Correlation between glucose concentration and reduced scattering coefficients in turbid media using optical coherence tomography

R. Poddar[1,2], S. R. Sharma[1], J. T. Andrews[1,3,*] and P. Sen[4]

[1]Department of Applied Physics, and [2]Department of Biotechnology, Birla Institute of Technology, Mesra, Ranchi 835 215, India
[3]Present address: Department of Applied Physics, Shri G.S. Institute of Technology and Science, Indore 452 003, India
[4]Laser Bhawan, School of Physics, Devi Ahilya University, Indore 452 017, India

Noninvasive, non-contact and *in vivo* monitoring of blood glucose is a long-needed pathology tool for saving patients from the recurring pain and hassle that can accompany conventional blood glucose testing methods. Optical coherence tomography known for its high axial resolution imaging modality is adopted in this article for monitoring glucose levels in tissue-like media, noninvasively. Making use of changes in reduced scattering coefficient due to refractive-index mismatch between the extracellular fluid and the cellular membranes and armed with a theoretical model, we establish a correlation between the glucose concentration and reduced scattering coefficient. The scattering coefficients are extracted from the deconvoluted interference signal using Monte Carlo simulation with valid approximations. A program code using NI LabVIEW™ has been developed for automation of the experiment, data acquisition and analysis.

Keywords: Blood glucose, diabetes, light scattering, noninvasive measurement, optical coherence tomography.

THE method of optical coherence tomography (OCT) was first introduced by Huang *et al.*[1]. It has been widely applied to medical imaging and diagnostics. OCT has the capability to acquire two- and three-dimensional tomographic images in biological tissues. These applications are limited by penetration depth, cross-sectional area, dynamic range and signal-to-noise ratio (SNR). However, it has been successfully applied to transparent ocular organs, where light scattering is minimum. Multiple scattering, which becomes dominant at large depths, is the fundamental limitation preventing OCT from reaching a large probing depth in turbid media[2]. OCT is widely used as a biomedical imaging modality. We extend this idea to a new direction where tomographic (cross-sectional) imaging is not the prime goal; instead we measure the optical properties of stratified media with better accuracy.

The optical properties themselves can potentially provide information to monitor tissue metabolic status or to diagnose diseases. Optical approaches to study turbid media in the presence of chiral[3] components have generated interest because of their potential use in noninvasive glucose monitoring for diabetic patients. Light scattering occurs in tissues because of the mismatch of index of refraction between the extracellular fluid (ECF) and the membranes of the cells composing the tissue. In the near-infrared region (NIR)[4–6], the index of refraction of the ECF is $n_{ECF} \approx 1.348–1.352$, while the index of refraction of the cellular membranes and protein aggregates is in the range $n_{cell} \approx 1.350–1.460$. It is well known that adding sugar to water increases the index of refraction of the solution. Similarly, adding glucose to blood in turn raises the refractive index of the ECF, which will cause a change in the scattering characteristics of the tissue as a whole. Hence, tissue glucose levels are correlated with scattering coefficients based on changes in the refractive index of the ECF. Of late, measurements on light scattering by blood show promising correlation between blood glucose and reduced scattering coefficient[7]. On the other hand, light scattering is not influenced by the red blood cells and other chemical composition of blood. At the same time, monitoring of glycemic status in patients with diabetes requires determination of blood glucose concentration. Significant efforts have been made by several groups in the past few decades to develop a biosensor for noninvasive blood glucose analysis.

Different optical approaches were proposed to achieve this goal. These approaches include polarimetry, Raman spectroscopy, NIR absorption and scattering, and photoacoustics[8–12]. Although these techniques are promising, they have limitations associated with low sensitivity, accuracy and insufficient specificity of glucose measurements at physiologically relevant levels. Larin and co-workers[13,14] have proposed a possible change in slope of the OCT signal due to changes in glucose in blood by OCT. However, the signal analysis procedure is quite complex and less accurate. They used a linear fit model to deduce OCT slope using the least-squares method. We employ a three-step procedure which is entirely different from their technique: (i) The OCT signal is deconvoluted from the source function. (ii) Extraction of optical properties of the

*For correspondence. (e-mail: jtandrews@sgsits.ac.in)

turbid media using Monte Carlo simulations with few valid approximations. (iii) Interpreting the changes in glucose concentration from the measured optical parameters.

Some theoretical models were developed to understand the governing physical process and to better interpret the OCT signal in highly scattering media. Pan *et al.*[10,15] established the relationship between the path-length resolved reflectance signal and the OCT signal using linear system theory. Monte Carlo technique was employed to simulate the path-length resolved reflectance, but it could not separate the effects of the singly scattered light and the multiply scattered light[16]. The OCT signal was split into: (i) summation of singly backscattered light (coherent) and (ii) multiply scattered light (partially coherent). The effect of multiple scattering on the formation of speckle patterns and the degradation of image contrast was demonstrated. In reality, light scattering in turbid media is a complex process, and it is only an approximation to assume that the OCT signal is from single backscattering alone. Photons still contribute to the OCT signal after a limited number of scattering events. The multiple scattering effects are clearly demonstrated in terms of the spreading of the point spread function (PSF).

