Journal of Diabetes Science and Technology
Volume 1, Issue 6, November 2007

ORIGINAL ARTICLES

Polarization-Based Diffuse Reflectance Imaging for Noninvasive Measurement of Glucose

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Abstract

Background:

The ability to measure glucose concentration through noninvasive approaches would impact the treatment of diabetes significantly. Polarization-based optical approaches have received considerable interest because of their potential medical applications. Glucose, a chiral molecule, has the ability to rotate the plane of linearly polarized light, commonly referred to as optical activity, as well as changing the refractive index of the media, which therefore affects the overall scattering coefficient in a given media. The magnitude of each effect is related to the concentration of glucose. Although most previous studies have reported on the use of polarimetry in the aqueous humor of the eye because of its nonscattering nature, one would also expect that glucose concentration could be measured in more turbid media such as tissue through a similar approach. This study investigated how each of these effects is correlated to glucose concentration in a physiological range for highly scattering biological media.

Methods:

A custom-designed imaging polarimeter was used to image highly scattering Intralipid-based media containing different concentrations of glucose. Model formation and glucose prediction were performed through the use of partial least squares (PLS) regression. Further insight into the differences between polarization-based image measurements and encoding of glucose information was provided through the use of principal component analysis (PCA).

Results:

When coupled with PLS regression, *in vitro* polarization measurements yielded highly correlated glucose predictions in both calibration and independent validation, 0.999 and 0.998, respectively. Through the use of PCA, it appears that the majority of the image-based signal yielding the most significant glucose information is attributable to changes in the overall scattering coefficient due to glucose concentration and, to a lesser degree, effects of optical activity.

continued →

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Abbreviations: (CCD) charge-coupled device, (PC) principal components, (PCA) principal component analysis, (PLS) partial least squares, (SEC) standard error of calibration, (SEP) standard error of prediction

Keywords: diabetes, glucose, imaging, polarization

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Abstract cont.

Conclusions:

This study showed how polarimetric-based imaging coupled with PLS regression can be used to quantify glucose concentration in highly scattering media. Such methods may potentially be able to extend the use of noninvasive *in vivo* polarimetric measurements, normally acquired in the anterior chamber of the eye, to other preferred sensing sites such as the skin.

J Diabetes Sci Technol 2007;1(6):873-878

Introduction

Diabetes is a common and serious chronic disease, which affects over 171 million people worldwide and was listed as the sixth leading cause of death based on U.S. death certificates in 2002.[1–3] Currently, only invasive methods for monitoring glucose levels are approved by the Food and Drug Administration, which usually require the lancing of a finger to obtain a blood sample for testing. Because this approach is often cumbersome and has associated pain, patient compliance is often below what is deemed acceptable, leading to poor glycemic control. Recently, however, great strides have been made in the noninvasive measurement of physiological glucose. Optical methods are on the forefront of approaches being applied for the detection and monitoring of physiological glucose concentrations. This article proposes and demonstrates a polarimetric image-based approach.

The effects of glucose concentration on the optical properties of tissue are well described in the literature. One of the more dominant effects is how glucose affects tissue scattering, whereby an increase of glucose concentration results in a decrease in the scattering coefficient.[4,5] The reasoning behind this is that the scattering coefficient is dependent on the mismatch between refractive indices of the extracellular fluids and the cellular membranes. Increasing glucose concentration in the extracellular fluids will increase its refractive index, which in turn decreases the overall tissue scattering coefficient. Esenaliev *et al.*[6] used optical coherence tomography to exploit this effect for the noninvasive measurement of glucose.

Another optical effect as a consequence of glucose, often considered in polarimetry, is that of optical activity. Glucose, a chiral molecule, has the ability to rotate the plane of linearly polarized light (i.e., the electric field), which is the basis for all the current highly sensitive polarimetric techniques, such as those reported by Cameron *et al.*[7–9] and Chou *et al.*[10] In these investigations, the proposed sensing site is the aqueous humor of the eye,[7–9,11] which is chosen because of its low scattering effects and glucose concentrations that are correlated with those of blood, which is very suitable for polarimetric type measurements. Mehrübeoğlu and colleagues[12] investigated the possibility of utilizing the diffusely reflected cross-polarization patterns from highly scattered media containing glucose and noted slight changes in the images; however, no quantification was performed. Wang and co-workers[13] reported on how optical activity contributions due to glucose were encoded in Mueller matrix patterns of turbid media through the use of a Monte Carlo simulation approach.

