Published in final edited form as:

Lasers Surg Med. 2018 March ; 50(3): 246–252. doi:10.1002/lsm.22786.

Noninvasive Optical Spectroscopy for Identification of Non-Melanoma Skin Cancer: Pilot Study

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Abstract

Objective: Optical spectroscopy offers a noninvasive alternative to biopsy as a first-line screening tool for suspicious skin lesions. This study sought to define several optical parameters across malignant and benign tissue types.

Study Design: Prospective pilot trial utilizing the Zenalux IM1 optical spectroscopy device from April 2016 to February 2017. For each skin lesion, provider pre-biopsy probability of malignancy was compared to histopathologic diagnosis. Optical data were characterized across basal cell carcinoma (BCC; $n=9$), squamous cell carcinoma (SCC; $n=5$), actinic keratosis (AK; $n=4$), scar tissue ($n=6$), nevus ($n=2$), and neurofibroma (NF; $n=1$). Across all patients, agreement was determined between control measurements collected adjacent to the lesion and from the upper extremity.

Methods: Prospective single center pilot study. The optical properties of 27 cutaneous lesions were collected from 18 adult patients presenting to Otolaryngology and Dermatology clinics with suspicious skin lesions warranting biopsy. Spectroscopy measurements were recorded for each lesion: two at the lesion site, two at an adjacent site (internal control), and one at the central medial

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Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and have disclosed the following: GMP, DSS, and Duke University have financial interest in Zenalux Biomedical, Inc. Research reported in this article was supported by the National Center For Advancing Translational Sciences of the National Institutes of Health under Award Number TL1TR001116. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

upper extremity (arm control). Variables of interest included absolute oxygenated hemoglobin (Hb), Hb saturation, total Hb concentration, and Eumelanin concentration. For each lesion, internal control averages were subtracted from lesion averages to provide delta parameter values, and lesion averages were divided by internal control averages to provide ratio parameter values.

Results: Mean percent difference between pre-biopsy probability of malignancy and histology was 29%, with a difference of 75% or greater seen in 5 of 25 lesions. Mean values for BCC, SCC, AK, and scar tissue varied most between extracted mean reduced scatter estimate (μ'_a ; cm^{-1}) delta values (BCC: -2.2 ± 3.8 ; SCC: -3.9 ± 2.0 ; AK: -3.3 ± 4.2 , Scar: -1.7 ± 1.2) and total Hb (μM) ratio (BCC: 2.0 ± 3.3 ; SCC: 3.0 ± 1.3 ; AK: 1.1 ± 0.6 ; Scar: 1.4 ± 1.1). Agreement between local and arm controls was poor.

Conclusion: This pilot trial utilizes optical spectroscopy as a noninvasive method for determining cutaneous lesion histology. Effect sizes observed across optical parameters for benign and malignant tissue types will guide larger prospective studies that may ultimately lead to prediction of lesional histology without need for invasive biopsy.

Keywords

optical spectroscopy; skin cancer

INTRODUCTION

Non-melanoma skin cancer (NMSC) is the most commonly diagnosed malignancy in white populations [1]. Non-melanoma skin cancer includes basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). The worldwide incidence of NMSC continues to increase at an alarming annual rate of 3–8% [2,3], with the worldwide incidence of BCC also increasing at a 10% [4]. The resultant health and economic burdens of skin cancer have been significant. An average of 4.9 million cases of skin cancer in the United States each year carried a direct annual cost of \$8.1 billion between 2007 and 2011, which is a 126% increase from 2002 to 2006 [5].

Skin biopsy based on clinical assessment is the current standard of care for screening skin lesions suspicious for malignancy. A recent systematic review found that clinical suspicion of NMSC had a sensitivity of 56–90% and a specificity of 75–90% when compared to biopsy [6]. A separate report of 2000 excised skin lesions showed that 40% of lesions suspected of malignancy were nonmalignant [7]. Common biopsy complications include bleeding, infection, and unsightly scars, while anaphylactic reactions and arrhythmias may also occur [7]. Risk factors for NMSC often mirror those for biopsy complications, including older age, anti-coagulants, smoking, and alcohol intake [8]. Improved noninvasive screening methods for non-melanoma skin cancer are needed.

