Optical imaging of aqueous humor to diagnose premature diabetes mellitus

Anil K. Gupta[*1], C. L. P. Gupta[2], Alok Singh[3] and P.Bharti[4]

ABSTRACT

In this investigation, a comparative study of the transmitted two dimensional16 Mueller polarization matrix element images, based upon the measurement of the glucose concentration in an aqueous humor sample using a polarimeter have been reported. The glucose solution and aqueous humor have been illuminated with a He-Ne (632.8 nm) laser in the vitro measurement. It is concluded that the images obtained by transmitted Mueller polarization matrix in forward direction are capable of giving information about the variation in polarization patterns to measure normal glucose level, impaired fasting glycaemia (IFG), impaired glucose tolerance (IGT) and diabetes mellitus.

***Key words** : Polarimeter, Mueller polarization matrix, Aqueous humor*

1. INTRODUCTION

Interest in frequent determination of glucose concentrations of diabetic patients has increased since the publication of the diabetes control and complications trial report [1, 2]. Use of non invasive (NI) techniques offers several advantages, One of them being relief from pain other major advantage is exposure to sharp objects and biohazard materials. The potential for increased frequency of testing, and hence, tighter control of the glucose concentrations, and hence for a closed loop monitoring system which include a monitor and insulin pump [3]. Recent, polarimetric measurements in turbid media have gained importance by their ability to provide information about photons. For instance, polarization imaging has been used to enhance spatial resolution in turbid samples [4] and to increase contrast for surface imaging by removing the depolarization intensity that consists of multiply scattered photons [5]. Non invasive monitoring of glucose has been of particular interest because of pain associated with invasive self monitoring. Several recent publications and reviews have discussed the importance of NI glucose testing and report on attempts at its measurement [6-10].

An NI body glucose monitoring device is defined as a device that comes in contact with, or remotely senses, a human body part, without protrusion through membranes or sampling a body fluid for analysis external to the body part. An NI glucose monitor processes optical signals transmitted through or reflected by the stratum corneum, dermis and epidermis layers, subcutaneous tissue, interstitial fluid, and blood vessels which represents independent compartments. Two research methodologies are used for NI glucose measurements. An empirical approach involves the following steps:

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(a) Collection of NI signals from non diabetic individuals as well as diabetic patients while performing an oral glucose tolerance test (OGTT) or a glucose challenge test;
(b) Simultaneously measuring blood glucose concentrations by an invasive method; and
(c) Using models based on the correlation between measured glucose values and NI optical signals for computation of results.

This approach does not measure the effect of other metabolites and interferences, biological noise, or variability in instrument-body interface, but attempts to compute-out those noise contributions. The number of variables and the complexity of data analysis necessitates the use of multivariate chemometric techniques such as principal component analysis, partial least squares, or artificial neural network methods [11, 12].

A second approach is the physical model approach. This method involves the following steps:

(a) Measurement of a glucose optical signal in a simple matrix;
(b) Progressive increases in the complexity of the matrix to mimic human tissues; (c) Demonstration of accuracy and precision at each step; and
(d) Correlation of the data with a mathematical model for light propagation in tissues.

Finally, the detection system and the measurement method are applied to body parts. The in vivo signals are again correlated with the invasive data by use of chemometric techniques. This stepwise approach allows for identification of noise components so that strategies may be derived to minimize their contribution to the signal before the use of chemometric techniques[14].

2. THEORY

A beam of light is composed of electromagnetic waves and obey Maxwell's equation at all points of light propagation. The equation that relates optical rotation to a medium specific rotation is given by the equation,

$$[\alpha]^{T}\lambda\text{ , pH} = \alpha / LC \qquad (1)$$

here, L is the specific rotation of an optically active compound in degree in a specific temperature T, and PH of the system, λ is wavelength, L is an optical path length in decimeter and C is the glucose concentration of sample (aqueous humor) in grams of mass per milliliter of solution.

The study of photons through any medium is a very complex phenomenon. The illuminating radiation scatters through random medium according to Rayleigh and Mie scattering theories depending on the size of the scatterer. The photons travelling through random medium may be classified into three types; ballistic components; snake components and diffused components.

In this experiment, we consider forward transmitted photons (ballistic components) for characterization of aqueous humor at different glucose levels. The transmitted photons preserve their polarization after passing through the aqueous humor sample. The Mueller matrix polarization component of transmitted photons provides useful information about the medium.

