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Glucose sensing in the anterior chamber of the human eye model using supercontinuum source based dual wavelength low coherence interferometry

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ABSTRACT

Supercontinuum source based dual wavelength low coherence interferometry (DWLCI) technique is proposed to measure glucose in the aqueous humor of the eye. Studies were conducted in the second overtone region, first overtone region and combination band to determine the favourable wavelength region for glucose measurement. Bandpass filters at centre wavelengths 1300 nm, 1580 nm, 2100 nm and 2250 nm were used to filter out appropriate bands of interest for the study from the broad wavelength range of 900–2800 nm. In the studies, human eye model was used to simulate the anterior chamber of the eye containing artificial aqueous humor with varying physiological concentrations of glucose ranging from 0 to 250 mg/dl. Studies based on spectral shift measurements and relative reflectivity were conducted. Enhancement in the resolution of glucose was obtained in the combination band at 2100 nm compared to the first overtone region at 1580 nm, due to the increase in the absorption of glucose in the combination band. Glucose resolution of ≈10.3 mg/dl was estimated in the first overtone region. Whereas, an enhancement in the resolution of glucose of ≈2.4 mg/dl was attained in the combination band.

1. Introduction

Effective glucose monitoring is essential for curtailing the mortality rates related to diabetes which affects multiple organs of the body causing several health complications such as diabetic retinopathy, neuropathy, nephropathy, cerebrovascular and cardiovascular diseases [1–3]. Conventional method of glucose measurement is invasive and requires puncturing the finger with a lancet to squeeze out a drop of blood for measurement. As it is required to monitor the glucose level several times per day, there is an increased risk of infection along with pain and patient noncompliance due to the discomfort caused by finger-prick [4]. Apart from blood, glucose sensing in other biological fluids such as interstitial fluid, tear, saliva, aqueous humor and sweat is being carried out [5].

Electrochemical approach for detecting glucose in the tear was reported. It was an invasive procedure wherein a miniaturized glucose oxidase electrode was inserted into the canaliculus of the lachrymal gland. A detection limit of 8 μM was achieved. Its invasive nature and biocompatibility issues are the main limitations [5,6]. Polymer crystalline colloidal arrays based optical approach for detecting glucose in tear was reported [7]. Optical response of the material while binding with glucose was detected at 1725 nm using a UV-VIS-NIR spectrophotometer. A detection limit of 6.1 μg/dl was reported [5]. Boronic receptor based sensor needs to be integrated with a contact lens for real time analysis. Its binding activity is dependent on the pH of tear. One of the limitations of implantable biosensors is biofouling, resulting in the improper functioning of the implanted biosensors [5,7].

Minimally invasive devices based on continuous glucose monitoring methods are developed in order to replace the invasive finger-prick method. Minimally invasive devices include needle type enzyme electrodes implanted subcutaneously to measure glucose levels in the interstitial fluid. Reverse iontophoresis is a minimally invasive technique used to measure glucose in the interstitial fluid of the dermis region. It requires application of electrodes to the skin. Inflammatory response due to biocompatibility issues, fluctuations in perspiration and temperature, motion artifacts caused due to breathing, body movements and blood flow are the limitations of reverse iontophoresis [5,8]. Infection, biocompatibility, long term stability, miniaturization, specificity and accuracy in measurements are the main concerns related to minimally invasive continuous glucose monitoring devices [9].

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On the other hand, extensive research on developing non-invasive continuous glucose monitoring system based on optical methods such as Raman spectroscopy, optical polarimetry, absorption spectroscopy, optical coherence tomography, photo acoustic spectroscopy is being carried out by several researchers [9–11]. Wearable sensors for detecting glucose are also being developed by serveral research groups [11,12]. A combination of dielectric spectroscopy for detecting conductivity of cell membrane based on changes in glucose level along with optical sensors are developed as a wearable multi-sensor glucose monitoring system [13]. Currently there are products being developed which detect glucose level based on the physical activities performed by integrating acceleration, position, temperature and sweat in the algorithms used to estimate glucose levels [14]. This may not benefit diabetic patients with poor physical condition [5]. Non-invasive measurement of acetone, one of the biomarkers for diabetes was carried out from the exhaled breath, using ultraviolet (10.6 eV) based differential mobility sensor [15,16]. Limitation is that the biomarkers are not specific for diabetes alone but for other complications as well. Hence it is required to test multiple biomarkers to diagnose a particular disease so as to improve the selectivity.

Glucose measurement in the artificial aqueous humor of the human eye was conducted using Raman spectroscopy at 785 nm in combination with partial least square model [17]. Although highly specific absorption bands due to reduced interference from water compared to NIR and MIR spectroscopy can be obtained with Raman spectroscopy, requirement of high power laser, highly sensitive detectors to measure weak signals and longer acquisition time are the limitations of Raman spectroscopy for real time monitoring [10,11]. In-vivo optical polarimetric method of glucose sensing based on polarization rotation of visible light in the anterior chamber of the eye of New Zealand rabbits using Helium Neon laser at 632.8 nm was reported [18]. However, the limitations associated to polarimetric method are the motion artifacts due to respiration, motion induced corneal birefringence, difficulty in coupling the light laterally through the anterior chamber of the eye, as well as confounding results due to the presence of other optically active components such as ascorbate and albumin in the aqueous humor of eye [7,9].

