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REVIEW

Laser-induced fluorescence made simple: implications for the diagnosis and follow-up monitoring of basal cell carcinoma

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Abstract. Noninvasive treatments are increasingly being used for the management of basal cell carcinoma (BCC), the predominant type of nonmelanoma skin cancer, making the development of noninvasive diagnostic technologies highly relevant for clinical practice. Laser-induced fluorescence (LIF) spectroscopy emerges as an attractive diagnostic technique for the diagnosis and demarcation of BCC due to its noninvasiveness, high sensitivity, real-time measurements, and user-friendly methodology. LIF relies on the principle of differential fluorescence emission between abnormal and normal skin tissues (*ex vivo* and *in vivo*) in response to excitation by a specific wavelength of light. Fluorescence originates either from endogenous fluorophores (autofluorescence) or from exogenously administered fluorophores (photosensitizers). The measured optical properties and fluorophore contributions of normal skin and BCC are significantly different from each other and correlate well with tissue histology. Photodynamic diagnosis (PDD) is based on the visualization of a fluorophore, with the ability to accumulate in tumor tissue, by the use of fluorescence imaging. PDD may be used for detecting subclinical disease, determining surgical margins, and following-up patients for residual tumor or BCC relapse. In this review, we will present the basic principles of LIF and discuss its uses for the diagnosis, management, and follow-up of BCC. [DOI: 10.1117/1.JBO.19.3.030901]

Keywords: fluorescence spectroscopy; nonmelanoma skin cancer; basal cell carcinoma; laser noninvasive diagnosis; follow-up monitoring.

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1 Introduction

Nonmelanoma skin cancer (NMSC) is a term used to encompass skin cancer forms other than malignant melanoma, and it most commonly refers to squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). BCC is the most common malignancy in humans and its incidence is on the rise with considerable public health implications.[1]

The optimal management of skin cancer relies on early and accurate diagnosis, appropriate treatment, and monitoring for potential relapse. Noninvasive treatments are increasingly being used for the management of BCC. Recent advances in the molecular pathophysiology of BCC have opened the way for new exciting targeted therapies, including oral hedgehog signalling inhibitors, in order to avoid the need of extensive, repetitive, or mutilating surgery.[2] A plethora of new developments in optical imaging techniques is available for the noninvasive diagnosis (photodiagnosis) of NMSC including fluorescence, diffuse reflectance, Raman and near-infrared spectroscopies, optical coherence tomography, and multiphoton and confocal laser scanning microscopies.[3,4] More interestingly, optical methods are also increasingly being used to monitor clearance of skin cancer after traditional treatments and screen for early relapse detection.

In this review, we will present the basic principles of laser-induced fluorescence (LIF) for the management of BCC, and we will discuss its use in early and advanced BCC diagnosis, the use in determining surgical margins, and the ability to detect residual cancer or tumor relapse.

2 LIF: Basic Principles Made Simple

In LIF, nonionizing radiation is delivered and collected with optical fibers that are placed in contact with the skin surface.

The excitation light passes through and explores the tissue under the probe noninvasively, and fluorescence light is emitted back to the surface where it is collected by the fibers at a fixed distance away from the source fiber. These optical measurements depend on the morphology, function and, biochemical composition of the tissue. They are also influenced by the fluorophore distribution, and by the tissue's optical properties, especially at the maximum imaging depth. Quantitative optical spectroscopy techniques constitute an objective diagnostic methodology, as they do not rely on the operator's experience.

Fluorescence emission from a skin lesion is excited with an irradiation source including coherent or incoherent broadband lights, e.g., laser, light-emitting diode (LED), a xenon lamp, or a halogen lamp. Fluorescence originates either from endogenous fluorophores (autofluorescence) or from exogenously administered fluorophores (photosensitizers).

