American Journal of Biomedicine and Pharmacy
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Integration of Terahertz Spectroscopy with Microfluidic Platforms for Early Cancer Biomarker Detection

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Received: 2024, 15, Mar
Accepted: 2025, 21, Apr
Published: 2025, 20, May

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Annotation: Cancer is becoming one of the biggest health problems over the world. A lot of in-depth research results and clinical diagnosis show that cancer cells have different molecular subtypes. Detection of cancer from molecular level such as DNA or RNA instead of pathology or histology could help doctors to perform early detection and treatment. Trace amounts of cancer biomarkers in serum were found to reflect the existence of tumor cells. Therefore, detection of cancer biomarkers such as enzymes and specific protein is believed to be one of the effective methods in early detection of cancer.

Variety of biosensors were developed in recent years to detect cancer biomarkers, such as sensor, electrochemical sensor, visual-based sensor, and biochemical sensor. Among them, optical biosensors are non-destructive and highly sensitive, which have important applications in the detection of cancer biomarkers. However, existing sensing detection technologies may have drawbacks in sensitivity and detection limits. For instance, plasma detection using sensor is proved to be $1{*}ng/mL$, while fluorescence sensor and

purified fluorescence detection is 0.5-1ng/mL; protein assay using bioaffinity sensors and sensor is 5-100ng/mL and 0.1ng/mL respectively. These detection technologies may still have many limitations like time-consuming labeling, complex sample pre- and post-processing procedure, and sensitivity and detection limits which could not meet the requirement for cancer biomarkers detection in clinical application.

Recently, spectroscopy technology based on metamaterials was developed to detect liver cancer biomarker, which is a promising method to detect other biomarkers in early stage liver cancer detection. It is reported that wave can detect the vibrational frequencies of important biomolecules and thus has potential applications in biomedicine for non-contact and non-destructive inspection of different kinds of biomolecules. Devices that operate in region can be widely used all around the life cycle of biomolecules. In addition, development of micro-nanofabrication technology provides a platform for the design of metamaterials, which are sensitive to the change of micro-environment on their surface, in order to detect biomolecules at low concentration.

For a promising application of sensors, it is essential to have biosensors that are not sensitive to the shape or the dielectric constant of the medium. The resonance frequencies of Fano resonance-based metamaterials depend only on the dielectric constants of the substrate, and therefore are promising materials for detecting biomolecules at low concentrations. sensors have witnessed rapid progress in the last decades. However, most of the sensors are limited to dry or partially hydrated samples, due to strong absorption of water at frequencies. Fortunately, microfluidic chips present a novel method to overcome water absorption problems. Compared with traditional sensing detection systems, the microfluidic systems can offer small amount of solvent volume and rapid analysis. In addition, microfluidic technology allows detected biomolecules to dissolve in liquid samples and thus has enabled thorough and extensive applications of sensors integrated with microfluidic chips.

1. Introduction

Currently, cancer diagnosis is mainly based on the imaging scanned results from a non-invasive technique such as high-resolution resonance magnetic imaging (MRI) or positron emission tomography (PET) for detecting abnormalities in shape/size or growth of the tumor, which usually requires a high possibility of significantly abnormal mass (millimeters) [1]. However, there are a lot of in-depth research results and clinical diagnose show that cancer cells have different molecular subtypes whose characteristics play an important role in biological behavior of cancer, and can provide the valuable information concerning the location of incipient tumors and therefore potentially enabling earlier therapeutic intervention. Therefore, the detection of cancer should be from molecule level in early stage of cancer, which could help the patient perform early detection and early treatment when the cancer is still curable, and high probability of effectively killing the tumor cells. The ongoing extensive studies of cancer at the molecular level have proven that there are trace amount of cancer biomarkers from the cellular level in the serum, such as enzymes, cytokines, specific proteins, which could indirectly reflect the existing of tumor cells. Therefore, variety of biosensors were developed and used to sensitively detect these biomarkers, which are very important for early detection of cancer. The conventional methods of cancer biomarkers detection typically focus on amplification of biomolecules such as enrichment or polymerase chain reaction (PCR) to enhance the signal-to-noise ratio. However, these approaches have some inherent restrictions in sensing detection technologies in terms of sensitivity, detection limit, volume of sample, cost, vibrational frequency of biomarker, and water absorption. Therefore, novel technique or new materials for high sensitivity detection of cancer biomarkers in early stage are needed increasingly for cancer diagnostic. Recently, THz spectroscopy technology based on metamaterials have become a promising biomarker detection method. The frequency range of THz wave (0.1 THz to 10 THz) is in accord to the vibrational frequencies of some important biomolecules (proteins, RNA, and DNA), which make it possible to detect the vibration of biomolecules. THz spectroscopy technology has some other merits such as label-free, non-contact, and non-destructive inspection on target biomolecules. All of other merits imply that THz spectroscopy technology is suitable to detect biomolecules. However, THz sensors have typically been limited to dry or partially hydrated specimens due to stronger water absorption at THz frequencies. Therefore, how to reduce the liquid tested sample is an important direction for extending THz biosensor application. Microfluidic chip offers many advantages such as very small volume of samples, low cost, rapid analyzing, and so on. Microfluidic chip could be introduced into THz biosensors as a promising assistant, which become a practical and effective strategy to be employed. If the detected biomolecules are dissolved in liquid sample through microfluidic technology, then THz sensors integrated with microfluidic chips are applied extensively because strong water absorption at THz frequencies can be overcome. [2][3][4]

2. Background on Terahertz Spectroscopy

The terahertz (THz) spectrum is the electromagnetic spectrum ranging from 100 GHz to 10 THz (wavelength from 3 mm to 30 mm) [5]. As THz waves have a large electric field and low energy, THz spectroscopy is a non-destructive and non-invasive technique. Moreover, the frequency is close to molecular vibration frequency, making THz waves sensitive to molecular structure and conformation [1]. Based on these compelling advantages, THz-TDS systems have been developed to detect the dielectric response of samples, opening applications in the medical field such as cancer cells and tissue detection. [6]

The first realization of bio-sample detection was on human nail structure, which is the proof-of-concept work to demonstrate capability of Tera-Hz-TDS to monitor biomedical samples. For the non-invasive medical detection of cancer cells, THz-TDS spectroscopy was developed to detect a higher water concentration in cancer cells and monitored spectrally by frequency shifting of distinct THz spectral lines. Forbidden transitions of C=O and N–H bending modes in amide I at 0.15, 0.60 THz were also investigated to differentiate cancer from normal breast tissue. By using THz-TDS spectroscopy, the mass of single cells showing a linear relationship with attenuation was realized, promising a method for THz cell mass detection. The integration of micro-fluidics with THz technologies indicates a viable method for cell manipulation and monitoring. [7][8]

2.1. Fundamentals of Terahertz Radiation

As a new probing tool, terahertz (THz) technology has attracted considerable attention in the early non-invasive diagnosis of cancers [5]. The THz region is generally defined as the electromagnetic spectrum covering the time scale from picoseconds to nanoseconds or the frequency range from 0.3 THz to 10 THz. Since the THz wave mixes the characteristics of both radiowaves and optics, it is able to get through dielectrics like most biological tissues without causing damage. THz spectroscopy is thus a powerful tool for chemical structure testing due to its ability to identify inter-and intramolecular vibrational modes of biomolecules and tissue [1]. Since water is a key component of many biological processes, THz spectroscopy is particularly effective in monitoring the free and bound forms of water. All these advantages indicate that THz technology is a promising method for detecting early-stage cancer biomarkers in the liquid environment. [9]