In this study, we extend these investigations and demonstrate how diffuse reflectance polarization imaging coupled with advanced chemometric techniques can be used for the noninvasive quantification of glucose over a physiological range in highly scattered media. In addition, multivariate image analysis techniques are explored to further investigate how both scattering and optical activity effects are correlated to the glucose polarization signature used in quantification.

Scientific Methods

Experimental Apparatus

The experimental setup constructed for this investigation is illustrated in **Figure 1**. The light source is provided by a 10-mW 514 nm (i.e., green) argon ion laser (Melles Griot, Irvine, CA). This wavelength is well suited for

polarimetric-based glucose measurements, as the specific rotation, [α], of glucose (i.e., its ability to rotate linearly polarized light) is relatively large at 514 nm compared to longer wavelengths [e.g., [α] = 73°/(dm g/ml) at 514 nm (green) versus [α] = 41.9°/(dm g/ml) at 656 nm (red)]. The laser is focused through a Glan–Thompson linear polarizer onto the surface of a sample at an incident angle of 45°. The resulting spot diameter on the sample surface is <2 mm. The diffusely backscattered light from the sample surface is imaged through another Glan–Thompson linear polarizer, serving as an analyzer, with its transmission axis oriented perpendicular with respect to the initial polarizer. The crossed polarization image is focused via a Nikon® zoom lens onto the charge-coupled device (CCD) array of a thermoelectric-cooled 16-bit CCD camera (Apogee CCD, CA).

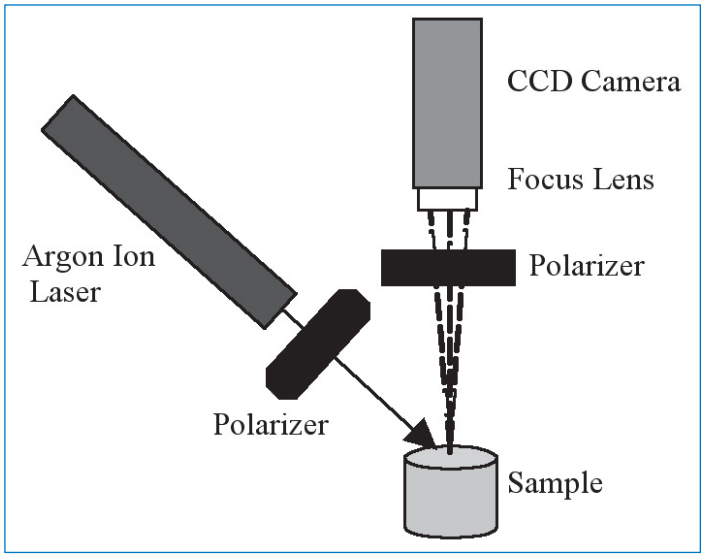

Figure 1. Experimental setup for the polarization imaging system.

In Vitro Experiments

Intralipid suspensions (Kabi Clayton, NC) with known optical properties and scattering centers of a known size distribution were used as a tissue phantom.[14] The scattering coefficient of the phantom is μ's = 10 cm^{-1}, which was obtained by appropriately diluting the 20% Intralipid suspension. The anisotropy factor (g) of the samples is approximately 0.8. From this base stock of Intralipid solution, a total of 16 separate 100-ml glucose samples were prepared. These samples were created by doping each sample with an appropriate amount of glucose from a concentrated stock solution. Each of the 16 samples contained glucose concentration levels ranging from 50 to 4000 mg/dl with 11 samples having glucose concentrations <1000 mg/dl. Concentrations above the normal physiological range were chosen in order to more fully investigate and demonstrate the optical activity and scattering effects of glucose. An additional set of samples was also prepared for independent validation.

Each sample was imaged with the polarimetric system, and the acquired images were cropped to 321 × 321 pixels with the center residing at the ideal geometric point of laser incidence. The approximate size of each image is 1.4 × 1.4 cm^2. To illustrate the type of images acquired, **Figure 2** shows an example of the diffusely reflected polarization pattern for a glucose concentration of 50 mg/dl. The unique pattern arises as a consequence of the distinctive distribution of various polarization states throughout the medium caused by scattering and optical activity effects. Each image is an average of three measurements to minimize possible laser fluctuations and other system noise.