Skin tissue autofluorescence, by near-ultraviolet (UV) and blue excitations, originates from endogenous fluorophores such as reduced nicotinamide adenine dinucleotide (NADH),[5–9] collagen, elastin,[9,10] and tryptophan.[8] Light-induced autofluorescence spectroscopy with laser source can be used to detect and quantify

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differences between healthy tissue and pathological lesions *in vivo*, in real time, with easy-to-use methodology for measurements, lack of need for contrast agents, precision, selectivity, and specificity (Table 1).[11–13] The use of lasers, instead of incoherent light sources in autofluorescence spectroscopy, has the advantage of using an exact wavelength and can deliver the excitation light with fibers to the selected body site, without significant loss of light power, and derive, in real time, medical information from naturally occurring endogenous fluorophores, without adding external (exogenous) fluorescent markers.[14] Tissue fluorescence can be improved by the application of exogenous fluorophores (photosensitizers) with selective absorption and fluorescence properties, which preferentially accumulate in cancer cells and, after the irradiation with light of specific wavelength, emit characteristic fluorescence delineating the site of NMSC.[15] Clinical studies have demonstrated that photosensitizer-induced skin fluorescence has significant diagnostic advantages compared with skin autofluorescence due to the increase in the fluorescence intensity by using exogenous fluorophores accumulating in the malignancy and the consequent better demarcation between the malignant and normal tissues (Table 2).

The use of exogenous fluorophores has been integrated in the clinical practice with the topical photodynamic therapy (PDT). Topical 5-aminolevulinic acid (ALA) and methyl aminolevulinic acid (MAL) are the most commonly used topical agents in PDT, while second-generation photosensitizers are under investigation. MAL-PDT has been approved by the European Medicines Agency as a treatment for actinic keratoses on the face and scalp when other therapies are considered less appropriate, for superficial and/or nodular BCC unsuitable for other available therapies, such as lesions on the mid-face or ears, lesions on severely sun damaged skin, large lesions, or recurrent lesions, and for SCC *in situ* (Bowen's disease) when surgical excision is considered less appropriate. PDT is increasingly being investigated in dermatology for a wide range of inflammatory and infectious cutaneous diseases.[16] It is based on the selective destruction of cancerous or affected cells without damaging the surrounding healthy tissue by combining three elements: oxygen, light, and a photosensitizing agent.

Light sources for PDT are coherent and incoherent broadband lights. Lasers as coherent sources are metal vapor lasers (copper and gold vapors), dye-pumped tunable (Ar^+-dye and Nd:YAG-dye) lasers, and diode lasers. Their advantages include the possibility to treat the lesion without affecting the surrounding healthy skin, the short illumination time without heating the surrounding tissue, and the use of monochromatic light.[17–22] Laser's capacity to emit high-flux monochromatic light and its focal precision, allows for small, demarcated lesions to be selectively treated within a short-time interval.[19,21,22] The advantage of dye lasers is the possibility to change the dye and thus the emission wavelength, making it possible to use the same laser in combination with various photosensitizers. Because of their large beam cross-section (typically 1 to 3 cm^2), the metal vapor lasers can be applied for PDT of large lesions, such as those occurring in the skin, without the need to use a beam expander and the alignment with the dye module is not critical as for argon lasers.[23] Diode lasers, besides having a convenient size, are also reliable, cheaper, and easy to use.[24] Incoherent light sources are fluorescent lamps, LEDs, filtered xenon arc, and metal halide light. LED are broadband sources, cheaper, more compact, and convenient for therapy of wider areas, larger or multiple tumors, and field cancerization therapy.[19,20,24–27] Photodynamic diagnosis (PDD) is a method for tumor demarcation that is based on the visualization of the fluorophore accumulating in the tumor tissue, by the use of fluorescence imaging.

3 Laser-Induced Autofluorescence for the Diagnosis of BCC

Noninvasive treatments are increasingly being used for the management of BCC, the predominant type of NMSC, making the

Table 1 Laser-induced fluorescence (LIF): pros and cons.

