Biomedical Optics EXPRESS

In vitro measurements of physiological glucose concentrations in biological fluids using mid-infrared light

Sabbir Liakat,[1,*] Kevin A. Bors,[1] Tzu-Yung Huang,[1] Anna P. M. Michel,[1,2] Eric Zanghi,[1,3] and Claire F. Gmachl[1]

[1]*Department of Electrical Engineering, Princeton University, Princeton, NJ 08540, USA*
[2]*Current address: Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA*
[3]*Current address: Sloan Automotive Lab, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*
**sliakat@princeton.edu*

Abstract: Mid-infrared transmission spectroscopy using broadband mid-infrared or Quantum Cascade laser sources is used to predict glucose concentrations of aqueous and serum solutions containing physiologically relevant amounts of glucose (50-400 mg/dL). We employ partial least squares regression to generate a calibration model using a subset of the spectra taken and to predict concentrations from new spectra. Clinically accurate measurements with respect to a Clarke error grid were made for concentrations as low as 30 mg/dL, regardless of background solvent. These results are an important and encouraging step in the work towards developing a noninvasive *in vivo* glucose sensor in the mid-infrared.

OCIS codes: (170.1470) Blood or tissue constituent monitoring; (300.1030) Absorption; (300.6340) Spectroscopy, infrared.

References and links

1. World Health Organization facts, http://www.who.int/mediacentre/factsheets/fs312/en/index.html.
2. O. S. Khalil, "Spectroscopic and clinical aspects of noninvasive glucose measurements," Clin. Chem. **45**(2), 165–177 (1999).
3. V. Tuchin, *Handbook of Optical Sensing of Glucose in Biological Fluids and Tissues* (CRC, 2009).
4. K. Maruo, M. Tsurugi, M. Tamura, and Y. Ozaki, "*In vivo* noninvasive measurement of blood glucose by near-infrared diffuse-reflectance spectroscopy," Appl. Spectrosc. **57**(10), 1236–1244 (2003).
5. R. Marbach, "A new method for multivariate calibration," J. Near Infrared Spectrosc. **13**(1), 241–254 (2005).
6. N. C. Dingari, I. Barman, G. P. Singh, J. W. Kang, R. R. Dasari, and M. S. Feld, "Investigation of the specificity of Raman spectroscopy in non-invasive blood glucose measurements," Anal. Bioanal. Chem. **400**(9), 2871–2880 (2011).
7. H. Ullah, B. Davoudi, A. Mariampillai, G. Hussain, M. Ikram, and I. A. Vitkin, "Quantification of glucose levels in flowing blood using M-mode swept source optical coherence tomography," Laser Phys. **22**(4), 797–804 (2012).
8. G. B. Christison and H. A. MacKenzie, "Laser photoacoustic determination of physiological glucose concentrations in human whole blood," Med. Biol. Eng. Comput. **31**(3), 284–290 (1993).
9. X. Guo, A. Mandelis, and B. Zinman, "Noninvasive glucose detection in human skin using wavelength modulated differential laser photothermal radiometry," Biomed. Opt. Express **3**(11), 3012–3021 (2012).
10. A. Hugi, R. Terazzi, Y. Bonetti, A. Wittmann, M. Fischer, M. Beck, J. Faist, and E. Gini, "External cavity quantum cascade laser tunable from 7.6 to 11.4 μm," Appl. Phys. Lett. **95**(6), 061103 (2009).
11. S. Liakat, A. Michel, K. Bors, and C. Gmachl, "Mid-infrared (λ=8.4-9.9 μm) light scattering from porcine tissue," Appl. Phys. Lett. **101**(9), 093705 (2012).
12. A. P. Michel, S. Liakat, K. Bors, and C. F. Gmachl, "*In vivo* measurement of mid-infrared light scattering from human skin," Biomed. Opt. Express **4**(4), 520–530 (2013).
13. M. Brandstetter, L. Volgger, A. Genner, C. Jungbauer, and B. Lendl, "Direct determination of glucose, lactate and triglycerides in blood serum by a tunable quantum cascade laser-based mid-IR sensor," Appl. Phys. B **110**(2), 233–239 (2013).
14. H. von Lilienfeld-Toal, M. Weidenmüller, A. Xhelaj, and W. Mäntele, "A novel approach to noninvasive glucose measurement by mid-infrared spectroscopy: The combination of quantum cascade lasers (QCL) and photoacoustic detection," Vib. Spectrosc. **38**(1-2), 209–215 (2005).

