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SPECTRAL IMAGING AND ANALYSIS TO YIELD TISSUE OPTICAL PROPERTIES

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An introduction to the basics of spectral imaging as applied to biological tissues is presented. An example of a spectral image of a face is used to demonstrate the data and spectral analysis that specify the melanin content (M), blood content (B), tissue oxygen saturation (S), water content (W), fraction of scattering due to Rayleigh scattering (f) and due to Mie scattering ($1-f$), and the reduced scattering coefficient at 500-nm wavelength ($\mu'_{s\,500\,nm}$). The sensitivity of reflectance spectra to variation in the various parameters is illustrated.

Keywords: Spectroscopy; spectral imaging; tissue optical properties; optical diagnostics.

1. Introduction

Spectral imaging involves the acquisition of two-dimensional (2D) images of reflectance, $R(x, y)$, at each of many wavelengths, λ. The data is combined into a "spectral cube", $R(x, y, \lambda)$. Each pixel has data at all wavelengths, and $R(\lambda)$ at x, y constitutes a reflectance spectrum. The spectrum $R(\lambda)$ is affected differently at different wavelengths by different tissue components. The analysis uses a library of absorption spectra from the literature for known quantities such as melanin, oxyhemoglobin, deoxyhemoglobin, and water, and models the wavelength-dependent tissue scattering properties by a combination of Rayleigh scattering (varies as λ^{-4}) and Mie scattering (varies as $\sim\lambda^{-1}$). A light transport model for one-dimensional (1D) reflectance is used.

This paper outlines the basics of spectral imaging and analysis. An example of a spectral image of a face is used to illustrate typical spectra and to demonstrate the spectral analysis. The sensitivity of reflectance spectra to variation in the various parameters is illustrated, which demonstrates that one may need to have independent specification of the factors f and W in order for the analysis to reliably yield values for M, B, S and $\mu'_{s\,500\,nm}$.

2. Measurement

A generic optical measurement can usually be described as the ratio of measurements of an unknown tissue sample, $M_{sample}(\lambda)$ [lab units], and a known reflectance standard, $M_{std}(\lambda)$ [lab units]. The ratio M/M_{std} cancels the wavelength dependence of the light source (S [W/nm]) and the wavelength responsivity of the detector (D [lab units/W/nm]):

$$\frac{M_{sample}}{M_{std}} = \frac{SRGD}{SR_{std}G_{std}D} = \frac{RG}{R_{std}G_{std}} = K\frac{R}{R_{std}}, \tag{1a}$$

where

$$K = \frac{G}{G_{std}}, \tag{1b}$$

and

$$R = \frac{M_{sample}}{M_{std}}\frac{R_{std}}{K}. \tag{1c}$$

The term G is the collection efficiency of the sample measurement, and G_{std} is the collection

efficiency of the standard measurement. For spectral imaging, G denotes the fraction of the light escaping from a pixel-sized area on the tissue surface, which is collected by a pixel on the camera. For spectral imaging, G and G_{std} are usually equal, since the standard and sample are placed in the same plane of focus for a measurement. They are also usually independent of wavelength. A factor K equals the ratio G/G_{std}, and typically K equals 1 and is wavelength independent. The value R is the unknown sample reflectance, and R_{std} is the known standard reflectance.

The geometry of collection is also affected by the angle of the sample surface, e.g., when the camera is viewing a face. The angle θ between the normal to the skin surface and the axis between camera and face causes the collection of reflectance to scale as $\cos(\theta)$. This $\cos(\theta)$ factor is considered later in the theory for predicting collected reflectance.

