Noninvasive in vivo optical characterization of blood flow and oxygen consumption in the superficial plexus of skin

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Abstract. Assessing the metabolic activity of a tissue, whether normal, damaged, aged, or pathologic, is useful for diagnosis and evaluating the effects of drugs. This report describes a handheld optical fiber probe that contacts the skin, applies pressure to blanch the superficial vascular plexus of the skin, then releases the pressure to allow refill of the plexus. The optical probe uses white light spectroscopy to record the time dynamics of blanching and refilling. The magnitude and dynamics of changes in blood content and hemoglobin oxygen saturation yield an estimate of the oxygen consumption rate (OCR) in units of attomoles per cell per second. The average value of OCR on nine forearm sites on five subjects was $10 \pm 5$ (amol/cell/s). This low-cost, portable, rapid, noninvasive optical probe can characterize the OCR of a skin site to assess the metabolic activity of the epidermis or a superficial lesion. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License.

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1 Introduction

Real-time monitoring of the cellular oxygen consumption rate [OCR, attomoles of $O_2$ per cell per s (amol/cell/s)] can characterize the metabolic activity of a tissue, providing physiological information with clinical relevance. For instance, the metabolic rate may provide a basis for comparing normal and pathological states. Prior studies have used other methods such as respirometry of extracted mitochondria ex vivo and in vivo multiphoton microscopy to assess the OCR of tissues. This paper focuses on assessing the OCR of the skin’s epidermis. Monitoring the epidermal OCR may characterize normal, diseased, and aged skin, detect malignancy, and monitor the effects of drugs and treatments.

Different values of OCR for various cells and tissues have been reported in the literature. Based on data from in vitro studies, Wagner et al. cataloged a wide range of OCR from 1 to 1000 amol cell$^{-1}$ s$^{-1}$, with a median value of 23. Balu et al. used multiphoton microscopy in vivo to image NADH fluorescence in the epidermis as a proxy for OCR. They reported an OCR of about $0.035 \mu$moles/10$^6$ cells/h in keratinocytes in the basal layer, which equals 9.7 amol cell$^{-1}$ s$^{-1}$. They also reported a nonuniformity of OCR across the epidermal thickness, with more superficial keratinocytes having lower OCR.

This paper presents an alternate method using a topical optical fiber probe measurement at the skin surface to assess the blood volume and hemoglobin oxygen saturation before, during, and after manually applied pressure to blanch the superficial vascular plexus that provides oxygen to the epidermis. The refill time after release of pressure specifies the blood flow in the blanched superficial plexus. The magnitude and dynamics of changes in blood content and hemoglobin oxygen saturation yield an estimate of the epidermal OCR in units of amol cell$^{-1}$ s$^{-1}$. The goal of this paper is to report a pilot study that illustrates the method and cites initial values for the in vivo epidermal OCR.

Previous work has used applied pressure to remove blood from a tissue for the purpose of improving the observation of other chromophores and tissue structures. For example, Ermakov and Gellermann detected carotenoids in the skin after using pressure to remove superficial blood. Such reports use pressure to remove the blood, which is regarded as an interfering factor. In this paper, we use pressure to identify the superficial vascular plexus, which is easily blanched by pressure. The spectroscopy of this blanchable plexus informs about the OCR of the epidermis.

2 Methods

2.1 Experimental Design

An optical fiber probe was used to spectrally measure the transport of light through in vivo forearm skin sites (Fig. 1). The probe consisted of a source fiber and a collection fiber with a center-to-center spacing of 1.88 mm (0.400-mm-core-dia fibers). The fibers were embedded in a flat plastic plate (three-dimensional-printed using polylactate) that allowed perpendicular fiber contact with the skin while distributing the pressure applied to the skin. The probe handpiece was a cylinder within which the optical probe assembly moved. As one manually pressed the probe against the skin, the outer cylinder pushed a spring against the inner probe assembly, hence applying pressure by the spring. The displacement of the spring was...
observable on a ruler attached to the inner assembly, which extended outside of the top of the cylinder to allow easy viewing. A reproducible pressure was applied by pressing the probe until the desired displacement was achieved. Repeated tests of the blanching force registered 610 ± 10 g on a scale. The flat surface of the probe tip distributed the force, hence, the probe could also be placed lightly on the skin to monitor refill while avoiding blanching the superficial plexus of blood.

