Photoacoustic technique for noninvasive glucose sensing : principles and implementations

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**PHOTOACOUSTIC TECHNIQUE FOR NONINVASIVE
GLUCOSE SENSING: PRINCIPLES AND
IMPLEMENTATIONS**

ZHANG RUOCHONG

SCHOOL OF ELECTRICAL & ELECTRONIC ENGINEERING

2020

**PHOTOACOUSTIC TECHNIQUE FOR NONINVASIVE
GLUCOSE SENSING: PRINCIPLES AND
IMPLEMENTATIONS**

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School of Electrical & Electronic Engineering

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2020

STATEMENT OF ORIGINALITY

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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A handwritten signature in black ink, consisting of the Chinese characters '张若冲' (Zhang Ruochong) in a cursive style.

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20 Jan 2020

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SUPERVISOR DECLARATION STATEMENT

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

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AUTHORSHIP ATTRIBUTION STATEMENT

This thesis contains material from 6 papers published in the following peer-reviewed journals and proceedings where I was the first author. Besides, another paper was just submitted and under review.

Chapter 2 was published as Zhang, Ruochong, Siyu Liu, Haoran Jin, Yunqi Luo, Zesheng Zheng, Fei Gao, and Yuanjin Zheng. "Noninvasive Electromagnetic Wave Sensing of Glucose." *Sensors* 19, no. 5 (2019): 1151. DOI: 10.3390/s19051151.

The contributions of the co-authors are as follows:

- I prepared the reference papers, analyzed, organized and drafted the review article.
- Mr Siyu Liu, Dr Haoran jin, Ms Yunqi Luo and Mr Zesheng Zheng helped to search literature.
- Prof Yuanjin Zheng supervised and coordinated the whole work.
- Prof Fei Gao helped to review and revise the manuscript.

Chapter 3 was published as Zhang, Ruochong, Fei Gao, Xiaohua Feng, Siyu Liu, Rahul Kishor, Yunqi Luo, and Yuanjin Zheng. "Noninvasive photoacoustic measurement of glucose by data fusion." *Analyst* 142, no. 16 (2017): 2892-2896. DOI: 10.1039/C7AN00743D.

The contributions of the co-authors are as follows:

- Prof Fei Gao and I initiated the idea.
- I did the experiment, processed the data and wrote the manuscript.
- Dr Xiaohua Feng, Mr Siyu Liu, Miss Yunqi Luo helped to review and improve the manuscript.
- Dr Rahul Kishor prepared the PDMS layer for experiment.
- Prof Yuanjin Zheng suggested on the whole work and provided funding support.

Chapter 4 was published as Zhang, Ruochong, Fei Gao, Xiaohua Feng, Haoran Jin, Shaohua Zhang, Siyu Liu, Yunqi Luo, Bengang Xing, and Yuanjin Zheng. "'Guide Star' Assisted Noninvasive Photoacoustic Measurement of Glucose." *ACS sensors* 3, no. 12 (2018): 2550-2557. DOI: 10.1021/acssensors.8b00905.

The contributions of the co-authors are as follows:

- Prof Fei Gao, Dr Xiaohua Feng and I discussed and proposed the method.

- I derived the theoretical analysis, verified the idea by experiment and drafted the manuscript.
- Dr Haoran Jin and Dr Shaohua Zhang helped to prepare the phantom for experiment.
- Mr Siyu Liu and Miss Yunqi Luo helped to go through the manuscript and check the theoretical derivation.
- Prof Bengang Xing helped on chemical part and Prof Yuanjin Zheng coordinated physical and engineering part of the work.

Chapter 5 was submitted to Photoacoustics as Zhang, Ruochong, Yunqi Luo, Haoran Jin, Siyu Liu, Fei Gao and Yuanjin Zheng. "Time-domain Photoacoustic Waveform Spectroscopy".

The contributions of the co-authors are as follows:

- I came out with the idea, developed the algorithm, verified it by experiment and prepared the draft.
- Miss Yunqi Luo suggested on the algorithm for data processing.
- Dr Haoran Jin checked the equation derivation and helped with simulation.
- Prof Fei Gao and Prof Yuanjin Zheng coordinated the whole work and suggested on the manuscript organization and writing.

Chapter 6 has been published as Zhang, R., Tang, K., Yang, C., Jin, H., Liu, S., & Zheng, Y. "Portable Photoacoustic Sensor for Noninvasive Glucose Monitoring" in 2019 IEEE International Symposium on Circuits and Systems (ISCAS) (pp. 1-4). IEEE, and Zhang, R., Qu, Z., Jin, H., Liu, S., Luo, Y., & Zheng, Y. "Noninvasive glucose measurement by microwave biosensor with accuracy enhancement" in 2018 IEEE International Symposium on Circuits and Systems (ISCAS) (pp. 1-4). IEEE.

The contributions of the co-authors are as follows:

- I came out with the idea, demonstrated it by experiment and prepared the draft.
- Dr Kai Tang fabricated the circuit board.
- Mr Chuanshi Yang developed the analog front end (AFE).
- Dr Haoran Jin programmed the FPGA.
- Mr Siyu Liu helped on the experiment verification.
- Dr Zilian Qu fabricated the resonance coils.
- Miss Yunqi Luo helped on the microwave sensing experiment.

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- Prof Fei Gao and I came out with the idea. I demonstrated it by experiment and prepared the draft.
- Dr Xiaohua Feng developed the scanner system.
- Mr Siyu Liu did the experiment with me.
- Dr Ran Ding helped to prepare the phantom.
- Prof Yuanjin Zheng supported the work and suggested on the manuscript construction.

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20 Jan 2020

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LIST OF ABBREVIATIONS

AFE	Analog front end
ANN	Artificial neural network
ANSI	American National Standards Institute
ARD	Absolute relative difference
BA	Bland-Altman
BS	Beam splitter
CEG	Clarke Error Grid
DAQ	Data acquisition
eGFR	Estimated glomerular filtration rate
EM	Electromagnetic
FDA	Food drug administration
FIR	Far-infrared
FPGA	Field programmable gate arrays
FWHM	Full width half maximum
GOx	Glucose oxidase
GSPA	“Guide star” assisted photoacoustic
GUI	Graphic user interface
HBS	Human blood serum
ISF	Interstitial fluid
LNA	Low noise amplifier
LOOCV	Leave one out cross validation
MAE	Mean absolute errors
MEMS	Microelectromechanical systems
MLR	Multilinear regression
MIR	Mid-infrared
MOSFET	Metal–oxide–semiconductor field-effect transistor
MPE	Maximum permissible exposure
MSPE	Mean squared prediction error
NIR	Near-infrared
OCT	Optical coherence tomography
OGTT	Oral glucose tolerance test
OPO	Optical parametric oscillator
PA	Photoacoustic
PBS	Phosphate-buffered saline
PCR	Principal Component regression
PD	Photodetector
PDMS	Polydimethylsiloxane
PLSR	Partial least square regression
PZT	Piezoelectric
QCL	Quantum cascaded laser
RMSE	Root-mean-square error
SD	Standard deviation
SFS	Skin fluorescence spectroscopy
SLR	Single linear regression
SNR	Signal-to-noise ratio
TEC	Thermoelectric cooling
THz	Terahertz
tPAWS	Time-domain photoacoustic waveform spectroscopy
UV	Ultraviolet
VGA	Variable gain amplifier
UST	Ultrasound transducer

SUMMARY

Diabetes Mellitus is a kind of chronic metabolic disorder that cannot be completely cured. It is caused by lack of insulin or insulin resistance and characterized by abnormally high blood glucose levels. It can lead to complications including cardiovascular disease, kidney disorder, eye damage and even result in death, if not properly treated. Hence, tight control of blood glucose level is crucial for diabetic patients. However, the commercially available glucose meters used for homecare are mainly invasive and based on glucose oxidase which is also costly. Although, several noninvasive devices are under development using fluorescent, ultrasonic, thermal and electromagnetic methods, none of them has passed the market test yet. It is still worth exploiting truly noninvasive glucose monitoring technique for clinical application.

Plenty of attempts had been devoted to noninvasive glucose monitoring employing different techniques. Most of them are optical methods with a few other exceptions using bio-impedance spectroscopy, and electromagnetic sensing. Among those traditional optical methods, major problems lie in sensitivity, specificity and penetration depth. Photoacoustic (PA) technique, on the other hand, compensates the diffusion and diffraction limits faced by pure optical methods by detecting the acoustic signal, induced by the tissue absorption and thermal expansion, since acoustic wave suffers $\sim 10^3$ less scattering than light. Meanwhile, the excellent contrast of optics is maintained in PA. This PhD thesis comprehensively investigates the fundamental physics behind photoacoustic phenomenon and combines the findings with glucose detection to realize the specific biomedical application. Furthermore, a fully integrated portable photoacoustic sensor was designed, developed and tested for noninvasive glucose sensing.

In the introduction, background and urgent need of the noninvasive glucose

monitoring device were described which are the motivations of this thesis. Then, several state-of-the-art noninvasive glucose monitoring techniques are comprehensively introduced and compared to show the advantage of photoacoustics in Chapter 2, followed by the detailed discussion about working principal and relationship between biological components and photoacoustic signal generation. In Chapter 3 to 5, novel findings and methods including accuracy enhancement by data fusion, sensitivity improvement by indirect photoacoustic method, multidimensional waveform analysis and their corresponding performance on noninvasive glucose measurement are presented. Next, a study on system design and development for noninvasive glucose measurement is conducted in Chapter 6, targeting for real clinic and homecare application. The final goal is to commercialize the research achievement and replace the current finger-prick glucose meters to improve the quality of diabetic management. In the last chapter, a conclusion is drawn and some future work is recommended.

In summary, this thesis comprehensively investigates photoacoustic effect and utilizes the novel findings on the application for noninvasive glucose sensing, which can be regarded as translational research. Interesting concepts are proposed and experiments on glucose in the physiological range are demonstrated. Implementation of developing a portable photoacoustic sensor is also carried out, which could be the potential replacement of the invasive devices in the market now.

Chapter 1. INTRODUCTION

This chapter briefly introduces the cause of Diabetes Mellitus and the common practice of daily blood glucose monitoring. Then, the motivation and objectives of the research are discussed, followed by the major contributions and organization of this thesis.

1.1 Background

Blood glucose level is one of the most important physiological parameters that is associated with the metabolic and homeostatic mechanism in the human body. Diabetes mellitus is a prolonged metabolic disorder due to insufficient insulin production or an improper cell response to insulin [1]. The diabetic population was projected to be 552 million by 2030, as reported by David R. Whiting et al. [2], based on data sources for 80 countries. Diabetes imposes a heavy economic burden on patients and their families. According to the American Diabetes Association, the total cost associated with diabetes in the U.S. was \$327 billion in 2017 [3]. Diabetes can be classified into three main categories: Type I, Type II, and gestational diabetes [4]. The first one is usually called “juvenile diabetes”, which is mainly diagnosed in children and results from a lack of insulin produced by beta-cells [5-7]. Only 5~10% of diabetic patients have this form. The second one is the most prevalent among diabetic patients, accounting for more than 90% [8], and is primarily caused by an unhealthy lifestyle and genes [9]. It is characterized by insulin resistance and sometimes combines with reduced insulin secretion [4, 10-12]. The last category usually occurs among pregnant women and will either disappear or develop to Type II diabetes after delivery [4, 13, 14]. Long-term abnormal levels of glucose (hyperglycemia when the glucose level is >200 mg/dL [15] and hypoglycemia when the glucose level is <70 mg/dL [16]) often lead to complications, including accelerated atherosclerosis [17], stroke [18], neuropathy [19],

nephropathy [20], and retinopathy [21]. In addition, it has been reported that diabetes also significantly increases the risk of cause-specific death [18, 22, 23]. Since there is no specific cure for diabetes [24], daily glycemic measures need to be carried out together with active treatments (insulin injection or bariatric surgery) to improve diabetic patients' quality of life. Since 1962, when Clark and Lyons proposed the electrochemical method [25], glucose oxidase (GOx) has been widely applied for glucose determination. The well-established glucose meters are mainly based on electro-enzymatic reactions, which require a finger-prick device to obtain a drop of blood ($\sim 1 \mu\text{L}$) and apply it onto a disposable testing strip [26, 27]. Although the accuracy of this

Figure 1. 1 Estimated Diabetes Population [2].

kind of invasive device has been proved and is accepted ($\pm 20\%$), physical pain and infection can be easily caused [28, 29] with a discrete readout only. Moreover, the annual cost of testing strips is estimated to be \$750 per patient [30]. Therefore, semi-invasive or minimally invasive devices have been developed with the aim of replacing those finger-prick devices and achieving continuous blood glucose monitoring (CBGM). They usually measure the glucose concentration in interstitial fluid (ISF) by implanting a tiny and relatively painless subcutaneous sensor. Nevertheless, the sensitivity gradually degrades as the protein builds up on the surface of the sensor, and

hence frequent calibration is required [31, 32]. To help the 500 million diabetic patients to further reduce pain and heavy economic burden, a truly noninvasive glucose monitoring technique or sensor is highly demanded.

1.2 Motivation and Objectives

Plenty of attempts have been devoted to solving this global challenge but limited success has been achieved. There are several limitations which hinders the noninvasive glucose monitoring techniques from mass clinical applications.

First of all, the one-dimensional data such as signal amplitude or phase delay information are usually employed to correlate with glucose concentration. Such data can be easily distorted due to instrument instability and noise which degrades the measurement accuracy and reliability. Hence, multi-dimensional data are preferred to compensate each other and improve the measurement performance.

Secondly, blood glucose only accounts for a small portion of human body. Only four grams glucose are circulating in a person weighing 70 kilograms [33]. Glucose levels range from 70 mg/dL to 180 mg/dL. Hypoglycemia or hyperglycemia happens when glucose levels are out of this region. Since the variation amount is tiny with other environment noise presented, sensitivity of measurement is usually a challenge. To ensure the signal-to-noise ratio (SNR) and dynamic range are good enough to identify glucose changes, improved measurement method and advanced signal processing algorithms are desired to suppress noise and maximize the useful information.

Besides, the ultimate target is to replace the current finger-prick glucose monitoring devices in the market. Two application scenarios are proposed: 1. Individual glucose sensor used for homecare; 2. General patients' usage in hospital. Either of them needs to consider portability and cost. To push the research to real application, a miniaturized and low cost sensor system is desired that can fulfill the daily healthcare needs of

patients or be used in the hospitals.

1.3 Major Contributions and Organization of the Thesis

To overcome the above mentioned limitations, this research work employs photoacoustic method, aiming to provide a potential solution for noninvasive glucose monitoring. The thesis concentrates on the comprehensive study on the existing noninvasive methods based on electromagnetic (EM) waves, fundamental physics of photoacoustics, its link to the specific application of noninvasive glucose monitoring and development of sensor system.

Chapter 2 is a general review of several noninvasive glucose sensing techniques from scientific and engineering points of view. The review mainly focuses on EM wave based methods which are the most widely studied ones. Advantages and limitations of each method will be stated. Chapter 3 describes the conventional photoacoustic sensing method from theoretical point of view. Then, a novel method for noninvasive glucose monitoring using photoacoustic method based on data fusion is proposed. In Chapter 4, a new method named “Guide star” assisted photoacoustic (GSPA) measurement is introduced which is able to significantly enhance detection sensitivity. Moreover, another novel method named time-domain photoacoustic waveform spectroscopy (tPAWS) is introduced in Chapter 5. It has a great potential to complement or surpass the conventional spectroscopy method for chemical analysis. Apart from the above mentioned novel methods which mainly focus on the conceptual study, system development is also conducted in Chapter 6 of this thesis. Portable sensor prototype for noninvasive and continuous glucose monitoring has been developed and functionally verified. At the end of this chapter, study is also extended to microwave sensing which can be incorporated in sensor system to realize sensor fusion in the future. A conclusion is drawn in Chapter 7 followed by recommendations for future work.

Chapter 2. NONINVASIVE GLUCOSE MONITORING TECHNIQUES BY ELECTROMAGNETIC WAVES: A REVIEW

During the past decades, much effort has been devoted to the development of truly noninvasive glucose sensors that employ various emerging technologies. Among those methods, electromagnetic (EM) wave sensing has drawn much attention due to its rich interactions, including absorption, scattering, and transmission, with particular compounds inside the body. As shown in Figure 2.1, EM waves can be classified into radio waves, microwaves, terahertz (THz) waves, visible/infrared (IR) light, ultraviolet, X-rays, and gamma rays [34] based on different frequencies or wavelengths. EM waves with higher frequencies carry higher photon energy, which can damage tissues and organs by ionizing radiation. In contrast, non-ionizing radiation is relatively safe and suitable for noninvasive biomedical sensing and imaging. The boundary between ionizing and non-ionizing radiation falls in the ultraviolet (UV) region with a photon energy between 10 eV and 33 eV, which is not well-defined. Herein, we will mainly discuss the utilization of non-ionizing radiation, including vis/IR, T-ray, and microwaves, regarding the application of noninvasive glucose measurement. A molecule's characterization is realized by its specific vibrational frequencies. Fundamental vibrations can be excited by the mid-infrared (MIR) light, known as the “fingerprint region” of many molecules, including glucose. Overtones and combinational bands usually fall into the near-infrared (NIR) region, while T-waves and microwaves can be used to detect tissue permittivity variations caused by glucose fluctuations. In the following section, we will review the EM-based noninvasive glucose sensing techniques; more specifically, optical methods, THz spectroscopy, and microwave sensing. Brief introduction of photoacoustic method will also be presented at the end of this chapter.

Figure 2. 1 A diagram of the electromagnetic (EM) spectrum and its relevance to glucose. NIR, near-infrared; MIR, mid-infrared; FIR, far-infrared.

2.1 Infrared (IR) Spectroscopy

There are several kinds of interactions between light and biological tissues depending on the properties of target tissues and the characteristics of illuminating sources. The interactions can be mainly categorized into absorption, transmission, emission, reflection, and scattering. Molecules with vibrational and rotational motions tend to absorb light at matched frequencies or wavelengths due to resonance. More specifically, chemical bonds can move in the form of bending, symmetrical stretching, asymmetrical stretching, et al. [35]. The quantum vibrational energy bands usually fall into the infrared (IR) region [36]. Thus, IR spectroscopy has been widely applied in analytical chemistry for characterizing samples in various states such as gases, liquids and solids. Based on different excitation sources, it can be classified into near-infrared (NIR) spectroscopy [37], mid-infrared (MIR) spectroscopy [38], and far-infrared (FIR)/THz spectroscopy [39].

NIR radiation was discovered by Sir William Herschel in 1800 [40], and the first NIR spectrum was obtained in 1881 by Abney and Festing in the range of 1000–1200 nm [41]. Nowadays, NIR spectroscopy utilizes an EM wave in the range of 700–2500 nm [37, 42–44], which covers several optical windows where photons have less

interactions with interfering tissue compounds, such as water, hemoglobin, and lipids, so that the penetration depth can achieve several millimeters [32, 45], where capillary beds locate [46-48]. The absorption in this wavelength range corresponds to the combination, the first overtone, the second overtone, or the higher-order overtone of a fundamental molecule's stretching and bending [37, 49, 50]. Glucose is a kind of monosaccharide with the molecular formula $C_6H_{12}O_6$ in the form of pyranose. It has several absorption peaks in the NIR region, which are listed in Table 2.1.

Table 2. 1 Absorption peaks of glucose in the NIR region and the corresponding functional groups.

No.	Wavelength (nm)	Functional Group
1	2273	Combination of O-H/C-O stretching [50]
2	2261	
3	1688	2vCH [51]
4	1638	First overtone [52]
5	1536	vOH + vCH [51]
6	1408	2vOH [51]
7	1126	3vCH [51]
8	1042	Combination of vCH [53, 54]
9	1018	Combination of vCH [55]
10	939	3vOH [51]
11	930	3vCH ₂ [53]
12	910	4vCH [53, 54]

The light attenuation due to analytes absorption only is governed by Beer-Lambert Law [56] and the transmitted light can be expressed as:

$$I = I_0 \exp(-\mu_a l) \quad (2.1)$$

I_0 is the initial photon energy, μ_a is the absorption coefficient and l is the effective optical path length. μ_a is proportional to εC (cm^{-1}), where ε represents the molar extinction coefficient and C is the molar concentration of the analyte.

Light transmission is usually measured by a spectrometer and processed by multivariate analysis to specifically extract glucose information. NIR spectroscopy allows for deep tissue (>1 mm) measurement with high sensitivity. The instrumentation

is simple and relatively low-cost. However, glucose absorption peaks are broad in this region and may overlap with other interferences, such as water and lipids. Besides this, temperature, humidity, and other environmental factors may also affect the measurement results.

MIR spectroscopy works in a similar way except that longer wavelengths ranging from 2500 to 10,000 nm [57] are employed, where the well-known “fingerprint region” of glucose locates. The featured absorption peaks in this region are sharper and provide better specificity than NIR spectroscopy does. The maxima of glucose absorption in the MIR region are listed in Table 2.2.

Table 2. 2 Absorption peaks of glucose in the MIR region and the corresponding functional groups.

No.	Wavelength (nm)	Functional Group
1	8000	C-H bending vibrations [57-60]
2	8244	[61]
3	8658	Pyranose ring [62, 63]
4	8680	[64]
5	9290	C-H bending vibrations [57-61, 64]
6	9551	C-H bending vibrations [57-60]
7	9680	$\nu(\text{C-O-H})$ or $\nu(\text{C-O-C})$ vibration [59-61, 64, 65]
8	9746	C-O-H bending vibration [57, 59, 60]

Despite the relatively good specificity or selectivity, penetration depth is severely limited due to the strong absorption of water and lipids [66]. Thus, MIR light can only pass through the first layer of skin—stratum corneum, with a thickness of 10–20 μm [67] and detect glucose of ISF found in the stratum spinosum layer [61, 68]. It was proved that ISF in epidermis has a strong correlation with blood glucose in spite of several minutes’ delay [69-72].

