Optical Coherence Tomography for blood glucose monitoring through signal attenuation

Lucas Ramos De Pretto[a,b], Tania Mateus Yoshimura[a,b], Martha Simões Ribeiro[a], Anderson Zanardi de Freitas[a]

[a]Center for Laser and Applications, Nuclear and Energy Research Institute, Av. Professor Lineu Prestes, 2242, Cidade Universitária, São Paulo, SP, Brazil, 05508-000; [b]Post-graduate program in Nuclear Technology, University of São Paulo, Av. Professor Lineu Prestes, 2242, Cidade Universitária, São Paulo, SP, Brazil, 05508-000

ABSTRACT

Development of non-invasive techniques for glucose monitoring is crucial to improve glucose control and treatment adherence in patients with diabetes. Hereafter, Optical Coherence Tomography (OCT) may offer a good alternative for portable glucometers, since it uses light to probe samples. Changes in the object of interest can alter the intensity of light returning from the sample and, through it, one can estimate the sample's attenuation coefficient (μ_t) of light. In this work, we aimed to explore the behavior of μ_t of mouse's blood under increasing glucose concentrations. Different samples were prepared in four glucose concentrations using a mixture of heparinized blood, phosphate buffer saline and glucose. Blood glucose concentrations were measured with a blood glucometer, for reference. We have also prepared other samples diluting the blood in isotonic saline solution to check the effect of a higher multiple-scattering component on the ability of the technique to differentiate glucose levels based on μ_t. The OCT system used was a commercial Spectral Radar OCT with 930 nm central wavelength and spectral bandwidth (FWHM) of 100 nm. The system proved to be sensitive for all blood glucose concentrations tested, with good correlations with the obtained attenuation coefficients. A linear tendency was observed, with an increase in attenuation with higher values of glucose. Statistical difference was observed between all groups (p<0.001). This work opens the possibility towards a non-invasive diagnostic modality using OCT for glycemic control, which eliminates the use of analytes and/or test strips, as in the case with commercially available glucometers.

Keywords: in vitro, diabetes, non-invasive, attenuation coefficient, optical coherence tomography, glycemic control, glycemia, mouse blood, blood dilution.

1. INTRODUCTION

According to the World Health Organization (WHO)[1], in 2008, 36 million people died due to noncommunicable diseases (NCDs). This number corresponded to 63% of all deaths in that same year. Ailments like diabetes, cardiovascular diseases (CVD), cancers and respiratory diseases account for almost 80% of all NCD deaths.

Responsible for nearly half of all NCDs deaths, CVDs and diabetes are preventable in 80% of the cases[2]. Their socioeconomic burden affects not only mortality and treatment costs, but also decreases quality of life and productivity. For an example, in 2005 Brazil lost USD 2.7 billion in the national income due to diabetes and CVDs, a financial loss that was expected to triplicate by 2015, as reported by WHO[3]. However, the International Diabetes Federation (IDF) estimates that the total diabetes-related expenditures in Brazil for that year were over USD 21 billion[4].

The Type-2 diabetes mellitus (T2DM) and CVD major risk factors are comprised in a medical condition known as metabolic syndrome (MetS). Dysglycemia, hypertension, atherogenic dyslipidemia and obesity (especially abdominal obesity) are strong predictors for the development and progression of the aforementioned diseases.

Optical Coherence Tomography and Coherence Domain Optical Methods in Biomedicine XX, edited by Joseph A. Izatt, James G. Fujimoto, Valery V. Tuchin, Proc. of SPIE Vol. 9697, 96973F · © 2016 SPIE · CCC code: 1605-7422/16/$18 · doi: 10.1117/12.2213347

The simultaneous occurrence of these risk factors is accompanied by an oxidative stress state and endothelial dysfunction[5]. Sustained high blood glucose levels, as observed in diabetic and pre-diabetic patients, greatly contributes to this scenario due to its direct relation to the impairment of vascular functions, since glucose is a highly reducing sugar and, in elevated concentrations, may form covalent links with endothelial proteins, changing their function[6, 7].

