microbeads (500 μL) were added. After gentle washing, the TECHNODISK was read using the CCD reader.

The TECHNODISK offers a measuring range overranking the ELISA method by 2 Log (Fig. 1B). The major advantage of TECHNODISK compared with the FIA is the elimination of the classical fluorescence interferences, thus offering a better sensitivity in low titers.

These preliminary experiments demonstrate many advantages over the conventional FIA and ELISA methods. The design of the TECHNODISK device allows the detection and quantification of up to seven different antigens simultaneously. Moreover, we have been able to apply the TECHNODISK technology to the quantification of cellular antigens, especially platelet glycoproteins. This could offer an attractive method for point-of-care monitoring of the new platelet glycoprotein IIb/IIIa antagonists.

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Lateral Tissue Inhomogeneity: A Missing Link in Photoplethysmographic Noninvasive Measurement of Arterial Blood Constituents, Lester A. Sodickson (Cambridge Research Associates, 263 Waban Ave., Waban, MA 02468; fax 617-964-4799, e-mail las.cra@mediaone.net)

Over the past decade, much effort has been directed at extending the success of oxygen saturation in pulse oximetry (1–4) to the noninvasive determination of other blood analyte concentrations (5). The tissue transmission window nestled between the strong absorption of hemoglobin in the visible and the high absorption of water in the mid-infrared is a potentially useful wavelength range because of increased tissue penetration. This work reports initial results from a new apparatus that directly compares light transmission through a finger at wavelengths in the pulse oximetry and transmission window regions. Wavelength-dependent changes as a function of applied finger pressure are documented, posing a challenge for instrumental stability and design.

The photoplethysmographic method isolates arterial blood effects by analyzing small waveform excursions induced by incremental blood flow in and out of monitored tissues during each heartbeat. Each signal is separated on a pulse-by-pulse basis into a steady or direct current (DC) portion and a pulsatile or alternating current (AC) portion (1). For a signal \( S \), the intent is that this AC/DC fraction, or modulation fraction, approximate \( dS/S \), which is proportional to the absorbance. The ratio of modulation fractions at two wavelengths then approximates the ratio of absorbances at those wavelengths. In pulse oximetry, the saturation is determined from a nonlinear calibration curve of oxygen saturation plotted against this ratio of modulation fractions (1).

The present apparatus departs from pulse oximetry practice in using fixed optics that largely eliminate ambient light interference and motion-induced probe-coupling artifacts (4). The hand is placed palm down on a flat surface beneath a light shield, with the examined finger pressed against a 15-mm diameter optical window that is illuminated from below by multiple light-emitting diodes (LEDs) located azimuthally around a 14° half-angle cone. Approximately 3-mm wide gaussian-shaped beams are projected to the center of the window via lenses. A 5-mm diameter InGaAs detector (Germanium Power Devices) mounted in a slight recess in a 25-mm diameter holder that passes through the light shield is pressed against the top surface of the finger to simultaneously monitor transmitted light at all wavelengths.

The near-infrared LEDs (Oriel Instrument and Opto-Diode) emit 1–3 mW of light centered on 820, 1000, 1220, and 1300 nm, with 25–40 nm full width at half maximum. The four wavelengths were selected for different absorbances of major hemoglobin variants and water to facilitate the simultaneous determination of total hemoglobin concentration and oxygen saturation (5). The 820 nm/1000 nm pair forms a surrogate pulse oximeter whose performance is compared directly here with that of the 1000 nm/1220 nm pair. The 1220 nm wavelength lies in the middle of the tissue transmission window.

The light sources are driven with four distinct sine waves generated by two SigLab Dynamic Signal Analyzers (DSP Technology) controlled and monitored by Matlab (The Mathworks). The Fourier-transformed detector signal amplitudes, their derivatives, and calculated ratios of the relative degree of modulation at different wavelengths are displayed in real time at 100 Hz to facilitate user analysis and control of the sample site location and applied pressure.

