Single LED-Based Device to Perform Widefield Fluorescence Imaging and Photodynamic Therapy

Clovis Grecco[a], Hilde H. Buzzá[a], Mirian D. Stringasci[a], Cintia T. Andrade[a], Jose D. Vollet-Filho[a], Sebastião Pratavieira[a], Anderson L. Zanchin[b], Aparecida M. Tuboy[b], Vanderlei S. Bagnato[a*]

[a] *São Carlos Institute of Physics, University of São Paulo, PO Box 369, 13560-970, São Carlos, SP, Brazil.*

[b] *MMOptics Ltda., São Carlos, SP, Brazil.*

ABSTRACT

Photodynamic therapy (PDT) is a treatment modality that can be indicated for several cancer types and pre-cancer lesions. One of the main applications of PDT is the treatment of superficial skin lesions such as basal cell carcinoma, Bowen's disease and actinic keratosis. Three elements are necessary in PDT, a photosensitizer (PS); light at specific wavelength to be absorbed by the PS, and molecular oxygen. A typical PS used for skin lesion is protoporphyrin IX (PpIX), which is an intrinsic PS; its production is stimulated by a pro-drug, such as 5-aminolevulinic acid (ALA). Before starting a treatment, it is very important to follow up the PpIX production (to ensure that enough PS was produced prior to a PDT application) and, during a PDT session, to monitor its photodegradation (as it is evidence of the photodynamic effect taking place). The aim of this paper is to present a unique device, LINCE (MMOptics - São Carlos, Brazil), that brings together two probes that can, respectively, allow for fluorescence imaging and work as a light source for PDT treatment. The fluorescence probe of the system is optically based on 400 nm LED (light emitting diodes) arrays that allow observing the fluorescence emission over 450 nm. The PDT illumination probe options are constituted of 630 nm LED arrays for small areas and, for large areas, of both 630 nm and 450 nm LED arrays. Joining both functions at the same device makes PDT treatment simpler, properly monitorable and, hence, more clinically feasible. LINCE has been used in almost 1000 PDT treatments of superficial skin lesions in Brazil, with 88.4% of clearance of superficial BCC.

Keywords: fluorescence imaging, photodynamic therapy, protoporphyrin IX, light emitting diodes

*vander@ifsc.usp.br; http://cepof.ifsc.usp.br

Biophotonics South America, edited by Cristina Kurachi, Katarina Svanberg, Bruce J. Tromberg, Vanderlei Salvador Bagnato, Proc. of SPIE Vol. 9531, 953121 · © 2015 SPIE
CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2185925

INTRODUCTION

Photodynamic Therapy (PDT) involves three key elements: a photosensitizer (PS), light with the appropriate wavelength to excite the PS, and molecular oxygen present in the tissue. In this process, the PS is administered and preferentially accumulates in abnormal cells. Light is delivered to the photosensitized tissue, and the excited PS molecules, in contact with molecular oxygen, produce reactive cytotoxic species that promote cell death either by necrosis, apoptosis or autophagy [1, 2].

There are many PS that can be used in clinical applications of PDT. Most of them are intravenously injected, and it is necessary to wait a specific time for the PS to concentrate within the lesion; this waiting time is known as Drug-Light Interval (DLI). Nevertheless, endogenous PS also exist, such as protoporphyrin IX (PpIX), which production can be stimulated in the tissue. PpIX is a precursor of heme in its biosynthetic pathway. Under normal conditions, heme is regulated so that there is no PpIX accumulation. However, when its production is stimulated aiming PDT applications, PpIX is produced in the mitochondria, accumulates within cells and works as an efficient PS.

The main precursors of PpIX are 5-aminolevulinic acid (ALA) and its variants as the methyl-aminolevulinate (MAL). A negative feedback control within mitochondria responds to changes in concentration of heme that depend on the available concentration of ALA. The increase of ALA in a biological tissue can lead to an increase of local PpIX production and, consequently, to an accumulation [3]. One of the options to increase the PpIX concentration in lesions is the topical application of ALA or MAL (the latter is clinically used in topical applications to favor ALA percolation through skin). Normal tissues usually eliminate very fast the excess of PpIX, while abnormal tissues have a different accumulation and elimination time.

