SURFACE ACOUSTIC WAVE (SAW) DESIGN AND APPLICATIONS IN MICROFLUIDICS

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INTERDISCIPLINARY GRADUATE SCHOOL

NANYANG ENVIRONMENT & WATER RESEARCH INSTITUTE (NEWRI)

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Abstract

Lab-on-a-chip (LOC) is the current trend towards developing point-of-care devices. LOC finds its application in fields of medicine, environmental monitoring and towards a multitude of industrial applications. It involves integrating all the functions that were done in a laboratory, from input samples to results delivery in an easy-to-handle device. This was realised due to the advent of microfluidics and the microelectronics technology. Physically, an LOC consists of four main parts: microfluidics, actuators, sensors and readout circuits. Many research groups along with startup companies have developed technologies to realise fluid actuation/control and signal detection with impressive capabilities. However, there is a dearth of LOC's available in the market. They are still confined within the spaces of the laboratory and regarded as a "chip-on-lab" functionality, justifiably due to the lack of an integrated platform that performs the different assay procedures in a seamless and automated fashion. This work is in pursuit of developing an integrated platform to realise an LOC utilising surface acoustic wave (SAW) devices.

SAW are nanometer amplitude vibrations that are generated on a piezoelectric substrate. During the last decade, SAW has been intensively used and researched for microfluidic applications majorly as an actuator. SAW capabilities as a sensor for immunoassay was also explored. This unique feature of SAW to act both as an actuator and sensor makes it easier for integration. In this thesis, we first study the use of

SAW as an actuator of the fluids by establishing a novel mechanism for characterising the SAW energy transmission in fluidic channels, which is essential for all the SAW microfluidics design, using a mixing structure. We developed analytical models in this work that could be used to optimise the power transmission coefficient and hence increase actuation efficiency.

Besides focusing on the application of SAW as an actuator, its usage as a sensor was primarily relying on the immunoassay technique which required complicated surface preparation steps. In the subsequent work, we proposed and demonstrated a new sensing methodology utilising photoacoustic induced surface acoustic wave (SAW-PA) for simultaneous optical and mechanical property characterization of analytes (including cells, nanoparticle and dyes). A nanosecond pulsed laser excitation on a sample triggers a longitudinal acoustic wave in the fluid which is mode-converted into a Rayleigh SAW on the piezoelectric substrate and detected using the metal electrodes (interdigital transducer, IDT). We further developed a numerical model to study the wave conversion process (longitudinal acoustic waves to SAW) and demonstrate that the PA generated in the microfluidic channel acts as a mechanical resonator dependent on the dimensions of the microfluidic channel. We experimentally verified that a SAW device matched to the channel resonant frequency could improve the sensitivity.

Finally, we propose a platform combining the SAW actuators and SAW-PA sensor, which is closer to realising the initial objective of an integrated platform for LOC. In this work, we have developed an integrated microfluidic system that combined high-efficiency (> 95%) tilted-angle standing surface acoustic wave (taSSAW) based particle separation, particle concentration inside an open microfluidic chamber and sensing, on a single LiNbO_3 substrate. The platform demonstrated real-time quantitative detection of $10\text{ }\mu\text{m}$ polystyrene beads down to 7 particles in $10\text{ }\mu\text{l}$ of the sample volume in 15 minutes.

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List of Abbreviations

IDT	Interdigital transducer
SAW	Surface acoustic wave
PA	Photoacoustics
LOC	Lab on a chip
taSSAW	Tilted-angle standing surface acoustic wave
LiNbO ₃	Lithium niobate
PDMS	Polydimethylsiloxane
FEM	Finite element method
GNP	Gold nanoparticle
GNR	Gold nanorod

