from common and dysplastic nevus, using DRS. The study was conducted on 120 pigmented lesions of which 64 were confirmed histopathologically, as melanoma. As a result, the variations in spectra between groups of lesions with different diagnoses were examined and reduced to features suitable for discriminant analysis. A classifier distinguishing between benign and malignant lesions was performed with sensitivity/specificity of between 64–69 % and 72–78 %. These results demonstrated that classifiers between pairs of the group common nevus, dysplastic nevus, in situ melanoma, and invasive melanoma show better or similar performance than the benign/malignant classifier	Murphy et al. (2005)
Melanoma Dysplastic nevi	Spectrophotometer (Ocean Optics, SB2000) Light source: tungsten–halogen light source (Ocean Optics, HL-2000); Fiber-optic probe: 6 concentrically arranged 200 μm core optical fibers, and for diffuse reflectance collection, a single 200 μm core central optical fiber (Ocean Optics, R200-7) $\lambda \in$ (460–1,000) nm	The study was conducted on 10 human subjects including 6 with dysplastic nevi, 1 with melanoma in situ, and 3 with malignant melanoma. The results of this study demonstrated that the optical absorption spectrum of in vivo melanin presents an exponential dependence on wavelength. The authors justify theoretically this exponential dependence on the basis of a recently proposed model for the structure of eumelanin protomolecules. Also, a new method for analyzing diffuse reflectance spectra, which identifies intrinsic differences in absorption spectra between malignant melanoma and dysplastic nevi in vivo, was reported. The authors have also found evidence that the histologic transition from dysplastic nevi to melanoma in situ and then to malignant melanoma is reflected in the melanin absorption spectra	Zonios et al. (2008)

Table 3 continued

Disease	Device/parameters	Results	Reference
Melanoma Basal cell carcinoma squamous cell carcinoma Benign lesions	–	Twenty-three patients with twenty-eight lesions (9 BCC, 4 melanoma, 2 SCC, and 13 benign lesions) were investigated in this study by DRS. Also, intraoperative margin assessments were also performed on the 28 biopsy samples. The results of the study showed that the sign of the spectral slope was positive for benign and negative for malignant tissues. This parameter was used to discriminate between malignant and benign lesions with a sensitivity and specificity of 87 and 85 %, respectively. Sensitivity and specificity of the system in detecting positive surgical margins on 14 excised biopsy samples were 80 and 90 %, respectively	Canpolat et al. (2012)
Basal cell carcinoma seborrheic keratosis fibroepithelial polyp Intradermal nevi	–	The study was conducted on 73 patients with facial skin lesions (basal cell carcinoma, seborrheic keratosis, fibroepithelial polyp, and intradermal nevi). The results of the study revealed that DRS can differentiate between normal and pathological skin conditions as well as benign and malignant skin conditions	Jiao et al. (2009)
Basal cell carcinoma	Diffuse reflectance spectroscopic system Light source: pulsed xenon-arc lamp Optical fiber probe: one illumination fiber (400 μm) and one fiber which collect the light reflected (200 μm) $\lambda \in (330–750)$ nm	Seventy-three patients were investigated in this study by DRS. Comparison of the histological diagnosis and DRS in the diagnosis of BCC resulted in a sensitivity 77.8 % and specificity 80.3 %	Upile et al. (2012)
Malign skin lesions	Diffuse reflectance spectroscopic system: UV spectrometer Single optical fiber probe	Eighteen patients with twenty lesions were investigated in this study by DRS and skin biopsy The results have demonstrated a good correlation between diffuse reflectance spectra and the pathology results with sensitivity and specificity of 82 and 89 %, respectively	Canpolat et al. (2007)

