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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 ± 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. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: [10.1117/1.JBO.22.11.115002](https://doi.org/10.1117/1.JBO.22.11.115002)]

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1 Introduction

Real-time monitoring of the cellular oxygen consumption rate [OCR, attomoles of O₂ 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.^{1–3} 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.⁴ cataloged a wide range of OCR from 1 to 1000 amol cell⁻¹ s⁻¹, with a median value of 23. Balu et al.¹ 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 μmoles/10⁶ 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.⁵ 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

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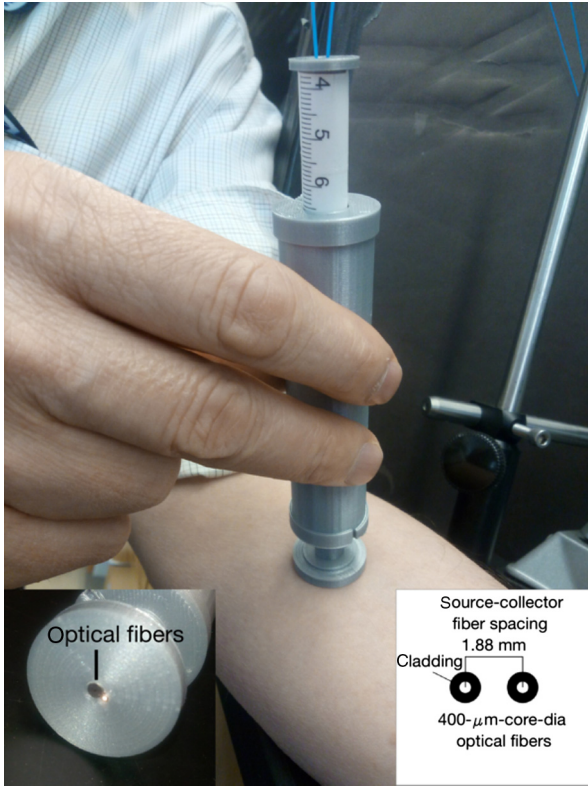


Fig. 1 The optical fiber probe placed on the forearm. Two optical fibers deliver and collect white light for measurement by a spectrometer. When the handpiece is pressed against the skin, pressure is applied by a spring whose displacement is monitored by reading the ruler. The inset shows the probe tip with two 0.400-mm-core-dia fiber separated center-to-center by 1.88 mm embedded in a broad circular plastic surface. The applied pressure is distributed over this circular surface, so the pressure is more easily controlled.

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 vascular plexus. Then light pressure was resumed, which allowed the superficial plexus to refill with blood.

Spectral analysis used the 500 to 1000 nm range of wavelengths. Light below 500 nm (blue light) is scattered by the epidermis and superficial papillary dermis before encountering significant blood content; therefore, spectra at such short wavelengths require a two-layer model for analysis. Light above 500 nm (green to near-infrared) penetrates into the dermis and samples the superficial plexus of microvasculature at about

200 to 400 μm depth. Hence, a simple diffusion theory that assumes uniform distribution of blood could be used (see Sec. 2.3).

The analysis of the spectrum at each time point specified the apparent average blood volume fraction (B) and the mixed arteriovenous oxygen saturation (S) (see Sec. 2.3). Hence, the time courses of $B(t)$ and $S(t)$ were specified. The values of B and S after applying pressure for about 10 s (t_1) and after the refilling of the plexus (t_2) were noted: t_1 = at maximum blanching, t_2 = 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 Internal Review Board at OHSU.

2.2 Calculation of OCR

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

1. Blood flow

$$J = (\Delta B / \Delta t) (B_{\text{plexus}} / B_{\text{sp}}) V_{\text{plexus}} \quad (\text{cc/s per cm}^2 \text{ surface area}) \quad (1)$$

where $\Delta B / \Delta t = [B(t_2) - B(t_1)] / (t_2 - t_1)$, change in apparent blood per unit time (s^{-1}); $B_{\text{plexus}} / B_{\text{sp}} = 2.5$ = 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 cm^2 surface area).