A spectral camera can be implemented in many ways. The light can be passed through a variable transmission filter to illuminate the sample with a narrow band of wavelengths, and the camera acquires an image using each choice of wavelength band. Another approach is to illuminate with white light while the camera views the sample through a transmission filter that passes light within one narrow wavelength band. Again, images are acquired for a series of filter settings to pass a series of narrow wavelength bands. A third approach is shown in Fig. 1. White light is delivered, but an optical system views only a line (x-axis) on the sample while a prism or diffraction grating disperses the wavelengths, yielding a 2D image of lateral position (x) and wavelength (λ) that is detected by a CCD camera. A galvo mirror scans the line across the sample in steps along the y-axis. The series of acquired x-λ images, one for each y, are compiled into a *spectral cube*, $R(x, y, \lambda)$. It is helpful to deliver the illumination light through a linear polarizer and place a cross-polarized linear polarizer in front of the camera, so that specular glare from the sample surface is rejected. Only light that enters the sample and undergoes multiple scattering will return cross-polarized light that can reach the camera. The white standard used for calibration must also be a volume scatterer so that it also reflects cross-polarized light.

Figure 2 shows an image obtained with a spectral camera as in Fig. 1. The image includes both a white and black reflectance standard for calibration. In this figure, the face is shown at one wavelength (579 nm). The figure also shows the spectra for the left and right cheeks, in both raw counts and in the normalized units of reflectance. The calculation of R is similar to Eq. (1), but now includes the additional measurement of a black standard. The expression for R is:

$$R = R_{\mathrm{StdBlack}} + R_{\mathrm{StdWhite}} \frac{M_{\mathrm{Skin}} - M_{\mathrm{StdBlack}}}{M_{\mathrm{StdWhite}} - M_{\mathrm{StdBlack}}}. \tag{2}$$

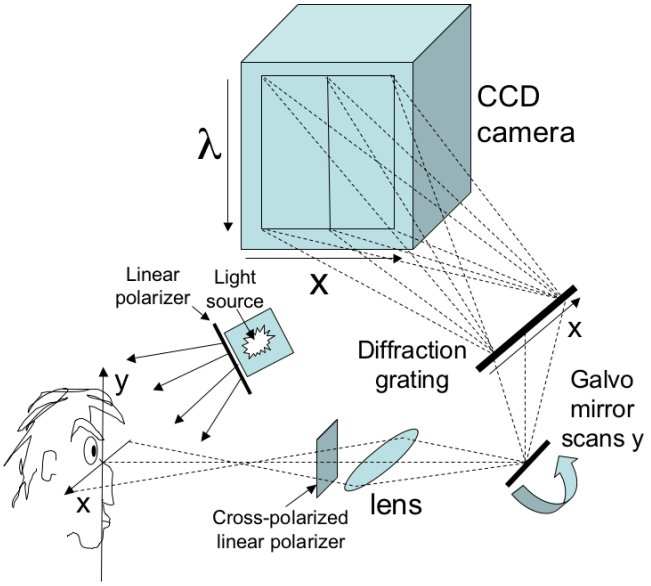
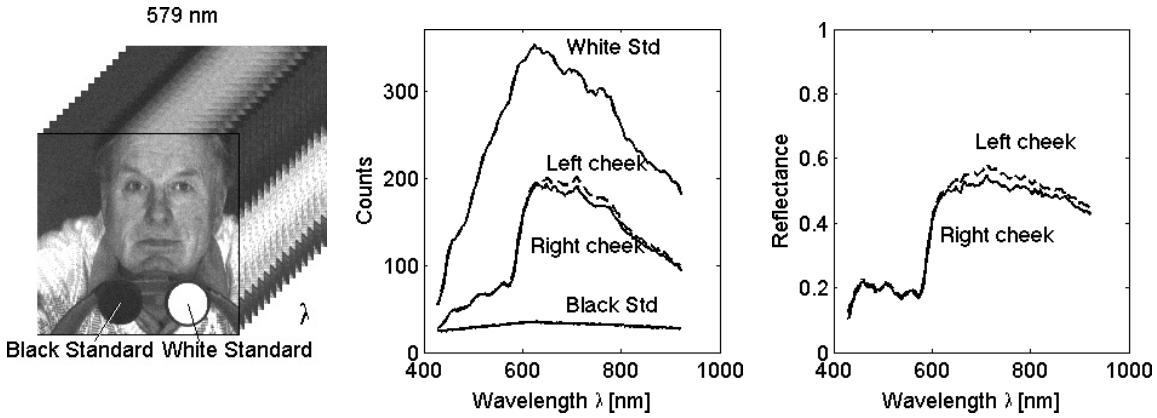


Fig. 1. One version of a spectral camera. A lens images a line along the x axis onto a CCD camera. A diffraction grating disperses the light along the λ axis of the CCD camera. An image acquires reflectance $R(\lambda, x)$. A galvo mirror scans along the y axis, and each image at one y yields $R(\lambda, x, y)$. (Note: this is a highly schematic drawing, not properly depicting the optical design.)