Optical spectroscopy has several attributes that can improve current NMSC screening practices. It can (i) sample multiple sites without the need for tissue removal; (ii) provide nearly immediate feedback; and (iii) identify subtle biophysical features that precede gross morphological changes. With these features, it provides important physiological and metabolic endpoints that can assess tumor response to therapy. Previous studies using the

Zenalux optical spectroscopy device (Zenalux Biomedical; Durham, NC) to distinguish between breast cancer and normal tissue have demonstrated promising results [9–11]. Most recently, when assessing for malignancy of surgical margins during breast conserving surgery, optical property estimation error was found to be less than 10% over a wide range of absorption and scattering coefficients [11].

In developing such devices for clinical application, is a significant limitation that the current device seeks to address. Namely, the pressure applied when placing the probe on the tissue site of interest can influence the physiology. Uncontrolled probe pressure for one can lead to inter-operator variability and directly influences the optical spectrum in a time and pressure dependent manner (most notably, applied pressure leads to tissue blanching due to blood drainage) [12–14]. In this manuscript, a novel probe was developed which integrates a pressure sensor and spring loaded probe to facilitate direct feedback and control over the target pressure range used for measurement.

Accurately distinguishing between benign and malignant skin lesions using optical spectroscopy could greatly reduce the frequency of skin biopsies. Our pilot study aimed to determine the optical spectroscopy characteristics of various skin lesions in patients undergoing skin biopsy and evaluation in otolaryngology and dermatology clinics. We hypothesized that noninvasive optical spectroscopic measurements of cutaneous lesions result in unique signatures that can differentiate between malignant and benign conditions as assessed by clinical and histopathological methods. If successful, optical measurements could be used to survey for NMSC in a noninvasive manner.

MATERIALS AND METHODS

Subject Selection

This prospective study was conducted at the Durham VA Medical Center (DVAMC) after obtaining IRB approval (01885). Eligible patients included those at least 18 years of age who were enrolled between April 2016 and February 2017 from two outpatient DVAMC clinics, dermatology ($n=13$) and otolaryngology ($n=5$), with at least one clinically suspicious skin lesion warranting biopsy. Lesions for which the optical probe could not form a complete seal against the skin were excluded ($n=2$), as were data from two patients who withdrew following data collection. Clinical variables of interest included age, gender, race, location of biopsy, biopsy result, and date of procedure. Primary spectroscopy markers of interest included: hemoglobin (Hb) saturation (%) estimate, total Hb (μM) estimate, absolute oxygenated Hb concentration (μM) estimate, absolute deoxygenated Hb concentration (μM) estimate, absolute eumelanin concentration (μM) estimate, mean extracted scattering coefficient (μ_a ; cm^{-1}), mean reduced extracted scattering coefficient (μ'_a ; cm^{-1}), μ'_a at 400nm (cm^{-1}) estimate, scatter power estimate, and vessel diameter * $[\text{Hb}_{\text{blood}}]$ ($\mu\text{m} * \mu\text{M}$) estimate.

Pre-Biopsy Probability

For 25 (93%) lesions, one of two attending physicians predicted a 0–100% pre-biopsy probability of malignancy. The absolute difference between pre-biopsy probability and histologic results (100%=malignant, 0%=benign) was averaged. As an approximate estimate

of sensitivity and specificity of clinical suspicion for malignancy in biopsied lesions, absolute differences were dichotomized with a <50% difference classified as a true positive (malignant) or negative (benign) prediction opposed to ≥50% difference (false positive/negative).

Optical Spectroscopy

The Zenascope IM1 is a diffuse reflective spectroscopy system intended to be used for quantitative measurement of bio-endpoints. The device was designed and manufactured at Zenalux Biomedical and utilizes patented intellectual property licensed from Duke University. The system has not yet been approved by the FDA as a medical device. The Zenascope IM1 is comprised of a probe assembly and base unit, controlled *via* a graphical user interface running on laptop PC. The probe assembly includes a 2 MP USB camera, a white light source for bright field imaging, a UV light source with filters for fluorescence imaging, a pressure sensor and an optical fiber core (Fig. 1). Measurements were acquired using an automated pressure triggered measurement, to record the optical spectra only when pressure hits a minimum threshold of 48mmHg, with any recording exceeding 120mmHg being discarded. This was empirically determined to be the range at which adequate probe contact was ensured, while minimizing the applied pressure, which can affect perfusion. The optical fiber core is composed of pair (one transmit, one receive) of 2m long silica 400µm optical fibers. At the distal end, the fiber pair is epoxied together with a 2mm center to center separation, and is covered by a 175mm long, 6.25mm diameter stainless steel ferule (Fig. 2). The common end of the probe is designed to deliver and collect light while in contact with patient tissue. The base unit houses a 5W halogen lamp, a spectrometer, and a custom PCB with embedded microcontroller. The visible wavelength light lamp output, a total power of 3mW and power density of 2.4W/cm², is coupled through the optical transmit fiber and delivered to the tissue. Reflected and scattered light from the tissue sample is coupled into the return fiber, which is connected to the spectrometer within the base unit.