In Stokes vectors-Mueller matrix approach, a polarization state of light is represented by a four component Stokes vector S = [I, Q, U, V]. The interaction of light with optical elements changes the polarization state of the light from s to s' .Light interaction with any optical element or medium is described as a multiplication of stokes vector with a 4×4 matrix, S' = MS. This sixteen element matrix is called Mueller matrix [15, 16].49 intensity measurements with various orientations of polarizer's and analyser's were made to obtain the 16 elements of the Mueller matrix. Table 1 shows the various orientations of 16 Mueller matrix elements.

Table :1. Calculation of the 16-image Mueller matrix. The notation is as follows: the first term represents the input polarization state, while the second term represents the output polarization state. The states are defined as H for horizontal, V for vertical, P for +45, M for -45, R for right circular, and L for left circular.

M11=HH+HV+VH+VV	M12=HH+HV-VH-VV	M13=PH+PV-MH-MV	M14=RH+RV-LH-LV
M21=HH-HV+VH-VV	M22=HH-HV-VH+VV	M23=PH-PV-MH+MV	M24=RH-RV-LH+LV
M31=HP-HM+VP-VM	M32=HP-HM-VP+VM	M33=PP-PM-MP+MM	M34=RP-RM-LP+LM
M41=HR-HL+VR-VL	M42=HR-HL-VR+VL	M43=PR-PL-MR+ML	M44=RR-RL-LR+LL

3. EXPERIMENTAL SYSTEM

The study of photons through any medium is a very complex phenomenon. The illuminating radiation scatters through random medium according to Rayleigh and Mie scattering theories depending on the size of the scatterer. The photons travelling through random medium may be classified into three types;

The polarimetric measurements are performed with the experimental setup as shown schematically in figure 1. The unpolarised He-Ne laser beam of approximately 25 mW of power at 632.8 nm in wavelength was passed through an optical element with aqueous humor. The optical element consists of two branches; input optical element (L_1,P_1,and W_1) and output optical element (P_2,R_2, and W_2). These optical elements are necessary to create the input and output polarization states required to derive the output 16 element Mueller matrix corresponding to the transmitted intensity.

The calibration of optical elements presented in the fig. 1 is done by a nulling technique involving the use of the input polariser (P_1) and output polariser (P_2) oriented with their principal axes at 90' with respect to each other. Each component is calibrated by adjusting the supplied intensity until the desired input and output polarisation states of H (Horizontal), V (Vertical) , P(+45^0) , M(-45^0) is obtained by the detection of a minimum intensity by the CCD camera. The calibration is done sequentially with the output retardation plate (W_2). The desired intensities to obtain the necessary degree of rotation from the output rotator for each of the desired polarization states, is determined by varying the state of the input polarizer(P_1) until the desired intensity was captured by the CCD camera. Retardation plate similar calibration is done for input polarization (W_1).

The laser beam is focused through lens L_1 (f = 15cm) to the polarizer P_1 to generate linearly polarized light. The linearly polarized light from P_1 is focused to retardation plate W_1 (λ/4 plate) behind the P_1 to generate circularly polarized light, with the retarder principal plane at 45^0 with respect to the plane of the incident linearly polarized beam. Light transmitted from aqueous humor is then pass through analyzer P_2 and retarder W_2 and focused by lens L_2 on the CCD camera.

The intensity is detected by a charge coupled device CCD camera (Pulnix TM-6X-CCIR, Imager (1/2) interline transfer CCD, pixel-752x582, scanning-625 line 625 line CCD, TV resolution-560x420). The obtained images on CCD camera are manipulated via a MAT LAB®programs which performs calculations shown in fig 2, 3, and 4 used to generate the 16 Mueller matrix element images.

Sample :

In our experiment, the sample is an aqueous humor filled in 1cm diameter cell. The composition of aqueous humor is indicated in Table2.The aqueous humor is analyzed with three different concentration of glucose level given below.

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(a) For normal glucose level, aqueous humor was prepared with 81 mg of glucose per deciliter of water and two dimensional image for this concentration is shown in fig.2.

(b) For impared fasting glycaemia (IFG), aqueous humor was doped with 110 mg of glucose per deciliter of water and 16 element of optical imaging is shown in fig.3.

(c) For impaired glucose tolerance (IGT), aqueous humor was doped with 128mg of glucose per deciliter of water and 16 element of optical imaging is shown fig. 4.