Sample data are shown in Fig. 1. The top panel plots the time course of three of the signals as a finger is pressed against the optical window with several steps of increasing pressure followed by two decreases in pressure. The curves are presented as the deviation of the signals from their values at \( t = 0 \). The pulsatile components of these deviations as well as the pressure-induced DC baseline shifts are smaller at 1220 nm than at 820 or 1000 nm. The middle panel presents the results of sliding window calculations of the ratio of modulation fractions for pairs of signals in the top panel. The pressure steps produce clearly correlated steps in the 1000 nm/1220 nm ratio with barely visible changes in the corresponding 820 nm/1000 nm ratio, which approximates the Red/Infrared ratio of pulse oximetry (1). Thus, site pressure changes for measurements at 1000 nm/1220 nm would degrade estimates of analyte absorbance ratios compared with measurements at 820 nm/1000 nm. The left two sections of the bottom panel of Fig. 1 provide an alternate view of the data, which confirms that pressure-induced offsets in the nonpulsatile background are similar at 820 and 1000 nm and markedly different at 1220 nm.

The fundamental concern raised by these data is that the sampled tissue volume is not the same at all wavelengths. In evaluating this, it is important to separate
internal tissue effects mediated by scattering from instrumental effects such as lateral displacements or angular differences between the incident beams. Diffusion theory models of pulse oximetry often rely on the rapid conversion of parallel incident light to an isotropic point source a short distance beneath the tissue surface (4). The short mean free paths of photons in tissue (6) and Monte Carlo calculations (7) of photon histories vs depth suggest that the use of off-axis beams in this study can produce separations a short distance beneath the tissue surface by fractions of a millimeter, amounts that are comparable to the emitter separations commonly used in pulse oximetry.

The potential impact of the off-axis illumination on the pressure dependence of the modulation ratios was assessed empirically by repeating the measurements with permuted azimuthal locations of the LEDs and with the finger pointed alternately toward the 1220 or 1300 nm LED positions indicated in the rightmost section of the lower panel of Fig. 1. The observation that the pressure dependence of the 1000 nm/1220 nm modulation ratio is much greater than that of the 820 nm/1000 nm ratio survives all the permutations and thus cannot be attributed merely to instrumental effects.

Additional experiments with narrow bands of reflective or black foil pressed against the sides of the finger show significant decreases (~20%) of the total transmission when reflective bands are removed, and similar increases when black bands are removed. A nonnegligible portion of the light is clearly traveling around the periphery, where it can be influenced by an external reflector.

Another experimental design concern is the possibility that pressure changes might themselves induce inhomogeneities in the distribution of pulsing blood vessels. The instrument’s optical interface to the finger is sufficiently robust that the high pressure range can be traversed with sufficient resolution to monitor the gradual shutdown of

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**Fig. 1. Variation of signals and modulation ratios with applied pressure.**

*(Top panel)*, the percentage of variation of the indicated signals about their initial values as a finger is pressed against the instrument’s optical window, with three increasing and two decreasing pressure steps at times indicated by the dashed lines. The curves are offset for clarity. *(Middle panel)*, the modulation ratio \( R = (dA/A)/(dB/B) \) for pairs of signals \( A \) and \( B \), following the pulse oximetry algorithm, with \( dA \) and \( dB \) the signal decrements as arterial blood enters the tissue. To improve precision, \( R \) was determined by a least-squares fit of one waveform, in its entirety, to the other in a several second-long sliding window, according to the equation: \( A = mB + k_2(t - t_c)^2 + k_1(t - t_c) + k_0 \), with \( t_c \) the center of the window, and \( m \) and \( k_0-2 \) the fitting parameters. The panel shows the calculated values of \( m \), scaled to their mean values across the whole 50-s data acquisition. The curves are offset for clarity. *(Left and middle bottom panels)*, plots of relative signal strengths with each scaled to its maximum value. The dashed lines show the expected results in the absence of scattering. *(Right bottom panel)*, azimuthal locations of the light sources around the 14° cone on which the LEDs are mounted. These azimuthal locations were permuted to rule out systematic errors in apparent pressure dependence attributable to angular differences in incident beam direction for the various wavelengths.
pulsing blood vessels. This shutdown appears as a decline in the AC signal magnitudes accompanied by both continued increase in DC signal and profound changes in the waveforms. For the data shown in Fig. 1, the pressure range was limited to maintain the presence of an apparent dicrotic notch in the waveform derivative whose disappearance has been observed to precede the shutdown of pulsing blood vessels.