The selectivity of MIR spectroscopy is better than that of NIR spectroscopy as it covers glucose’s “fingerprint region”. The major drawback of this method is that the penetration depth is limited to several microns and only ISF can be reached. Besides

this, the illuminating source, such as a QCL, is bulky, expensive and weak.

2.2 Fluorescence

Fluorescence refers to a phenomenon in which substances emit light (usually at a longer wavelength) when they absorb EM radiation. Fluorescence spectroscopy is useful in medical and biochemical analysis of chemical groups. The excitation source usually falls in the ultraviolet (UV) region with high photon energy. Fluorescence has the advantage of high sensitivity, which even allows for single molecule detection [73]. In addition, the characteristic emission of a certain fluorophore also guarantees high specificity. Fluorescent methods for glucose measurement can be based on intrinsic skin fluorescence spectroscopy (SFS) or specially designed molecule reporters. For the design of extrinsic fluorophores, several factors, including quantum yield, photostability, absorption, and wavelength, have to be considered [74]. Both fluorescence intensity and lifetime can be evaluated to provide sufficient information.

Fluorescence-based methods have excellent sensitivity. However, the excitation source, which falls in the UV region, suffers from strong scattering and it usually measures body fluids, such as tears and sweat, which have relatively poor correlation with blood glucose.

2.3 Raman Spectroscopy

Raman spectroscopy relies on the inelastic scattering of photons, named after C.V. Raman [75], to identify different molecules. There are two types of Raman scattering: stokes scattering, in which incident photons transfer energy to molecules, resulting in scattered photons with lower energy; and anti-Stokes scattering, which leads to increased photon energy when molecules transfer energy to incident photons. Raman spectroscopy system consists of a coherent and monochromatic light source, a grating to disperse the light, filters, and a photodetector to obtain Raman spectra. Several

advanced Raman techniques have been developed, such as surface-enhanced Raman spectroscopy (SERS), resonance Raman spectroscopy (RRS), tip-enhanced Raman spectroscopy (TERS), and their combinations. Glucose has characteristic scattering features in the range of 400–1500 cm^{-1} as mentioned in [76].

Owing to the sharp and distinct peaks, Raman spectroscopy has excellent selectivity. However, the long acquisition time makes it sensitive to laser intensity fluctuation, and the signal is weak.

2.4 Optical Coherence Tomography (OCT)

OCT is able to provide depth-resolved information on skin layers by detecting coherently backscattered photons. It was developed by Huang et al. in 1991 [77]. The system usually consists of a two-beam interferometer and a photodiode. An envelope of an interferometric signal can be used to reconstruct a cross-sectional, two-dimensional (2D) image and the light attenuation information can be calculated thereafter. Noninvasive glucose measurement by OCT is based on the fact that glucose variation in extracellular fluid (ECF) will induce a refractive index mismatch change between ECF and cellular components. Thus, the scattering coefficient will change, which is reflected by the backscattered signal strength. The effect of the refractive index mismatch induced by glucose is higher than that of other body osmolytes [78].

OCT provides good resolution; however, it is based on light scattering for noninvasive glucose sensing. Thus, the obtained value can be affected by the presence of other interfering components, which results in poor selectivity.

2.5 Terahertz Spectroscopy

EM waves with frequencies ranging from 0.3 to 3 THz are named terahertz waves, which fall between far-infrared and microwaves. They are sensitive to not only the molecule itself but also intra- and inter-molecular rotational and vibrational transitions

[79]. The excitation sources include, but are not limited, to QCLs, Gunn and tunnel transit-time (TUNNETT) diodes, solid-state electronic devices, such as uni-travelling-carrier photodiodes (UTC-PDs), and optical rectification. Photoconductive antenna, Schottky barrier diodes (SBDs), and bolometers have been widely used as terahertz detectors [80]. Moreover, electro-optic (EO) techniques can be also employed with nonlinear crystal [81]. Terahertz time-domain spectroscopy (THz-TDs) was developed for the characterization of chemical compounds and medical imaging by non-ionizing radiation. Both amplitude and phase information can be obtained in the time domain. Although many nonmetallic or nonpolar materials are transparent to T-rays, the penetration depth is limited to hundreds of micron in biological tissues, due to the strong water absorption [82].

THz is sensitive to molecules and intra/inter-molecule transitions. Nevertheless, it requires a dedicated excitation source and suffers from poor penetration depth, which means that a T-wave can only reach ISF with a reflection setup.

2.6 Microwave Sensing

EM wave that ranges from 1 mm to 1 m, which corresponds to frequencies between 300 GHz and 300 MHz is called microwave. Microwaves can easily penetrate homogeneous tissue with a thickness of millimeters, especially in a low-frequency range, which remains a challenge for most of the optical-based methods. Besides this, the cost of microwave sensors is usually low and fabrication is relatively easy. The reflection, transmission, and absorption of a millimeter wave are closely related to the dielectric property or relative permittivity of skin [83, 84], which varies with glucose fluctuations [85-87]. The complex permittivity varies with frequency, and can be expressed by the Cole–Cole equation [88]:

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (j\omega\tau)^{1-\alpha}} \quad (2.2)$$

where ε_∞ and ε_s are dielectric constants at infinite and static frequencies, respectively, and τ is a relaxation time constant. These three parameters are related to glucose concentration [89]. α is a value between 0 and 1. Scattering or S parameters describing the two-port networks are usually investigated to infer glucose change.

Microwave-based glucose sensors are low-cost and can be easily miniaturized. The penetration depth is also appreciated, especially at a low-frequency range. However, it lacks selectivity as the dielectric constant is strongly affected by other blood components as well.

2.7 Photoacoustic Sensing

The PA effect refers to a phenomenon where an object absorbs heat from light and undergoes thermal expansion to generate acoustic wave, which was firstly discovered by Alexander Graham Bell in 1880 [90]. Combining the high contrast of an EM wave with the deep penetration of an acoustic wave in biological tissue, the PA technique is able to achieve prominent performance in bio-sensing and bio-imaging applications[91-95]. Although, theoretically, any kind of EM wave can generate a PA signal, vis/IR lasers are the most frequently reported due to the wide availability of sources, the convenience of manipulation, and the rich functionality. Compared to IR spectroscopic methods for glucose detection, PA spectroscopy collects acoustic wave, which is $\sim 10^3$ times more immune to tissue scattering and directly related to the laser energy deposited in skin, yielding deeper penetration and better sensitivity. Besides pure optical properties like absorption, a PA signal also contains information related to mechanical or acoustic properties of the tissue [96, 97], which are affected by glucose concentration. Similar to optical spectroscopy, laser wavelengths ranging from NIR to MIR have been adopted in various studies [61, 98-101].

Photoacoustic technology detects a laser-induced pressure change by an acoustic

transducer with relatively good sensitivity. The acoustic wave is less-scattered in tissue, resulting in a better penetration depth. A detailed discussion about PA mechanism will be given in the Chapter 3.

2.8 Summary

Researchers have not stopped pursuing the ultimate solution for noninvasive blood or ISF glucose monitoring, driven by the tremendous academic and market values. EM-wave-based methods are the most attractive ones owing to their wide spectral region and being information-rich. We listed several representative techniques herein and compared in Table 2.3.

Table 2. 3 Comparison of different EM-wave-based methods.

Technology	Penetration	Target	Sensitivity	Selectivity	System Size	Cost
NIR	>1 mm	ISF, blood	High	Good	Portable	Low
MIR	Several μm	ISF	High	Good, better than NIR spectroscopy	Large	High
Fluorescence	<1 mm	Tear, ISF, blood	High	Excellent	Portable	Low
Raman	<1 mm	ISF, tears	Low	Excellent	Portable	Medium
OCT	<1 mm	ISF	High	Poor	Portable	Medium
THz	$\sim 100 \mu\text{m}$	ISF	High	Good	Large	High
Microwave	>1 mm	Blood, ISF	High	Poor	Portable/wearable	Very Low
PA	Better than IR spectroscopy	ISF, blood	High	Good	Portable	Low

Although a number of groups have demonstrated in-vitro and in-vivo applications, there is no well-recognized method that has conquered the great difficulty so far. Global challenges include sensitivity, specificity, system stability, and calibration. For example, IR spectroscopy often rely on a powerful light source with a wide wavelength range as well as advanced calibration methods, such as PCR and ANN, to achieve specificity. Moreover, the penetration depth is also limited due to the strong tissue scattering and absorption in this region. Microwaves can reach deeper tissue, but there is no specific

absorption for glucose. In other words, they lack specificity. Raman spectroscopy possesses favorable specificity. Nevertheless, its sensitivity is poor and it is sensitive to laser stability. Fluorescence-based methods have prominent sensitivity and specificity; however, most of them require exogenous markers and are not truly noninvasive or measure such body fluids as tears. PA combines the excellent contrast related to optical absorption and deep penetration of acoustic wave. Thus, the sensitivity, penetration depth and selectivity are favorable.

For clinical application and device development, effort should also be devoted to miniaturization and cost reduction of the instrumentation to convert these technologies into commercial products, since portability and price are the major concerns of end-users, which determine the potential market shares. Regarding the cost, researchers never stop their effort to reduce the system cost for each technique. However, due to restrictions of excitation sources like MIR and THz, as well as the dedicated detectors such as spectrometer, some of the system costs are relatively high. On the contrary, NIR, fluorescence, microwave and PA system costs can be largely shrunk [102-104]. Based on the above considerations, NIR spectroscopy, fluorescence, Raman spectroscopy, microwave sensing and PA sensing have great potential to be turned into affordable products and used in daily healthcare. There are several tentative products employing the abovementioned technologies, such as Glucotrack, Glucosense, Aprise, and SugarTrac. Despite that, the requirements have yet to be met, and there is still a long way to go for these novel approaches to replace the current finger-prick glucose meters. Overall, it can be seen from the table that PA is quite potential to be developed into commercial product regarding various performance indicators. This thesis will concentrate on this multi-wave emerging technology for noninvasive glucose measurement.

Chapter 3. NONINVASIVE PHOTOACOUSTIC MEASUREMENT OF GLUCOSE BY DATA FUSION

In this chapter, PA phenomenon will be introduced regarding pressure generation, collection and the fundamentals of PA spectroscopy. Then, based on conventional PA spectroscopy, we proposed a novel method by data fusion to achieve accuracy enhancement for noninvasive glucose monitoring.

3.1.1 Photoacoustic Spectroscopy

3.1.2 Photoacoustic Signal Generation

The PA generation involves the following physical procedures: modulated laser light is guided to illuminate on the target, leading to optical absorption and the target will undergo heating and thermoelastic expansion, which generates the acoustic wave propagating outwards and inwards. Ultrasound transducer (UST) or microphone is employed to receive the photoacoustic signal for further interpretation. The whole process is illustrated in Figure 3.1.

Figure 3. 1 Figure illustration of the process of photoacoustic signal generation.

The photoacoustic wave generation and propagation in an inviscid medium is mainly governed by the general photoacoustic equation in homogenous media [105]:

$$\nabla^2 p(r,t) - \frac{1}{v_s^2} \frac{\partial^2}{\partial t^2} p(r,t) = -\frac{\beta}{C_p} \frac{\partial H(r,t)}{\partial t} \quad (3.1)$$

where v_s is sound velocity, β is volume thermal expansion coefficient, C_p is heat capacity at constant pressure and $H(r,t)$ is the source term. When the laser pulse is ultrashort which satisfies the thermal confinement condition (thermal relaxation time is much longer than laser pulse) and stress confinement (laser pulse is shorter than stress relaxation time), the initial pressure p_0 generated by laser beam can be estimated as

$$p_0 = \eta_{th} \Gamma \mu_a F \quad (3.2)$$

where η_{th} is heat conversion efficiency. Γ represents Gruneisen coefficient which is defined as $\Gamma = \beta v_s^2 / C_p$, μ_a is total absorption and F denotes laser fluence in mJ/cm^2 .

3.1.3 Photoacoustic Signal Collection

There are several kinds of detectors to collect PA signals and two of them which are most widely used in literature are introduced here.

3.1.3.1 Microphone and Photoacoustic Cell

PA spectroscopy is established in gas, liquids and solids sensing where microphone together with an acoustic resonator (PA cell) are normally used as detector with superior sensitivity [106]. In conventional PA spectroscopy, several wavelengths are selected and each wavelength is fired N times and the duration between each firing is T . T is determined by the resonance frequency of the photoacoustic cell so that the photoacoustic signal sequence would have a frequency coinciding with the resonance frequency of the cell. Moreover, a lock-in amplifier is needed to suppress wide-band noise and further increase SNR. The typical setup of PA spectroscopy with microphone and PA cell is shown in Figure 3.2.

Figure 3. 2 Typical setup of frequency-domain PA spectroscopy. (a) Schematic of the instrument arrangement. (b) Measurement procedure, S_i : PA spectrum at concentration i , λ_i : i -th wavelength, T : the laser repetition period, N : number of laser firing.

For this kind of narrow band frequency-domain detection, design of PA cell plays an important role in optimizing sensitivity. First of all, the microphone's frequency response needs to be known so that the resonance frequency of PA cell can be designed to coincide with the maximum amplitude point in microphone's spectrum. A typical frequency response of a commercial MEMS microphone (ICS-40720) is shown in Figure 3.3 (a) where the response peak locates at ~ 22 kHz. The performance of PA cell can be simulated by COMSOL Multiphysics using thermoviscous acoustic, frequency domain interface, which helps to optimize the parameters design. Take the well-known H-cell as an example which consists of two buffer volumes and one main cavity as shown in Figure 3.3 (b) and (c) [107], assuming a pulsed Gaussian laser beam heats the air in the resonator chamber along the symmetry axis. For the modified PA cylindrical resonator, since the cross-sectional dimensions of the resonators are much smaller than the acoustic wavelength, the excited sound field develops a spatial variation only along the length of the resonator, that is, the resonators are primarily longitudinal resonators. The effective length of the simple cylindrical resonator is modified from real length. The dominant resonance frequency of a cylindrical resonator without loss can be

estimated as [108]:

$$f_{jmq} = \frac{c}{2} \left[\left(\frac{\alpha_{jm}}{R_{res}} \right)^2 + \left(\frac{q}{L_{res}} \right)^2 \right]^{1/2} \quad (3.3)$$

where R_{res} and L_{res} are the radius and length of the main cylinder where resonance occurs. The jmq indices are non-negative integers refer to the eigenvalues of the radial, azimuthal, and longitudinal modes, respectively, and α_{jm} is the j th zero of the derivative of the m th Bessel function divided by π .

Figure 3. 3 (a) Microphone frequency response. (b) 2D axis-symmetrical view of H-cell. R_{buf} : radius of buffer volume, L_{buf} : length of buffer volume, L_{res} : length of resonant cell, R_{res} : radius of resonant cell. (c) 3D revolution view of H-cell.

An example is briefly discussed here. To decide the resonance frequency, simulation was run in COMSOL with parametric sweep of L_{res} . R_{res} , R_{buf} and L_{buf} are firstly fixed at 1 mm, 1.5 mm and 3.75 mm, respectively. L_{res} was swept from 7.5 to 8 mm with a step size of 0.1 mm. The simulated frequency response was shown in Figure 3.4 from which it can be seen that when L_{res} equals to 7.7 mm, the resonant frequency falls at 22 kHz which matches microphone's resonant frequency. The pressure distribution is shown in the inset and it is mainly the first longitudinal mode (1 0 0). Although the resonant frequency is largely decided by the length of resonant cell, if only considering longitudinal mode, the buffer volume's dimensions can be also adjusted to

further optimize the PA cell performance. Besides H-cell, T-cell is also widely applied in literature [61, 98, 109].

Figure 3. 4 Frequency response of the PA cell with parametric sweep of L_{res} . Inset: pressure distribution at 22 kHz when $L_{res} = 7.7$ mm.

3.1.3.2 Piezoelectric (PZT) Transducer

PZT effect refers to the property of certain kind of materials to convert stress to electrical signal and vice versa. Hence, PZT materials are suitable to be ultrasonic transmitters, receivers and transceivers. Typically, the PZT ultrasound transducer consists of several functional parts as shown in Figure 3.5 (a). Backing material is to absorb excessive ringing and enhance the axial resolution. The most essential part of the UST – PZT crystal is sandwiched between two electrodes, whose thickness affects the center frequency and geometry shapes the signal. The matching layer is used to adjust the acoustic impedance mismatch to reduce reflection and optimize the received acoustic pressure. The hole in the center of the customized transducer is the light pass which is specially designed for PA application. Figure 3.5 (b) shows an example of echo signal received by the UST which is centered at ~ 2.2 MHz with $\sim 60\%$ bandwidth. Its frequency response is shown in (c).

Figure 3. 5 (a) Structure of PZT transducer. (b) Example of echo signal. (c) Frequency spectrum of the 2MHz transducer with 60% -6dB bandwidth.

Despite that PA cell enhances the detection SNR significantly, it operates in frequency-domain to lock-in the laser repetition rate. In practice, the direct EM coupling which may be much stronger than real PA signal also possesses the same frequency component. Hence the PA cell and lock-in amplifier may detect this undesired interference. Moreover, the time-domain information will be wasted which also contains rich glucose-related information. Time-domain PA signal needs to be utilized in the following studies, which cannot be provided by frequency-domain measurement. Besides, PA cell can be easily contaminated due to increasing humidity caused by skin transpiration [109]. With the ultimate goal to deliver a real applicable sensor, another consideration is that incorporation of PA cell and lock-in amplifier may increase the system complexity as well as cost for a miniaturized portable sensor. Above all, UST will be employed instead of microphone with PA cell and lock-in amplifier in this thesis.

3.1.4 Working Principle of Conventional Photoacoustic Spectroscopy

It is normally assumed that only the received PA signal amplitude is dependent on the total absorption of the sample at certain wavelength. Hence, it is equivalent to obtain an absorbance spectrum. To achieve specificity and selectivity, multiple wavelengths are usually employed to excite the sample. Wavelength selection is crucial in this case and normally falls in NIR and MIR region where characteristic absorption band lies in

for most chemicals. The simplified equation is as follows:

$$\begin{aligned} & \begin{bmatrix} PA(\lambda_1) & PA(\lambda_2) & \dots & PA(\lambda_M) \end{bmatrix} \\ &= k \begin{bmatrix} \varepsilon_1(\lambda_1) & \varepsilon_1(\lambda_2) & \dots & \varepsilon_1(\lambda_M) \\ \varepsilon_2(\lambda_1) & \varepsilon_2(\lambda_2) & \dots & \varepsilon_2(\lambda_M) \\ \dots & \dots & \dots & \dots \\ \varepsilon_N(\lambda_1) & \varepsilon_N(\lambda_2) & \dots & \varepsilon_N(\lambda_M) \end{bmatrix} \times \begin{bmatrix} C_1 & C_2 & \dots & C_N \end{bmatrix} \end{aligned} \quad (3.4)$$

where k is a coefficient depending the system that can be known by calibration. $\varepsilon(\lambda)$ is the molar extinction coefficient of a certain analyte at certain wavelength and C is molar concentration. The concentration matrix can be obtained by solving:

$$\mathbf{C} = (\boldsymbol{\varepsilon}^T \boldsymbol{\varepsilon})^{-1} \boldsymbol{\varepsilon}^T (\mathbf{PA} / k) \quad (3.5)$$

However, this conventional way ignores other useful information contained in PA signal except optical absorption. In the following sections, a novel method will be introduced which utilizes other information that is inherently contained in time-domain PA signal.

3.2 Novel Prediction Method by Data Fusion

Conventional glucose detection methods rely on the PA peak-to-peak value, yet phase difference or time delay information of the PA signal is seldom utilized. Moreover, most of methods depending on single indicator can either be easily distorted by laser energy fluctuation and other surrounding noise, or employ complex system consisting of lock-in amplifier and PA cell which is a specially designed acoustic resonator with high quality factor to enhance signal to noise ratio.

Herein, we report a novel noninvasive PA glucose measurement method by extracting both amplitude and time delay information from single PA signal and predict glucose level through data fusion which improves the accuracy of measurement without using extra and complicated instrumentation, since PA signals inherently consist of both optical absorption and acoustic propagation velocity information. We demonstrated *in*

vitro that the accuracy is improved compared to traditional single-parameter-based measurement, which can be easily distorted by laser energy fluctuation and other surrounding noise. A pulsed laser with 7 ns pulse width was adopted considering its higher signal generation efficiency than the continuous laser [110], hence PA cell and lock-in amplifier can be avoided to reduce system complexity and cost.

3.3 Experimental Section

3.3.1 Experimental Setup

The schematic of experimental setup is shown in Figure 3.6. The collimated laser beam from a tunable pulsed laser source (Opolette 355, OPOTEK Inc., California) with a 20 Hz repetition rate, was weakly focused in the quartz cuvette with a 2-mm diameter focal spot. The NIR laser fluence during the measurement was controlled to be around 30 mJ/cm² which is well within ANSI standard [111]. The tuning range of the laser in NIR region covers 700 nm to 2000 nm with a pulse duration around 7 ns (FWHM). A small portion of light was split and fed into the photodetector for laser intensity monitoring. A broadband ultrasonic transducer (fractional bandwidth 75%, V303SU, Olympus, Japan) with center frequency of 1 MHz was placed in contact with bottom of the cuvette. The customized 1 MHz transducer was selected mainly because the center frequency of generated PA signal is around 1 MHz. Polydimethylsiloxane (PDMS) of ~4mm thick was used as the coupling media. The received PA signal was then passed to a low noise amplifier (Olympus, 5662) with 54 dB gain before collected by the oscilloscope (Teledyne Lecroy, New York) with 100 MS/s sampling rate. Then, the acquired data was sent to a personal computer for post processing by MATLAB. Temperature was kept constant throughout the experiment so that PA amplitude and speed of sound were not affected.

