

PROCEEDINGS OF SPIE

SPIDigitalLibrary.org/conference-proceedings-of-spie

Label-free multispectral lifetime fluorescence to distinguish skin lesions

Renan A. Romano, Ramon G. T. Rosa, Javier A. Jo, Cristina Kurachi

Renan A. Romano, Ramon G. T. Rosa, Javier A. Jo, Cristina Kurachi, "Label-free multispectral lifetime fluorescence to distinguish skin lesions," Proc. SPIE 10890, Label-free Biomedical Imaging and Sensing (LBIS) 2019, 108902L (4 March 2019); doi: 10.1117/12.2510498

SPIE.

Event: SPIE BiOS, 2019, San Francisco, California, United States

Label-free multispectral lifetime fluorescence to distinguish skin lesions

Renan A. Romano^a, Ramon G.T. Rosa^a, Javier A. Jo^b, Cristina Kurachi^a

^a University of São Paulo – Sao Carlos Institute of Physics - Av. Trabalhador São-Carlense, 400 – São Carlos/SP –Brazil; ^b Department of Biomedical Engineering – Texas A&M University, US

ABSTRACT

Skin lesions are the most common human cancer diseases, usually, is it diagnosed by clinical visual inspections followed by biopsy. Early detection of these diseases is critical, depending on an accurate and trained dermatologist and can increase the survival rate. Aiming for screening and early diagnose skin lesions many techniques are presented, however, optical techniques are highlighted since they are fast and noninvasive. In this context, fluorescence steady-state and lifetime imaging show potential by being able to image metabolic changes using endogenous contrast. Here it is demonstrated an in vivo label-free multispectral fluorescence lifetime imaging system to distinguish between two types of clinically similar lesions. A pulsed Nd:YAG laser emitting at 355 nm is used to excite the endogenous fluorophores and three channels of acquisition bands are used to imaging the skin. Preliminary results showed differences in the fluorescence lifetime between Bowen and Actinic Keratosis as well as the lesion and the skin around, demonstrating a potential tool to identify the lesion and its edges.

Keywords: Fluorescence Lifetime, Multispectral, Skin Lesions, Label-free, in vivo

1. INTRODUCTION

Skin cancer diagnosis is a great challenge nowadays since it has the major incidence rates among all cancer types. Non-melanoma cancer is the most incident even being less aggressive, while melanoma is one of the most lethal cancer types due to its high metastatic potential. Early diagnosis is the main goal in new techniques development since it is responsible not only by increasing life expectancy, as well as decreasing treatment morbidity¹⁻³.

Based on changes on roughness, skin color, exophytic areas, and anamnesis visual clinical inspection is the most common method for skin cancer diagnosis, it is usually followed by biopsy and histopathological validation. In spite of this type of detection be very fast and, if performed by a well-trained dermatologist, well accurate, it has some problems. They are in general difficulty in determining borders of the tumor, leading to incomplete treatment, as well as errors in diagnosing clinically similar lesions. Furthermore, a two-session diagnostic procedure is not efficiently managed at the public health system.¹⁻³

In this context, early diagnosis label-free fluorescence is gaining prominence since it does not depend on exogenous fluorophores. Main native fluorophores are collagen, elastin, NADH, and FAD, and they are related to morpho-functional and metabolic activities they can act as intrinsic optical biomarkers. Since mitochondrial anomalies and dysfunction can be caused by many diseases (such as cancer, diabetes and neurodegenerative diseases) these molecules present a great potential as endogenous agents for diagnosis.^{4,5}

Aiming to distinguish clinically similar lesions this study used a multispectral fluorescence lifetime imaging system. Actinic Keratosis (benign) and Bowen disease (malignant) were imaged and lifetime optical biomarkers were analyzed. Preliminary results show regions of interest where it was possible to distinguish both surrounding healthy tissue and discriminate between these lesions.

2. MATERIAL AND METHODS

Malignant (Bowen disease) and benign (Actinic Keratosis) skin lesions of patients from Amaral Carvalho Hospital from Jau – SP were imaged. Lesions were chosen by a dermatologist according to both size and clinical diagnosis. Biopsy and histopathology were performed by the physician decision. Classification was based either on clinical or histological diagnosis.