Table 4 Application of Raman spectroscopy for the diagnosis of skin cancer

Disease	Device/parameters	Results	Reference
Melanoma	Raman spectrometer system	Raman spectra of 289 skin cancers and benign skin lesions were analyzed using partial least-squares regression and linear discriminant analysis. The results have revealed that skin cancers could be well differentiated from benign skin lesions (sensitivity 91 % and specificity 75 %) and malignant melanoma from benign pigmented lesions (sensitivity 97 %, specificity 78 %)	Zhao et al. (2008)
Melanoma Basal cell carcinoma Squamous cell carcinoma Actinic keratoses Atypical nevi Melanocytic nevi Blue nevi Seborrheic keratoses	Integrated real-time system of Raman spectroscopy	The study was performed on 453 patients with 518 benign and malignant skin lesions. The results revealed that Raman spectroscopy can be used to distinguish malignant from benign skin lesions with good diagnostic accuracy. The sensitivity of the method was reported to be between 95 and 99 %, while specificity ranges from 15 and 54 %	Lui et al. (2012)
Melanoma Pigmented nevi Basal cell carcinoma Seborrheic keratoses	NIR-FT-Raman spectrometer	In this paper, the authors investigated the possibility of using chemical changes in the melanoma tissue detected by Raman spectroscopy and neural networks for diagnostic purposes. In this respect, FT-Raman spectra from samples of melanoma ($n = 22$) were compared to pigmented nevi ($n = 41$), BCC ($n = 48$), seborrheic keratoses ($n = 23$) using neural network analysis. The results revealed that the band alterations (amide I protein band (1,660 cm^{-1}) and lipid-specific band peaks (1,310 and 1,330 cm^{-1}) can be independently identified by neural network analysis of Raman spectra. The authors report a sensitivity and specificity of this method for the diagnosis of melanoma of 85 and 99 %, respectively	Gniadecka et al. (2004)
Melanoma Basal cell carcinoma Squamous cell carcinoma Actinic keratoses Seborrheic keratoses Various nevi		A Raman spectroscopy study was carried out on 274 skin lesions including cancers (31 melanomas, 18 basal cell carcinomas, and 39 squamous cell carcinomas), precancerous lesions (20 actinic keratoses), and benign lesions (48 seborrheic keratoses and 118 various nevi). The result of this study showed that precancerous lesions and cancerous lesions can be differentiated from benign lesions with a sensitivity of 90 % and specificity of 75 %. These results can provide the basis for introducing Raman spectroscopy to clinical use	Zeng et al. (2011)
Melanoma Pigmented nevi	FT-Raman spectrometer (Bruker RFS 100, Karlsruhe, Germany) Excitation source: Nd:YAG laser $\lambda = 1{,}064$ nm $P = 300$ mW resolution = 4 cm^{-1}	In this study, 10 samples of cutaneous melanoma, 9 samples of pigmented nevi, and 10 samples of normal skin were investigated by FT-Raman spectroscopy in order to differentiate cutaneous melanoma and pigmented nevus. The results of this study have shown that the normal skin did not show a significant variation between the spectra; the spectral standard of the pigmented nevi group showed significant variation, and the cutaneous melanoma group also showed variation. The discriminatory analysis of the Raman spectra showed a 75.3 % efficiency of the differentiation between the three groups studied	Cartaxo et al. (2010)