Figure 3. 6 Experimental setup. BS: beam splitter, UST: ultrasound transducer, PD: photodetector, LNA: low noise amplifier. A PC is used to control the laser shooting, receive data from oscilloscope and implement data fusion. c_{glucose1} and c_{glucose2} : predicted glucose concentrations by amplitude and time delay. c_{glucose} : predicted glucose concentration after data fusion.

3.3.2 Sample Preparation

Firstly, to prove the feasibility of the proposed method, highly concentrated solution was prepared and tested. 10 g glucose powder (α -D-glucose, Sigma-Aldrich, Singapore) was dissolved in 100 mL deionized water. At the beginning, 4 mL pure water was added in quartz cuvette and the glucose concentration was increased by extracting 0.5 mL sample solution and adding the same amount of glucose solution (10 g/dL) before each measurement. The procedure was repeated 10 times and at the end, 10 different glucose levels were obtained and tested (0, 1.25, 2.343, 3.3, 4.138, 4.871, 5.512, 6.073, 6.564, 6.993 g/dL). Then, to further verify the concept in lower concentrated glucose solution, the sample container was replaced by a flowing quartz cuvette filled with 4 mL pure water and then, 350 mg/dL glucose solution was uniformly and continuously injected into the cuvette by a syringe pump until it totally replaced the pure water.

3.3.3 Wavelength Selection

Glucose has relatively strong absorption peaks in NIR region attributed to overtones and combinational bands of -CH and -OH [51, 112]. Apart from wide availability of high power laser sources, wavelengths falling in this region are able to penetrate deeper

in tissue compared to those in MIR region so that glucose in capillaries and interstitial fluids are detectable. In this experiment, 1600 nm was selected for glucose detection as it is one of the absorption peaks in NIR region [113-115].

3.4 Results and Discussion

3.4.1 Hybrid Photoacoustic Signal

According to literature [61, 116-119], glucose concentration is directly proportional to PA peak-to-peak value [120] which was also mentioned before, that is:

$$c_{glucose} \propto p_0 \propto \eta_{th} \Gamma \mu_a F \quad (3.6)$$

Apart from PA peak-to-peak based prediction, ultrasound has also been used to detect glucose level based on that ultrasound propagation velocity linearly increases with glucose concentration [121, 122]. Thus, keeping the distance from the source to transducer constant, arrival time of laser induced ultrasound signal will vary with glucose concentration. The reference time t_0 was selected as the common starting point of recording data traces. Velocity can then be reflected by calculating the delay between arrival time of signal peak t_{peak} and the reference time t_0 . When t_{peak} varies in a small range around a certain value, based on linear approximation (Eq. 3.7), we can derive Eq. 3.8 by substituting $f(x) = 1/x$.

$$f(x) \approx f(a) + f'(a)(x-a) \quad (3.7)$$

$$\frac{1}{x}(x=a) \approx \frac{1}{a} - \frac{1}{a^2}(x-a) \propto -x \quad (3.8)$$

By replacing x with t_{peak} , we can derive Eq. 3.9 and Eq. 3.10 can also be obtained since velocity is proportional to concentration.

$$\frac{d}{dt_{peak}}(t_{peak} = b) \approx \frac{d}{db} - \frac{d}{db^2}(t_{peak} - b) = m - nt_{peak} = p - qt_{delay} \quad (3.9)$$

$$c_{glucose} \propto v = \frac{d}{t_{peak}} \approx p - qt_{delay} \propto -t_{delay} \quad (3.10)$$

where d is fixed propagating distance and a, b, m, n, p, q are constants.

3.4.2 Data Fusion

Owing to the fact that PA inherently incorporate optical absorption and sound propagation information, not only peak-to-peak value but also time delay variation can be obtained from the PA waveform. Glucose level can be predicted by peak-to-peak value and time delay information separately and then linearly combined by competitive data fusion based on Central Limit Theorem. Weighting factors ω_1, ω_2 assigned to each kind of predictions are determined by the corresponding variances. After calibration, variances of the two kinds of predictions, σ_1^2 and σ_2^2 , can be obtained and glucose level is calculated by Eq. 3.11 and 3.12. Final result is more accurate than single-parameter prediction because of the imprecision and uncertainty reduction [122] as well as instrumentation instability compensation. The variance σ^2 of fused data is proved to be smaller than any of the single-parameter based predictions' (Eq. 3.13). The overall data fusion architecture is shown in Figure 3.6.

$$w_i = \frac{\sigma_1^2 \sigma_2^2}{(\sigma_1^2 + \sigma_2^2) \sigma_i^2}, \quad i = 1, 2 \quad (3.11)$$

$$c_{glucose} = \sum w_i c_{glucose_i}, \quad i = 1, 2 \quad (3.12)$$

$$\sigma^2 = \frac{\sigma_1^2 \sigma_2^2}{(\sigma_1^2 + \sigma_2^2)} < \sigma_i^2, \quad i = 1, 2 \quad (3.13)$$

3.4.3 High Concentration Measurement

The experiment was firstly done on the glucose solution at high concentration to prove the feasibility of the proposed method. Owing to the fact that PA effect is related to generation and detection of acoustic wave induced by laser, both amplitude and time delay information can be obtained simultaneously from time-domain PA signal. Not only amplitude variation, but also time shift can be observed from the PA waveforms

generated at different glucose concentrations, which are shown in Figure 3.7.

Figure 3. 7 PA waveforms of glucose solution at different concentrations.

Calibration was firstly conducted prior to prediction and the corresponding coefficients were determined by training data. Measurement was repeated five times for each concentration. The prediction results by peak-to-peak value and time delay are shown in Figure 3.8 from which their linear correlation with glucose concentrations can be clearly identified. The coefficients of determination (R^2) for peak-to-peak value and time delay versus glucose concentration are 0.9416 and 0.9668 respectively, indicating a strong linear correlation. As expected, the PA peak-to-peak value increases with glucose concentration while the time delay is decreasing as the ultrasound wave travels faster. 1 g/dL glucose variation leads to ~14% PA amplitude and ~11% time-delay change, corresponding to 0.27% velocity change when propagation distance is fixed at ~3.1 cm.

Figure 3. 8 (a) PA amplitude and (b) time delay of signal peak versus glucose concentration at high level.

The distinct peak-to-peak value and time delay data were firstly used to predict glucose concentration separately, followed by the weighted average combination described in Eq. 3.11 and Eq. 3.12. The results of the three prediction methods are depicted in Figure 3.9 and their performances are evaluated by root-mean-square error (RMSE) of each method. The hybrid PA detection (Figure 3.9(c)) yields a smaller RMSE of 0.3909 g/dL, indicating an accuracy improvement of 29.5% and 5.24% compared to single-parameter predictions (shown in Figure 3. 9(a), (b) with an RMSE of 0.5546 g/dL and 0.4152 g/dL for the peak-to-peak value and time delay correlation respectively). In addition to RMSE values, mean absolute errors (MAEs) with standard deviations (SDs) for peak-to-peak based, time-delay based predictions and data fusion are calculated as 0.48 ± 0.28 , 0.35 ± 0.2206 and 0.32 ± 0.2201 g/dL respectively, which also reflect the accuracy enhancement.

Figure 3. 9 Correlation between predicted glucose concentration and reference

concentration using (a) PA amplitude, (b) time delay and (c) data fusion. The unit of RMSE is g/dL.

3.4.4 Low Concentration Measurement

To further prove the potential of proposed hybrid PA measurement for clinical application, experiment was repeated on glucose solution at low concentration ranging from 0 mg/dL to 350 mg/dL which covers physiological range of diabetic patients. A flowing quartz cuvette filled with 4 mL pure water was used for the experiment. Glucose solution at a concentration of 350 mg/dL was uniformly and continuously injected into the cuvette by a syringe pump until it totally replaced the pure water. 35 data points were acquired and saved throughout the procedure and the experiment was repeated for five times. The linear trend over the whole range can be observed in Figure 3.10 by curve fitting, which indicates that the response of hybrid PA measurement is sensitive for low milligram per deciliter glucose measurement. Previous work [112] using an optimal time-domain filter achieved 38% accuracy enhancement in NIR spectroscopy measuring glucose concentrations ranged from 51 mg/dL to 493 mg/dL. Our proposed method achieved accuracy improvements of 33.63% and 55.80% respectively, which are reflected by the decrease of RMSEs (shown in Figure 3.11's caption) after data fusion.

Figure 3. 10 (a) PA amplitude and (b) time delay of signal peak versus glucose concentration at low level.

Furthermore, Clarke's error grid (CEG) analysis is also adopted for performance

evaluation at glucose level within physiological range. CEG is able to show the linear trend of prediction and assess the monitoring accuracy from clinical point of view by taking into account not only the difference between predicted and reference values, but also the clinical significance behind it [123]. Correlations between predicted and reference glucose concentrations mapped in CEG are presented in Figure 3.11. For peak-to-peak-dependent prediction, 17.14% of the data points fall in zone B, 2.86% falls in zone D and the others are in zone A. For time-delay-dependent prediction, 25.71% of the measured data is in zone B, 11.43% is in zone C, and zone D contains 2.86% of the data while the others fall in zone A. After data fusion, all of the points are well within clinically acceptable regions, among which 94.29% falls in zone A and 5.71% falls in zone B, indicating a significant improvement in detection accuracy.

Figure 3. 11 Correlations between preset and predicted glucose concentrations shown in CEG by using (a) PA amplitude, (b) time delay and (c) data fusion. The RMSEs are 36.48, 54.77 and 24.21 mg/dL respectively. The MAEs±SDs are 29.49±21.47, 39.01±38.45 and 16.09±18.10 mg/dL.

3.5 Summary

In conclusion, we proposed a novel method for convenient noninvasive glucose measurement employing PA technique. By inherent integration of optical absorption and acoustic wave propagation information, PA signal's peak-to-peak value and time delay can be simultaneously obtained and utilized to predict glucose concentration. The

sensing accuracy is improved by combining the single-parameter (peak-to-peak value and time delay) predicted glucose concentrations. The accuracy enhancement has been demonstrated on aqueous glucose solution at both high (0~7g/dL with 29.5% and 5.24% improvement) and low (0~350mg/dL with 33.63% and 55.80% improvement) concentrations, showing great potential of this novel noninvasive glucose sensing technique for self-monitoring of blood glucose. The data fusing process can be further optimized by precisely knowing error distribution function of each single-parameter-based prediction, which requires a large amount of training data. Despite that, the proposed method provides a new solution for accurate glucose measurement which may improve the diagnosis and treatment quality of Diabetes Mellitus. However, for *in vivo* measurement, interfering factors including temperature variation and movement should be identified and minimized. Moreover, individual calibration for each patient must be carefully done since the different skin properties and measurement sites could affect the amplitude and time delay information. Future work will be focused on development of a more reliable noninvasive glucose sensor using multi-dimensional data fusion with improved sensitivity, coupling efficiency and signal processing algorithm. After that, *in vivo* experiment and clinical human trial will be conducted.

Chapter 4. “GUIDE STAR” ASSISTED NONINVASIVE PHOTOACOUSTIC MEASUREMENT OF GLUCOSE

As mentioned before, glucose variation in physiological region is very small and hard to capture. Hence, the sensitivity of glucose sensor is crucial which strongly affects the proper functionality. One of the solutions is to adopt PA resonant cell and lock-in amplifiers to detect the specific narrow band signals [61, 124] at the cost of system size and complexity. Moreover, the strong electromagnetic direct coupling which shares the same repetition rate with the laser shooting will also fall in the detection band and causes severe interference in such frequency domain detection.

4.1 Working Principle

In this chapter, we propose a “guide star” assisted PA (GSPA) measurement method to overcome the above mentioned challenges by combining the fundamental working principles of NIR spectroscopy – Beer-Lambert’s Law [56] and PA measurement. A comparison of the conventional NIR spectroscopy, conventional PA measurement and proposed method are illustrated in Figure 4.1. In NIR spectroscopy (Figure 4.1(a)), the chromophore’s concentration can be determined by measuring light attenuation after penetrating the target, which is dominated by Beer-Lambert’s Law [125]:

$$A = \log\left(\frac{I_0}{I}\right) = \varepsilon(\lambda)Cl \quad (4.1)$$

where I and I_0 are transmitted and incident light intensities, $\varepsilon(\lambda)$ is molar extinction coefficient depending on wavelength λ , C represents chromophore’s concentration and l is optical path length that the photons traveled through. It is known that NIR spectroscopy mainly depends on the relative transparency of tissues in NIR region to attain penetration depth and the total absorption of the targeted compounds [126] is related to the tissue thickness in transmission mode, ignoring the low scattering in the

long wavelength region.

Figure 4. 1 Illustration and comparison of (a) NIR spectroscopy, (b) traditional PA measurement and (c) proposed GSPA measurement. LS: laser source; PD: photodiode; l : optical path length; UST: ultrasound transducer; VPD: virtual photodiode (“guide star”).

In traditional PA spectroscopy (Figure 4.1(b)), target’s absorption at certain wavelength will lead to energy deposition followed by thermal expansion, the generated acoustic pressure amplitude of the point source is then recorded and correlated to glucose concentration as described in Eq. 4.2 [127]:

$$p_0 = \eta F \Gamma \varepsilon(\lambda) C \quad (4.2)$$

where η is heat conversion efficiency, F is the fluence of laser beam reaching the target and Γ denotes Gruneisen coefficient which is equal to $\beta v^2 / C_p$. Here, β represents for thermal expansion coefficient, v means acoustic velocity and C_p represents for heat capacity at constant pressure. It can be seen that PA signal not only relates to wavelength dependent absorption but also depends on other mechanical properties such as acoustic velocity, thermal expansion coefficient and etc. Thus, other information like time of flight can also be utilized to predict glucose concentration, as proposed in our previous work [101]. Moreover, acoustic wave is usually more immune to scattering and attenuation in deep tissue and thus results in deeper penetration compared to pure optical methods. However, traditional PA spectroscopy relies on signal amplitude directly generated by the point source target whose difference caused

by glucose variation can be extremely weak, since its concentration is typically low in complex component matrix. Moreover, one of the major interfering compounds – water also has strong absorption peaks in NIR region. Because of these obstacles, the measurement sensitivity is usually poor.

To deal with the above-mentioned difficulties, GSPA measurement of glucose as shown in Figure 4.1(c) was proposed. In the proposed method, a virtual photodiode (“guide star”) is arranged in transmission configuration. Instead of investigating pressure directly generated from glucose-containing tissue, PA signal generated by the “guide star” is monitored to infer glucose absorption. The emitted light travelling from laser source to virtual photodiode is the same as that in NIR spectroscopy. However, the ultrasound transducer arranged in reflection mode replaces the photodiode to receive PA signals generated from the strong, broad-band and featureless absorbing object. Since PA signal is proportional to the fluence shining on the object, it essentially measures the transmitted light intensity after penetrating the glucose-containing tissue, exactly like utilizing a photodiode in transmission mode. The PA amplitude generated by the virtual photodiode can be estimated by modifying the heating source with Beer—Lambert’s Law:

$$p_0 = \eta\mu \frac{\beta v^2}{C_p} F_0 \exp[-\varepsilon(\lambda)Cl] \quad (4.3)$$

where F_0 is the fluence shooting on the surface of tissue, μ represents for the strong and invariant absorption of the “guide star”. Besides optical information, the received PA signal from “guide star” also contains acoustic information. In general, generation and propagation of PA wave can be described by the following one-dimensional wave equation [128] along the z-axis:

$$(\nabla^2 - \frac{1}{v^2} \frac{\partial^2}{\partial t^2})p(z,t) = -\frac{\beta}{C_p} \frac{\partial H(z,t)}{\partial t} \quad (4.4)$$

where H represents for the heating function defined as the deposited thermal energy per unit time and per unit volume. The above equation could be solved by Green's function as follows [129]:

$$p(z,t) = \frac{\beta}{4\pi C_p} \int dt' \int dz' [g(z,t|z',t')] \frac{\partial H(z,t)}{\partial t} \quad (4.5)$$

in which $g(z,t|z',t') = 2\pi v u(t-t' - \frac{z-z'}{v})$ and $u(x)$ is Heaviside function [130].

Normally, the PA signal is induced by a short pulsed laser source, the heating function in GSPA is thus in the form of $H(z',t') = \eta\mu F_0 \exp[-\varepsilon(\lambda)Cl]\delta(t')$. Substituting these into Eq. 4.5, we can obtain the generated and propagated pressure wave at the transducer surface as follows:

$$p(z,t) = \frac{\beta v^2}{2C_p} \eta\mu F_0 \exp[-\varepsilon(\lambda)Cl] \delta(t - \frac{z}{v}) \quad (4.6)$$

z is known as the transducer's position. From Eq. 4.3 and 4.6, it can be seen that GSPA method provides additional scalability of total absorbance by glucose and thus enhanced sensitivity owing to the tunable optical path length l . In addition, it is worth noting that glucose concentration variation not only leads to PA amplitude change but also causes sound propagation velocity variation [131]. Namely, sound velocity can be also expressed as a function of glucose concentration: $v = v_0 + kC$, where v_0 is initial sound velocity without glucose and k is a constant. Therefore, Eq. 4.6 can be further written as

$$p(z,t) = \frac{\beta(v_0 + kC)^2}{2C_p} \eta\mu F_0 \exp[-\varepsilon(\lambda)Cl] \delta(t - \frac{z}{v_0 + kC}) \quad (4.7)$$

where peak arrival time is $z/(v_0 + kC)$, representing the time it takes for the pressure wave to reach transducer surface. Based on linear approximation [132], values in the closeness of a certain point can be well approximated by the linear tangent curve to the

graph of the function. Namely, it can be linearized by $L(x) \approx f(a) + f'(a)(x - a)$ when $x \approx a$. Since glucose varies in a small range, linear approximation can be applied without loss of generality. Hence, , amplitude and arrival time of pressure wave are linearly proportional to glucose concentration [101] within the small range. The received waveform can be represented as the convolution of $p(z, t)$ and transducer's response, that is, $p_r(z, t) = p(z, t) * h(z, t)$. The simulated waveforms of 5 different glucose concentrations are shown in Figure 4.2 and the correlations of amplitude and peak position with glucose concentrations are also presented in insets. Due to the increased total absorption as glucose goes up, the number of photons reached the “guide star” decreases. Travelling time of pressure wave also decreases as the speed of sound increases. Therefore, it can be observed that both of the PA signal amplitude and peak delay time decrease with increasing glucose level.

Figure 4. 2 Simulated PA waveforms at 5 glucose concentrations (1~5 in the order of increasing glucose concentrations). Insets: correlation between PA amplitudes and glucose concentrations (blue); correlation between peak positions and glucose concentrations (red).

4.2 Materials and Methods

The proposed glucose measurement method was validated by in vitro experiment.

PA signals generated from glucose sample with concentrations over the range of 50 ~ 350 mg/dL, which fall into physio-logical range in different media, were measured and analyzed. Aqueous glucose solutions were firstly tested and followed by the solutions of HBS.

4.2.1 Sample Preparation

Pure glucose solutions were prepared for *in vitro* experiment. Commercially available high-purity α -D-glucose powder (α -Dextrose, 96%, Sigma Aldrich, 158968) was dissolved in distilled water to obtain the solutions with different concentrations ranging from 50 ~ 350 mg/dL with a step size of 50 mg/dL. Variations of PA waveforms were observed during measurement.

The proposed method was also verified with HBS (Sigma Aldrich, H4522) solutions. In order to reduce the glucose concentration adjustment error [133], 10 mL HBS was diluted in 90 mL PBS solution (0.05 M, pH = 7.4) and glucose powder was then added in the 10 v/v % HBS-PBS solution. Glucose concentrations were controlled to be from 50 to 350 mg/dL in steps of 50 mg/dL.

4.2.2 Glucose Absorption Spectrum in NIR Region

To select the proper wavelength used in the following experiment, absorption spectrum of glucose was measured by a commercial UV/Vis/NIR spectrophotometer (Lambda 950, PerkinElmer). A pair of highly transparent quartz cuvettes were used to contain the testing samples. The wavelengths from 1000 nm to 1800 nm were swept with a step size of 1 nm and the obtained spectra were plotted in Figure 4.3. Although the pattern is dominated by water absorption, it still can be seen that there are two major different absorbing regions. One falls at ~1453 nm where water exhibits stronger absorption resulting from the combination of H-O-H symmetric and asymmetric stretch mode transitions [134]. At this wavelength, total absorbance will decrease as the glucose

concentration increases due to the reported water displacement effect. The other region is wide including wavelengths from $\sim 1580\text{ nm}$ to $\sim 1700\text{ nm}$ which is assigned as $-\text{OH}$ and $-\text{CH}$ combination band ($\nu\text{OH} + \nu\text{CH}$) [51]. The absorption is increasing with glucose concentrations in this region. In our experiment, 1600 nm was selected as the illuminating light source considering the absorption and penetration requirement in GSPA. The absorption of 3M glue which is used to fix the guide star is much lower than glucose and water absorptions at this wavelength. Moreover, its position and thickness were kept unchanged throughout the experiment. Hence, the effect from the glue can be neglected.