#188486 - $15.00 USD Received 8 Apr 2013; revised 31 May 2013; accepted 9 Jun 2013; published 11 Jun 2013

15. W. B. Martin, S. Mirov, and R. Venugopalan, "Middle infrared, quantum cascade laser optoelectronic absorption system for monitoring glucose in serum," Appl. Spectrosc. **59**(7), 881–884 (2005).
16. W. L. Clarke, D. Cox, L. A. Gonder-Frederick, W. Carter, and S. L. Pohl, "Evaluating clinical accuracy of systems for self-monitoring of blood glucose," Diabetes Care **10**(5), 622–628 (1987).
17. A. Guyton and J. Hall, "Insulin, glucagon, and diabetes mellitus," in *Textbook of Medical Physiology* (W.B. Saunders Co., 1996), Chap. 78, pp. 971–983.
18. American Diabetes Association, "Living with diabetes: blood glucose control," http://www.diabetes.org.
19. S. de Jong, "SIMPLS: an alternative approach to partial least squares regression," Chemom. Intell. Lab. Syst. **18**(3), 251–263 (1993).

1. Introduction

Diabetes is a disease that over 340 million people worldwide [1] live with. Daily blood glucose monitoring is essential to management of the disease, as it impacts diet and required medication. Currently, the most accurate method of monitoring involves an often painful finger prick and the drawing of blood. The development of a non-invasive *in vivo* glucose concentration monitor would make this routine daily practice much more convenient for diabetics. Methods of noninvasive *in vivo* glucose detection have been studied for decades [2,3], but traditional invasive monitors still remain the standard.

Prior optical noninvasive *in vivo* glucose detection studies have been focused on the near-infrared (near-IR) [4] due to the presence of resonant glucose overtone and combination bands combined with low water absorption in that region, which allows for greater penetration of light into skin. However, absorption features of other biological absorbers such as hemoglobin and amides are also relatively broad and strong in the near-IR, leading to the necessity of complex multivariate analysis to extract the impact of only glucose on the spectrum obtained from backscattered light. The unpredictability of concentrations of these other absorbers leads to chance temporal correlations and the need to calibrate data using complex sets recorded over multiple days [5]. Work using Raman spectroscopy with near-IR light has also been reported, but the method has its own obstacles to overcome, such as the relatively weak signal associated with Raman scattering [6]. Furthermore, recent work using optical coherence tomography in the near-IR to sense glucose *in vitro* has been reported [7], but so far there have been no reports of *in vivo* work with this method.

The mid-infrared (mid-IR) is promising for the field of noninvasive *in vivo* glucose detection, as the glucose molecule contains fundamental vibrational resonances between 8 - 10 μm [8] which are not overlapped by other biological absorbers except water. Water is a broad featureless absorber throughout the near and mid-IR, but its absorption coefficient is roughly four orders of magnitude greater at 10 μm than at 1 μm, which has been the biggest challenge for researchers focusing on noninvasive *in vivo* glucose detection in the mid-IR regime [9]. However, recent developments in mid-IR light source technology, including pulsed Quantum Cascade (QC) lasers able to provide high peak powers on the order of hundreds of milliwatts while maintaining average powers on the order of a few milliwatts [10] have provided the capacity to obtain more robust signals from skin regions where mid-IR light had previously been considered to be undetectable.

Two major steps towards the realization of the noninvasive *in vivo* sensor are the ability to detect light that penetrates deep enough into the tissue region of interest in order to reach bio-fluids with glucose content, and the demonstration of accurate glucose concentration predictions *in vitro* within the physiologically relevant range for humans. Interstitial fluid (ISF) in the dermis layer of skin has the best correlation of glucose concentration to blood glucose levels when compared to ISF in other skin layers [3,4]. We have recently reported work demonstrating that backscattering of mid-IR light from a QC laser can indeed be detected from the dermis layer of porcine [11] and human [12] skin, based on observed scattering patterns versus angle and wavelength and signal attenuation at the detector.