Optical probe measurements were completed in the clinical setting immediately prior to skin biopsy. The tissue sample of each corresponding site was analyzed according to tissue type upon histopathologic diagnosis: basal cell carcinoma (BCC), squamous cell carcinoma (SCC), actinic keratosis (AK), benign scar tissue, neurofibroma (NF), and nevus. Absolute Hb, total Hb, and μ'_a were predicted to show the greatest distinction between tissue types to reflect the changes in vascularity and hypoxia that correspond to tumor development (Figs. 3 and 4).

Data Analysis

Data were analyzed using a scalable Monte Carlo inverse model of light transport in tissue [15,16]. Absorption is modeled as the sum of the known component absorber extinction coefficients multiplied by their unknown concentrations. The reduced scattering, μ'_s was modeled using the power law dependence:

$$\mu'_s = \mu'_s(\lambda_0) \left(\frac{\lambda}{\lambda_0} \right)^{-b}$$

where, λ_0 was set to 400nm, and $\mu_s'(\lambda_0)$ and b determine scattering magnitude and spectral line shape, respectively. Briefly, this model performs a non-linear least squares fit of the measured and modeled diffuse reflectance spectra to extract the absorber concentrations (oxy-hemoglobin, deoxy-hemoglobin, and eumelanin), scattering parameters (scattering amplitude, power coefficient), and the pigment packaging factor of van Veen et al. to account for non-heterogeneous dispersion of hemoglobin in tissue [17]. The reflectance spectra were calibrated by first subtracting the dark background, collected with the shutter closed, and then normalized to that taken from a Spectralon reflectance standard provided with the instrument, which was acquired following completion of the clinical data collection for each session. The model was applied over the wavelength range of 500–600nm, which includes relevant spectral features for the chromophores of interest.

Spectroscopy measurements were recorded for each lesion: two at the lesion site, two at a control site adjacent to the lesion (local control), and one at the central medial upper extremity (arm control). Lesion and local control measurement pairs were averaged for each patient. Spectroscopy parameters were reported across all patients as lesion average, the difference between lesion average and local control average (delta), and lesion average divided by local control average (ratio). To account for variance in optical properties of tissues of various thicknesses as well as variance of tissues at identical locations across different patients, optical variables of control tissue were characterized across cutaneous locations, while agreement between local control paired averages and arm control values was determined using Bland Altman plots for absolute (local control–arm control) differences and percent ($[(\text{local} - \text{arm control}) / 0.5 (\text{local control} + \text{arm control})]$) differences.

RESULTS

Eighteen patients from procedural otolaryngology and dermatology clinics were recruited in this study, and 27 lesions were analyzed. Patient demographics, clinical characteristics, and pathological results from 27 lesions analyzed are provided in Table 1. Our study population was predominately Caucasian (14/18), male (16/18), and above 65 years of age (13/18). Skin lesions were included from a wide variety of cutaneous locations. Histopathologic diagnoses included BCC, ($n=9$), SCC ($n=5$), AK ($n=4$), scar tissue ($n=6$), nevus ($n=2$), and NF ($n=1$).

To estimate the clinical utility of optical spectroscopy as a screening tool for cutaneous cancer, biopsy results (100%=malignant; 0%=benign) were compared to physician pre-biopsy percent suspicion of malignancy, where available ($n=25$). An absolute difference of 29% was observed between pre-biopsy suspicion and biopsy results. Lesion pathology from 12/27 (44%) were correctly predicted to within 5% certainty of benign or malignant classification, while 5/27 (19%) demonstrated a predictive difference of 75% or greater. Dichotomizing the pre-biopsy predictions from percentages to benign (<50% suspicion of malignancy) and malignant ($\geq 50\%$) predictions, clinical suspicion demonstrated a sensitivity of 92% and a specificity of 54% (Table 2). False positives per clinical suspicion included AK ($n=4$) and scar ($n=2$) lesions, while the single false negative was a BCC lesion.