3. General Spectral Curve Fitting

In general, a reflectance spectrum is specified as $R(\lambda)$, which can be calculated using a variety of computations, such as Monte Carlo simulations[1] or diffusion theory.[2] Equation (3a) cites a subroutine called $getR(\mu_{\mathrm{a}}, \mu'_{\mathrm{s}}, M\mu_{\mathrm{a.mel}})$ to represent a generic computation of a reflectance spectrum from skin with a pigmented epidermis and underlying dermis. It has three arguments: the absorption coefficient μ_{a} [cm^{-1}], the reduced scattering coefficient μ'_{s} [cm^{-1}], and the epidermal melanin absorption described as $M\mu_{\mathrm{a.mel}}$ [cm^{-1}], in which M is the volume fraction of melanosomes in the pigmented epidermis and $\mu_{\mathrm{a.mel}}$ is the absorption spectrum of the interior of a typical melanosome ($\mu_{\mathrm{a.mel}}$ [cm^{-1}]).[3] The factor $\cos(\theta)$ is included to account for the



(a) (b) (c)

Fig. 2. (a) A spectral cube, showing a face image taken at a series of 120 wavelengths from 430 nm to 920 nm, using a spectral camera like Fig. 1. (b) The raw counts of white and black standards, and left and right cheek. (c) The reflectance spectra for the left cheek and right cheek.

efficiency of collection of flux escaping the tissue. In summary,

$$R = \cos(\theta)\, getR(\mu_{\rm a}, \mu'_{\rm s}, M\mu_{\rm a.mel}), \tag{3a}$$

where

$$\mu_{\rm a} = B(S\mu_{\rm a.oxy} + (1-S)\mu_{\rm a.deoxy}) + W\mu_{\rm a.water}, \tag{3b}$$

$$\mu'_s = \mu'_{\rm s\,500\,nm}\left(f\left(\frac{\lambda}{500\,\rm nm}\right)^{-4} + (1-f)\left(\frac{\lambda}{500\,\rm nm}\right)^{-1}\right). \tag{3c}$$

The parameters are:

- $\cos(\theta)$ depends on the angle between normal to skin and camera/face axis
- $\mu_{\rm a}(\lambda)$ the absorption coefficient [$\rm cm^{-1}$] for each wavelength (λ)
- $\mu'_{\rm s}(\lambda)$ the reduced scattering coefficient [$\rm cm^{-1}$] for each wavelength λ, $\mu'_{\rm s} = \mu_{\rm s}(1-g)$ where $\mu_{\rm s}$ is the scattering coefficient and g is the anisotropy of scattering
- M the volume fraction of melanosomes in the epidermis (60-μm thick)
- $\mu_{\rm a.mel}(\lambda)$ the $\mu_{\rm a}$ for the interior of a melanosome, $\approx 680(\lambda/500\,\rm nm)^{-3.33}$ [$\rm cm^{-1}$]
- B average volume fraction of whole blood in skin [dimensionless] (whole blood = 150 g hemoglobin/liter)
- S tissue oxygen saturation (mixture of arterial and venous vessels), $0 < S < 1$
- $\mu_{\rm a.oxy}(\lambda)$ the absorption coefficient of oxygenated whole blood
- $\mu_{\rm a.deoxy}(\lambda)$ the absorption coefficient of deoxygenated whole blood
- W average volume fraction of water in tissue [dimensionless]
- $\mu_{\rm a.water}(\lambda)$ the $\mu_{\rm a}$ for pure water [$\rm cm^{-1}$]
- $\mu'_{\rm s\,500\,nm}$ the $\mu'_{\rm s}$ value at 500-nm wavelength [$\rm cm^{-1}$], about 43 $\rm cm^{-1}$ from experiments
- f the fraction of scattering due to Rayleigh scattering (scattering structures much smaller than the wavelength of light), ≈ 0.62
- $1-f$ the fraction of scattering due to Mie scattering (scattering structures close to or greater than the wavelength of light), ≈ 0.38.

