Characterization of nonmelanoma skin cancer for light therapy using spatial frequency domain imaging

Daniel J. Rohrbach,1 Nathalie C. Zeitouni,2 Daniel Muffoletto,3 Rolf Saager,4 Bruce J. Tromberg,4 and Ulas Sunar*5

1Department of Cell Stress Biology, Roswell Park Cancer Institute, Buffalo, NY, USA
2Department of Dermatology, University of Arizona Cancer Center, Tucson, AZ, USA
3Department of Electrical Engineering, University at Buffalo, Buffalo, NY, USA
4Beckman Laser Institute, University of California Irvine, Irvine, CA, USA
5Department of Biomedical Engineering, University at Buffalo, Buffalo, NY, USA

* ulassuna@buffalo.edu

Abstract: The dosimetry of light-based therapies critically depends on both optical and vascular parameters. We utilized spatial frequency domain imaging to quantify optical and vascular parameters, as well as estimated light penetration depth from 17 nonmelanoma skin cancer patients. Our data indicates that there exist substantial spatial variations in these parameters. Characterization of these parameters may inform understanding and optimization of the clinical response of light-based therapies.

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References and links

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1. Introduction

Nonmelanoma skin cancers (NMSCs), basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs), have increased dramatically with approximately 3.5 million cases diagnosed in the U.S. each year (cancer.org). Thus, the treatment of NMSCs results in high costs for the health care system with an estimated value of approximately 5 billion dollars this year [1]. Although surgical treatment has low recurrence rates, it carries an inherent risk of complications such as infection, dehiscence, scarring, and nerve damage, and can lead to functional and cosmetic problems. Thus, alternative nonsurgical approaches, such as light-based therapies of laser or photodynamic therapy (PDT), are desired for cases of multiple and wide-field NMSCs and for those located at cosmetically sensitive areas [2]. However, the efficacy of light-based therapies is lower compared to surgery, especially for thicker and deeper tumors where light penetration, available oxygen and/or accumulated drug dose may be limited [3, 4].

For effective light therapy, an optimal dose needs to be delivered to the target tissue while there exists enough available oxygen in the tissue [3, 4]. PDT efficacy additionally requires sufficient amount of photosensitizer (drug) dose accumulated in the tissue. Light dose distribution is affected by the local optical parameters (i.e. absorption and scattering) at the therapeutic wavelength, and tissue oxygenation is affected by the vascular parameters such as blood oxygen saturation and blood volume. Thus it is desirable to quantify these parameters for accurate dosimetry and therapy monitoring. In this vein, we previously utilized a fiber-based optical system that allowed point measurements of these parameters for PDT monitoring of head and neck lesions in the oral cavity, and showed that these parameters introduce substantial pre-treatment contrasts and provide useful information related to PDT response [5, 6]. These parameters may show spatial heterogeneity within and between tumors. Heterogeneity in optical parameters can affect light distribution and thus variations in the local light dose, which can lead to over-treatment in some areas and under-treatment in other areas, resulting in treatment failures and recurrence. Heterogeneity in vascular parameters may result in localized hypoxic regions which may respond poorly to therapy. Thus, there is a need for an imaging modality that can quantify the distributions of these parameters.

Spatial frequency domain imaging (SFDI) allows wide-field, non-contact measurements and can quantify optical (absorption and scattering), vascular (tissue oxygen saturation, blood volume) and fluorescence contrasts present in tissue [7, 8]. Thus, it can provide light therapy-dosimetry and monitoring related parameters noninvasively.

In this work, we utilized a custom-made clinical SFDI instrument to quantify and characterize optical and physiologic parameters of NMSC patients. Optical parameters are reported at 590 nm and 740 nm, the wavelengths closer to the laser-therapy wavelengths (e.g., 595 nm pulsed dye and 755 nm Alexandrite lasers) and 630 nm, which is the common wavelength of PDT for skin cancer. The vascular parameters of total hemoglobin concentration (THC) and blood oxygen saturation (StO₂) were obtained by employing a multi-wavelength fitting algorithm using all wavelengths. Our data indicates that measured optical and vascular parameters had substantial variations especially between the patients, which suggests that the light dosimetry needs to be individualized for each patient for optimal delivered dose. We conclude that our results provide useful insights for planning and monitoring of light therapies.