Figure 4. 3 Absorption spectra of water, glucose and 3M glue

4.2.3 Experimental Setup

The experimental setup of GSPA is depicted in Figure 4.4 (a). The heating source is a tunable laser system (OPOTEK, Opolette 355), which is able to provide laser wavelengths from 400 nm to 2000 nm with a pulsed duration of 7 ns at 20 Hz . The widely available flowing quartz cuvettes with different optical path lengths (1 mm and 2 mm) were adopted to demonstrate the scalable and enhanced sensitivity of our proposed method. Sample solutions were injected through the silicone tube by syringe to minimize the setup instability. A single-element piezoelectric ultrasound transducer

(Doppler Electronic Technologies Co., Ltd) with a 2 mm-diameter hole in the center was customized so that the laser beam can pass through the hole and shine on the target. The customized transducer's frequency is centered at 2 MHz with ~80% bandwidth. It was arranged as reflection mode and fixed at one side of the sample container by 1 mm thick 3M glue which is also used as acoustic coupling medium. A black tape (3M, Heavy Duty Vinyl Electrical Tape 22) with broadband, featureless and strong absorption was served as the "guide star". Because of these properties, the black tape is usually employed to quantify and evaluate performance in PA imaging studies [135, 136]. It was attached by a 2 mm thick 3M glue and PDMS at the other side. PDMS is used as acoustic coupling [137] and delay layer so that PA signal can be collected and differentiated from directly coupled electromagnetic interference. Moreover, PDMS is also transparent at 1600 nm which will not attenuate laser energy [138]. The output energy of the laser beam was measured to be 0.5 mJ and focused by a condenser lens to an area with diameter of 1 mm. After passing through the transducer's hole, the fluence at the surface of the sample is calculated to be $\sim 16 \text{ mJ/cm}^2$, which is well within ANSI standard [139]. The received signal was fed into a pre-amplifier (Olympus, 5662) with 54 dB gain and digitized by an oscilloscope (Teledyne LeCroy, Waverunner 8000) with a sampling rate of 250 MHz. The signals were averaged 100 times to improve the SNR by 20 dB. Captured data were then sent to a computer for post processing. The data refreshing time is around $\sim 7\text{s}$. Temperature was kept being $23 \pm 1^\circ\text{C}$ throughout the experiment to eliminate the effect on PA amplitude and sound velocity.

Figure 4. 4 (a) Schematic of experimental setup. GS: guide star; UST: ultrasound transducer; PDMS: polydimethylsiloxane; ConL: condenser lens; PD: photodiode; BS: beam splitter; LNA: low-noise amplifier; DAQ: data acquisition system. (b) Obtained PA waveforms at different glucose concentrations.

4.2.4 Calibration and Evaluation

The 10 data sets obtained with each cuvette were equally divided into calibration and validation sets. Amplitude and peak position information of calibration sets were extracted and fitted by MATLAB R2015b (MathWorks, Inc.) curve fitting toolbox. The coefficients of fitted curves were then returned and used to predict glucose level of validation sets and compare with known reference glucose levels. The performance was evaluated in terms of coefficient of determination (R^2), root mean square error (RMSE) of prediction. However, correlation indicated by R^2 is only used to show how strongly a pair of data are related. To further assess the degree of agreement between new method and golden standard, BA plot (also called difference plot) was employed to investigate the difference between predicted values and references[140]. In a BA plot, mean of difference is to show consistent bias of the new measurement results compared to standard. Limits of agreement, which are the mean difference plus and minus 1.96 time

of standard deviation (SD) represent the range of 95% normally distributed differences. Generally speaking, narrower intervals reflect smaller difference variations and better measurement quality of the new method. Furthermore, to be clinically meaningful, the CEG analysis was also adopted which divides the entire prediction range of glucose values into five regions, taking into account the clinical considerations [123]. Typically, Zone A and B are considered as clinically acceptable regions, whereas data in C-E will potentially result in erroneous and even dangerous treatment. The more data fall in Zone A, the better accuracy the proposed method achieves.

4.3 Results and Discussion

The experiment was first carried out using the pure aqueous glucose solution to prove the concept of GSPA. Figure 4.4(b) shows the example of collected PA waveforms at different glucose concentrations, from which the variation trend is seen to be well coincident with the simulated results (Figure 4.2). As glucose concentration rises, PA amplitude generated from the guide star is decreasing and the wave travels faster leading to the shorter arrival time of its peak. To demonstrate the effect of optical path length in GSPA, experiments were done with both 1-*mm* and 2-*mm* quartz cuvettes. The amplitude and peak position fitting results are presented in Figure 4.5.

Figure 4. 5 Measurement results of **aqueous glucose solution** in **1-mm** cuvette: (a) Amplitude fitting and (b) peak position fitting with glucose concentrations. (c) BA plot. (d) Clarke's Error Grid analysis. Measurement results in **2-mm** cuvette: (e) Amplitude fitting and (f) peak position fitting with glucose concentrations. (g) BA plot. (h) Clarke's Error Grid analysis.

For the 1-mm quartz cuvette, by extracting the amplitudes and peak positions followed by correlation with glucose concentrations, the R^2 values are calculated to be 0.9025 and 0.931 respectively as presented in Figure 4.5(a) and (b). The linearly fitted RMSE values are $1.647\text{E-}03$ V and $3.312\text{E-}09$ s for the amplitude and peak position. It can be regarded as good correlation regarding these two evaluation coefficients. The

sensitivities are reflected as the slopes of the fitting curves. In this case, every 1 *mg/dL* of glucose concentration variation leads to 47.34 μV change in amplitude and 0.1029 *ns* change in peak arrival time. By using the data fusion method proposed by us previously [101], individual weighting factor of amplitude and peak position fittings were estimated through calibration and employed to combine prediction results of the two indicators. The obtained final results were evaluated by BA plot and CEG analysis. The BA plot shows a mean difference of 0.0081 *mg/dL* and the limits of agreement are 37.3185 and -37.3023 *mg/dL* (0.0081 $\pm$ 37.3104) respectively. In the CEG chart, the correlation coefficient R^2 improves to 0.9699 after data fusion and RMSE value is 20.85 *mg/dL* for final prediction. Among all the data points, 85.71% falls in Zone A (clinically accurate) and 14.29% is in Zone B (clinically acceptable). The performance can be regarded as acceptable based on the common criteria. Hence, it shows that validity and feasibility of our proposed GSPA glucose measurement were well verified.

To demonstrate the sensitivity scalability of GSPA, experiment was repeated with the 2-*mm* quartz cuvette. The amplitude and peak position fitted coefficients of determination are 0.9811 and 0.9867 shown in Figure 4.5(e) and (f), together with RMSE values of 9.52E-04 *V* and 2.318E-09 *s*. Based on these two evaluation parameters and fitting curves, the better correlation of 2-*mm* optical path can be visualized compared to the previous 1-*mm* one. Moreover, improvement can also be found regarding sensitivities. 1 *mg/dL* concentration change leads to 58.02 μV amplitude and 0.1686 *ns* peak position changes in this case, which will result in easier detection of glucose variation, especially in low concentrations. After processed with data fusion, the mean difference between final predicted glucose levels and know references is -0.0923 *mg/dL* and BA plot in Figure 4.5(g) reveals the variation interval is [-12.8540 12.6694], which is calculated as $Mean \pm 1.96 SD$ (-0.0923 $\pm$ 12.7617). In addition, from

clinical point of view, final prediction result achieved 0.9964 and 7.13 *mg/dL* of R^2 and RMSE values. 100% of data points fall in Zone A as plotted in the CEG, indicating the extraordinary accuracy of the GSPA measurement of glucose.

To better visualize it, performances regarding all the evaluation parameters of different optical path lengths are summarized and compared in Table 4.1. It is worth noting that apparent performance improvement was achieved by using longer optical path length under the same experimental conditions. Firstly, the coefficients of determination R^2 have been increased while the RMSE values have been reduced which represent better correlation quality. The slopes of amplitude and peak position measurements were increased by 22.6% and 64.1% respectively, indicating the enhancement of measurement sensitivity. This is due to the increased total absorbance and travelling distance before the photons reach and illuminate guide star, as illustrated in Eq. 4.7. Under the circumstances, every unit of glucose concentration change will result in larger variations in the corresponding parameters which enhances the measurement quality. More interestingly, the prediction accuracy reflected by BA plot and CEG analysis also shows the advance of using 2-*mm* cuvette. Variation interval in BA plot narrows down for nearly 3 times, from [37.3185 -37.3023] to [-12.8540 12.6694] and more prediction data in CEG fall in Zone A. From the above discussion, combining with advantage of NIR spectroscopy, scaling factor of the optical path length l offers the adjustability for sensitivity and accuracy optimization, compared to traditional direct PA glucose measurement method. Despite that longer optical path length tends to provide higher sensitivity, this improvement is restricted in a certain range. The penetration depth achieved is more than 2 *mm* such that enough photons can reach the “guide star” to generated PA signal. It should be noted that the number of photons reaching virtual photodiode is inversely proportional to optical path length, just

like the transmitted mode NIRS. Hence, PA amplitude generated by “guide star” will decrease as l increases, which is reflected in Figure 4.5(a) and (e). In other words, optical path length should be selected as long as possible to achieve better sensitivity while making sure PA signal generated by “guide star” not falling below noise level. Due to the dual effect of l , an optimized value regarding measurement performance exist which could be determined by calibration for individual patients.

Table 4. 1 Performance comparison of 1-*mm* and 2-*mm* optical path length

Parameters	Correlation						Prediction						
	Amplitude			Peak Position			BA	CEG					
	R ²	RMSE (V)	Slope	R ²	RMSE (s)	Slope	Mean± 1.96SD (mg/dL)	R ²	RMSE (mg/dL)	A	B	C	D
1- mm	0.9025	1.65E-03	-4.73E-05	0.9310	3.31E-09	-1.03E-10	0.0081±37.3104	0.9699	20.85	85.71%	14.29%	0%	0%
2- mm	0.9811	9.52E-04	-5.80E-05	0.9867	2.32E-09	-1.69E-10	-0.0923±12.7617	0.9964	7.13	100%	0%	0%	0%

To further demonstrate the potential of proposed GSPA measurement of glucose, experiment was carried out on prepared HBS. HBS is the fluid portion of blood clot removing red cells, white cells or fibrinogen [141]. It’s relatively transparent and more similar to interstitial fluid which makes up 55% of blood volume and contains various proteins, glucose and lipids [142]. The 2-*mm* was selected because of its better performance as discussed previously. The results are presented in Figure 4.6. Curve fitting results show strong correlation of PA amplitude and peak position versus glucose concentration. The coefficients of determination achieved 0.9581 and 0.9557 respectively. In BA plot analysis, the mean value of the references minus data fusion results is -0.1507 *mg/dL* which varies in the range of [30.7942 -31.0956]. In CEG analysis, the R^2 and RMSE values are 0.9791 and 17.3 *mg/dL*, respectively. Moreover, 100% data points fall in clinically accuracy region A. Although the performance of HBS measurement is a bit worse than that of pure glucose solution owing to more complex component matrix, it still can be regarded as a precise one, based on all the evaluation parameters. This result further proves feasibility and potential of the proposed method

for practical application. The short sensing time for each readout enables the continuous and real time monitoring. However, due to the stronger scattering and absorption of biological tissue, laser power, optical path length (tissue thickness) and the sensitivity have to be carefully calibrated and optimized in real clinical application.

Figure 4. 6 Measurement results of **HBS** in **2-*mm*** cuvette. (a) Amplitude fitting and (b) peak position fitting with glucose concentrations. (c) BA plot. (d) Clarke's Error Grid analysis. $R^2 = 0.9791$, $RMSE = 17.3 \text{ mg/dL}$.

4.4 Summary

In conclusion, we proposed and demonstrated a “guide star” assisted method for noninvasive glucose measurement combining merits of PA and NIRS. First of all, glucose-related information including optical absorption and sound velocity can be acquired by PA signals. Besides, adjustment of tissue thickness (optical path length), which photons need to transmit before illuminating the “guide star”, enables ability of sensitivity and accuracy enhancement. *In vitro* detection was evaluated by correlation quality, degree of agreement and clinical accuracy. The above indices proved feasibility

of the GSPA method to measure glucose in physiological range. Moreover, using 2-*mm* quartz cuvette shows more reliable measurement result compared to 1-*mm* one, as expected. It is because that larger l amplifies the signal difference caused by glucose variation, as indicated in Eq. 4.7. The performances were compared in Table 4.1 regarding sensitivity (slope), goodness of correlation (R^2) and prediction errors, which demonstrated the scalability and sensitivity enhancement offered by optical path length adjustment. For clinical trial, the quartz cuvette is to be replaced by the tissue at suitable measurement site such as earlobe and purlicue. These measurement sites are usually hairless and without bones whose thickness can be adjusted by a personal clip. The laser fluence at 1600 *nm* is far below the MPE of skin specified by ANSI standard (1 J/cm^2) which indicates the true non-invasiveness of the GSPA method for clinical application. To develop portable and low-cost sensors, compact laser diodes are usually adopted to replace bulky laser sources. However, the output power is low, leading to poor sensitivity. Previous work [143, 144] employed multi-pass gas cell with all-optical interferometric detection or dedicated fiber amplifier, did achieve promising result regarding sensitivity enhancement. However, system complexity has also been significantly increased, preventing them to be used for real application. Our approach here provides promising solution to enhance the sensitivity without increasing system complexity, which is one of the common challenges for noninvasive glucose monitoring, facilitating future diabetes care. Besides, it can be also incorporated into other PA sensing applications such as blood oxygen saturation measurement and *et al.* Despite that longer optical path leads to higher sensitivity, a limit of enhancement exists where the PA signal from “guide star” is just detectable from noise floor. The optimized tissue thickness is related to system parameters and patients’ conditions. Therefore, similar to other glucose monitoring devices, individual calibration for this method is an

essential procedure to find out the optimized tissue thickness and set the baseline. This method is undergoing a patent procedure.

Chapter 5. TIME-DOMAIN PHOTOACOUSTIC WAVEFORM SPECTROSCOPY FOR GLUCOSE MEASUREMENT

PA effect is the product of light-ultrasound interactions and its time-domain waveform contains rich information. Besides absorption, the waveform inherently consists of other mechanical properties of the sample including acoustic propagation velocity, thermal expansion, viscosity and *et al.*, which are all related to glucose variation but have not been utilized in conventional PA spectroscopy. In this chapter, we propose a novel method named time-domain PA waveform spectroscopy (tPAWS). It is achieved by multivariate analysis of time-domain PA waveform generated at single wavelength to quantify glucose concentration from highly noisy background. Fundamental theory was analyzed to show the superiority of tPAWS over conventional peak-amplitude based PA prediction. In vitro experimental on HBS were conducted to prove the feasibility of the novel method and demonstrated its significant gain in sensitivity and accuracy (more than 10 times regarding RMSE) without increasing apparatus and system complexity. Moreover, commercial multi-wavelength infrared (IR) spectrometer was also adopted for performance comparison to further demonstrate the superiority of tPAWS. Overall, it shows the great potential of tPAWS to complement or surpass the current spectroscopic method as a new sensing concept and technique for glucose monitoring and other chemical analysis.

5.1 Working Principle

Noninvasive glucose measurement remains “the holy grail” [145] for decades. It is challenging in terms of specificity, sensitivity and accuracy. Various technologies have been studied to solve these issues by measuring either blood or interstitial fluid (ISF) [146]. Among those technologies, PA spectroscopy has shown promising capability, as it overcomes the diffraction limit faced by optical methods. Meanwhile, it also utilizes

the specific absorption of glucose in near-infrared/mid-infrared (NIR/MIR) region. As mentioned before, Traditional IR spectrometer acquires absorption information based on Beer-Lambert law [56]:

$$A = \log\left(\frac{I_0}{I}\right) = \varepsilon(\lambda)Cl \quad (5.1)$$

where A is total absorbance, I and I_0 are transmitted and incident light intensities, $\varepsilon(\lambda)$ is molar extinction at wavelength λ . Chromophore's concentration C can be calculated when optical path length l is known. Being different from traditional optical techniques involving only optical properties, PA effect is a multi-physical process and its waveform is related to various parameters including optical absorption, heat capacity, viscosity, sound velocity, etc. However, traditional PA spectroscopy typically chooses the strongest peak in transient signal and uses its magnitude to infer absorber's concentration. This simplified approximation assumes that only optical absorption is related to glucose concentration, as reflected in the commonly used equation:

$$Amp(\lambda) = k\Gamma F \varepsilon_g(\lambda)C_g \quad (5.2)$$

where k represents light-to-heat conversion efficiency, Γ is Gruneisen parameter describing thermodynamic properties, F is laser fluence in mJ/cm^2 , $\varepsilon_g(\lambda)$ is glucose extinction coefficient at wavelength λ , and C_g is glucose concentration. Similar to IR spectroscopy, PA amplitude at a certain wavelength is one-dimensional datum. Multiple wavelengths must be used for selectivity and sensitivity compensation which increases the system complexity and cost. Peak-amplitude-based approach may work well when signal-to-noise ratio (SNR) is good. However, it can be easily distorted by the surrounding noise and interference which may severely deteriorate measurement accuracy. The rich information in PA waveform obtained at single wavelength is not fully utilized in this case. It has been noted that parameters like viscosity, thermal

properties, acoustic propagation velocity and acoustic impedance are also affected by blood or ISF composition [101, 147], which will contribute to the PA waveform generation. In this letter, we propose to take full advantage of single wavelength PA waveform to enrich the data dimensions so that measurement sensitivity and accuracy can be dramatically improved. This will be realized by multivariate analysis of time-domain PA waveform (tPAWS).

Taking into account glucose-related parameters, time-domain PA waveform can be expressed as a damped sinusoidal signal [148], whose shape depends on the target density, viscosity and acoustic velocity:

$$p(t) = A \exp(-Bt) \sin(\sqrt{a^2 v^2 - B^2} t) \quad (5.3)$$

where A is determined by initial condition and related to absorption $\mu = \varepsilon C$. v is acoustic velocity. B relates to propagation phase constant a , shear viscosity η , bulk viscosity ξ and density ρ in the way that $B = 0.5a^2(\xi + 4\eta/3)/\rho$. It is worth noting that apart from absorption, the above mentioned mechanical properties are all affected by glucose change which are usually neglected. Namely, the shape of PA waveform is directly affected by glucose concentration or other components. More interestingly, PA waveform can be seen as the linear superposition of individual absorber's partial wave [149].

In practice, the acquired waveform is more complex. Instead of the typical N-shape bipolar pulse, ringing is often noticed due to the limited transducer bandwidth. Moreover, the reverberation due to acoustic impedance ($z = \rho v$) mismatch at boundaries will further distort the waveform. The received pressure wave is also determined by laser pulse duration, illumination area, transducers' geometry, sample size and other noise from electrical components. Assuming the measurement condition is fixed and all other factors are relatively stable, we can say without loss of generality,

the variation of PA waveform is mainly caused by target's property change resulted from concentration variation and random noise. Considering the changes are subtle and within physiological range, the obtained waveform can be regarded as linear response of the component concentration.

Univariate analysis or simple linear regression (SLR) using PA amplitude cannot make appropriate prediction presenting interfering signals. On the other hand, multivariate analysis of the waveform is possible to recover useful information from noisy signals [150]. Multilinear regression (MLR) finds out the linear combination of variables in original matrix to estimate concentrations using calibration data set. Nevertheless, the trained model may incorporate too much redundant information. On the contrary, principal component regression (PCR) is able to decompose the multi-dimensional data into a set of uncorrelated orthogonal components based on their variances, before regress it to response. It is widely applied in spectroscopic analysis done by multiple wavelengths. In the proposed method, we creatively adopt it to describe time-domain PA waveform. The expression is as follows [151]:

$$\mathbf{p}(\mathbf{t}) = \begin{bmatrix} p_1(t_1) & \cdots & p_1(t_n) \\ \vdots & \ddots & \vdots \\ p_m(t_1) & \cdots & p_m(t_n) \end{bmatrix} = \mathbf{S}\mathbf{L}^T + \mathbf{E} \quad (5.4)$$

where $\mathbf{p}(\mathbf{t})$ is a $\mathbf{m} \times \mathbf{n}$ training data matrix containing predictors, whose $\mathbf{m}$ rows are observations at different concentrations and $\mathbf{n}$ columns are variables, in this case, PA signals at each time point. $\mathbf{S}$ is score matrix ($\mathbf{m} \times \mathbf{q}$) and $\mathbf{L}$ is loading matrix ($\mathbf{n} \times \mathbf{q}$) with $\mathbf{q}$ principal components in columns. $\mathbf{E}$ is formed of residuals. The loading and score matrices are obtained by calibration data set. Then, MLR is applied to regress $\mathbf{S}$ to concentration vector $\mathbf{C}_g$ ($\mathbf{m} \times 1$) and obtain regression coefficients $\hat{\boldsymbol{\beta}}$ ($\mathbf{q} \times 1$):

$$\hat{\boldsymbol{\beta}} = \arg \min_{\boldsymbol{\beta} \in \mathbb{R}^q} \{(\mathbf{C}_g - \mathbf{S}\boldsymbol{\beta})^T (\mathbf{C}_g - \mathbf{S}\boldsymbol{\beta})\} = (\mathbf{S}^T \mathbf{S})^{-1} \mathbf{S}^T \mathbf{C}_g \quad (5.5)$$

such that $C_g = S\hat{\beta} + E$ [152]. When validation data $p_{m+1}(t)$ come in, its corresponding glucose concentration can be estimated by multiplying the loadings and regression coefficients obtained from calibration data:

$$C_{g,m+1} = p_{m+1}(t)L\hat{\beta} \quad (5.6)$$

It can be said that PCA is a subcategory of unsupervised learning. Its counterpart in supervised learning - partial least squares regression (PLSR) can realize the similar function, given the known concentration vector as response during calibration. While trying to explain the variance of original data matrix, PLSR also maximize the covariance between score matrix and concentration matrix [136] to achieve a more meaningful and efficient prediction.