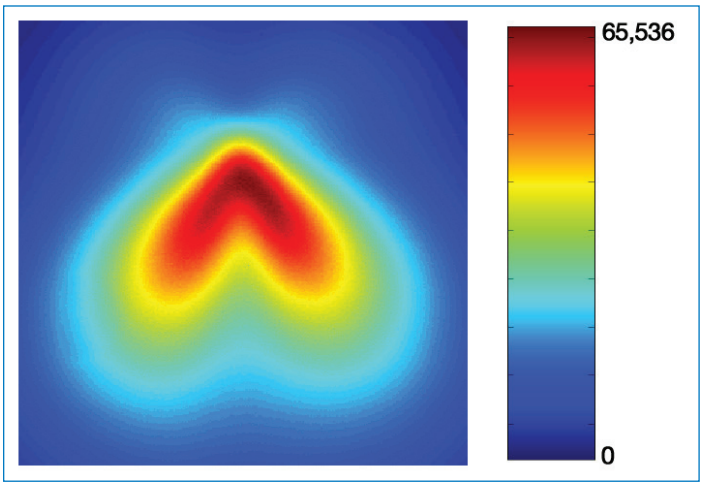

Figure 2. Raw image obtained for a glucose level of 50 mg/dl with a pseudo-color map applied. The color bar represents respective levels of light intensity.

To quantify glucose concentration in the tissue phantoms, partial least squares (PLS) regression[15] was employed to form a calibration model. To achieve this, each two-dimensional polarization image is reshaped into a one-dimensional vector/array. This operation in essence converts image data to a spatial-based spectrum to facilitate the application of the PLS technique. Before forming the PLS regression calibration model, transformed data were first corrected with multiplicative signal correction[15] to remove any scaling artifacts. A PLS model was then formed with four latent variables. The number 4 was selected in order to capture the most significant variance in data while preventing overfitting in the model formation. Performance of the calibration model was evaluated through computing the standard error of calibration (SEC) and was further validated with

an independent collected data set, which determined the standard error of prediction (SEP). SEC and SEP were calculated as follows:

$$SEC = \sqrt{\sum_{i=1}^{n_c} \frac{(\hat{c}_i - c_i)^2}{n_c - 1}}, \quad (1)$$

$$SEP = \sqrt{\sum_{i=1}^{n_p} \frac{(\hat{p}_i - p_i)^2}{n_p - 1}}, \quad (2)$$

where c_i and p_i are the glucose concentrations in the calibration and validation data set, respectively. The parameters $\hat{c}_i$ and $\hat{p}_i$ represent the predicted glucose concentration in calibration and validation, and the parameters and denote the number of calibration and validation samples.

Results

The resulting calibration and validation prediction plots are shown in **Figure 3**. As can be seen, the estimated glucose concentrations in both calibration and validation are highly correlated with correlation coefficients of 0.999 and 0.998, respectively, for the full concentration range. For concentrations up to 400 mg/dl, the correlation coefficients are slightly lower at 0.9615 and 0.9298, respectively. In addition, the SEC and SEP are 48.7 and 74.0 mg/dl, respectively. Although it is recognized that these errors are high in respect to acceptable error levels for the *in vivo* application of this technique, it should be noted that this approach is only the initial step at applying polarimetry for glucose detection in highly scattered samples, such as tissue. Through the use of advanced electro-optic techniques, similar to those applied to eye-based polarimetry[7–10] and other light-based scattering measurements,[16] it is expected that these error levels can be reduced considerably. For example, the cross-polarization approach, as described in this article, could be extended to provide the complete image-based sample Mueller matrix.[16] This type of measurement utilizes multiple types/states of polarized light to provide a more complete characterization of polarization and scattering effects, which should allow for a further reduction in predictive error.

To further understand the mechanisms behind this approach, a principal component analysis (PCA)[17] approach can be applied to these image data. The loading plots of the first four principal components (PC) are shown in **Figure 4**. The sample numbers correspond