Monitoring glucose levels has, therefore, a major role on prevention and diagnosis of MetS. Currently, the most common method is the use of glucometers[8], which requires collection of blood samples through skin puncture. However, studies indicate that among the barriers to patient monitoring are the fear of needle, pain, and discomfort during the exams[9]. Thus, non-invasive approaches are of great interest to improve adherence to treatment and, also, to prevent generation of waste such as test strips. Avoiding the use of test strips also reduces the costs associated with self-monitoring, another barrier reported in the literature[9]. In the United States, in 2012, the cost for each patient was, approximately, USD 770 per year[10].

Optical Coherence Tomography (OCT) technique has been widely used for clinical applications[11], namely ophthalmology[12-15] and dermatology[16-18], and its ability to image and analyze blood samples has been previously demonstrated[19-21]. In this study, we aim to explore the effects of blood glucose levels, *in vitro*, in the attenuation of OCT signal, assessing the viability for the use of OCT as a noninvasive tool for glucose monitoring in future studies. To our knowledge, there are no reports of the total attenuation coefficient (μ_t) of *in vitro* glucose concentrations varying on a physiological range, and such was the motivation for the present work.

2. METHODOLOGY

2.1 Sample preparation

Blood samples were drawn from C57BL/6 female mice aging from 6 to 8 weeks through cardiac puncture. Animals were under deep anesthesia during the procedures, which were conducted in accordance with the Guideline for the Care and Use of Laboratory Animals[22] and were approved by the institutional animal ethics committee. Euthanasia was performed by overdose of chemical anesthetics, and death was confirmed by vital signs evaluation.

Whole blood samples were heparinized (166 IU/mL) to prevent coagulation and 90 µL aliquots were distributed, in triplicate, over a 96 wells microtiter plate. Four different groups with variations in glucose concentrations were obtained by adding different glucose solutions. To achieve the desired variations in glucose levels, four solutions of PBS (Phosphate-Buffered Saline, 1 M, pH 7.4) were prepared with increasing concentrations of glucose (D - (+) - Glucose, Sigma-Aldrich) between 16 mg/dL and 31 mg/dL, with addition steps of 5 mg/dL. Volumes of 10 µL of such solutions were added to the wells previously filled with heparinized blood, resulting in a final volume of 100 µL per well.

On a second experiment, only one glucose solution was prepared (53 mg/dL in concentration) for addition to new heparinized blood samples, since we have previously noticed a relevant time-decay in glucose concentrations after the solution was added to the diluted blood. Again, blood samples were placed in a 96-wells microtiter plate resulting in final volumes of 100 µL per well. The glucose solution was prepared with an isotonic saline solution for blood dilution. Following the study by Popescu *et al*[23] we aimed to check the effect of a higher multiple scattering component on the ability of the technique to differentiate glucose levels based on attenuation of OCT signal, since multiple scattering is present when probing through biological tissues[24, 25], as is the case when applying the method *in vivo*. The blood was, then, diluted to a 40:60 blood:saline-glucose ratio.

2.2 Blood glucose assessment

For reference, blood glucose levels were assessed, in duplicate, by a portable glucometer (OneTouch® Ultra®, Johnson & Johnson Medical Devices & Diagnostics) using the appropriate test strips. Strips were positioned directly over the blood samples' surface inside the wells.

2.3 OCT measurements

Wells were positioned for individual image acquisition with the OCT system. For each sample, 100 images were obtained sequentially in the same position. Small samples from each group (0.5 µL), were placed on glass slides and imaged by OCT for determination of the refractive index (RI). With the RI it was possible to correct the depth probed on each sample. The B-Scans were, finally, analyzed via software, and attenuation coefficient values were calculated.

The OCT system used for those analysis was a commercial Spectral Radar OCT OCP930SR (Thorlabs Inc.) with a central wavelength of 930 nm, spectral bandwidth (FWHM) of 100 nm and spatial resolution of 6 μm (lateral and axial) in the air. B-Scans acquired from the blood samples were 2000x512 pixels (frame rate of, approximately, 8 frames per second) covering 4 mm of the sample (lateral).

A custom software was developed to analyze the acquired B-Scans. The analysis uses a simple model to calculate the attenuation coefficient of OCT signal, from an exponential decay based on Beer-Lambert's law, as follows:

$$I(z) = I_0 . e^{-2\mu_t z} + C \quad (1)$$

with $I(z)$ the intensity at depth z, I_0 the source intensity, μ_t the attenuation coefficient and C a constant accounting for background noise.