Table 3 provides lesion average, delta (lesion average–local control average), and ratio (lesion average/local control average) means and standard deviations for spectroscopy

parameters across all lesions ($n=27$). The means and standard deviations were plotted for absolute Hb, total Hb, and μ'_a (Fig. 1).

To evaluate the consistency of optical parameters for normal skin controls across cutaneous locations, Bland Altman graphs were plotted for total Hb values between local (i.e., adjacent to lesion site) and arm controls for all patients (Fig. 2). The bias of total Hb difference (local–arm control) was $-3.0 \pm 13.2 \mu\text{M}$ (95% CI -28.8 to $22.8 \mu\text{M}$), while the bias of total Hb % difference ($[\text{local} - \text{arm control}] / 0.5[\text{local control} + \text{arm control}]$) was $-17 \pm 52\%$ (95% CI -119% to 85%).

DISCUSSION

Optical spectroscopy determines the scattering and absorption properties of tissue, providing valuable information about the morphology of the normal or abnormal tissue as well as its hemoglobin, melanin, water content [18]. The emergence of fiber-optic technology and advances in sensitive detection schemes have enabled the development of remote sensing systems that can measure optical spectra rapidly and noninvasively from several millimeters to several centimeters deep within human tissue.

Over 7,200 skin biopsies per 100,000 US adults over age 65 were performed in 2001, representing a 2.5-fold increase in biopsies over 15 years [19]. The current number of annual biopsies is likely much higher due to increasing NMSC incidence [2]. To address this growing need, optical spectroscopy may be of most use when ruling out biopsies of lesions with low suspicions for malignancy. Provider suspicion of malignancy differed almost 30% from pathological diagnosis, particularly in cases of suspected malignancy. This difference justifies the observed practice of obtaining biopsy even where the clinical probability of malignancy is thought to be of 5% or less, which accounted for 20% of all biopsies. Providers likely obtain biopsies at low percentages of suspected malignancy due to the risk-benefit ratio of a missed malignant lesion. Although these findings are not powered to draw statistical conclusions, nor is a 50% dichotomization a robust depiction of clinical decision-making, these data appear consistent with sensitivity and specificity of clinical prediction for NMSC from larger studies [6,7].

The hemoglobin-derived parameters of our pilot data suggest that SCC and BCC are hypoxic relative to normal skin (Table 2, Fig. 1). This trend is consistent with previous literature of optical detection of NMSCs that has been promising for distinguishing between BCC and benign skin, [6,20–25] between actinic keratosis and benign skin [20,23,26,27], and between actinic keratosis and malignancies [21,26,27]. In a similar study, Rajaram et al. reported the use of diffuse reflectance spectroscopy and fluorescence spectroscopy for the diagnosis of non-melanoma skin cancer [28]. They found that diffuse reflectance spectroscopy alone was able to discriminate between BCC and normal at sensitivity and specificity of 89%, however specificity in particular was lower for distinguishing AK from normal or BCC. Across these studies, identifying specific subtypes of atypia and malignancy has proved challenging [18]. One of the key experimental variables in any such technique is the applied pressure. To address these limitations, the Zena-scope IM1 probe collects a wide

variety of spectroscopic parameters, and importantly controls for pressure for consistent measurement conditions.

In addition to diffuse reflectance spectroscopy, a variety of other optical approaches have been taken which could also provide complimentary information. These include confocal imaging, multiphoton imaging, optical coherence tomography, Raman spectroscopy, and photoacoustic imaging [18,29]. Recent advances in optical spectroscopy have demonstrated promising results as sensitive and specific means of *in vivo* identification of nerve tissue [30], colorectal liver metastases [31], and oral squamous cell carcinoma [32]. Although a comprehensive review is outside the scope of this article, the imaging techniques listed in general are able to provide a high resolution tomographic view of the lesion of interest, using either endogenous or exogenous contrast. The primary limitation is the relative lack of clinical validation, the cost and complexity of operating these devices, as well as the need for a person skilled in image analysis or development of an automated diagnostic system. All of these limitations have limited clinical availability and adoption. The primary advantage of the Zenascope system are its simplicity, relatively low cost components and ease of use, while retaining the ability to extract quantitative physiologic data from the tissue sites. Simplicity is important both in terms of rapid acquisition and analysis, and limited influence of user expertise and bias in placing the probe due to the automated pressure sensing system. This facilitates the use of such devices also at point of care and low resource settings [33].