The absorption coefficient $\mu_{\rm a}(\lambda)$ is a combination of the blood and water absorption. The reduced scattering coefficient $\mu'_{\rm s}(\lambda)$ is a combination of Rayleigh and Mie scattering. The absorption spectrum for the melanosome interior $\mu_{\rm a.mel}(\lambda)$ is obtained from a report on the threshold for explosively vaporizing melanosomes with pulsed lasers,[3] and is an average spectrum used for reference. Melanosomes actually vary in their melanin content. The analysis determines the product $M\mu_{\rm a.mel}(\lambda)L_{\rm epi}$, where $L_{\rm epi}$ is the accumulated pathlength [cm] of photons in the epidermis before they escape to be detected. The analysis cannot separately specify the values M, $\mu_{\rm a.mel}(\lambda)$ and $L_{\rm epi}$ since the epidermis is too thin to allow separation of these factors. As a convention, the

thickness of the epidermis is assumed to be 60 μm, which is the typical thickness of forearm skin. The value M is the apparent volume fraction of melanosomes in a 60-μm-thick epidermis. We report values of M in this manner in an effort to communicate to doctors and biologists a more meaningful value than the optical depth $M\mu_{\mathrm{a.mel}}L_{\mathrm{epi}}$, which has optical significance but little biological meaning.

The function $getR(\mu_{\mathrm{a}}, \mu'_{\mathrm{s}}, M\mu_{\mathrm{a.mel}})$ was implemented based on multiple Monte Carlo simulations using various optical properties (MCML, Monte Carlo Multi-Layered[1]), but alternative methods can be used.

One can use Eq. (3) for *least squares fitting* to match a fit for $\cos(\theta)$, B, S, M, W, $\mu'_{\mathrm{s}}\,500\,\mathrm{nm}$ and f that match the experimental reflectance spectrum. One matches the experimental data expressed as R in Eq. (1c) or Eq. (2) with the prediction of R by theory in Eq. (3a). This matching of experiment and theory is summarized below

$$\frac{M_{\mathrm{sample}}}{M_{\mathrm{std}}}\frac{R_{\mathrm{std}}}{K} = \cos(\theta)getR(\mu_{\mathrm{a}}, \mu'_{\mathrm{s}}, M\mu_{\mathrm{a.mel}}), \quad (4)$$

in which the left-hand side of the equation represents the experiment and the right-hand side represents the theoretical value. However, since K is not directly known, it is practical to multiply both sides of Eq. (4) by K to yield an expression with the left side composed of measurements and the right side composed of theory and fitting parameters:

$$\frac{M_{\mathrm{sample}}}{M_{\mathrm{std}}}R_{\mathrm{std}} = K\cos(\theta)getR(\mu_{\mathrm{a}}, \mu'_{\mathrm{s}}, M\mu_{\mathrm{a.mel}}). \quad (5)$$

4. Analysis of Spectra

The analysis uses least squares fitting to fit for the parameters M, B, S, W, f and $\mu'_{\mathrm{s}500\,\mathrm{nm}}$. A light transport theory is needed, which is generically called $getR(\mu_{\mathrm{a}}, \mu'_{\mathrm{s}}, M\mu_{\mathrm{a.mel}})$. The program listed here assumes $K\cos(\theta) = 1$, but in general the fitting can include this factor. The μ_{a} and μ'_{s} are the absorption and reduced scattering coefficients for the background melanin-free skin tissue, with epidermis and dermis treated as having the same optical properties. This assumption is not strictly true, but the epidermis is so thin that the error in this assumption is minor. The factor $M\mu_{\mathrm{a.mel}}$ denotes the product of the apparent average melanosome volume fraction M [dimensionless] and the average absorption coefficient of the interior of a typical cutaneous melanosome, $\mu_{\mathrm{a.mel}}$ [cm^{-1}].