Pros	Cons
Noninvasiveness: not every clinically suspect NMSC lesion turns out to be cancerous	
No need for a lesional biopsy, i.e., an invasive method, which requires the evaluation from a trained dermatopathologist, and the result may take some days to be available or may be inconclusive (due to an error of biopsy sampling or tissue mishandling)	Malignant tissues normally exhibit weak fluorescence with small features that are difficult to observe
High sensitivity	The multitude and the variability of clinical forms and fluorescence properties of benign and malignant skin lesions limit its specificity
User-friendly methodology	The interpretation of results may depend on patient-related characteristics such as skin, age, and phototype
Real-time measurements	
Use for photodynamic diagnosis (PDD) of BCC	
Fluorescence diagnosis allows an accurate assessment of BCC borders to determine excision margins prior to surgical removal	
LIF may be used to follow up after PDT in deciding whether repetition of the treatment is necessary when the response to therapy is difficult to ascertain	

Table 2 Differences in fluorescence between BCC and healthy skin depending on excitation wavelengths and autofluorophores.

Laser/light source, excitation wavelength	Autofluorophores	Differences in fluorescence between BCC and healthy skin tissue	References
Nitrogen laser, 337 nm	Collagen reduced nicotinamide adenine dinucleotide (NADH)	BCC: lower fluorescence intensity than healthy skin	3, 38
LED, 365, 385, and 405 nm	Endogenous porphyrins	BCC: lower fluorescence intensities than healthy skin	8
		Appearance of porphyrin is typically pronounced in advanced sate of BCC	
Dye laser pumped by a nitrogen laser, 365 nm	NADH collagen keratin	No significant differences between the fluorescence of control sites and nonmelanoma skin tumors	11, 33
Low-pressure mercury arc lamp, 365, 405,436, 546, and 577 nm			
HeCd laser, 442 nm		BCC: lower fluorescence signal	34
125 W xenon arc lamp, 295 nm	Epidermal tryptophan residues	BCC: higher fluorescence intensity compared with normal skin	9, 35
	Dermal collagen cross-links	The measured fluorescence intensity was reduced in BCC relative to normal skin	9, 35
Xenon lamp (75 W), 370 nm	NADH, collagen, and elastin	BCC: lower autofluorescence than in surrounding normal skin	36
	PpIX	BCC: higher PpIX fluorescence than in surrounding normal skin	
Nitrogen/dye laser, 410 nm	Collagen elastin crosslinks	BCC: lower fluorescence emission than in normal skin	37
A frequency tripled ultrafast Yb: glass fiber laser, 355 nm		There is no clear trend in the lifetime shifts of BCC observed, with 355-nm excitation, while the fluorescence lifetime of BCC is consistently lower than that of the surrounding peri-lesional skin, under 445-nm excitation	39
Diode laser, 445 nm		No significant difference in the mean emission wavelength between BCC and healthy skin for either excitation wavelengths	

development of noninvasive diagnostic technologies highly relevant for clinical practice. Lesional biopsy and histopathologic evaluation (Fig. 1) have been the gold standard for the diagnosis of NMSC and the differential diagnosis of SCC and BCC. However, the biopsy is an invasive method; it requires histopathologic evaluation from a trained dermatopathologist, and the result may take some days to be available or the result may not be conclusive due to an error of biopsy sampling or mishandling of the tissue. Also, not every clinically suspect NMSC lesion turns out to be cancerous. LIF spectroscopy is a very attractive diagnostic technique for early diagnosis and demarcation of basal skin carcinoma due to its high sensitivity, user-friendly methodology for real-time measurements, and noninvasiveness.

Skin tissues under excitation with UV light (in the spectral region 260 to 400 nm) demonstrate differences in their biochemical content and metabolic state, with higher autofluorescence intensities in healthy skin compared with BCC, allowing diagnostic differentiation. In malignant tissue, fluorescence spectral changes are due to a decrease in collagen and elastin and a decrease in NADH levels, mainly due to the shifted equilibrium between the highly fluorescent NADH and the less-fluorescent oxidized-form NAD^+ in the malignant tissue. In the spectral region of 500 to 600 nm, the reduction of the fluorescence is attributed to hemoglobin absorption.[28–32] Similar autofluorescence patterns have been shown for both superficial and nodular BCCs.[28]

The feasibility of autofluorescence spectroscopy for skin cancer detection using excitation at 375 nm has been investigated. No significant differences in the shape of fluorescent spectra or in fluorescence intensity values between tumor and normal skin has been found.[33] Autofluorescence spectra from BCC lesions, excited with HeCd laser at 442 nm, showed decreased fluorescence intensity as compared with the surrounding normal skin, a trend that has also been confirmed by *in vivo* autofluorescence imaging of BCC lesions.[34]

