ABSTRACT

REAL-TIME DIFFERENTIAL REFRACTOMETRY WITHOUT INTERFEROMETRY AT A SENSITIVITY LEVEL OF $10^{-6}$

by Michael Steven McClimans

We present a refractometer based on the principle of total internal reflection (TIR) that can sensitively record in real-time the refractive index of fluids over a wide range of refractive indices. The device uses a divergent laser beam and linear diode array, and has no mechanical or optical moving parts, enabling us to achieve the measurement of refractive index at a sensitivity level of $10^{-6}$. Our refractometer does not rely on interferometry, thus enabling the device to be compact, portable, and inexpensive. To the best of our knowledge, this is the first time a non-interferometric device that performs real-time differential refractometry with a sensitivity better than $10^{-5}$ has been demonstrated in the literature. We show that our experimental results agree very well with Fresnel theory. We establish a theoretical limit on the sensitivity of this class of refractometers.
REAL-TIME DIFFERENTIAL REFRACTOMETRY WITHOUT INTERFEROMETRY AT A
SENSITIVITY LEVEL OF $10^{-6}$

A Thesis

Submitted to the
Faculty of Miami University
in partial fulfillment of
the requirements for the degree of
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Department of Physics
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Miami University
Oxford, Ohio
2006

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I would like to dedicate this work to my wife Betsie and son Micah. There is no question that without Betsie’s love and support, my accomplishments would not have been possible. Micah, I did this all for you. I wanted you to be able to look up to a father with an education. I know that a college education is in your future. I wish you to have even more success than I.

I would also like to send a special thanks to Samir. It was your excitement for research that drew me to your lab, and your wisdom that guided me through. You helped shape my outlook on research and strengthened my love for science.
List of publications

“Real-time differential refractometry at $10^{-6}$ level without interferometry”
*Applied Optics* - Accepted for publication February 2006
*Applied Optics* is rated number three for number of citations.

“A simple method to stably float a coupled system of optics tables”
*Optics and laser Technology* - accepted October 28, 2005

“Large atom-density change at constant temperature by varying trap anisotropy in a dilute magneto-optical trap”
*Optics Communications* 248 (2005) 173-178
*Optics Communications* is rated number four for number of citations.
1. Background and Motivation

The purpose of this thesis is to summarize my accomplishments while working with Dr. Samir Bali. The forthcoming is the paper, as it will appear in Applied Optics. It should also be noted that Applied Optics is the number three rated refereed optics journal. This rating is based on how often articles with in the journal are cited in other published peer reviewed journals. I would also like it to be noted that this is my third publication while studying at Miami University. I was the second author on a paper accepted to Optics Communications for work done with our magneto optical trap (MOT). I was first author on a paper accepted to Optics and Laser Technology describing our method for interlocking two floating optics tables, stably. Now, on with focus of my work, the Refractometer.

Differential refractometry refers to the measurement of small changes in the refractive index of liquids and gases. Such measurement has found increasing use in recent years, with diverse applications ranging from imaging in biological cells and tissues, and detection of cancerous pre-cursors, to thin film characterization for the semiconductor industry, and chromatography, and even regulation of the fermentation process in the wine industry. For many environmental remediation applications such as the monitoring of pollutants in ground water, or toxins in air, the real-time measurement of changes in refractive index with a sensitivity of $10^{-5}$ or better is desirable, even necessary. Differential refractometry with a sensitivity of $10^{-5}$ and better has traditionally been achieved using refractometers based on interferometry. However, such refractometers are generally not as portable and compact as non-interferometric refractometers, and not as amenable to fully automated operation. On the other hand, non-interferometric refractometers are typically limited to a $10^{-4}$ sensitivity level, with a sensitivity of $10^{-5}$ being only barely achieved by some expensive, state-of-the-art, commercial refractometers. Furthermore, we note that the most commonly used type of refractometer, namely the Abbe refractometer, is designed for steady-state measurements of isotropic samples, and is incapable of recording real-time changes in the refractive index.

In this paper, we demonstrate a simple optical device that records real-time changes in the refractive index of fluids with sensitivity better than 3x10-6. The device design relies upon the principle of total internal reflection, and is not based on interferometry. Our refractometer consists of no moving mechanical parts, not even a moving optical beam, and is rugged,
inexpensive, user-friendly, and compact. Specifically, the design is based on the detection of the angular distribution of intensity of a divergent laser beam that is reflected from the sample onto a linear diode array. Thus, the center of each pixel corresponds to a unique angle of incidence on the sample surface. This design is currently used in some new commercially available refractometer models\textsuperscript{14}. However, the resolution of these commercial refractometers is limited to 2x10\textsuperscript{-5} or less. Moreover, the theoretical limit on the sensitivity of this type of refractometer has never been previously explored in the literature. Here, we show that the fundamental limit on the sensitivity of this refractometer is not determined by the pitch of the diode array as one may initially suspect, but by the smallest intensity change that can be measured by an individual pixel in the array. We derive a theoretical expression for this fundamental limit in terms of the pixel size and dark current, and various other design parameters. We find that, in principle, the sensitivity of this class of refractometers can be better than 10\textsuperscript{-8}, comparable with the best interferometric refractometers\textsuperscript{9,10}. In practice, though, the sensitivity is expected to be limited by the intensity fluctuations in the laser to about 10\textsuperscript{-7}. Furthermore, we identify other sources of optical noise that may significantly degrade the performance of the refractometer, and describe methods to suppress their contributions. In this way, by a detailed experimental and theoretical analysis of the different sources of mechanical, electrical, and optical noise, we have been able to demonstrate non-interferometric refractometry at a sensitivity level better than 3x10\textsuperscript{-6}, i.e., one to two orders of magnitude more sensitive than the commercial models mentioned earlier in this paragraph\textsuperscript{14}. The demonstrated sensitivity is an order of magnitude less than the expected sensitivity of 10\textsuperscript{-7}, due to our inability to produce in a controlled manner samples with closely-lying values of refractive index. To the best of our knowledge, this is the first time a non-interferometric device that performs real-time refractometry with sensitivity better than 10\textsuperscript{-5} has been demonstrated in the literature.