The decay profile used for analysis is the arithmetic mean of A-scans adjacent in space over a region of interest, manually selected in the B-Scan. Such A-Scans are aligned through a peak-finding function, prior to the calculation, to avoid influence of curvature or displacement of the samples.

A fitting of the model in (1) is performed, and the parameters I_0, μ_t and C are obtained. For the proposed study, only the value of μ_t is of interest. This process is repeated for each consecutive B-Scan.

2.4 Statistical analysis

For glucose measurements, arithmetic means were calculated with their respective standard deviations. Mean attenuation coefficients values are presented as a function of their corresponding glucose concentrations. Kolmogorov-Smirnov test attested our data were not drawn from a normally distributed population, so we performed the Kruskal-Wallis with Dunn's for multiple mean comparisons. Diluted blood attenuation coefficients were also submitted to K-Means cluster analysis to identify groups of similar data. All data analysis were performed with GraphPad Prism 5 and OriginPro 9.1 softwares, and we assumed as statistically significant values for $p<0.001$.

3. RESULTS AND DISCUSSION

Following the indicated methodology, different groups of whole blood were analyzed using the portable glucometer, to obtain the reference values for glucose concentration. The values presented in Table 1 are the mean ± standard deviation for four measurements with the portable glucometer. Notwithstanding our expectations, the obtained blood glucose concentrations range surpassed our previous calculations after glucose addition, presumably due to propagation of errors during sample preparation (dilution and weighing).

B-Scans were acquired while the samples were in room temperature (approx. 25 ºC). After analysis, the system proved to be sensitive for all blood glucose concentrations tested, with good correlations with attenuation coefficients as reported in Figure 1.

Groups	Blood glucose (mg/dL)
1	229.75±09.55
2	294.83±06.03
3	384.78±11.91
4	517.50±31.82

Table 1 – Blood glucose concentrations for each group. Values are presented as means ± SD.