A future trial guided by the listed effect sizes will likely require a machine-learning algorithm that combines multiple spectroscopic parameters to accurately predict tissue type. Additional trials may also allow for more accurate distinctions between benign and malignant lesions, which could guide clinicians toward proceeding with biopsy or continuing observation.

Testing for agreement between total hemoglobin (Hb) levels of normal skin of the upper medial arm and adjacent to the lesion was performed to explore which controls may be required in future spectroscopy trials. Agreement between local and arm controls did not consistently correspond to lesion location: the four closest measures of total Hb agreement included local control measurements of the temple, back, wrist, and arm. The observed variance in optical parameters across cutaneous locations may suggest that a larger subsequent trial should consider stratification by lesion site and incorporating lesion depth into a machine-learning predictive algorithm.

Limitations

This pilot study provides effect sizes across a broad range of lesion locations and types, but is not itself powered for predictive analysis. Future multivariate analysis of optical parameters alongside patient parameters including age, ethnicity, and skin thickness will be performed in a subsequent trial. Pre-biopsy probabilities were collected from just two specialist physicians; while these data have limited generalizability, further predictive variance between practitioners would further reinforce the clinical need for standardization in efforts toward reduction of false-positive biopsies through noninvasive screening measures. Applicability of the observed parameters and effect sizes are limited to future

studies using the Zenascope IM1 probe. The patient demographics of this study may have limited applicability to the general population. This study did not include any patients with cutaneous melanoma, which may present an additional unique and clinically relevant optical signature to incorporate in distinguishing between tissue types.

CONCLUSION

This pilot trial utilizes the Zenascope IM1 optical probe as a noninvasive method for detection cutaneous lesions. The effect sizes observed across optical parameters for benign and malignant tissue types will guide larger prospective studies that may ultimately lead to prediction of lesional histology without need for invasive biopsy.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Zenalux Biomedical, Inc. for providing the IM1 probe. We also acknowledge NIH for funding(5R42CA156901,awardedtoZenaluxwith subcontract to Duke University). GMP, DSS, and Duke University have financial interest in Zenalux Biomedical, Inc.

Contract grant sponsor: National Center for Advancing Translational Sciences; Contract grant number: TL1TR001116; Contract grant sponsor: NIH; Contract grant number: 5R42CA156901.

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Fig. 1.
Zenoscope IM1 block diagram.

Fig. 2.
Optical fiber core mechanical drawing.

Fig. 3.

Optical characteristics across lesion types. Means and standard deviations are plotted for reduced extracted mean scatter estimate (μ_{S_0}'), Hemoglobin (Hb) saturation estimate, and Hb total estimate across lesion averages, deltas (lesion average local control average), and ratios (lesions average/local control average).

A. Total Hb: Absolute Agreement of Local to Arm Controls **B. Total Hb: Percent Agreement of Local to Arm Controls** **Fig. 4.**

Agreement between control measurements. Bland–Altman plots of Total Hb agreement between local and arm control measurements for **(A)** (local control–arm control) difference with respect to total Hb average ($[\text{local control} + \text{arm control}]/2$) and **(B)** ($[\text{local control} - \text{arm control}]/(0.5[\text{local control} + \text{arm control}])$) percent difference with respect to total Hb average.

TABLE 1.

General Patient and Lesion Characteristics

Lesion characteristics (<i>n</i> = 27)			Patient demographics (<i>n</i> = 18)		
	<i>N</i>	(%)		<i>N</i>	(%)
Lesion site			Age, years		
Ear	6	22	Mean	66	–
Temple	4	15	SD	13	–
Back	3	11	Gender		
Cheek	2	7	Male	16	89
Forearm	2	7	Female	2	11
Nose	2	7	Race		
Proximal arm	2	7	Caucasian	14	78
Scalp	2	7	African American	3	17
Abdomen	1	4	Hispanic	1	6
Calf	1	4			
Chest	1	4			
Wrist	1	4			
Histology					
BCC	9	33			
Scar tissue	6	22			
SCC	5	19			
AK	4	15			
Nevus	2	7			
Neurofibroma	1	4			

TABLE 2.