The paper is organized as follows. In Section 2 below, we describe the principle of application of total internal reflection (TIR) to refractometry. In Section 3, we describe the design and construction of our device and the advantages over previous TIR -based refractometers. In Section 3, we also derive a theoretical expression for the fundamental limit on the device sensitivity owing to pixel dark current and laser intensity fluctuations. Further, we indicate how we suppress noise from other sources, for example, from stray laser light scattered into the pixels. Section 4 describes the calibration of the device. Next, in Section 5 we
demonstrate the sensitivity of our refractometer to changes in the sample's refractive index at few parts in $10^{-6}$. Finally, we state our conclusions and outlook in Section 6.

2. Device Principle. Application of Total Internal Reflection (TIR) to refractometry

Consider a glass prism of refractive index $n_{\text{prism}}$, as shown in Fig. 1(a), with a sample of unknown refractive index $n_{\text{sample}}$ placed on the top, where $n_{\text{sample}} < n_{\text{prism}}$. Suppose a laser beam of intensity $I_i$ is incident on the prism-sample interface with an angle of incidence $\theta_i$. TIR occurs if $\theta_i$ exceeds the critical angle $\theta_c$ given by

$$\theta_c = \sin^{-1} \left( \frac{n_{\text{sample}}}{n_{\text{prism}}} \right)$$

for the prism-sample interface. In this case, one simply obtains unity for the ratio of the reflected intensity $I_r$ to the incident intensity $I_i$. On the other hand, if $\theta_i < \theta_c$, a transmitted beam $I_t$ refracts into the sample, causing the reflection coefficient $I_r/I_i$ to sharply decrease from unity in accordance with the well-known Fresnel equations of reflection and refraction\textsuperscript{15}. For an incident beam that is polarized parallel to the plane of incidence, as is the case for our device, we have by Fresnel theory that\textsuperscript{15}

$$\frac{I_r}{I_i} = \frac{\tan^2 (\theta_i - \theta_r)}{\tan^2 (\theta_i + \theta_r)}$$

where by Snell's law we have $\sin \theta_r = (n_{\text{prism}}/n_{\text{sample}})\sin \theta_i$. A representative plot of the ratio $I_r/I_i$ as a function of $\theta_i$ is shown in Fig. 1(b). The point of sudden departure from unity marks the sharp boundary between TIR and refraction, and occurs when $\theta_i = \theta_c$. Thus, a careful determination of the location of this boundary enables, in principle, a sensitive measurement of $\theta_c$ for the sample and hence from Eqn 1 the value of $n_{\text{sample}}$. In practice, however, as described in Section 3, the exact location of the critical angle for the $I_r/I_i$ curve is obscured by technical noise (mechanical and/or electrical and/or optical in origin). This prevents discrimination between close-lying values of $n_{\text{sample}}$. In this context, we draw attention to the encircled region shown in Fig. 1(b), where $\theta_i$ is slightly less than $\theta_c$. In this region, we see that for a small change
in $\theta$ there is a large change in $I/I$, much larger than any changes that may be caused by noise. In other words, in order to achieve a relatively noise-free discrimination between close-lying values of $n_{\text{sample}}$, one may examine the region $\theta_i < \theta_c$ in the immediate neighborhood of $\theta_c$ rather than just detect the one point $\theta_i = \theta_c$. In Sec. 4, we shall use this method to sidestep technical noise ripples at the critical angle.

3. Device Design and Construction. Advantages over existing TIR-based refractometers

A. Suppression of mechanical noise

The sensitivity of most critical angle based refractometers\textsuperscript{4,5,13} is ultimately limited by the signal-to-noise ratio with which one detects the exact point where the transition from TIR to non-TIR occurs. The most common of these devices is the Abbe refractometer\textsuperscript{13} in which the sample is placed between adjacent prisms and the intensity of the refracted beam passing through the sample from the first prism to the second is measured. Alternatively, the intensity of the reflected ray from the interface of a prism\textsuperscript{4} or lens\textsuperscript{5} with the sample is monitored as $\theta_i$ is varied by rotating a turntable on which the prism or lens is placed. This need for mechanical adjustment of the incident angle in order to satisfy the requirement $\theta_i = \theta_c$ introduces mechanical noise (which limits device sensitivity), and time-delays (which preclude the possibility of real-time refractometry), and often necessitates the presence of an operator (which obviates possibilities for remote control).

By contrast, in our device all mechanical sources of noise have been practically eliminated. Specifically, we incorporate in our design the following two components. 1) a divergent laser beam instead of a collimated beam, thereby automatically enabling access to many different incident angles simultaneously, and 2) a linear diode array to provide position-resolved detection of the angular distribution of light reflected from the sample.