5.2 Materials and Methods

5.2.1 Sample Preparation

Aqueous glucose solution was prepared using α -D glucose powder (158968, Sigma Aldrich) and distilled water. 10 sample solutions ranging from 50 mg/dL to 500 mg/dL, at a step size of 50 mg/dL were prepared and used in the later IR spectrometer measurement. Then, sample solution with HBS (HBS, H4522, Sigma Aldrich,) were also prepared in the same concentrations. Phosphate-buffered saline (PBS) was used to dilute HBS and reduce glucose adjustment error. After that, α -D-glucose powder was added into the 10 v/v % HBS-PBS solution to obtain the 50 mg/dL ~ 500 mg/dL sample solution. The obtained reference concentrations are within human physiological range.

5.2.2 Experimental Setup

PA measurement was carried out by a self-built system on the HBS solutions. For comparison purpose and show the advantages of tPAWS, commercial IR spectrometer was employed to quantify aqueous glucose solution.

5.2.2.1 Photoacoustic Measurement

In vitro experiment was carried out to prove the feasibility of proposed glucose prediction by PA waveform analysis and evaluate the performance of different multivariate analysis. The experimental setup is depicted in Figure 5.1. Laser beam from a wavelength-tunable laser (Opolette 355, OPOTEK) was fed into a fiber by a lens group. The fiber output was connected to a reflective collimator and directed to illuminate the sample solution contained in quartz cuvette. A syringe connected with plastic tube was used to change the solution. The laser was tuned to 1600 nm because of strong glucose absorption at this wavelength [153]. The fluence was controlled to $\sim 30\text{mJ/cm}^2$ which is well within the maximum permissible exposure (MPE) of skin, regulated by ANSI standard [154]. A ring-shaped 2 MHz ultrasound transducer with 60% bandwidth was arranged in reflection mode, putting on the sample. In between, a 3 mm thick transparent PDMS slice was employed as gel-free acoustic coupling and delay layer. The ultrasound signal was amplified by a low-noise amplifier (54 dB), sampled by an oscilloscope with 100MS/s sampling rate and sent to computer for post-processing. Two data sets were prepared for calibration and validation by repeating the measurement.

Figure 5. 1 Schematic of experimental setup. PDMS: polydimethylsiloxane; UST: ultrasound transducer; QC: quartz cuvette.

5.2.2.2 Infrared Spectroscopy

Aqueous glucose samples were measured by commercial UV/Vis/NIR

spectrophotometer (Lambda 950, PerkinElmer) for further comparison. A pair of quartz cuvettes with effective optical path length of 1 mm were placed in the spectrometer. One of them was empty and served as reference and the other contained sample solution under test. Wavelength was swept from 800 nm to 1875 nm with 5 nm interval.

5.3 Results and Discussion

5.3.1 Traditional Amplitude-based Photoacoustic Measurement

The original PA waveforms of calibration set without any data processing are presented in Figure 5.2(a). It can be seen that the waveforms are severely distorted by noise and reverberation caused by the system instability and complex boundary conditions. Using traditional peak-to-peak amplitude prediction fails to reflect glucose variation trend (Figure 5.2(b)). The coefficient of determination R^2 is normally used to measure the prediction power of the fitted model. In this case, R^2 is only 0.5387, indicating there is no obvious linear relationship between amplitudes and glucose concentrations. This kind of failure often happens in practice under unpredictable and fluctuating conditions, which hinders the accurate and effective measurement.

Figure 5. 2 (a) Time-domain PA waveforms of glucose solution at different concentrations (50 – 500 mg/dL) presenting noise and reverberations. Inset: zoom-in views of positive and negative peaks. (b) Peak-to-peak amplitude versus glucose concentrations.

5.3.2 Time-domain Photoacoustic Waveform Spectroscopy (tPAWS)

PA waveform inherently consists of many concentration-related parameters. Since it is much more informative than amplitude only, MLR, PCR and PLSR were applied to analyze the waveform and predict glucose concentration respectively, by MATLAB statistics and machine learning toolbox. The non-informative data before the first N-shape pulse were removed to reduce redundancy. Generally speaking, MLR may work well under ideal situation. However, this blind regression model may incorporate irrelevant variances which are not due to glucose change [155]. Different from MLR, PCR sequentially extracts components that contribute to the largest variations of the waveforms in an unsupervised manner. Normally, in ISF, the component matrix is much simpler than that in blood. The assumption that the largest variation is due to glucose change can be justified since lipid, melanin are not expected to fluctuate in a relatively short time, especially in the 2-hour oral glucose tolerance test (OGTT) [61]. Hence, it is anticipated that the first few principal components of PA waveform are related to glucose. Apart from PCR, PLSR can realize the similar function in a supervised manner. It is used to explain the variance of the PA waveform and meanwhile, maximize its covariance with response, which is glucose concentration.

In this demonstrative experiment, 9 principal components were obtained as there are 10 different concentrations. The total variance in predictors explained by different number of components are plotted in Figure 5.3(a), where PCR curve is higher than PLSR. It's not surprising that PLSR explains less variance in predictors than PCR does, with the same number of components. Because the subspace defined in PCR maximizing the variance rotates to a direction that can better describe response in PLSR. The variance explained in response versus number of components used in PLSR is plotted in Figure 5.3(b). It shows that using 3 components can almost explain all the

variance in response vector. Next, to determine the appropriate number of components used to build up the regression models, mean squared prediction errors (MSPE) of PCR and PLSR with accumulated number of components were plotted in Figure 5.3(c).

Figure 5. 3 (a) Total percentage variance explained in predictors using different number of principal components obtained by PCR and PLSR. (b) Percentage variance explained in response with different number of principal components used in PLSR. (c) MSPE of PCR and PLSR with accumulated number of components.

Leave one out cross validation (LOOCV) was applied to reduce overfitting problem. The explained variances and fitting MSPEs of PCR and PLSR with accumulated number of components are summarized in Table 5.1, where **Q** means total number of components used. The fitting results indicate that using 3 components in PLSR can achieve relatively good fitting, with ~59% of total variance in predictors and ~100% variance in response explained. However, in PCR, at least 5 components are needed to obtain similar prediction accuracy with ~85% of total variance explained. The less variance explained in predictors using PLSR does not weaken its prediction power, since a large portion of variance may not result from glucose change. PCR, because of no supervision, tends to incorporate this redundant information.

Table 5. 1 Summary of fitting performance obtained with accumulated number of components Q

Q	PCR		PLSR		
	Explained predictor variance	MSPE	Explained predictor variance	Explained response variance	MSPE
1	40%	1279	40%	96%	713
2	56%	1273	51%	99%	71
3	69%	496	59%	100%	2
4	79%	494	74%	100%	1
5	85%	16	79%	100%	0
6	89%	16	88%	100%	0
7	93%	16	92%	100%	0
8	97%	11	96%	100%	0
9	100%	0	100%	100%	0

5.3.3 Comparison of Conventional Photoacoustic Amplitude Measurement and tPAWS

The trained regression models by SLR, MLR, PCR and PLSR were applied on validation data set and the prediction results were evaluated by CEG analysis, which is “gold standard” to quantify clinical accuracy of glucose measurement. Region A contains values that are within 20% of the references. Region B means the prediction is not so accurate but will not lead to wrong treatment. These two regions are considered as clinically acceptable. Moreover, BA plot was also employed to assess the level of agreement between references and measured values. Presence of potential bias or outliers can be identified through this plot. CEG and BA plots are presented in Figure 5.4.

Figure 5. 4 Performance evaluation by CEG analysis and BA plots. (a)-(d) are CEGs for PA amplitude-based SLR, waveform-based MLR, PCR and PLSR, respectively. (e)-(h) are the corresponding BA plots.

Apparently, the novel prediction method by PA-waveform analysis is much more superior to traditional amplitude based one. Single-dimensional amplitude data can be easily distorted resulting in weak correlation with glucose concentration. On the contrary, multivariate analysis is able to recover glucose information from waveform, especially when employ PCR and PLSR. Time-domain PA waveform inherently involves multiple glucose-concentration-related parameters which should not be neglected. The multiple variables can compensate each other to increase information richness and suppress noise which cannot be achieved by bandpass filter, since bandpass filter only blindly removes signal in certain frequency range without considering information effectiveness. However, some of the variables in the waveform may describe overlapping information or noise. To improve data parsimony and prediction accuracy, dimension reduction techniques including PCR and PLSR can be applied.

5.3.4 IR Spectrometer Measurement on Aqueous Glucose Solution

To further show the merits of tPAWS, commercially available dual-beam spectrometer Lambda 950 was employed to measure aqueous glucose samples at physiological concentrations. The absorption spectra were recorded from 800 *nm* to 1875 *nm* which are shown in Figure 5.5.

Figure 5. 5 IR absorption spectra of aqueous glucose solution (50 ~ 500 mg/dL) obtained by Lambda 950.

Since the concentration variation is very small, the differences in absorption spectra can be hardly seen without any data processing. Then, multivariate analysis was applied to further analyze the obtained spectra at different glucose concentrations. For a fair comparison, 5 principal components in PCR and 3 in PLSR were incorporated to predict glucose concentrations. The obtained results were also evaluated by CEG and BA plot, as shown in Figure 5.6.

Figure 5. 6 IR spectroscopy performance evaluation. (a) and (b) are CEG analysis and BA-plot of prediction by PCR. (c) and (d) are CEG and BA-plot of prediction by PLSR.

Compared to the proposed tPAWS shown in Figure 5.4, IR absorption spectra with multivariate analysis does not perform well, especially at low concentration range. The first three points deviates the reference line a lot and fall in region B and D, indicating

the sensitivity which only relies on optical absorption is relatively low even with multi-wavelength compensation. On the contrary, PA waveform is decided by multiple glucose-related parameters and hence may be more sensitive to tiny variation. To better compare each method and perceive the advantage of tPAWS, Table 5.2 summarizes the performance indicators of PA amplitude prediction by SLR, tPAWS using MLR, PCR, PLSR and IR spectroscopy.

Table 5. 2 Summary of prediction performance of different methods

Method Indicator	Single-wavelength PA system				IR spectrometer	
	Amplitude	tPAWS			Absorption spectra	
	SLR	MLR	PCR	PLSR	PCR	PLSR
Q	-	-	5	3	5	3
R²	0.4760	0.8947	0.9955	0.9956	0.9285	0.9087
RMSE (mg/dL)	173.40	44.56	9.93	9.84	41.36	46.25
A	20%	70%	100%	100%	70%	50%
B	20%	10%	0%	0%	20%	40%
C	50%	0%	0%	0%	0%	0%
D	10%	20%	0%	0%	10%	10%
Mean ±1.96SD (mg/dL)	34.97 ±320.54	-7.97 ±97.50	2.26 ±29.52	2.49 ±29.18	9.88e-12 ±79.32	-2.61e-12 ±89.67

TPAWS shows its outstanding prediction power among traditional methods. The accuracy improvements regarding RMSE are more than 10 times and 4 times respectively, compared to traditional amplitude-based PA sensing and IR spectroscopy. Although the experiment was demonstrated on glucose measurement, the proposed method can be extended to other laboratory analyte quantitation or in vivo noninvasive physiological parameter measurement. The advantages of proposed method are multi-facet: 1. Differences among various samples are amplified as the original data matrix is rotated to maximize variance. Hence, sensitivity can be significantly enhanced. 2. The trivial information is removed and noise is further suppressed in a more reasonable way, which cannot be realized by simple noise filter. 3. Since sensitivity is improved, bulky

powerful laser, which is employed to guarantee SNR and stable peak-to-peak values can be replaced by lower power laser diode. Miniaturization of the whole system can be realized. 4. Unlike IR spectrometer requiring multiple wavelengths, the proposed method is cost-effective since the waveform containing multi-dimensional data can be easily obtained using single wavelength for post-processing. It does not need additional apparatus or experiment procedure compared to existing PA setup.

However, there are also several limitations of the proposed method. First of all, it may not contribute to selectivity enhancement. In infrared spectroscopy, selectivity is realized by distinct and sharp absorption peaks of individual analyte, while overlap of these peaks will degrade selectivity. For the proposed method, PA waveforms of different components are overlapped as most of them are mixed in the solution or tissue and cannot be resolved in time domain. Derivative of the waveform may be studied and incorporated in the future to improve waveform resolution, like what is done in derivative spectrophotometry [156]. TPAWS works best when the largest variation is caused by target analyte. If stronger interfering components exist, the first few principal components will tend to describe them other than target analyte. In that case, lower order of principal components should be considered instead. Secondly, the violent movement and boundary condition change will distort the waveform pattern and invalidate the calibrated coefficients. This does not present a problem for in vitro experiment as testing conditions can be well controlled and fixed. However, for noninvasive glucose measurement on human, it is difficult to exactly copy the testing condition every time. Therefore, a mechanical structure like a finger clip for fixation must be designed to alleviate the problem.

5.4 Summary

To summarize, we proposed to utilize time-domain PA waveform by multivariate

analysis to estimate glucose concentration in a brand new way, which is named as tPAWS. In vitro experiment on HBS demonstrated the feasibility and the preliminary results clearly showed its superiority over traditional PA sensing. Moreover, tPAWS using homemade PA system was also compared with commercial multi-wavelength IR spectrometer measurement. Its sensitivity and prediction accuracy prevails the latter as well. Although the proposed method cannot fully overcome the global challenge of specificity at this stage, it considerably surpasses widely applied amplitude-based PA predictions and traditional IR spectroscopy. Apart from glucose test, tPAWS is equally applicable for other laboratory substances quantification using the same or less complex apparatus to achieve better result. It has the potential to complement or surpass the existing multi-wavelength spectroscopy and opens up new possibilities for chemical sensing and analysis.

Chapter 6. DESIGN AND DEVELOPMENT OF MINIATURIZED AND PORTABLE PHOTOACOUSTIC SENSOR FOR NONINVASIVE AND CONTINUOUS GLUCOSE MONITORING

In the previous chapters, fundamental working principles of PA sensing technique for noninvasive glucose monitoring and several novel methods have been introduced. The demonstrative experiments were conducted by laboratory setup which employed bulky apparatus including wavelength tunable pulsed optical parametric oscillator (OPO) laser, lens group, low-noise amplifier (LNA), oscilloscope and *et al.* To push it one step forward to meet the ultimate goal of replacing the current invasive glucose sensors with noninvasive glucose sensor for real clinical and healthcare, miniaturized systems with all components in a portable size have been developed.

In this chapter, the portable sensor system is presented with detailed design and performance characterization.

6.1 System Design

The schematic overview of the portable sensor is presented in Figure 6.1 (a). The system consists of the following parts: power management circuits, optical components, system control and processing unit, analog front-end (AFE), sensor head frame together with graphical user interface (GUI) on personal laptop or mobile devices. A compact fiber-coupled 1550nm laser diode (SemiNex) is incorporated in the system with an optical power of 3.5W. The wavelength is selected as 1550nm because of glucose' strong absorption in this region and the wide availability of laser sources. To drive and modulate the laser diode, a power MOSFET controlled by high-speed gate drive circuit is adopted to provide transient high current at 14A. The output is modulated at 1MHz with 500ns pulse width and 400Hz repetition rate. In this case where the duty cycle is quite low: $1 / (500 \text{ ns} \times 400\text{Hz}) = 0.02\%$, thermoelectric cooling (TEC) can be neglected. Triggering signal is generated by FPGA which is also used as reference of data

averaging. A thermoelectric cooling is added to protect the laser diode. A power bank with one 12V output is fed into combinations of DC/DC converters so that it can provide the +28V and +12V to the laser driver as well as $\pm 5\text{V}$ to FPGA and AFE. The fiber output is fed into a reflective collimator (Thorlabs, Inc) and focused on the target tissue by a condenser lens through the hole of an ultrasound transducer (UST) (Doppler Electronic Technologies). The UST is customized with a center frequency of 1MHz and 60% bandwidth. A 5mm thick PDMS layer is used as acoustic and optical coupling medium between the UST and skin because of its transparency and suitable acoustic impedance [137, 138], so that PA signal can be collected with less attenuation. Fiber output, collimator, lens, UST and PDMS are fixed by a 3D printed frame as sensor head. The received PA signal is firstly filtered by a 5th order Butterworth low-pass filter with 0~2MHz bandwidth and then amplified by a low noise amplifier (LNA) with ~35 dB gain, followed by a cascaded 2-stage variable-gain amplifier (VGA) to adjust the voltage level with a ~ 35 dB gain. Next, it is digitized by the analog-to-digital converter (ADC) with a 50Ms/s sampling rate. More details of design and performance of the homemade AFE will be presented elsewhere by other team members. The digitized signal is transferred into FPGA for post processing. 400 waveforms are filtered and averaged so that the SNR can be improved by at least 32dB. After averaging, the peak-to-peak amplitude of PA signal is extracted and mapped to glucose level. The predicted values are then transferred and displayed on the GUI. Users are able to start, pause and exit the operation anytime by clicking the corresponding buttons. The total weight of the sensor is around 650g with the size of 17cm(L) x 14cm(W) x 10cm(H) as shown in Figure 6.1 (b). However, the 12800mAh power bank currently used as power supply occupies ~300g of the total weight which can be further reduced.

The PA waveforms are transmitted out through ZigBee (DL-20) wireless modules to mobile device and shown in the GUI. The baud rate is 57600Bd and data refreshing time is 3s. This is limited by the number of average for 1 datum which is 400 at the current stage to ensure SNR. Besides, a fixation frame enclosing optical and acoustic components was designed to suit for finger testing. With simple modification, it can be also used for multiple measurement sites including purlicue, ear lobe, wrist and et al, as shown in Figure 6.1 (c). Finger was chosen here for demonstration because it's rich in capillaries and hairless.

6.1.1 Power Management Circuit Design

To drive the high power laser diode and the homemade AFE with portable power bank, stable power supply is required. Several DC-DC converters are designed including 12V to 5V low dropout regulator (LDO), 12V to -5V inverting power supply and 12V to 28V boost converter. 5V output is based on commercial chip LTC1771 and the others are using LT3757 with adjusted peripheral circuit as shown in Figure 6.2 (a)-(c). LTSpice was used to simulate the performance of DC-DC converters and the results are listed in Figure 6.2 (d)-(f). It takes ~2.1 ms, ~3.5 ms and ~1.5 ms for the 5V, -5V and 28V power supply circuit to reach a steady state output, respectively. Compared to the whole testing procedure, the stabilization time can be neglected. The average DC current limitations are 2A, 3A~5A and 2A which are far than enough to drive the whole system when laser diode is operated in pulsed mode with low duty cycle.

Figure 6. 2 Diagrams of (a) 5V low dropout regulator, (b) -5V inverting power supply, (c) 12V to 28V boost converter and their corresponding transient simulation results (d)-(f).

6.1.2 Laser Diode Driver

The commercial laser diode in a 4-pin package requires a 14A current source to maximize the output power at 3.5W. Moreover, the modulation frequency is quite high (1MHz square wave). Thus, a fast switch is needed. Considering the IP problem, only simplified circuit design diagram of the laser diode driver is shown in Figure 6.3 (a). The voltage-controlled current source provides constant current to feed into the laser diode which is decided by input voltage V_{in} , voltage-divide resistors R_1 , R_2 , and the current limit resistor R_{limit} :

$$I_{LD} = \frac{V_{\pm} R_2}{R_1 R_{limit}} \quad (6.1)$$

Then, a power MOSFET was employed as the switch to provide square-wave modulated current. Gate input was used to control the on-off of the switch.

Figure 6. 3 (a) Simplified circuit diagram of laser diode driver.

6.1.3 Buffer Circuit Design for Gate Input

FPGA provides a 1MHz, 3.3V square wave as modulation signal to control the on and off the switch. Since the driving power of FPGA is limited to high impedance input, buffer is needed to drive the LD driver. The voltage buffer employed is in traditional common collector or emitter follower configuration, which is well-known as a voltage buffer. The circuit diagram is shown in Figure 6.4 (a) and the simulation result is presented in (b) with 50 Ω load impedance.

Figure 6. 4 (a) Circuit diagram of modulation buffer. (b) Simulation result of the buffer

output when modulated at 1MHz with 50 Ω loading.

6.1.4 Graphical User Interface (GUI)

Currently, a laptop is employed as mobile device for demonstration and display. The GUI was designed and programmed using MATLAB GUIDE function. As shown in Figure 6.5(a), the historical waveform traces for generated PA are displayed in the top windows, to monitor the signal quality. Only when the signal amplitudes reach a certain level can we say the measurement is valid. Otherwise, the coupling condition is not optimized and needs to be adjusted again. The glucose measurement result for the current time point is shown in text window in both mg/dL and mmol/L (1 mmol/L = 18 mg/dL). Moreover, glucose trend is displayed for the patients to understand their condition to realize the continuous monitoring.

6.1.5 Operating Procedure

Though the sensor has not been ready for production yet, the current version is easy to operate. The operating procedure is listed in Figure 6.5 (b). Patients or clinicians can open GUI and turn on power supply of power bank, press start button and wait for the self-calibration which takes 10s until the first waveform appears in GUI.

Figure 6. 5 (a) Example of sensor GUI. (b) Flowchart of operating procedure.

