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Sensitive real-time measurement of the refractive index and attenuation coefficient of milk and milk-cream mixtures

W. R. Calhoun,* H. Maeta,† S. Roy,‡ L. M. Bali,† and S. Bali†[1]
*Department of Physiology, Virginia Commonwealth University, Richmond 23298
†Department of Physics, Miami University, Oxford, OH 45056
‡Department of Mathematics, Computer Science, and Physics, Davis and Elkins College, Elkins, WV 26241

ABSTRACT

We demonstrate a first simultaneous measurement of both the refractive index and the attenuation coefficient (defined as the sum of the scattering and absorption coefficients) of highly turbid milk and milk-cream mixtures. We achieve this by observing the real-time reflectance profile of a divergent laser beam made incident on the surface of the milk sample. The experiments were carried out on commercial milk samples with fat volume concentrations of 0.5 or less, 1.6, and 3.3%, and on milk-cream mixtures with fat volume concentrations of 10 and 33.3%, without any dilutions of these samples. We find that the reflectance data are well described, for the first time without any empirical fit-parameters, by Fresnel theory that correctly includes the effect of angle-dependent penetration into the turbid medium on the total internally reflected signal. Therefore, our method provides the most accurate determination to date of the refractive index and attenuation coefficient of milk and milk-cream mixtures. Our sensor is compact, portable, and inexpensive.
Key words: refractive index, scattering coefficient, attenuation coefficient, milk

INTRODUCTION

Real-time accurate optical measurement of the refractive index and attenuation coefficient (i.e., the sum of the scattering and absorption coefficients) offers an attractive, noninvasive method for the monitoring of milk quality and fat content. It is well known that the refractive index of milk is difficult to estimate because of the turbidity (i.e., multiple light scattering) caused by casein micelles, air bubbles, and fat globules (see, for example, Walstra et al., 1999). Attempts have been made to suppress multiple scattering by diluting the milk with a solution that disperses the casein micelles (Walstra et al., 1999) and then estimating the fat content by measurement of the light scattered by the colloidal fat particles in milk. However, these measurements are limited in accuracy by experimental error in the diluting process and theoretical error in modeling the effect of dilution on the optical properties of milk.

A basic question naturally arises: Is there an experimental method to sensitively measure the basic optical properties, such as refractive index and attenuation coefficient, of a highly turbid sample directly without the need to dilute the sample? And, if so, can the data be explained with a valid theoretical model to extract accurate values for the refractive index and attenuation coefficient? We believe, for reasons outlined below, that despite receiving considerable attention for many decades (see, for example, Meeten 1997; Reyes-Coronado et al., 2005; Niskanen et al., 2007, and references therein) the answer to both questions is, surprisingly, no. Specifically, for milk and milk-cream mixtures, the literature suggests that these questions have been of interest for some time (see, for example, Rangappa 1948; Räty and Peiponen, 1999; Jääskeläinen et al., 2001) but remain unanswered.

Traditionally, for transparent fluid samples, the most commonly used method for measuring the refractive index is to determine the critical incident angle at which total internal reflection (**TIR**) occurs for light rays striking the sample. Indeed, this is the principle on which the widely used Abbe refractometer is based: one detects the light transmitted through the sample and observes a dark and a bright region adjacent to each other in the transmitted spot. The dark region is caused by TIR of the incident rays so that the dividing line between the 2 regions yields the critical angle and hence the refractive index. On the other hand, several other critical angle-based refractometers reported recently measure the light reflected from the sample surface (see, for example, Meeten and North, 1995; Bali et al., 2005; McClimans et al., 2006). A distinct "knee" appears in the reflectance versus incident angle curve at the critical angle.

However, no matter whether one measures transmission or reflection, these critical angle-based methods

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[1]Corresponding author: balis@muohio.edu

fail for highly turbid samples such as milk and milk-cream mixtures because the critical angle is not a well-defined concept for turbid media, as was pointed out recently (Räty and Peiponen, 1999; Niskanen et al., 2007). Experimentally, one observes in the case of the transmission-based Abbe refractometer that the dividing line between the TIR and non-TIR regions becomes indistinct and blurry. In the case of reflection-based critical angle refractometry, a smoothing out of the sharp knee in the reflectance versus incident angle curve is observed (Meeten and North, 1995; Räty and Peiponen, 1999; Jääskeläinen et al., 2001), which precludes a precise determination of the location of the critical angle. It is important to understand the reason behind this smoothening of the reflectance curve in the vicinity of the expected location of the critical angle to satisfactorily model the observed reflectance. An appropriate model has not been proposed to date. Merely empirically defining an effective critical angle that is equal to the angle where maximum change of slope of the reflectance versus incident angle curve occurs, determined by differentiating the reflectance curve (Mohammadi, 1995; this method is also employed in various commercial devices such as the Reichert AR-6 series; Reichert Analytical Instruments, Depew, NY), has been shown to yield significantly erroneous values for the refractive index (Meeten and North, 1995; Meeten 1997). See, for example, Figure 1b in this paper.