Optical spectrosopic techniques in the near infrared region (NIR) and mid infrared region (MIR) are also widely researched. Glucose has broad absorption bands in the near infrared region. NIR (750–2500 nm) spectroscopy studies can reach from 1 to 100 mm deep within the tissues, whereas, MIR (2500–10,000 nm) spectroscopy has a limitation of light penetration only upto a few micrometers within the tissues. Compared to the first overtone region of wavelength range from 1500 nm to 1800 nm, glucose absorption is expected to be 5 times higher in the combination band of 2000 nm to 2400 nm with significant absorption bands at 2123 nm, 2272 nm and 2325 nm [19]. In-vitro glucose measurement in aqueous solution performed based on balanced arm spectroscopy method was reported. Conventional tungsten or halogen lamps used in infrared spectroscopic techniques were replaced by supercontinuum source (wavelength extending from 1500 to 2400 nm) in order to improve the signal to noise ratio of the system [20]. These methods depend on bulk tissue optical properties and lack layer specific information from the sample by evading the interference from surrounding tissues, which is possible by optical coherence tomography.

Optical coherence tomography (OCT) is a non-invasive, low coherent interferometric technique that provides information from a specific layer within the sample based on the difference in the refractive index of the interfaces within the sample [21,22]. It is clinically employed in ophthalmology for diagnosing vascularization, various layers of retina, and pathological conditions of iris in the anterior chamber of the eye [23]. Time domain OCT for measurement of glucose by indirect measurement of scattering coefficient are reported [24]. Limitations are the motion artifacts caused due to translation of the reference mirror and the presence of fat, protein and water which also amounts to scattering. Hence enhancement in selectivity and specificity are the main concerns.

In order to measure glucose in-vivo, it is essential to selectively detect glucose from various interferants. Differential absorption based on dual wavelength approach proposed in this technique promotes selective detection of glucose with an enhanced resolution of glucose in the combination band around the wavelength range from 2100 to 2300 nm. Differential absorption based OCT for measuring water concentration at 1312 nm and 1488 nm, from human cornea was reported [24]. Time domain low coherence interferometry technique based on differential absorption approach was carried out using SLED sources at wavelengths 1625 nm and 1310 nm to measure glucose in a non-scattering medium of the anterior chamber of the eye, considering iris as a reflector. A resolution of 26.8 mg/dl was reported in eye model and a resolution of 69.6 mg/dl was reported in pig eye experiment [25].

Frequency domain differential absorption optical coherence tomography (FD-DAOCT) technique using superluminescent light emitting diode (SLED) to measure glucose of concentration ranging from 0 to 4000 mg/dl in skin phantoms of concentration 2%–10% intralipid was also reported [26]. Such studies were also conducted in a scattering medium constituting intralipid of concentrations 0.25% and 0.5% to mimic the optical properties of oral mucosa and glucose resolution of 15 mg/dl in 0.25% and 19 mg/dl in 0.5% intralipid was reported at wavelengths 1589 nm and 1310 nm using superluminescent light emitting diode (SLED) source [27]. For multi-wavelength measurements, a single supercontinuum light source is also suited as it provides continuous and broadband near infrared output of few hundreds of nanometer width. The source is robust and no tuning mechanism is involved. Further studies were also reported on sensing glucose from ex vivo human gingival tissue using supercontinuum source based FD-DAOCT technique at 1300 nm and 1580 nm with a resolution of 23 mg/dl [28]. Advantages of frequency domain LCI over time domain LCI are the faster scanning time, improvement in the resolution of glucose, access to spectral features and elimination of motion artifacts due to stationary components [24–27].

In this study, supercontinuum source based differential absorption glucose measurements in the aqueous humor of the eye model have been performed and a comparison at multiple wavelengths at 1300 nm (where glucose absorption is insignificant), 1580 nm, 2100 nm and 2250 nm (where glucose absorption is prominent) have been analysed. Measurement of glucose from the aqueous humor of the eye using the frequency domain - dual wavelength low coherence interferometry (FD-DWLCI) technique is advantageous because it is non-invasive, non-contact, does not require puncturing of the skin to draw out blood, ease in accessing aqueous humor of the eye and does not necessitate placement of any sensors in the eye. Dual-wavelength approach in a combination band around 2100 nm is expected to improve selective measurement of glucose. Supercontinuum source is employed for this study due to its simplicity in switching wavelengths with a bandpass filter rather than using multiple SLED sources of different wavelengths [28–30]. Studies have been conducted in the combination band in order to attain enhancement in the resolution of glucose. Improved selectivity in measuring glucose in the presence of interfering constitutents, mainly water has been attained by dual wavelength approach.