Sensitivity and Specificity of Clinical Suspicion for Malignancy for 25 Lesions

Provider suspicion for malignancy	Biopsy	
	Positive	Negative
Positive	11	6
Negative	1	7

TABLE 3.

Optical Parameters are Listed as (I) Averages, (II) Deltas (Lesion—Local Control), and (III) Ratios (Lesion/Local Control)

	BCC (<i>n</i> = 9)	SCC (<i>n</i> = 5)	AK (<i>n</i> = 4)	Scar (<i>n</i> = 6)	Nevus (<i>n</i> = 2)	NF (<i>n</i> = 1)
I. Averages						
Extracted mean $m\mu'_a$ (cm ⁻¹)	15.7 ± 3.7	11 ± 3.1	14 ± 4.9	12.1 ± 5	9.2 ± 0.7	9.4
Scat- $m\mu'_a$ at 400 nm (cm ⁻¹) estimate	26.3 ± 5.7	26 ± 5.8	28.1 ± 3.9	25.7 ± 10.4	21.7 ± 0.8	22.3
Abs-eumelanin Conc. (μM) estimate	1,096 ± 788	1,338 ± 688	637 ± 279	1,669 ± 617	1,719 ± 516	1,363
Hb saturation (%) estimate	56.1 ± 22.0	57 ± 12.5	48.6 ± 13.9	66.8 ± 20.9	59.1 ± 0.6	36.9
Hb total (μM) estimate	36.5 ± 27.6	42.6 ± 9.6	23.6 ± 11.3	36.7 ± 26.5	36.7 ± 6.2	11.7
II. Deltas						
Extracted mean $m\mu'_a$ (cm ⁻¹)	-2.2 ± 3.8	-3.9 ± 2	-3.3 ± 4.2	-1.7 ± 1.2	0.7 ± 1.1	0.2
Scat- $m\mu'_a$ at 400 nm (cm ⁻¹) estimate	-3.7 ± 5.7	-4 ± 5.8	-1.9 ± 3.9	-0.1 ± 0.8	-1.3 ± 10.7	-7.7
Abs-eumelanin Conc. (μM) estimate	271 ± 880	146 ± 426	17 ± 261	188 ± 520	672 ± 340	177
Hb saturation (%) estimate	-21 ± 26	-3.8 ± 30.1	-25.3 ± 31.1	-2.9 ± 20.9	16.9 ± 16.8	-5.4
Hb total (μM) estimate	6.4 ± 33.1	25.7 ± 11.3	1.6 ± 13.3	8.2 ± 28.9	21.4 ± 12.2	-3.2
III. Ratios						
Extracted mean $m\mu'_a$ (cm ⁻¹)	0.9 ± 0.2	0.7 ± 0.1	0.8 ± 0.3	0.9 ± 0.1	1.1 ± 0.1	1
Scat- $m\mu'_a$ at 400 nm (cm ⁻¹) estimate	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.5	0.7
Abs-eumelanin Conc. (μM) estimate	1.7 ± 1.7	1.1 ± 0.3	1 ± 0.4	3.8 ± 6.6	1.6 ± 0.2	1.1
Hb saturation (%) estimate	0.8 ± 0.3	1.1 ± 0.5	0.7 ± 0.4	1 ± 0.3	1.5 ± 0.6	0.9
Hb total (μM) estimate	2.0 ± 3.3	3 ± 1.3	1.1 ± 0.6	1.4 ± 1.1	2.7 ± 1.4	0.8

BCC, basal cell carcinoma; SCC, squamous cell carcinoma; AK, actinic keratosis; NF, neurofibroma; $m\mu_a$, mean reduced scattering coefficient; $m\mu'_a$, mean corrected scattering coefficient; Hb, hemoglobin; Abs⁻, absolute; Oxy⁻, oxygenated; Deoxy⁻, deoxygenated; Conc⁻, concentration.