Recently, there have been 2 noteworthy attempts (Reyes-Coronado et al., 2005; Niskanen et al., 2007) at measuring the refractive index of turbid media. Reyes-Coronado et al. (2005) demonstrated a first simultaneous real-time measurement of both the refractive index and the attenuation coefficient of a turbid medium by measuring the transmittance of a light beam through a thin prism filled with a colloidal suspension of polystyrene spheres. However, Reyes-Coronado et al. (2005) point out a limitation of their transmission-based approach that is a major drawback from the point of view of milk characterization: their method works only for moderately turbid media with an attenuation coefficient of less than 40 cm^{-1} and is thus inadequate for working with milk and milk-cream mixtures for which the attenuation coefficient ranges from about 40 cm^{-1} for milk with less than 0.5% fat (trade name in the United States: fat-free or skim milk) to almost 1,200 cm^{-1} for a milk-cream mixture with 33% fat (trade name: heavy whipping cream). On the other hand, Niskanen et al. (2007), building upon earlier work by the same group (Räty and Peiponen, 1999; Jääskeläinen et al., 2001), have demonstrated a unique spectrophotometric device capable of measuring the refractive index as well as the attenuation coefficient (though not simultaneously in real-time, unlike the present work) of milk. But this work has the following major drawback (discussed below in more detail in Theoretical Considerations in Materials and Methods): the theory used to model the data is, in the authors' words, empirical. This, in itself, is not necessarily a weakness. However, little or no scientific justification is offered by the authors for their model other than that it has been "observed to be useful" in making their fits agree with the data whenever their measurement "departs strongly" from traditional Fresnel theory. As such, it would not be surprising if the values of the refractive index and attenuation coefficient extracted from their data-fits turn out to be significantly inaccurate. Further, we note that the sensitivity to differential changes in refractive index in Niskanen et al. (2007), Räty and Peiponen (1999), and Jääskeläinen et al. (2001) is limited to 1 part in 10^4 or less, at least an order of magnitude less than what we demonstrate in the present work. Finally, Räty and Peiponen (1999) and Jääskeläinen et al. (2001) present data only for milk, not for milk-cream mixtures.

In this paper, we present a first real-time simultaneous measurement of both the refractive index and the attenuation coefficient of milk and milk-cream mixtures with unprecedented sensitivity. We achieve this by observing the real-time reflectance profile of a divergent laser beam made incident on the surface of the milk sample. The experiments were carried out with commercial milk samples that had fat volume concentrations of 0.5 or less, 1.6, and 3.3% and on samples of highly turbid milk-cream mixtures with fat volume concentrations of 10 and 33.3%, without any dilutions of these samples. The sensitivity of our method to differential changes in refractive index is demonstrated to be at least 1 part in 10^5 (i.e., an order of magnitude better than previous measurements by past workers). It is important to note that we have developed a new model of total internal reflection from a turbid medium based on Fresnel theory that, for the first time, is shown to fit the reflectance data without any empirical fit parameters. Therefore, our method provides a much more accurate determination than ever before of the refractive index and attenuation coefficient of milk and milk-cream mixtures.

MATERIALS AND METHODS

Theoretical Considerations

The basic principle of TIR-based refractive index measurement is depicted in Figure 1a. A sample of refractive index n_{sample} is placed on top of a glass prism of known refractive index n_{prism} ($>n_{sample}$) and a light ray of intensity I_i is made incident on the prism-sample interface so that the angle of incidence at the interface

