English Research Express@NCKU Volume 22 Issue 9 - November 30, 2012
[http://research.ncku.edu.tw/re/articles/e/20121130/1.html]

Chromophore concentrations, absorption and scattering properties of human skin *in-vivo*

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Optics Express 17(17), 14599-14617, 2009
(Also appeared in the October 2, 2009 issue of Virtual Journal for Biomedical Optics)

Near infrared diffuse reflectance spectroscopy (DRS) is commonly used to determine *in-vivo* tissue absorption coefficient μ_a and reduced scattering coefficient μ_s', from which tissue functional information, such as hemoglobin concentration, oxygen saturation, water concentration, and averaged scatter size and density can be deduced.[1-2] Both of these parameters are important in noninvasive tissue diagnostics, since the scattering coefficient of tissue can provide information about the mean size of the tissue scatterers, while the absorption coefficient of tissue can be used to determine chromophore species and concentrations. [3-4] When applying DRS technique to study human skin, these parameters can provide essential information for many aesthetic, therapeutic, and diagnostic applications such as monitoring of skin blood oxygenation, melanin concentration, detection of cancer with fluorescence, laser surgery, and photodynamic therapy. [5]

We have developed a new fiber-based probe that can be used to determine the optical properties of superficial *in-vivo* skin using DRS in conjunction with a two-layer diffusion model. [6] The facilitating technologic innovation of this probe is the presence of a highly diffusing Spectralon layer at the input side that enables a diffusion model for small source-detector distances (less than three transport mean-free-paths), and thus, the absorption and reduced scattering coefficients of the superficial volumes of samples can be separated and quantified using a very simple photon diffusion theory. Measurements using this probe were carried out *in-vivo* at two anatomical locations (volar forearm and palm) on fifteen subjects (five subjects of African descent, five Asians, and five Caucasians). [7] Optical properties obtained using the superficial diffusing probe correlate closely with the layer structure of skin and demonstrate similarities to those of ex-vivo skin determined from integrating sphere measurement techniques.

In this study, the probe design has been modified into multiple source-detector pairs as shown in Fig. 1. This design allows the employment of a white light source for obtaining continuous spectra of absorption and reduced scattering coefficients. The advantages of this multi source-detector separation probe include relative low instrument cost and self-calibration for instrument response (by using the reflectance of one source detection pair as the reference and normalizing the reflectance of other source detector separation pairs to the reference). A two-layer photon diffusion model is then fit to the normalized reflectance versus source-detector separation by using a least square minimization algorithm to determine the absorption and reduced scattering spectra. The

recovered absorption spectra are linearly fit with known absorption spectra of chromophores to extract chromophore concentrations, and the reduced scattering spectra are fit to a scattering power law to obtain the scattering power. [8]

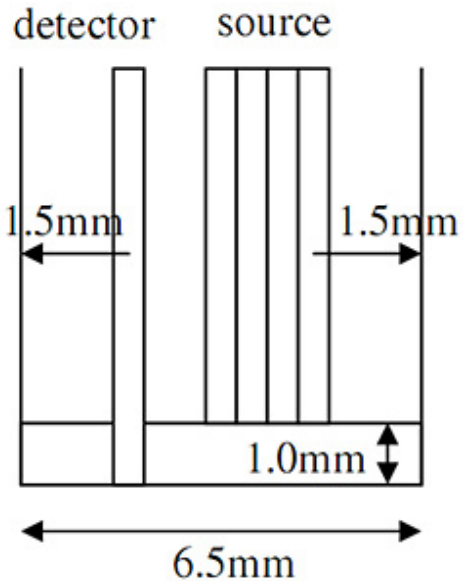

Fig. 1. Configuration of the diffusing probe.

From our *in-vivo* skin data obtained from 18 subjects of different skin phototypes, it is found that performing the "two-region chromophore fitting" to the absorption spectrum would result in the best fit with minimal residuals as illustrated in Fig. 2. "Two-region chromophore fitting" means that the skin absorption spectrum is fit to a set of known chromophore absorption spectra at wavelengths between 500 nm and 600 nm and again fit separately between 600 nm and 1000 nm. The rationale for performing the two-region fitting is that the skin has very different optical properties in the visible and the NIR wavelength regions, and thus the sampling volumes at these two regions are quite different. We generated Monte Carlo simulation results to support this assumption. It can be seen in Fig. 3 that, at a same source-detector separation, the longer the source wavelength the deeper the interrogation depth. Likewise, the best fittings for reduced scattering coefficients of skin were obtained when the reduced scattering spectra were fit in the region below and above 600nm separately. This suggests that the average scatterer sizes of skin determined in the visible and the NIR regions are much different. Results from the measurements carried out here also indicate that the scattering power is not only dependent to anatomical location but also on skin phototype. In addition, experimental results obtained from measuring 10 subjects with forearm venous occlusion were provided. We recovered significantly different hemoglobin concentration at the region below and above 600nm. We obtained that the deoxy-hemoglobin concentration at upper dermis and oxy-hemoglobin concentration at deeper dermis increased when venous occlusion of 50mmHg was applied on the forearm. We demonstrated that our diffusing probe combined with the two-region fitting method was capable of monitoring the variation in hemoglobin concentration of *in-vivo* skin at different depths simultaneously. Our results agree with those reported by other researchers and thus further support and validate our proposed method. In the future, we will employ this probe to study skin photoaging and *in-vivo* skin melanin synthesis. We will be able to study the modulation of mean scatterer size as well as chromophore concentrations in the epidermis and dermis introduced by UV radiation.