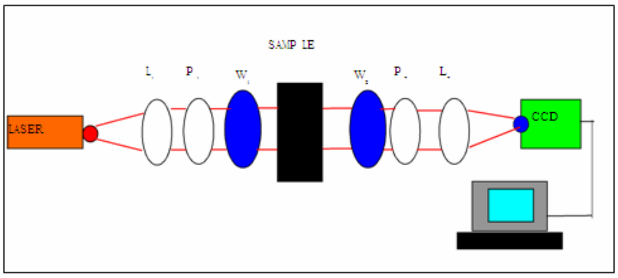


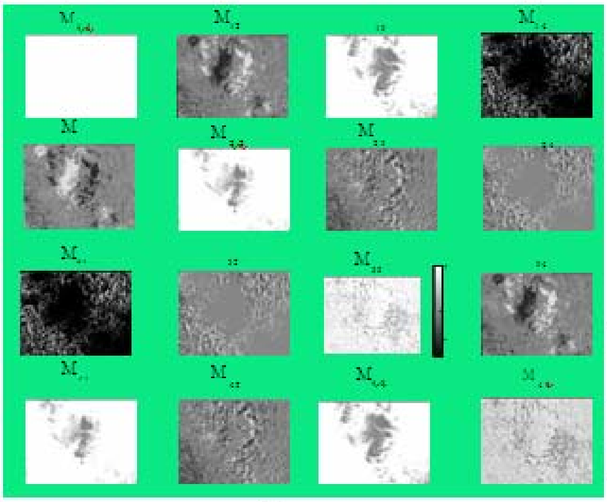
Fig. 1. Experimental setup for measurements of transmitted Mueller matrix elements.

Table : 2

Items	Quantity
Protein	100 mmol/l
Sodium	90 mmol/l
Glucose	50 mmol/l
Ascorbic acid	75 mmol/l

3. RESULTS AND DISCUSSION

A total of 10 experiments were conducted with a 632.8 nm He-Ne laser using a hyperglycemic concentration range for both the glucose-doped water and aqueous humor media. The solution concentrations are illuminated to determine the glucose level in aqueous humor. Optical images show the output intensity and polarization states for different solution concentration. The decrease in the intensity of aqueous humor solution is due to its birefringent behavior. In this measurement we used a linear light source.



(a)

1.04	0.502	0.258	-0.059
0.643	0.750	0.532	0.541
0.485	0.024	0.325	0.321
0.0653	0.325	-0.036	0.065

(b)

Fig. 2. (a) The 2 dimensional images of Mueller matrix transmitted intensity elements for aqueous humor at 4.5 mmol/l glucose concentration.

(b) Mueller matrix data for a transmitted polarized laser beam from aqueous humor at 4.5 mmol/l glucose concent

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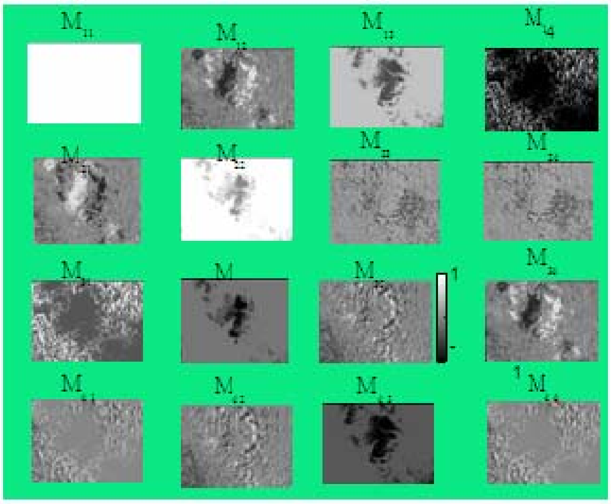


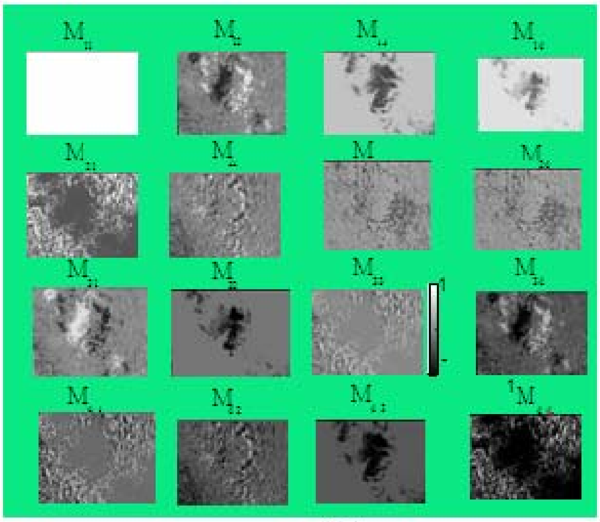
(a)