The proposed technique is a non-contact and a non-destructive method of sensing glucose by both spectral shift measurements and layer wise reflectivity studies. Spectral shift induced by the refractive index change with increase in the concentration of glucose from 0 to 250 mg/dl in steps of 50 mg/dl was measured from the interference signal obtained using low coherence interferometry at wavelengths 1300 nm, 1580 nm and 2100 nm, individually. Whereas, ratio of relative reflectivity based on the attenuation of light by varying the concentrations of glucose from 0 to 250 mg/dl was estimated from the depth resolved axial scan obtained from the interference signal for λ_{ON} (1580 nm, 2100 nm and 2250 nm) and λ_{OFF} (1300 nm). First part of Section 2 explains the transmission studies conducted with water and glucose in aqueous humor mimicking solution and the second part

includes the explanation of the dual wavelength low coherence interferometry method. In Section 3, the principle of dual wavelength low coherence interferometry is explained. In Section 4, the results obtained based on spectral shift measurements and reflectivity studies in the second overtone region, first overtone region and the combination band are discussed.

2. Material and methods

2.1. Transmission spectroscopy

Transmission studies based on transmission spectroscopy were conducted in the second overtone, first overtone and combination band using supercontinuum laser source of wavelength range 900–2800 nm. The transmission spectroscopy set up consisted of the supercontinuum laser source (Leukos-SM-250-IR), 2 mm pathlength cuvette (Suprasil 300 Quartz, Starna) filled with the sample, a microscope objective lens (Newport M-20 × 040) and a Tmc300 spectrometer (Bentham). Transmission spectrum of water (Type-1 Milli-Q) and aqueous humor mimic containing physiological concentration of glucose ranging from 0 to 250 mg/dl prepared in saline using D glucose powder (738360 Sigma-Aldrich, molecular weight: 182.17, CAS number: 18991-62-3). Artificial aqueous humor was considered for the studies with saline as the scattering medium as in the case of real aqueous humor. Preliminary studies were conducted with water in order to ascertain the significant water absorption regions. Attenuation coefficient of water was estimated from the transmission spectrum for the wavelength range of 1200–2400 nm as shown in Fig. 1(a).

Noticeable water absorption bands were observed at 1440 nm and 1940 nm. The attenuation coefficient of the artificial aqueous humor with physiological concentration of glucose estimated from the transmission spectrum at 1300 nm, 1580 nm and 2100 nm using the Beer Lambert's law, $\alpha = (1/l) \times \ln(I_0/I)$, where, α is the attenuation coefficient (absorption + scattering) (mm^{-1}), l is the pathlength (mm), I_0 and I are the incident and transmitted intensity, respectively, was estimated as shown in Fig. 1(b). An increase in the attenuation coefficient with increase in the concentration of glucose was observed at 2100 nm due to the higher absorptivity of glucose at 2100 nm compared to 1580 nm and 1300 nm. Water absorption is significant at wavelengths, 1440 nm and 1940 nm [19]. Nevertheless, comparatively lower water absorption is observed in the glucose absorption bands around 1580 nm (first overtone) and 2200 nm (combination bands). Hence, these wavelength ranges were considered suitable for glucose measurements.

2.2. Dual wavelength low coherence interferometry

The anterior chamber of the eye model was designed and fabricated in the laboratory as shown in Fig. 2(a). The eye model was fabricated using an optical dome (N-BK7 Glass, Edmund Optics) of 25 mm diameter, outer height of 11.5 mm and a thickness of 1 mm, allowing NIR light to pass through, representing cornea. The glass dome was fixed on a holder with an inner diameter of 22 mm and an outer diameter of 45 mm, consisting of three M3 screws. Glass dome along with the holder was clamped to an elevated flat dome of 8.85 mm height, in order to obtain a path length of 1.65 mm, representing the anterior chamber of the eye (distance between the inner cornea and the iris). It was fabricated using Teflon material of 45 mm diameter consisting of a flat dome surface on top and a 25 mm diameter protrusion at the bottom in order to fix it to the sample holder of the LCI set up. The Teflon holder with the flat surface represents the iris, which acts as the reflecting surface within the anterior chamber. As the incident light passes through the eye model, reflections due to the refractive index change of structures is expected at the air/cornea interface, cornea/aqueous humor interface and aqueous humor/iris interface as described in the schematic in Fig. 2(b).

Artificial aqueous humor with glucose concentration ranging from 0 to 250 mg/dl was prepared using D glucose powder (738,360 Sigma-Aldrich, molecular weight: 182.17, CAS number: 18991–62-3) in saline and the sample was filled using a spinal needle in the optical dome that was clamped on the sample holder in the sample arm of the FD-DALCI set up as shown in Fig. 3. Supercontinuum laser source (Leukos-SM-250-IR) used as the incident light source in the studies, covers all NIR wavelengths ranging from 900 to 2800 nm with an average power of 1.3 W. The supercontinuum light source was with a pulse width of 1 ns and pulse repetition rate of 250 kHz. Light from the source through the optical fiber was collimated using a collimating lens and filtered to specific bandwidths of interest using a band pass filter. Band pass filters (Edmund Optics) with centre wavelengths at 1300 nm, 1580 nm, 2100 nm and 2250 nm, with bandwidth of 50 nm were employed in the experimental set up. Filtered light from the band pass filter was directed towards a 50/50 metallic beam splitter, which splits the light towards the fixed reference mirror and the sample placed at equal distances from the beam splitter.