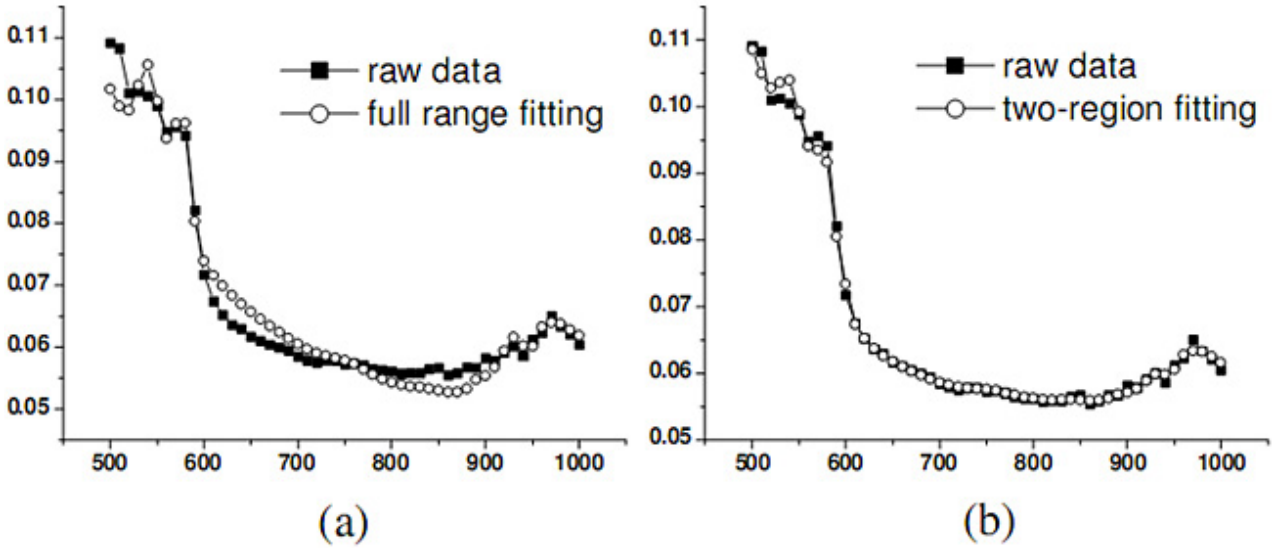

Fig. 2. Typical chromophore fitting examples with full range fitting method and two-region fitting method. (a) and (b) demonstrate the fitting results of two fitting methods with the same raw data.

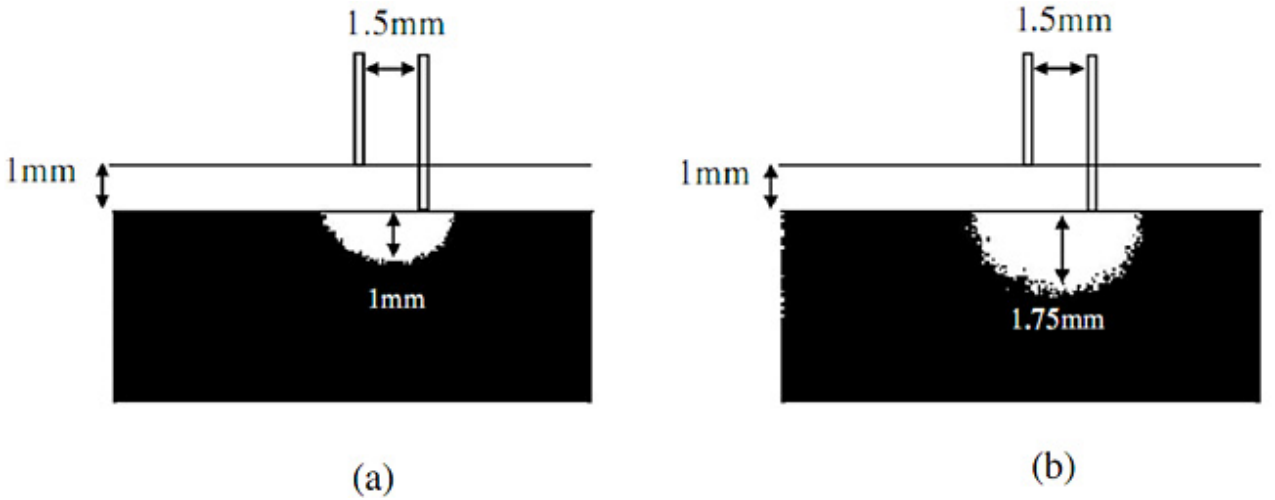

Fig. 3. 50% threshold maps for the diffusing probe of 1.5mm source-detector separation with (a) 500nm light source and (b) 900nm light source.

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