Chapter 1

Introduction

A lab-on-a-chip (LOC) is a class of device which integrates the multitude of laboratory tasks into a single-chip, whose size varies from a few square millimetres to square centimetres. The whole purpose of an LOC is to perform an automated and a high throughput analysis of the samples for in situ/point-of-care diagnostics. The history of LOC is essentially linked to microfluidics, which is associated to the microtechnology used in semiconductors. The fabrication of MEMS devices in the early 60's subsequent to the micro technologies fostered the development of the first LOC- a gas chromatography, in 1979 by S.C. Terry at the Stanford University [1]. Subsequently, in the 80's there was a spur in the research towards LOC with the development of micropumps, sensors and also towards the production of polymer chips with ease in fabrication compared to the silicon technologies. In the 90's research exploration on microfluidics and miniaturisation of the biochemical process such as PCR by integrating various steps from sample collection to analysis, called generally as the Micro total analysis system (μ Tas) [2]. Further, the improvement in individual components and its application to non-analysis lab processes inspired the term *Lab-on-a-chip*. Today for certain applications including chemical analysis [3], medical diagnostics [4] and en-

vironmental monitoring [5], the LOC receives growing interests. The current trends in the research and product developments highlight that LOC will transform the diagnostics in the future. The challenge would be to integrate the maximum amount of functionalities to reduce cost, size and improve the speed of operation. Presently the technologies are not unified and hence impose the restriction on the ease of integration, research needs to address this point for their wide applicability [6, 7]. As we explore in this thesis, certain technologies have the capability to perform a multitude of functionalities and therefore possess the potential to realise an LOC.

1.1 Motivation for this thesis

Microfluidics is the core component of any LOC system. It is the microscale conduit in an LOC. Whitesides [8] defines microfluidics as the "science and technology of systems that process or manipulate small (10^{-9} to 10^{-18} litres) amount of fluids, using channels with dimensions of tens to hundreds of micrometres". Various features including reduced reagent and sample consumption, system miniaturisation and low cost become attractive in adhering to the requirements of an LOC. However, microfluidics possesses certain challenges, which limits a self-contained system, and requires integration with various active components to actuate and manipulate fluids. Some of the limitations include [9]:

- The increased surface area-volume ratio due to reduced dimensions causes the surface and viscous forces to dominate and reduce fluid flow.
- The Reynolds number in a microfluidic system where ρ and μ are the density and the fluid viscosity, while L and v denotes the characteristic length and velocity, respectively. The low Re creates a laminar flow in the microfluidic channels and presents challenges in fluid mixing.
- Further, the application of LOC in bioanalysis requires the capability for addi-

tional microfluidic manipulations including sample preparation steps such pre-concentration and separation, with the ultimate goal of analyte detection.

The restrictions mentioned above emphasise the point that additional fluid control (actuator) units and sensors with microfluidics are essential to realise an LOC. Depending on the concerned applications, the components may vary. However, in general, a typical LOC would include sample handling, sample and reagent mixing, separation and finally detection [6, 10]. In this thesis, we will discuss about using surface acoustic wave (SAW), which are nanomechanical waves propagating in a piezoelectric substrate, for microfluidic actuation and sensing and finally integrate them to actualize an LOC. Despite the current advancement in SAW microfluidics for actuation (to be discussed in detailed in Sec 2.3.1), their downstream detection still relies on using microscopes and cell counters like haemocytometers which are performed off-chip. The off-chip transferring involves the risk of sample loss, which is quite detrimental, especially for detection of rare specimens. However, a fully integrated LOC incorporating the SAW fluidic actuation with downstream detection is not realised.

1.2 Objectives

The broad scope of the thesis is to advance the state of art in SAW based LOC's by integrating SAW based- sensors and actuators with microfluidics on a single piezoelectric substrate, which has not been performed yet. Towards this goal, the thesis work focusses on the following:

- (i) SAW actuator- There is a growing interest to couple SAW into disposable superstrates, which involves acoustic wave propagation through the various layer. Due to the multilayer propagation, the energy for fluid actuation is reduced. Coupling of SAW to superstrates was reported previously (details in Chapter 2) which did not address the effect of frequency and coupling layer thickness on the acoustic

energy transmission efficiency. In this work, we perform a detailed characterization by proposing new analytical models to optimise the power transmission coefficient and experimentally demonstrated using the application of the fluid mixing phenomenon.