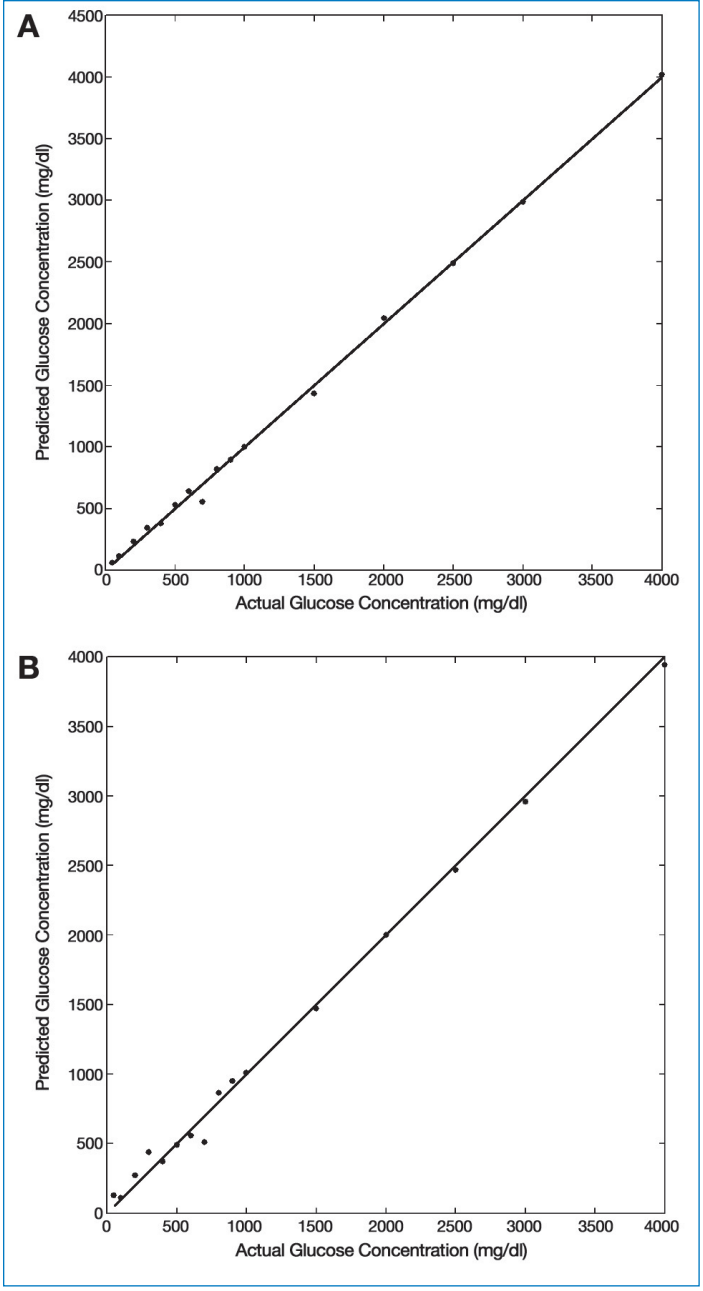

Figure 3. Glucose concentration prediction results: (a) calibration and (b) validation.

to increasing glucose concentration. The loadings can be interpreted as weights for each variable when calculating the PC or, in other words, they contain information on how the variables relate to each other. The loadings for the first PC indicate that all glucose samples contribute nearly equally to the variance seen. In addition, the slight negative slope in these loadings is expected, as the scattering coefficient decreases for increased glucose concentration. This observation could potentially correlate to changes in the scattering coefficient due to glucose, which is a dominant effect. The loadings for PC #2 indicate minimal contribution to the overall variance,

with the exception of what appears to be an outlier at the glucose concentration level of 1000 mg/dl, which was also apparent in the raw images. The loadings for PC #3 indicate that the variables corresponding to the samples in the range of 1500–4000 mg/dl contribute largely to the variations seen. One possible explanation is that this describes variations due to optical activity, as this would be more dominant for larger glucose concentrations (i.e., the amount of rotation in the linear polarization vector is directly proportional to the glucose concentration). The loadings for PC #4 appear uncorrelated and significantly noisier (i.e., somewhat random variation as glucose concentration increases), which probably describe mostly measurement error.

In PCA, the scores contain information on how the individual samples relate to each other. Regarding the scores, as can be seen in **Figure 5a**, the scores of the first PC capture the nature of original data, as compared to **Figure 2**, which are mainly attributable to the overall scattering coefficient. The other plot of interest is that of the scores for PC #3 (**Figure 5b**), which depict apparent variations among samples, especially for locations that are distant from the point of optical incidence, which are hypothesized to be because of optical rotation. The interdependency between the scores of PC #1 and PC #3 is illustrated in **Figure 5c**. As can be seen from this plot, the bright horizontal red-orange band is thought to be attributed to variations in the scattering coefficient as a consequence of changes in the refractive index, whereas vertical deviations are thought to be because of optical rotation, which has a significantly lower contribution.