ALA does not show observable fluorescence when excited in the visible range while PpIX has a high fluorescence. The emitted fluorescence signal allows visual follow-up of the accumulated PpIX, requiring only a light source for PpIX excitation at about 400 nm, and filters to cut off the backscattering light. With such a simple setup, it is possible to evidence and observe PpIX fluorescence emission, which can be used as a tool in order to discriminate normal from abnormal tissue, to increase the visual contrast, and to determine the lesion margins for treatment [4].

An important issue about de use of widefield imaging systems is the ability of monitoring PDT treatment. Firstly, the margins of treatment can be determined by fluorescence visualization only. Secondly, it makes possible to monitor PpIX consumption during the PDT procedure by the decrease in fluorescence emission, caused by the PpIX photodegradation, which is an indicative of the reactive oxygen species activity [5, 6]. After the treatment application, presence of little to none signal of PpIX fluorescence is also an indication that enough fluence was delivered to the lesion.

With this improvement in mind, our research group proposed to provide a tool that conjugates the necessary instrumentation for PpIX irradiation aiming PDT application and monitoring in tissue. To achieve this purpose, a

device should contain two setups composed by light sources: one for tissue illumination to perform the treatment, and another to assess the PpIX formation and its bleaching. For PDT treatment, red light (630 nm) is usually is the best option because it can penetrate deeper in the tissue, while violet light (400 nm), although more efficient to excite PpIX and to allow a better visualization of PpIX fluorescence, is limited in penetration and thus reaches very superficial layers of tissue only. There are PDT applications, though, for which shorter wavelengths are perfectly suitable, such as microbiological control and very superficial lesions (e.g., keratoses). These applications usually have also to be delivered to large areas, since such lesions easily disseminate on tissue surface, and small probes make the clinical treatment time frequently impracticable. All this considered, our proposal for this equipment does include two treatment probe options, presenting different arrangement of the LED arrays, to provide proper illumination of either small or large lesions, and offering the possibility of treatment of large areas using any of those wavelength ranges (red or blue).

The present article thus describes a dual device, aimed for both lesion visualization/treatment monitoring and PDT treatment, LINCE (MMOptics, São Carlos - Brazil) which was developed by the company in a partnership with the team from the Biophotonics Lab of the São Carlos Institute of Physics, at the University of São Paulo, partiality financed by FINEP agency grant. Main components, a suggested application protocol, and a brief description of the main results achieved with this device are herein presented.

MATERIAL AND METHODS

LINCE is a LED-light source device for irradiation of biological tissues *in vivo*, aiming to be both a PDT source and a fluorescence generation source. For this purpose, two different probes are available in the device platform: a fluorescence visualization probe and a treatment probe (Figure 1). Both use light-emitting diodes (LED) arrays as light source, in different wavelengths.