Assembled FLIM operates on time-domain by measuring the fluorescence decays with a fast detector and digitalizer. Light from a Q-switched pulsed Nd:YAG laser (<1.5 ns pulses) emitting at 355 nm was used to excite native fluorophores. Fluorescence beam is divided into three channels by dichroic mirrors and filters (F1 – 390 ± 20 nm; F2 – 452 ± 22 nm; F3 > 496 nm). Each one being coupled by lenses to fibers and guided to a broadband amplifier and then to the same MCP-PMT (Microchannel plate – Photo Multiplier Tube) in different times where the signal is recorded in sequence at 2.5 Gs/s, allowing resolution of 0.4 ns. Control and acquisition are performed by a LabVIEW code in a computer, being able to acquire images in 2.5 s (140 x 140 pixels). Filters were chosen to cover major spectral band of collagen and elastin (F1), NADH (F2), and FAD (F3).⁶

Data was processed by correcting electronic baseline of fluorescence decay, as well as filter and fiber transmissions percentage average. Then, instrument response function deconvolution was performed to extract the signal of fluorescence decay from each emission channel. Decays were fitted by bi-exponential decay functions where parameters were called optical biomarkers. Exponential weights were adjusted and normalized, then only the second exponential weight was analyzed since it is correlated to the first exponential weight. Furthermore, area under the decay curve was calculated by integrating fluorescence signal on time. Integrated fluorescence ratios were also analyzed as optical biomarkers. Histograms of the images regions of lesion were performed to visualize differences on malignant and benign lesions.

3. RESULTS AND DISCUSSION

Figure 1 (a) and (b) show images of different optical biomarkers generated with FLIM data for Actinic Keratosis and Bowen disease respectively. Images show integrated intensity per channel in the first column, darker pixels indicate lower intensity values. While second column shows on false colors the average lifetimes per pixel in each fluorophore channel. Furthermore, third column shows ratio between channels integrated intensities.

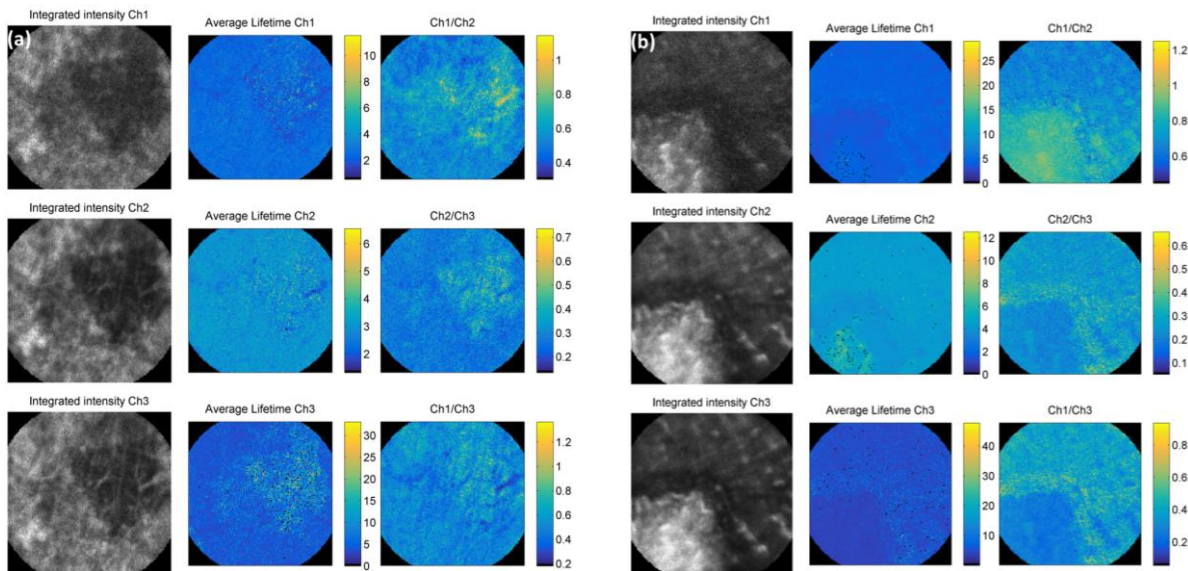


Figure 1. Maps of optical biomarkers for actinic keratosis (a), and Bowen disease (b) respectively. Images show integrated intensity (first column), and average lifetime (second column) for each channel (rows). Moreover, ratios between integrated intensities are shown in the third column. Pixel lighter colors represent higher values for each optical biomarker.