In the present article, we employ OCT to monitor the reduced scattering coefficient for different values of glucose concentration in an aqueous solution with Intralipid as the scatterers. Monte Carlo simulation technique with valid approximations was adopted to understand the contribution of the multiple scattered light obtainable from OCT. The experimental observations are supported by the theoretical analysis based upon the transport theory. A strong correlation between reduced scattering coefficient and glucose concentration has been established. Maximum measurement error of 5% was observed at hypoglycemic range.

Theory

Michelson interferometer is the basis of any OCT set-up (as schematically shown in Figure 1). A motor-controlled reference mirror and sample with focusing assembly were kept in the two arms of the interferometer. Light scattered from the sample arm and light reflected from the reference arm interfere and were detected by a photodiode. The Doppler frequency generated by constant scanning speed of the mirror was modulated by a coherence function of the low coherent light source. A bandpass filter centred at the Doppler frequency acted as a coherence gate for signal detection. The sample in the OCT set-up was a turbid medium such as tissue, plants, composites, etc. Light scattered from a turbid medium may be broadly classified under two categories, viz. (i) the least scattered light, which undergoes only single or very little scattering, and (ii) diffusely scattered light, which undergoes multiple scattering events. From the principle of classical theory of scattering, least scattered light maintains coherence, whereas multiply scattered light loses coherence. Due to finite width of the coherence gating employed in the OCT, multiple scattered light with path-length difference falling within the coherence length of the source was detected. The optical irradiance at the detector was the superimposition of all the light fields reflected from within the scattering sample and the reference mirror and is given by,

$$I_{\mathrm{d}}(\tau) = \left\langle \left| \int_{L_{\mathrm{s}}}^{\infty} E'_{\mathrm{s}}(t, L_{\mathrm{s}})\mathrm{d}L_{\mathrm{s}} + E_{\mathrm{r}}(t, \tau) \right|^2 \right\rangle, \quad (1)$$

where τ [$= (L_{\mathrm{s}} - L_{\mathrm{r}})/c = \Delta L/c$] is the time delay corresponding to the round-trip optical path length between two beams and L_{s} [$= L_{\mathrm{s}0} + \sum_{i=0}^{\infty} L_{si}$] takes care of the round-trip path length to the sample surface and the total path length within the sample that accumulates during each scattering. $E'_{\mathrm{s}}(t, L_{\mathrm{s}})$ is the path length resolved field intensity. The first part of eq. (1) is important for the present study since it contains information regarding optical properties of turbid media.

The OCT signal obtainable from a turbid medium is a convolution of path length resolved diffuse reflectance (arises from turbidity of the media) and the low coherence function arises from the coherence property of the laser source. Accordingly, the OCT signal may be rewritten as,

$$I_{\mathrm{d}}(L_{\mathrm{r}}) = 2\sqrt{I_{\mathrm{s}} I_{\mathrm{r}}}[R(L_{\mathrm{s}})^{1/2} \otimes C(L_{\mathrm{s}})], \quad (2)$$

where $I_{\mathrm{d}}(L_{\mathrm{r}})$ is the OCT signal detected by a photodiode, I_{s} and I_{r} are the signals from the sample and reference arms respectively, $R(L_{\mathrm{s}})$ is the path length resolved diffuse reflectance and $C(L_{\mathrm{s}})$ is low-coherence function of the source. In order to extract the scattered light ($R(L_{\mathrm{s}})$) from

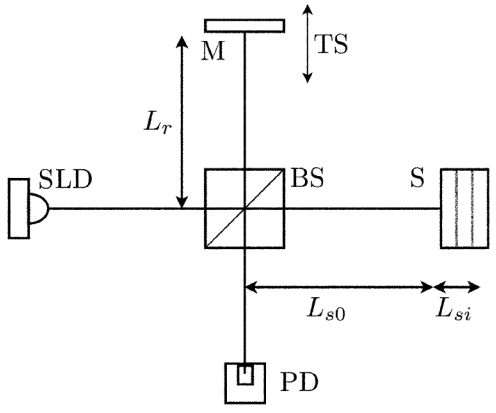

Figure 1. Schematic of the experimental set-up. SLD, Superluminescent diode; M, Mirror; BS, Beam splitter cube; TS, Stepper motor-controlled translation stage; PD, Photodiode; S, Sample.