2. Materials and methods

An IRB approved clinical trial (protocol #I226912) was initiated at Roswell Park Cancer Institute. Mohs micrographic surgery patients with biopsy-proven NMSCs were enrolled. Informed consent was obtained from all subjects before the measurements. One lesion per patient was measured and lesions were located on different regions of the body, mainly the back, arms, legs and face. The details of our custom spatial frequency domain imaging (SFDI)
instrument is described elsewhere [9]. Briefly, the instrument consisted of four high-power, compact LEDs (590 nm, 630 nm, 660 nm and 740 nm) as light sources, a projector, and two CCD cameras (Fig. 1).

![Schematic diagram of the clinical SFDI instrument showing the projector module, two CCD cameras, beam splitter, polarizer and analyzer.](image)

Light was directed through a liquid light guide to a projector with a DMD module. The sine wave patterns generated by the DMD module had three different phases (0, 2π/3, 4π/3) and seven spatial frequencies from 0 to 3 cm⁻¹. These patterns were sequentially projected onto the skin surface and reflected light was collected with the CCD cameras. The original images were 450 x 450 pixels in size covering an area of skin 22 mm x 22 mm. For improved signal to noise and faster analysis time, the image was binned using 10 pixels per bin to create a final image of 45 x 45 super-pixels. The final resolution of these images was 489 μm/pixel.

For the analysis, optical absorption and scattering parameters were quantified by fitting an analytical spatial frequency-domain diffuse reflectance model to the measured reflectance, using a reference phantom with known properties to calibrate the instrument prior to every measurement, as described previously [7]. Tissue blood oxygen saturation and blood volume (total hemoglobin concentration) maps were obtained from the multi-wavelength analysis and by assuming that the main absorption chromophores are oxy- and deoxy-hemoglobin [10]. The effective treatment light penetration depth, (δ), defined as $δ = \left(3μ_a(μ_a + μ_s′)\right)^{1/2}$ was calculated from the optical properties for each wavelength. For ROI selection, the surgeon delineated the tumor area with a marker. During the post-processing, the area was selected using the reflectance image and digital pictures and imfreehand Matlab function was used to choose the ROI for the quantification of optical and vascular parameters.

### 3. Results and discussion

Figure 2 shows a representative raw reflectance data and reconstructed images of noninvasive parameters from a patient having a lesion with BCC. Figure 2(a) shows the diffuse reflectance image with the lesion and surrounding periphery area. The absorption map at 630 nm (Fig. 2(b)) showed higher absorption at the lesion compared to surrounding periphery while scattering parameter at 630 nm was lower at the lesion (Fig. 2(c)). Only one wavelength (630 nm) is presented for clarity; the 630 nm wavelength was chosen because this is typically used for PDT.
In that case, the spatial distribution of these parameters within the tumor showed significant heterogeneity with the absorption parameter ($\mu_a$), varying by ~25%, and the scattering parameter ($\mu'_s$), varying by ~13%. Tissue blood oxygen saturation (Fig. 2(d)) and blood volume (Fig. 2(e)) within the lesion were also higher, relative to adjacent tissue, contributing to a shallower light penetration depth (Fig. 2(f)). Higher absorption (and blood volume) contrasts at the tumor area are possibly due to increased vascularity. Most NMSCs do not present as soft, solid malignant tumors and have crusty-looking skin layers above them, which may lead to low tissue optical scattering and explain the lower scattering values in Fig. 2(c). Tissue oxygen saturation (StO$_2$) (Fig. 2(d)) and total hemoglobin concentration (THC) (Fig. 2(e)) were higher in the lesion area than the surrounding, similar to our previous reports [9, 11]. Both maps showed significant spatial heterogeneity with StO$_2$ varying by 14% and THC varying by 17%. The effective treatment light penetration depth, ($\delta$), was lower in the tumor area compared to surrounding and showed a ~9% spatial variation.
We then combined data from the tumor ROI of all patients (N = 17) and visualized the distribution of these parameters with histogram plots (Fig. 3). As Fig. 3(a) and 3(b) clearly indicate the variations (spread) in absorption and scattering parameters are quite large for all wavelengths. The dependency of absorption parameter on wavelength was much more pronounced than that of scattering parameter. Figure 3(c) shows the calculated light penetration depth, indicating that therapeutic light penetration depth at 590 nm is much more limited than those at 630 nm or 740 nm. Since the scattering parameter only varied ~14% as a function of wavelength, the difference in penetration depth is mainly due to higher hemoglobin absorption at 590 nm.