The source fiber delivered white light. The collection fiber returned light to a spectrometer (QE Pro, Ocean Optics Inc., Dunedin, Florida) which measured the light in the 300 to 1100 nm range. Light in the 500 to 1000 nm range yielded strong signals and was used for the analysis. The spectra were acquired continuously by a digital chart recorder (implemented using a MATLAB® program with a GUI interface). Spectra with light pressure were acquired for about 30 s to establish a baseline. Then, strong pressure was applied for about 10 s, which blanched the superficial blood volume, i.e., the superficial baseline. Then, strong pressure was applied for about 10 s, and after the refliling of the plexus (t2) were noted: t1 = at maximum blanching, t2 = after refill of the plexus upon release of pressure. The data at these time points were used to calculate the OCR.

The experiment was conducted on ventral forearm skin sites; nine measurements on five subjects. For each subject, the probe was placed on the forearm, and pressure was applied then released. Then, the probe was moved to a different site on the forearm and the experiment repeated, eventually measuring three to six sites. Measurements over a visible large blood vessel were avoided. Measurements were approved by the Institutional Review Board at OHSU.

2.2 Calculation of OCR

Calculation of the OCR (amol/cell/s) was accomplished by the following:

1. Blood flow

\[ J = (\Delta B/\Delta t)(B_{\text{plexus}}/B_0)V_{\text{plexus}} \]

(cc/s per cm² surface area) (1)

where \( \Delta B/\Delta t = [B(t_2) - B(t_1)]/(t_2 - t_1) \), change in apparent blood per unit time (s⁻¹), \( B_{\text{plexus}}/B_0 = 2.5 \) = ratio of blood in plexus versus blood observed spectrally (see below); \( D_{\text{plexus}} = 0.0200 \) (cm) thickness of superficial vascular plexus (estimate); \( V_{\text{plexus}} = D_{\text{plexus}}/(1 \text{ cm}^2) = 0.0200 \) (cc per cm² surface area).

2. Oxygen extraction

\[ O_{2,\text{ex}} = 4\Delta SJC_{\text{Hb}} \text{ (moles O}_2\text{/s per cm}^2\text{ surface area)}, \]

(2)

where 4 = four \text{O}_2 \text{ molecules per hemoglobin molecule}; \( f = V_v/V_s \), the ratio of venous volume to arterial volume (assumed); \( f_s = 1/(1 + f) \), volume fraction of blood that is arterial; \( f_v = f/(1 + f) \), volume fraction of blood that is venous; \( S_s = 0.97 \), arterial oxygen saturation (assumed); \( S_v = (S - f_sS_a)/f_v \), venous oxygen saturation; \( \Delta S = S_s - S_v \), difference in oxygen saturation between arterial and venous; \( C_{\text{Hb}} = C_{\text{blood}}/MW \), concentration (moles/cc); \( C_{\text{blood}} = 0.150 \) g Hgb/cc, hemoglobin concentration of whole blood; \( MW = 64,500 \) g/mole, molecular weight of hemoglobin.

3. Number of cells in epidermis per cm² surface area

\[ n_{\text{cells}} = V_{\text{epidermis}}/V_{\text{cell}}, \]

(3)

where \( V_{\text{epidermis}} = (0.0100 \text{ cm}^2)/(1 \text{ cm}^2) \) (cc per cm² surface area); \( V_{\text{cell}} = \pi D^2/6 \text{(cc/cell)} \); \( D = 10 \times 10^{-4} \) cm diameter of spherical epidermal cell (approximation).
4. OCR

\[
\text{OCR} = \frac{O_{2,ex}/n_{\text{cells}}}{10^{18} \text{ moles/amol}}
\]

\[
(\text{amol/cell/s}).
\]  \hspace{1cm} (4)

The factor \( B_{\text{plexus}}/B_{sp} = 2.5 \) is the ratio of blood in the plexus versus the apparent average blood deduced by diffusion theory from the observed spectrum. The spectral analysis uses a simple diffusion theory [see Eq. (4)] that assumes a homogeneous skin tissue with an overlying pigmented epidermis. Any observed blood absorption is assumed to be due to blood distributed evenly throughout a semi-infinite homogeneous skin. But the superficial vascular plexus has a higher local blood content \( (B_{\text{plexus}}) \) than the average \( (B_{sp}) \) and is located at about 200 to 400 \( \mu \)m depth \( (200-\mu \)m thickness assumed here). The spectrally observed average \( B_{sp} \) does not equal the true local \( B_{\text{plexus}} \).