Table 4 continued

Disease	Device/parameters	Results	Reference
Melanoma Lymph node metastasis	FT-Raman spectrometer (Bruker RFS 100/S, Karlsruhe, Germany) Excitation source: Nd:YAG laser $\lambda = 1{,}064$ nm $P = 300$ mW Resolution $= 4$ cm^{-1}	A total of 371 Raman spectra from 10 normal human skin samples (105 spectra), 10 cutaneous melanoma fragments (140 spectra), and 9 lymph node metastasis samples (126 spectra) were acquired and analyzed in this study in order to obtain a differential diagnosis. The discriminative analysis of these spectra has demonstrated that phenylalanine, DNA, and amide I spectral variables stood out in the differentiation of the three groups. The percentage of correctly classified groups based on these three biochemical constituents was 93.1 %. This result demonstrated that FT-Raman spectroscopy is capable of differentiating melanoma from its metastasis, as well as from normal skin	Oliveira et al. (2010)
Melanoma Basal cell carcinoma	Near-infrared Raman spectrometer with Raman probe $\lambda = 830$ nm $P = 50$–200 mW $t_{exp} = 20$ s exposure time	A total 145 Raman spectra from biopsy fragments of normal, BCC, and melanoma were analyzed to identify differences in the biochemical constitution of these samples. Raman spectra of some compounds which are expected to be represented in human skin spectra were collected and a linear least-squares fitting model to estimate the contributions of these compounds to the tissue spectra was developed. The results have shown that actin, collagen, elastin, and triolein were the most important biochemicals representing the spectral features of skin tissues. A classification model applied to the relative contribution of collagen III, elastin, and melanin using Euclidean distance as a discriminator could differentiate normal from BCC and melanoma	Silveira et al. (2012)
Basal cell carcinoma	–	The potential use of Raman microspectroscopy for automated evaluation of excised skin tissue during Mohs micrographic surgery was investigated. A multivariate supervised classification model was developed and validated on 329 Raman spectra of skin tissue acquired from 20 patients. The results have shown that BCC can be discriminated from healthy tissue with 90 ± 9 % sensitivity and 85 ± 9 % specificity. When this model was applied on tissue sections from new patients, the Raman images have shown an excellent correlation with histopathological sections, BCC being detected in all positive sections	Larraona-Puy et al. (2009)
Basal cell carcinoma	FT-Raman spectrometer (RFS 100/S-Bruker Inc., Karlsruhe, Germany) Excitation source: Nd:YAG laser $\lambda = 1{,}064$ nm $P = 300$ mW resolution ~ 4 cm^{-1}	Eight set of samples histopathologically diagnosed as BCC and five set of samples diagnosed as benign tissue were analyzed by FT-Raman spectroscopy in order to detect spectral changes between benign and malignant skin tissues. As a result of this study, the authors demonstrated that by applying principal components analysis over all 13 samples, tissue type could be identified with sensitivity and specificity of 100 %	Nunes et al. (2003)

analysis of the Raman spectra was performed with various methods, from partial least-squares regression (Zhao et al. 2008; Lui et al. 2012; Silveira et al. 2012), discriminative analysis (Larraona-Puy et al. 2009; Oliveira et al. 2010; Cartaxo et al. 2010; Lui et al. 2012), comparison of relevant spectral bands (Nunes et al. 2003) to more complex approaches like neural network analysis (Gniadecka et al. 2004). Most of the articles relied on more than one analyzing technique in order to obtain reliable results, demonstrating that a complex analysis is far superior to limited ones, because changes in the spectra are rarely punctual.

Basal cell carcinomas were addressed in many studies (Gniadecka et al. 2004; Lieber et al. 2008; Larraona-Puy et al. 2009; Zeng et al. 2011; Lui et al. 2012; Silveira et al. 2012). In vivo diagnostic approaches showed great sensitivities (all above 90 %), with lesser specificity (ranging from 54 to 95 %). These results prove this method to be a valuable adjunct to clinical examination in planning the surgical excision. Sample studies (Nunes et al. 2003; Gniadecka et al. 2004; Larraona-Puy et al. 2009; Silveira et al. 2012) gave equally good results with sensitivities near to 100 % and specificities above 85 % (Nunes et al. 2003; Larraona-Puy et al. 2009). Therefore, RS is soon expected to compete with histological examination during surgery, being faster and easier to perform, even in a future adaptation of Moh's micrographic surgery (Larraona-Puy et al. 2009). In terms of single-band discrimination, amid I proteins (around 1,660 cm^{-1}) proved to be an important element in differentiation between normal skin or melanoma (Nunes et al. 2003; Larraona-Puy et al. 2009), although a more complex analysis seems to be more reliable (Gniadecka et al. 2004; Silveira et al. 2012). In regard to differential diagnosis between BCC and SCC, the reports are not conclusive, this direction of investigation being one of the great importance and needing further research, as long as excision planning is generally based on preoperative data concerning this matter.

Differentiating benign pigmented lesions from melanoma is a matter of great concern for the surgeon, being known that this is the most aggressive skin cancer. Therefore, many studies addressed this subject (Gniadecka et al. 2004; Zhao et al. 2008; Cartaxo et al. 2010; Oliveira et al. 2010; Zeng et al. 2011; Lui et al. 2012; Silveira et al. 2012). In vivo examination (Zhao et al. 2008; Zeng et al. 2011; Lui et al. 2012) is of the upmost importance, as it directs the surgical therapy. The results were outstanding, showing sensitivities of nearly 100 % and specificities ranging from 70 to 78 % (Zhao et al. 2008; Zeng et al. 2011). As dermoscopy is in current use in preoperative evaluation of pigmented lesions, research comparing it to RS is mandatory before bringing the latter into standard medical practice.