Previous work on detection of glucose *in vitro* via transmission measurements show the promising capabilities of mid-IR glucose sensing but leave room for improvement. The studies are either confined to narrower boundaries of concentrations (40 to 140 mg/ dL versus

#188486 - $15.00 USD Received 8 Apr 2013; revised 31 May 2013; accepted 9 Jun 2013; published 11 Jun 2013

our range from 1 to 400 mg/ dL) [13], do not use techniques conducive to *in vivo* measurements (photoacoustic spectroscopy requires more incident power than our method, which may lead to problems with tissue heating and/or damage during *in vivo* measurements) [9,14], or had too few measured samples to establish the technique's sensitivity limit with respect to detectable concentration (only 8 concentrations in the physiological range were used in the regression fit) [15]. In this work, we show that glucose concentrations can be predicted with clinical accuracy throughout and below the entire physiological concentration range (50-400 mg/dL) with respect to a Clarke Error grid [16] in aqueous, serum, and Intralipid solution using mid-IR transmission spectra. Clinical accuracy is defined as predictions having less than 20% deviation from the actual concentration when outside the hypoglycemic range (< 70 mg/dL blood glucose level) or a prediction value in the hypoglycemic range when the actual concentration is also in the hypoglycemic range. Serum is used here to simulate ISF, as it has been reported to be a valid ISF proxy [17]. Intralipid (combination of linoleic and alpha-linoleic fatty acids) is used as another biological background solvent. Through the use of these background solvents, we establish that glucose can be isolated in various biological fluids using mid-IR light.

2. Experiment and data collection

Solutions with different glucose concentrations were created from 10% (equivalent to a 10,000 mg/dL concentration) glucose solution obtained from TekNova, which was serially diluted with the background material (water, serum, or Intralipid) to create concentrations ranging from 1 mg/dL to 10,000 mg/dL. Serum was obtained from Animal Technologies, Inc., while Intralipid was obtained from Sigma Aldrich.

In vitro transmission spectra of glucose solution samples were acquired in the following manner; light from a mid-IR source was transmitted through a 100 μm path length pressure-sealed liquid cell containing the solution. A liquid nitrogen cooled mercury cadmium telluride (MCT) detector was used to collect the transmitted light. The two mid-IR light sources used were an IR source contained in a Nicolet Fourier Transform Infrared Spectrometer (FTIR), which emits incoherent broadband (650 - 4000 cm^{-1}) continuous wave light with an integrated power of 7 mW and a Daylight Solutions External Cavity QC Laser, tunable from 1000 to 1200 cm^{-1} which emits pulsed (100 kHz with 1-5% duty cycle) coherent light single mode (full width-half maximum < 1 cm^{-1}) with a beam diameter on the order of a millimeter and average power of approximately 5 mW and peak powers on the order of 100 mW (dependent on wavenumber). Spectra were acquired using software that was interfaced to each source - OMNIC for the FTIR and LabView for the QC Laser. The spectrum collected for each trial run resulted from the averaging of 100 voltage readings from the MCT's preamplifier collected for each wavenumber.

Ideal blood glucose concentrations in the human body range from 70 to 140 mg/dL before meals and peak at approximately 200 mg/dL for two hours after meals. Diabetics can reach the hyperglycemic range (considered by the American Diabetes Association to be a blood glucose concentration greater than 240 mg/dL), in which case they would have to take medication and/or exercise to bring glucose levels down [18]. Calibration and prediction using transmitted mid-IR spectra of concentrations too high to be physiologically relevant (500, 1000, 5000, and 10000 mg/dL) and concentrations of 1, 5, 10, 50, and 100 mg/dL were initially done to gauge the feasibility limits of using this technique. Later, experiments were conducted using only physiological concentrations, with a denser set of nominal concentration values - at least 18 unique concentrations calibrated between 1 to 400 md/dL.

The method of analysis used for quantifying the predictability of the different glucose solutions was partial least squares regression (PLSR), more specifically the SIMPLS algorithm for PLSR [19] built into MATLAB. We used a subset of two or more spectra of a given known concentration for calibration, and the rest of the spectra taken were used for prediction. The accuracy of each individual prediction was determined based on their

#188486 - $15.00 USD Received 8 Apr 2013; revised 31 May 2013; accepted 9 Jun 2013; published 11 Jun 2013

placement on a Clarke grid, with the aforementioned definition for clinical accuracy. The mean predicted value for a given subset of prediction spectra (with the same expected concentration) was the data point plotted on the grid, while error bars represented the standard deviations in predicted values. Along with analyzing how similar prediction values of a given subset of spectra were to each other, we also analyzed how similar the mean predicted value was to its expected value. To accomplish this, we used the standard error of prediction (SEP) metric, defined as the following:

$$SEP = \sqrt{[\frac{1}{N}\sum_{1}^{N}(C_a - C_p)^2]} \quad (1)$$

Here, the N represents the number of predictions for a given concentration, the C_a denotes the actual concentration, and C_p denotes the predicted concentration.