A Monte Carlo simulation (MCMC) was used to simulate the reflectance spectra (15 wavelengths from 500 to 1000 nm) when such a plexus was given different blood volume fractions \( (B_{\text{plexus}} = 0.001 \) to 0.05). The spectra were then analyzed to specify an apparent \( B_{sp} \), using the same diffusion theory analysis that was applied to the experimental data. There was a linear relationship between \( B_{\text{plexus}} \) and \( B_{sp} \) with a slope of 2.5. Hence, when spectral analysis of a measured spectrum specified \( B_{sp} \) to be 0.01, the true \( B_{\text{plexus}} \) was 0.025.

The relative sizes of the arterial and venous vascular volumes are an important parameter in the calculation of OCR. The ratio of volumes, \( f = V_{\text{venous}}/V_{\text{arterial}} \), was assumed to be 1.0 in this paper. Equation (4), however, allows for \( f \) to deviate from 1.0.

#### 2.3 Spectral Analysis

The measurements yielded a reflectance spectrum, \( M_{\text{skin}}(\lambda) \), a spectrum from a white standard (99% reflectance standard, SpectralonTM, Labsphere Inc., New Hampshire) with the probe held 1 cm above the standard, \( M_{99\%}(\lambda) \), and a spectrum with the probe held in air within a dark room, \( M_{0\%}(\lambda) \). The dark spectrum was subtracted from the other spectra, then a ratio was taken

\[
M_R = \frac{M_{\text{skin}} - M_{99\%}}{M_{99\%} - M_{0\%}} = \frac{SR_{\text{skin}}C_{\text{skin}}D}{SR_{99\%}C_{99\%}D} = \frac{R_{\text{skin}}C_{\text{skin}}}{R_{99\%}C_{99\%}}
\]

or

\[
M_R R_{99\%} = KR_{\text{skin}},
\]  \hspace{1cm} (5)

where \( K = C_{\text{skin}}/C_{99\%} \). This ratio cancels the wavelength dependence of the light source (S) and detector responsivity (D). The left side of Eq. (4) is known from measurements and \( R_{99\%} = 0.99 \). The right side of Eq. (4) is fit by least squares fitting using the modified diffusion theory described in Eq. (4). After the fitting, the skin reflectance \( R_{\text{skin}} = M_R/K \) is in units of (mm^-2). The factor \( K \) depends on the ratio of collection efficiency when the probe held about 1 cm above the standard versus collection efficiency when the probe contacted the skin.

Figure 2(a) shows the raw reflectance spectra in units of (counts): \( M_{99\%} \), \( M_{0\%} \), and \( M_{\text{skin}} \pm \) applied pressure. Figure 2(b) shows the reflectance \( R_{\text{skin}} \) \( K R(\lambda) \pm \) applied pressure. The factor \( K \) equals 2.5 based on the average value fit for all acquired spectra on the five subjects.

The spectrum \( KR_{\text{skin}} \) was matched by least squares fitting using a simple light transport expression based on diffusion theory [Eq. (4)]. A predicted spectrum \( pKR \) was determined by specifying the fitting parameters: blood content \( (B) \), oxygen saturation \( (S) \), epidermal melanin \( (M) \), scattering \( (a) \), and a calibration factor \( (K) \). These parameters are defined below. The parameter \( a \) scales the magnitude of the reduced scattering coefficient, \( \mu'_a(\lambda) \), whose spectral shape is described below. The expression for \( pKR \) (counts) was

\[
pKR = KT_{\text{epi}} g Rr(\mu_a, \mu'_a, r, n_a),
\]  \hspace{1cm} (7)

where \( K = \text{calibration constant (counts mm}^{-2}) \); \( T_{\text{epi}} = \exp(-M_{\text{a,al}} L_{\text{epi}}) \), the attenuation due to the pigmented epidermis; \( M = \text{volume fraction of melanosomes in } 60-\mu\text{m-thick forearm epidermis; } M_{\text{a,al}} = \text{absorption coefficient of interior of cutaneous melanosome;} \) \( L_{\text{epi}} \approx 3\times \text{epidermal thickness} = 0.180 \text{ mm}; \mu_a \) is the absorption coefficient (mm^-1)