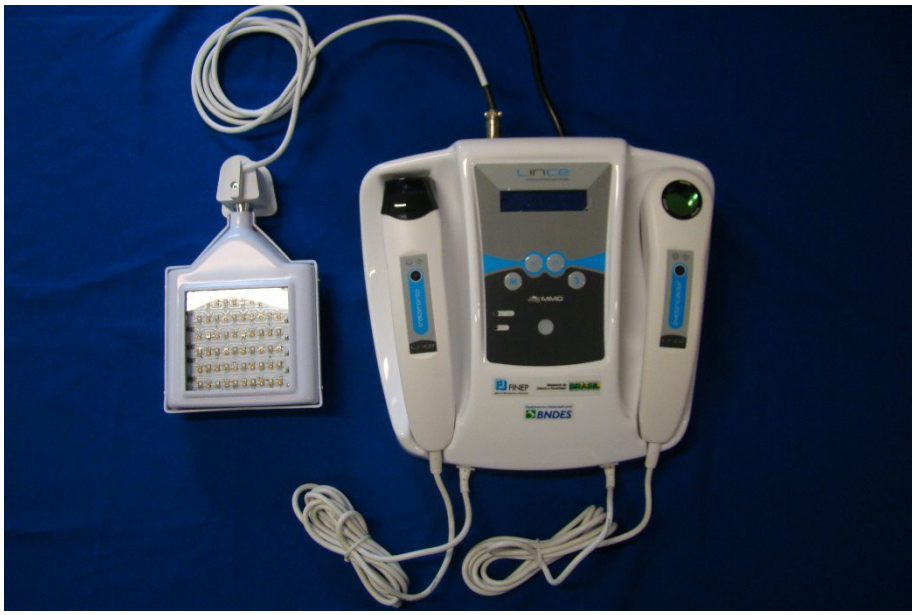

Figure 1 - View of the device platform: a) standard treatment probe; b) large area treatment probe.

Platform Main Board

This board is the main platform to which both probes are pigtailed. It includes probes sockets, an "on/off" button, and a pigtailed power cable that feeds the device. A digital display and buttons are also present to allow for selection of either the evidencing or the treatment probes, and to determine the treatment parameters for irradiation. The platform also includes a light sensor to be used every time each of the probes is turned on, and it allows one to verify if the probe in use is emitting light enough for sufficient fluorescence visualization or appropriate treatment.

Fluorescence Visualization Probe

The fluorescence probe is an optical setup composed of LED arrays, the LED electronics, light collimator components, a dichroic mirror, and a longpass filter (similar setup is found elsewhere [7]). Those LED arrays emit at about (400±10) nm, with maximum output power of 50±10 mW/cm². Figure 2 shows the spectrum of the LED used at visualization probe. These wavelengths are suitable for excitation of most of the biomolecules present at skin, and of most of the PS molecules that are currently used in PDT. The collimators are used to help focusing and directing the LED light upwards to the main cavity in the probe. At this point, the dichroic mirror splits light, allowing undesired wavelengths to go through and redirecting a narrower LED emission band to the objective output of the cavity. At the target tissue, light is absorbed and fluorescence generated. All light collected at this output returns from the investigated tissue to the cavity, and collected light at the excitation range is again deviated from the cavity visualization axis. The remaining light, which includes the generated

fluorescence, goes through the dichroic to the eyepiece, where another filter improves the contrast between green fluorescence and red fluorescence.

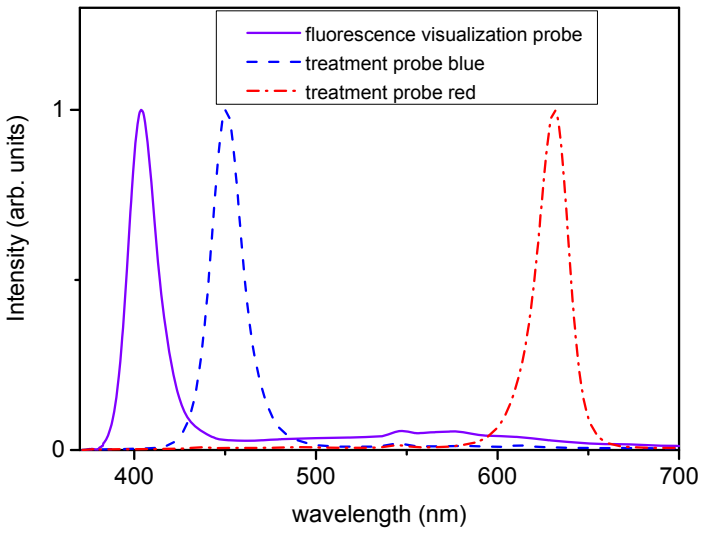

Figure 2 - Emission light spectra.

The arrangement of the light sources and filters suits the aimed application, since normal tissue fluoresces at the green range of the electromagnetic spectrum, due to the presence of highly organized collagen fibers, and abnormal skin tissue presents an important reduction in this fluorescence due to the absence of such an organized collagen[8]. In addition, the main PSs currently used for PDT and marked fluorescence techniques so far mostly fluoresce in the red range of the spectrum. Hence, the abnormal tissue does not fluoresce importantly at green, but as the PS accumulates in cells, strong fluorescence signal in the red range is observed, which makes that contrast an important achievement for proper optical monitoring of PS uptake and clearance during/after PDT [9].