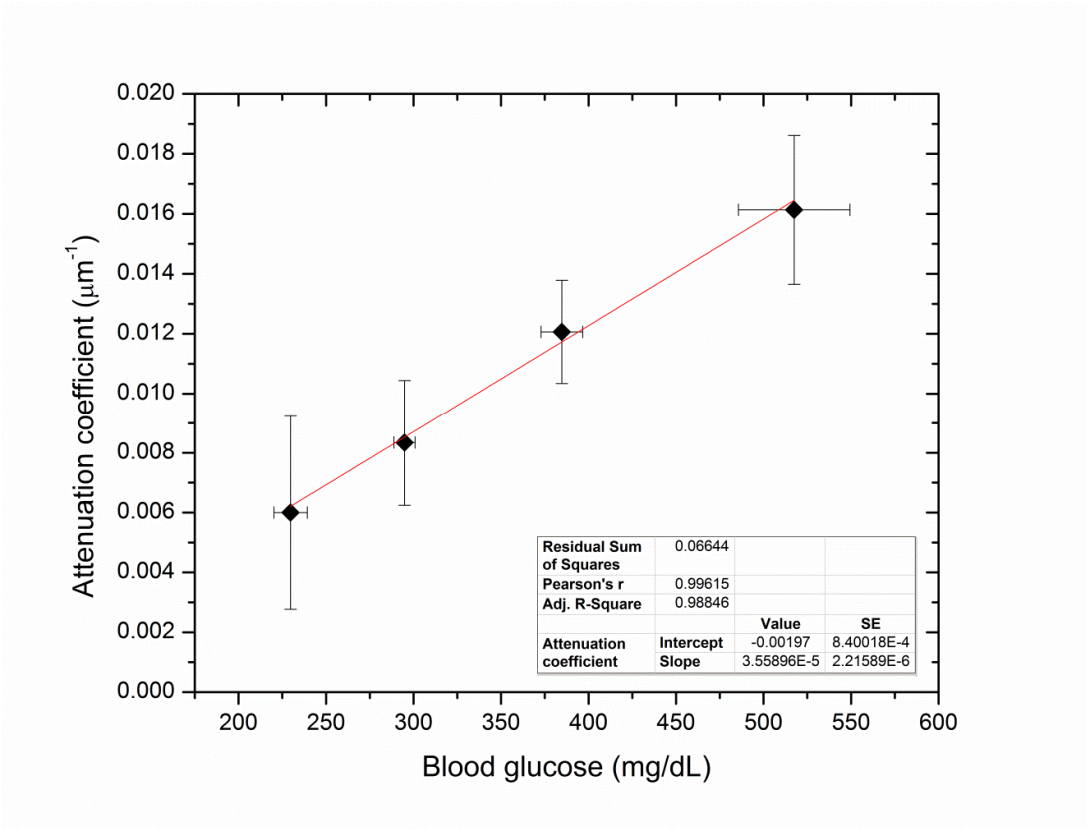

Figure 1 - Attenuation coefficients calculated in relation to average blood glucose concentrations, after refractive index correction (IR=1.26). Multiple comparison analysis showed $p<0.001$ between every group (Kruskall-Wallis with Dunn's as *post hoc*). Red line represents the best linear correlation obtained, with slope of 3,55e-5 with an adjusted R-Squared of 0.98, which indicates that a linear model is suitable for our data.

It is possible to observe a trend of increase in the coefficient of attenuation (i.e. greater signal attenuation) with increasing blood glucose values. This may be explained due to greater glucose concentrations resulting in a higher density of scatterers in the sample, decreasing the mean free path of the photons traveling through such a medium. However, many studies in the literature reported the use of glucose as an optical clearing agent[26, 27] due to index matching of the refractive index of plasma and erythrocytes[28], in addition to aggregation and changes in size of red blood cells (RBC), as suggested by Tuchin *et al*[29], which enhances the transmittance of OCT signal. Nevertheless, Tuchin has also reported an augment of the total attenuation coefficient of blood when mixed with glucose[29], and a related work showed an increase tendency in μ_t with glucose concentrations up to 10,000 mg/dL[30]. Those values, nonetheless, reside outside the normal values of blood glucose levels recommended for euglycemic subjects (between 70 and 100 mg/dL as the latest Standards of Medical Care in Diabetes[31]). Our results, therefore, show that the increasing tendency in μ_t is also true for a range that is closer to normal glucose concentrations, compared to those above discussed.

Statistical tests reported significant differences among all groups with $p<0.001$ when blood glucose concentrations are between 230 and 517 mg/dL. As the spacing (in glucose concentration) between our samples varies greatly, it's not possible to define a differentiating resolution for the technique. However, differences as low as 65 mg/dL (between groups 1 and 2) could be detected.

Even though the tested range surpasses targeted glycemia values for normoglycemic subjects, our results show a glimpse of possibility for differentiating glucose levels from signal attenuation, enabling the use of the OCT technique for this goal, considering that patients with diabetic ketoacidosis present plasma glucose levels above 250 mg/dL and severe cases of hyperglycemia crises can even reach values exceeding 600 mg/dL[32].

We were able to extract more data points of glucose concentrations from our diluted blood samples. This time, the previously observed linear correlation between glycemia and attenuation coefficient was no longer exhibited. As reported by Popescu *et al*[23], solely diluting blood to 40% with isotonic saline solution (without adding glucose or any other solute) results in a stronger multiple-scatter component in OCT signal, which slows its decrease with respect to depth. Associated with that explanation is the fact that the dilution affects the mean free path of the photons, as the scatterers will be more disperse in the medium. In that way, small changes in the concentration of glucose will not significantly change the light attenuation of the diluted blood, as was the case in our prior tests with whole blood. Therefore, only greater differences in glucose levels will be noticeable through this approach, i.e, it lowers the resolution for blood glucose differentiation.

Aiming to discover any correlations between attenuation coefficients and glucose concentrations in these diluted samples, we have performed K-Means clustering analysis on our results in order to observe the necessary change in glucose concentrations for appreciable differences in μ_t to occur. That enables the ascertainment of how many distinct groups there are throughout our range of different glucose levels in diluted blood. Results are presented in Figure 2.