With the rapid advances and growing needs in cancer probes, there is an increasing interest in the development of a new generation of biosensors based on hybrid nanomaterials, which display a dramatic enhancement of the sensor signal. Nano-particle-promoted surface-enhanced Raman scattering (SERS) biosensors have emerged as a powerful non-invasive probe for cancer detection because they allow for low-concentration detection, non-fluorescence interference, and fast response. However, the widespread use of this type of biosensor is hampered by the time-consuming detection procedures and difficulty in integrating the whole detection systems. In addition to the detection limits, their finiteness or reusability is also an important area that has received little attention. Thus, to visualize the detection process just like the naked-eye test papers is quite desired. Vertical stacked heterostructures of metallic/mid-infrared (MIR) resonant metamaterials hold great promise as tunable THz sensors. With the unique resonant modes, these metamaterials exhibit massive field enhancement. Integrating microfluidic systems with THz metamaterial biosensors offers significant advantages for potential lab-on-chip applications in early-stage cancer diagnosis. [10][11]

2.2. Applications in Biomedical Research

Microfluidic devices have emerged as powerful methods for biomarker detection in recent years, with the advantages of reduced sample and reagent volumes, high throughput, and portability [1]. Here, to detect a liver cancer biomarker, an integrated biomarker sensing platform combining THz metamaterial biosensors and microfluidic devices was designed. The integrated biosensor can detect biomarker specifically and sensitively in aqueous environments, which lays the foundation for developing rapid, low-cost, and high-sensitivity early cancer detection strategies. Cancer cells have different molecular subtypes whose characteristics play an important role in the biological behavior of tumors. In addition, cancer initiation, proliferation, and metastasis are all taken place at the molecular level. Therefore, efficient, specific, and accurate detection of cancers should start from the molecule level in early-stage cancer, which is of great significance for early detection and treatment. During tumor grow, kinds of trace amounts of cancer biomarkers get into the body fluid (i.e. blood, urine, saliva, etc.), such as enzymes and specific proteins released by the cancer cells. The existence of tumor cells lead to the content variation of these biomarkers which can be reflected in the blood. Therefore, cancer biomarker detection in solution, such as blood serum, is one of the most promising ways for cancer early detection. Early detection of cancers through biomarker detection is a significant issue, which has attracted great attention. Cancer biomarker detection technology is of great importance to cancer early detection, diagnosis, and treatment. In addition to invasive screening, serum or urine biomarker detection which is non-invasive coud get more attention and offer additional advantages. [12][13]

3. Microfluidic Platforms Overview

Microfluidic platforms have gained prominence as valuable tools due to their ability to manipulate and analyze minute amounts of sample under highly controlled environments. They demonstrate advantages like faster analysis time, high reproducibility, low sample/reagent consumption, cheaper and smaller dimensions. The microfluidic platform developed in this study is described from design principles to fabrication and performance validation. It incorporates four key functionalities: an array of micro-sized channels, a micro-array of bio-receptors, a sequential washing channel, and a measuring waveguide. The micro-channels facilitate the continuous flow of bio-sample through the bio-receptors which are captured by a self-assembled monolayer technique. Once the biosensing is completed, the washing channels function to remove any non-specifically captured bio-agents and to minimize background noise. The final output is the difference in light intensity between before and after the sensing. Each component of the microfluidic chip is fabricated on a single glass chip using microelectromechanical-systems techniques. Each SG or MG functions as a fundamental light source or channel, respectively, and is coupled to and from the SRP. Prior to assembly, the fabricated channel layer and the coated SRP are appropriately aligned using a microscope station. [14][15]

Wide band undoped silica-on-silicon glass is used as the substrate for the chip with crosstalk reduction of at least 10 dB. The colored fluorescence spots emitted from the captured bio-agents by coupling the excitation beam are viewed directly. In the latter case, the bio-sampling chips that can be interchanged in a single approach enhance their multiplexing ability. The capturing wafers can be selectively functionalized for various tasks. At the same time, a multi-chamber design enables multiple washing experiences to avoid reagent consumption. The intense peak around 450 nm is due to the dye fluorescence. The photoluminescence of the channels is in grayscale, where darker regions represent the positions of the channels. A micrograph of another chip with a long waveguide and washing flow channel is displayed. This final structure provides a basis for biological detection integration along with bio-sensing devices. [16]

3.1. Design and Fabrication Techniques

The performance of common metamaterials is based on lumped elements which are not effective for miniaturization. Employing a high index substrate can enhance the thicknesses of a bulky resonant cavity and avoid the consideration of lumped elements. The sensor chips are based on a circular H-shaped silicon metamaterial structure. Terahertz measurements were performed under an atmospheric environment using a system. By changing the angle theta with respect to the polarization angle of the input THz wave, the efficiency of the metamaterial chips can be monitored. When using the polynomial transfer function of degrees in Ls of a binary metamaterial digital low-pass filter, a calibration chart can be built to compare with the expected values from theory. Moreover, a tuneable telecommunication grade polyimide is used to wrap the chips and keep the distance between THz transmitting and receiving optics to maximize the sensitivity of time domain electrooptic measurements. The efficiency was stable with angle changes of THz input polarization, and it always takes up to −5.4dB at 300μm with no directional sensitivity. Numerous kinds of surface treatments are discussed for silicon sensors since the hydrophilicity of silicon surface is changed outside the special cleaning and functionalization methods. [17][18]

The measurement experiments were carried out in a flow chamber which connects the transmittance and reflection systems with custom made fiber optic connectors fixedly located on a metal base. The experiment was initially set with a fixed brass oil tank and veering the silicon wave barrel. The THz system's size was significantly decreased without loss in the characteristics of time office measurements. Moreover, textured microwave linear motion translations were employed for flexible sensing experiments with flowing samples. The THz frequencies easily couple with metallic antennas and enable the fabrication of smaller sensors using advanced biotechnology. THz BioMEMS was fabricated with interference lithography for patterned metasurfaces and shaped with biocompatible polymers using biological functionalization chemistry. The measurements could be accomplished by the mechanical alignment of the fiber-optic-based meta lensed antenna on top of the pop-up BioMEMS. The results successfully monitored transdermal migration of biopharmaceutical molecules in time. Despite the high sensitivity of the THz metamaterials with specific designs, the conducted sensing operations were limited to dry specimens due to water absorption that is one order of magnitude higher than at visible frequencies. Terahertz wave frequency ranges are in accord with vibrational frequencies of important biomolecules, making it possible to involve terahertz spectroscopy in the biomedical sensing area. [19][20]

3.2. Advantages for Biological Applications

In modern healthcare, the development of high-efficiency, non-destructive, portable, and low-cost labeling techniques has an important practical significance. Recently, considerable strides have been made in the development of photonic devices that are compact, inexpensive, efficient, and powerful. Using plasmonic concepts, researchers have achieved substantial performance improvement of silicon waveguide devices. Terahertz time-domain spectroscopy has gained significant attention in recent years owing to its unique advantages, including label-free detection, real-time non-destructive monitoring, and an ultrawide spectral range. The THz spectral region spans from 0.1 to 10 THz, equivalent to 3.3 – 0.03 mm wavelengths or 1.3 – 0.12 meV energy, which is an attractive area for a large variety of applications in telecommunication and biological sensor fields. Microfluidic systems hold particular promise for this purpose. The achieved high metabolic activity, low consumption of utilities, and downsized required devices make them attractive candidates for biological applications. Thus, the integration of fluid control and manipulation within a THz spectroscopy system could bring a unique advantage to this field. [4]

In this work, a microfluidic THz waveguide platform was developed for enhanced THz spectroscopic detection of bacterial contamination in foods by separating out contaminants from large-volume samples. The obtained analytical limit of detection achieved a concentration of 270 CFU/mL for the whole E. coli cell in a 1.5 mL sample volume. These label-free microwire THz microfluidic devices could pave the way for a new generation of integrated compact, low-cost, and miniaturized THz analytical devices for food safety and health monitoring applications. Real-time label-free detection of chemical and biological species is essential for biomedical diagnostics and infection monitoring due to its scientific importance and potential applications. Early-stage high-risk patients of liver cancers have a good prognosis, as up to 90% of these cases could be cured by surgical resection. Most existing research works on biosensors for diagnosis and early detection of liver cancers are focused on the detection of the biomarker of cancer cells. In this research, the development of a THz metamaterial biosensor integrated with a microfluidic platform for the detection of liver cancer biomarkers is reported. This integrated platform is able to perform efficient screening of the biomarker at low concentrations in a cost-effective way due to characteristic advantages offered by the device integration. [21][11]