Treatment Probe - Small Areas

The small probe area illumination is composed of a set of five LED arrays, emitting at (630±10) nm (figure 2), arranged in a circle at the tip of the probe, and a piece of light collimator to focus the produced beam. There is also a support to keep the probe at the ideal distance for irradiation (the one for which the device was calibrated), since PDT applications depend on the fluence rate delivered, which in turn depends on the distance of the light source. The diameter of the irradiation tip (3.3 cm - for 20 ± 2 mm spots) was designed to privilege small lesions, such as basocelular and nodular carcinomas up to 2 cm in diameter. These lesions are far more common than others, and respond more adequately to the treatment using the protocols this device was devised for.

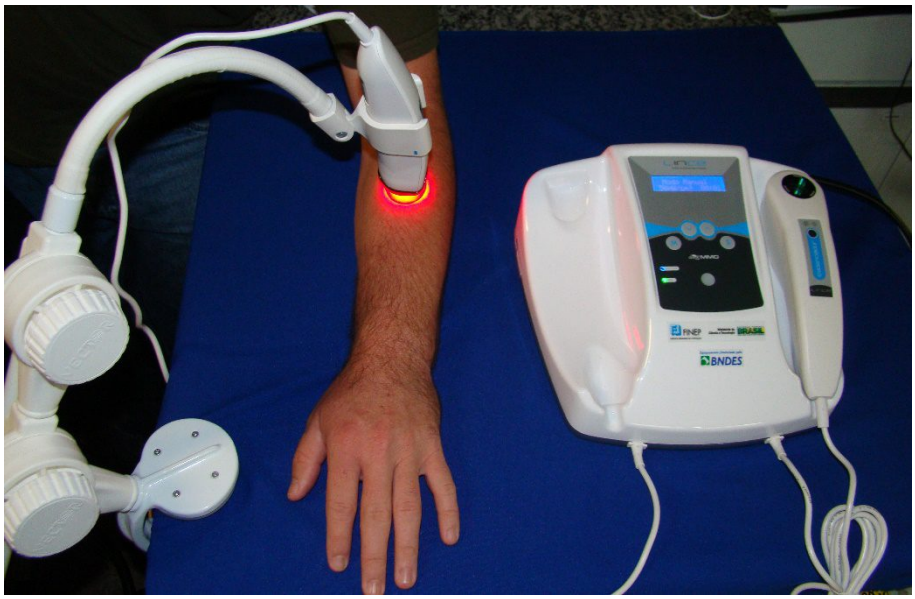

Figure 3 - Illustration of PDT treatment using the small area probe.

Fluence rate for this probe, rather than fixed, is controlled by its electronics, which is comprised in the main board of the platform and changed using the digital display. There are fluence rate options ranging from 50 mW/cm² to 150 mW/cm², with increments of 25 mW/cm², and time control that allows from 1 min up to 90 min of uninterrupted irradiation. Such freedom of choice allows for a skilled professional to set proper parameters of fluence rate and time intervals to apply any appropriate total fluence during treatment, which is a major parameter of PDT treatment. There are also three different standard protocols for which the treatment time is also automatically set: P1 is set as 50 mW/cm² and 67 min, P2 as 100 mW/cm² and 33 min, and P3 as 150 mW/cm² and 22 min (total fluence 200 J/cm² each).

Treatment Probe - Large Areas

In clinical applications, large lesions that are eligible for PDT treatment were also contemplated with a larger irradiation probe, since the small probe would require long periods of time to properly range the full extension of such lesions, which might make clinical situations impracticable (Figure 3b).

This larger probe is composed by a larger number of LED arrays for irradiation of larger areas. This probe is rectangular (8.0 x 7.6 cm), with 60 LED *arrays*. Thirty *arrays* emit at wavelengths centered at (630±10) nm, in the red range of the spectrum, and 30 *arrays* emitting centered at (450±10) nm range (figure 2), in the blue range of the spectrum. The *arrays* for each wavelength are arranged juxtaposed, to ensure an equivalent irradiation profile for both wavelengths, and to maximize the profile homogeneity in order to ensure equally delivered light fluences.