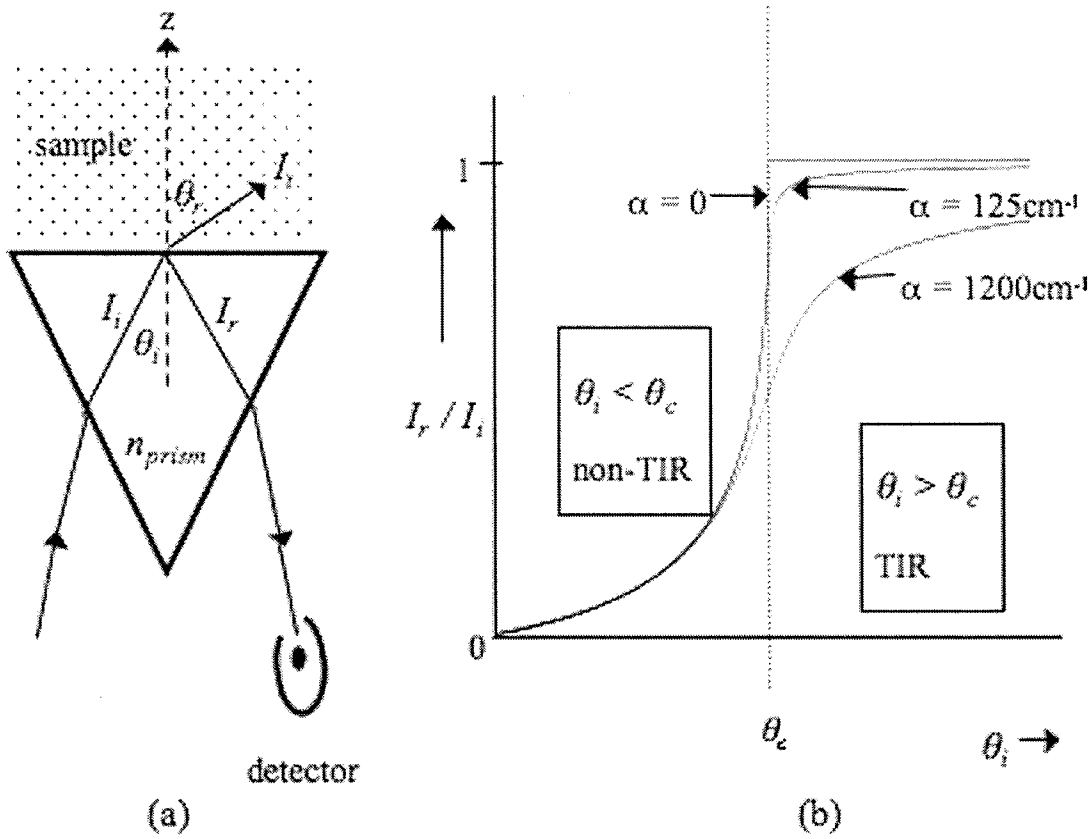

Figure 1. a) The principle of refractive index measurement based on total internal reflection (TIR). b) Plots of I_r/I_i (θ_i) from Equation 6 for 1 transparent medium ($\alpha = 0$) and 2 highly turbid media ($\alpha = 125$ cm^{-1} and 1,200 cm^{-1}) that have the same value of n_r as the transparent medium. Here, I_r and I_i are the reflected and incident intensities at the sample interface, n_r is the real part of the sample refractive index, and α is the attenuation coefficient of the sample.

is θ_i and the angle of refraction is θ_r. The sample refractive index is written in terms of a real part n_r and an imaginary part n_i as

$$n_{sample} = n_r + in_i, \quad [1]$$

where n_i is related to the attenuation coefficient α, the laser frequency ω, and the speed of light c, as (see, for example, Heald and Marion 1995)

$$\alpha = 2n_i\,\omega/c. \quad [2]$$

The attenuation coefficient α measures the loss of directed radiation per unit length through the sample owing to scattering, or absorption, or both; that is, the intensity $I(z)$ of a light beam propagating in the z-direction through the medium can be written as

$$I(z) = I_0 \exp(-\alpha z), \quad [3]$$

where I_0 is the intensity at $z = 0$. One may consider α to be the quantitative measure of turbidity.

If the sample is transparent ($\alpha = 0$), we find upon varying the incident angle θ_i that we encounter a critical angle θ_c at which the angle of refraction θ_r becomes 90°. One may simply apply Snell's law for refraction, namely, $\sin\theta_r = (n_{prism}/n_{sample})\sin\theta_i$, at $\theta_i = \theta_c$, to determine the sample refractive index:

$$n_{sample} = n_{prism}\sin\theta_c. \quad [4]$$

In Figure 1b the curve marked $\alpha = 0$ shows a theoretical reflectance profile I_r/I_i (θ_i) for a transparent medium, where I_r is the reflected ray intensity. When $\theta_i > \theta_c$, one simply obtains unity for the ratio I_r/I_i (i.e., TIR at the prism-sample interface is observed). On the other hand, when $\theta_i < \theta_c$, a transmitted beam I_t refracts into the sample, causing the reflection coefficient I_r/I_i to sharply decrease from unity as a function of θ_i in accordance with the usual Fresnel relation,

$$\frac{I_r}{I_i} = \frac{\tan^2\left(\theta_i - \theta_r\right)}{\tan^2\left(\theta_i + \theta_r\right)}, \quad [5]$$

where we assume the incident beam to be polarized parallel to the plane of incidence (see, for example, Heald and Marion, 1995). The vertical dashed line in Figure 1b marks the sharp transition between the TIR and non-TIR regions, thus locating the critical angle θ_c and hence the value of n_{sample}. Recently, we demonstrated real-time refractometry at the one part per million level in transparent samples (McClimans et al., 2006).