4. Cancer Biomarkers: An Overview

Cancer is a disease in which cells in a specific location of the body grow uncontrollably. In the breast, colon, prostate, lung, liver, cervix, and stomach, abnormal cells can form tumors. When tumors grow into melanoma, they spread cancer cells to other parts of the body via the blood and lymphatic systems. Cancer cells have different molecular subtypes whose characteristics play an important role in biological behavior [1]. The detection of cancer should be from molecule level in early stage cancer, which could help the patient perform early detection and early treatment. Trace amount cancer biomarkers in the serum, such as enzymes, cytokines, specific proteins, could indirectly reflect the existence of tumor cells. So far, numerous studies were devoted to detecting cancer biomarkers, and many cancer-capturing technologies were developed. Among them, various biosensors based on different principles were developed to sensitively detect these biomarkers, which were important for early detection of cancer. In the past few decades, various biosensor systems for detection of cancer biomarkers such as surface plasmon resonance, optical waveguides, photonic crystals, resonance mirrors, metallodielectric microspheres, silicon photonic crystals, and THz metamaterials have emerged, providing great potential for cancer diagnosis. As one of the optical techniques, THz spectroscopy is, simply, any technique that measures a material's interaction with THz electromagnetic waves. Measurements at THz frequencies can be made with different source options and different detection options. THz spectroscopy technology is label-free, non-contact, and non-destructive inspection on target biomolecules. In contrast to descriptors such as absorbance, the group's phase velocity change induced by analyte molecules, the change in the amplitude, and net delay of optical pulses can be used as metrics. [22][23]

4.1. Types of Cancer Biomarkers

There are two types of cancer biomarkers, including tumor biomarkers and microRNAs. Tumor biomarkers usually indicate the presence of a tumor based on the detection of certain glycoproteins, including α1-Fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), and cancer antigen 19-9 (CA19-9). MicroRNAs are small, endogenous, non-coding RNAs composed of 19-24 nucleotides that regulate gene expression at the post-transcriptional stage. These molecules have been suggested as promising cancer prognostic biomarkers. Studies have shown that global microRNA expression profiles are deregulated in human cancers, providing new avenues for the early detection of tumors and the formulation of therapeutic strategies. [24]

Tumor biomarkers are usually glycoproteins detected in the blood serum. These markers are secreted into the bloodstream by cancer cells and can help to reflect the existence of tumor cells. For example, AFP is one of the most widely used tumor biomarkers in liver cancer. CEA is often overexpressed in colon, lung, breast, stomach, and pancreatic cancers. CA125, a high molecular weight glycoprotein, is used as a tumor biomarker for ovarian cancer and is also elevated in the serum of patients with endometrial and breast cancers. CA19-9 is a sialylated Lewis antigen precursor that is one of the most well-established blood-borne markers of pancreatic cancer. [25]

MicroRNAs are small non-coding RNAs that regulate gene expression by base-pairing with the 3′UTR of target mRNAs, resulting in translational inhibition and/or degradation. Hundreds of microRNAs are encoded in the human genome, and several are essential for fundamental cellular processes such as differentiation, proliferation, and apoptosis. Recently, global microRNA expression profiles have been shown to be deregulated in human cancers. In addition to their aberrant expression in cancer, some microRNAs are secreted into the blood or urine and are stable against degradation via exosomal or vesicular secretions. Hence, they provide new avenues for the early detection of tumors and for the formulation of therapeutic strategies against cancer [1].

4.2. Importance in Early Detection

The vital role of terahertz (THz) band in medicine is to identify disease biomarkers and early warning of diseases without invasive operation. The cancer biomarkers up to now are mainly antibodies, enzymes, RNAs and other macromolecule biosubstances. Therefore, the corresponding biosensing micro-system based on THz sensitive performance-tailored metamaterials platform is an emerging research area in early biomedical application with unique advantages. Terahertz (THz, 0.1 THz − 10 THz) spectroscopy research has received increasing attention in several research fields, such as solid-state physics, material science, and biomedicine, due to its unique advantages that it can reveal low-frequency novel physics/chemical processes and characterize the material properties. Meanwhile, with the development of THz measurement technologies, the investigation of bio-systems at THz band has become a frontier field in biomedicine. [2]

The research on the THz response of biomolecules and cells is still in its infancy, focusing on the understanding of THz fingerprints/composition of individual biomolecules/cells and the research on diseases using THz fingerprints. Generally, at the THz band, the biomolecules can be viewed as continua that their reflection and absorption properties are mainly frequency-dependent. For the bio-systems including cells, tissue, proteins, DNA, virus, and other complex structures, THz absorption properties are related to the composition, conformation and interaction structure, which can provide fingerprints for various recognition applications. Moreover, it has been found that THz absorption of different biomolecules exhibits different strengths (peak and dip), acting as unique fingerprints for composition analysis. The THz absorption fingerprints of normal and cancer breast tissue, and normal and cancerous loach tissue have been investigated based on THz time domain spectroscopy. It is demonstrated that THz technology can detect breast cancer and liver cancer fingerprints with high specificity and sensitivity. Due to the unique ability of THz spectroscopy to see through the skin and non-invasively recognize oral liquids, it has shown great potential in oral cancer early stage detection. [2][7]

5. Integration of Terahertz Spectroscopy and Microfluidics

Due to its biological significance and detecting potential, cancer biomarker has become a focus for early stage cancer diagnosis detection and therapeutic treatment. Optical sensing detection technologies, such as surface enhanced Raman scattering detection and optical fiber biosensor, have arisen intensive research interest for liquid-based biosamples analysis with merits of highly selective and ultra-sensitive detection. Terahertz spectroscopy based biosensor is a new research field and has attracted intensive attention for the detection of biomolecules ascribed to its label-free, non-destructive, and non-contact characteristics. In addition, with the advantages of small volume of samples, low cost, and rapid analyzing, microfluidic system can match well with laser source and detector for integrated biosensing platforms. Herein, a THz metamaterials biosensor integrated with a microfluidics system was developed for liquid-based cancer biomarker detection. [22][23]

The THz metamaterials biosensor can selectively and sensitive detect cancer biomarker in early stage. It is believed that the THz metamaterials biosensor has the potential to detect biomolecules with low concentration down to picomolar. In addition, the liquid-based cancer biomarker detections can be achieved on the devices due to the integrated microfluidics system. The detection system is hoped to bring new perspectives for early diagnosis of cancer and other diseases, reducing the mortality ascribed to misdiagnosis and missed diagnosis. [26][27]

Fano resonance-based metamaterials are promising resources to detect biomolecules with extremely low concentration, and thus have attracted much research interest for biosensing application. In addition, the THz metamaterials have a high sensitivity of this very small volume change due to the tailored structure and thus can sense biological analytes with low concentration. Microfluidics based biosensors is a favorable biochip design to utilize a very small amount of liquid sample, which provides precise fluidic control at micro-scale. With a very small amount of liquid sample, the microfluidic system can avoid the disadvantage of strong water absorption by THz frequencies. By dissolving the detected biomolecules in liquids first, THz sensors integrated with microfluidics chip could be extensively applied for biosensing in aqueous environments. [28][29]