For this large probe, different fluence rates are used for the standard protocols, ranging between 10 and 30 mW/cm² for both wavelengths. The total irradiation area is 60.8 cm², in contrast to the area of the small probe

from the original LINCE, which is approximately 8.5 cm². That equals about seven times the original LINCE treatment area.

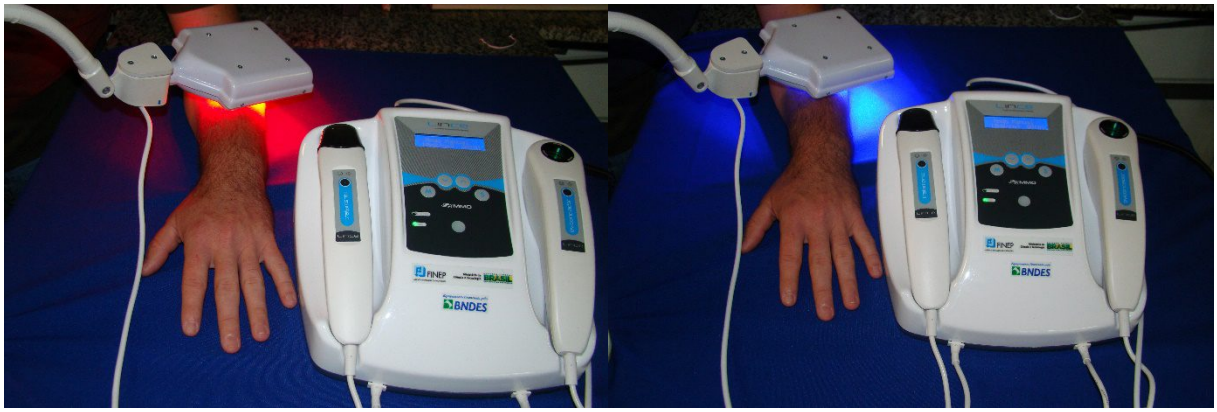

Figure 4 - Illustration of PDT treatment using the large area probe: a) red; b) blue.

Blue light has been added to this probe because there are PDT applications for the treatment of very superficial lesions, such as actinic or seborrheic keratoses, which may strongly benefit from using light within this range of the spectrum region combined to the most common PSs, such as PpIX. These PS are more efficiently activated with blue light than with red light, but it cannot be used for deeper lesions due to the low penetration of the shorter wavelengths in biological tissue [10].

In addition, other non-oncological applications of PDT use blue light, such as microorganisms' inactivation on infected lesions, which require very short penetration of light to be effective.

RESULTS AND DISCUSSION

Research and Clinical Application

As mentioned before, a device that brings together a platform to evidence lesions and a treatment light source would allow one to monitor the PpIX production and its clearance throughout the treatment. The process of evidencing increases the ability of the physician to fully observe the lesion, since different tissues have different fluorescence.

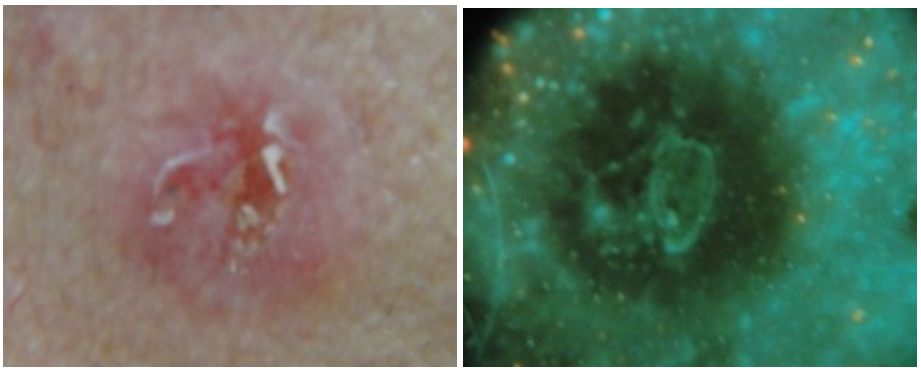

Figure 5 – Figure shows a basal cell carcinoma: a) standard white light image; b) autofluorescence of the lesion, showing the lesions borders with improved accuracy by the contrast of different fluorescent regions, when compared to white light imaging.