A schematic of our refractometer is shown in Fig. 2. An infrared laser diode (785 nm) is pigtailed to a single-mode optical fiber of mode field diameter 5.8 $\mu$m. The numerical aperture of the fiber is 0.12, meaning the divergent Gaussian TEM\textsubscript{00} beam emerging from the fiber has a half-angle of $\phi = 6.89^\circ$. This beam is incident on an equilateral F2-glass prism (we calculate $n_{\text{prism}} = 1.608925$ for F2 glass at 785 nm using a dispersion formula supplied by Schott-Optical.
Glass Technologies) of side = 2.5 cm, such that the center ray of the divergent beam strikes the first prism-face at normal incidence. This is accomplished by aligning the retro reflection from the entrance face of the prism back upon the fiber. The angle $\theta$ represents the angle of incidence of an arbitrary ray within the diverging beam at the prism-sample interface. The fluid sample whose refractive index $n_{\text{sample}}$ is to be determined is placed on top of the prism, so that the prism-base forms the prism-sample interface. The reflected rays at the prism-sample interface exit from the prism and are allowed to fall on a diode array (Hamamatsu S3903-512Q), driven by a C7884 Hamamatsu circuit driver which is controlled by LabView through a GPIB interface. The array has 512 pixels. Each pixel has a diameter 25 $\mu$m. Thus, the center of each pixel may be associated with a unique incident angle $\theta_i$. Note that in our device there is no moving mechanical component, not even a moving light ray, to keep track of. Data taking merely consists of recording the intensity registered by each pixel on the diode array. Thus, all mechanical noise is practically eliminated.

B. Fundamental limits on refractometer sensitivity owing to pixel dark current and laser intensity fluctuations

In the absence of mechanical noise, the sensitivity of our refractometer is limited by electrical and optical noise arising from the dark current in each pixel, and from intensity fluctuations in the laser, respectively.

Fig. 2 shows that when the critical angle $\theta_c$ for the prism-sample interface lies in the range of angles subtended by the divergent beam, a clear demarcation appears in the reflected beam spot between a lighted portion corresponding to TIR ($\theta > \theta_c$) and a dark portion corresponding to non-TIR ($\theta < \theta_c$). This “dark edge” marks the location on the pixel array of the critical angle, which moves across the array as the sample's refractive index changes. At first glance, it may seem that a given pixel is either bright or dark, meaning that the pitch of the diode array (i.e., the pixel diameter, which is 25 $\mu$m in our case) determines the spatial resolution for the location of the dark edge. However, this is not true, for a variation in the location of the dark edge within a single pixel leads to measurable changes in the intensity recorded by this pixel. Therefore, the resolution of the refractometer is not limited by the pixel diameter but by the
smallest intensity change discernable by this pixel; this change, in turn, is clearly limited by the pixel dark current and laser intensity fluctuations.

We first find the geometrical relation between pixel-center position and the incident angle \( \theta \) at the prism-sample interface. The central ray of the divergent beam is perpendicular to the entrance face of the prism, and hence also to the emergent face because the prism is equilateral. We find by purely geometrical considerations that

\[
\theta_i = \beta + \delta_i + \tan^{-1}\left[a_i \left(b + n_{\text{prism}} \left(c + d\right) + \delta_2\right)\right],
\]

where \( \beta \) is the angle of the prism and is 60° in our case, \( a_i \) is the lateral displacement in the detector plane of an arbitrary ray (with incident angle \( \theta \)) from the central ray (see Fig 2) and can be expressed in terms of the pixel number \( N_i \) (see Eqn. 4 below), \( b \) is the total path length of the central ray inside the glass prism (which one may readily verify from geometrical considerations to be equal to the dashed line AB drawn in the figure perpendicular to the input face of the prism; \( b = 21.7 \) mm in our case), \( c \) and \( d \) are the total path lengths in air of the central ray from the source to the prism and from the prism to the detector plane respectively (\( c = 11 \) mm and \( d = 20.5 \) mm for our particular device), and \( \phi_i \) is the angle of refraction of the boundary ray of the divergent beam cone at the air-prism interface (calculated from Snell's Law to be 4.2760170 in our case) as shown. In Sec. 4 the calibration procedure helps determine the symbols \( \delta_{i1} \) and \( \delta_{i2} \), where \( \delta_{i1} \) is the error in degrees denoting the departure from perpendicular alignment of the central ray onto the entrance face of the prism, and \( \delta_{i2} \) is the error in the measurement of the total path length of the central ray from the source to the detector. If we denote as \( N_i \) the pixel on whose center the emergent ray corresponding to angle \( \theta \) is incident, and \( N_0 \) the pixel on whose center the central ray is incident, then we also have the following simple relation between \( a_i \) and \( N_i \).

\[
a_i = \frac{N\alpha}{2} \left(\frac{N_0 - N_i}{N_0}\right),
\]

where \( N \) is the total number (\( N = 512 \)) of pixels in the diode array and \( \alpha \) is the pixel diameter (25 \( \mu \)m), meaning that \( N\alpha/2 \) is simply equal to half the length of the diode array.
To estimate the least intensity change discernable by a pixel, we assume for convenience that the dark edge corresponding to the critical angle $q_c$ initially lies at the center of a pixel which we denote by $N_c$ (see Figs. 2 and 3). Suppose the refractive index of the sample now changes by a small amount, leading to a slight displacement $\Delta a$ of the dark edge away from the center of this pixel, as shown in Fig. 3. The corresponding change in power $\Delta P$ detected by this pixel is given by $\Delta P \approx I \alpha \Delta a$, where $I$ is the total internal reflected laser intensity ($\approx 5.1 \text{ mW/cm}^2$ in our case) and $\alpha \Delta a$ is the approximate change in illuminated area. If the response (in mA/mW) of the pixel is denoted by $R$, the small change in detected photocurrent $\Delta i$ due to the displacement $\Delta a$ of the dark edge is given by

$$\Delta i = (I \alpha \Delta a)R. \quad (5)$$

We find from Eqns. 3 and 5 that the corresponding change in the critical angle is

$$\Delta \theta_c \approx \frac{\Delta i}{I \alpha R} \left( b + n_{\text{prism}} \left( c + d \right) \frac{\cos \phi_c}{\cos \phi} \right)^{-1} \quad (6)$$

where we have set $\delta_{1,2} = 0$ for the purpose of this theoretical analysis. Further, we have used the approximation $\tan(\theta_c - \beta) \approx (\theta_c - \beta)$ in radians, because the maximum possible range for $\theta_c - \beta$ is simply given by the beam divergence $\pm \phi$ inside the prism, meaning that $\theta_c - \beta$ is small. The relation between the measured change in critical angle $\Delta \theta_c$ and the change in the sample refractive index, denoted by $\Delta n_{\text{sample}}$, is straightforwardly derived from Eqn. 1 as.