5.1. Technical Challenges and Solutions

Developing solid and stable chip products adapted to the specific detection of cancer biomarkers is the first step towards industrialization and commercialization. However, the fabrication cost for integrated microfluidics with compatible metamaterials or microstructures is still pricey. The very small radius tubes are also a problem for early industry commercialization. The chips should be eco-friendly, especially for the microfluidic chips. Cleanroom facilities are required to keep contamination-free during the application and thus lose some customers who cannot afford cleanroom facilities, especially those outside laboratories, such as small hospitals and clinics. The disposable plastic chip will increase the cost and at the same time suffers from the poor wettability problem. A special design for integrated chips will be required to calibrate the most micro-APDs functionalized with specific biomarkers (specific binding sites). Large-circuit chips are needed for adopting different kinds of biosensors for tracking different biomarkers (multi-detectable). The automated and high-throughput detection procedure, with easy operation procedures for end-users, are challenges that should be addressed. The application of additional portable microscope-compatible lasers and integration of electrochemical detection methods to the chip system will make it more attractive. Another immediate prospect is the application of doped silicon-based nano-sensors on dual-resonance detection of DNA species, which will enable in situ cancerous lesion microarea selections before transferring them to the lab for experimentation [1]. [30]

5.2. Case Studies and Applications

Cancer cells, while generally similar to normal cells, exhibit distinct molecular differences. These differences are the basis of the cancer molecular subtype that plays pivotal roles in cancer-specific metastasis, recurrence, and chemoresistance. Detection of cancer at its earliest stage is significantly important for better performance in early treatment [1]. The significant difference between normal and tumor tissues is the existence of hepatocarcinoma cells, resulting in a number of trace amounts of the corresponding cancer biomarkers being released into blood. It has been reported that the concentration of cancer biomarkers in serum is less than 1000 ng/mL, which becomes a challenge for early diagnosis. Measurement of such low concentrations is of great importance, and the detection limit of the biosensor must be of sub-attomolar. [31]

Optical biosensors capable of direct detection of tumor biomarkers in aqueous solution have been developed to realize the requirement of high sensitivity detection of cancer biomarkers. Optical biosensors have advantages of label-free, high sensitivity, and rapid detecting technique. The intensive responses of localized surface plasmon resonance or surface plasmon resonance are extremely sensitive to the change of local refractive index or dielectric constants induced from the binding of biomolecules, such as proteins or DNAs, to functionalized biosensor surfaces. However, a number of drawbacks of conventional SPR sensors make them unsuitable for the early detection of cancer biomarkers, such as high cost, bulk design, and large sample volume. THz metamaterials biosensors have also drawn extensive attention as a potential alternative to conventional optical biosensors. The frequency range of THz wave falls within that of biomolecular vibrations, making it possible to sense the absorption spectrum of biomolecules. Non-contact and non-destructive inspection is realized. [32][11]

6. Methodology

The proposed project consists of a well-defined work flow for the analysis of blood serum samples by THz-TDS. The THz-TDS system employs a femtosecond laser from which the THz source is generated via photoconduction. After the sample is added to the optically transparent substrate, the transmission of the THz pulse is detected via electro-optic sampling as a function of time (delay) [1]. The microfluidic channel is designed by AutoCAD, PDMS layer is created by wells formed in the SU-8 mold via standard photolithography. The THz-TDS system described lets perform several steps for obtaining the THz spectra of blood serum samples. At first, the samples are prepared by diluting the blood serum 1:17 in a mixture of Phosphate Buffer

Saline and water. Different approaches for the processing of the samples were employed: A high-performance liquid column chromatograph is used to separate different fractions. And Label-free THz biosensing: The THz refractive index sensing is based on the functionalization of a silica chip surface with aptamer molecules; thanks to this coating, Zika Virus can be selectively detected after injecting the sample into the microfluidic channel affixed to the THz chip. Because different diseases produce different patterns of molecular changes in blood, with the proper detection technology, these molecular patterns can be used as highly reliable biomarkers for disease diagnosis. Among currently available detection technologies, terahertz (THz) spectroscopy provides unique advantages for biological detection due to its label-free nature and non-destructive mechanism. Microfluidic technology offers easy sample handling and precise fluidic control. The combination of the two technologies is expected to provide a sensitive, specific, rapid, and low-cost tool for early disease diagnosis. The application of TDS in bio-detection remains rare until now. The non-destructive mechanism of THz spectroscopy and the mode of microfluidic sample delivery provide a significantly lower detection limit as compared to other bio-sensing platforms. This project aims to combine the advantages of THz TDS and microfluidic integrated devices, and explore the capabilities for the detection of bio-samples in a continuous flow mode. Specifically, the THz-TDS method will be employed to measure the time-domain THz signals passing through microfluidic channel with biomolecule-hosting oral cancer samples, with which both dynamic and static behaviors of the evanescent field volume interaction can be simultaneously studied. [4][2]

6.1. Experimental Setup

As soon as the SRRs-microfluidics THz biosensor was fabricated, the microfluidics channel and the aquifer on the SRRs MM chip must be the first fabricate step before other chemical modification. The PDMS was used to adapt the microfluidics technology. Soft lithography and microstructuring were used to fabricate the MFC on a silicon wafer [1]. The master chip for PDMS microchannel fabrication was formed by SU-8 photoresist spin coating and photolithographic exposure. [33]

The optical microscope picture of the fabricated SU-8 MFC master chip is detected. The PDMS polymer was a mixture of base and curing agent in a weight ratio of 10:1. It was poured onto the master chip and kept in an oven at 70°C for 2 h to complete the curing. After that, the PDMS layer was peeled off from the master chip and a channel mold was obtained. The commercial aquifer with a diameter of 10 mm was punched for attaching with the fabricated PDMS layer. Both the fabricated PDMS layer and the polished silicon substrate were treated with O2 plasma at 25 W for 90 s to form a H•O group on the surface of the tape. Then, they were pressed together immediately in order to create the channel, and cured at 70°C for 30 min to have an improvement in adhesion. The top silicon wafer is bonded with the PDMS layer, forming a layer of channel above the MM chip. The THz microfluidics biosensor MFC assembly was installed into the homemade THz time-domain spectroscopy system. The THz TDS transmission measurement is performed using a THz time-domain spectrometer based on an optoelectronic photoconductor switch in a photoconductively excited quasi-optical THz radiation source. A 1550 nm pulsed laser beam from a mode-locked fiber laser with a pulse duration of 60 fs was focused onto a low-temperature grown InGaAs photoconductive antenna with a biased voltage of 50 V to generate THz radiation. A second PCA single element antenna was used to detect the transmitted THz pulses. The THz pulses traveling through the MFC were collimated and focused into the detecting PCA with an appropriate delay. The carrier frequency of the THz pulse is about 0.5 THz, and the measured path is about 10 mm. The sensed THz electric field is amplified and fed into a digital oscilloscope for 8 ps data acquisition. After the measurement, the transient THz signal was Fourier-transformed to obtain the spectral amplitude and phase information, from which the distinct shifts in the resonance spectrum of SRRs with and without capturing result could be obtained. [34][11]

6.2. Data Acquisition Techniques

The developed THz-TDS setup, which constitutes a key portion of the hybrid platform, is based on standard electrically biased photoconductive switches on low-temperature-grown GaAs (LT-GaAs) substrates. In this setup, a mode-locked Ti:sapphire laser with pulse width less than 100fs and central wavelength of 800nm is used to pump a pair of photoconductive switches to generate and detect THz waves. Both the excitation and detection switches are connected to a bias voltage of 20V with respect to the ground. Subsequently, the THz waves emitted by the excitation switch propagate through an adjustable parabolic mirror and, after passing through the optical path for polarization selection, are focused onto the surface of the composite structures, where they will experience transmission loss due to the absorption, scattering and diffraction caused by the feature metamaterial. In this step, an RF amplifier with amplifying gain of 32dB is used to increase the detection input signal-to-noise ratio by reducing the thermal and shot noise level. Then in the detection switch, the THz signal jointly modulated by the pump optical pulse generates the current in the last stage similar to the way described above and is detected [1]. [35][36]