It is possible to note differences between lesion region and surrounding tissue in both benign and malignant lesion images. Integrated intensities and its ratios show higher differences to distinguish lesion edges, while average lifetimes for the three channels demonstrates lower potential for margins detection.

Histograms of each optical biomarker were generated from the regions of interest to compare pixel values between malignant and benign regions of interest of two images. Figure 2 show the histograms in counts of pixel values for each optical biomarker extracted. Benign lesion (Actinic Keratosis (AK)) histograms are represented in red, while malignant

(Bowen disease) histograms are represented in black. Figure 2 shows not only integrated channels ratios but temporal lifetime bi-exponential decay parameters, i.e., weights of free (longer) exponential fraction (a_2); longer lifetime (lt) and shorter lifetime (st) for each channel in rows.

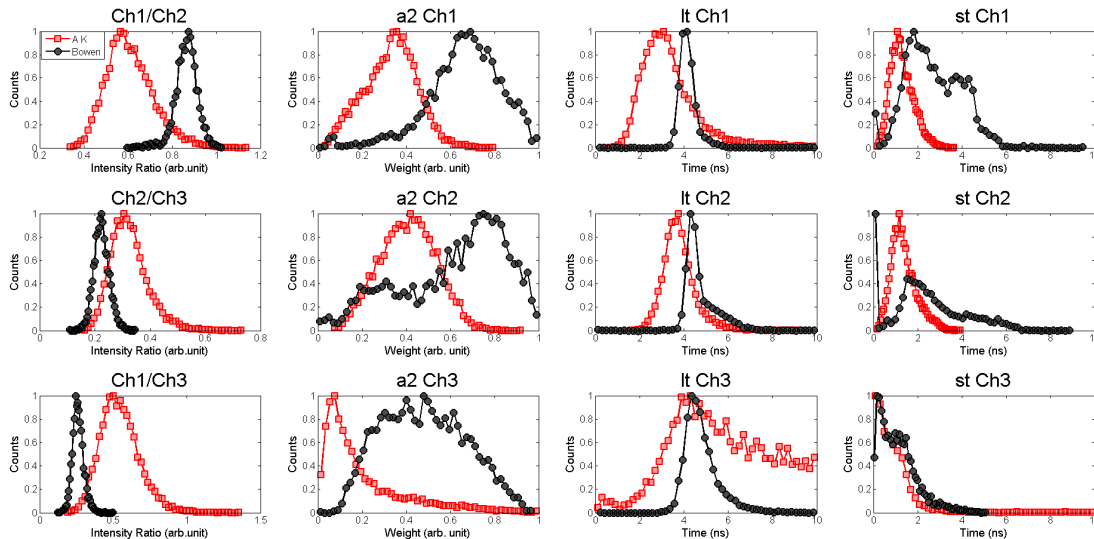


Figure 2. Comparison of Actinic Keratosis (AK), and Bowen disease optical biomarkers histograms. Benign (red squares and line), and malignant (black circles and line). First column shows ratio between integrated intensities of each channel. While the other three columns represents the bi-exponential decay parameters, i.e., weights (a_2); longer lifetimes (lt) and shorter lifetimes (st).

Comparing histograms higher differences can be observed not only in integrated intensity ratios, as well as in bi-exponential decay weights (a_2), and longer lifetimes for each channel. While shorter lifetimes shows lower differences. This result demonstrates the potential in FLIM to distinguish between malignant and benign lesions.

Recent works demonstrated differences between different lesions and healthy tissue. Shorter lifetimes showed higher differences between basal cell carcinoma and healthy tissue, as well as between squamous cell carcinoma (an advanced form of Bowen disease) and surrounding tissue. Furthermore, using these differences lesion borders were also delineated⁷⁻¹¹. Although it was demonstrated in animal models, human skin cancer biopsies, and *in vivo* in human patients, understand such differences in clinical studies with a higher number of patients is still missing. The present study showed higher differences in longer lifetimes, these results demonstrates that different lesions can have changes in metabolic optical biomarkers. Further studies with a higher number of patients must be conducted to increase results significance.