More promising results reported higher fluorescence intensity in nonmelanoma tumors (BCC and SCC) compared with healthy skin using UV excitation at 295 nm for the tryptophan residues, which could be a result of epidermal thickening in tumor site. In contrast, the fluorescence intensity associated with collagen cross-links was lower in tumors, because of the erosion and degradation of the connective tissue after excitation

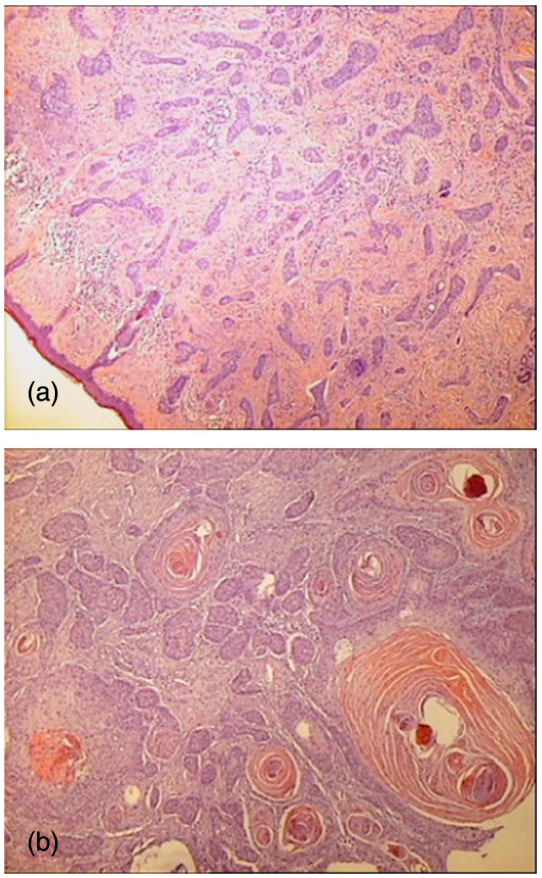

Fig. 1 Histopathologic images of (a) basal cell carcinoma (BCC) and (b) squamous cell carcinoma (SCC).

with 350 nm.[35] Similar results have been shown with lower fluorescence signal in BCC compared with normal skin.[36] Fluorescence spectroscopy with a nitrogen/dye laser tuned at 410-nm excitation has been used for the detection of BCC *in vivo*.[37] A correlation was found between the cancer detection diagnostic accuracy and the skin phototype in 49 patients. The diagnostic accuracy for tumor detection was lower in patients with darker skin types, with a diagnostic accuracy of 93% for phototype I, while it was 78% for phototype III.

Spectroscopic assessments of autofluorescence during experiments on unstained human skin samples (BCC, SCC, and healthy tissues) were carried out with a homemade nitrogen laser ($\lambda = 337$ nm).[37] The fluorescence intensity of malignant tumor was weaker than that of the normal tissue, especially in the skin tissue with SCC, which showed a larger displacement in the red spectrum. Moreover, there were significant differences in the spectral signatures between BCC and SCC, which facilitated their differential diagnosis.

Another laser-induced spectroscopic method used in skin tissues is the investigation of the fluorescence lifetime of endogenous fluorophores. Time-resolved autofluorescence spectra of BCC have been studied with *in vivo* measurements prior to surgical excision.[38] This method is relatively independent of the factors of fluorophore concentration and signal attenuation by the sample and has been shown that both *ex vivo* and *in vivo* effectively differentiate between healthy and affected tissues in various types of skin cancer.[38–40] The paired difference in fluorescence lifetime in each spectral channel between healthy skin and BCC has been investigated. The fluorescence lifetime did not vary significantly with emission wavelength over the spectral range containing the peak fluorescent signal (425 to 540 nm for UV excitation and 475 to 550 nm for blue excitation). There was no significant difference in the mean emission wavelength between healthy skin and BCC for either excitation wavelengths. There was no clear trend in the lifetime shifts in BCC observed with UV excitation; however, for blue light excitation (445 nm), the fluorescence lifetime in BCC was consistently lower than that of the surrounding nonaffected peri-lesional skin (i.e., $t_{\text{lesion}} < t_{\text{normal}}$).[38]