$$\Delta n_{\text{sample}} \approx \sqrt{n_{\text{prism}}^2 - n_{\text{sample}}^2} \Delta \theta_c \quad (7)$$

It is clear from Eqns. 5, 6 and 7 that if we can estimate the smallest measurable value of $\Delta i$ we would be able to calculate the sensitivity for our refractometer. The question then is. What is the smallest detectable change in photocurrent $\Delta i$? This smallest value is limited by the pixel dark current and/or the fluctuations in the laser intensity.
Role of pixel dark current: If we assume the optical noise to be negligible for now, a reasonable lower limit for $\Delta i$ is given by the pixel dark current (denoted as $i_D$). Setting $\Delta i = i_D$ corresponds to a light measurement with 1:1 signal-to-noise ratio. For our device, the pixel dark current $i_D$ is specified by the manufacturer to be 0.1 pA or less, and the response $R$ is specified to be 0.15 mA/mW. Substituting these values in Eqn. 5, we obtain $\Delta a_{\text{min}}$ to be $\approx 0.5$ nm, which is over four orders of magnitude smaller than the pixel diameter - a remarkable result (which suggests the potential applicability of this simple device to the detection of subnanometric motion of a light beam). From Eqn. 6 we find that $\Delta \theta_{c_{\text{min}}}$ is predicted to be $\approx 7 \times 10^{-9}$ radians.

Then, from Eqn. 7 we find that the theoretical limit imposed by the pixel dark current on the sensitivity of our refractometer to changes about $n_{\text{sample}} = 1.333$ (water), say, is approximately $6 \times 10^{-9}$, comparable to the most sensitive interferometric refractometers $^9, ^{10}$.

Role of laser intensity fluctuations. In many cases, instead of the dark current in pixel $N_c$, it is the fluctuations (denoted by $f$) in the laser intensity $I$ that may be the dominant factor limiting the sensitivity of the photocurrent to small changes in the sample's refractive index. The fluctuations in the detected photocurrent $\Delta i$ caused by these laser intensity fluctuations may be written as $\Delta i = \frac{1}{4} f I \pi \alpha^2 R$. In our case, we have measured $f$ in each pixel to be no more than 0.05% by taking 50 measurements of the laser beam power on the diode array and examining the variance of the voltage signals recorded by each pixel. Thus, using previously mentioned values for $I$, $\alpha$, and $R$, we find that $(\Delta i) \approx (5 \times 10^{-4}) (5.1 \text{mW/cm}^2) (\pi/4) (25 \times 10^{-4} \text{cm}^2) (0.15 \text{mA/mW}) \approx 2 \times 10^{-9}$ mA. Substituting this value for $\Delta I$ in Eqn. 5 we find that the smallest value of $\Delta a$ that is detectable by the pixel $N_c$, with a signal-to-noise of 1:1, is given by $f \pi \alpha / 4 \approx 10$ nm, which is over three orders of magnitude smaller than the pixel diameter. From Eqns. 6 and 7 we find that the smallest measurable value for $\Delta \theta$ is predicted to be $\approx 1.4 \times 10^{-7}$ radians, and the theoretical limit imposed by the laser intensity fluctuations on the sensitivity of our refractometer to changes about $n_{\text{sample}} = 1.333$ (water), say, is approximately $1.2 \times 10^{-7}$, a factor 20 higher than the lower limit imposed by the pixel dark current.

Therefore, in our case, the optical noise owing to laser intensity fluctuations dominates over the electrical noise owing to pixel dark current, and is responsible for setting a theoretical limit of about $10^{-7}$ on the sensitivity of this class of diode-array based refractometer.

In Sec. 3C below we now describe how data for the intensity distribution reflected from the prism-sample interface are collected and analyzed.
C. Device operation and data analysis. Further suppression of optical noise

We start by measuring the reflected intensity distribution for air (i.e., with no sample present). In this case, the incident angles $\theta$ at the prism-sample interface are such that the entire Gaussian beam suffers TIR at the prism-air interface, yielding a reflected intensity profile that reproduces the input Gaussian beam, meaning that for all practical purposes the reflected intensity distribution for air is equal to $I_i$ for the diverging beam. The Gaussian beam is centered upon the diode array so that $N_o = 255$. Curve A in Fig. 4(a) shows the reflected intensity distribution for air (which we refer to as the $I_r$-curve from now on) measured by the diode array as a function of the pixel number.

Next, we measure the reflected intensity distribution $I_r$ for water and various transparent samples of different refractive indices. The samples used are solutions that consist of different concentrations of dimethyl sulfoxide (DMSO) in water. DMSO and water were chosen for their excellent intermiscible properties. DMSO has a refractive index of 1.4780 - 1.4790 in the temperature range 20° - 25°C. We used sample volumes of about 0.5 ml for this work, which was enough to cover the entire prism-base. In principle, one merely needs to cover the laser beam spot (approximate area 0.14 cm$^2$) on the prism-base, meaning far smaller sample volumes may be used if desired. As an auxiliary check of our device, and as a calibration reference, we use a commercial Abbe refractometer which, however, has a resolution limited to only 5 x 10$^{-4}$. The Abbe refractometer is calibrated by the manufacturer to provide refractive index values for $\lambda = 589$ nm.