Then, clip the sensor head on finger to start the measurement result. It will take 2-3

minutes to be stabilized for the first readout and after that, datum will be refreshed every 3s. The historical trend will be recorded which is crucial for diabetes screening in oral glucose tolerance test (OGTT).

6.1.6 In vitro Experiment

6.1.6.1 Sample Preparation

Both high and low concentrations of glucose solution were prepared for *in vitro* experiment. Commercially available α -D-glucose powder (Sigma Aldrich) was dissolved in distilled water and the concentrations were adjusted to 1 ~ 8 g/dL with step size of 1 g/dL. Besides, 50 ~ 350 mg/dL which is human physiological range of glucose level were also prepared with step of 50 mg/dL. All the sample solution is kept in room temperature (23°C) until consumed.

6.1.6.2 Experimental Procedure

The sample of glucose solution was filled in a transparent tygon tube and fixed by the sensor head. A syringe was used to change the solution at different concentrations. As mentioned in previous chapter, one-dimensional PA signal generated by glucose along z-axis can be expressed by the following equation:

$$p(z,t) = \frac{\beta v^2}{2C_p} \eta F \epsilon C_g \delta(t - \frac{z}{v}) \quad (6.1)$$

where β , v , C_p represent for thermal expansion coefficient, acoustic velocity in sample solution and heat capacity at constant pressure, respectively. η is optic-heat conversion efficiency and F represents for local fluence of laser source. ϵ is molar absorption coefficient which is dependent on wavelength and C_g is glucose concentration. These two parameters form the total absorption. Therefore, PA signal amplitude is directly proportional to glucose concentration. The coefficients of the linear relationship can be found by calibration during *in vitro* experiment.

6.1.6.3 Performance Evaluation

The obtained data were evaluated both statistically and clinically. First of all, linear regression was performed by Matlab Curve Fitting Toolbox and coefficient of determination R^2 as well as root-mean-square error (RMSE) were used to represent the goodness of correlation between glucose concentration and PA signal amplitude. Besides, BA plot was also performed to show the agreement between predicted glucose levels and references. As introduced previously, the centered solid line represents for their mean difference ($\bar{d}$) and the two dotted lines are defined as “limits of agreement” with the value of $\bar{d} \pm 1.96 \times$ standard deviation. Apart from the above statistical evaluation, CEG analysis was performed on low concentration measurement. It takes into account clinical interpretation of the data and prediction errors. To recap, there are five regions in CEG: A contains data within 20% difference of reference; B includes those with larger error but will not lead to harmful treatment; Data in region C will cause unnecessary treatment or overcorrection of glucose level; D indicates fatal failure to detect abnormal glucose level and E are those predictions leading to dangerous treatment. Usually, region A and B are regarded as clinically acceptable regions for glucose sensors.

6.1.6.4 Data Analysis

The obtained linear equation of high concentration solution by calibration is listed in (6.2):

$$PA_{high}[V] = 0.002933 \times C_{glucose}[g/dL] + 0.1649 \quad (6.2)$$

The correlation between predicted glucose levels and references are shown in Figure 6.6 (a) together with BA plot in Figure 6.6 (b), from which it can be seen the good correlation at first glance. The R^2 value achieved is 0.9526 and RMSE is only 0.5903 g/dL. In BA plot, the mean difference is only 0.0114 g/dL and the limits of agreement

are 1.0826g/dL and -1.0598g/dL, respectively. The functionality of the portable PA sensor was therefore well proved.

Figure 6. 6 (a) Correlation between measured glucose levels and reference levels at high concentrations. (b) BA plot for **high concentration** glucose measurement results.

To further demonstrate its potential in real clinical application, glucose solution covering physiological range from 50 ~ 350 mg/dL was also tested. The calibrated equation is as follows:

$$PA_{low}[mV] = 1.956 \times 10^{-3} \times C_{glucose}[mg / dL] + 9.526 \times 10^{-3} \quad (6.3)$$

The linear correlation is shown in Figure 6.7 (a) where the R^2 value is calculated to be 0.8966 and the RMSE is 40.18 mg/dL. Although a bit worse than high concentration test, the performance still can be regarded as good. The mean difference between predicted values and references is only 0.0825 mg/dL which can be seen from BA plot (Figure 6.7 (b)). The differences vary in the range of [-71.80 71.97] mg/dL. As the controlled concentrations fall in the physiological range of blood glucose level, the measurement results can be evaluated by CEG analysis as shown in Figure 6.7 (c). Among all the obtained data points, 71.43% fall in region A and the 28.57% are in region B. None of them are in other regions. Note that both A and B are defined as clinically acceptable regions. The above-mentioned parameters obtained by statistical and clinical evaluation methods validated the performance of our portable PA sensor.

Figure 6. 7 (a) Correlation between measured glucose levels and reference levels at low concentrations. (b) BA plot for **low concentration** glucose measurement results. (c) CEG analysis.

6.2 Preliminary Human Trial

The preliminary *in vivo* (human trial) experiment was conducted on a 25-year-old healthy male volunteer. The measurement started 45 mins after volunteer's dinner in McDonald's and data points were saved every 5 minutes. The overall trend of the glucose-related PA signal change can be clearly seen from Figure 6.8. Around 1 hour after meal, the glucose level reaches its highest value and after that, it decreases rapidly within the next 1 hours because of insulin effect. The PA signal change was well coincident with the reference glucose level (maximum error < 20%, which is the data point at 65 min, (137-142)/142) indicating great potential of our sensor for real healthcare application.

6.3 Discussion and Summary of the Portable System

To summarize, a portable noninvasive glucose sensor utilizing PA technique with miniaturized laser diode has been developed and demonstrated. Compared to NIR-spectroscopy-based sensors, our proposed PA sensors utilizes deep penetration provided by acoustic wave to realize capillary blood glucose measurement. *In vitro* experiment was performed with glucose solution at both high and low concentrations. *In vivo* experiment on human volunteer was also conducted in laboratory. Functionality and

accuracy of our sensor were well demonstrated indicating its potential to replace the current invasive glucose sensors. However, in real application, calibration is necessary for each patient and every measurement. In above-mentioned pre-clinical study, the first few points were used to obtain prediction coefficients for the volunteer. In future clinical trial, we propose to establish data base for each individual user and retrieve it when use. Periodic calibration may be required to adjust the coefficients because of measurement condition drift.

Figure 6. 8 Measured PA signal change after dinner of a healthy volunteer compared with reference glucose levels after meal.

6.4 Explore Other Possibilities for the Sensor System

From practical point of view, the study can be expanded to explore more possibilities to solve the ultimate challenge of noninvasive glucose monitoring although this thesis is centered on PA sensing. Apart from employing multiple wavelengths to generate PA signals and enhance selectivity and accuracy, we can also consider to incorporate other techniques in the sensor system as auxiliary method. For example, Glucotrack combines ultrasound, thermal and electrical impedance measurements. Though it has not been well proved as a commercial product, the way inspires us to

consider sensor fusion in the sensor development. Sensor fusion merges the information acquired by multiple sensors using linear or nonlinear models to make decision, prediction and et al [157]. Uncertainty reduction and accuracy improvement can be achieved theoretically.

6.4.1 Microwave Sensing

As mentioned in Chapter 2, microwave sensing is low-cost and easy to miniaturize which makes it most suitable to be incorporated in our sensor system without increasing much cost and complexity. A tentative trial of developing microwave sensor is discussed in details as follows.

6.4.1.1 Sensor Design and Theory

The proposed microwave sensor used to detect glucose concentration mainly consists of two inductive coils with the same configuration (7 turns copper wire with 13 mm diameter, the coil length is 7.5 mm). The surfaces of the coils which need to be in contact with samples are conductive and the proposed measurement setup is shown in Figure 6.9 (a).

Figure 6. 9 (a) Schematic of experimental setup. (b) The simplified equivalent circuit of the sensor prototype.

The sample solution holding by a dielectric container was placed tightly between the two coils, which behaves like a capacitor whose capacitance will vary with the glucose concentration [158, 159]. The simplified equivalent circuit is shown in Figure 6.9 (b). A bridging capacitor connects two parallel inductors where L represents for the inductance of the two coils with the same configurations, C_g is the capacitance of the glucose solution. R_l and R_c are due to parasitic effect. The S_{11} magnitude is defined as Eq. 6.4 [160]

$$S_{11} = 20 \log \left| \frac{Z_{in} - R}{Z_{in} + R} \right| \quad (6.4)$$

where R is the source impedance 50Ω and Z_{in} is the input impedance which can be estimated as Eq. 6.5:

$$Z_{in} = \{[(j\omega L + R_l) \parallel R] + (\frac{1}{j\omega C_g} \parallel R_c)\} \parallel (j\omega L + R_l) \quad (6.5)$$

where $C_g \propto \varepsilon$ is related to the glucose concentration since the dielectric constant ε will change with it [161-163] and the resonance peaks appearing at the poles of Eq. (6.4) will also be affected.

It is known that glucose is a kind of a small, non-ferromagnetic metabolite. Hence, the change of glucose concentration will not affect the mutual inductance between the two coils [164]. In a relatively small range of variation, the relationship among S_{11} magnitude change, resonance peak position shift and the dielectric constant variation induced by different glucose concentrations can be treated as linear, according to linear approximation. The increasing glucose concentration leads to increasing dielectric constant and hence the increasing capacitance C_g [162, 165, 166]. Within the certain small range, the reflected S-parameter's (S_{11}) magnitude (absolute value) will decrease

and the resonance frequency will shift down as the capacitance increases. Although the exact values of the parameters are not known, this trend can be estimated in circuit simulation (Figure 6.10). This kind of correlation between S-parameter and glucose concentration has also been proven experimentally in several literature [159, 163, 167-169].

Figure 6. 10 (a) S_{11} variation with capacitance C_g by circuit simulation using LTSpice.

6.4.1.2 Data Fusion

Traditional microwave glucose monitoring methods are based on single parameter (either the S-parameter's magnitude or the resonance frequency shift), this kind of single parameter based prediction can be easily distorted by the surrounding noise such as instrumentation instability and uncertainty. Herein, we proposed to utilize both of the information related to glucose concentration by data fusion method to improve the prediction accuracy which is able to reduce uncertainty and imprecision [170]. The data fusion method has been discussed in Chapter 3. First of all, the glucose level can be predicted by the S_{11} magnitude and resonance frequency shift separately ($C_{glucose1}$ and $C_{glucose2}$) and then linearly combined by competitive data fusion for the final predicted value $C_{glucose}$. The weighting factors w_1 and w_2 of each predicting method can be determined by their corresponding variance (σ_1 and σ_2) obtained in calibration

procedure. The variance of the final prediction result σ is shown to be less than any of the single parameter based predictions.

6.4.1.3 Experimental Setup

To verify the functionality of our proposed microwave sensor, we performed in vitro experiment on the aqueous glucose solution. The powder of α -D-glucose (Sigma-Aldrich, Singapore) was dissolved in deionized water to obtain 0.5 ~ 7 g/dL sample solution. During measurement, the two coils are connected to the two ports of network analyzer (Agilent Technologies, USA, E5061B). The temperature was kept to be constant ($\sim 25^\circ\text{C}$) throughout the experiment to minimize interference. The frequency was swept from 10 MHz to 2 GHz and each spectrum was averaged for 128 times before storing, where the resonance peak was found at around 1.23 GHz. The magnitude and resonance frequency position of reflected S-parameter (S_{11}) were extracted for further processing by MATLAB R2015a (MathWorks, Inc., USA).

Calibration was firstly done by curve fitting toolbox to determine the coefficients of linear equations of the obtained data and reference glucose concentration. The variances of magnitude based prediction and resonance frequency position based prediction were calculated to determine the weighting factors of these two predictions.

6.4.1.4 Results and Discussion

The recorded S_{11} data are shown in Figure 6.11 from which we can clearly identify that the magnitude of S_{11} will increase and the resonance frequency will shift down as the glucose concentration increases. The trend of the measurement results match well with our circuit simulation results in Figure 6.10.

Figure 6. 11 S_{11} parameters of the glucose solution in the range of 0.5 ~ 7 g/dL.

The linear regression equations obtained by calibration are listed in Eq. 6.6 and 6.7 which are used to convert measured S_{11} magnitude and resonance frequency position back to glucose concentration. The coefficients of determination (R_2) for the two equations are 0.9827 and 0.9941 respectively, indicating a strong correlation for the magnitude and resonance frequency position of S_{11} versus glucose concentration.

$$S_{11}(dB) = 1.157 \times c_{glucose}(g/dL) - 33.38 \quad (6.6)$$

$$Freq(MHz) = -1.304 \times c_{glucose}(g/dL) + 1230 \quad (6.7)$$

The predicting results by distinct magnitude and resonance frequency position of S_{11} are depicted in Figure 6.12 (a) and (b) respectively.

The single parameter based predicting results were linearly combined by data fusion as described before. The final result by data fusion is presented in Figure 6.12 (c), from which the accuracy enhancement can be seen. The performance of each kind of prediction method was evaluated by their corresponding root-mean-square error (RMSE). The RMSE values are 0.2887 g/dL and 0.1681 g/dL respectively for magnitude based prediction and frequency position based prediction.

Data fusion method attained a RMSE value of 0.1364 g/dL representing significant accuracy improvement of 52.75% and 18.86% compared to single parameter (magnitude

and frequency) predictions, which proves the functionality and robustness of our proposed sensor with enhanced accuracy.

The preliminary study of microwave sensing shows the great potential for it to be incorporated in the current sensor system to further improve the overall performance.

Figure 6. 12 (a) Prediction results by magnitude and by (b) frequency shift and by (c) data fusion.

6.5 Summary

In this chapter, detailed implementation for noninvasive and continuous glucose

monitoring by PA technique has been discussed, which is a large step towards real application and commercialization. Great effort has been devoted to translational research in this study. A portable prototype was firstly developed and verified. Preliminary human trial in laboratory successfully acquired the glucose variation trend after meal on a healthy volunteer. Furthermore, microwave sensor has also been studied and preliminarily verified to enhance the performance using sensor fusion in the future work.

Despite the promising performance achieved, there are several issues to be considered in the future development. First of all, the size and weight of the sensor prototype can be further reduced for better portability. Secondly, the specificity issue during real application must be solved as there are other interfering components such as water, lipid and temperature variation which can affect the generated PA signal. To address this problem, multiple sensors can be integrated so that multi-dimensional data can be obtained to calibrate out the noisy components, since different components tend to distribute in different groups, given multi-dimensional data. Multi-dimensional data analysis including PCR and PLSR is also able to leave out redundant information, like what has been mentioned in Chapter 5. The challenge of combining multiple types of sensors will lie in hardware integration, software control and data analysis. Take microwave sensor as an example, the accommodation of coils, network analyzer circuit and power supply have to be compatible with the current sensor. Meanwhile, the device size has to be maintained portable enough for personal healthcare. Source control and data acquisition have to be integrated in a single user interface. Moreover, data analysis algorithm for multiple sensors have to be developed. Last but not least, the total power management of the whole system has to be better designed in order to replace the heavy power bank with smaller battery while maintaining the durability of the sensor.

Chapter 7. CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

This thesis conducted a comprehensive study on noninvasive glucose monitoring employing PA technique, from theory investigations to implementations. Several novel methods have been proposed and verified by laboratory setup. Moreover, a portable sensor prototype was delivered at the end. More specifically, a literature survey of state-of-arts EM noninvasive glucose monitoring techniques was reviewed and compared in Chapter 2 to show the merits of PA. Then, generation and collection of PA were briefly introduced followed by the working principle of traditional PA spectroscopy in Chapter 3. A novel and convenient method utilizing both PA signal amplitude and peak arrival time by data fusion was proposed as well. In Chapter 4, “guide star” assisted indirect method aiming to enhance measurement sensitivity was discussed and analyzed. Furthermore, in Chapter 5, it was proposed to utilize the entire time-domain PA signal by multivariate analysis to predict glucose achieving superior sensitivity and accuracy. The novel method was named as time-domain PA waveform spectroscopy (tPAWS) and demonstrated to be more sensitive than commercial NIR spectrometer. Last but not least, the effort to develop a miniaturized portable sensor system was discussed in Chapter 6. The prototype employed miniaturized laser diode was functionally verified on a healthy volunteer. In addition, a preliminary study on microwave sensing was conducted which can be incorporated in the future development to realize more reliable measurement by sensor fusion.

In conclusion, this thesis introduced several novel sensing methods and to our best knowledge, was the first one to look deep into the shape of time-domain PA waveform which reflects a lot of sample properties other than optical absorption, and correlate them to glucose concentration. This study may inspire the future development of

spectroscopy, motivate new sensing technique and lead to real solution of noninvasive blood parameter monitoring. Besides, the implementation was carried out by delivering a sensor prototype with compact size and promising laboratory result, which is a crucial step forward to real clinical or homecare application.

The above mentioned novel methods and sensor prototype are under filing process for a patent.

7.2 Recommendations for Future work

Although promising results have been achieved in this thesis, the sensor prototype can be further improved to incorporate more novel methods introduced in previous chapters including data fusion, GSPA and tPAWS. Sensitivity and specificity are expected to improve which will lead to more accurate and robust measurement. However, GSPA may not be suitable for finger measurement as it is too thick to be penetrated. In that case, ear lobe or purlicue measurement needs to be adopted with modified personal clip. Other techniques like microwave sensing, diffused optics, IR spectroscopy can be also considered for sensor fusion to reduce measurement uncertainty in real application.

Despite that pilot laboratory study has been carried out, more systematic and larger scale clinical validation are still required after the sensor design is optimized in order to seek FDA approval and proceed to mass production. In future work, the accuracy and reliability of the prototyped sensor will be tested in clinical studies over 2 phases.

Phase I will involve 48 healthy volunteers based on the following demographic categorization:

Table 7. 1 Demographic categorization of healthy volunteers in Phase I test

Ethnicity	Gender	Category		Number of subjects
		Age	Body mass index	
Chinese	Male	18-60	<27.5	2
			≥27.5	2
		>60	<27.5	2
			≥27.5	2
	Female	18-60	<27.5	2
			≥27.5	2
		>60	<27.5	2
			≥27.5	2
Malay	Male	18-60	<27.5	2
			≥27.5	2
		>60	<27.5	2
			≥27.5	2
	Female	18-60	<27.5	2
			≥27.5	2
		>60	<27.5	2
			≥27.5	2
Indian	Male	18-60	<27.5	2
			≥27.5	2
		>60	<27.5	2
			≥27.5	2
	Female	18-60	<27.5	2
			≥27.5	2
		>60	<27.5	2
			≥27.5	2

The healthy volunteers will be studied with 75g oral glucose tolerance test (OGTT) after an 8-hour overnight fast. Thirty minutes prior to testing, participants will be fitted with the prototype device. For reference glucose concentrations, an IV catheter will be placed into a warmed peripheral hand vein to obtain arterialized blood, from which plasma glucose will be measured by an YSI 2300 STAT glucose analyzer. Blood will be sampled at -15, -1, 15, 30, 45, 60, 90, 120, 150, 180 minutes. At time 0, participants will drink 75g of glucose. The prototype device will be used to measure the blood glucose concentration from the dorsum of the right hand at the same time points and the glucose readings will be compared to the reference plasma glucose concentrations. During the experiment, the participants will be kept indoors.

Phase II will involve 30 subjects with type 2 diabetes. Exclusion criteria include pregnant and nursing mother as well as participants with chronic kidney disease (i.e. eGFR < 60 ml/min/1.73m²) and fasting plasma glucose >11.0 mM. The subjects will

undergo the same 75g OGTT test protocol as the healthy volunteers. They will not be prevented from using their oral glucose lowering drugs or insulin during the test.

The performance of the prototype device will be evaluated using clinical and numerical accuracy methods [171]. Clinical accuracy will be assessed using CEG analysis that evaluates medical importance of the differentiations between the prototype device and the established invasive blood glucose reference method [172]. Numerical accuracy will be assessed using absolute relative difference (ARD) of paired prototype device-YSI 2300 STAT measurement readings, calculated as follows: $ARD = \frac{|\text{Prototype device} - \text{YSI 2300 STAT}|}{\text{YSI 2300 STAT}} \times 100(\%)$, where prototype device refers to measurement result of prototype device and YSI 2300 STAT refers to measurement result of YSI 2300 STAT. The distribution of ARD values will be used to gain statistical insights on the device performance across different patient characteristics.

After clinically verified, Food and Drug Administration (FDA) approval can be applied.