The sample studies were even more encouraging, showing that RS is well suited to distinguish melanoma from other pigmented lesions (Gniadecka et al. 2004; Cartaxo et al. 2010), from normal skin or metastatic tumors (Oliveira et al. 2010; Silveira et al. 2012) or BCCs (Gniadecka et al. 2004; Silveira et al. 2012). Spectral bands that can differentiate melanoma from normal skin, pigmented nevi, or BCC include lipids (Gniadecka et al. 2004), polysaccharides, tyrosine and amide I (Cartaxo et al. 2010), collagen III, elastin and melanin (Silveira et al. 2012), phenylalanine, DNA, and amide I (Oliveira et al. 2010), showing a great deal of variability among articles, a fact that points out the need for complex analysis of the spectra in order to generate reliable results.

Two of the most important advantages of this method are its applicability both in vivo and in vitro as well as its rapidity in examination and results. Together with the good sensibility and specificity figures, these make RS one of the most valuable optical diagnostic methods in differentiating benign, premalignant, and malignant skin lesions with a good chance to enter common medical practice.

Confocal microscopy

Confocal microscopy (CM) is an optical imaging technique for noninvasive tissue imaging with a higher resolution and contrast than the conventional microscopy. These main features derive from the usage of a point source of light for the illumination of specimen and the placement of a pinhole between tissue specimen and detector which rejects multiply scattered out-of-focus light allowing only the in-focus light from the specimen to reach the detector. The image created in this way is an optical section representing one focal plane within the examined specimen. By changing the plane of focus or moving the specimen, a series of images at different positions can be produced through the thickness of the specimen and a three-dimensional representation of the specimen can be produced by the optical sectioning.

This technique has become a valuable tool in dermatology during the last decade being applied in both basic research (Rigby and Goldie 1999; Lima et al. 2009) and clinical diagnosis including the assessment of benign and malignant skin lesions (Gerger et al. 2006; Gonzalez 2009; Ulrich et al. 2008; 2012a, b), tumor margin mapping (Curiel-Lewandrowski et al. 2004; Scope et al. 2010), monitoring response to medical treatments (Ulrich et al. 2010, 2012a, b), and diagnosis of inflammatory and infective skin diseases (Hicks et al. 2003; Swindells et al. 2004). The performance of CM applications in the skin cancer diagnosis as reported in the literature over the past decade is presented in Table 5.

We can see from Table 5 that the great majority of the cited studies have used devices from the same producer (Vivascope 1000; Lucid Inc., Rochester, NY, USA or

Table 5 Clinical applications of confocal microscopy technique (CM) for the diagnosis of skin cancer