Fig. 4(a) shows $I_r$-curves measured for two DMSO-water solutions, namely S1 and S2, of concentrations 50% and 30% respectively (where the concentration value refers to the percentage by volume of DMSO in water), and for distilled water W. The vertical dotted lines in this figure show the location of the transition from the TIR to non-TIR regime for S1, S2, and W. To give the reader a feel for the refractive indices of these samples, we state the values of $n_{sample}$ as measured by the auxiliary Abbe refractometer for S1, S2, and distilled water respectively: 1.4065, 1.3770, and 1.3330. Each reflected intensity curve displayed in Fig. 4(a) is an average over 50 scans, and the time taken for each scan is 8.5 ms, implying that our measurements are real-time for most practical purposes.

The data in Fig. 4(a) has visible contributions from two additional sources of optical noise.
First, the curves in Fig 4 (a) for air A, S1, S2, and water W do not start and end at nearly zero intensity, sitting instead atop an offset of about 0.5 Volts, despite the fact that in our setup, the reflected beam spot size at the diode array is less than the length of the array. This is because of the presence of stray scattered laser light from nearby optical components and their mounting hardware. It is important to suppress this noise, otherwise the contribution from noise dominates at small values of $I_r$. This noise-floor is approximately determined for each curve in Fig 4 (a) by finding the minimum $I_r$-value for that curve, which is then subtracted from each measured $I_r$-value to yield a corrected $I_r$-curve. These corrected $I_r$-curves for S1, S2, and W are divided by the corrected $I_r$-curve for air A, and the resultant $I_r/I_i$-curves are plotted in Fig. 4 (b), which closely resemble the representative $Ir/Ii$ curve drawn from the Fresnel theory in Fig. 1(b). Superposed on the data in Fig. 4(b) are shown solid-line curves derived from Fresnel theory, which does not take into account technical noise in any form. An explanation of how these theoretical curves are obtained is provided in Sec. 4 below. It is clear from Fig. 4(b) that the agreement between experiment and theory deteriorates only at lower values of reflected intensity, where technical noise due to stray light is expected to dominate, hence the worst agreement for W. However, at values of $I_r/I_i$ that are above 0.8, in Fig. 4(b), the agreement between theory and experiment is excellent for all three curves S1, S2, and W.

Second, it is apparent in Fig. 4(a), more so in (b), that noise ripples appear in all the data curves near the TIR-non TIR boundary culminating in a large “spike” at the critical angle. We found empirically that the ripple frequency depends upon the sample thickness, leading us to speculate that these ripples possibly arise from interference between the front and back reflections in the sample. At any rate, no matter what the noise source may be, it is vital to circumvent this problem in order to create a sensitive refractometer. Figs. 5 shows a magnified view of the experimental $I_r/I_i$-curves in the critical TIR-non TIR transition region for three different close-lying DMSO-water solutions, named F1 through F3, of concentrations 19.012 %, 18.0775 %, and 17.2689 %, respectively (with refractive index values, as measured with the Abbe refractometer, of 1.3615, 1.3600, and 1.3590 respectively). The solid lines are merely drawn as an aid to the eye. On this magnified scale, the “spike” looks like a “hump.” Clearly, the precise pixel-location of the critical transition is ambiguous - does the transition occur at the peak of the hump, or at the beginning or at the end? We decided to alleviate this problem by taking a cue from the comment in Sec. 2 regarding the encircled region shown in Fig. 1(b), and
examining the region immediately after the TIR-non TIR transition in Figs. 5(a), where $I_r/I_i$ falls smoothly from unity in a noise-free manner. In Sec. 4 below, we show how we use this noise-free region to calibrate our device for use as a sensitive refractometer.

4. Device Calibration

We calibrate the pixel number $N_i$ in terms of refractive index by the following three steps. First, we choose an $I_r/I_i$-curve (say, F1) in Fig. 5(a), and draw a horizontal dotted line (from now on referred to as the calibration axis) that passes through a pixel data-point (say, $N_i = 391$) lying on F1. We postulate that the pixel number at the point of intersection of the calibration axis with the $I_r/I_i$-curve for a given sample corresponds to the critical angle for that sample. Thus $N_i = 391$ is taken to correspond to the critical angle for sample F1. This explains why our refractometer is differential, and not absolute, for it is evident from Fig. 5(b) that the true critical angle would always lie at a smaller (but indeterminate, owing to excessive noise) pixel value. The axis of calibration is chosen to lie close to unity ($I_r/I_i = 0.923$), yet stay well clear of the noise ripples in the data in the TIR region. The exact y-location of the axis of calibration is unimportant. Second, we measure the refractive index of the chosen sample F1 with the reference Abbe refractometer ($n_{F1} = 1.3615$ as mentioned earlier) and calculate the critical angle $\delta_{F1}$ from this measured value using Eqn. 1. Third, we substitute $\theta_{F1}$ for $\theta_i$ and 391 for $N_i$ in Eqn. 3 to obtain an equation in which the misalignment error-constants $\delta_1$ and $\delta_2$ are the only two unknowns (the values for all other symbols were specified in subsection 3 B). Of the many possible combinations of values that $\delta_1$ and $\delta_2$ may take, yet satisfy Eqn. 3, we choose that combination for which both $\delta_1$ and $\delta_2$ are nearly minimized, namely, $\delta_1$ is equal to $0.349111^\circ$, and $\delta_2$ is equal to $0.394915$ cm. Now all the parameters on the right-hand side of Eqn. 3 are known. Substituting the various pixel numbers into the right-hand side of this equation yields a range of incident angles, each of which transforms into a critical angle for a given sample at the point of intersection of that sample's $I_r/I_i$-curve with the calibration axis. A refractive index may be straightforwardly inferred from each critical angle. Thus, the axis of calibration in Fig. 5 is now calibrated in terms of refractive index.