1.023	0.502	-0.258	0.059
0.583	0.651	0.832	0.061
0.385	0.324	0.519	0.321
0.0653	0.325	-0.136	0.509

(b)

Fig. 3. (a) The 2 dimensional images of Mueller matrix transmitted intensity elements for aqueous humor at 6.1 mmol/l glucose concentration.

(b) Mueller matrix data for a transmitted polarized laser beam from aqueous humor at 6.1 mmol/l glucose concent



(a)

1.1	0.502	0.258	0.591
0.643	0.325	0.502	0.511
0.485	0.024	0.065	0.321
0.0453	0.325	-0.036	-0.059

(b)

Fig. 4. (a) The 2 dimensional images of Mueller matrix transmitted intensity elements for aqueous humor at 7.0 mmol/l glucose concentration.

(b) Mueller matrix data for a transmitted polarized laser beam from aqueous humor at 7.0 mmol/l glucose concentration.

The two dimentional images of Mueller matrix corresponding to transmitted intencity (Figure 2, 3 and 4) of the glucose concentration are taken for aqueous humor solution by a polarimetric setup and the resultant matrix provids comprehensive details about the sample. The M_{11} element of the matrix shows the direct intensity pattern and the characteristic of the illuminating light. The other matrix is the result of linear and circular polarization output. These images and the matrices were obtained by using 49 different orientations, and the measurements of the polarized optics are given in Table 1. Mueller matrices M_{22}, M_{33} and M_{44} are unitary and represent the horizontal, vertical, ±45, L-circular and R-circular polarization. The diagonal matrix of the Mueller train can estimate the glucose concentration in aqueous humor. The Mueller matrix value of M_{23} and M_{41} increase with the concentration of glucose in aqueous humor shown in fig.2, and fig.3, but a further increase in concentration of glucose in aqueous humor decreases the value of Mueller matrix M_{23} and M_{41} shown in fig.3, and fig.4. It is Concluded thatthe change in polarization states of transmitted photon is due tothe variation of glucose concentration in aqueous humor. Similarly, continuous variation of M_{24} and diagonal Mueller matrix in fig.2, fig.3, and fig.4, can be used to differentiate the glucose concentration in aqueous humor.

4. CONCLUSION

We demonstrate transmitted photon characterization and develop an analytical experimental model of aqueous humor characterization technique with different glucose concentrations to distinguish between normal glucose level, impaired fasting glycaemia (IFG), impaired glucose tolerance (IGT). This paper presents the use of a polarized incident beam and the analysis of various polarization components in transmitted light, to observe a 2-dimensional intensity pattern. However by introducing the stokes –vector and Mueller matrix concept, we provide a framework to select subsets of measurement that comprehensively describe the optical properties of transmitted media. All 16 elements can describe the optical properties of medium in terms of intensity and polarization states .The detailed experimental work shows Mueller matrix approach for analyzing glucose concentration in aqueous humor by transmitted light and its potential for use in biomedical diagnostics.

The comparative study of optical images at different glucose levels (Fig. 2, Fig. 3, and Fig. 4) shows that, as the glucose concentration in aqueous humor increases, the intensity of diagonal matrix of Mueller train at different glucose level decreases. Fasting plasma glucose should be below 6.1 mmol/l. Fasting levels between 6.1 and 7.0 mmol/l are borderline of impaired fasting glycaemia (IFG), and fasting levels repeatedly at or above 7.0 mmol/l are diagnostic of diabetes. It can be assumed that material in the sample will also possess a unique optical rotation spectrum.

5. FUTURE PLANS AND IMPROVEMENTS

The size of the experimental design can be reduced by the recent developments in micro and nano fabrication technologies. Consequently, after miniaturization, this non invasive glucose sensor can be used to monitor glucose concentrations in diabetic patients regularly. This device can also be used to determine optical properties of various biological phantoms using software programs. This design may prove to be quick, simple, sensitive, and easy to handle.

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