- (ii) **SAW sensor**- Acoustic wave based sensors for evaluating mechanical parameters of fluid samples such as density, viscosity, etc. exists. However, devices for the simultaneous optical and mechanical characterization of fluids remains unexplored. Herein we propose a novel sensor combining photoacoustics (PA) and SAW to extract optical and mechanical characterization of fluids in micro volumes. The microfluidic channel acts as an acoustic resonator and numerical simulation using the finite element method (FEM) was used to explain the working principle.
- (iii) **SAW enabled LOC**- Towards effectuating an integrated platform with sensors and actuators on the same chip, we designed and fabricated an integrated device which incorporates a SAW based separation technique and downstream detection on the same chip, moving closer to the expected goal of a true-LOC (as shown in chapter 5).

A common theme that runs through this thesis involves the study and optimisation of acoustic wave propagation: to impart energy into the fluid for actuation (discussed in chapter 3) and to receive the acoustic wave to sense the analyte properties (addressed in Chapter 4). From the SAW actuator (relying on acoustic streaming) and SAW sensor (photoacoustic induced SAW) studies in Chapter 3 and 4 respectively, we established the principles to finally incorporate a novel LOC based on SAW in Chapter 5.

1.3 Thesis outline

Chapter 2: [Literature Review](#).

In this chapter, we begin with a comprehensive introduction into the field of LOC by discussing the various processes involved in its realisation. We also review the various actuation and detection methods available in the microfluidic devices and stress on the significance of the acoustic based techniques. We then focus on an emerging optoacoustic sensing methodology- photoacoustics (PA) and its challenges in incorporating into microfluidics. Finally, an overview of the surface acoustic wave (SAW) based microfluidic actuation techniques is provided.

Chapter 3: [Acoustofluidic mixing: The study of an acoustically coupled multi-layered microfluidic platform on SAW substrate](#).

In Chapter 3, we report a comprehensive study of SAW induced mixing on a reusable superstrate using extensive experiments and analysis. The study reveals a novel mechanism for characterising the SAW energy transmission in fluidic channels, using a mixing structure. The main contribution of the work comes from the discussion of the effect of thickness of coupling layer and frequency on the mixing efficiency in a Y-shaped microchannel and development of analytical models.

Chapter 4: [Photoacoustic induced surface acoustic wave \(SAW-PA\) sensor for sensing analytes in a microfluidic channel](#)

In this section, we introduce a novel sensor utilising the SAW IDTs to detect an acoustic signal generated by the optoacoustic resonance inside a microfluidic channel. We first demonstrate through experiments, the use of this SAW-PA platform for investigating the optical and acoustical properties of standard dye solutions and gold nanoparticles. We present analytical models to explain the acoustic mode conversion from the photoacoustic induced longitudinal waves to SAW. In this chapter, we explore the

physics behind the SAW-PA sensing by performing a detailed numerical simulation using finite-element method. We show the existence of acoustic resonance inside the microfluidic channel and efficient conversion of a longitudinal wave generated inside the channel into SAW at eigen modes existing parallel to the piezoelectric substrate. We validate the numerical simulation by performing experiments to determine the sensitivity improvement of the SAW-PA sensor by choosing SAW IDTs with different centre frequencies.

Chapter 5: [Integrated microfluidic system using SAW for real-time separation and detection of particles on a single substrate](#)

This chapter binds the concepts of the SAW actuation and SAW-PA sensing to implement an integrated microfluidic platform. We combined the tilted angle standing surface acoustic wave based (taSSAW) separation, particle concentration and sensing on a single LiNbO₃ substrate. The platform was used to demonstrate real-time quantitative detection of polystyrene beads. We first show high efficiency (> 95 %) separation of polystyrene beads (3 μm and 10 μm) using an SSAW. The separated particles were concentrated on an open PDMS cavity using a single SAW IDT. The microfluidic channel was designed to meet the conditions for the formation of a standing wave inside the fluid, to concentrate the particles. Experiments were conducted to prove the validity of the concept. The aggregated particles were further sensed using the SAW-PA sensor. The choice of optimum frequency was the design challenge, which was established using numerical modelling and experiments. Finally using experiments we demonstrate the performance of the sensor.