In MATLAB$^{\mathrm{TM}}$ notation, the least squares fitting is accomplished by the following:

```
% for nm = wavelengths, and spectra
   Msample, Mstd, Rstd
% using the array MU(:,1:4) that holds
   absorption spectra
B = 0.002; % initial guesses of values
S = 0.075;
W = 0.65;
M = 0.02;
f = 0.62;
musp500nm = 43; % cm^-1
RK = Msample./Mstd.*Rstd;
start = [B S W M f musp500nm];
        % the initial guess
result = fminsearch('fitspectrum',start,
       [ ], nm, RK, MU);
B = result(1);
S = result(2);
W = result(3);
M = result(4);
musp500nm = result(5);
f = result(6);
```

where MU is an array of optical absorption spectra, $\mu_{\mathrm{a}}(\lambda)$ [cm^{-1}], from the literature, for oxygenated and deoxygenated whole blood, water, and the interior of a melanosome:

$$MU(:,1) = \mu_{\mathrm{a.oxy}},$$
$$MU(:,2) = \mu_{\mathrm{a.deoxy}},$$
$$MU(:,3) = \mu_{\mathrm{a.water}},$$
$$MU(:,4) = \mu_{\mathrm{a.mel}}.$$

The program `fitspectrum.m` that is used by *fminsearch*( ) is written as follows:

```
function err = fitspectrum(start,
               nm, RK, MU)
B = start(1);
S = start(2);
W = start(3);
M = start(4);
musp500nm = start(5);
f = start(6);
X = [B*S B*(1-S) W]';
mua = MU(:,1:3)*X;
musp = musp500nm*(f*(nm/500).^-4
       + (1-f)*(nm/500).^-1);
pRK = getR(mua, musp, M*muamel, f,
      musp500nm);
err = sum( (pRK - RK).^2 );
```

When doing such least squares fitting, it is important to only use the wavelengths of spectral signal that are strong and well behaved. Often the signal below 450 nm is too noisy, unless one has a light source with a strong blue component. The signal above 900 nm may be noisy due to loss of detector response at long wavelengths.

5. An Approximate Light Transport Theory

The development of a light transport theory, expressed as an algorithm `getR(mua, musp, M*muamel, f, musp500nm)`, is still an on-going project for the author. A practical expression was developed for the 1D reflectance used in spectral imaging by running Monte Carlo simulations over a range of optical properties, and the results are summarized as:

$$R = \cos(\theta) e^{-M\mu_{\mathrm{a.mel}} L_{\mathrm{epi}}} e^{-\mu_{\mathrm{a}} L_{\mathrm{skin}}}, \qquad (6)$$

in which the factor $e^{-\mu_{\mathrm{a}} L_{\mathrm{skin}}}$ describes the reflectance from a melanin-free skin, in which the epidermis and dermis are considered as one homogeneous tissue. The factor $e^{-M\mu_{\mathrm{a.mel}} L_{\mathrm{epi}}}$ describes the extra attenuation due to the pigmented epidermis. The factor $\cos(\theta)$ accounts for the angle of the skin surface. The total 1D diffuse reflectance is a function of the ratio $\mu_{\mathrm{s}}'/\mu_{\mathrm{a}}$, and hence a single parameter, L_{skin}, can characterize the light transport at a particular wavelength. The pathlength L_{skin} is approximated by a polynomial, and implemented as a subroutine:

```
function Lskin = getLskin(mua, musp)
% function Lskin = getLskin(mua, musp)
% mua, musp can be vectors or matrices.
% MATLAB subroutine by Steven Jacques.
   April 18, 2007.
% based on Monte Carlo simulations
% with n_tissue:n_air interface
  of 1.4:1.
fitAvsNp = [ % log(A) vs log(Np)]
-8.208055e-08
2.201098e-06
1.191870e-06
-3.201765e-04
1.148715e-03
1.838374e-02
-8.925491e-02
2.098984e+00];
Np = musp./mua;
A = exp( polyval(fitAvsNp,log(Np)) );
delta = 1./sqrt(3*mua.*(mua + musp));
Lskin = A.*delta;
```