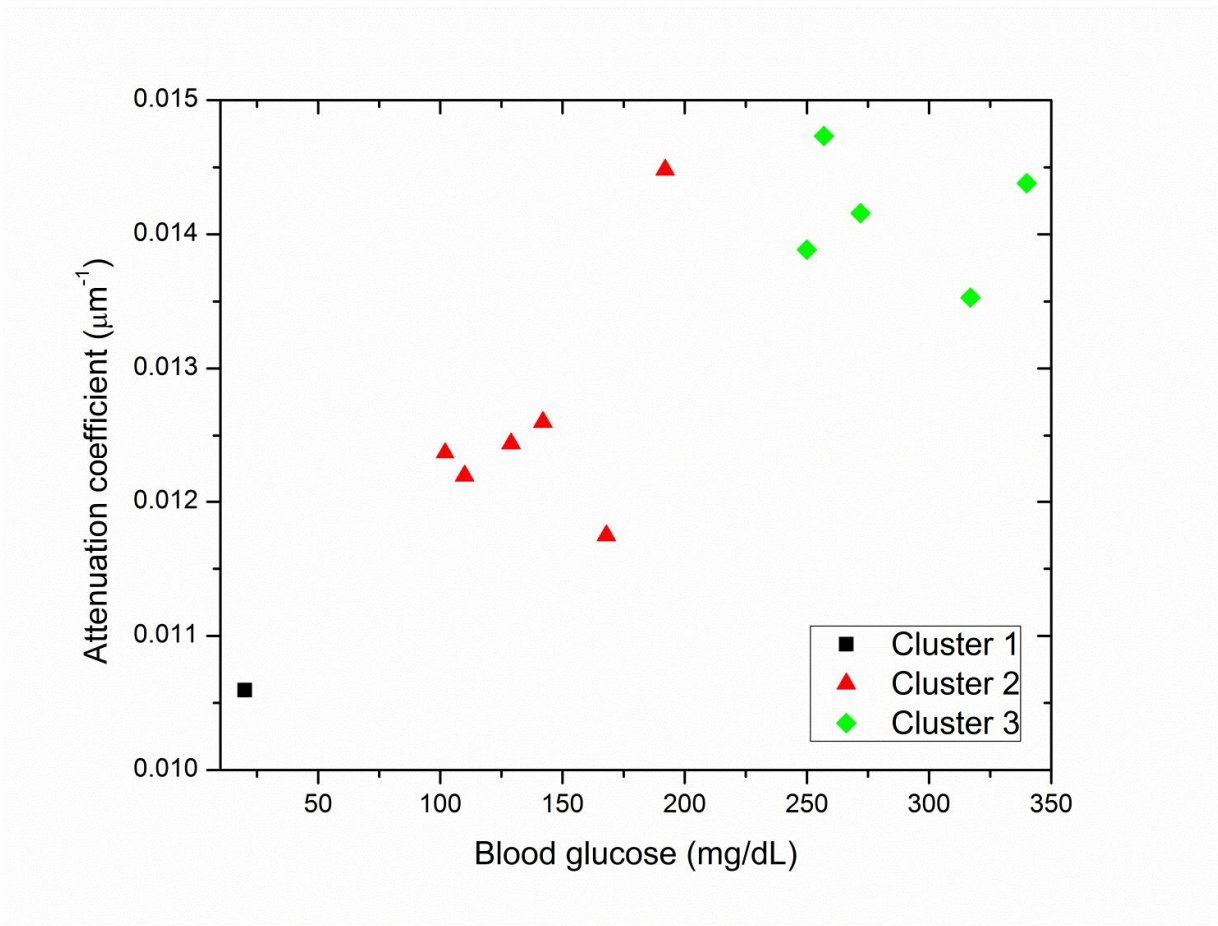

Figure 2 - After refractive index depth correction (IR=1.45), attenuation coefficients were calculated in relation to glucose concentrations from diluted blood, and grouped in clusters by K-Means method

The K-Means algorithm identified 3 clusters in our results, with centers separated by 120 mg/dL (Cluster 1 to 2) and 146 mg/dL (Cluster 2 to 3). This gives out an average resolution for differentiation of about 133 mg/dL for those samples, inferior to the one previously obtained for whole blood. The average glucose concentration of each cluster was

calculated and assigned as different data points, as presented in Table 2. Those new values were plotted as a function of glucose concentration and are reported in Figure 3.

Groups	Blood glucose (mg/dL)
Cluster 1	20.0±0.0
Cluster 2	140.5±34.5
Cluster 3	287.2±34.4

Table 2 - New groups resulted from our K-Means analysis of diluted blood samples. Data are presented as means +/- SD.