Fig. 4(a) represents the source spectrum ranging from 1275 nm to 1325 nm after passing throught the band pass filter with a centre wavelength of 1300 nm, Fig. 4(b) represents the source spectrum obtained using the band pass filter with a centre wavelength of 1580 nm, with a spectral width ranging from 1550 nm to 1615 nm. The supercontinuum source spectrum is a flat spectrum except for a spike that occurs at the wavelength 1560 nm which can be seen in Fig. 4(b). The spike of the source that occurs at 1560 nm has resulted in the high power of the source spectrum with the centre wavelength 1580 nm compared to the source spectra with centre wavelengths at 1300 nm, 2100 nm and 2250 nm.

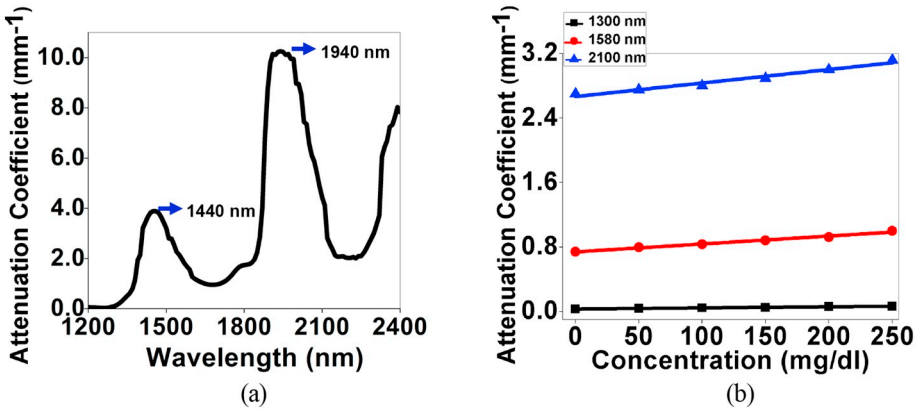

Fig. 1. (a) Attenuation coefficient of water in the wavelength range 1200–2400 nm and (b) Attenuation coefficient of aqueous humor mimicking solution containing glucose ranging from 0 to 250 mg/dl at wavelengths 1300 nm, 1580 nm and 2100 nm.

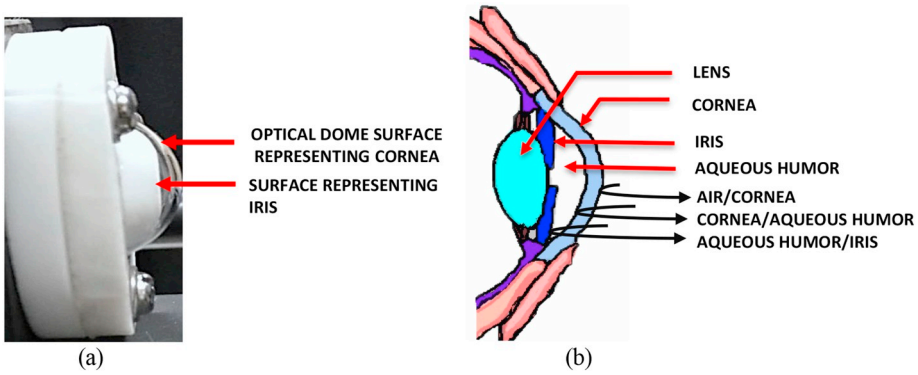

Fig. 2. (a) Eye model representing the anterior chamber of the human eye (b) Schematic representing the different structures and the reflecting surfaces of the anterior chamber of the human eye.

This peak around 1560 nm was attributed to the laser output at the fundamental wavelength. Fig. 4(c) and (d) represent the source spectrum measured in front of the sample after passing through the band pass filters with centre wavelengths at 2100 nm and 2250 nm, respectively. Suitable neutral density filter was placed in the reference arm so as to reduce the intensity of light getting reflected back from the reference mirror so that it matches with the intensity of light reflected back from the sample. A calcium fluoride focusing lens of focal length 50 mm was located on the sample arm to focus the light on to the sample.

Low coherence interferometry technique was carried out in the second overtone (around 1300 nm), first overtone (around 1580 nm) and combination bands (around 2100 nm and 2250 nm) by choosing the appropriate band pass filter based on the wavelength region of interest. When the optical path length difference of the front surface of the optical dome representing the cornea (without sample) and the reference mirror was equal, the interference signal was obtained. Neutral density filters of appropriate optical density (Holmarc) were used to reduce the intensity of the light reflected from the reference mirror to match with the intensity level of light reflected back from the sample after aqueous humor was injected into the eye model. The signal of light reflected back from the sample was obtained by blocking the reference arm and the signal of light obtained from the reference mirror was obtained by blocking the sample arm. Light reflections from both the arms were directed towards the beam splitter and focused on to the optical spectrum analyser (OSA 203C, Thorlabs) using a focusing lens of 100 mm and a single mode fiber of core/cladding diameter, 9/125 µm. When the optical path difference was equal and within the coherence length, interference signal was obtained using the optical spectrum analyser, at a resolution of 0.15 nm. Further extraction of depth information from the interference signal was performed by applying inverse Fourier transform (IFT).