Tissue fluorescence analysis with a 442-nm HeCd laser light was used to illuminate and excite unstained skin tissue sections in order to demarcate the normal and BCC areas.[41] The fluorescence images of the samples were recorded by a CCD camera though a microscope. In the cancerous regions, the epidermis showed a very weak fluorescence signal, while the stratum corneum exhibited fluorescence emissions peaking at about 510 nm. In the dermis, the basal cell islands and a band of surrounding areas showed a very weak fluorescence signal, while distal dermis above and below the basal cell island showed a greater fluorescence signal with different spectral shapes.

Wide-field false-color images of fluorescence lifetimes of unstained biopsies of 25 BCCs were studied, following excitation of autofluorescence with a 355-nm pulsed UV laser to clearly discriminate areas of BCC from the surrounding uninvolved skin and to allow localization and delineation of the malignant areas.[39] The maximum visual information could be obtained by merging the lifetime and intensity images to produce intensity-weighted lifetime maps. The resultant image combined two complementary dimensions of information and allowed the lifetime information to be related to the anatomical intensity image without loss of information from either parameters.[39]

The excitation wavelengths, autofluorophores, and differences in fluorescence between healthy and cancer tissues are summarized in Table 2.

4 PDD for BCC

LIF by exogenous fluorophore agents has been used in the context of PDT as a diagnostic method. PDD is a method for tumor demarcation that is based on the visualization of a fluorophore, with the ability to accumulate in tumor tissue, by the use of fluorescence imaging. ALA and MAL are nonfluorescent precursors of fluorescent protoporphyrin IX (PpIX). Visualization of PpIX represents the basis of the PDD for skin tumors, and it may be used with fluorescence imaging systems. Image-processing methods widely used for demarcation of BCC after ALA-induced PpIX include the ratio imaging method and threshold-based imaging.[42] Ratio imaging method uses the division of either autofluorescence intensity versus PpIX fluorescence intensity after ALA application, or the fluorescence intensity of tumor marked area versus the ALA-treated normal skin area, or the fluorescence intensities between red over other spectral areas after administration of ALA-induced PpIX.[42–44] Threshold imaging method uses either a total emission photon count in fluorescence spectra as the discriminating index where a threshold value is calculated to separate normal tissue indices from indices of cancer tissues or an intensity ratio between normal and control skin tissues' fluorescence spectra.[37,42] This threshold divides the signal intensity scale into two zones: normal and cancer zones. The optimal threshold value, however,

depends on the intensity of the acquired fluorescence image.[42] Those two methods can reduce geometric effects, influence from the incident light distribution, and provide a quantitative measurement for correlation with subsequent histological assessments, although when intensity of the fluorescence is fluctuating, the threshold-based methods introduce errors in tumor demarcation.[42,44]

Five superficial and 10 nodular BCCs in 15 patients were studied with fluorescence measurements prior to the topical application of ALA, 2-, 4-, and 6-h post-ALA application, immediately post-PDT (60 J cm^{-2} at 635 nm), and 2 h after treatment. Superficial BCC showed a maximum PpIX fluorescence 6 h post ALA application, whereas in nodular BCC the maximum occurred 2 to 4 h after the application.[45] This variability of the fluorescence intensity may be due to the duration of the ALA/MAL application time, the concentration of the ALA/MAL cream, and/or the intensity of the illuminating light. There are various approaches to achieve robust tumor demarcation with derivation of novel unsupervised image segmentation methods that are not dependent on the variability of the fluorescence intensity, and LIF may be used for tumor demarcation, i.e., determination of the tumor boundaries.[42]

Fluorescence diagnosis allows an accurate assessment of BCC borders to determine excision margins prior to surgical removal. LIF with a diode laser at 633 nm was used to monitor the buildup of the ALA/methyl-esterified δ-ALA (ALA-ME)-induced PpIX in BCCs.[46] A clear demarcation between the lesion and the normal skin was detected with LIF for both PpIX precursors before PDT treatment.[46]