Figure 5 shows clearly how fluorescence makes the borders more easily identified than using white light imaging only. Skin abnormal tissue usually presents biochemical changes such as reduction in collagen fibers organization and modification in the amounts of NAD(H) and FAD(H), which make abnormal tissue much less fluorescent than normal skin tissue, providing high contrast and improving margins determination.[8]

It is also possible to perform the so-called "marked" fluorescence to detect the lesions, using an agent that has affinity with the abnormal cells and that provides a different color of fluorescence to improve this visualization. Fortunately, because PpIX accumulates preferentially in those cells and presents high fluorescence yield, in a contrasting wavelength when compared to normal skin, the photosensitizer itself contributes to best determine legion borders. As a matter of fact, the low fluorescence of unhealthy skin tissue improves that even further, because photosensitized lesion cells emit mostly red fluorescence, and normal tissue emits green fluorescence, enhancing the visual contrast.

Since the abnormal cells will produce more photosensitizer and its fluorescence is in the red range, the lesion becomes very prominent by observation with the evidencing probe even during treatment. After the illumination, however, photobleaching of PpIX will have taken place, and the red fluorescence will be decreased, as showed in the Figure 6. This important reduction in the PpIX fluorescence from the irradiated tissue is a strong evidence of the consumption of the drug, and thus of a successful delivery of the required PDT light fluence.

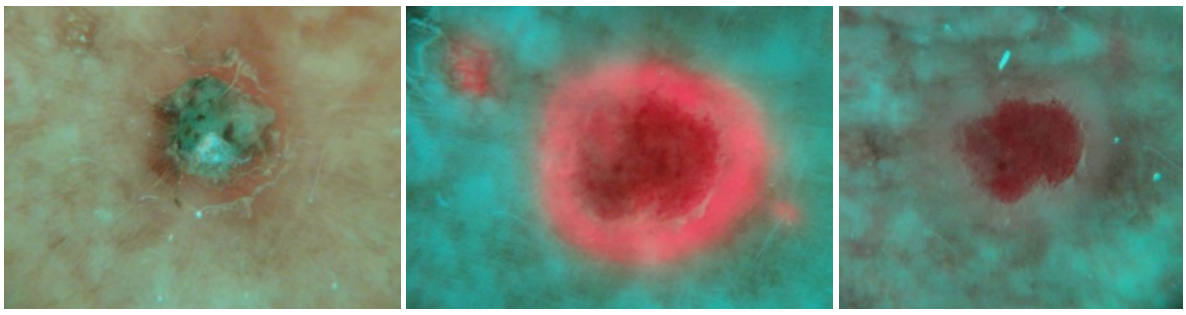

Figure 6 – The monitoring of PpIX: a) before the ALA application; b) 3 hours after ALA application, with PpIX production; c) after illumination.

The possibility to follow, in real time, every step of the treatment using a single tool gives more confidence to the professionals that are applying the PDT as a treatment, because it provides a method to infer immediately the outcome of the application based on the information provided by fluorescence assessment.

The use of LINCE under clinical trials has been exhaustively performed so far in treating primary superficial and nodular BCC with diameters up to 20 mm, and maximum thicknesses of 2 mm, as well as Bowen's diseases. Approximately a thousand BCC lesions, including superficial and nodular ones, were treated during four years through a Brazilian government-funded PDT clinical trial project, with 88.4% of complete lesion remission. The evidencing probe has been successfully used to monitor the formation and the degradation of PpIX. The visualization of PpIX fluorescence greatly improved physicians' ability to follow treatment sessions from the beginning to the end. The evidencing probe has effectively provided a high-contrast between PpIX and normal skin fluorescence, permitting determination of the exact location of lesions and a customized treatment.

The additional proposal of the large area probe for irradiation has increasingly allowed treatment of a large number of skin lesions. Different fluence rate values can be adjusted, depending on the PDT protocol, and thermal effects are positively avoided by using the provided protocols, under adequate medical indication. The large area version of LINCE, besides application for large neoplastic lesions, has also been used to treat pre-malignant lesions such as actinic keratosis with large extensions, and for aesthetics procedures.