3. Principle of DWLCI

Based on the Beer Lambert's law, the following equation can be used to estimate the reflected intensity from the sample medium [24,25].

$$I_s(\lambda, d) = I_o(\lambda) e^{-(\mu_{as}(\lambda)+\mu_{ss}(\lambda)+\mu_{ag}(\lambda)+\mu_{sg}(\lambda))d} \quad (1)$$

where I_s is the intensity from the sample. Considering round trip in LCI, after traveling through twice of the thickness z of the sample, $d = 2z$, λ is the wavelength of the incident light, I_o is the incident intensity, μ_{as} is the absorption coefficient of saline, μ_{ss} is the scattering coefficient of saline, μ_{ag} is the absorption coefficient of glucose and μ_{sg} is the scattering coefficient of glucose. As we consider wavelengths, λ_{ON} (1580 nm, 2100 nm and 2250 nm), where glucose absorption is prevalent and λ_{OFF} (1300 nm), where glucose absorption is trivial, the difference in the LCI equations for λ_{ON} and λ_{OFF} can be written as [25–29],

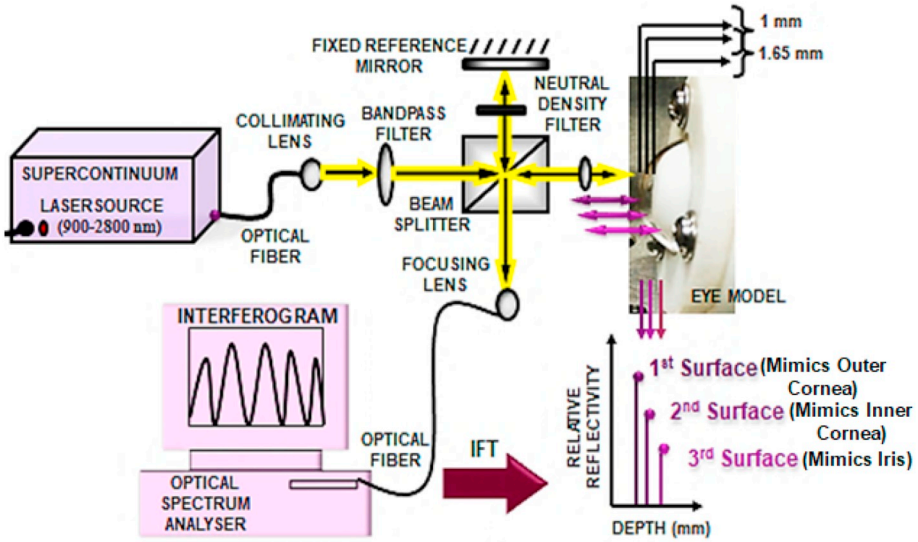

Fig. 3. Schematic of in-house developed DWLCI system.

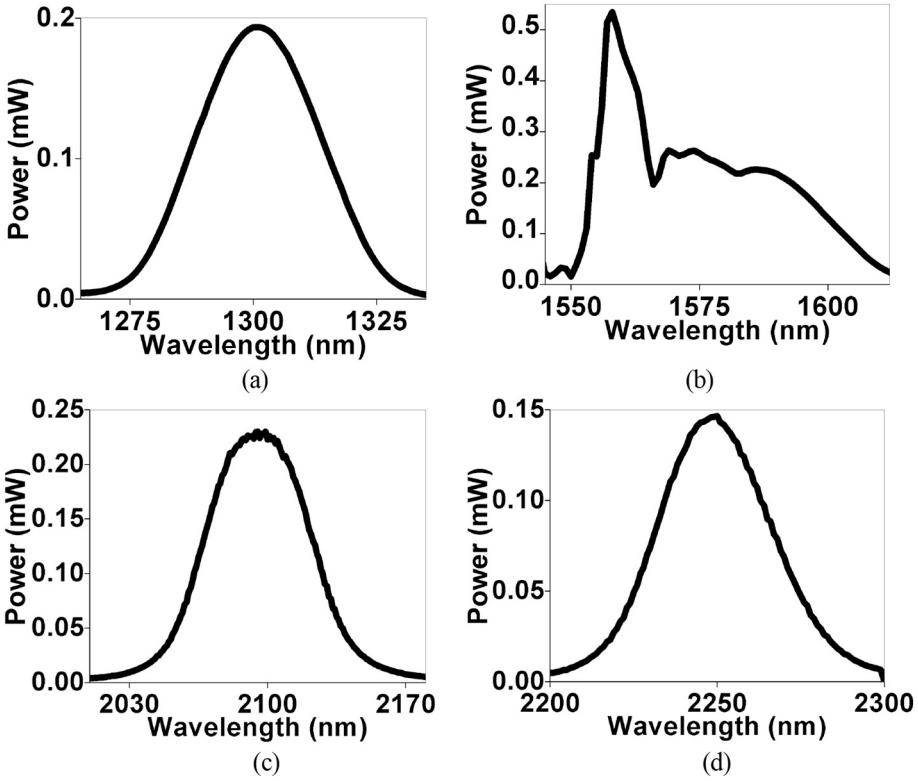

Fig. 4. Source spectrum obtained using band pass filters with centre wavelengths at (a) 1300 nm, (b) 1580 nm, (c) 2100 nm and (d) 2250 nm.