3. Results and analysis

Initial FTIR transmission spectra for aqueous, serum, and intralipid solutions with concentrations in a range from 10 to 10,000 mg/dL (specifically 10, 50, 100, 500, 1000, and 10000 mg/dL) show glucose absorption features that become more apparent with higher concentrations (Fig. 1a). Since the spectra shown are normalized to a water background, dips in the signal seen around 1040, 1080, 1125, and 1160 cm^{-1} are attributed to glucose absorption. When prediction spectra were analyzed with the calibration vector, we obtained a nearly 1:1 linear relationship between predicted concentrations and expected concentrations; R^2 values of 0.999 (water – Fig. 1b), 1.00 (serum – Fig. 1c), and 1.00 (Intralipid – Fig. 1d) were obtained. However, when using only concentrations up to 100 mg/dL for prediction, R^2 values deviated by up to 10%, showing that the near-perfect linear relationship stemmed from the overwhelming signal to noise ratio of the absorption features of the higher concentrations.

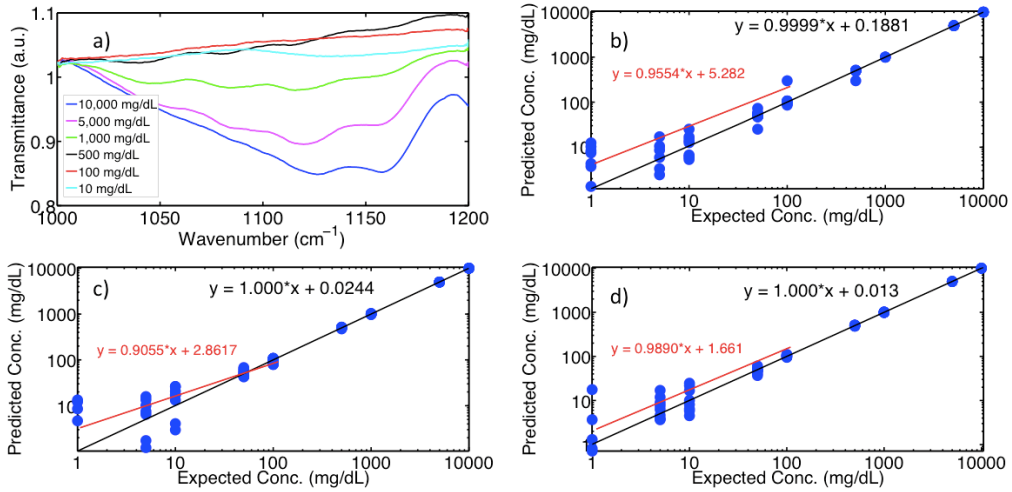

Fig. 1. a) Representative transmission spectra of aqueous glucose at concentrations from 10 – 10,000 mg/dL measured by FTIR spectroscopy. b-d) Prediction of glucose concentrations using calibrations ranging from 1 through 10,000 mg/dL in water (b), serum (c), and Intralipid (d). The red lines and corresponding fitting equations show changes to the linear fit when only concentrations between 1 and 100 mg/dL are used for calibration and prediction.

For FTIR experiments focusing in on the physiologically relevant range of glucose concentrations (50-400 mg/dL) in aqueous and serum solutions, a denser range of nominal concentration values was used. This was done because a more robust calibration matrix consisting of more spectra improves prediction accuracy. The concentrations used for

#188486 - $15.00 USD Received 8 Apr 2013; revised 31 May 2013; accepted 9 Jun 2013; published 11 Jun 2013

calibration and prediction of glucose in aqueous solution can be seen in Table 1. Some intermediate concentrations (112, 135, 225, and 337 mg/dL) were created using mixtures of two other concentrations in order to make a denser prediction concentration set. The expected concentrations that are listed in bold are the ones used for calibration. Each concentration listed had three prediction spectra tested. From the table, one can see that for every respective concentration > 50 mg/dL, the average values of the three predicted concentrations were between 80 - 120% of the expected value, and the SEP were within 20%. Since the SEP of both concentrations that are included in calibration and those that are just used for prediction are under 20% of the known concentration value, we show that our calibration set is robust enough for this concentration range. Furthermore, we show that there is no prediction bias favoring concentrations that were used for calibration versus those only used for prediction, which is important for eventual *in vivo* predictions in humans because one cannot expect de facto concentrations to be the same as the ones used in calibration.