2. Oxygen extraction

$$O_{2,\text{ex}} = 4 \Delta S J C_{\text{HGb}} \quad (\text{moles O}_2 / \text{s per cm}^2 \text{ surface area}), \quad (2)$$

where 4 = four O_2 molecules per hemoglobin molecule; $f = V_v / V_a$, the ratio of venous volume to arterial volume (assumed); $f_a = 1 / (1 + f)$, volume fraction of blood that is arterial; $f_v = f / (1 + f)$, volume fraction of blood that is venous; $S_a = 0.97$, arterial oxygen saturation (assumed); $S_v = (S - f_a S_a) / f_v$, venous oxygen saturation; $\Delta S = S_a - S_v$, difference in oxygen saturation between arterial and venous; $C_{\text{HGb}} = C_{\text{blood}} / \text{MW}$, concentration (moles/cc); $C_{\text{blood}} = 0.150$ g.HGb/cc, hemoglobin concentration of whole blood; $\text{MW} = 64,500$ g/mole, molecular weight of hemoglobin.

3. Number of cells in epidermis per cm^2 surface area

$$n_{\text{cells}} = V_{\text{epidermis}} / V_{\text{cell}}, \quad (3)$$

where $V_{\text{epidermis}} = (0.0100 \text{ cm}) (1 \text{ cm}^2)$ (cc per cm^2 surface area); $V_{\text{cell}} = \pi D^3 / 6$ (cc/cell); $D = 10 \times 10^{-4}$ cm diameter of spherical epidermal cell (approximation).

4. OCR

$$\text{OCR} = (O_{2,\text{ex}}/n_{\text{cells}})(10^{18} \text{ moles/amol}) \quad (\text{amol/cell/s}). \quad (4)$$

The factor $B_{\text{plexus}}/B_{\text{sp}} = 2.5$ 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. (7)] 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_{plexus}) than the average (B_{sp}) and is located at about 200 to 400 μm depth (a 200- μm thickness assumed here). The spectrally observed average B_{sp} does not equal the true local B_{plexus} .

A Monte Carlo simulation (MCML⁶) 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_{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_{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 (2), 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

$$M_{\text{R}} = \frac{M_{\text{skin}} - M_{0\%}}{M_{99\%} - M_{0\%}} = \frac{SR_{\text{skin}}C_{\text{skin}}D}{SR_{99\%}C_{99\%}D} = \frac{R_{\text{skin}}C_{\text{skin}}}{R_{99\%}C_{99\%}} \quad (5)$$

or

$$M_{\text{R}}R_{99\%} = KR_{\text{skin}}, \quad (6)$$

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. (6) is known from measurements and $R_{99\%} = 0.99$. The right side of Eq. (6) is fit by least squares fitting using the modified diffusion theory described in Eq. (7). After the fitting, the skin reflectance $R_{\text{skin}} = M_{\text{R}}/K$ is in units of (mm^{-2}). The factor K depends on the ratio of collection efficiency by the probe when 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 $R_{\text{skin}}KR(\lambda) \pm$ applied pressure. The factor

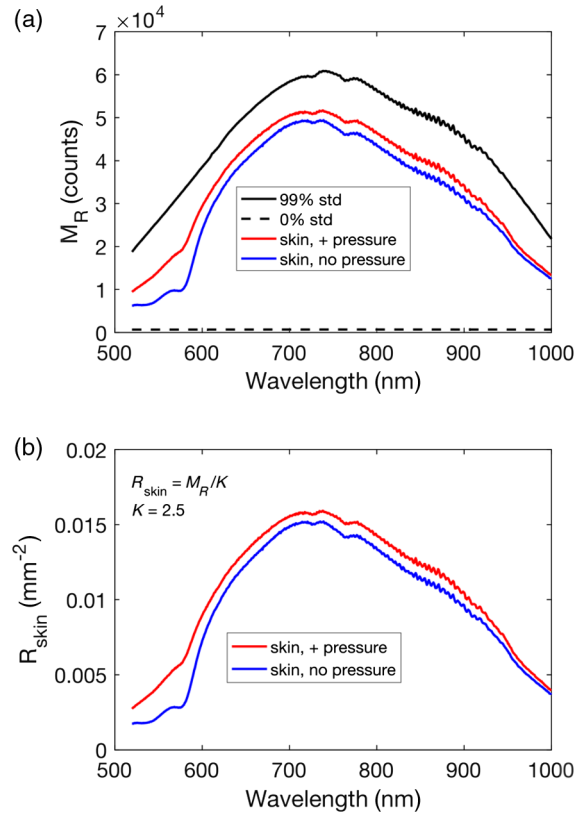


Fig. 2 (a) The raw spectra $M_{\text{skin}} \pm$; pressure, $M_{99\%}$, and $M_{0\%}$ (counts). The $M_{99\%}$ is scaled to fit on graph. (b) The reflectance spectrum, where source and collection fiber contact the skin.