On the other hand, for turbid media such as milk, the transition between the TIR and non-TIR regions of the reflectance profile is significantly more gradual, owing to multiple scattering as explained below. This is depicted in Figure 1b by the 2 curves marked $\alpha = 125$ and $\alpha = 1{,}200$; these values of α were chosen because they are close to the attenuation coefficients of milk with 3.3% fat (trade name in the United States: whole-fat milk) and 33.3% fat (trade name: heavy whipping cream), respectively. The traditional approach in Fresnel theory to turbid media is to simply allow n_{sample} to be complex in Equation 5, yielding (Meeten and North, 1995; Meeten, 1997; Niskanen et al., 2007)

$$\frac{I_r}{I_i} = \frac{M + P^2\cos^2\theta_i - \sqrt{2}\cos\theta_i\left(M + \sin^2\theta_i\right)\sqrt{M+L}}{M + P^2\cos^2\theta_i + \sqrt{2}\cos\theta_i\left(M + \sin^2\theta_i\right)\sqrt{M+L}}, \quad [6]$$

where we have used for convenience the notation

$$P = \left(n_r^2 + n_i^2\right)/n_{prism}^2,$$

$$L = \left[\left(n_r^2 - n_i^2\right)/n_{prism}^2\right] - \sin^2\theta_i, \text{ and}$$

$$M = \sqrt{P^2 - 2L\sin^2\theta_i - \sin^4\theta_i}. \quad [7]$$

The reflectance plots in Figure 1b are drawn using Equations 6 and 7 for 2 turbid media of different (constant) values for n_i and α but the same value of the real refractive index n_r as the transparent medium. These plots emphasize that to simultaneously determine the real and imaginary components of the refractive index of a turbid medium, it is important to make detailed reflectance measurements over a range of angles around the TIR/non-TIR transition, not just at the transition itself. For angles just below the TIR/non-TIR transition, the overlap in the non-TIR region for the 3 media with identical n_r suggests the reflectance there is dominated by n_r. On the other hand, for angles just above the TIR/non-TIR transition it is α (and therefore n_i) that dominates. This is because in TIR the ray actually penetrates into the sample, so the reflected ray does not originate from the point at which the incident ray strikes the sample face, emerging instead from a laterally shifted point (the Goos-Hanchen shift; Snyder and Love, 1976). During this penetration the loss incurred because of scattering in the turbid medium causes an attenuation of the total internal reflected intensity. We see below that Equation 6, as used above, succeeds qualitatively but fails quantitatively in predicting this attenuation.

A cursory glance at the turbid reflectance profiles in Figure 1b reveals the flaw in early empirical attempts (Mohammadi 1995; still used by commercial devices such as the Reichert AR-6 series) to associate the maximum change of slope of the I_r/I_i curve with an effective critical angle, thereby claiming to measure the real part n_r of the refractive index via Equation 4. All 3 media depicted have been assigned the same value for n_r in Equation 6, yet the point of maximum slope change of the reflectance profile moves progressively to the right as the turbidity increases.

However, the above usage of the Fresnel Equations 6 and 7 for turbid media is still not completely correct. An important, and ultimately incorrect, assumption implicit in drawing the turbid reflectance plots in Figure 1b is that the value of n_i, defined and measured via Equations 1, 2, and 3 at normal incidence, is taken to be a constant. This is an important point that has eluded the attention of recent workers (Jääskeläinen et al., 2001; Niskanen et al., 2007). Note that the penetration depth, defined as the depth at which the intensity of the evanescent wave decreases to $1/e$ of its initial value, varies with θ_i, going from a few nanometers at incident angles far above the TIR/non-TIR transition to a few hundred nanometers at incident angles in the vicinity of the transition. The attenuation coefficient α is just the reciprocal of this penetration depth, thus ascribing an angular dependence to α and therefore also to n_i (Calhoun, et al., 2010). From the Fresnel theory for reflection and refraction into a scattering-absorbing medium (see, for instance, Heald and Marion, 1995), we find that this new angular-dependent $n_i(\theta)$ can be written in terms of the original n_i (which was defined at normal incidence) as

$$n_i\left(\theta\right) = n_i\left[4\pi\sqrt{\left(M - L\right)/2}\right]^{-1}. \quad [8]$$