I_d, it is deconvoluted with the coherence function $C(L_s)$. The scattering coefficients are obtained by fitting the deconvoluted signal with the following equation obtainable from transport theory

$$\nabla^2 U_d(r) - K_d^2 U_d(r) = -\frac{3}{4\pi}\rho\mu_{tr}P_0\delta(r). \quad (3)$$

Here $U_d(r)$ is the average diffuse intensity, P_0 is the total radiating power and K_d $(= \sqrt{3\rho^2\mu_a\mu_{tr}})$ is a fluid constant which varies with concentration of the fluid. Here, μ_a, μ_s and μ_{tr} are the absorption, scattering and transport coefficients respectively, and ρ is the number density of the scatterers.

Light scattering in turbid media depends strongly on the value of anisotropy parameter g, which is the average cosine of the scattering angle. Total forward scattering occurs for $g = 1$, while isotropic scattering occurs for $g = 0$. In tissue-like media the light scattering is generally highly forward peaked since the anisotropy parameter $g \geq 0.8$. Since the OCT signal composed of backscattered photons, the scattering coefficient is replaced with the reduced scattering coefficient

$$\mu_s' = (1 - g)\mu_s,$$

instead of the conventional scattering coefficient, to take care of changes arising due to anisotropy in the system. Also, the wavelength of the light source can be appropriately chosen[17] to minimize the light absorption such that $\mu_a^2 << \mu_s^2$. Accordingly, the transport coefficient is now redefined in terms of the absorption and scattering coefficients as

$$\mu_{tr} = \mu_a + (1 - g)\mu_s = \mu_a + \mu_s'. \quad (4)$$

After simplification, the solution of eq. (3) is found to be

$$\frac{r}{r_0}\frac{U_d(r)}{U_{d0}(r_0)} = \exp[-K_d(r - r_0)]. \quad (5)$$

From eq. (5) and radial distribution of diffuse light relative change of scattering coefficient and glucose concentration can be measured.

Experiment

We use an IR superluminescent diode (SLD) emitting at 810 nm (Hamamatsu) as the light source. The wavelength of the light source was appropriately chosen such that tissue absorption can be ignored[18]. The scattered light was detected by a photodiode (Hamamatsu) and the signal was measured using a lock-in amplifier (EG&G 7265).

The motor-controlled stages, data-acquisition system and the lock-in amplifier were controlled through a PC. After deconvolution of the OCT signal (I_d) with reference to the source function ($C(L_s)$) of light source, diffuse reflectance signal ($R(L_s)$) was obtained. It was further used with the transport theory to calculate the reduced scattering coefficient of the turbid media. The data collected for different concentrations of glucose were analysed and the scattering coefficient estimated for each measurement with valid Monte Carlo approximations[19].

Sample description

Glucose solutions prepared with different concentrations were mixed with turbid media (Intralipid™ 0.1 v/V, values of scattering coefficient and anisotropy parameter used for the tissue phantom being 50 cm^{-1} and 0.8 respectively) and used as sample. The exact values of the glucose concentration used in experiment ranged from 20 to 2000 mg/dl to establish the correlation between the hypoglycemic stage (≤64 mg/dl) and hyperglycemic stage (≥100 mg/dl). One should note here that the normal blood glucose level range is 64–100 mg/dl. The time allotted for settlement and interaction of glucose with Intralipid was 2 min, as addition of glucose takes some time to change the scattering properties of the medium.

Results and discussions

In order to carry out the simulation a program code was written using LabVIEW™ with 1000 iterations (with option for N number of iterations). The program window is shown in Figure 2. The program makes use of eqs (2)–(5) for simulation and data-fitting. The method adopted by Larin and co-workers[13,14] to monitor glucose concentration in tissue phantom, provides tomographic images

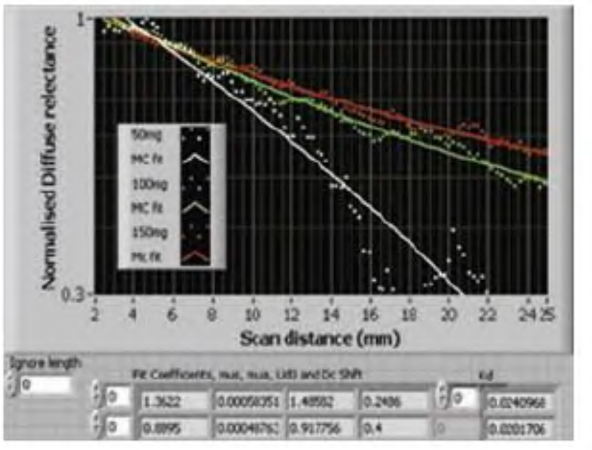

Figure 2. Front panel of Monte Carlo simulation program written using LabVIEW™. Dotted curves are obtained from experimental data while the solid lines are fit data using simulation. The simulation constants are displayed at the bottom. The results are displayed after 1000 iterations (optimized).