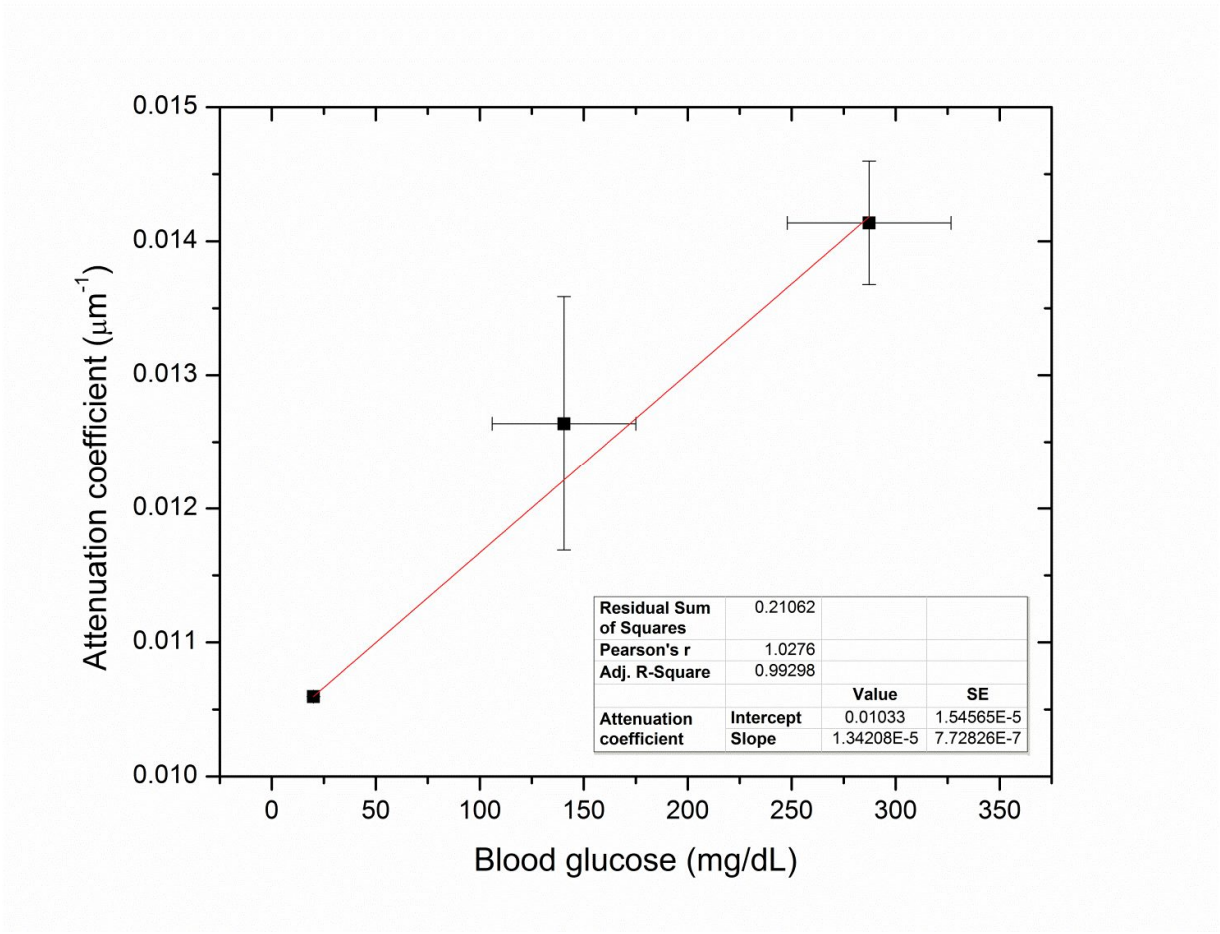

Figure 3 - Mean attenuation coefficients calculated in relation to average blood glucose concentrations. Multiple comparison analysis showed $p<0.001$ between every group (Kruskall-Wallis with Dunn's as *post hoc*). Red line represents the best linear correlation obtained, with an adjusted R-Squared of 0.99 indicating our data can be represented by a linear model.

Following clustering, our new groups were analyzed using Kruskall-Wallis combined with Dunn's for multiple mean comparisons and significant differences between all groups were observed ($p<0.001$). Furthermore, after re-groupment the increasing tendency in μ_t is once more noticeable, and we were able to establish a linear correlation between glucose concentration and attenuation coefficients for our diluted blood samples through a fitting. The fit reported a slope of 1.34e-5 and an R-Squared value of 0.99, which indicates, once again, that the linear correlation may be a good model for the presented data.