It is this $n_i(\theta)$ that must be substituted for n_i into Equation 6, not a constant. Figure 2 illustrates the

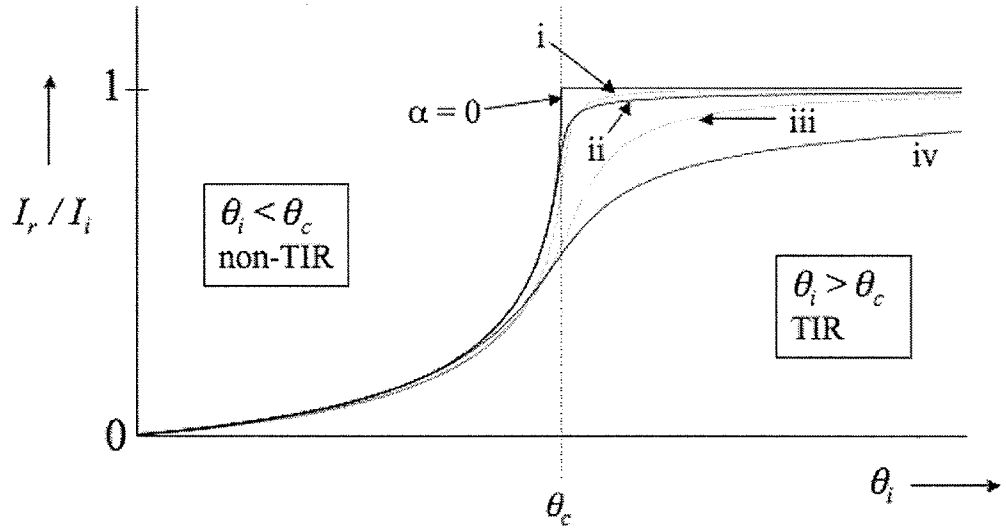

Figure 2. The gray lines i and iii are plots of Equations 6 and 7 that incorporate angle-dependent penetration of the incident beam into the turbid medium by using the new relation Equation 8. The solid lines are plots of Equations 6 and 7 with the same n_r values as the gray lines but without incorporating Equation 8 in the theory. Here, I_r and I_i are the reflected and incident intensities at the sample interface, n_r is the real part of the sample refractive index, and α is the attenuation coefficient of the sample; TIR = total internal reflection.

difference between the theoretical predictions for the reflectance I_r/I_i in the case of 2 turbid media. The gray lines i and iii are plots of Equation 6 for the same 2 turbid media depicted in Figure 1b, but this time corrected using Equation 8. For $\alpha = 0$ this correction is zero. By using this corrected Fresnel relation for TIR from a turbid medium, we find we can fit the reflectance data without the need to introduce any extraneous empirical fitting parameters. This makes sense because the correction embodied by Equation 8 is deduced and applied in a manner that is consistent with Fresnel theory. On the other hand, the solid lines ii and iv in Figure 2 reproduce the turbid curves from Figure 1b—they plot Equation 6 without the correction and do not agree with the reflectance data observed in the present work or with data observed by previous workers (Meeten and North 1995; Jääskeläinen et al., 2001; Niskanen et al., 2007). To explain the departure of the uncorrected Equation 6 from their data, Niskanen et al. (2007) speculated their reflectance data was contaminated by light diffusely scattered from the turbid medium into their detector. Building upon an idea originally proposed by Meeten and North (1995), Niskanen et al. (2007) proposed the following empirical formula for the modified reflectance R^+:

$$R^+ = R + \phi\, R^m\, (1 - R), \qquad [9]$$

where R is the reflectance I_r/I_i in Equation 6, whereas ϕ and m represent free parameters, arbitrarily introduced to describe the diffuse scattering. Both ϕ and m are allowed to freely vary so as to bring R^+ into agreement with the data. No scientific justification is given for the introduction of these 2 parameters, nor of the values they are allowed to attain ($m = 1.5$ in Niskanen et al., 2007) during the fitting process. We believe that the physics described by Equation 9 are incorrect. The values of these parameters suggest that the diffuse scattering entering the detector may be as much as several percent of the light transmitting into the turbid medium, which is unrealistically large.