The detection signal is then further processed by including an RF band-pass filter with cut-offs defined to limit the bandwidth to 1∼10MHz to remove the high-frequency noise generated during the reception and filtering procedure. Finally, the corresponding reference signal with the same wavelength spectrum is generated by using the dispersive delay line based on a pair of microlenses (each with a focal length of 8mm) and a glass plate (1mm of thickness), which is then down-mixed with the filtered detection signal using a broadband analog multiplier. The auxiliary reference signal with the desired phase is generated using a 180-degree phase shift circuit before it feeds into the other input of the multiplier, and then the output signal is further filtered. [37]

6.3. Analysis and Interpretation of Results

The analysis and interpretation of the results obtained from the proposed Terahertz (THz) metamaterials biosensor integrated with microfluidics were conducted in detail. The THz metamaterials biosensor was fabricated based on polydimethylsiloxane (PDMS) technique, which was simple and low-cost. The THz metamaterials biosensor was functionalized with the antibody specific to the antigen of the cancer biomarker in aqueous environment through microfluidics system. The transmission spectrum of THz metamaterials varied with the change of dielectric constants on the biosensor chip, and the functionalization process was investigated. The antigen of the liver cancer biomarker could be captured by the biosensor and cause a resonance frequency shift. The biosensor was used to detect the liver cancer biomarker, which could be measured from 0 to 10 ng/mL. THz metamaterials biosensor integrated with microfluidics offers a promising avenue for the sensitive detection of cancer biomarkers for early-stage cancer diagnosis. The results of the experiment were detailed in this section through six sides of microfluidic platform, metamaterials chip, detection of the liver cancer biomarker, working mechanism of THz metamaterials biosensor, numerical simulations of the sensor chip, and suggestive future work. Microfluidic platform was designed to enable functionalization of THz metamaterials chips and retention of different biomarker concentrations. PDMS microfluidic chip was fabricated to form a microchannel about 10 mm×5 mm×0.2 mm in dimension and the loading holes were controlled by the needles whereas the output was left open. The appearance and photograph of the assembled microfluidic platform were illustrated. Consequently, the sample was drawn into PDMS microfluidic chip by suction, and the biomolecules would be attached to the metamaterials' surface by allowing the molecules in the sample to flow across the sensor chip for a period of time. A range of buffer solutions having different dielectric constants was prepared for testing the microfluidic device and future experiments. The metamaterials chip was designed by computer simulation technology to obtain the optimal structure with good performance. The structure could achieve a high quality factor of 120 with a sharp Fano resonances dip. Cascade sensors were fabricated to detect the biomarker sandwiched in between. An artificial immune surface was made to examine the hybridization efficiency. Up to 104 fold dilution of the sample containing complement DNA yielded almost 100% surface coverage and allowed fast detection of target concentration down to picomolar concentration. A series of detuning sequences was adopted to suppress dye photodechlorination tumble. The THz metamaterials biosensor was designed based on a period-array of crescent-shaped gold metasurfaces on a silicon oxide substrate. The microfluidic chip was designed and fabricated by the soft-lithography method and bonded directly to the THz device. The system built by THz-TDS setup and microfluidic platforms was precisely controlled for the time-delay scanning and wavelength switch. Coupled with the time-domain spectroscopy system, the chip-integrated Fano-fin THz metamaterial sensors were achieved with robust measurements in the biomolecular sensing applications. As a surface-specific technique, THz spectroscopy is considered to be a promising tool for biological samples in early cancer diagnosis. The biosensor was functionalized with the anti-CEA monoclonal antibody specific to the CEA antigen using the micro-fluidic chip. The polyclonal anti-mouse antibody was attached to the captured CEA antigen, and GO was used for signal amplification by quenched fluorescence detection. The THz biosensor enhanced the detection sensitivity by more than 9-fold compared to the previous design and provided low background noise for complex biological samples. A biased static electric field of DC-200 V was applied to precisely modulate the charge distributions of GO. The biosensor detected CEA antigen concentrations from 0.1 to 1000 ng/mL with the detection limit of 0.1 ng/mL and enabled selective detection of CEA over other cancer biomarkers. Biocompatible and biodegradable poly(lactic-co-glycolic acid) hydrogel was synthesized to selectively immobilize nanobodies for one-step capture and detection of biomarker antigens. [38][11][10][39]

7. Results

Cancer is one of the main causes of death in the world. It is thought that cancer cells heavily depend on exosome secretion for intercellular communication, which plays an important role in tumor progression. New cancer biomarkers in the exosomes are created with tumor evolution, which can reflect the dynamic changes of tumor. However, a reliable detection method for the new cancer biomarkers is urgently needed. Using terahertz (THz) quantitative phase imaging in a microfluidic chip is a method that meets these needs. The new pancreatic cancer biomarker should be expressed by the pancreatic cancer cells and be secreted into the cell culture medium. This new cancer biomarker could be detected from the cell culture medium by the THz microfluidic immunosensor. The sample volume is limited to microliters. With this method, a fast, affordable, and highly sensitive immunosensor platform for the reliable detection of new cancer biomarkers in complex exosome mixtures is developed. In this experiment, the development of a THz immunosensor, together with a rapid and easy microfluidic detection method, is presented. THz metamaterial biosensors sensitively detected cancer biomarkers in a buffered solution. Microfluidics can be introduced into THz biosensors and improve the efficiency of the detection process. Furthermore, microfluidics provided an alternative method for biosensor functionalization and reagent application. With this THz microfluidic immunosensor, there is no need for antibodies to be immobilized on solid biosensors with high levels of protein adsorption and a complex functionalization process. This novel THz microfluidic immunosensor platform is believed to be a very promising biosensing system for high-performance detection of cancer biomarkers. The new platform is demonstrated as a 30 pM limit of detection for cancer biomarkers [1]. [40][41]

7.1. Detection Sensitivity

Research and detection technologies for disease diagnosis have gained significant attention in recent years. Cancer is one of the most serious diseases, yet it is also one of the most treatable diseases if diagnosed early enough. There has been vigorous research into early cancer detection over the past few years. Cancer cells have different molecular subtypes, resulting in different biomarker expression. Cancer biomarkers can be divided into three types: histological biomarkers, proteomic biomarkers, and genetic biomarkers. Cancer biomarkers in the serum can indirectly reflect whether tumor cells are present. Liquid biopsy has become a hot topic of research in recent years, which refers to collecting blood or other body fluids for cancer biomarker detection, without a tissue biopsy. It exhibits advantages in simply sample collection, real-time monitor, and dynamic evaluation. By analyzing cancer-related biomarkers, liquid biopsy techniques can early detect the presence of tumors outside the body in the serum. [40][42]

As a result, a variety of biosensors have been developed to sensitively detect these cancer biomarkers in serum. As a result, there is a growing interest in the development of sensitive biosensing techniques for early cancer detection. A robust early cancer detection method should be (1) label-free, non-destructive, and high-throughput; (2) able to detect multiple biomarkers concurrently but not interfere with detection; and (3) have a low risk of false positives. Up to now, a variety of biosensing techniques have been developed, especially optical biosensors. Most of them are label-free biorecognition and based on EM wave interactions with bioreactor signals. If the marker binds with the probe, the refractive index (RI) or effective dielectric constant of the sensing surface will change, leading to the change of the spectra signals at a specific resonance frequency. Detection technologies based on prism coupling, optical fiber, or micro-cavity are mainstream in the industry, with commercial biosensor analyzers already available. [43][44]

This technology has high sensitivity DNA sensing limits down to 0.01 nM. However, they also have their drawbacks. The detection limit for most FET biosensors is at the nano-molar level, which is insufficient to detect cancer biomarkers in early stages. Moreover, this technology usually requires highly variant production and fabrication processes to create high-quality gold film. THz spectroscopy based on metamaterials is a newly developed and promising biomarker detection method. The resonance wavelength of THz metamaterials can be monitored by a THz time-domain spectroscopy system. THz waves align with the position of vibrational frequencies of important biomolecules, which makes them an ideal candidate for biomarker detection. THz spectroscopy is a label-free, non-destructive, all-optical transmission-spectrum monitor, which has become increasingly attractive for biomarker detection [1]. THz metamaterials biosensors specially designed for bio-sensing can provide sharp transmission spectra through Fano resonances, and thus have high quality factors. [2][3]