The pathlength L_{epi} is approximated by the function:

```
function Lepi = getLepi(Lskin, Mmuamel)
% Lskin = getLskin(mua, musp)
% Mmuamel = M*muamel
Depi = 0.0060; % assumed epidermal
       thickness
Lepi = 2*Depi*(4.51 - log10(Mmuamel));
```

This L_{epi} function is also based on the Monte Carlo simulations and is only approximate. Equation (6) is only a preliminary result, and work continues on refining this description. Epidermal melanin actually slightly decreases L_{skin} and thereby slightly decreases the effect of blood and water on the observed reflectance. Blood in the dermis decreases L_{epi} and hence reduces the effect of melanin on reflectance. The above algorithms *getLskin*( ) and *getLepi*( ) summarize the results to a first approximation, and are used with Eq. (6) to provide an approximate light transport model for rapid analysis.

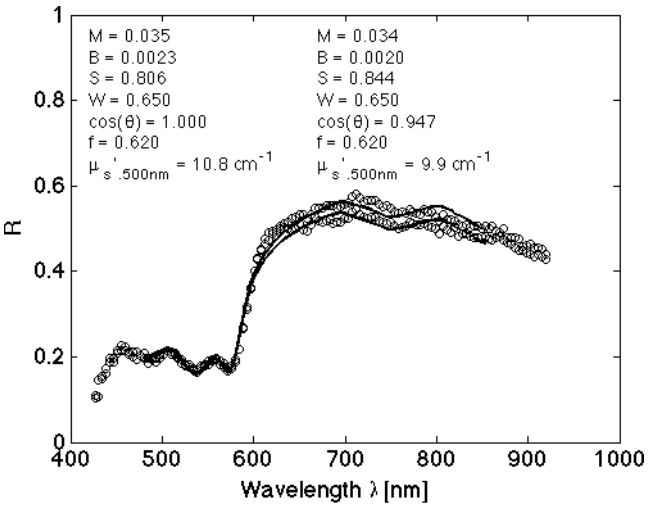


Fig. 3. Analysis of the reflectance spectra from left and right cheek of face in Fig. 2. Circles are original data. Solid line is fit between 480 nm and 850 nm. The values for fitting parameters are listed. M = melanosome volume fraction in 60-μm-thick epidermis. B = average skin blood volume fraction. S = mixed arterio-venous oxygen saturation of hemoglobin. W = water content (held constant). θ = angle of face relative to camera, f = Rayleigh fraction of scattering (and $1-f$ is Mie fraction of scattering), $\mu_{\mathrm{s}\,500\,\mathrm{nm}}'$ = reduced scattering coefficient at 500 nm.

6. The Effects of Optical Properties on Reflectance Spectra

Figure 3 shows the analysis of the spectra from the cheek sites on the face of Fig. 2, using Eq. (6) for $getR(\,)$. The optical parameters used in the fitting were M, B, S, $\cos(\theta)$ and $\mu'_{s\,500\,\mathrm{nm}}$. The other factors were set at constant values, $W = 0.65$, $f = 0.62$, with $K = 1$. The original spectra are shown as circles and the fits between 480–850 nm are shown as solid lines.

Figure 4 shows the variation in reflectance spectra when one parameter is varied, while holding the other parameters constant. In all figures, the solid line is the spectrum from the left cheek. The thin lines are the spectra when one parameter is varied. Different portions of the spectrum are affected by the various parameters.