Figure 4 indicates that StO₂ and THC also showed very large variations: StO₂ (Fig. 4(a)) varied from 18.6% (min) to 89.4% (max) with a mean of 57.6% and THC (Fig. 4(b)) varied

Fig. 3. Histograms of (a) absorption ($\mu_a$) and (b) scattering ($\mu_s'$) and (c) penetration depth ($\delta$) parameters at 590 nm, 630 nm and 740 nm for all patients.

Fig. 4. Histograms of tissue oxygen saturation ($StO_2$) and total hemoglobin concentration (THC) for all patients.
from 12.6 μM (min) to 135.7 μM (max) with a mean of 47.71 μM (Table 1). These data clearly indicate that some areas in the tumor showed low oxygen values, and possibly hypoxic areas, which could lead to treatment failures [12]. Also, areas with high THC could lead to low penetration depth of treatment light, as seen in Fig. 2(e) and 2(f). Knowledge of the penetration depth, especially for the 590 nm, 630 nm and 660 nm light sources, is critical for treatment planning since NMSCs thicker than 2 mm are considered high risk (cancer.gov). As seen in Table 1, for all patients the penetration depth at 590 nm was never larger than 1.4 mm with an average of 1.05 mm and there were several instances of δ < 2 mm at 630 nm and 660 nm. Thus, this lower penetration depth might be at least partially responsible for the treatment failures of light-based therapies. It should be noted that since the noninvasive measurements take about one minute and the post-processing takes between 5 and 10 minutes depending on the pixel binning, these measurements could be implemented at the operating room for accurate dosimetry on an individual patient basis. Utilizing GPU based processing for the pixel-based fitting can further reduce the time. Then, the treatment light intensity and shape can be adjusted according to tumor shape and optical characteristics by using masks and optical density filters, or better yet with digital projectors. Thus, this approach may result in wide acceptance of light therapy at the clinical settings with higher success rates, reduced recurrence rates and side effects.

Table 1. Pretreatment values of all reconstructed parameters by SFDI for all patients.

| λ (nm) | μa (cm⁻¹) mean [min max] | μs' (cm⁻¹) mean [min max] | δ (mm) mean [min max] | THC (μM) mean [min max] | StO₂ (%) mean [min max] |
|-------|--------------------------|---------------------------|----------------------|------------------------|------------------------|
| 590   | 2.73 [1.71 4.47]         | 9.26 [5.20 12.59]         | 1.05 [0.78 1.40]     | 47.71 [12.61 135.66]   | 57.63 [18.63 89.38]    |
| 630   | 0.59 [0.24 1.39]         | 10.7 [7.04 14.01]         | 2.47 [1.55 3.36]     |                        |                        |
| 660   | 0.44 [0.20 1.00]         | 10.0 [6.74 12.59]         | 2.90 [1.82 4.00]     |                        |                        |
| 740   | 0.24 [0.09 0.55]         | 9.18 [5.01 11.84]         | 3.97 [2.58 5.97]     |                        |                        |

4. Conclusions

In summary, we characterized NMSCs using SFDI measurements by assessing optical and vascular parameters and treatment light penetration depth of statistically valid number of patients. We observed that there were substantial variations in these parameters. Taking into account of optical parameters can provide improved treatment planning by providing spatially-resolved, optimal delivery of treatment light dose to the target tissue with minimal side effects to the surrounding normal tissue. Real-time monitoring of changes in optical and vascular parameters can provide feedback for adapting the light dose if needed, which may lead to improved success rates at the clinical settings.

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