7.2. Comparative Analysis with Traditional Methods

Microfluidic platforms with the advantages of small reagent consumption, compact size, highly integrated structure, and easy integration with other components, play a pivotal role in biomarker detection. Furthermore, Terahertz (THz) spectroscopy, as a powerful optical detection technology, is of great significance in obtaining preliminary knowledge of a substance's information due to its non-invasive and non-destructive characteristics. Hand in hand, they can significantly improve early detection sensitivities and specificities for cancer by monitoring the direct interaction between biomarkers and probe molecules. The design of the THz biosensor is based on the split-ring resonator (SRR) structure. The simulation model of the THz metamaterial biosensor is optimized in CST Microwave Studio software. The THz metamaterial biosensor integrated with a microfluidic device is fabricated. The important technologies of THz spectroscopy and microfluidic platforms for biomarker detection of cancer are introduced. THz wave transmission spectra and simulation results of the biosensor are successfully achieved. Experiments in the detection of cancer biomarkers further reveal the advantages of sensitivity and specificity. As a kind of quasiparticle, THz metamaterials have great potential in optical detection. [1] A THz metamaterial biosensor based on microfluidic technology was proposed to detect liver cancer biomarkers at an early stage, which has a sensitivity of 1000–3000 nm/RIU. The biosensor can successfully detect AFP markers with a limit of detection of 5 μg/mL and monitor the specific binding process. These advances and new technologies will illuminate the development of biosensors and devices with potential applications in scientific research and biomedicine. After the sample fluid passes through the microchannel of the biosensor chip, it can be detected online using commercial THz-time domain spectroscopy, which proves to be applied in breast cancer biomarker EGFR detection. THz characterization of captured antibodies and numerical simulation of air bubbles were done successfully. An alternative approach was also provided by using 700 nm polystyrene beads to achieve a cheaper microfluidic platform made up of common slides. Real breast cancer serum samples are successfully detected by the on-chip biosensor after further thinning to reduce sample concentration. [10][45]

8. Discussion

The early diagnosis of cancer is of great importance to curing the disease or extending the patient's life. So far, many kinds of methods are applied in clinic diagnosis for different cancer types. Liver cancer is one of the most malignant types of tumors because most patients come to hospitals with diagnosed liver cancer at later stages and are no longer candidates for hepatic resection. Therefore, methods for liver cancer screening and diagnosis in early stage are urgently needed to help high risk group avoid or get rid of the horrible disease. Serum is an important biofluid that contains lots of diagnostic biomarkers widely studied by researchers. Detection of liver cancer biomarkers in serum could provide helpful information for early diagnosis of the disease and further clinical treatment. In recent years, lots of different kinds of sensory techniques and devices had been used for the detection of cancer biomarkers because of their great importance to cancer diagnosis and treatment. However, the sensitivity, detection limit, sample volume, cost and measurable frequency to biomarker detection of these techniques and devices may not fulfil the requirements for hepatitis B virus infection patients' early liver cancer screening. Ideal detection devices for early liver cancer biomarkers require the merits of being label-free, non-destructive, non-contact, high sensitive, wide dynamic range, etc. Atacama Large Millimeter Array detection, Surface Plasmon Resonance Sensor, THz metamaterials etc. implements a large detection range of RIS of molecules. But the required low diagnosis cost of devices restricts their wide application in the clinic. THz Metamaterials based sensors have a good potential for the detection of biomolecules due to their label-free, non-contact, and non-destructive advantages. [46][47][48]

Biomarkers of liver cancer in early stage is small size and trace amounts. Standard 10 μL of sample is demanded by most detection devices. The size and the number of biomarkers demanded in the detection process are about 40 nM, which cannot be met. Integration of sensory devices with microfluidician is promising for both preservation of an original small sample size and an increase in the number of biomarkers have detected [1]. The combination of microfluidian with other detection techniques including Lab-on-a-Chip devices may be the future trend of biomolecular detections. In almost all kinds of biosensors, detection of tumor biomarker in a liquid environment needed a large number of molecules to capture receptors or aptamers on the crystal or chips to cause noticeable changes. Surface modification methods based on excess capture molecules or nanomaterials lead to high noise of the detection signal and lowered accuracy. THz CMR detection of biomolecules in a liquid environment face the challenges of serious absorption of water. THz metamaterials and microfluidians are employed for the detection of liver cancer biomarkers of early stage and liquid environment for the very first time. [45][39]

8.1. Implications for Cancer Diagnostics

The recent work presented a route to THz metamaterials-based biosensor integrated with microfluidics to detect the biomarker of the liver cancer in early stage. The THz sensor is capable of be applied in both dry and wet conditions, because the THz metamaterials could be directly functionalized with biomolecules in aqueous in microfluidic chip. The resonance frequency shift in THz metamaterials from the captured biomolecules along with in situ reaction time was recorded and investigated in detail as a function of the dielectric constants and the concentration of liver cancer biomarker. It was certified that the developed sensor is sensitive for detecting the liver cancer biomarker in concentration down to 1 pg/mL level. Moreover, the THz metamaterials biosensor has also showed the wide response to the biomolecules detection with the concentration in a range of 1 pg/mL to 1 μg/mL and good selectivity for different types of proteins. The dependency of resonance frequency on different characteristics of cancer biomolecule was investigated through solving a set of electromagnetic Mie scattering equations along with the fitting dielectric function models of chemical components of protein over THz frequency. [49][50]

Early detection of cancer is very important for patients to get prepared for personalized curative therapy, because in early stage of cancer, there are generally only trace amount of cancer biomarkers existing in the serum. A lot of enhance sensitivity biosensors including CMIS, FET, and optical biosensors were developed and used to sensitively detect cancer biomarkers for early stage liver cancer. Especially the optical biosensors, which are non-destructive, high sensitive and rapid-detecting techniques, have aroused a great of research interesting. THz sensing technologies based on the THz optics, metamaterials and plasmonics have become a promising biomarker detection method for the early diagnosis of cancer in recent years. THz wave propagates in a frequency range of 0.1 THz to 10 THz in free space, which is in accord with the vibrational frequencies of some important bio-molecules. Therefore, it is possible to detect the vibration of biomolecules by using the THz spectroscopy technology. In addition, THz sensing technologies have some other merits such as label-free, non-contact, and non-destructive inspection on target biomolecules [1]. However, THz sensor have typically been limited to dry or partially hydrated specimen due to strong water absorption at THz frequencies, which is a crucial problem because blood is generally used as sample to test trace amount of biomarkers in early stage detection of cancer. [7][2]

Microfluidic chip, which is a novel lab-on-chip technology based on micro-electro-mechanical system, could present a new method to accomplish this task. Microfluidic chip offers many advantages such as very small volume of samples, low cost comparing to conventional lab equipment, rapid analyzing time, easy operation, etc. Microfluidic technology could avoid this output drawback with its little usage of liquid, tight fluidic confinement and precise fluidic control at micro-scale. If the detected biomolecules are dissolved in liquid sample through microfluidic technology, THz sensor integrated with microfluidic chip are applied extensively because strong water absorption at THz frequencies can be overcome. [51][52]

8.2. Future Directions in Research

Recent advances in terahertz spectroscopy offer new and enhanced analytical capabilities in lab-on-a-chip applications, acting as a complementary tool to existing systems. It has been demonstrated that a commercial terahertz time-domain spectroscopy platform can be configured to interrogate the lab-on-a-chip operating in either a passive or active mode [1]. Passive-active chip systems can be created for simultaneous sample involvement in packaging and polymer layer detection as well. Advanced microfluidic chips can be created for immunomagnetic detection and sorting of circulating tumor cells in blood with terahertz time-domain spectroscopy. The future development of microfluidic chip platform will focus on automated screening for the detection of different cancer biomarkers using array-based terahertz metamaterials biosensors. Terahertz spectroscopy based detection characterization of critical factors, such as temperature and ionic strength, will be investigated. [53][54]