K equals 2.5 based on the average value fit for all acquired spectra on the five subjects.

The spectrum KR_{skin} was matched by least squares fitting using a simple light transport expression based on diffusion theory [Eq. (7)]. 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, $\mu'_s(\lambda)$, whose spectral shape is described below. The expression for pKR (counts) was

$$pKR = KT_{\text{epi}} \text{getRr}(\mu_a, \mu'_s, r, n_r), \quad (7)$$

where K = calibration constant (counts mm^{-2}); $T_{\text{epi}} = \exp(-M\mu_{a,\text{mel}}L_{\text{epi}})$, the attenuation due to the pigmented epidermis; M = volume fraction of melanosomes in 60- μm -thick forearm epidermis; $\mu_{a,\text{mel}}$ = absorption coefficient of interior of cutaneous melanosome;⁷ $L_{\text{epi}} \approx 3 \times \text{epidermal thickness} = 0.180 \text{ mm}$; μ_a is the absorption coefficient (mm^{-1})

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

where $\mu_{a,\text{oxy}}$ = absorption spectrum of oxygenated whole blood;⁸ $\mu_{a,\text{deoxy}}$ = absorption spectrum of deoxygenated whole blood;⁸ μ'_s = reduced scattering coefficient (mm^{-1}) of skin, based on literature;⁹ $a = \mu'_s$ at $\lambda = 500 \text{ nm}$, which scales the shape of $\mu'_s(\lambda)$ such that; $\mu'_s(\lambda) = a[0.32(\lambda/500 \text{ nm})^{-4} + 0.68(\lambda/500 \text{ nm})^{-0.91}] = \text{Rayleigh plus Mie scattering}$;⁹