Taken together, these data indicate that the nonpulsatile DC baseline signals against which the percentage of modulation is measured do not track each other well at all wavelengths when the applied pressure changes. One possible explanation is that there is a wavelength-dependent “extra” light component traveling around the pulsing blood vessels, offsetting the denominators in the modulation fraction calculations so that the comparison between wavelengths is also skewed.

An alternative and perhaps equivalent possibility is that light in the transmission window region spreads more broadly across the finger because the longer multiple-scattering paths are not truncated by high absorption along the way. In this interpretation, pressure increments decrease the tissue thickness and drive blood from the site. Both of these effects increase the fraction of surviving photons that follow longer paths and sample larger volumes. Indeed, measurements on thinner fingers showed offsets similar to those produced by increased pressure. The net effect of pressure changes and tissue thickness is thus expected to be higher where the tissue absorption is higher to start with, i.e., below 1000 nm.

It is likely that in pulse oximetry the signal backgrounds track well enough compared with oxy- and deoxyhemoglobin absorbance changes over the clinical range that the results are accurately accurate. The situation changes, however, in the transmission window region, where sensitivity to location and pressure are increased at the same time that the analyte absorbances are much smaller. Clearly, greater attention to instrumental design will be required to decrease the pressure dependence to observe such smaller analyte absorbances reliably.

Efforts are underway to explore a variety of improved optical designs to minimize the fluctuations with pressure and orientation, directed at obtaining stable, finger-independent, high-precision ratio measurements between multiple wavelengths. Control of the pressure dependence of the pulsatile data and detailed comparison of the waveforms at different wavelengths is expected to be a key tool in improving the precision and accuracy of photoplethysmography.

References

Materials for Fabricating Biosensors for Transdermal Glucose Monitoring. Man-Shueung Chan,1* Donald Kuty,2 John Pepin,1 Norman Parris,2 Russ Potts,2 Mike Reidy,2 Mike Tierney,2 Chris Uhegbu,2 and Yalia Jayalakshmi2 (1 DuPont Photopolymer & Electronic Materials, 14 T.W. Alexander Dr., Research Triangle Park, NC 27709-4425, and 2 Cygnus Inc., 400 Penobscot Dr., Redwood City, CA 94063; * author for correspondence)

Most amperometric biosensors based on the metal-catalyzed electrooxidation of hydrogen peroxide (H2O2) combine an electrochemical sensor with an analyte-specific oxidase-type enzyme, e.g., glucose oxidase for the detection of glucose. H2O2 produced by the glucose oxidation is measured at a working electrode that has a platinum group metal catalyst.

Improved long-term outcomes for diabetic subjects is improved by controlling blood glucose with frequent monitoring, i.e., more than five times per day. Transdermal extraction of interstitial fluid for glucose monitoring is an attractive approach to achieving this goal without the need to frequently draw blood samples by finger-pricking.

Materials to fabricate blood glucose biosensors are often based on electron-transfer mediators that are not suitable for transdermal extraction because of their potential toxic effects. Furthermore, the glucose concentration in transdermally extracted fluid is typically ~5 μmol/L vs ~5 mmol/L in whole blood. This places a far greater demand on detection methods. A biosensor fabricated with pure platinum, printed on a ceramic substrate using high temperature processing, typically provides good H2O2 sensitivity (~70 nA per μmol/L per cm2) and low background current (~10 nA) to meet the needs for accurate detection of glucose in extracted interstitial fluid. A platinum ceramic biosensor is not commercially viable because of the prohibitively high cost of platinum and the fabrication processes. A polymer thick film (PTF) ink containing platinum/carbon (Pt/C) electrocatalyst with a low percentage of platinum, which can provide good sensor performance similar to that of a platinum ceramic sensor, is a preferred material to meet the needs for fabricating disposable biosensors for transdermal glucose monitoring.

The challenge in developing such a Pt/C ink lies in overcoming the conflicts of material limitations vs functional requirements, e.g., (a) a low Pt loading of <5% vs a high H2O2 sensitivity of >50 nA per μmol/L per cm2; (b) hydrophobic graphite particle surfaces vs a water wettable electrode surface needed for fast electrochemical reactions; and (c) sufficient polymer binder to hold graphite and platinum