Disease	Device/parameters	Results	Reference
Melanocytic skin lesions benign nevi malignant melanomas basal cell carcinomas seborrheic keratoses	NIR reflectance confocal microscope (Vivascope 1000; Lucid Inc., Rochester, NY, USA). light source: diode laser $\lambda = 830$ nm; $P < 35$ mW numerical aperture: 0.9 lateral resolution: (0.5–1.0) μm axial resolution: (3.0–5.0) μm	In this study, 117 melanocytic skin lesions and 45 non-melanocytic skin lesions (90 benign nevi, 27 malignant melanomas, 15 basal cell carcinomas, and 30 seborrheic keratoses) were examined by CM. The acquired images were rated by 4 independent observers. The results of the evaluation have shown that: the differentiation between melanoma and all other lesions can be achieved with a positive predictive value of 94.22 %; malignant lesions (melanoma and basal cell carcinoma) can be diagnosed with a positive predictive value of 96.34 %; assessment of distinct confocal microscopy features showed a strong interobserver correlation; classification and regression tree analysis has facilitated a correct classification in 96.30 % of melanomas, 98.89 % of benign nevi, and 100 % of basal cell carcinomas and seborrheic keratoses. These results demonstrate that CM may be considered as a promising method for the noninvasive assessment of melanoma and non-melanoma skin tumors	Gerger et al. (2006)
Malignant melanomas benign naevi	NIR reflectance confocal microscope (Vivascope 1000; Lucid Inc., Rochester, NY, USA)	A total of 3,709 tumor images obtained from 20 malignant melanomas and 50 benign naevi were evaluated by independent observers. The results have shown that sensitivity and specificity of 97.5 and 99 % could be achieved by the independent observers (positive predictive value 97.5 %, negative predictive value 99 %). Classification tree analysis has conducted to a correct classification in 92.4 % of the benign nevus images and 97.6 % of melanoma images. Consequently, CM could be used as a screening tool in skin oncology	Gerger et al. (2008)
Melanoma nevi		In this study, the sensitivity and specificity of confocal features for the diagnosis of equivocal melanocytic lesions (136 melanomas and 215 nevi) were evaluated. Microscopy image analysis by χ^2 test, multivariate discriminant analysis, and binary logistic regression revealed that: melanomas are mostly characterized by epidermal disarray and pagetoid cells in the epidermis, non-edged papillae, and cellular atypia at the junction, and atypical nests and bright nucleated cells in the upper dermis; benign lesions are characterized by regular dermal–epidermal architecture, and absence of pagetoid infiltration and atypical cells; five out of the 136 melanomas, with mildly atypical melanocytes and occasional pagetoid cells at histopathology, were not diagnosed by confocal microscopy. These results lead to the conclusion that CM could be useful for second level examination of clinical equivocal lesions	Pellacani et al. (2007)
Melanoma nevi Spitz or Reed nevi	NIR reflectance confocal laser scanning microscopes (Vivascope 1000 and Vivascope 1500; Lucid Inc., Rochester, NY, USA)	A total of 202 melanocytic lesions (76 melanomas, 114 nevi, and 12 Spitz or Reed nevi) were investigated in this study in order to establish a correlation between dermoscopic patterns in melanocytic lesions and confocal microscopic findings and conventional histopathologic findings. Some characteristic architectural and cytologic substrates were identified by confocal microscopy and correlated with histopathological examination. The results of this study could be useful for the identification of specific substrates in melanocytic lesions and consequently the interpretation of the dermoscopic features	Pellacani et al. (2008)

Table 5 continued

Disease	Device/parameters	Results	Reference
Melanoma melanocytic nevi		A total of 125 patients with 125 lesions (88 melanocytic nevi and 37 melanomas) were examined by CM and dermoscopy. The results of this study revealed that the dermoscopy had a sensitivity of 89.2 %, a specificity of 84.1 %, a positive predictive value of 70.2 %, and a negative predictive value of 94.9 % while the CM was found to have a sensitivity of 97.3 %, a specificity of 83.0 %, a positive predictive value of 70.6 %, and a negative predictive value of 98.6 %. These results suggest that these two diagnostic methods are complementary	Langley et al. (2007)
Basal cell carcinoma squamous cell carcinoma	NIR reflectance confocal microscope (Vivascope 1000; Lucid Inc., Rochester, NY, USA)	In this study, the CM and histopathology were used as methods for the examination of the difficult-to-diagnose skin lesions in 137 patients. The results have proved that 106 lesions (out of a total of 129 lesions histologically proven to be malignancies) were diagnosed as "malignant" by CM. A difference of 23 lesions were diagnosed as "normal" by CM (6 basal cell carcinoma and 17 squamous cell carcinoma) demonstrating a false negative rate of 23/129 (17.83 %) or a sensitivity of 82.17 %. These results show that CM can provide diagnostic information which is reliable for over 82 % of clinically difficult to diagnose	Amjadi et al. (2011)
Basal cell carcinoma actinic keratosis	Fluorescence microscope (OptiScan Ltd., Melbourne, Australia)	In this study, fluorescent confocal microscopy was used for the monitoring of actinic keratoses and basal cell carcinoma response to topical therapy using Imiquimod as an immune-response modifier. The results demonstrated that this optical technique allowed a monitoring of the local immune response following therapy with Imiquimod and demonstrated a continuous normalization of diseased skin on repeated evaluations over time. These results are only preliminary and further investigations are required in the future	Astner et al. (2008)
Actinic keratosis	NIR reflectance confocal microscope (Vivascope 1000; Lucid Inc., Rochester, NY, USA)	Forty-six AKs from 44 patients were included in this study. The evaluation of these lesions was performed by clinical examination, CM, and routine histology. The results of this study have shown that the sensitivity/specificity values of CM features ranged from 80 to 98.6 %. These results show that CM could be considered as an adjunct tool to clinical diagnosis and monitoring	Ulrich et al. (2008)
Lentigo maligna solar lentigo ephelis actinic keratosis flat seborrheic keratosis	NIR reflectance confocal microscope (Vivascope 1000; Lucid Inc., Rochester, NY, USA)	In this study, a total of 64 CM features were scored retrospectively and blinded to diagnosis in a series of CM sampled, clinically equivocal, macules of the face ($n = 81$ lentigo maligna, $n = 203$ benign macules). In addition to describing CM diagnostic features for lentigo maligna, an algorithm was developed (LM score) to distinguish lentigo maligna from benign macules. The results have shown that a LM score of X2 resulted in a sensitivity of 85 % and specificity of 76 % for the diagnosis of lentigo maligna	Guitera et al. (2010)
Lentigines lentigo maligna		Ten patients suspected with lentigines ($n = 6$) and lentigo malignas ($n = 4$) were included in this study. The characteristics of these pigmented lesions were qualitatively described by CM and compared with histopathologic findings. The results showed the following: the benign lentigines presented distinct architectural and cytologic features compared with melanomas; an increase in the density of dermal papillae surrounded by a bright monomorphic layer of cells was seen in all cases of lentigines, but not for melanomas; lentigines presented an absence of atypical melanocytes; melanomas presented bright, atypical, polymorphous cells present in a pagetoid pattern with coarse, branching dendrites observed throughout the epidermis. These characteristics of lentigines could help in the diagnosis of melanoma and discrimination from benign lesions	Langley et al. (2006)