CONCLUSIONS

A conveniently designed device, providing conjugated treatment light source for PDT and an evidencing probe to properly allow for accurate determination of lesion margins and PDT follow-up was presented in literature for the first time. The main advantages of such a platform were explained, mainly concerning the convenience of a light device using LED arrays as light source and, especially, the important improvement in adequate monitoring of a PDT application for skin lesions throughout the whole process, from margin delimitation to assurance of sufficient light fluence delivery. The LED arrays used as light sources are also cheap and compact, which reduces the device weight and makes it very convenient during clinical procedures - even portable, provided power outlets.

This article has also described an additional probe for the same device, designed for large lesions, which are usually greatly deprived from fast and efficient PDT applications due to the inappropriate light sources available to date. Partial results of treatments using the device have been described elsewhere [11], and have been overviewed here. Reports showing the efficacy of this device shall increase as it comes out to the PDT community, as the important contribution it is, by leading to improved clinical conditions for PDT application in skin lesions.

ACKNOWLEDGMENTS

The authors acknowledge the support provided by the Brazilian funding agencies: CAPES; CNPq (INOF – INCT grant: 573587/2008-6); São Paulo Research Foundation (FAPESP), particularly by the grant 2013/07276-1 (CEPOF); BNDES (grant: 09.2.1458.1) and FINEP.

CONFLICT OF INTEREST

The authors Anderson L. Zanchin and Aparecida M. Tuboy are employees at MM Optics Ltda.

REFERENCES

[1] P. Agostinis, K. Berg, K. A. Cengel *et al.*, "Photodynamic Therapy of Cancer: An Update," Ca-a Cancer Journal for Clinicians, 61(4), 250-281 (2011).

[2] B. C. Wilson, "Advanced Photodynamic Therapy," Biophotonics, 315-334 (2008).

[3] J. C. Kennedy, and R. H. Pottier, "Endogenous Protoporphyrin-Ix, a Clinically Useful Photosensitizer for Photodynamic Therapy," Journal of Photochemistry and Photobiology B-Biology, 14(4), 275-292 (1992).

[4] S. L. Marcus, R. S. Sobel, A. L. Golub *et al.*, "Photodynamic therapy (PDT) and photodiagnosis (PD) using endogenous photosensitization induced by 5-aminolevulinic acid (ALA): Current clinical and development status," Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy V, Proceedings Of, 2675, 32-42 (1996).

[5] J. D. Vollet-Filho, P. F. C. Menezes, L. T. Moriyama *et al.*, "Possibility for a full optical determination of photodynamic therapy outcome," Journal of Applied Physics, 105(10), (2009).

[6] S. Pratavieira, P. L. A. Santos, P. F. C. Menezes *et al.*, "Phototransformation of hematoporphyrin in aqueous solution: Anomalous behavior at low oxygen concentration," Laser Physics, 19(6), 1263-1271 (2009).

[7] S. Pratavieira, C. T. Andrade, A. G. Salvio *et al.*, [Optical Imaging as Auxiliary Tool in Skin Cancer Diagnosis] InTech - Open Access Publisher, (2011).

[8] I. Pavlova, M. Williams, A. El-Naggar *et al.*, "Understanding the biological basis of autofluorescence imaging for oral cancer detection: High-resolution fluorescence microscopy in viable tissue," Clinical Cancer Research, 14(8), 2396-2404 (2008).

[9] C. T. Andrade, J. D. Vollet, A. G. Salvio *et al.*, "Identification of skin lesions through aminolaevulinic acid-mediated photodynamic detection," Photodiagnosis and Photodynamic Therapy, 11(3), 409-415 (2014).

[10] L. A. Sordillo, Y. Pu, S. Pratavieira *et al.*, "Deep optical imaging of tissue using the second and third near-infrared spectral windows," Journal of Biomedical Optics, 19(5), (2014).

[11] D. P. Ramirez, C. Kurachi, N. M. Inada *et al.*, "Experience and BCC subtypes as determinants of MAL-PDT response: Preliminary results of a national Brazilian project," Photodiagnosis and Photodynamic Therapy, 11(1), 22-26 (2014).