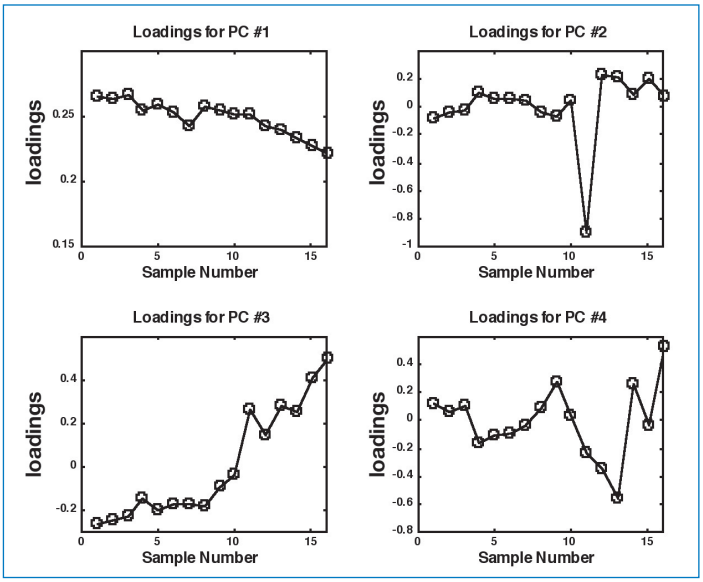

Figure 4. Loading plots for the first 4 PCs

Conclusion

In this investigation, a polarimetric-based approach used to quantify glucose concentration in highly scattered media was demonstrated. This approach entails the use of diffuse reflectance polarization imaging coupled with the use of PLS regression. It appears that two key factors could possibly alter the diffusely reflectance polarization patterns when glucose is present. The first is the change of the refractive index of the medium as related to glucose concentration. This change in effect will alter the overall scattering coefficient of the medium. Second, glucose as a chiral molecule has the ability to rotate linearly polarized light in proportion to glucose concentration. Regarding the ability to quantify glucose at physiological levels through the presented approach, it appears that the scattering effect is the more dominant signal of the two. The optical activity effect, however, appears to also be present as revealed through the PCA. In highly scattered media, the effect of optical activity would be expected to

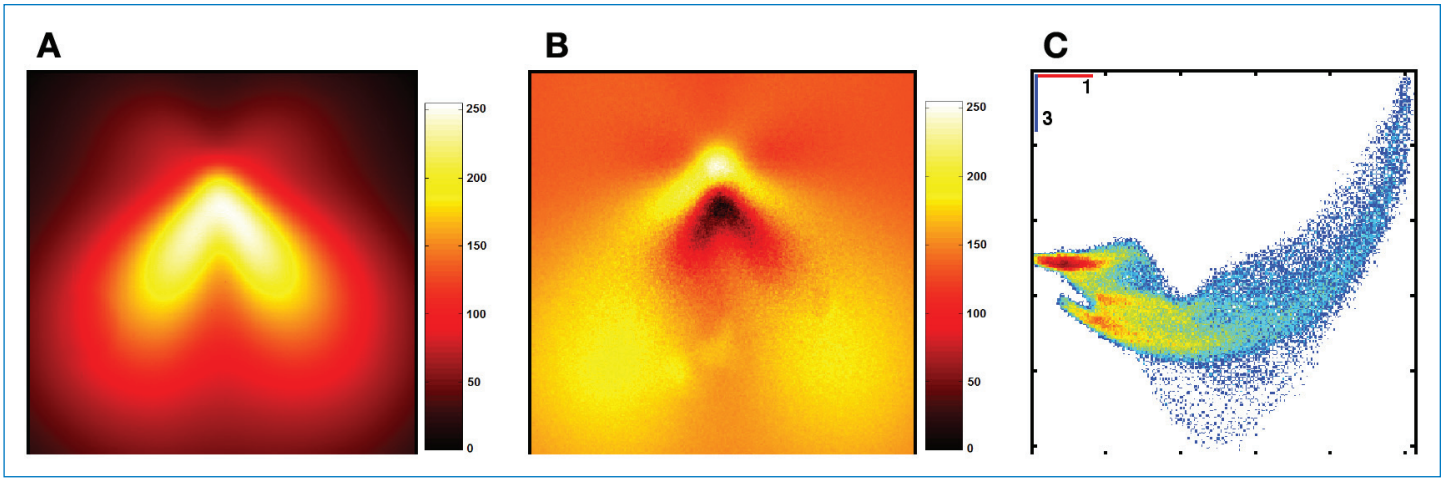

Figure 5. (a) Score image for principal component #1, **(b)** score image for principal component #3, and **(c)** principal component score #1 versus principal component score #3.

be lessened as the numerous scattering events will tend to alter this signal. Finally, this study represents an initial step of using image-based polarimetry as a potential means for *in vivo* noninvasive glucose monitoring. It is suspected that other substances present in skin and surrounding tissues (i.e., proteins and biological analytes), including variations within polarization-sensitive structural components such as collagen and elastin, could potentially confound measurements of this type. Therefore, substantial development in the approach will still be required. The outcomes of this investigation, however, show considerable potential for the proposed method, especially in the alternative application site (i.e., skin) as compared to traditional polarimetry in the eye.

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