We now check the quality of our calibration by comparing the Abbe measurements for other samples besides F1 (for example, samples F2 and F3 in Fig. 5(a), and water W) with our own device's calibrated measurements of $n_{sample}$. Fig. 5(b) depicts a vertically expanded view
near the axis of calibration (which forms the x-axis for this figure) of the three $I_r/I_i$ curves F2, F3, and W. The measured refractive indices corresponding to these three curves are read off from their respective points of intersection with the axis of calibration. The comparison between the reference Abbe readings and our calibrated device measurements is shown in Table 1. It is gratifying to note that in each case our refractometer agrees with the Abbe reading to within the resolution of the Abbe refractometer, namely $5 \times 10^{-4}$. In this way, the calibration of our device over the refractive index range 1.3330 to 1.3615 is complete. Actually, the range of measurable refractive indices extends well beyond 1.3615. A cursory glance at Fig. 4 reveals that while the TIR-non TIR transition for water appears near the lower limit of the observable range, we obtained clearly observable critical transitions for refractive indices up until about 1.43 (the refractive index for sample S1 in Fig. 4 is measured on the Abbe refractometer to be 1.4065). Thus, we may achieve the calibration of our device over a rather wide range of refractive indices. Furthermore, in Sec. 5 below we demonstrate that, despite this wide range, our refractometer is sensitive to changes in the refractive index at better than the $10^{-5}$ level. This is the reason for quoting our refractometer's calibrated readings to the sixth place of decimal in Table 1, even though the x-axis in Fig. 5(b), which was chosen to display the wide range of calibration, is too coarse to reveal changes on that fine a scale.

Table 1 also shows the theoretical values for $n_{\text{sample}}$ predicted by Fresnel theory. To obtain these values we first express Fresnel's formula (Eqn. 2) for $I_r/I_i$ in terms of the pixel number $N_i$ instead of $\theta_i$ using the calibration performed just above. Now, for any given sample, we examine Fig. 5(a) and plug into Fresnel's formula the value of $N_i$ at which the sample's $I_r/I_i$-curve intersects with the axis of calibration. We then calculate the value of $n_{\text{sample}}$ from Eqn. 2 that yields $I_r/I_i$ equal to 0.923 corresponding to our choice of y-location of the axis of calibration. For example, for sample F1, we calculate that $n_{\text{sample}} = 1.361556$ at $N_i = 391$ yields 0.923 for $I_r/I_i$. Note that it is typical for the $N_i$-value at the point of intersection of an $I_r/I_i$-curve with the axis of calibration to be fractional since a data point will rarely lie at this intersection point. In all such cases, the fractional value of $N_i$ is determined by interpolating between the two pixel data points on the $I_r/I_i$-curve nearest to the calibration axis. We see in Table 1 that the theoretical and experimental values for the refractive indices of F2, F3 and W agree within the resolution of the reference Abbe. Thus, the Fresnel theory offers good support to our experimental results. Once $n_{\text{sample}}$ is calculated in this way, the entire theoretical $I_r/I_i$-curve may be generated from Eqn. 2.
Such theoretical $I_r/I_i$-curves are plotted for samples S1, S2 and water in Fig. 4. Again, we find for S1 and S2 the theoretical refractive index values of 1.406444 and 1.376977 agree with the Abbe measurements of 1.4065 and 1.3770 within the resolution of the Abbe refractometer. To conclude this section on device calibration we note that instead of using an arbitrary sample as our reference point for calibration, an alternative could be to choose the refractive index of water as our reference point. Extensive tables exist in the literature that cite the refractive index of water at different temperatures and wavelengths\textsuperscript{16,17}. However, in our case, we were unsure about the quality of our distilled water, and preferred to do the calibration based on a concrete measurement albeit with poor resolution. We also note that if we had a high-resolution calibration refractometer we would use two, not one, reference points for calibration. We would then have two equations for the two unknowns $\delta_1$ and $\delta_2$, meaning we could have then solved for the unknowns exactly, thus enhancing the quality of our calibration.

5. Sensitivity of our Refractometer

We now demonstrate measurements with our refractometer of changes in the refractive index that are significantly smaller than $10^{-5}$. Fig. 6 shows the $I_r/I_i$ curves for twenty-four different DMSO-water solutions. These solutions were prepared systematically so that there are several samples for which the refractive indices are not distinguishable on the $10^{-4}$ scale. For example, none of the 12 samples with refractive index between 1.3591 and 1.3594 in Fig. 6 were resolvable by the reference Abbe refractometer, least of all the closest-lying samples denoted by A and B, and C and D. On the other hand, Fig. 7 demonstrates that our refractometer is capable of clearly distinguishing these close-lying samples. The fact that the lines no longer look vertical is irrelevant because only their points of intersection with the axis of calibration (the x-axis in Fig. 7) matter. Note that the least count of the x-axis in Fig. 7 is $10^{-6}$ units of refractive index. Thus, samples A and B are demonstrated to be separated in refractive index by $4 \times 10^{-6}$, and samples C and D are separated by less than $3 \times 10^{-6}$. The reason we could not probe even smaller refractive index changes was that we had no method for preparing in a controlled manner samples with ever-decreasing differences in refractive index.

The data in Figs. 4 through 7 clearly show that our refractometer performs highly sensitive measurements of changes in refractive index over a wide range of indices. The current data was taken with no temperature stabilization on the device leading to a temperature
fluctuation of 0.5 °C over about half an hour. As a result it was difficult to prepare in a controlled fashion DMSO-water solutions for which the refractive index differed by a few parts in a million or less.