\[
\mu_a = B_S \mu_{a,\text{oxy}} + B(1-S) \mu_{a,deoxy} + W \mu_{a,\text{water}}
\]

where \( \mu_{a,\text{oxy}} = \text{absorption spectrum of oxygenated whole blood;} \mu_{a,deoxy} = \text{absorption spectrum of deoxygenated whole blood;} \mu'_a = \text{reduced scattering coefficient (mm}^{-1}) \) of skin, based on literature\[ \mu_a \) at \( \lambda = 500 \text{ nm, which scales the shape of } \mu'_a(\lambda) \text{ such that: } \mu'_a(\lambda) = a[0.32(500/500)^{-4} + 0.68(500/500)^{-0.91}] = \text{Rayleigh plus Mie scattering.} \]
\[ \text{getRr}(\mu_a, \mu_0s, r, r_i) = \text{local reflectance (mm}^{-2}) \text{ (diffusion theory, Farrell et al.10); } r = 1.88 \text{ mm center-to-center distance between source and collection fibers.} \]

\[ n_r = \frac{1.4}{1.5}, \text{ ratio of the approximate refractive indices, } n_{\text{skin}}/n_{\text{probe}}. \]

Hence, the spectral fitting yielded values for \( B, S, M, \alpha, \text{ and } K. \) After an initial analysis of all spectra on the five subjects, a common value of \( K \) was determined to be 2.5. The same \( M_{\text{site}} \) was used to analyze all the spectra. Subsequent analysis fit for \( B, S, M, \text{ and } \alpha \) only. The water content was assumed to be \( W = 0.65 \) for all subjects. Figure 3 shows the spectral fitting of the nine spectra from five subjects.

### 3 Results

First, the calculation of OCR is illustrated by applying the calculation to one of the pressure-release data from one subject in Fig. 4. The calculation steps are listed in Table 1. The mean \( \pm \) standard deviation values of OCR for the six pressure releases on that subject yielded an OCR = 9.1 \pm 2.7 amol/cell/s, which is close to the value of 9.7 amol/cell/s reported by Balu et al.1

Second, the analysis of the nine skin sites on five subjects is summarized in Table 2. The means and standard deviations of the OCR on each of the nine skin sites are listed. The average coefficient of variation for measurements on a site was 34%, which is site-to-site variation on the forearm during one sitting of a subject. The range of mean OCR values extended from 0.9 to 18.0 amol/cell/s. The average mean OCR value was 10 \pm 5 amol/cell/s \( (n = 9 \text{ skin sites}). \) There was significant sitting-to-sitting variation on subjects 1, 2, and 5, who were measured more than once.

Figure 5 places the OCR data of this report on a histogram drawn using the tabulated data in the review of OCR values for various tissues and cell cultures by Wagner et al. A couple of the skin site data are on the edge of the histogram but most are clustered in the middle. Hence, the skin OCR values are in the more common range found by various investigators.
4 Discussion

Topical placement of an optical fiber probe on the skin followed the dynamics of blanching by applied pressure then refilling upon release of the pressure. A spectrogram of reflectance spectra versus time was acquired and analyzed by a simple diffusion theory model to yield the time course of blood volume fraction \( B(t) \) and mixed arteriovenous oxygen saturation of hemoglobin \( S(t) \). The \( B(t) \) and \( S(t) \) recordings specified the blanched value at \( t_1 \) and the refilled values at \( t_2 \), which yielded values for the blanchable blood volume and the blood flow to this volume, which is presumed to be the superficial plexus just under the epidermis. This plexus exclusively provides oxygen for the epidermis and does not supply significant oxygen to the dermis. So, the oxygen delivery of the plexus is an indicator of the metabolic consumption of oxygen by the epidermis.

Balu et al.\(^\text{B}^\) used two-photon NADH fluorescence to quantify the gradient of OCR across the epidermis. More work is needed to understand the difference between the average OCR measured by the optical fiber measurement and the OCR gradient that they observed. One of the assumptions in the fiber probe analysis is that the rate of cellular oxygen extraction from the blood is equal to the cellular OCR, which assumes that all of the oxygen used by the epidermis comes from the blood. On the other hand, some studies have reported a

\[ \text{OCR} = \frac{\Delta O_2}{n_{\text{cell}}} \times 10^{18} \]