AUTHOR'S PUBLICATIONS

Journal papers:

- **Zhang, R.**, Gao, F., Feng, X., Liu, S., Ding, R., & Zheng, Y. (2019). Photoacoustic resonance imaging. **IEEE Journal of Selected Topics in Quantum Electronics**, 25(1), 1-7.
- **Zhang, R.**, Liu, S., Jin H., Luo Y., Zheng, Z., Gao F., & Zheng, Y. (2019). Noninvasive Electromagnetic Wave Sensing of Glucose. **Sensors** 19(5), 1151.
- **Zhang, R.**, Gao, F., Feng, X., Jin, H., Zhang, S., Liu, S., ... & Zheng, Y. (2018). "Guide Star" Assisted Noninvasive Photoacoustic Measurement of Glucose. **ACS sensors**, 3(12), 2550-2557.
- **Zhang, R.**, Gao, F., Feng, X., Liu, S., Kishor, R., Luo, Y., & Zheng, Y. (2017). Noninvasive photoacoustic measurement of glucose by data fusion. **Analyst**, 142(16), 2892-2896.
- **Zhang, R.**, Luo, Y., Jin, H., Liu, S., Gao, F., & Zheng, Y. Time-domain Photoacoustic Waveform Spectroscopy for Glucose Measurement. **Sensors and Actuators B: Chemical**, under review
- Ai, X., Wang, Z., Cheong, H., Wang, Y., **Zhang, R.**, Lin, J., ... & Xing, B. (2019). Multispectral optoacoustic imaging of dynamic redox correlation and pathophysiological progression utilizing upconversion nanoprobes. **Nature Communications**, 10(1), 1087.
- Jin, H., **Zhang, R.**, Liu, S., & Zheng, Y. (2019). Fast and High-resolution Three-dimensional Hybrid-domain Photoacoustic Imaging Incorporating Analytical Focused Transducer Beam Amplitude. **IEEE transactions on medical imaging**.
- Jin, H., **Zhang, R.**, Liu, S., Zheng, Z., & Zheng, Y. (2019). A Single Sensor Dual-Modality Photoacoustic Fusion Imaging for Compensation of Light Fluence Variation. **IEEE Transactions on Biomedical Engineering**, 66(6), 1810-1813.
- Liu, S., Feng, X., Jin, H., **Zhang, R.**, Luo, Y., Zheng, Z., ... & Zheng, Y. (2019). Hand-held photoacoustic imager for theranostics in 3D. **IEEE transactions on medical imaging**.
- Wang, Z., Upputuri, P. K., Zhen, X., **Zhang, R.**, Jiang, Y., Ai, X., ... & Zheng, Y. (2019). pH-sensitive and biodegradable charge-transfer nanocomplex for second near-infrared photoacoustic tumor imaging. **Nano Research**, 12(1), 49-55.
- Duan, T., Lan, H., Zhong, H., Zhou, M., **Zhang, R.**, & Gao, F. (2019). Optical Spectroscopic Ultrasound Displacement Imaging: A Feasibility Study. **IEEE Journal of Selected Topics in Quantum Electronics**, 25(1), 1-8.
- Jin, H., **Zhang, R.**, Liu, S., & Zheng, Y. (2018). Passive ultrasound aided acoustic resolution photoacoustic microscopy imaging for layered heterogeneous media. **Applied Physics Letters**, 113(24), 241901.
- Duan, T., Lan, H., Zhong, H., Zhou, M., **Zhang, R.**, & Gao, F. (2018). Hybrid multi-wavelength nonlinear photoacoustic sensing and imaging. **Optics letters**, 43(22), 5611-5614.
- Liu, S., **Zhang, R.**, Zheng, Z., & Zheng, Y. (2018). Electromagnetic–Acoustic Sensing for Biomedical Applications. **Sensors**, 18(10), 3203.
- Liu, S., Feng, X., Gao, F., Jin, H., **Zhang, R.**, Luo, Y., & Zheng, Y. (2018). GPU-accelerated two dimensional synthetic aperture focusing for photoacoustic microscopy. **APL Photonics**, 3(2), 026101.

- Gao, F., **Zhang, R.**, Feng, X., Liu, S., Ding, R., Kishor, R., Qiu, L. and Zheng, Y., (2017). Phase-domain photoacoustic sensing. *Applied Physics Letters*, 110(3), p.033701.
- Gao, F., Feng, X., **Zhang, R.**, Liu, S., & Zheng, Y. (2017). Adaptive photoacoustic sensing using matched filter. *IEEE sensors letters*, 1(5), 1-3.
- Liu, S., **Zhang, R.**, Luo, Y., & Zheng, Y. (2017). Magnetoacoustic microscopic imaging of conductive objects and nanoparticles distribution. *Journal of Applied Physics*, 122(12), 124502.
- Gao, F., Feng, X., **Zhang, R.**, Liu, S., Ding, R., Kishor, R. and Zheng, Y., (2017). Single laser pulse generates dual photoacoustic signals for differential contrast photoacoustic imaging. *Scientific Reports*, 7.
- Gao, F., Kishor, R., Feng, X., Liu, S., Ding, R., **Zhang, R.** and Zheng, Y., (2017). An analytical study of photoacoustic and thermoacoustic generation efficiency towards contrast agent and film design optimization. *Photoacoustics*.
- Gao, F., Bai, L., Liu, S., **Zhang, R.**, Zhang, J., Feng, X., Zheng, Y. and Zhao, Y., (2017). Rationally encapsulated gold nanorods improving both linear and nonlinear photoacoustic imaging contrast in vivo. *Nanoscale*, 9(1), pp.79-86.
- Gao, F., Bai, L., Feng, X., Tham, H.P., **Zhang, R.**, Zhang, Y., Liu, S., Zhao, L., Zheng, Y. and Zhao, Y., (2016). Remarkable In Vivo Nonlinear Photoacoustic Imaging Based on Near-Infrared Organic Dyes. *Small*, 12(38), pp.5239-5244.

Conference papers:

- **Zhang, R.**, Tang, K., Yang, C., Jin, H., Liu, S., & Zheng, Y. (2019, May). Portable Photoacoustic Sensor for Noninvasive Glucose Monitoring. In *2019 IEEE International Symposium on Circuits and Systems (ISCAS)* (pp. 1-4). IEEE..
- **Zhang, R.**, Qu, Z., Jin, H., Liu, S., Luo, Y., & Zheng, Y. (2018, May). Noninvasive glucose measurement by microwave biosensor with accuracy enhancement. In *2018 IEEE International Symposium on Circuits and Systems (ISCAS)* (pp. 1-4). IEEE.
- Jin, H., **Zhang, R.**, Liu, S., & Zheng, Y. (2019, May). A Miniaturized Dual-Modality Photoacoustic Fusion Imaging System. In *2019 IEEE International Symposium on Circuits and Systems (ISCAS)* (pp. 1-5). IEEE.
- Liu, S., Feng, X., **Zhang R.**, & Zheng, Y. (2018, May). Portable photoacoustic system for noninvasive blood temperature measurement. In *2018 IEEE International Symposium on Circuits and Systems (ISCAS)* (pp. 1-5). IEEE. (**Best paper**)
- Gao, F., Feng, X., Liu, S., **Zhang, R.**, Ding, R., Kishor, R. and Zheng, Y., 2016, November. Nonlinear electromagnetic-acoustic sensing and imaging. In *Region 10 Conference (TENCON), 2016 IEEE* (pp. 2017-2020). IEEE.
- Ding, R., Gao, F., Feng, X., Kishor, R., Sun, H., **Zhang, R.**, Liu, S., Yang, C. and Zheng, Y., 2016, November. High-performance hybrid organic-inorganic perovskite nanoparticles based piezoelectric energy harvester. In *Region 10 Conference (TENCON), 2016 IEEE* (pp. 3257-3260). IEEE.

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BIBLIOGRAPHY

- [1] D. G. Gardner, D. Shoback, and F. S. Greenspan, *Greenspan's basic & clinical endocrinology*. McGraw-Hill Medical, 2007.
- [2] D. R. Whiting, L. Guariguata, C. Weil, and J. Shaw, "IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030," *Diabetes research and clinical practice*, vol. 94, no. 3, pp. 311-321, 2011.
- [3] C. f. D. Control and Prevention, "National diabetes statistics report, 2017," *Atlanta, GA: Centers for Disease Control and Prevention, US Dept of Health and Human Services*, 2017.
- [4] A. D. Association, "Standards of medical care in diabetes--2007," *Diabetes care*, vol. 30, p. S4, 2007.
- [5] P. Concannon, S. S. Rich, and G. T. Nepom, "Genetics of type 1A diabetes," *New England Journal of Medicine*, vol. 360, no. 16, pp. 1646-1654, 2009.
- [6] D. Daneman, "Type 1 diabetes," *The Lancet*, vol. 367, no. 9513, pp. 847-858, 2006.
- [7] M. A. Atkinson, G. S. Eisenbarth, and A. W. Michels, "Type 1 diabetes," *The Lancet*, vol. 383, no. 9911, pp. 69-82, 2014.
- [8] D. Atlas, "International Diabetes Federation. IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation, 2015," ed, 2015.
- [9] U. Risérus, W. C. Willett, and F. B. Hu, "Dietary fats and prevention of type 2 diabetes," *Progress in lipid research*, vol. 48, no. 1, pp. 44-51, 2009.
- [10] D. G. Gardner, D. Shoback, and F. S. Greenspan, *Greenspan's basic & clinical endocrinology*. McGraw-Hill Medical, 2007.
- [11] S. Vijan, "Type 2 diabetes," *Annals of internal medicine*, vol. 152, no. 5, pp. ITC3-1-1, 2010.
- [12] S. Chatterjee, K. Khunti, and M. J. Davies, "Type 2 diabetes," *The Lancet*, vol. 389, no. 10085, pp. 2239-2251, 2017.
- [13] C. Kim, K. M. Newton, and R. H. Knopp, "Gestational diabetes and the incidence of type 2 diabetes: a systematic review," *Diabetes care*, vol. 25, no. 10, pp. 1862-1868, 2002.
- [14] A. D. Association, "Gestational diabetes mellitus," *Diabetes care*, vol. 27, p. S88, 2004.
- [15] G. E. Umpierrez, S. D. Isaacs, N. Bazargan, X. You, L. M. Thaler, and A. E. Kitabchi, "Hyperglycemia: an independent marker of in-hospital mortality in patients with undiagnosed diabetes," *The Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 3, pp. 978-982, 2002.
- [16] P. E. Cryer, "Hypoglycemia, functional brain failure, and brain death," *The Journal of clinical investigation*, vol. 117, no. 4, pp. 868-870, 2007.
- [17] E. Bonora *et al.*, "HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects: prospective data from the Verona Diabetes Complications Study," *Diabetes care*, vol. 25, no. 7, pp. 1135-1141, 2002.
- [18] N. Morrish, S.-L. Wang, L. Stevens, J. Fuller, H. Keen, and W. M. S. Group, "Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes," *Diabetologia*, vol. 44, no. 2, p. S14, 2001.
- [19] P. J. Dyck *et al.*, "The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population - based cohort The Rochester Diabetic Neuropathy Study," *Neurology*, vol. 43, no. 4, pp. 817-817,

- 1993.
- [20] J. L. Gross, M. J. De Azevedo, S. P. Silveiro, L. H. Canani, M. L. Caramori, and T. Zelmanovitz, "Diabetic nephropathy: diagnosis, prevention, and treatment," *Diabetes care*, vol. 28, no. 1, pp. 164-176, 2005.
 - [21] R. Klein, B. E. Klein, S. E. Moss, M. D. Davis, and D. L. DeMets, "The Wisconsin Epidemiologic Study of Diabetic Retinopathy: II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years," *Archives of ophthalmology*, vol. 102, no. 4, pp. 520-526, 1984.
 - [22] J. M. Baena-Díez *et al.*, "Risk of cause-specific death in individuals with diabetes: a competing risks analysis," *Diabetes care*, vol. 39, no. 11, pp. 1987-1995, 2016.
 - [23] L. N. McEwen *et al.*, "Risk factors for mortality among patients with diabetes: the Translating Research Into Action for Diabetes (TRIAD) Study," *Diabetes Care*, vol. 30, no. 7, pp. 1736-1741, 2007.
 - [24] J. B. Buse *et al.*, "How do we define cure of diabetes?," *Diabetes care*, vol. 32, no. 11, pp. 2133-2135, 2009.
 - [25] L. C. Clark and C. Lyons, "Electrode systems for continuous monitoring in cardiovascular surgery," *Annals of the New York Academy of sciences*, vol. 102, no. 1, pp. 29-45, 1962.
 - [26] A. Ciudin, C. Hernández, and R. Simó, "Non-invasive methods of glucose measurement: current status and future perspectives," *Current diabetes reviews*, vol. 8, no. 1, pp. 48-54, 2012.
 - [27] K. Tonyushkina and J. H. Nichols, "Glucose meters: a review of technical challenges to obtaining accurate results," *Journal of diabetes science and technology*, vol. 3, no. 4, pp. 971-980, 2009.
 - [28] M. Sharon, "Infection transmission associated with point of care testing and the laboratory's role in risk reduction," *EJIFCC*, vol. 25, no. 2, p. 188, 2014.
 - [29] F. Inayat and A. B. Rai, "Acute hepatitis C virus infection related to capillary blood glucose meter," *Saudi Medical Journal*, vol. 37, no. 1, p. 93, 2016.
 - [30] J. Yeaw, W. C. Lee, M. Aagren, and T. Christensen, "Cost of self-monitoring of blood glucose in the United States among patients on an insulin regimen for diabetes," *Journal of Managed Care Pharmacy*, vol. 18, no. 1, pp. 21-32, 2012.
 - [31] J. L. Smith, "The pursuit of noninvasive glucose: Hunting the deceitful turkey," *Revised and Expanded*, copyright, 2015.
 - [32] S. K. Vashist, "Non-invasive glucose monitoring technology in diabetes management: A review," *Analytica chimica acta*, vol. 750, pp. 16-27, 2012.
 - [33] D. H. Wasserman, "Four grams of glucose," *American Journal of Physiology-Endocrinology and Metabolism*, vol. 296, no. 1, pp. E11-E21, 2009.
 - [34] A. Ishimaru, *Electromagnetic Wave Propagation, Radiation, and Scattering: From Fundamentals to Applications*. John Wiley & Sons, 2017.
 - [35] B. Stuart, *Infrared spectroscopy*. Wiley Online Library, 2005.
 - [36] E. B. Wilson, J. C. Decius, and P. C. Cross, *Molecular vibrations: the theory of infrared and Raman vibrational spectra*. Courier Corporation, 1955.
 - [37] H. W. Siesler, Y. Ozaki, S. Kawata, and H. M. Heise, *Near-infrared spectroscopy: principles, instruments, applications*. John Wiley & Sons, 2008.
 - [38] A. B. Seddon *et al.*, "Mid-infrared Spectroscopy/Bioimaging: Moving toward MIR optical biopsy," *Laser Focus World*, vol. 52, no. 2, pp. 50-53, 2016.
 - [39] P. R. Griffiths and C. C. Homes, "Instrumentation for Far - Infrared Spectroscopy," *Handbook of Vibrational Spectroscopy*, 2006.
 - [40] W. Herschel, "XIII. Investigation of the powers of the prismatic colours to heat

and illuminate objects; with remarks, that prove the different refrangibility of radiant heat. To which is added, an inquiry into the method of viewing the sun advantageously, with telescopes of large apertures and high magnifying powers," *Philosophical Transactions of the Royal Society of London*, vol. 90, pp. 255-283, 1800.

- [41] W. Abney and E. Festing, "Near Infrared Spectral of Organic Liquids [J]," *Philos Trans R Soc*, vol. 172, p. 887, 1881.
- [42] Y. Ozaki, "Near-infrared spectroscopy—its versatility in analytical chemistry," *Analytical Sciences*, vol. 28, no. 6, pp. 545-563, 2012.
- [43] J. Yadav, A. Rani, V. Singh, and B. M. Murari, "Prospects and limitations of non-invasive blood glucose monitoring using near-infrared spectroscopy," *Biomedical signal processing and control*, vol. 18, pp. 214-227, 2015.
- [44] D. A. Burns and E. W. Ciurczak, *Handbook of near-infrared analysis*. CRC press, 2007.
- [45] S. Perrey, "Non-invasive NIR spectroscopy of human brain function during exercise," *Methods*, vol. 45, no. 4, pp. 289-299, 2008.
- [46] I. M. Braverman, "The cutaneous microcirculation: ultrastructure and microanatomical organization," *Microcirculation*, vol. 4, no. 3, pp. 329-340, 1997.
- [47] A. C. Shore, "Capillaroscopy and the measurement of capillary pressure," *British journal of clinical pharmacology*, vol. 50, no. 6, pp. 501-513, 2000.
- [48] R. E. McCall and C. M. Tankersley, *Phlebotomy essentials*. Lippincott Williams & Wilkins, 2008.
- [49] W. F. McClure, "Near-infrared spectroscopy the giant is running strong," *Analytical Chemistry*, vol. 66, no. 1, pp. 42A-53A, 1994.
- [50] L. Weyer and J. Workman Jr, *Practical guide to interpretive near-infrared spectroscopy*. CRC press, 2007.
- [51] O. S. Khalil, "Spectroscopic and clinical aspects of noninvasive glucose measurements," *Clinical chemistry*, vol. 45, no. 2, pp. 165-177, 1999.
- [52] W. Yang, N. Liao, H. Cheng, Y. Li, X. Bai, and C. Deng, "Determination of NIR informative wavebands for transmission non-invasive blood glucose measurement using a Fourier transform spectrometer," *AIP Advances*, vol. 8, no. 3, p. 035216, 2018.
- [53] M. Golic, K. Walsh, and P. Lawson, "Short-wavelength near-infrared spectra of sucrose, glucose, and fructose with respect to sugar concentration and temperature," *Applied spectroscopy*, vol. 57, no. 2, pp. 139-145, 2003.
- [54] B. G. Osborne, T. Fearn, and P. H. Hindle, *Practical NIR spectroscopy with applications in food and beverage analysis*. Longman scientific and technical, 1993.
- [55] S. Šašić and Y. Ozaki, "Short-wave near-infrared spectroscopy of biological fluids. 1. Quantitative analysis of fat, protein, and lactose in raw milk by partial least-squares regression and band assignment," *Analytical Chemistry*, vol. 73, no. 1, pp. 64-71, 2001.
- [56] D. Swinehart, "The beer-lambert law," *J. Chem. Educ*, vol. 39, no. 7, p. 333, 1962.
- [57] O. S. Khalil, "Non-invasive glucose measurement technologies: an update from 1999 to the dawn of the new millennium," *Diabetes technology & therapeutics*, vol. 6, no. 5, pp. 660-697, 2004.
- [58] P. D. Vasko, J. Blackwell, and J. Koenig, "Infrared and raman spectroscopy of carbohydrates: Part I: Identification of O · H and C · H-related vibrational

- modes for D-glucose, maltose, cellobiose, and dextran by deuterium-substitution methods," *Carbohydrate Research*, vol. 19, no. 3, pp. 297-310, 1971.
- [59] P. Vasko, J. Blackwell, and J. Koenig, "Infrared and raman spectroscopy of carbohydrates.: Part II: Normal coordinate analysis of α -D-glucose," *Carbohydrate Research*, vol. 23, no. 3, pp. 407-416, 1972.
- [60] V. V. Tuchin, *Handbook of optical sensing of glucose in biological fluids and tissues*. CRC press, 2008.
- [61] M. A. Pleitez, T. Lieblein, A. Bauer, O. Hertzberg, H. von Lilienfeld-Toal, and W. Mäntele, "In vivo noninvasive monitoring of glucose concentration in human epidermis by mid-infrared pulsed photoacoustic spectroscopy," *Analytical chemistry*, vol. 85, no. 2, pp. 1013-1020, 2012.
- [62] M. Ibrahim, M. Alaam, H. El-Haes, A. F. Jalbout, and A. d. Leon, "Analysis of the structure and vibrational spectra of glucose and fructose," *Ecletica quimica*, vol. 31, no. 3, pp. 15-21, 2006.
- [63] S. Kino, S. Omori, T. Katagiri, and Y. Matsuura, "Hollow optical-fiber based infrared spectroscopy for measurement of blood glucose level by using multi-reflection prism," *Biomedical optics express*, vol. 7, no. 2, pp. 701-708, 2016.
- [64] M. Pleitez, H. von Lilienfeld-Toal, and W. Mäntele, "Infrared spectroscopic analysis of human interstitial fluid in vitro and in vivo using FT-IR spectroscopy and pulsed quantum cascade lasers (QCL): Establishing a new approach to non invasive glucose measurement," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 85, no. 1, pp. 61-65, 2012.
- [65] C. Petibois *et al.*, "Determination of glucose in dried serum samples by Fourier-transform infrared spectroscopy," *Clinical chemistry*, vol. 45, no. 9, pp. 1530-1535, 1999.
- [66] R. Waynant and V. Chenault, "Overview of non-invasive fluid glucose measurement using optical techniques to maintain glucose control in diabetes mellitus," *LEOS newsletter*, vol. 12, no. 2, pp. 3-6, 1998.
- [67] P. Elsner, *Skin bioengineering: techniques and applications in dermatology and cosmetology*. Karger Medical and Scientific Publishers, 1998.
- [68] H. von Lilienfeld-Toal, M. Weidenmüller, A. Xhelaj, and W. Mäntele, "A novel approach to non-invasive glucose measurement by mid-infrared spectroscopy: The combination of quantum cascade lasers (QCL) and photoacoustic detection," *Vibrational spectroscopy*, vol. 38, no. 1-2, pp. 209-215, 2005.
- [69] M. Pfohl, A. Pfeiffer, and H. Schaltz, "Spot glucose measurement in epidermal interstitial fluid—an alternative to capillary blood glucose estimation?," *Experimental and clinical endocrinology & diabetes*, vol. 108, no. 01, pp. 1-4, 2000.
- [70] P. C. O'brien, S. D. Wise, S. Ness, and S. M. LeBlanc, "Dermal interstitial glucose as an indicator of ambient glycemia," *Diabetes care*, vol. 20, no. 9, pp. 1426-1429, 1997.
- [71] S. Gebhart *et al.*, "Glucose sensing in transdermal body fluid collected under continuous vacuum pressure via micropores in the stratum corneum," *Diabetes technology & therapeutics*, vol. 5, no. 2, pp. 159-166, 2003.
- [72] S. N. Thennadil, J. L. Rennert, B. J. Wenzel, K. H. Hazen, T. L. Ruchti, and M. B. Block, "Comparison of glucose concentration in interstitial fluid, and capillary and venous blood during rapid changes in blood glucose levels," *Diabetes Technology & Therapeutics*, vol. 3, no. 3, pp. 357-365, 2001.
- [73] S. Weiss, "Fluorescence spectroscopy of single biomolecules," *Science*, vol. 283, no. 5408, pp. 1676-1683, 1999.