6. Conclusions

In conclusion, we have built a proof-of-principle refractometer, which we have demonstrated is sensitive to changes in the refractive index at a level less than $3 \times 10^{-6}$. The determination of the refractive index of an unknown sample is completed in less than a second, and is hence near real-time. Most biological imaging applications focus on samples for which the refractive index is close to that of water. The refractive index for water lies too close to the end of our useful range in the setup described in this work. However, by simply changing the prism to a lower refractive index (for example, BK7 glass with a refractive index of 1.517) one may center the usable range of refractive indices upon water for bio-imaging purposes. Sample sizes need not be larger than a fraction of 0.5 ml. The high quality of the data in Fig. 7, and the theoretical analysis in subsection 3B of the fundamental limit on the sensitivity of this class of refractometer, suggests that the device is capable of resolving differences in refractive index even smaller than $10^{-6}$. However, we could not test the sensitivity of the device at that level because of our inability to produce samples with refractive indices lying that close. Temperature stabilization, and increasing the number of pixels from 512 to 1024, are obvious steps to improve the sensitivity of the device. Even so, this is the first time, to the best of our knowledge, that a non-interferometric refractometer capable of real-time measurements with better than $10^{-5}$ sensitivity has been demonstrated. From the point-of-view of bio imaging, the measurement of refractive index of turbid and absorbing samples is of great interest. Our refractometer offers the potential for highly sensitive differential refractometry at the $10^{-4}$-$10^{-5}$ level. Traditional critical angle-based refractometers fail with such samples because they examine only the one point $\theta_i = \theta_c$. It is well known that near the critical angle the beam experiences substantial penetration into the sample before undergoing reflection back out (this is the Goos-Hanchen effect). The scattering during this penetration causes a nonzero imaginary component of the refractive index, and has been shown to have a smoothing effect on the sharp knee in the reflectance-versus-incident angle curve. Furthermore, in this case, the “critical” angle at which the maximum reflectance change occurs has been shown to yield erroneous values for the real part of the refractive index. In our
refractometer design we examine not just $\theta_i = \theta_c$ but the nearby region $\theta_i < \theta_c$. As demonstrated in subsection 3C and Sec. 4 one may perform exceptionally sensitive differential refractometry by steering clear of the noisy TIR-non TIR region and instead working in a nearby noise-free region.

We gratefully acknowledge invaluable discussions with Professor L. M. Bali. Financial support from the Research Corporation and the Petroleum Research Fund is gratefully acknowledged. An application for a patent has been filed for the refractometer described in this work.
References


16. The inevitable small error between the postulated and actual value is of no concern to us because our goal is to build a differential refractometer. We reiterate that we are only concerned with accurately measuring differences in refractive index, not the absolute refractive index.


Fig. 1 (a) A sample of unknown refractive index $n_{\text{sample}}$ is placed on the top of the prism (refractive index $n_{\text{prism}}$). A collimated laser beam of intensity $I_i$ is incident on the prism-sample interface with an angle of incidence $\theta_i$. The emergent laser beam has intensity $I_r$. (b) A representative plot of $I_r/I_i$ versus $\theta_i$ is shown, for two different samples. The regions of total internal reflection (TIR) and frustrated total internal reflection (FTIR) are indicated. $\theta_{c1}$ and $\theta_{c2}$ are the critical angles corresponding to the two different samples. The region where small changes in $\theta_i$ cause drastic changes in $I_r/I_i$ is encircled.

Fig. 1 McClimans et al.
Fig. 2 McClimans, et al.

Fig. 2. Schematic outline of the refractometer. The shaded portion on top of the prism represents the sample. A divergent laser beam is incident on the prism-sample interface, and the reflected rays fall on a linear diode array. See Sec. 4 and 5 for an explanation of the symbols.
Fig. 3 McClimans, et al.

Fig. 3. Calculating the least intensity change discernable by a pixel: The dark edge demarking the TIR and non-TIR regions moves to a different location within the pixel $N_c$ when the samples refractive index $n_{\text{sample}}$ changes slightly. Assuming the dark edge to be initially located at the center of $N_c$, the change in illuminated area is $\approx \alpha \Delta a$ where $a$ is the pixel diameter and $\Delta a$ is the spatial shift of the dark edge.
Fig. 4. (a) The reflected intensity distribution across the diode array for a diverging Gaussian laser beam incident on the prism-sample interface for four different samples: 1) A – the “sample" is air. TIR occurs for all incident angles. 2) S1 - the sample is a 51 % DMSO - water solution. TIR occurs from pixel numbers 1 up to 230. 3) S2 - the sample is a 30 % DMSO - water solution. TIR occurs from pixel numbers 1 up to 335. 4) W - the “sample" is distilled water. TIR occurs from pixel numbers 1 up to 484. (b) The $I_r/I_i$ curves for W and samples S1 and S2 are obtained by dividing the respective reflected intensity distributions in (a) by that for air. Note the “spikes" in $I_r/I_i$ at the critical angle.
Fig. 5. a) A magnified view of the $I_r/I_i$-curves in the TIR-FTIR transition region for three different DMSO-water solutions F1 through F3 of concentrations 19.012%, 18.0775%, and 17.2689%, respectively. The noise spikes in Figs. 3 now appear clearly as humps of varying sizes and shapes. The axis of calibration is the horizontal dotted line drawn in a noise-free region near, but not at, the TIR non-TIR transition point (see Sec 4 for further explanation). The reference curve is F1, with all other refractive indices being measured relative to the Abbe value of 1.3615 for F1. The solid lines are merely drawn in to aid the eye.

b) A vertically expanded view of the $I_r/I_i$-curves for F1, F2, F3, and distilled water (W) near the axis of calibration, which forms the x-axis in this figure. Note the break in the x-axes to accommodate water in the figure.
Fig. 6. Twenty-four different DMSO-water samples were systematically prepared so that a wide range of differences in refractive index may be measured and displayed. All the lines are parallel to each other. Note the “band” formed by the twelve samples with close lying values of refractive index. These samples are not distinguished by the Abbe refractometer, yet are clearly resolved by our device (see Fig. 7).