Pharmacokinetic studies investigated the borders of tumor growth and the intensity of accumulation of radachlorin in 32 patients with BCC and the intensity of accumulation of Photosense in 81 patients.[47] The discrimination between normal and malignant tissues was done by spectral-fluorescent complex and spectra analyzer LESA-01 (He-Ne-laser, $\lambda = 633$ nm). There was fluorescence from all tumors, and additional fluorescence zones were found, while a cytological confirmation of BCC was available in most cases. Two-dimensional images of fluorescence signs of radachlorin in normal skin were detectable up to 5 days after injection.

In an attempt to increase this PpIX-based fluorescence tissue contrast in normal skin in seven patients with nodular BCC, a multichannel fluorescence imaging system was developed to collect PpIX (635 nm), autofluorescence (470 and 600 nm), and photobleached products (670 nm) emission from both cancerous lesions and surrounding normal skin before and after PDT, after excitation with a small sealed-off nitrogen laser pumping a dye laser emitting at 405 nm.[48,49] The photosensitizer's fluorescence was monitored to distinguish normal skin from malignant lesions, as well as to track the accumulation of photodegraded products during PDT. The malignant region 1 week after PDT was limited to the area delineated by the multicolor fluorescence imaging system, suggesting that the tumor did not, to a large extent, infiltrate the surrounding tissue.

Optical spectroscopy may noninvasively monitor disease progression in real time based on its ability to collect optical parameters that correspond to distinct morphology, function, and biochemical composition of the tissue, which may change over time.[50] LIF may be used to follow up after PDT in deciding whether the repetition of the treatment is necessary when the response to therapy is difficult to ascertain.

5 Optimizing Results: Processing and Interpretation of Spectral Data

In general, the LIF spectra of normal and malignant tissues exhibit certain differences at several wavelengths. However, it is difficult to observe subtle but consistent differences in the raw data, because these differences are often masked by large variations in intensity. Intrapatient variability in the fluorescence intensity response is typically large and affects the diagnostic accuracy.[51,52] In addition, the autofluorescence spectra of malignant tissues have usually very low intensity of fluorescence radiation emitted by endogenous fluorophores, above excitation of 300 nm.[3,15,34,36,53]

Fluorescence is a highly promising and attractive technique for the diagnosis and demarcation of BCC. However, the multitude and the variability of clinical forms and fluorescence properties of benign and malignant skin lesions pose issues that limit its specificity. This is also the case with BCC that may present as nodular, superficial, morpheic, cystic, or ulcerative type.[13] Also, the interpretation of results may depend on patient-related characteristics such as skin age and phototype.[54,55] In order to account for intrapatient and intralesion variations in the evaluation of the results from LIF spectra, mathematical models and specific statistical analysis techniques have been applied. Some researchers focused on the spectral profile of each spectrum containing specific characteristics that are more consistent. These have been amplified and compared by the use of effective diagnostic algorithms with some form of normalization. This is especially important for malignant tissues which normally exhibit weak fluorescence with small features that are difficult to observe.[56] Nevertheless, the simple normalization, namely, the division with respect to the integrated intensity of the entire spectrum, is redundant and inefficient.[52,57] Fluorescence spectra of NMSC[37] underwent statistical process using total emission photon count as the discriminating index. A threshold value was calculated to separate normal tissue indices from indices of cancer tissues. The classification accuracy of each data point was determined using the threshold value.

Rajaram et al.[50] fit the observed spectra to models and were able to extract optical parameters of the tissue such as the absorption and scattering coefficients, hemoglobin concentration, and the relative contributions of the constituent fluorophores. Using these parameters in a leave-one-out cross-validation, they were then able to diagnose BCCs with a sensitivity of 94% and a specificity of 89%.[50]

Various data analysis methods have been devised and employed to differentiate between fluorescence spectra of normal and cancerous tissues for the purpose of cancer diagnosis. There are several methods for the analysis of autofluorescence tissue spectra. Evaluated methods include principle peak ratio, differential normalized fluorescence (DNF), bivariate DNF (2-D-DNF), principal component analysis, and correlation coefficient mapping.[56–64]