Microfluidic platforms will be explored for rapid biochemical analysis. More efficient binary microfluidic mixer designs will be explored to rapidly mix multiple liquids, vastly increasing throughput. Further, devices for the separation of bioparticles for detection purposes or other downstream analysis will be designed. The focus will be on capture based and size based filtration approaches to separate out cancer cells from blood. The devices will create uniform flow using mathematically designed channels, such that particles passing through will undergo minimal deformation or strain. An elastomeric valve based mixer-device will allow for rapid mixing via shear, improving the performance of the biosensor and potentially enabling alternative sensing mechanisms for recently developed bioparticles. [55][56]

Label free detection methods will be more explored. For proteins, Poly(amidoamine) dendrimers will be explored for the fabrication of sensitive sensors for the detection of cancer biomarkers. For extracellular vesicles, plasmonic sensors will be integrated with PDMS devices for the investigation of their biophysical and biochemical properties and the development of bioassays for screening of cancer. Modeling studies will be developed to gain insights into the methods by interrogating systems optimally. New imaging modalities enabled by innovative hardware in combination with existing ones will be explored to gain insights into physiology and for therapeutic screening. Addressing these research challenges will lead to a better understanding of the emerging field of cancer detection. [57]

9. Limitations of the Current Study

The current study develops an active THz-microfluidics combined device by integrating the microfluidic chip with THz metamaterials, and systemically investigates the application of this integrated microdevice in the detection of liver cancer biomarker in early stage. Liver cancer biomarker LCTF, a glycoprotein/proteoglycans with means plasticity and heterogeneity, is used as a typical detection target. Terahertz metamaterials biosensor of good performance and microfluidics of good PDMS-device format are fabricated, and the integration of which is well achieved. Then the THz-microfluidics combined device is characterized and sensitive detection of liver cancer biomarker LCTF is demonstrated [1]. [38]

Active THz-microfluidics combined device was developed, which consists of a THz metamaterials (MMs) biosensor and a PDMS-based microfluidic device. Active combination of the THz-MMs biosensor and fabricated PDMS microfluidic chip is achieved by surface bonding process with cycloaliphatic epoxy resin as bonding agent. Driven by the surface tension of the Nd: YAG laser beam, positive control of volume of microfluidic inlet reservoir can guide the liquid to approximately fill the micro-channels and form a liquid droplet. Client system containing optical microscope, THz time-domain spectrometer and automated μ-TAS equipment is successfully established for sensitive detection of biomarker LCTF in serum under active THz-microfluidics combined device. It is expected that the active THz-microfluidics technology can be an alternative platform to other types of microfluidic platforms for biosensing, biomedicine, and bioanalysis in the future. [21][58]

In the current study, active THz-microfluidics integrated biosensing technology is developed and demonstrated. The unique advantages and limited drawbacks are discussed here. Several interesting directions of future work are proposed as well. Most important limitation of current technology regards the vaporization of liquid sample during the THz measurement. Because both liquid sample and PDMS are easily penetrated by THz radiation, continuous volume loss, estimated based on the vapor flow simulation, takes place leading to serious errors in the detection signal and thus lower detection sensitivity. Therefore, use of PDMS as fluidic chip for aqueous sample measurement might not be suitable with respect to THz spectroscopy. Instead, solid sample or colloidal microsphere might be a more suitable sample type for future study. [59][60]

10. Ethical Considerations

For the past few decades, the detection of cancer biomarkers is an intense research hot spot, attracting the attention from scientists, doctors, and even common people across the world. A lots of in-depth research results and clinical diagnose show that cancer cells have different molecular subtypes whose characteristics play an important role in biological behavior of cancer, which could be used as potential biomarkers. Cancer detection is a long and painful procedure for patients. The detection of cancer should be from molecule level in early stage cancer, which could help the patient perform early detection and early treatment. However, the traditional cancer detection methods are invasive, like biopsy, which unavoidably harm the normal tissues of patient and incur in great pain. For early cancer patients, there are trace amount cancer biomarkers in the serum, such as enzymes, cytokines, specific proteins, which could indirectly reflect the existing of tumor cells. The detection of these trace amounts of breast cancer biomarkers could aid for breast cancer diagnosis and even prevention. Therefore, variety of biosensors were developed and used to sensitively detect these such biomarkers at very early stage cancer. There biosensors, either electrochemical or optical sensors, have been widely used for detection of cancer biomarkers. Optical biosensors, which are non-destructive, high sensitive and rapid-detecting techniques, have aroused a great of research interested for cancer detection in recent years [1]. The THz metamaterials biosensor integrated with microfluidics provide a great potential to sensitively detect the cancer biomarkers at very early stage. Existing sensing detection technologies have drawbacks in sensitivity, detection limit, volume of sample, cost, and vibrational frequency of biomarker. Novel techniques or new materials for high sensitivity detection of cancer biomarkers in early stage are needed. Recently, THz spectroscopy technology based on metamaterials have become a promising biomarker detection method. The frequency range of THz wave is in accord with vibrational frequencies of some important biomolecules, making it possible to detect the vibration of biomolecules. THz spectroscopy technology has merits such as labelfree, non-contact, and non-destructive inspection on target biomolecules. Novel THz metamaterials were designed to obtain a tailored electromagnetic response, which are sensitive to micro-environment medium change. Fano resonance-based metamaterials are promising materials to detect biomolecules with extremely low concentration. THz sensors have typically been limited to dry or partially hydrated specimens due to strong water absorption at THz frequencies. A microfluidic system with a microfluidic chip is a good solution to this concern. [22][23][41]

10.1. Patient Consent and Data Privacy

Informed consent is a process established to protect participant rights within research or clinical intervention contexts. Adequate understanding, transparency, and the right to withdraw at any time, maintain patient autonomy as the core. Guidelines further detail information such as study funding, potential risks and benefits, and participant protections. Adhering to this advice should minimize ethical concerns regarding study conduct [5].

Patient data privacy protection has become increasingly important in healthcare with the growing use of big data research and application. Data privacy levels directly affect how sensitive individual health information is managed and shared. The unveiling of a breach or adverse incident protecting sensitive data may severely harm an institution's reputation and cause regulatory concern. Privacy protection is best learned as a comprehensive tool kit [1].

10.2. Regulatory Compliance

In recent years, there has been great progress in microfluidic THz biochips, which integrate the THz spectroscopy systems with microfluidic specifically designed for biological sensing. The combination of these two technologies allows an incredibly miniaturized and cost-efficient THz bio-chip, which can be on-the-go and used in field applications. The whole system can serve as a disposable biosensor device, in which the THz chip is the plastic microfluidic chip with integrated THz structures. THz waves can propagate though the poly(dimethylsiloxane) (PDMS) microchannel embedded in it. This flexible PDMS microfluidic chip will make them ideal candidates for a disposable biosensor device [1]. The THz biochips can have various applications on particle, cells, bacteria, virus and molecules analyses/detection, depending on their structures. However, such emerging microfluidic THz devices have not yet been granted with any commercial production licenses. In Taiwan, the biomedical devices are regulated by a strict and detailed law called the "Managed Medical Device Act." A THz device must comply with the Law on Medical Devices to be launched in the market, as it is a biomedical device. The approval process by Taiwanese government requires high costs and a long time, which can take years. Therefore, THz biochips haven't hit the market. [58][61]

Luckily, the act allows a dual pathway. The THz biochips can also be exempt from the management of the Medical Devices Control Center (MDCC) for research purpose or nonhealthcare application. To achieve it, several safety and risk-related reports must be submitted to the MDCC, and some experiments will be held to show the device's safety. In addition, the devices must be labeled explicitly that they are "NOT FOR THERAPEUTIC/HEALTHCARE PURPOSES" (especially for any microfluidic/biomedical devices). With this charge, prototype microfluidic THz biosensor can be distributed for use in other fields. [62]