Table 5 continued

Disease	Device/parameters	Results	Reference
Lentigo maligna lentigo maligna melanoma		Twelve patients with 17 lesions (lentigo maligna and lentigo maligna melanoma) were examined by confocal microscopy and histological examination. The results of this study showed that main features of these lesions observed in the microscopic images are related to a focal increase in atypical melanocytes and nests surrounding adnexal openings, sheets of mainly dendritic melanocytes, cord-like rete ridges at the dermoepidermal junction and an infiltration of adnexal structures by atypical melanocytes. These results suggest that the in vivo assessment of equivocal skin lesions at a cellular level could be done by CM	Ahlgrimm-Siess et al. (2009)

Vivascope 1500, Lucid-Tech Inc., Henrietta, NY, USA), the only problem that the researchers had to answer being the criteria in image analysis is that it can give the most accurate result. Only one article (Astner et al. 2008) used a fluorescence microscope that allowed also the evaluation of the effectiveness of the treatment with Imiquimod for the selected lesions.

Differentiating in a safe and reliable mode benign pigmented lesions from melanoma remains the main concern of the researchers (Gerger et al. 2006, 2008; Langley et al. 2006, 2007; Pellacani et al. 2007, 2008; Ahlgrimm-Siess et al. 2009; Guitera et al. 2010). While the technique seems to be the same in most studies, it is the interpretation that makes the difference. While some authors used assessment from more than one investigator (Gerger et al. 2006, 2008; Pellacani et al. 2007), others relied on only one expert (Langley et al. 2006, 2007; Pellacani et al. 2008; Ahlgrimm-Siess et al. 2009; Guitera et al. 2010). Features that lead to a melanoma or lentigo maligna diagnostic include: epidermal disarray and pagetoid cells in the epidermis, non-edged papillae and disarrangement of the dermoepidermal junction, cellular atypia, atypical nests, and bright nucleated cells in the dermis or papillae (Pellacani et al. 2007, 2008; Guitera et al. 2010) and so on. Most researchers have tried to find a reliable interpretation algorithm that may be applicable to all cases (Gerger et al. 2006, 2008; Pellacani et al. 2007; Guitera et al. 2010) or at least point out the main significant aspects (Langley et al. 2006; Pellacani et al. 2008; Ahlgrimm-Siess et al. 2009). Confocal microscopy proved to be a valuable adjunct to dermoscopy (Langley et al. 2007; Pellacani et al. 2008). Sensitivity and specificity indices were high (above 80 %) (Gerger et al. 2006, 2008; Pellacani et al. 2007; Guitera et al. 2010), proving that the method may become a useful tool in preoperative decisions. It has the advantage of being quick and it does not need complex mathematical interpretation (like other cited optical methods do), but it still relies on expert opinion, as long as a proven algorithm is difficult to establish even with meta-analysis.