It is worth noting that, even though the higher glucose concentration in this test intercepts with the first two concentrations observed for whole blood, the diluted samples behave differently regarding to the calculated slopes from our linear adjustments: increments in glucose concentrations will promote less impact on the attenuation coefficient when compared to whole blood samples, which may be due to the stronger multiple-scatter component in OCT signal resulted by dilution with saline solution, as previously discussed.

4. CONCLUSIONS

Our results suggest that the attenuation coefficient is a feasible way to differentiate glucose levels on blood samples using OCT. That translates as a positive indicator for possible clinical implementation of such a technique in the future, enabling noninvasive glucose monitoring.

Although OCT has already been reported *in vivo* using the optical clearing effect of glucose for glycemic differentiation, the attenuation coefficient may, also, be a viable approach, but further tests should be performed to validate the technique.

A great variety of glucose concentrations may reveal the differentiating resolution of the method for whole blood, but with the data from the performed tests, differences of 65 mg/dL were detected. However, in the presence of a great multiple scattering component, as in the case of saline-diluted blood, this resolution was greatly impacted, and the average resolution was calculated to be of 133 mg/dL, half of the sensitivity reported for whole blood. This may pose as an impediment for *in vivo* applications, and additional studies are necessary.

Finally, even though OCT systems currently are restricted to clinical and research environments, a trend to make those systems available for domestic use is observed in the literature[33, 34]. Therefore, OCT may be used as a self-monitoring device by patients, and having a variety of analysis techniques already developed for this purpose is of great importance for the social impact of this technology.

Acknowledgment: the authors would like to thank the Brazilian funding agencies São Paulo Research Foundation-FAPESP (grant #2013/05492-9, grant #2013/09311-9 and grant #2014/02564-1) and The National Council for Scientific and Technological Development (CNPq) – Brazil (grant #449440/2014-1 and grant#309367/2013-1).

5. REFERENCES

[1] W. H. Organization, [Burden: mortality, morbidity and risk factors] WHO Press, Geneva, 1 (2010).

[2] W. H. Organization, [Preventing chronic diseases: a vital investment. WHO global report] WHO Press, (2005).

[3] D. Abegunde, A. Stanciol, and W. H. Organization, [An estimation of the economic impact of chronic noncommunicable diseases in selected countries] WHO Press, (2006).

[4] I. D. Federation, [IDF Diabetes Atlas] International Diabetes Federation, Brussels, Belgium(2015).

[5] R. S. Xu, "Pathogenesis of diabetic cerebral vascular disease complication," World J Diabetes, 6(1), 54-66 (2015).

[6] R. Bucala, "Diabetes, aging, and their tissue complications," J Clin Invest, 124(5), 1887-8 (2014).

[7] H. Vlassara, and J. Uribarri, "Advanced glycation end products (AGE) and diabetes: cause, effect, or both?," Curr Diab Rep, 14(1), 453 (2014).

[8] S. K. Vashist, "Continuous glucose monitoring systems: a review," Diagnostics, 3(4), 385-412 (2013).

[9] W. M. Ong, S. S. Chua, and C. J. Ng, "Barriers and facilitators to self-monitoring of blood glucose in people with type 2 diabetes using insulin: a qualitative study," Patient Prefer Adherence, 8, 237-46 (2014).

[10] J. Yeaw, W. C. Lee, M. Aagren *et al.*, "Cost of self-monitoring of blood glucose in the United States among patients on an insulin regimen for diabetes," J Manag Care Pharm, 18(1), 21-32 (2012).

[11] A. M. Zysk, F. T. Nguyen, A. L. Oldenburg *et al.*, "Optical coherence tomography: a review of clinical development from bench to bedside," Journal of Biomedical Optics, 12(5), 051403-051403-21 (2007).

[12] J. A. Izatt, E. A. Swanson, M. R. Hee *et al.*, "Quantitative assessment of cataract development with optical coherence domain reflectometry and optical coherence tomography," Investigative Ophthalmology & Visual Science, 33(4), 1300-1300 (1992).

[13] J. A. Izatt, M. R. Hee, D. Huang *et al.*, "Ophthalmic diagnostics using optical coherence tomography," Proceedings of the Society of Photo-Optical Instrumentation Engineers (Spie). 1877, 136-143 (1993).