$$\ln\left(\frac{S_{LCI}(z,\lambda_{ON})}{S_{LCI}(z,\lambda_{OFF})}\right) = \ln\left(\frac{I_r(\lambda_{ON})I_o(\lambda_{ON})}{I_r(\lambda_{OFF})I_o(\lambda_{OFF})}\right) - (\Delta\mu_{as} + \Delta\mu_{ss} + \Delta\mu_{ag} + \Delta\mu_{sg})z \quad (2)$$

where, $\Delta\mu_{as}$ is the difference in the absorption coefficient of saline, $\Delta\mu_{ss}$, is the difference in the scattering coefficient of saline, $\Delta\mu_{ag}$ is the difference in the absorption coefficient of glucose and $\Delta\mu_{sg}$ is the difference in the scattering coefficient of glucose at the two wavelengths λ_{ON} and λ_{OFF}. Subsitituting the ratio of relative reflectivity obtained from low coherence interferometry studies, along with known values of difference in the absorption cross section of glucose $\Delta\sigma_{ag}$ and difference in the absorption coefficient of glucose $\Delta\mu_{ag}$, the concentration of glucose C, can be estimated as,

$$C = \frac{\Delta\mu_{ag}}{\Delta\sigma_{ag}} \quad (3)$$

4. Results and discussion

Fig. 5 shows the typical plot representing a spectral shift of ≈2.6 nm in the interference signal for the glucose concentrations 0 (red trace) and 200 mg/dl (black trace), for the wavelength range 2050–2150 nm. Spectral shift induced by variation in the refractive index, was estimated from the interference signals for varying concentrations of glucose ranging from 0 to 250 mg/dl as shown in Fig. 6. The values of slope estimated from the spectral shift measurements were ≈0.0166 nm/(mg/dl) for 1300 nm, ≈0.0152 nm/(mg/dl) for 1580 nm, ≈0.0128 nm/(mg/dl) for 2100 nm and ≈0.0125 nm/(mg/dl) for 2250 nm. Detection limit was obtained as the ratio of resolution of the detector to the sensitivity of measurement for each wavelength [27]. The detection limits estimated were ≈9.2 mg/dl, ≈9.86 mg/dl, ≈11.5 mg/dl and ≈12 mg/dl for the centre wavelengths 1300 nm, 1580 nm, 2100 nm and 2250 nm, respectively. The temperature was maintained constant under laboratory conditions throughout the studies.

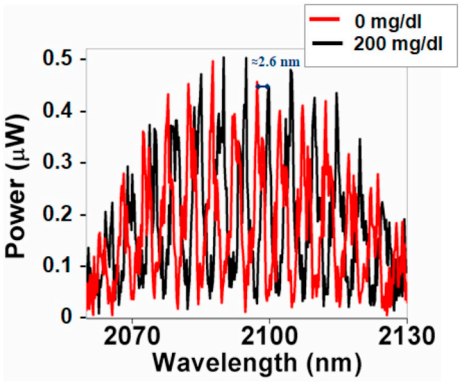

Fig. 5. A typical interference signal with spectral shift for the glucose concentrations 0 mg/dl (red trace) and 200 mg/dl (black trace) for the wavelength range 2050–2150 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

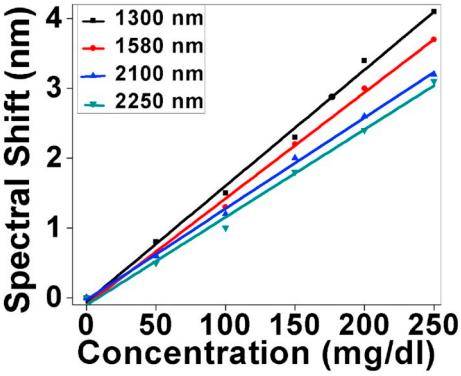

Fig. 6. Spectral shift Vs concentration of glucose for wavelengths 1300 nm, 1580 nm, 2100 nm and 2500 nm.