Experiment

The experimental setup for real-time measurement of the reflectance profile is basically the same as already described in McClimans et al. (2006), except that the diode laser and pixel array used are a different model as described below. As indicated in Figure 1a, the sample is placed on top of a prism (equilateral, side 2.5 cm, F2 glass; $n_{prism} = 1.614805$ at 660 nm is calculated using a dispersion formula supplied by Schott-Optical Glass Technologies, Elmsford, NY). The light source is a diode laser (660 nm) pigtailed to a single-mode fiber (numerical aperture: 0.12). The fiber outputs a divergent beam (angle of divergence equal to twice $\sin^{-1}0.12$, or 13.78°) with a Gaussian profile, which is made incident on the prism such that the center ray of the beam strikes the first prism-face at right angles. The beam is reflected from the prism-sample interface onto a 1-dimensional pixel array (1,024 pixels, pixel diameter: 14 μm; Perkin-Elmer LD3522PGK-022, Perkin-Elmer, Waltham, MA).

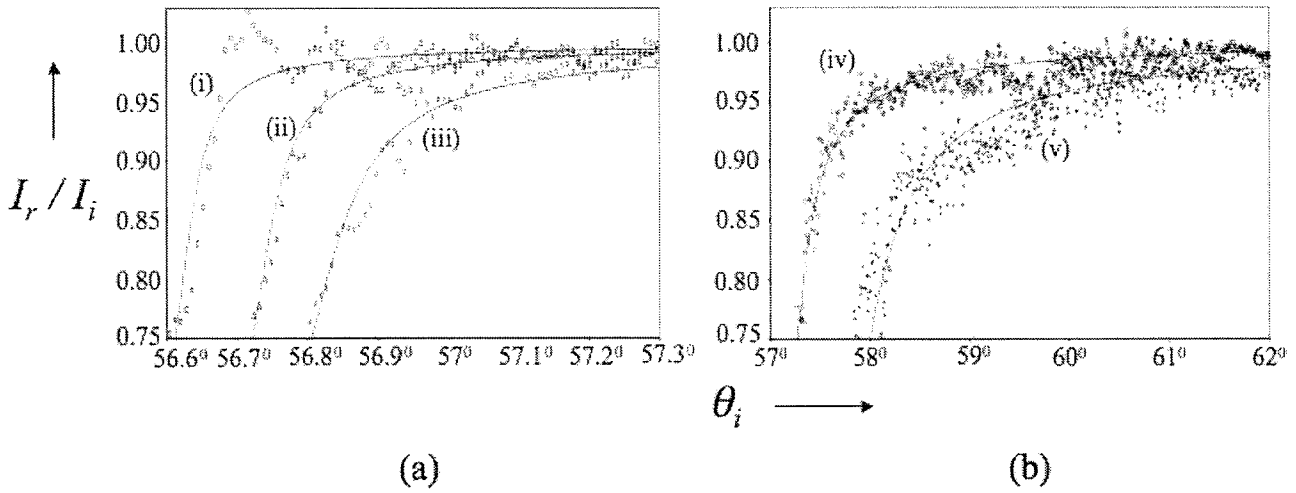

Figure 3. Measurements of the ratio I_r/I_i at 660 nm as a function of θ_i for a) milk with fat volume concentration of i) less than 0.5%, ii) 1.6%, and iii) 3.3%, and b) milk-cream mixtures with fat volume concentrations of iv) 10% and v) 33.3%. In both figures, the solid lines are theoretical fits from Equations 6, 7, and 8 in which the 2 unknowns n_r and n_i (and hence α from Equation 2) are fit parameters determined by a least chi-squared fit to the data. The fitted values are presented as a table in Figure 4. Here, I_r and I_i are the reflected and incident intensities at the sample interface, n_r and n_i are the real and imaginary parts of the sample refractive index, and α is the attenuation coefficient of the sample.

Where TIR occurs the pixels are brightly illuminated, otherwise not, leading to an edge between dark and light regions of the reflected beam profile. For a transparent sample this dark edge is sharply defined and is confined within a single pixel, in which case simply monitoring the intensity readout of this pixel yields a sensitive measurement of real-time changes in the refractive index of the sample (McClimans et al., 2006). But, in the case of turbid samples, the dark edge is manifested as a gradual reduction of intensity spread over many pixels. The profile I_r/I_i (θ_i) is obtained in 3 steps using a Labview program (McClimans et al., 2006). We first associate a unique angle of incidence θ_i with the center of each pixel by calibrating our refractive index measurements to those of a state of the art commercial refractometer (Reichert Analytical Instruments, AR6 Series). To perform the calibration we prepared 2 separate (transparent) water-glycerin mixtures, measured their refractive indices on the commercial refractometer, and defined these values as the refractive index readings for our own refractometer. Next, we obtain I_i (θ_i) by measuring the reflectance curve for air (no sample present), for which TIR occurs over the entire range of incident angles subtended by the laser, thus reproducing the Gaussian laser beam on the pixel array. Finally, we place the sample on the prism, measure I_r (θ_i), and compute the ratio I_r/I_i (θ_i).