**Table 1** Example calculation from the fourth pressure release in Fig. 4

| \( B(t_1) \) | \( B \) measured while blanched | 0.00121 |
| \( B(t_2) \) | \( B \) measured after refill | 0.00313 |
| \( S(t_2) \) | \( S \) measured after refill | 0.655 |
| \( \frac{dB}{dt} \) | \( \frac{[B(t_2) - B(t_1)]}{(t_2 - t_1)} \) | \( 5.99 \times 10^{-4} \) s\(^{-1} \) |
| \( B_{\text{plexus}} \) | \( B_{\text{plexus}} / B_{\text{sp}} \) from Monte Carlo sims. | 2.5 |
| \( V_{\text{plexus}} \) | 0.02 cc/cm\(^2\) skin surface |
| \( J \) | \( \frac{(dB/dt)B_{\text{plexus}}V_{\text{plexus}}}{\Delta S_{\text{cell}}} \) | \( 2.99 \times 10^{-6} \) cc/s |
| \( C_{\text{blood}} \) | conc. of HGB in whole blood | 0.150 moles/cc |
| MW\(\text{HGB} \) | 64,500 moles/cc |
| \( C_{\text{HGB}} \) | \( C_{\text{blood}} / \text{MW} \) | \( 2.33 \times 10^{-6} \) moles/cc |
| \( f \) | \( V_{\text{venous}} / V_{\text{arterial}} \) | 1.0 |
| \( f_a \) | \( 1 / (1 - f) \) | 0.5 |
| \( f_v \) | \( f / (1 - f) \) | 0.5 |
| \( S_a \) assumed | 0.97 |
| \( S_v \) | \( \frac{(S(t_2) - S_a f_a)}{f_v} \) | 0.34 |
| \( \Delta S \) | \( S_a - S_v \) | 0.66 |
| \( \Delta O_2 \) | \( 4 \Delta SJC_{\text{cell}} \) | \( 1.75 \times 10^{-10} \) |
| \( D_{\text{cell}} \) | 0.0010 cm |
| \( V_{\text{cell}} \) | \( \pi D_{\text{cell}}^3 / 6 \) | \( 5.24 \times 10^{-10} \) |
| \( n_{\text{cell}} \) | \( V_{\text{cell}} / V_{\text{cell}} \) | 1.91 \times 10^7 |
| OCR | \( \frac{\Delta O_2}{n_{\text{cell}}} \times 10^{18} \) | 9.2 amol/cell/s |

**Table 2** Summary of OCR for nine sites on five subjects.

<table>
<thead>
<tr>
<th>Site</th>
<th>Subject</th>
<th>Mean ± std (n pressure releases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>9.1 ± 2.7 (n = 6)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>12.9 ± 2.3 (n = 4)</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>15.8 ± 5.0 (n = 3)</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.9 ± 0.2 (n = 3)</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>9.9 ± 2.5 (n = 4)</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>11.4 ± 5.7 (n = 5)</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>4.1 ± 2.0 (n = 3)</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>9.1 ± 5.8 (n = 3)</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>18.0 ± 4.3 (n = 3)</td>
</tr>
</tbody>
</table>
Gaining a better understanding of epidermal oxygen consumption may prove useful in differentiating normal from pathological states. The optical fiber probe is a low-cost portable system that offers a convenient, rapid, noninvasive assessment of the metabolic activity of the epidermis, or any pathologic growth near the skin surface. Such a probe may prove useful in clinical dermatology, which was the motivation for this study.

Disclosures
The authors have no conflicts of interests to disclose.

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References

Faezeh Talebi Liasi is a medical doctor and an alumni of the University of Washington School of Medicine. She worked on this project at the Oregon Health and Science University (OHSU) while she was still in medical school. Her research interests include optical characterization of tissue and its use in diagnostics.

Ravikant Samatham received his MS degree in mechanical engineering from the University of Nevada, Reno, in 2004 and a PhD in biomedical engineering from OHSU in 2012. He is now a senior research scientist in the Department of Dermatology at OHSU. His research focuses are second harmonic generation microscopy, optical spectroscopy, and optical imaging.

Steven Jacques received his BS degree in biology from MIT in 1972, his MSEE degree in electrical engineering and computer science, and his PhD in biophysics and medical physics from the University of California-Berkeley in 1984. He has served at Massachusetts General Hospital/Harvard Medical School, the University of Texas M.D. Anderson Cancer Center, the OHSU, and now at Tufts University. His research focuses on light propagation and interactions with tissues.