Fig. 6 McClimans, et al.
The refractive index for an unknown sample is read from the point of intersection of the $I_r/I_i$-curve for the sample with the x-axis, which is the calibration axis. Our device clearly resolves the two pairs of lines A and B, and C and D, for which the differences in refractive index are $4 \times 10^{-6}$ and $3 \times 10^{-6}$ respectively.

Fig. 7 Mc Climans, et al
Table 1. A table of the refractive indices for samples F2, F3, and water W as measured by our Abbe refractometer and our calibrated device. Both refractometers agree to within the accuracy of the Abbe refractometer.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration ( % by volume of DMSO in water )</th>
<th>Refractive index $n_{\text{sample}}$</th>
<th>Discrepancy ($\times 10^{-4}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reference Abbe</td>
<td>Calibrated Device</td>
</tr>
<tr>
<td>F1</td>
<td>19.0120</td>
<td>1.3615</td>
<td>1.361500</td>
</tr>
<tr>
<td>F2</td>
<td>18.0775</td>
<td>1.3600</td>
<td>1.359871</td>
</tr>
<tr>
<td>F3</td>
<td>17.2689</td>
<td>1.3590</td>
<td>1.358635</td>
</tr>
<tr>
<td>W (water)</td>
<td>0</td>
<td>1.3330</td>
<td>1.332929</td>
</tr>
</tbody>
</table>

Table 1. Mcclimans, et al
Appendix A - Set-Up Tips

- When working with the Hamamatsu diode array DO NOT START THE LABVIEW PROGRAM WITHOUT FIRST TURNING ON THE 12 VOLT POWER SUPPLY. It is best to never turn it off. If the program is run with no power to the driver circuit, the circuit and possibly the diode array will be destroyed.

- Before any components are set it is wise to be sure that the optical breadboard you are working on is level. This helps you through the alignment process to avoid not catching your miss-alignments thus causing problems to multiply.

- Use stops to mark positions. Each component needs to be able to be adjusted on at least 3 axis. It often helps to lock two posts together. The benefit to this is for example; you have the prism and the beam aligned perpendicularly, but now the height needs to be adjusted. If you loosen the post to adjust the height you will certainly loose your perpendicularity.

- Regardless of the chosen wavelength, complete the initial set-up with a visible beam. Once all alignments are set, the fiber can be replaced without loosing your position.

- Do as much of the initial alignment as possible with a collimated beam. Finding the retro-reflections, the center of the beam, and center of the diode array is much easier with a collimated beam. Use one of the beam collimators with an FC connection to do this. After everything is set the collimator can be removed.

- When setting up the diode array remember that you need to give yourself freedom to make adjustments in 7 different dimensions. The axes are as follows: 1) toward and away from the prism. 2) Horizontally to the left and right of the beam. 3) vertically, up and down. 4) The diode array must be able to rotate on the axis of the centroid of the beam. 5) Course adjustment of the angle of incidence with the beam left and right. 6) Fine adjustment of the angle of incidence w/beam left and right. 7) Coarse and fine adjustment of the angle of incidence w/beam top to bottom. Ok, that is 8 dimensions.
Appendix B - Calibration

For the calibration, we use equation (2) from section 3 of this paper. The point here is to solve for $\delta_1$ and $\delta_2$. To simplify things $\beta$ is combined with $\delta_1$ to become $A$ then re-write the values with in the $\tan^{-1}$ brackets as $\tan^{-1}[a_i/d]$. Now equation 2 looks like

$$\theta_i = A + \tan^{-1}[a_i/d]$$ (4)

We now have two unknowns, $A$ and $d$, to solve for. Remember that $\theta_i = \sin^{-1}[n_i/n_{\text{prism}}]$ and $a_i = N/2[(N_c-N_i)/N_c]$. You now have enough to create two different equations out of eqn. 4, one for each of the two samples chosen. We still have a bit of work left. The first tough part here is determining the value of $N_i$. First you must determine where the horizontal calibration line will be, .923, .951, .99345 ….etc. I suggest you have Samir make that decision because regardless of the line you choose, he will want you to change it. Then you will have to do all this work over again.

Once you have determined the horizontal line, look at the $I_i/I_r$ graph for the two calibration solutions. $N_i$ will be the pixel value where the graph crosses the chosen horizontal line. This will not be a whole number. The more decimal places you keep the better. Start by finding the x and y coordinates of the two pixels before and after the crossing. Find the slope of the line between them then determine the pixel value at the crossing.

Do this for both solutions. The values of $A$ and $d$ will be the same for both equations. The other terms will dependent on the solutions parameters. Isolate the $A$ in both eqns then set them equal to each other. It should look something like this

$$\sin^{-1}(n_1/n_p) - \tan^{-1}(a_1/d) = \sin^{-1}(n_2/n_p) - \tan^{-1}(a_2/d).$$ (5)

One equation, one unknown, that is looks much nicer. This is still a transcendental equation. Fortunately, I have a TI-89 that will solve it for me. Use whatever method works best for you. Have someone else check your work when finished.

You should now have values for $A$ and $d$. Now you can enter equation 4 into the Excel spreadsheet. Be sure to expand $a_i$ so the $N_i$ term indexes for each pixel. Copy this equation down
through all the pixels. In the next column over convert all of the previous columns values to radians –Excel only accepts radians. The next column is the index. Enter in Snell’s Law, 
\[ n_{\text{prism}} \sin(\text{Cell to the left}) \] Copy it down through and you have your calibrated values of \( n \).

**Appendix C**

**Angle Theory Expanded**

This expanded view of the beam path can aid in the interpretation of equations 3 and 4. I believe it to be less confusing than the bent version depicted in figure 2.