RESULTS AND DISCUSSION

In Figures 3a and b, we show measured reflectance profiles at 660 nm for milk and milk-cream mixtures, with values for the attenuation coefficient α ranging from about 40 cm^{-1} for milk with less than 0.5% fat to about 1,200 cm^{-1} for cream with 33.3% fat. The values of n_r and n_i (and hence α from Equation 2) are determined by curve-fitting these reflectance profiles using Equations 6 and 8, with n_r and n_i as the best-fit parameters. The large difference in scale of the horizontal axes in Figures 3a and b is attributable to the fact that the refractive index and attenuation coefficient values for the 2 milk-cream mixtures in Figure 3b are vastly different as compared with the 3 types of milk in Figure 3a for which the refractive indices and attenuation coefficients are close-lying. The refractive indices and attenuation coefficients for the 3 types of milk in Figure 3a and the 2 milk-cream mixtures in Figure 3b are tabulated in Figure 4.

We compared our measurements of n_r for 3 transparent ($n_i = 0$) water-glycerin mixtures with n_r values 1.36380, 1.37767, and 1.40093 (separate from the transparent water-glycerin mixtures referred to in the last paragraph of the previous section that we used for calibration, which had n_r values 1.34792 and 1.43374) with those obtained from the commercial refractometer (Reichert AR-6 series) and found perfect agreement to 1 part in 10^5, which is the limit of sensitivity for the commercial device. This demonstrates the sensitivity of our device for n_r measurement to be at least 1 part in 10^5. Because the value for n_i is obtained, together with n_r, from a least-squares fit to the data, we expect our device to have the same sensitivity, 1 in 10^5, for n_i measurement. Of course, the Reichert device fails to yield reasonable n_r values for milk and milk-cream mixtures

Fat Volume	Commercial Devices			Jääskeläinen, et al (2001)					This Work		
	n_r	n_i	α (cm^{-1})	n_r (A)	n_r (B)	n_r (C)	n_i (A)	α (cm^{-1})	n_r	n_i	α (cm^{-1})
< 0.5%	1.34808	0.00002	4	1.3463	1.3444	1.3469	0.0002	38	1.34846	0.00021	41
1.6%	1.35160	0.00009	17	1.3467	1.3444	1.3483	0.0004	76	1.35012	0.00031	59
3.3%	1.35379	0.00015	29	1.3475	1.3462	1.3525	0.0006	114	1.35123	0.00066	126
10%	1.36665								1.35692	0.00277	528
33.3%	1.38810								1.36541	0.00618	1177

Figure 4. The table shows our measurements of n_r and compares them with previous measurements. Blank entries indicate that meaningful measurements are not available. In all cases, the attenuation coefficient α is determined from a measurement of n_i using Equation 2. See Results and Discussion for an explanation of the superscripts A, B, and C and of the data presented. Here, n_r and n_i are the real and imaginary parts of the sample refractive index.

and, indeed, any turbid media because it finds n_r by defining an effective critical angle based on the second derivative (Mohammadi, 1995) of the reflectance profile as explained above (see Theoretical Considerations in Materials and Methods). The Reichert device does not measure n_i.

Figure 4 tabulates our results and compares the values we obtained for the refractive index and attenuation coefficient of various types of milk with the values, where available, obtained by Räty and Peiponen (1999) and Jääskeläinen et al. (2001) and by the use of commercial devices. We also show measurements of n_r by Jääskeläinen et al. (2001). Entries left blank in the "commercial devices" column indicate that meaningful values could not be obtained. Similarly, blank entries in the "Jääskeläinen et al., 2001" columns indicate that these values were not measured in Jääskeläinen et al. (2001) and that we have not been able to find reliable measurements for these values in the literature. Jääskeläinen et al. (2001) used 3 different methods for measuring n_r, each of which we discuss below.