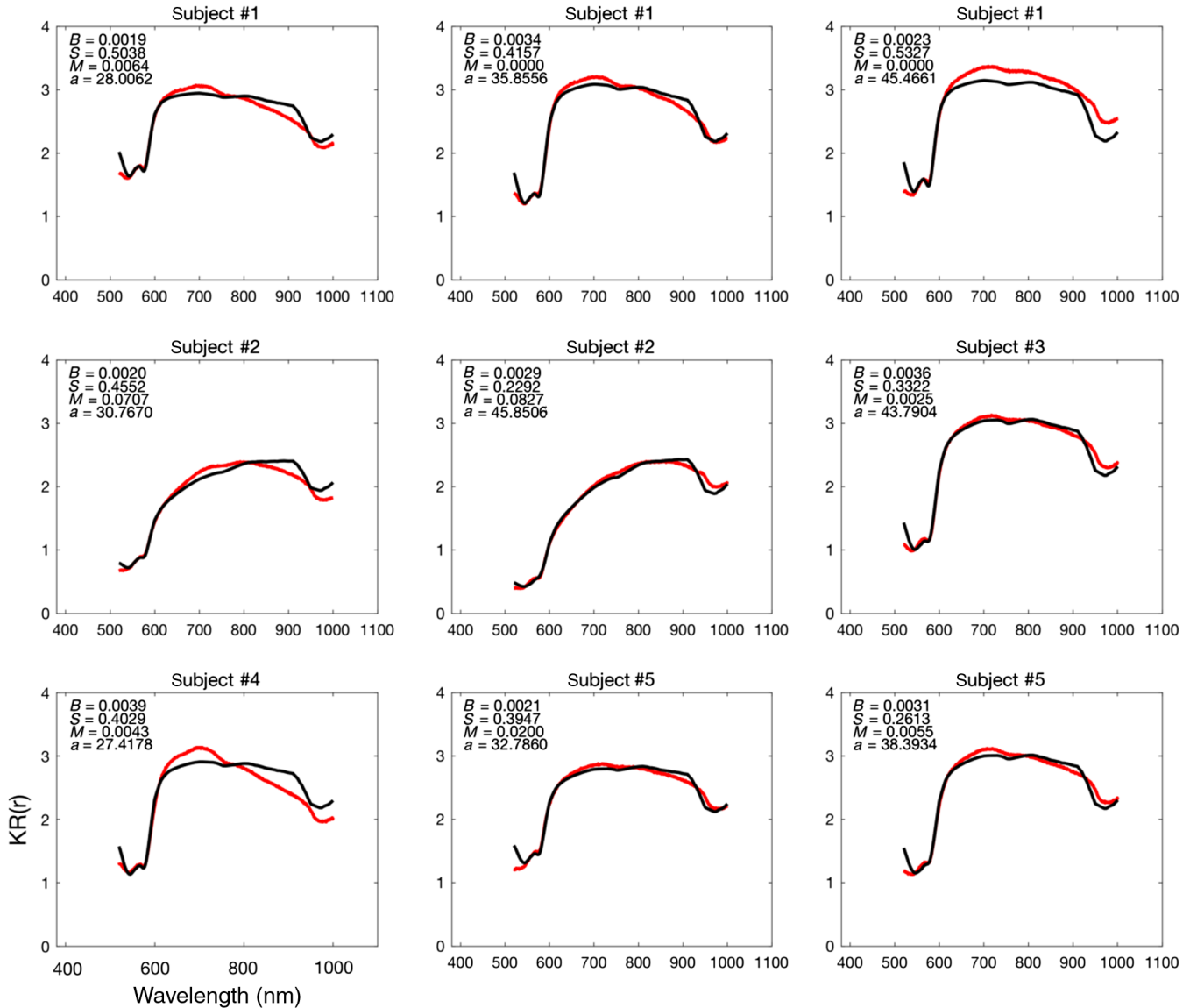


Fig. 3 Measurements of reflectance spectra, KR_{skin} (cm^{-2}), from the forearms of five subjects (red), and the diffusion theory fit to the spectra (black). Subject 2 was the most strongly pigmented subject. (calibration $K = 2.5$).

$\text{getRr}(\mu_a, \mu_s', r, r_i)$ = local reflectance (mm^{-2}) (diffusion theory, Farrell et al.¹⁰); $r = 1.88$ mm center-to-center distance between source and collection fibers. $n_r = 1.4/1.5$, ratio of the approximate refractive indices, $n_{\text{skin}}/n_{\text{probe}}$.

Hence, the spectral fitting yielded values for B , S , M , a , 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 $M_{99\%}$ was used to analyze all the spectra. Subsequent analysis fit for B , S , M , and a 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 $\text{OCR} = 9.1 \pm 2.7$ 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 ± 5 amol/cell/s ($n = 9$ 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.⁴ 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.

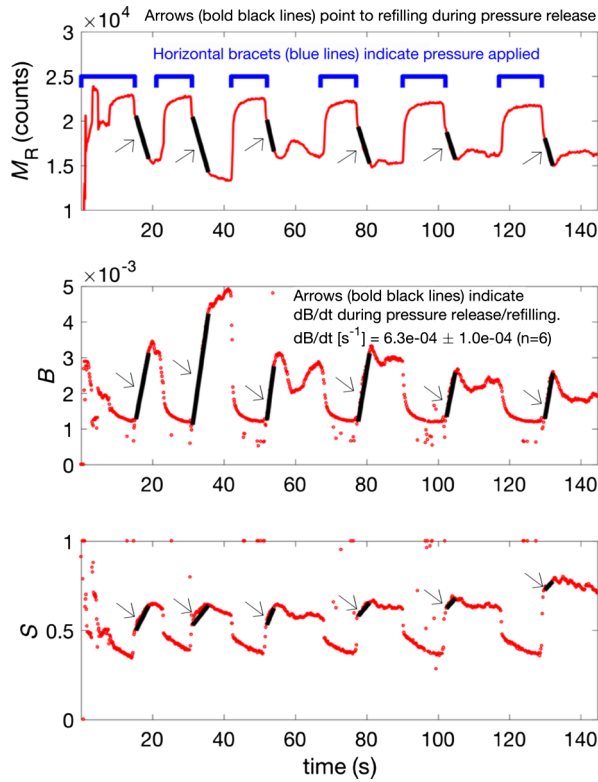


Fig. 4 The time course of signals. (a) $M_R(t)$, showing how applied pressure blanches the skin, causing an increase in reflectance. (b) $B(t)$, showing how pressure reduces the blood content, i.e., blanches the skin, and release of pressure yields an initial linear rise in B , specifying the slope dB/dt . (c) $S(t)$, showing the modulation of the oxygen saturation of hemoglobin in the mixed arteriovenous vasculature. In all three figures, the arrows point to bold black lines that show the same period t_1 to t_2 , during which there is linear refilling of the plexus.