4. FINAL REMARKS

Potential of assembled label-free FLIM in screening skin lesions was shown in this study. *In vivo* clinical measurements were performed in patient human skin lesions. Where a very early form of squamous cell carcinoma named Bowen disease was imaged, as well as Actinic Keratosis. These two lesions were chosen since they are clinically similar, even being malignant and benign respectively, it leads to lower accuracy of diagnosis by the physician. Furthermore, both lesions can be very difficult for the physician to determine borders for surgery.

Regions of the lesion and normal skin were proven to have different endogenous fluorescence lifetime, showing, in general, increases in longer lifetime values. Which corroborates with literature results of *ex vivo* human biopsies measurements, as well as animal models studies. Furthermore, ratios between integrated intensity channels showed higher differences being able to be used as potential markers for borders detection.

When comparing malignant and benign images, only in the regions of interest of the lesions, it can be inferred higher differences in longer lifetime component, as well as longer lifetime for all the three channels. Showing potential of label-free fluorescence lifetime imaging in distinguish between malignant and benign skin cancer lesions. Moreover, a higher

number of patients and different lesions must be imaged in order to obtain more complete results and classify between lesions.

REFERENCES

- [1] Siegel, R. L., Miller, K. D., Jemal, A., “Cancer statistics, 2018,” *CA. Cancer J. Clin.* **68**(1), 7–30 (2018).
- [2] MOGENSEN, M., JEMEC, G. B. E., “Diagnosis of Nonmelanoma Skin Cancer/Keratinocyte Carcinoma: A Review of Diagnostic Accuracy of Nonmelanoma Skin Cancer Diagnostic Tests and Technologies,” *Dermatologic Surg.* **33**(10), 1158–1174, John Wiley & Sons, Ltd (10.1111) (2007).
- [3] Madan, V., Lear, J. T., Szeimies, R.-M., “Non-melanoma skin cancer” (2010).
- [4] Croce, A. C., Bottioli, G., “Autofluorescence spectroscopy and imaging: a tool for biomedical research and diagnosis,” *Eur. J. Histochem.* **58**(4) (2014).
- [5] Heikal, A. A., “Intracellular coenzymes as natural biomarkers for metabolic activities and mitochondrial anomalies,” *Biomark. Med.* **4**(2), 241–263 (2010).
- [6] Shi, L., Lu, L., Harvey, G., Harvey, T., Rodríguez-Contreras, A., Alfano, R. R., “Label-Free Fluorescence Spectroscopy for Detecting Key Biomolecules in Brain Tissue from a Mouse Model of Alzheimer’s Disease,” *Sci. Rep.* **7**(1), 2599 (2017).
- [7] De Beule, P. A. A., Dunsby, C., Galletly, N. P., Stamp, G. W., Chu, A. C., Anand, U., Anand, P., Benham, C. D., Naylor, A., et al., “A hyperspectral fluorescence lifetime probe for skin cancer diagnosis,” *Rev. Sci. Instrum.* **78**(12), 123101, American Institute of Physics (2007).
- [8] Galletly, N. P., McGinty, J., Dunsby, C., Teixeira, F., Requejo-Isidro, J., Munro, I., Elson, D. S., Neil, M. A. A., Chu, A. C., et al., “Fluorescence lifetime imaging distinguishes basal cell carcinoma from surrounding uninvolved skin,” *Br. J. Dermatol.* **159**(1), 152–161 (2008).
- [9] Miller, J. P., Habimana-Griffin, L., Edwards, T. S., Achilefu, S., “Multimodal fluorescence molecular imaging for *in vivo* characterization of skin cancer using endogenous and exogenous fluorophores,” *J. Biomed. Opt.* **22**(6), 066007, International Society for Optics and Photonics (2017).
- [10] Sinsuebphon, N., Rudkouskaya, A., Barroso, M., Intes, X., “Comparison of illumination geometry for lifetime-based measurements in whole-body preclinical imaging,” *J. Biophotonics* **11**(10), e201800037, Wiley-Blackwell (2018).
- [11] Cheng, S., Hwang, D. Y., Cuenca, R., Malik, B., Maitland, K. C., Wright, J., Cheng, L., Jo, J. A., “In Vivo Detection of Oral Epithelial Pre-Cancer and Cancer by Endogenous Fluorescence Lifetime Imaging (FLIM) Endoscopy,” 3–5 (2016).