Less attention was given to non-melanoma skin cancers (Astner et al. 2008; Ulrich et al. 2008; Amjadi et al. 2011), but the results proved CM to be a good pretreatment assessment tool, both in diagnostic (Amjadi et al. 2011) or even in orienting the surgical excision margins (Amjadi et al. 2011). A derivative method that uses fluorescence has shown its value in monitoring noninvasive therapies on this type of skin malignancies (Astner et al. 2008).

Discussion

Optical techniques (OCT, DFT, DRS, RS, and CM) have shown promising results in the diagnosis of skin cancer.

They do not usually require tissue removal and allow the real-time diagnosis, but some of them (RS, CM) can also be applied on tissue samples, being more rapid than pathological examination.

Optical coherence tomography proved to be an effective imaging technique for the investigation of skin morphology alongside with fluorescence and DRS. The last two spectroscopic techniques have been widely applied to acquire information not only about the structure of tissue but also about biochemical composition of tissue. Raman spectroscopy has the same goal of biochemical characterization of tissues, but has shown better results in establishing differential diagnosis between benign, premalignant, and malignant skin lesions. Confocal spectroscopy allows for a more direct visual evaluation of skin tumors, being closest to pathological examination and does not rely on complicated analyzing models.

In OCT, diagnostic accuracy in clinical diagnosis of AK ranges from 79 to 86 % in sensitivity and 83 to 100 % in specificity. Several reports have demonstrated for BCC and AK an excellent correlation between fluorescence pattern and histopathology. The fluorescence spectroscopy could be therefore used in clinically ill-defined (malignant, premalignant, or benign) cutaneous lesions in order to better delimitate neoplastic tissue. Diffuse reflectance spectroscopy has the potential to provide the means to identify precancerous and cancerous lesion. Tissue information obtained by this spectroscopic technique could be useful in tissue classification and in the detection and characterization of a large number of pathologic disorders including cancer. Raman spectroscopy showed even better results than other cited spectroscopic techniques, its sensitivities rising high above 90 % both for differential diagnosis between benign and malignant tumors or even assessing tumoral margins. Confocal microscopy has the advantage of providing direct images of tumoral tissue and it does not need complex mathematical interpretation; nevertheless, it needs expert interpretation and is probably better accepted by the physicians, because peers are directly involved in its applications.

Widespread clinical application of these optical methods in the diagnosis of skin cancer is conditioned by certain factors such as the cost of equipments and their maintenance, personnel training for the acquisition, processing and interpretation of the data, and time of investigation. The cost of equipments for these optical methods is different. The portable diffuse reflectance spectrometers are relatively cheap while the fluorescence spectrometers require a large investment because of the special light source (laser or lamp) necessary to generate fluorescence. The complexity and requirements for OCT systems concerning the parameters of excitation light source and of the detection module make the costs of the equipments to be quite high. All these equipments require minimal maintenance, especially related to their periodic calibration. Operating all these equipments is not difficult and the staff training is quite easy. The application of algorithms for data analysis and the interpretation of results however require highly qualified personnel and special attention should be paid for their adequate training. Unlike other techniques for the diagnosis of skin cancer that are time-consuming (e.g., histopathological test), all these optical diagnostic techniques allow for real-time diagnosis.

Conclusions

In conclusion, the noninvasive optical techniques presented in this study (OCT, DFT, DRS, RS, CM) proved to be effective in the diagnosis of both benign and malignant diseases of the skin. Based on the presented results, we can anticipate that these techniques will find their place in medical practice as well as new advanced equipment for optical diagnostics will be developed and released on the market. To achieve this, the efforts of the scientific community, the medical community, and manufacturers should converge.

Acknowledgments This work was financed by the Ministry of Education, Research, Youth and Sport by means of the Research Program no. PN II PCCA 184/2012.

Conflict of interest We declare that we have no conflict of interest.

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