The effects of varying blood B and scattering $\mu'_{s\,500\,\mathrm{nm}}$ on the reflectance spectrum are similar. The factor B dominates the absorption coefficient μ_a, and $\mu'_{s\,500\,\mathrm{nm}}$ scales the entire scattering spectrum μ'_s. Since the reflectance is specified by the ratio μ'_s/μ_a, varying either of these two fitting parameters has similar effects. However, varying the water content W only affects the longer wavelengths. Hence, one can use the water content as an internal calibration to specify the scattering, based on the longer wavelength behavior of the spectrum. One assumes a particular water

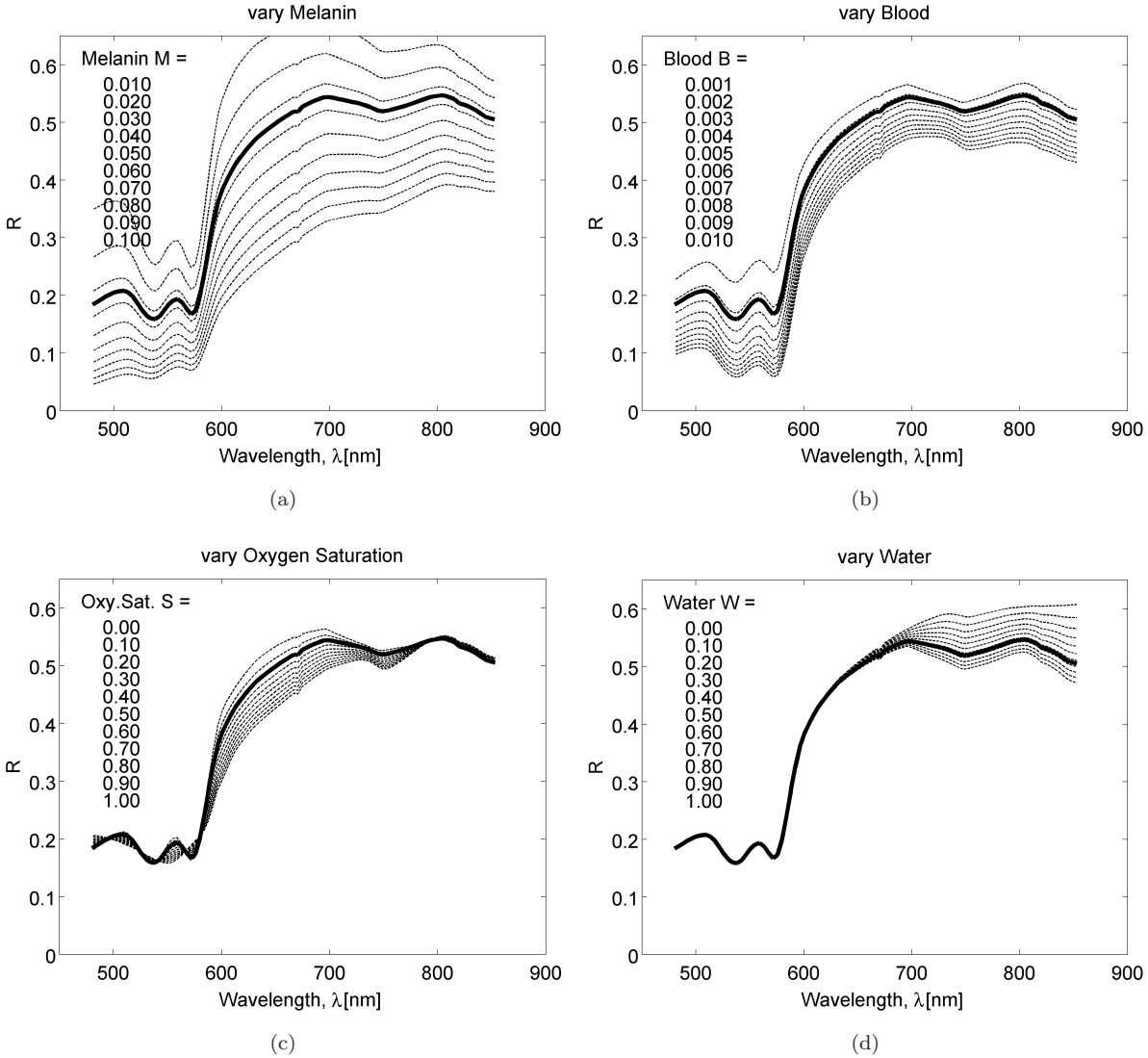
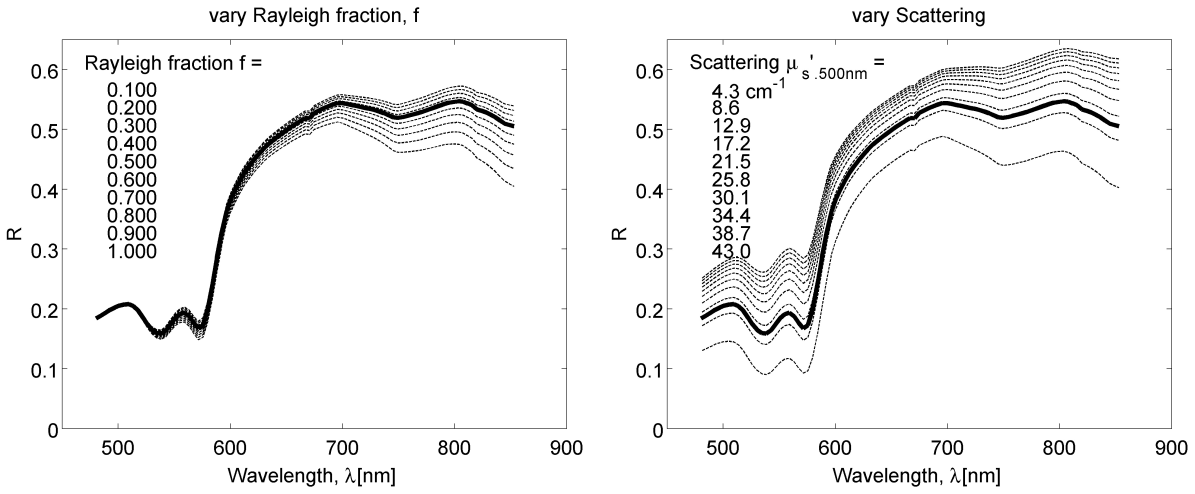