An additional measurement was conducted to determine the significance of errors introduced from imprecise serial dilution. Since serial dilution results in each sample containing portions of a larger concentration sample, an error in mixing introduced at some point would propagate throughout the rest of the entire range of concentrations. While this would not change the deviation between predicted values of a given concentration, it would mean that the nominal values of the concentrations themselves would not be what we expected them to be.

In order to obtain a gauge of the mixing error present in our experiment and to ascertain that our results were not the cause of an unexpected bias, we made a new batch of solutions for 500, 400, 300, and 200 that were independent of both the solutions used for the data set in Table 1 and each other (they were not created through serial dilution). Two separate, completely independent mixtures for each concentration were made. We then made a third mixture for each concentration by making 1:1 mixtures of the two. Spectra were taken for the three mixtures of each concentration and then predicted using the calibration of the data set in Table 1 (from 1 to 400 mg/dL). As seen in Table 2, the predicted concentration for the third mix was always between the predicted concentrations of the two independent mixes. Furthermore, the SEPs for the independent data set were comparable to those obtained in Table 1 for the respective concentrations. This shows that the noise in the spectra generated from imprecise mixing is much smaller than the noise generated from the electronic and mechanical interferences present in the experimental setup. Based on the fact that these predictions were obtained using an independent calibration set, we can eliminate mixing error and unwanted bias resulting from serial dilution as significant sources of error in the data.

The accuracy in the data was extracted via plotting the predicted concentration versus the expected concentration on a Clarke error grid. Figure 2 (top left) shows the Clarke plot of the FTIR data for aqueous glucose solutions. The following are the descriptions for the respective regions:

- A: clinically accurate readings
- B: results that would lead to benign action or inaction by the user
- C: results that would lead to unnecessary corrections
- D: results that would lead to action when inaction is necessary
- E: results that would lead to treatment that is opposite to what should be called for

Average values of predictions were clinically accurate throughout the entire 1-400 mg/dL region, and we obtained accurate and practical (non-negative) concentration values for solutions containing as low as 20 mg/dL concentrations of glucose.

#188486 - $15.00 USD Received 8 Apr 2013; revised 31 May 2013; accepted 9 Jun 2013; published 11 Jun 2013

FTIR experiments on glucose solution in serum were conducted in the same manner as those for the aqueous solutions. Due to the increased viscosity of the serum, which sometimes contained small solid particles, it was more difficult to mix with equal precision to that of the

Table 1. Average Prediction Values and Standard Errors of Prediction (SEP) for FTIR Transmission Spectra on Aqueous Glucose Solutions of Respective Concentrations*

Expected Concentration (mg/dL)	Average Predicted Value (mg/dL)	Standard Error of Prediction (mg/dL)
400.00	369.80	35.53
350.00	342.44	22.56
337.00	334.68	12.97
300.00	289.86	22.12
250.00	237.76	20.79
225.00	232.91	16.10
200.00	199.35	21.19
180.00	167.87	17.96
150.00	149.76	15.32
135.00	135.37	8.68
120.00	109.24	13.72
112.00	110.02	11.34
100.00	110.39	13.17
90.00	94.80	13.75
80.00	89.21	11.51
60.00	53.78	11.20
50.00	67.48	18.88
40.00	53.35	19.27
30.00	45.36	18.09
20.00	25.82	7.10
10.00	0.06	19.97
5.00	–2.65	20.21
1.00	4.64	9.91

*Bolded concentrations indicate that two samples of that concentration were used for calibration. Note that the SEP was calculated with each individual predicted value, not the average.