Among these methods, DNF is a simple, straightforward method and provides excellent classification.[56] In the DNF analysis, the diagnostic features are extracted from the difference between the averaged cancerous and averaged normal tissue spectra. Thus, in this analysis,[53,65] first, a normalization process is utilized in order to amplify and compare the spectral characteristics of normal and malignant tissues. The LIF spectra of normal and tumor tissues can be normalized to 1, at a particular wavelength, where the normalization wavelengths can

be chosen at the point corresponding to maximum fluorescence intensity. The resulting normalized intensity for each spectrum has a dimensionless value and consequently becomes less dependent on the intensity factor. In addition, a baseline curve, as the mean average of normalized fluorescence spectra from a reference set of normal tissue samples, can be determined, due to the fact that the normalized spectra of normal tissues have similar spectral profiles. Finally, a DNF curve for a specific tissue sample can calculated as the difference between the normalized fluorescence spectrum and the baseline curve. An excellent feature of the DNF method is its efficiency in extracting the diagnostic index. The DNF index, which is the spectral intensity of each tissue spectrum at the feature wavelength subtracted by the averaged normal tissue spectrum at the same wavelength, serves as the diagnostic index in cancer detection. The accuracy of the DNF method in a proper discrimination of BCC and normal skin tissues can be specified by calculating the percentage of correctly classified spectra, according to histopathology results.[53] The advantages of the DNF method, especially how to best perform cancer diagnosis based on the rich information in the extracted spectral features, have not been fully exploited. In DNF analysis of tissue spectra, usually two or more spectral features become apparent. In practice, either one of them was used in diagnosis or two spectral features were used independently to yield their own results. The sensitivity and specificity obtained for LIF in skin malignancies by DNF depend on the selected wavelength in the peak-near areas and can reach values from 80% to 98%,[64] whereas in other tissue malignancies, like tissue pathologies of the esophagus, authors reported a sensitivity of 100% and a specificity of 98%.[10]

Table 3 LIF: take home pearls.

1. Optical measurements including optical scattering, absorption, and fluorescence of the skin depend on its morphology, functional properties, and biochemical composition.
2. The photosensitizer-induced skin fluorescence has significant diagnostic advantages compared with skin autofluorescence due to the increase in fluorescence intensity by using exogenous fluorophores accumulating in skin cancer tissue, providing better demarcation between the malignant and normal tissues.
3. The measured optical properties and fluorophore contributions of normal skin and nonmelanoma skin cancers (NMSCs) are significantly different from each other and correlate well with tissue histology.
4. Intrapatient variability in the fluorescence intensity response is typically large and affects the diagnostic accuracy. In order to account for intrapatient and intralesion variations in the evaluation of results from LIF spectra, mathematical models and specific statistical analysis techniques have been applied with differential normalized fluorescence (DNF).
5. LIF spectroscopy is a very attractive diagnostic technique for the early diagnosis and demarcation of BCC due to its high sensitivity, user-friendly methodology for real-time measurements, and noninvasiveness.
6. In patients with BCC, photodynamic diagnosis may be used for detecting subclinical disease, determining surgical margins, and following up for residual tumor or BCC relapse.

6 Conclusions and Future Perspectives

Laser technology based on optical spectroscopy is a diagnostic tool for BCC, emerging to be integrated in the clinical setting. LIF spectroscopy is a very attractive diagnostic technique for early diagnosis and demarcation of BCC due to its high sensitivity, user-friendly methodology for real-time measurements, and noninvasiveness. The measured optical properties and fluorophore contributions of normal skin and NMSCs are significantly different from each other and correlate well with tissue histology (Table 3).

In patients with BCC, PDD may be used for detecting subclinical disease and determining surgical margins and following up for residual tumor or BCC relapse. Further research may investigate new photosensitizers and laser sources for the accurate diagnosis and follow-up monitoring of BCC in clinical practice.

The combination of optical techniques such as LIF spectroscopy and diffuse optical spectroscopy offer promise as a useful multimodal approach with considerable superiority for differentiating between normal and malignant tissues than each method alone.

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