Fig. 4. The variation in reflectance spectra as one parameter is varied (thin lines), while holding other parameters constant. Solid line is the spectrum for the left cheek. (a) Vary melanosome volume fraction, M. (b) Vary blood volume fraction B. (c) Vary oxygen saturation S. (d) Vary water content W. (e) Vary fraction of scattering due to Rayleigh scattering, f. (f) Vary the reduced scattering coefficient at 500 nm, $\mu'_{s\,500\,\mathrm{nm}}$.



(e) (f)

Fig. 4. (*Continued*)

content, which is often known to within 10%, and fits for $\mu'_{s\,500\,nm}$. Note also the similar effects of varying W and f, which suggest that fitting for f complicates the ability to assume W and fit for $\mu'_{s\,500\,nm}$. So it may often prove practical to assume values for both W and f, followed by fitting for the other parameters. A problem arises if the W or f are not known. Nevertheless, for a particular tissue type where one has learned the typical values of W and f using more robust experimental methods, spectral imaging can reliably follow variations in M, B, S, $\cos(\theta)$ and $\mu'_{s\,500\,nm}$.

7. Conclusions

A 1D reflectance spectrum can be analyzed by least squares fitting using the fitting parameters M, B, S, W, $\cos(\theta)$, f, and $\mu'_{s\,500\,nm}$. When analyzing the optics of skin on the face, the factor $\cos(\theta)$ is helpful in accounting for variation in the orientation of the skin surface. The factors W and f may be best assumed, for example $W \approx 0.65$ and $f \approx 0.62$ for skin, allowing the fitting to more reliably fit the other parameters. There is great interest in quantifying the melanin, blood content, and scattering of the face. Spectral imaging allows the efficient acquisition of the spatial distribution of tissue parameters and optical properties.

Acknowledgments

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