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) and mixed arteriovenous oxygen saturation of hemoglobin (S). 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.¹ 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

Table 1 Example calculation from the fourth pressure release in Fig. 3.

$B(t_1) = B$ measured while blanched	0.00121
$B(t_2) = B$ measured after refill	0.00313
$S(t_2) = S$ measured after refill	0.655
$dB/dt = [B(t_2) - B(t_1)]/(t_2 - t_1)$	$5.99 \times 10^{-4} \text{ s}^{-1}$
$B_{\text{ratio}} = B_{\text{plexus}}/B_{\text{sp}}$ from Monte Carlo sims.	2.5
V_{plexus}	0.02 cc/per cm^2 skin surface
$J = (dB/dt)B_{\text{ratio}}V_{\text{plexus}}$	$2.99 \times 10^{-5} \text{ cc/s}$
$C_{\text{blood}} = \text{conc. of HGb in whole blood}$	0.150 moles/cc
MW_{HGb}	64,500 moles/cc
$C_{\text{HGb}} = C_{\text{blood}}/MW$	$2.33 \times 10^{-6} \text{ moles/cc}$
$f = V_{\text{venous}}/V_{\text{arterial}}$	1.0
$f_a = 1/(1 - f)$	0.5
$f_v = f/(1 - f)$	0.5
S_a assumed	0.97
$S_v = [S(t_2) - S_a f_a]/f_v$	0.34
$\Delta S = S_a - S_v$	0.66
$\Delta O_2 = 4\Delta S J C_{\text{HGb}}$	1.75×10^{-10}
D_{cell}	0.0010 cm
$V_{\text{cell}} = \pi D_{\text{cell}}^3/6$	5.24×10^{-10}
$n_{\text{cells}} = V_{\text{epi}}/V_{\text{cell}}$	1.91×10^7
$\text{OCR} = (\Delta O_2/n_{\text{cells}})10^{18}$	9.2 amol/cell/s

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

Site	Subject	Mean \pm std (n pressure releases)
1	1	9.1 ± 2.7 ($n = 6$).
2	1	12.9 ± 2.3 ($n = 4$)
3	1	15.8 ± 5.0 ($n = 3$)
4	2	0.9 ± 0.2 ($n = 3$)
5	2	9.9 ± 2.5 ($n = 4$)
6	3	11.4 ± 5.7 ($n = 5$)
7	4	4.1 ± 2.0 ($n = 3$)
8	5	9.1 ± 5.8 ($n = 3$)
9	5	18.0 ± 4.3 ($n = 3$)

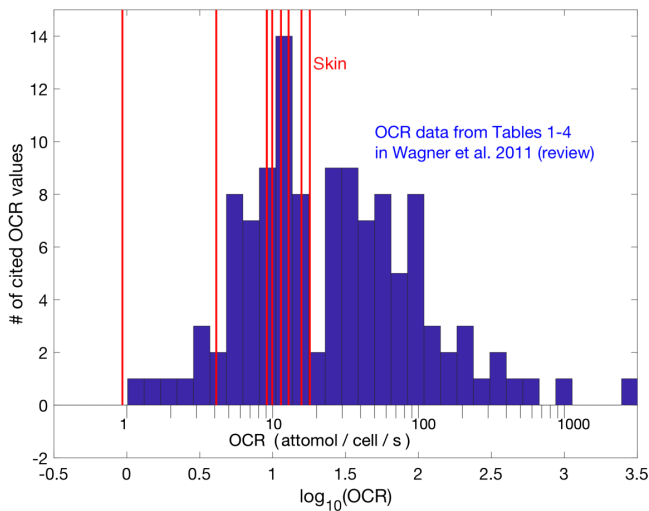


Fig. 5 The distribution of OCR (amol/cell/s) for various cells and tissues, as compiled in the review by Wagner et al. 2011. The red lines indicate the OCR of the nine skin sites measured in this report.

contribution of atmospheric oxygen to the epidermal oxygen supply.^{11–13}

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.

Acknowledgments

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