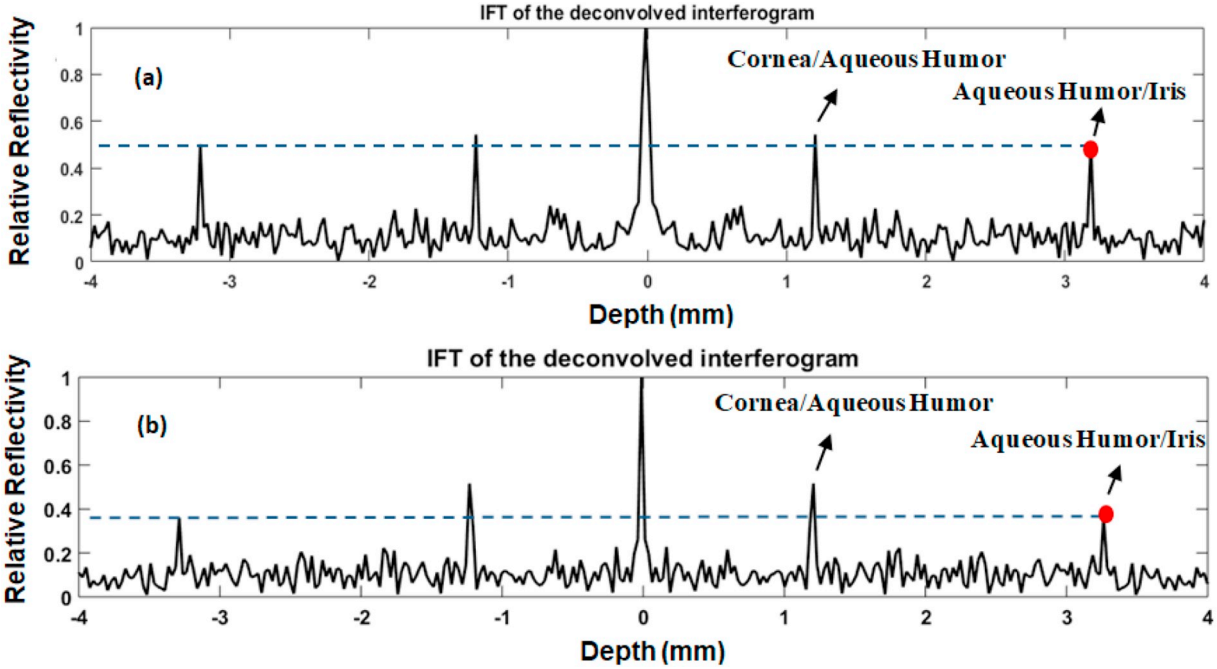

Fig. 7. (a) A typical depth scan obtained by applying inverse Fourier transform to the acquired interference signal at 2100 nm for the glucose concentration of (a) 50 mg/dl and (b) 200 mg/dl.

The change in the slope observed in Fig. 6 for various wavelengths could be explained based on the equation, phase difference, $\Delta\delta = {}^{2\pi l(n_1 - n_2)}/_{\lambda}$, where l is the pathlength, λ is the centre wavelength, n_1 and n_2 are the refractive indices for two different concentrations of glucose. As the phase difference is inversely proportional to the wavelength, a decrease in the slope of the spectral shift with increase in the wavelength could be observed as a result of refractive index change with varying concentrations of glucose. This was confirmed by estimating the phase difference for varying refractive index value corresponding to glucose concentrations for the wavelengths 1300 nm, 1580 nm, 2100 nm and 2250 nm.

In order to obtain depth information of the cornea/aqueous humor interface and aqueous humor/iris interface, inverse Fourier transform was applied to the interference signal acquired using the optical spectrum analyser at a resolution of 0.15 nm. Depth scan was obtained by applying IFT code developed in Matlab [27]. Fig. 7(a) and (b) show the typical depth scan obtained by applying inverse Fourier transform to the interference signal acquired at 2100 nm using OSA for the glucose concentrations of 50 mg/dl and 200 mg/dl, respectively.

An optical pathlength of ≈2.2 mm was obtained between the cornea/aqueous humor interface and aqueous humor/iris interface. Considering the refractive index of the aqueous humor as ≈1.33 with 50 mg/dl glucose concentration, the physical pathlength of ≈1.65 mm was estimated by dividing the optical pathlength with refractive index (using the formula optical depth = (refractive index) × (physical depth)). The relative reflectivity for various physiological concentrations of glucose was measured at the aqueous humor/iris interface. With increase in the concentration of glucose, a decrease in the relative reflectivity of ≈0.5 and ≈0.36 was observed around the wavelength 2100 nm as a result of attenuation due to glucose at the aqueous humor/iris interface for glucose concentrations of 50 mg/dl and 200 mg/dl, respectively.

Relative reflectivity was estimated at the aqueous humor/iris interface for the wavelengths 1300 nm, 1580 nm, 2100 nm and 2250 nm as shown in Fig. 8(a). The slope of the relative reflectivity was observed to be steeper at 2100 nm and 2250 nm due to increase in the attenuation of glucose compared to the wavelengths 1300 nm and 1580 nm. Although the relative reflectivity decreases with increase in the concentration of glucose for 1300 nm, the effect is attributed to scattering. Further, the ratio of relative reflectivity ($\lambda_{ON}/\lambda_{OFF}$) was estimated for λ_{ON} (1580 nm, 2100 nm and 2250 nm), and λ_{OFF} (1300 nm) as shown in Fig. 8(b).

Resolution of glucose was determined using the formula, $\partial C = (\Delta C/\Delta R) \times \partial R$ where, ΔC is the difference in glucose concentration, which is 50 mg/dl considered in the studies, ΔR is the mean difference of the ratio of relative reflectivity R, and δR is the mean value of the standard deviation in each measurement [25]. An increase in resolution of glucose of ≈2.4 mg/dl was estimated at 2100 nm, ≈6.9 mg/dl at 2250 nm and ≈10.34 mg/dl at 1580 nm, using FD-DWLCI technique. An R-square value of 0.97 was estimated for wavelengths 1580 nm and 2100 nm and 0.98 was estimated for 2250 nm. The enhanced resolution of glucose obtained at 2100 nm is due to the higher absorptivity of glucose at 2100 nm compared to 1300 nm and 1580 nm, while the scattering coefficient reduces with increase in wavelength.