[14] J. A. Izatt, M. R. Hee, E. A. Swanson *et al.*, "Micrometer-scale resolution imaging of the anterior eye in-vivo with optical coherence tomography," Archives of Ophthalmology, 112(12), 1584-1589 (1994).

[15] E. A. Swanson, J. A. Izatt, M. R. Hee *et al.*, "In-vivo retinal imaging by optical coherence tomography," Optics Letters, 18(21), 1864-1866 (1993).

[16] S. H. Han, C. H. Yoon, L. Conroy *et al.*, "OCT monitoring of cosmetic creams in human skin in vivo," Proceedings of SPIE. 8207(2012).

[17] E. Sattler, R. Kästle, and J. Welzel, "Optical coherence tomography in dermatology," Journal of Biomedical Optics, 18(6), 061224-061224 (2013).

[18] A. Sergeev, V. Gelikonov, G. Gelikonov *et al.*, "In vivo optical coherence tomography of human skin microstructure," Proceedings of the Society of Photo-Optical Instrumentation Engineers (Spie). 2328, 144-150 (1994).

[19] X. Q. Xu, J. H. Geng, and X. S. Teng, "Monitoring the blood coagulation process under various flow conditions with optical coherence tomography," Journal of Biomedical Optics, 19(4), 7 (2014).

[20] D. J. Faber, M. C. G. Aalders, E. G. Mik *et al.*, "Oxygen saturation-dependent absorption and scattering of blood," Physical Review Letters, 93(2), 4 (2004).

[21] F. F. Fu, X. Q. Xu, and J. H. Geng, "Characterization of flowing blood optical property under various fibrinogen levels using optical coherence tomography," Ieee Transactions on Biomedical Engineering, 59(9), 2613-2618 (2012).

[22] N. R. Council, [Guide for the care and use of laboratory animals: eighth edition] The National Academies Press, Washington, DC(2011).

[23] D. P. a. S. B. a. H. M. D. a. F. J. a. S. M. G. Popescu, “Propagation properties of 1300-nm light in blood-saline mixtures determined through optical coherence tomography,” Proc. SPIE, 6864, 686408-686408-9 (2008).
[24] J. M. Schmitt, “Optical coherence tomography (OCT): A review,” Ieee Journal of Selected Topics in Quantum Electronics, 5(4), 1205-1215 (1999).
[25] R. K. Wang, “Signal degradation by multiple scattering in optical coherence tomography of dense tissue: a Monte Carlo study towards optical clearing of biotissues,” Physics in Medicine and Biology, 47(13), 19 (2002).
[26] E. I. a. T. V. V. a. L. Q. a. C. H. Galanzha, “Effects of different doses of glucose on scattering properties of skin,” Proc. SPIE, 4707, 244-247 (2002).
[27] R. K. a. T. V. V. Wang, “Enhance light penetration in tissue for high-resolution optical imaging techniques by the use of biocompatible chemical agents,” Proc. SPIE, 4956, 314-319 (2003).
[28] M. Brezinski, K. Saunders, C. Jesser *et al.*, “Index Matching to Improve Optical Coherence Tomography Imaging Through Blood,” Circulation, 103, 5 (2001).
[29] V. V. Tuchin, X. Q. Xu, and R. K. Wang, "Sedimentation of immersed blood studied by OCT," Proceedings of the Society of Photo-Optical Instrumentation Engineers (Spie). 4241, 357-369 (2001).
[30] O. S. Zhernovaya, E. Jonathan, V. V. Tuchin *et al.*, "Study of optical clearing of blood by immersion method," Proceedings of SPIE-The International Society for Optical Engineering. 7898(2011).
[31] A. D. Association, “Standards of Medical Care in Diabetes-2015: Summary of Revisions,” Diabetes Care, 38, S4-S4 (2015).
[32] A. E. Kitabchi, G. E. Umpierrez, J. M. Miles *et al.*, “Hyperglycemic crises in adult patients with diabetes,” Diabetes Care, 32(7), 1335-1343 (2009).
[33] S. Tomczewski, and S. L., [Mobile optical coherence tomography (OCT) system with dynamic focusing and smart-pixel camera] Springer Berlin Heidelberg, Berlin, Germany(2012).
[34] M. S. Hrebesh, “Smartphone based multiple reference optical coherence tomography system,” Biomedical Optics 2014, BT3A.72 (2014).