(2D), which were averaged to 1D distribution of light in depth. The 1D distributions were plotted in a logarithmic scale to find the slope of the distribution at different depths using least squares method. This led to less accurate results with large error in measurement. We adopted a different direction, where the OCT signal was deconvoluted from the source function of the SLD. The deconvoluted signal was fitted using MC simulation with the PSF as defined earlier. The following parameters were estimated from the program code: μ_s', μ_a and fit constants arising due to experimental dark noise and background noise.

The OCT signals obtained for different concentrations of glucose are exhibited in Figure 3 for a fixed concentration of Intralipid. With increase in glucose concentration, the amplitude of the OCT signal decreases[16]. Also, one

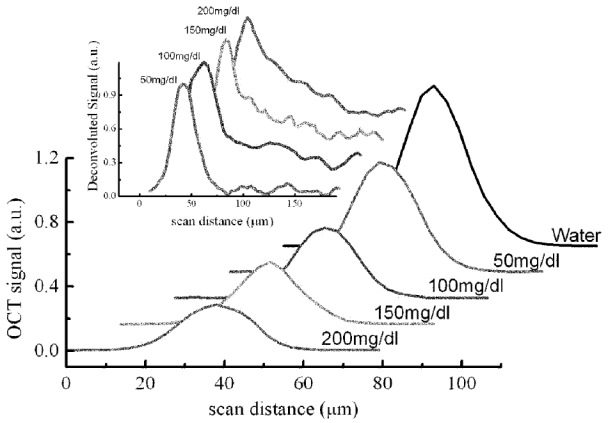

Figure 3. OCT signal for different glucose concentrations. For higher concentrations the amplitude of the signal decreases while the width of the curve increase. (Inset) Deconvoluted signals. The OCT signal obtained for water is taken as the source coherence function. The deconvoluted signals are normalized.

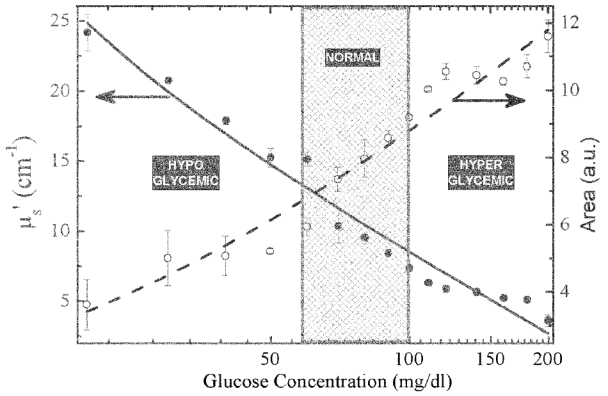

Figure 4. Semilog plot of reduced scattering coefficient and curve area with glucose concentration. Data were extracted from Monte Carlo simulation and fitted to the experimental data. The error bars were obtained after 25 measurements.

can notice that the Gaussian width of the curve increases. However, one cannot extract much information about the scattered light from these curves, since a strong coherence function from the low coherent light source is convoluted on the scattered signal. The coherence data obtained from an OCT signal of the non-scattering medium like water was used as the source function ($C(L_s)$). Other OCT signals obtained for different concentrations of glucose are deconvoluted and shown in Figure 3 (inset). The deconvoluted signals contain more information about light scattering. With increasing concentration the area as well as the tail part of the signal increase. This is a clear indication of increasing value of reduced scattering coefficient with decreasing glucose concentration.

The measured scattering coefficients obtained after Monte Carlo simulation are depicted as a semilog plot in Figure 4. The curve (solid circles) exhibiting the nature of reduced scattering coefficient for different concentrations of glucose indicates a near logarithmic nature. In the hypoglycemic region it shows a sharp change, while in the hyperglycemic region it has a smaller slope leading to lesser accuracy. This is also observed while measuring the area of the deconvoluted signal (open circles), and it also experiences a near logarithmic behaviour. Repeated measurements show that the slope of the curves is a constant. Also, the amount of Intralipid added does not change this behaviour. However, in order to find the value of the unknown concentration of glucose, one needs an initial value at a particular concentration of glucose. Since the slope remains a constant, this method could be better for noninvasive, non-contact, *in vivo* monitoring of blood glucose concentration. In order to realize this technique as a clinical tool, more efforts are required.

Conclusion

To conclude, armed with light scattering technique and OCT, we made an attempt to study the correlation between glucose concentration and reduced light scattering coefficient, which is better and accurate compared to that by Larin and co-workers[13,14]. A semilog plot of glucose concentration with reduced scattering coefficient suggests a linear relation. The value of reduced scattering coefficients are determined using Monte Carlo simulation. The technique promises to be a successful clinical tool for noninvasive and non-contact monitoring of blood glucose. However, to predict the exact value of blood glucose without feeding any initial parameter, a large number of trials are needed to calibrate the equipment.

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