- [74] P. W. Barone, R. S. Parker, and M. S. Strano, "In vivo fluorescence detection of glucose using a single-walled carbon nanotube optical sensor: design, fluorophore properties, advantages, and disadvantages," *Analytical chemistry*, vol. 77, no. 23, pp. 7556-7562, 2005.
- [75] C. V. Raman and K. S. Krishnan, "A new type of secondary radiation," *Nature*, vol. 121, no. 3048, p. 501, 1928.
- [76] J. Dong, Q. Tao, M. Guo, T. Yan, and W. Qian, "Glucose-responsive multifunctional acupuncture needle: a universal SERS detection strategy of small biomolecules in vivo," *Analytical Methods*, vol. 4, no. 11, pp. 3879-3883, 2012.
- [77] D. Huang *et al.*, "Optical coherence tomography," *Science*, vol. 254, no. 5035, pp. 1178-1181, 1991.
- [78] K. V. Larin, M. Motamedi, T. V. Ashitkov, and R. O. Esenaliev, "Specificity of noninvasive blood glucose sensing using optical coherence tomography technique: a pilot study," *Physics in Medicine & Biology*, vol. 48, no. 10, p. 1371, 2003.
- [79] P. H. Siegel, "Terahertz technology," *IEEE Transactions on microwave theory and techniques*, vol. 50, no. 3, pp. 910-928, 2002.
- [80] M. Tonouchi, "Cutting-edge terahertz technology," *Nature photonics*, vol. 1, no. 2, p. 97, 2007.
- [81] G. Gallot and D. Grischkowsky, "Electro-optic detection of terahertz radiation," *JOSA B*, vol. 16, no. 8, pp. 1204-1212, 1999.
- [82] E. Pickwell and V. Wallace, "Biomedical applications of terahertz technology," *Journal of Physics D: Applied Physics*, vol. 39, no. 17, p. R301, 2006.
- [83] M. Zhadobov, N. Chahat, R. Sauleau, C. Le Quement, and Y. Le Drian, "Millimeter-wave interactions with the human body: State of knowledge and recent advances," *International Journal of Microwave and Wireless Technologies*, vol. 3, no. 2, pp. 237-247, 2011.
- [84] S. Alekseev and M. Ziskin, "Human skin permittivity determined by millimeter wave reflection measurements," *Bioelectromagnetics: Journal of the Bioelectromagnetics Society, The Society for Physical Regulation in Biology and Medicine, The European Bioelectromagnetics Association*, vol. 28, no. 5, pp. 331-339, 2007.
- [85] J.-H. Park, C.-S. Kim, B.-C. Choi, and K.-Y. Ham, "The correlation of the complex dielectric constant and blood glucose at low frequency," *Biosensors and Bioelectronics*, vol. 19, no. 4, pp. 321-324, 2003.
- [86] A. Caduff, E. Hirt, Y. Feldman, Z. Ali, and L. Heinemann, "First human experiments with a novel non-invasive, non-optical continuous glucose monitoring system," *Biosensors and Bioelectronics*, vol. 19, no. 3, pp. 209-217, 2003.
- [87] Y. Hayashi, L. Livshits, A. Caduff, and Y. Feldman, "Dielectric spectroscopy study of specific glucose influence on human erythrocyte membranes," *Journal of Physics D: Applied Physics*, vol. 36, no. 4, p. 369, 2003.
- [88] K. S. Cole and R. H. Cole, "Dispersion and absorption in dielectrics I. Alternating current characteristics," *The Journal of chemical physics*, vol. 9, no. 4, pp. 341-351, 1941.
- [89] V. Turgul and I. Kale, "Characterization of the complex permittivity of glucose/water solutions for noninvasive RF/Microwave blood glucose sensing," in *Instrumentation and Measurement Technology Conference Proceedings (I2MTC), 2016 IEEE International*, 2016: IEEE, pp. 1-5.

- [90] A. G. Bell, "ART. XXXIV.--On the Production and Reproduction of Sound by Light," *American Journal of Science (1880-1910)*, vol. 20, no. 118, p. 305, 1880.
- [91] J. Kim *et al.*, "Programmable real-time clinical photoacoustic and ultrasound imaging system," *Scientific reports*, vol. 6, p. 35137, 2016.
- [92] S. Jeon *et al.*, "In vivo photoacoustic imaging of anterior ocular vasculature: a random sample consensus approach," *Scientific reports*, vol. 7, no. 1, p. 4318, 2017.
- [93] D. Lee *et al.*, "In Vivo Photoacoustic Imaging of Livers Using Biodegradable Hyaluronic Acid - Conjugated Silica Nanoparticles," *Advanced Functional Materials*, vol. 28, no. 22, p. 1800941, 2018.
- [94] W. Choi, E.-Y. Park, S. Jeon, and C. Kim, "Clinical photoacoustic imaging platforms," *Biomedical engineering letters*, vol. 8, no. 2, pp. 139-155, 2018.
- [95] J. W. Baik, J. Y. Kim, S. Cho, S. Choi, J. Kim, and C. Kim, "Super Wide-field Photoacoustic Microscopy of Animals and Humans In Vivo," *IEEE transactions on medical imaging*, 2019.
- [96] F. Gao, X. Feng, Y. Zheng, and C.-D. Ohl, "Photoacoustic resonance spectroscopy for biological tissue characterization," *Journal of biomedical optics*, vol. 19, no. 6, p. 067006, 2014.
- [97] R. Zhang, F. Gao, X. Feng, S. Liu, R. Ding, and Y. Zheng, "Photoacoustic resonance imaging," *IEEE Journal of Selected Topics in Quantum Electronics*, vol. 25, no. 1, pp. 1-7, 2019.
- [98] J. Kottmann, J. M. Rey, J. Luginbühl, E. Reichmann, and M. W. Sigrist, "Glucose sensing in human epidermis using mid-infrared photoacoustic detection," *Biomedical optics express*, vol. 3, no. 4, pp. 667-680, 2012.
- [99] P. P. Pai, P. K. Sanki, and S. Banerjee, "A photoacoustics based continuous non-invasive blood glucose monitoring system," in *MeMeA*, 2015, pp. 106-111.
- [100] J. Y. Sim, C.-G. Ahn, E.-J. Jeong, and B. K. Kim, "In vivo Microscopic Photoacoustic Spectroscopy for Non-Invasive Glucose Monitoring Invulnerable to Skin Secretion Products," *Scientific reports*, vol. 8, no. 1, p. 1059, 2018.
- [101] R. Zhang *et al.*, "Noninvasive photoacoustic measurement of glucose by data fusion," *Analyst*, vol. 142, no. 16, pp. 2892-2896, 2017.
- [102] K. B. Walsh, J. A. Guthrie, and J. W. Burney, "Application of commercially available, low-cost, miniaturised NIR spectrometers to the assessment of the sugar content of intact fruit," *Functional Plant Biology*, vol. 27, no. 12, pp. 1175-1186, 2000.
- [103] F.-B. Yang, J.-Z. Pan, T. Zhang, and Q. Fang, "A low-cost light-emitting diode induced fluorescence detector for capillary electrophoresis based on an orthogonal optical arrangement," *Talanta*, vol. 78, no. 3, pp. 1155-1158, 2009.
- [104] H. Zhong, T. Duan, H. Lan, M. Zhou, and F. Gao, "Review of low-cost photoacoustic sensing and imaging based on laser diode and light-emitting diode," *Sensors*, vol. 18, no. 7, p. 2264, 2018.
- [105] B. Cox and P. C. Beard, "Modeling photoacoustic propagation in tissue using k-space techniques," in *Photoacoustic Imaging and Spectroscopy*: CRC Press, 2017, pp. 25-34.
- [106] A. A. Kosterev, Y. A. Bakhirkin, R. F. Curl, and F. K. Tittel, "Quartz-enhanced photoacoustic spectroscopy," *Optics letters*, vol. 27, no. 21, pp. 1902-1904, 2002.
- [107] L. Duggen, R. Frese, and M. Willatzen, "FEM analysis of cylindrical resonant photoacoustic cells," in *Journal of Physics: Conference Series*, 2010, vol. 214, no. 1: IOP Publishing, p. 012036.

- [108] A. Miklós, P. Hess, and Z. Bozóki, "Application of acoustic resonators in photoacoustic trace gas analysis and metrology," *Review of scientific instruments*, vol. 72, no. 4, pp. 1937-1955, 2001.
- [109] J. Kottmann, J. Rey, and M. Sigrist, "New photoacoustic cell design for studying aqueous solutions and gels," *Review of Scientific Instruments*, vol. 82, no. 8, p. 084903, 2011.
- [110] J. Yao and L. V. Wang, "Sensitivity of photoacoustic microscopy," *Photoacoustics*, vol. 2, no. 2, pp. 87-101, 2014.
- [111] *American national standard for the safe use of lasers*. American National Standards Institute, L. I. o. America, 1993.
- [112] F. M. Ham, I. N. Kostanic, G. M. Cohen, and B. R. Gooch, "Determination of glucose concentrations in an aqueous matrix from NIR spectra using optimal time-domain filtering and partial least-squares regression," *IEEE transactions on Biomedical Engineering*, vol. 44, no. 6, pp. 475-485, 1997.
- [113] K. Maruo, M. Tsurugi, M. Tamura, and Y. Ozaki, "In vivo noninvasive measurement of blood glucose by near-infrared diffuse-reflectance spectroscopy," *Applied spectroscopy*, vol. 57, no. 10, pp. 1236-1244, 2003.
- [114] R. H. Clarke and Q. Wang, "Infrared glucose sensor," ed: Google Patents, 1993.
- [115] M.-S. Chou, "Method and apparatus for noninvasive measurement of blood glucose by photoacoustics," ed: Google Patents, 1999.
- [116] P. P. Pai, P. K. Sanki, S. Sarangi, and S. Banerjee, "Modelling, verification, and calibration of a photoacoustics based continuous non-invasive blood glucose monitoring system," *Review of Scientific Instruments*, vol. 86, no. 6, p. 064901, 2015.
- [117] S. Camou, T. Haga, T. Tajima, and E. Tamechika, "Detection of aqueous glucose based on a cavity size-and optical-wavelength-independent continuous-wave photoacoustic technique," *Analytical chemistry*, vol. 84, no. 11, pp. 4718-4724, 2012.
- [118] H. MacKenzie *et al.*, "Blood glucose measurements by photoacoustics," in *Biomedical Optical Spectroscopy and Diagnostics*, 1998: Optical Society of America, p. BTuC1.
- [119] K. Quan, G. Christison, H. MacKenzie, and P. Hodgson, "Glucose determination by a pulsed photoacoustic technique: an experimental study using a gelatin-based tissue phantom," *Physics in medicine and biology*, vol. 38, no. 12, p. 1911, 1993.
- [120] L. V. Wang and H.-i. Wu, *Biomedical optics: principles and imaging*. John Wiley & Sons, 2012.
- [121] Z. Zhao, *Pulsed photoacoustic techniques and glucose determination in human blood and tissue*. University of Oulu Finland, 2002.
- [122] I. Harman-Boehm, A. Gal, A. M. Raykhman, J. D. Zahn, E. Naidis, and Y. Mayzel, "Noninvasive glucose monitoring: a novel approach," *Journal of diabetes science and technology*, vol. 3, no. 2, pp. 253-260, 2009.
- [123] 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*, vol. 10, no. 5, pp. 622-628, 1987.
- [124] J. Kottmann, U. Grob, J. M. Rey, and M. W. Sigrist, "Mid-infrared fiber-coupled photoacoustic sensor for biomedical applications," *Sensors*, vol. 13, no. 1, pp. 535-549, 2013.
- [125] A. Bakker, B. Smith, P. Ainslie, and K. Smith, "Near-infrared spectroscopy," in *Applied Aspects of Ultrasonography in Humans*: InTech, 2012.

- [126] C. Elwell, "A practical users guide to near infrared spectroscopy," ed: Hamamatsu Photonics KK, 1995.
- [127] Y. Zhou, J. Yao, and L. V. Wang, "Tutorial on photoacoustic tomography," *Journal of biomedical optics*, vol. 21, no. 6, pp. 061007-061007, 2016.
- [128] L. V. Wang, "Tutorial on photoacoustic microscopy and computed tomography," *IEEE Journal of Selected Topics in Quantum Electronics*, vol. 14, no. 1, pp. 171-179, 2008.
- [129] G. Diebold and T. Sun, "Properties of photoacoustic waves in one, two, and three dimensions," *Acta Acustica united with Acustica*, vol. 80, no. 4, pp. 339-351, 1994.
- [130] G. Diebold, T. Sun, and M. Khan, "Photoacoustic monopole radiation in one, two, and three dimensions," *Physical review letters*, vol. 67, no. 24, p. 3384, 1991.
- [131] S. Zhao, W. Tao, Q. He, H. Zhao, and W. Cao, "A non-invasive photoacoustic and ultrasonic method for the measurement of glucose solution concentration," *AIP Advances*, vol. 7, no. 3, p. 035313, 2017.
- [132] E. W. Cheney, *Approximation theory III*. Academic Press New York, 1980.
- [133] J.-Y. Tang, N.-Y. Chen, M.-K. Chen, M.-H. Wang, and L.-S. Jang, "Dual-wavelength optical fluidic glucose sensor using time series analysis of d (+)-glucose measurement," *Japanese Journal of Applied Physics*, vol. 55, no. 10, p. 106601, 2016.
- [134] S. Jacquemoud and S. Ustin, "Application of radiative transfer models to moisture content estimation and burned land mapping," in *4th International Workshop on Remote Sensing and GIS Applications to Forest Fire Management*, 2003, vol. 312.
- [135] B. Rao *et al.*, "Real-time four-dimensional optical-resolution photoacoustic microscopy with Au nanoparticle-assisted subdiffraction-limit resolution," *Optics letters*, vol. 36, no. 7, pp. 1137-1139, 2011.
- [136] A. Biancolillo and F. Marini, "Chemometric methods for spectroscopy-based pharmaceutical analysis," *Frontiers in chemistry*, vol. 6, 2018.
- [137] I. Leibacher, S. Schatzer, and J. Dual, "Impedance matched channel walls in acoustofluidic systems," *Lab on a Chip*, vol. 14, no. 3, pp. 463-470, 2014.
- [138] D. Cai, A. Neyer, R. Kuckuk, and H. Heise, "Optical absorption in transparent PDMS materials applied for multimode waveguides fabrication," *Optical materials*, vol. 30, no. 7, pp. 1157-1161, 2008.
- [139] A. N. S. Institute, "Z136. 1-2014," 2000.
- [140] J. M. Bland and D. Altman, "Statistical methods for assessing agreement between two methods of clinical measurement," *The lancet*, vol. 327, no. 8476, pp. 307-310, 1986.
- [141] J. N. Adkins *et al.*, "Toward a human blood serum proteome analysis by multidimensional separation coupled with mass spectrometry," *Molecular & Cellular Proteomics*, vol. 1, no. 12, pp. 947-955, 2002.
- [142] O. N. Dennis, "Blood Components." https://www2.palomar.edu/anthro/blood/blood_components.htm (accessed Nov 4, 2018).
- [143] K. Krzempek *et al.*, "Multi-pass cell-assisted photoacoustic/photothermal spectroscopy of gases using quantum cascade laser excitation and heterodyne interferometric signal detection," *Applied Physics B*, vol. 124, no. 5, p. 74, 2018.
- [144] M. E. Webber, M. Pushkarsky, and C. K. N. Patel, "Fiber-amplifier-enhanced photoacoustic spectroscopy with near-infrared tunable diode lasers," *Applied*

- optics*, vol. 42, no. 12, pp. 2119-2126, 2003.
- [145] C. Offord. "Will the Noninvasive Glucose Monitoring Revolution Ever Arrive?" <https://www.the-scientist.com/news-analysis/will-the-noninvasive-glucose-monitoring-revolution-ever-arrive-30754> (accessed Mar 22, 2019).
 - [146] R. Zhang *et al.*, "Noninvasive Electromagnetic Wave Sensing of Glucose," *Sensors*, vol. 19, no. 5, p. 1151, 2019.
 - [147] C. Lou and D. Xing, "Photoacoustic measurement of liquid viscosity," *Applied Physics Letters*, vol. 96, no. 21, p. 211102, 2010.
 - [148] F. Gao, X. Feng, and Y. Zheng, "Photoacoustic elastic oscillation and characterization," *Optics express*, vol. 23, no. 16, pp. 20617-20628, 2015.
 - [149] X. L. Deán-Ben and D. Razansky, "On the link between the speckle free nature of optoacoustics and visibility of structures in limited-view tomography," *Photoacoustics*, vol. 4, no. 4, pp. 133-140, 2016.
 - [150] A. C. Olivieri, "Analytical advantages of multivariate data processing. One, two, three, infinity?," *Analytical Chemistry*, vol. 80, no. 15, pp. 5713-5720, 2008.
 - [151] S. Wold, K. Esbensen, and P. Geladi, "Principal component analysis," *Chemometrics and intelligent laboratory systems*, vol. 2, no. 1-3, pp. 37-52, 1987.
 - [152] S. Chatterjee and A. S. Hadi, "Influential observations, high leverage points, and outliers in linear regression," *Statistical science*, vol. 1, no. 3, pp. 379-393, 1986.
 - [153] R. Zhang *et al.*, "'Guide Star' Assisted Noninvasive Photoacoustic Measurement of Glucose," *ACS sensors*, vol. 3, no. 12, pp. 2550-2557, 2018.
 - [154] ANSI, "American National Standard for Safe Use of Lasers. ANSI Z136. 1–2014," vol. Z136.1, 2014.
 - [155] K. R. Beebe and B. R. Kowalski, "An introduction to multivariate calibration and analysis," *Analytical Chemistry*, vol. 59, no. 17, pp. 1007A-1017A, 1987.
 - [156] A. T. Giese and C. S. French, "The analysis of overlapping spectral absorption bands by derivative spectrophotometry," *Applied Spectroscopy*, vol. 9, no. 2, pp. 78-96, 1955.
 - [157] F. Gustafsson, *Statistical sensor fusion*. Studentlitteratur, 2010.
 - [158] M. Venkatesh and G. Raghavan, "An overview of microwave processing and dielectric properties of agri-food materials," *Biosystems Engineering*, vol. 88, no. 1, pp. 1-18, 2004.
 - [159] N. Kim, R. Dhakal, K. Adhikari, E. Kim, and C. Wang, "A reusable robust radio frequency biosensor using microwave resonator by integrated passive device technology for quantitative detection of glucose level," *Biosensors and Bioelectronics*, vol. 67, pp. 687-693, 2015.
 - [160] D. M. Pozar, *Microwave engineering*. John Wiley & Sons, 2009.
 - [161] W. M. Arnold, "Positioning and levitation media for the separation of biological cells," *IEEE Transactions on Industry Applications*, vol. 37, no. 5, pp. 1468-1475, 2001.
 - [162] D. R. Lide, "CRC Handbook of Chemistry and Physics 2004-2005: A Ready-Reference Book of Chemical and Physical Data," ed: CRC press Boca Raton, 2004.
 - [163] J. Kim, A. Babajanyan, A. Hovsepyan, K. Lee, and B. Friedman, "Microwave dielectric resonator biosensor for aqueous glucose solution," *Review of Scientific Instruments*, vol. 79, no. 8, p. 086107, 2008.
 - [164] C. B. Kriz, K. Rådevik, and D. Kriz, "Magnetic permeability measurements in bioanalysis and biosensors," *Analytical Chemistry*, vol. 68, no. 11, pp. 1966-1970, 1996.

- [165] K. Lee, A. Babajanyan, C. Kim, S. Kim, and B. Friedman, "Glucose aqueous solution sensing by a near-field microwave microprobe," *Sensors and Actuators A: Physical*, vol. 148, no. 1, pp. 28-32, 2008.
- [166] A. Babajanyan, J. Kim, S. Kim, K. Lee, and B. Friedman, "Sodium chloride sensing by using a near-field microwave microprobe," *Applied physics letters*, vol. 89, no. 18, p. 183504, 2006.
- [167] A. Mason *et al.*, "Non-Invasive In-situ Measurement of Blood Lactate using Microwave Sensors," *IEEE Transactions on Biomedical Engineering*, 2017.
- [168] M. Fok, M. Bashir, H. Fraser, N. Strouther, and A. Mason, "A novel microwave sensor to detect specific biomarkers in human cerebrospinal fluid and their relationship to cellular ischemia during thoracoabdominal aortic aneurysm repair," *Journal of medical systems*, vol. 39, no. 4, p. 37, 2015.
- [169] S. Kim *et al.*, "Noninvasive in vitro measurement of pig-blood D-glucose by using a microwave cavity sensor," *Diabetes research and clinical practice*, vol. 96, no. 3, pp. 379-384, 2012.
- [170] I. Harman-Boehm, A. Gal, A. M. Raykhman, J. D. Zahn, E. Naidis, and Y. Mayzel, "Noninvasive glucose monitoring: a novel approach," ed: SAGE Publications, 2009.
- [171] B. Kovatchev, S. Anderson, L. Heinemann, and W. Clarke, "Comparison of the numerical and clinical accuracy of four continuous glucose monitors," *Diabetes care*, vol. 31, no. 6, pp. 1160-1164, 2008.
- [172] W. L. Clarke, "The original Clarke error grid analysis (EGA)," *Diabetes technology & therapeutics*, vol. 7, no. 5, pp. 776-779, 2005.