Method A is similar in principle to our approach in that Jääskeläinen et al. (2001) measure the reflectance profile I_r/I_i as a function of θ_i. Jääskeläinen et al. (2001) used method A to find the value of n_r as well as n_i, as shown in the table in Figure 4. They fit the reflectance profile by minimizing chi-squared to find n_r and n_i just as we did, and call this the optimization method. However, the similarity ends there, for n_r and n_i are not the only fitting parameters used by Jääskeläinen et al. (2001); they also use 2 other free parameters (ϕ and m in Equation 9), which are assigned arbitrary values to make the theory (they used Equations 6, 7, and 9) agree with the turbid data. These parameters are based on an earlier treatment by Meeten and North (1995) and Meeten (1997) and are introduced as part of an empirical attempt to model the diffuse scattering that they claim enters the detector. However, the amount of scattering entering the detector would have to be unrealistically large for their model to agree with the data, as stated earlier. By contrast, our modification, embodied in Equation 8, of Fresnel's relation Equation 6 correctly describes the basic physics of TIR from a turbid medium and uses no extraneous free parameters save for n_r and n_i. Furthermore, unlike the sensors described in Niskanen et al. (2007), Räty and Peiponen (1999), and Jääskeläinen et al. (2001), our sensor has no moving parts, thus eliminating all mechanical noise (McClimans et al., 2006). For these reasons our data are more reliable and the extracted values of n_r and n_i are on a sound theoretical footing. On comparing the results obtained by Jääskeläinen et al. (2001) using method A for the 3 types of milk (shown in columns $n_r^{(A)}$ and $n_i^{(A)}$ of Figure 4) with our results obtained in this work, we notice that our n_r values are consistently higher whereas the n_i values are approximately similar.

In method B, Jääskeläinen et al. (2001) attempted to define an effective critical angle by differentiating the reflectance curve (Mohammadi, 1995) and then deduceing n_r using Equation 4. This same method is also employed by the commercial device (Reichert AR6) indicated in the n_r column under "commercial devices" in Figure 4. As pointed out previously (see Theoretical Considerations in Materials and Methods) and by others (Meeten and North, 1995; Meeten, 1997), this method

is manifestly flawed and therefore these values should be ignored. This method does not address the question of what values n_i or α may have. We attempted to measure these for the milk and milk-cream samples by using a state of the art spectrophotometer (Nanodrop, Thermo Scientific, Waltham, MA), which measures the light transmitted through a thin sample and requires sample sizes of only 1 μL. The device then uses Equation 3 to compute n_i and α. We failed to obtain any value for the milk-cream mixtures because practically no light transmits through despite the sample thickness being only 0.1 mm. For the milk samples, we did obtain n_i and α values, which are shown in Figure 4 under the "commercial devices" column. However, we see in Figure 4 that these values are far less than those obtained using our method and method A by Jääskeläinen et al. (2001), with the discrepancy increasing as the turbidity increases.

In method C, Jääskeläinen et al. (2001) employed surface plasmon resonance in a silver film that is deposited on the prism surface in between the prism and the sample. Interestingly, we note that the discrepancy between the n_r values measured by Jääskeläinen et al. (2001) using methods A and C grows with increasing turbidity from 0.0006 (i.e., 0.05%) for the lowest-fat milk (0.004% fat) to 0.0016 (i.e., 0.1%) for milk with 1.53% fat to 0.005 (i.e., 0.4%) for milk with 3.55% fat. By contrast, the discrepancy between our n_r values and the values obtained by Jääskeläinen et al. (2001) using method C stays at a constant 0.1% throughout for the 3 types of milk. This constant discrepancy could be attributable to the slight differences in fat volume between the Finnish and American milk samples, but more probably arises from the different calibration methods employed. Jääskeläinen et al. (2001) calibrated their surface plasmon resonance sensor to deionized water; however, their calibration drifts owing to oxidation and continual wear of the silver film. They mention that constant recalibration using a fresh silver film for each sample is time consuming. In our case, as mentioned earlier, we use commercial calibration fluids to calibrate our sensor to a state of the art commercial refractometer that works very well for transparent fluids. Unlike the silver film used by Jääskeläinen et al. (2001), none of the elements in our sensor are subject to wear and tear, and the calibration can be quickly checked at any time by simply replacing the milk sample with the calibration fluid. Furthermore, once calibrated, our sensor does not require frequent recalibration.

CONCLUSIONS

We made accurate and sensitive measurements of both the refractive index and the attenuation coefficient of different grades of commercial milk with varying fat concentrations and of milk-cream mixtures. We compared our measurements in milk with previous work and analyzed the differences. For milk-cream mixtures, such measurements have never before been reported. Our sensor is compact, robust, and capable of real-time, online, simultaneous monitoring of both n_r and α without any mechanical adjustments. Our measurements are accurate because the Fresnel theory, when corrected to properly account for the angle-dependent penetration of the light into the turbid medium while undergoing total internal reflection, describes our reflectance data well without the need to introduce any empirical fitting parameters.

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