Table 2. Predicted Concentration Versus Expected Concentration for an Independent Batch of Solutions Calibrated Using the Data set Shown in Table 1*

Expected Concentration (mg/dL)	Average Predicted Value (mg/dL)		Standard Error of Prediction (mg/dL)
500	Mix 1:	523	39.06
	Mix 2:	445	
	Mix 3:	468	
400	Mix 1:	337	38.50
	Mix 2:	421	
	Mix 3:	406	
300	Mix 1:	276	17.32
	Mix 2:	300	
	Mix 3:	282	
200	Mix 1:	205	15.81
	Mix 2:	174	
	Mix 3:	193	

*Mix 1 and 2 denote separate, unrelated mixtures for each concentration, while Mix 3 is a 1:1 mixture of mixes 1 and 2.

#188486 - $15.00 USD Received 8 Apr 2013; revised 31 May 2013; accepted 9 Jun 2013; published 11 Jun 2013

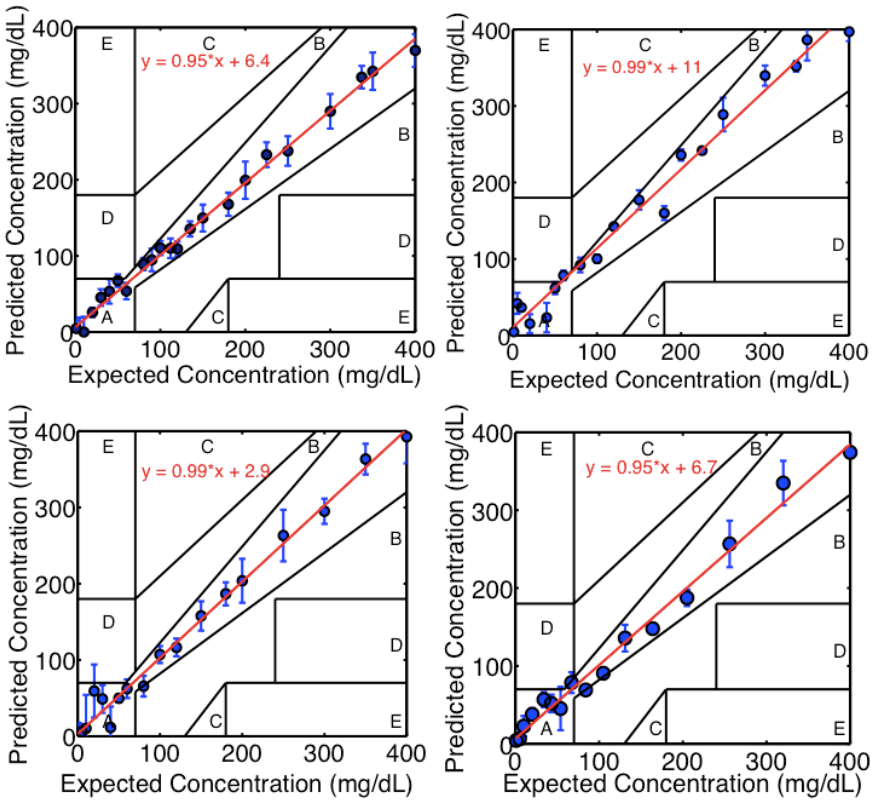

Fig. 2. Predicted glucose concentrations versus expected (actual) concentrations plotted on Clarke error grids [11]. **Top row**: aqueous solution (left) and serum solution (right) measured with FTIR. **Bottom row**: aqueous solution (left) and serum solution (right) measured with QC laser spectroscopy. The individual regions labeled A-E are described as follows: A - clinically accurate reading, B - result that would lead to benign action or inaction, C - results that would lead to unnecessary corrections, D - results that would lead to inaction when action is necessary, and E - results that would lead to treatment opposite to what should be given.