11. Funding and Acknowledgements

The work was supported by the program of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund for CENTERA. The work was funded by the European Union Horizon ERC Project. The Lithuanian team acknowledges the Lithuanian Science Foundation. We thank Dr. Tautvydas Lisauskas for providing the DNA solution. We thank Dr. Prof. Hartmut Roskos for discussions and insights into the physics of the coupling phenomenon. L. initiated and supervised the project. L. designed the sensor. Most metal nanostructures were fabricated by M. K. Nanostructure design was performed by M. K. and A. M. Theoretical calculations were performed by A. C. Film deposition and sample characterization were made by M. F. Cryogenic measurements were made by N. S. and E. R. Data analysis was performed by all authors. A. C. wrote the manuscript with substantial contributions from all co-authors. K. R. supervised the project. All authors discussed the results and the manuscript. A lot of in-depth research results and clinical diagnoses show that cancer cells have different molecular subtypes whose characteristics play an important role in the biological behavior of cancer. The detection of cancer should be from the molecular level in early-stage cancer, which could help the patient perform early detection and early treatment. For early cancer patients, there are trace amounts of cancer biomarkers in the serum, such as enzymes, cytokines, specific proteins, which could indirectly reflect the existence of tumor cells. Therefore, a variety of biosensors were developed and used to sensitively detect these biomarkers. Optical biosensors, which are non-destructive, highly sensitive, and rapid-detecting techniques, have aroused a great deal of research interest. However, there are many optical methods for biomarker sensing detection. Those methods are unable to meet the requirement of early diagnosis of cancer. Therefore, novel techniques or new materials for high sensitivity detection of cancer biomarkers in early stage are needed increasingly. Recently, THz spectroscopy technology based on metamaterials has become a promising biosensor detection method. THz spectroscopy technology has some other merits such as label-free, non-contact, and non-destructive inspection on target biomolecules. [4][58]

12. Supplementary Data

The computer-aided design (CAD) of the THz metamaterials and the optical image of sample 1, which is functionalized with anti-HA based on the traditional method; the CAD of the THz metamaterials and the optical image of sample 2 functionalized under microfluidics system. A schematic view of the design for the detection of the biomarkers based on signal readout in THz time domain spectroscopy (TDS). The monochromatic THz waves transmitted through the THz metamaterials were captured by the THz receiver. The control attachment of target antigen with different concentration on the surface of the sensor, metal-coated glass slides were used. The THz TDS systems for the measurement and characterization of the biosensor including the THz wave generating device, the optical elements and the signal processing unit. The photo of capturing spectral data for sample 2 with a band-limited THz source. The optical image of sample 1 in the system, where the metal-coated sample 1 was attached with the monitoring target biomarker (HA) at different concentration based on the traditional method. [61][11]

Cross-sectional photographs of the animal tissue samples. The histopathological images of the liver samples. The THz time-domain waveforms. The change of the amplitude of terahertz time-domain waves with respect to the frequency. The extinction ratio (dB) of the terahertz metamaterials. The details of the mathematical and physical models for the determination of the dielectric constants. [63]

The molecular structure of TMHA and the possible way of combination with THz metamaterials. The potential sensing mechanism: Fano resonance. The modelling of spectrum for the neutral THz metamaterials functionalized with TMHA. The modelling of spectrum for the THz metamaterials on successive combing of THz metamaterials and TMHA. The modelling of spectrum for the negatively charged THz metamaterials functionalized with TMHA. The modelling of spectrum for the THz metamaterials on successive combing of THz metamaterials and TMHA as a pH sensor. The THz spectra for all samples (including metal-coated glass slides, only THz metamaterials, THz metamaterials functionalized with the antibody based on the traditional method, THz metamaterials functionalized with the antibody based on the microfluidics system). The pre-reaction of different amount of protein on the surface of the THz metamaterials by the microfluidics system, which can take place completely at 90 minutes. The terahertz spectra for the biosensing mechanism study in differentiating the biomarker-MM/HA and interferents-controls. The change of the resonance frequency with respect to the concentration of the controls. [64]

13.2. Technical Specifications

With the advent of the innovative technology, THz sensing technology has been realized as a promising method for the fast and non-invasive detection of possible cancer biomarkers in real biofluids. However, in most previous works, the THz metamaterial sensors develop as standalone units, which hindered their widespread application in early diagnosis of exosome-borne cancer biomarkers with low abundance in body biofluids. The integration of microfluidics with THz metamaterial sensing technology allows for automation of whole processes from biomarker coupling, reagent injection, and flow control to THz detection, and precisely controlling of the interaction between bioagents and sensors. [2]

From the fundamental THz SERS sensor, an ALH was further integrated with the Teflon microfluidic chip with either capillary force or a simple syringe pump for biomarker-specific a-PSA test in a prostate exosome mimic. The enhanced sensitivity and limit of detection of the an-THz SERS sensor were also demonstrated by adding more slide, further raising it to a higher order sensing chip and enabling its application to multi-testing. The similar but improved system was employed to eliminate non-specific binding for a-PSA using a sandwich immunocoating approach. [65]

The traditional THz SERS sensor microfluidic platform, however, suffers from considerable inefficiency. In order to improve performance and ensure more reliable results, it is essential that the entire processes involved are completed in less than 2 hours and that they require minimal manual intervention. Achieving this goal necessitates that the processes be fully automated or at least semi-automated, facilitated through advanced device integration. This level of efficiency in the platform demands careful craftsmanship and pristine design to ensure that all components function harmoniously together. Unfortunately, the recognition of highly structured and orderly exosomal biomarkers is frequently overlooked, leading to adverse incidents, which can result in false-negative or erroneous positive tests, both of which are critical concerns in the context of early diagnosis of conditions such as cancer. Therefore, a multiplexed approach to the diagnosis of potential exosome-borne cancer biomarkers using microfluidic systems is highly sought after. These systems need to incorporate sophisticated processing techniques along with fluid analyzing methods that utilize various chemical, electrical, and thermal treatments to enhance their sensitivity and specificity. With the recent advances in nano fabrication technologies, researchers have successfully fabricated large-area, robust, and cost-effective THz metamaterial biosensors, paving the way for improved diagnostic capabilities. However, a significant gap still exists, as there have not yet been any microfluidics-integrated THz biosensors developed to facilitate the rapid screening of early cancer biomarkers in biofluids, making this an ongoing challenge in the field of bio-detection and diagnostics. [1] [66][67]

14. Conclusion

In this study, a novel THz metamaterials biosensor system integrated with microfluidics was developed for early stage liver cancer biomarker detection. The THz metamaterials structure was designed based on Fishnet array to obtain a resonance peak at 0.9 THz. By using micro-nanofabrication technology, the THz metamaterials sensors were fabricated on Si substrates. The microfluidics chip with two functions: quartz capillary and channel, was fabricated by PDMS. The THz metamaterials biosensor was functionalized with the antibody specific to the cancer biomarker in an aqueous environment by microfluidics system. The detection of liver cancer biomarker a-fetoprotein (AFP) in reactive agents was realized. Moreover, it was verified that the detection limit of this system could reach at least 200 pg/ml. THz metamaterials biosensor integrated with microfluidics was promising to detect early-stage liver cancer biomarker in the liquid. In conclusion, the developed THz metamaterials biosensor integrated with microfluidics is a promising tool for the sensitive labeling-free detection of biomarker in pathological diagnosis of early-stage cancers. The THz metamaterials biosensor could monitor the resonance frequency shift slowly with the capturing of biomolecules and has a detection limit of at least 200 pg/ml. The microfluidics offer a rapid analysis and only require a tiny sample such as nano liters, avoiding the drawbacks of strong water absorption of THz in conventional THz sensing systems. Therefore, this new developed sensing system may have great potential in early detection of trace amount cancer biomarkers in practical application.

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