An improved resolution of glucose was obtained using reflectivity studies compared to spectral shift studies as the reflectivity studies include absorption along with scattering characteristics of glucose unlike spectral shift which is purely based on the change in the refractive index. The detection limit of glucose using spectral shift method could be further enhanced by improving the resolution of the detector. The maximum permissible exposure limits at the cornea for the wavelength range 1500 to 1800 nm and for the wavelength range 1800 to 2600 nm for an exposure time from 10^{-13} to 10^{-9} s are 10^{13} W/m^2 and 10^{12} W/m^2, respectively [31]. Considering the average power of ≈0.5 mW measured using the power meter infront of the sample and a repetition rate of 250 kHz of the supercontinuum source, the pulse energy was estimated as 0.02 μJ. The diameter of the focussing spot size measured on the sample was ≈2 mm. For a source pulse width of 1 ns, the irradiance (i.e., the pulse energy per unit area) was estimated as ≈0.637 MW/m^2 which was within the recommended safe limit.

FD-DWLCI based glucose sensing would improve diabetic patient compliance in effective measurement of glucose clinically, due to its non-invasive nature, providing high sensitivity in the combination band along with its faster sensing capability using frequency domain LCI. The proposed system is advantageous for clinical application without the requirement of the test strips and lancets that need replacement after

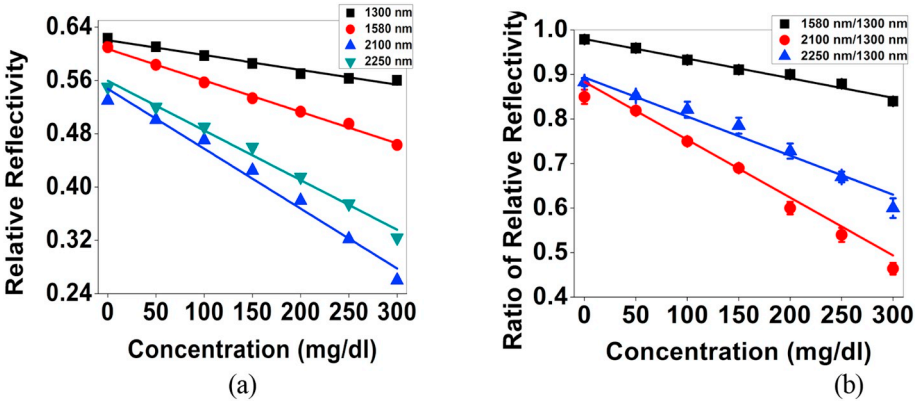

Fig. 8. (a) Relative reflectivity versus concentration of glucose for wavelengths at 1300 nm, 1580 nm, 2100 nm and 2250 nm. (b) Ratio of relative reflectivity versus concentration of glucose at λ_{ON} (1580 nm, 2100 nm and 2250 nm), and λ_{OFF} (1300 nm).

each measurement. Real-time measurement of glucose requires upgradation of the proposed free-space FD-DWLCI system into a compact and portable hand-held fiber-based system with a probe to focus the light onto the measurement site. As supercontinuum source is a broad band source, low cost broad band array multiplexers would be required to select particular wavelength range of interest for the studies in the case of fiber-based system, thereby evading the need for multiple superluminescent light emitting diode sources for measurements at various wavelength ranges. Reduced beam powers by attenuators are possible for real time analysis.Instantaneous recording and display of glucose level could be made possible with a compact low cost spectrometer connected to a mobile device with a simultaneous signal processing capability.

5. Conclusion

The viability of using a non-contact and a non-invasive FD-DWLCI technique to detect glucose in the aqueous humor of the eye model in the near infrared (1280–1320 nm), first overtone region (1560–1620 nm) and combination band (2000–2300 nm) was studied. As a preliminary step towards in vivo glucose studies which are planned for future, human eye model representing the structural features of the anterior chamber of the eye (representing cornea and iris with a pathlength of 1.65 mm, filled with artificial aqueous humor with physiological concentrations of glucose ranging from 0 to 250 mg/dl) and optical characteristics (attenuation coefficient and refractive index of aqueous humor) of real eye has been considered for the studies, providing promising results. Improvement in the sensitivity of glucose sensing was achieved in the combination band using the proposed technique. Enhancement of selectivity of glucose detection from interfering constituents, mainly water in the case of aqueous humor and measurement of glucose by a layer-wise analysis from the sample was accomplished by combining differential absorption approach with low coherence interferometry. Based on reflectivity studies, glucose resolution of ≈2.4 mg/dl was obtained at 2100 nm, which makes it a favourable wavelength for glucose detection. Resolution of glucose of ≈6.9 mg/dl and ≈10.34 mg/dl were estimated at 2250 nm and 1580 nm, respectively. Layer-wise detection capability of low coherence interferometry could also pave the way towards simultaneous glucose sensing and diagnosis of deformities in the structural layers of the eye (in the anterior chamber) of diabetic patients. Further studies could be extended towards developing a fiber based OCT system for in vivo sensing of glucose by considering ultra short pulse fiber lasers of safe power levels compared to nanosecond pulsed lasers, thereby elimating the chance of thermal effects and damage to the structures of the eye.

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Conflict of interest

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