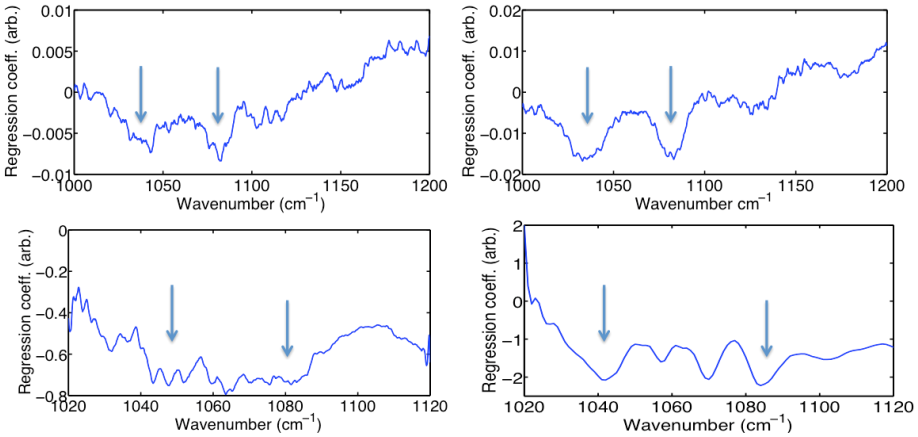

Fig. 3. First loading vectors for calibration sets in serum (left) and water (right), for FTIR (top row) and QC laser (bottom row) spectra, with arrows denoting wavenumber regions of the most prominent absorption features.

#188486 - $15.00 USD Received 8 Apr 2013; revised 31 May 2013; accepted 9 Jun 2013; published 11 Jun 2013

aqueous solution. Figure 2 (top right) shows the predicted versus expected value of serum solutions plotted on the Clarke grid. The results resemble those obtained for aqueous solutions, with slightly smaller standard deviations for 150 and 200 mg/dL, and slightly larger deviations for concentrations under 50 mg/dL (hypoglycemic range). Once again, we observe a cutoff at 30 mg/dL, below which we do not always obtain non-negative prediction concentrations. PLSR analysis of the first loading vectors of calibrations for both aqueous and serum solutions can be seen in Fig. 3. The first loading vectors highlight the wavenumbers where glucose absorption was most prominent, and one can see that these locations remained constant regardless of background solvent.

Lastly, we predicted aqueous glucose concentrations from QC laser spectra. This was necessary because of our determination that our cooled detectors could not detect FTIR light scattered from within the dermis layer of skin, but that pulsed QC laser light with just over 100 mW of peak power (yet average power on the order of the FTIR source's integrated power, which complies with ANSI standards for acceptable skin exposure) incident onto the skin could be detected from such depths [9]. As such, it was important that we established an ability to accurate predict glucose concentrations using the QC laser as well.

Experiments were conducted in the same manner as for the FTIR predictions, yet over a narrower range of wavenumbers than for the FTIR because of the limited laser tuning range. Figure 2 (bottom row) shows the predicted versus expected glucose concentration using the QC laser for both aqueous (left) and serum (right) solutions on a Clarke error grid. We observe slightly larger error bars on the QC laser data (compared to the FTIR data for both aqueous and serum solutions). We attribute this to the weaker signal to noise ratio seen in our QC laser data, which is a product of some mode-hopping artifacts caused by the laser tuning mechanism. Even though the standard deviation between individual prediction values for a respective concentration was slightly greater than for FTIR data, we see clinically accurate predictions to as low as 40 mg/dL. Furthermore, comparisons of the first loading vectors in Fig. 3 for FTIR (top) and QC laser (bottom) show similar regions of prominent glucose absorption features with respect to wavenumber.

4. Conclusion and outlook

In summary, we show that glucose concentrations spanning the entire range of human hypo- to hyperglycemia can be predicted to clinical accuracy (less than 20% deviation from the actual concentration when outside the hypoglycemic range or a prediction value in the hypoglycemic range when the actual concentration is also in the hypoglycemic range) *in vitro* when contained in biological fluids by using mid-infrared transmission spectra. Mid-infrared glucose absorption features are strong enough to where concentrations as low as 30 mg/dL can be predicted consistently using either a broadband mid-infrared source or a QC laser. The optical powers we use in this study (7 mW integrated for the FTIR broadband source and around 5 mW average for the QC laser), as well as the path length of the liquid cell (100 μm), are kept in accordance with acceptable power levels and expected propagation path lengths in human skin. Thus, it is an essential step towards consistently successful and sustainable noninvasive *in vivo* glucose detection in humans using mid-IR spectroscopy.

Acknowledgments

The authors would like to thank the Wendy and Eric Schmidt Foundation, the National Science Foundation (under Grant No. EEC-0540832), and the PEI/Grand Challenges Program. We would also like to acknowledge Robert Tona, Neal Zheng, and Jessica Doyle for their contributions to this research.

#188486 - $15.00 USD Received 8 Apr 2013; revised 31 May 2013; accepted 9 Jun 2013; published 11 Jun 2013