Chapter 6: [Conclusions and future work](#)

In the final part of the thesis, a summary of the work conducted will be presented with the list of key contributions. The chapter and thesis will conclude with potential future works.

Chapter 2

Literature Review

In this chapter, a comprehensive introduction to the field of lab-on-a-chip (LOC) devices is presented by an overview of different steps involved in its realisation. Following, an overview of the two important functionalities in realising an LOC: actuation and sensing are discussed. A distinctive preference is given to acoustic based actuation methods by highlighting its advantages over other active techniques, which is the method to be considered in this thesis. A brief overview of the application of the different microfluidic actuation techniques by surface acoustic wave (SAW) is reviewed. A consideration of the various detection methods in microfluidics is summarised, with a focus on an emerging optoacoustic sensing methodology- photoacoustics (PA) and its challenges in incorporating into microfluidics.

2.1 Defining Lab-on-a-chip

A lab-on-a-chip (LOC) is a class of device which integrates the multitude of laboratory tasks into a single-chip, whose size varies from a few square millimetres to square centimetres. The whole purpose of an LOC is to perform an automated and a

high throughput analysis of the samples for in situ/point-of-care diagnostics.

2.2 Processes involved in an LOC

Fluid control (actuator) units and sensors with microfluidics are essential to realise an LOC. Depending on the concerned applications, the components may vary. However, in general, a typical LOC would include sample handling, sample and reagent mixing, separation and finally detection [6, 10]. In the following section, we review these steps.

2.2.1 Sample handling

2.2.1.1 Sample injector

Sample injection is the first step involved in an LOC, where the sample to be analysed is introduced from the external world into the chip using either syringe pumps or automated pipetting systems. Apart from an external world, the fluid has to be precisely transferred between different components of the LOC, such as to move fluids to the sensing sites where the target immobilise and bind to the surface bound sites. A class of devices called micropumps were developed by researchers initially with the goal of delivering therapeutic agents into a human body, such as an insulin delivery system [11]. However, with miniaturisation of the chemical and biological analysis, different types of micropumps have been realised for LOC devices. They are classified by fluid flow and pressure generated into either a displacement or dynamic micropumps [12]. A displacement micropump works by applying the pressure created by a moving boundary to drive the fluid. Some examples of the displacement micropumps includes piezoelectric [13], pneumatic [14], rotary [15], electromagnetic [16], electrostatic [17] and recently expanding field of electrowetting [18]. The use of complicated structures and requirement of large areas for the controlling the elastic elements in

a displacement micropump renders them unsuitable for miniaturisation [19]. On the other hand, constant energy is imparted to the fluid to increase its momentum in a dynamic pump. Dynamic micropumps are classified by the driving field involved into acoustics (Flexural waves, the bubble generated streaming and SAW), electrokinetics (including electro-osmosis, electrophoresis, dielectrophoresis), optics (tweezers) and magnetohydrodynamics [20].

2.2.1.2 Sample pretreatment

Depending on the application, this step becomes essential to isolate or treat the required analyte from the complex sample. This phase becomes mandatory in certain molecular diagnostics involving DNA/RNA, as the nucleic acid is extracted from the whole cell. The extraction process improves the efficiency of the polymerase chain reaction (PCR). The challenge involved in integrating the sample preparation step in microfluidics apart from handling the different formats of raw samples is the large variation in the preparatory step for various type of analysis. For example, extraction of DNA samples from whole blood requires a dilution, lysis and pre-concentration [21], separating plasma from red blood cells requires only pre-concentration [22] step while separating white blood cells from whole blood needs a filtration stage [23]. Hence, it becomes difficult to generate a general framework for the separation process. Despite the lack of a universal frame for the sample preparation step, sample preparation is done on-chip which reduces the time (in few mins) [21] and manual intervention compared to the conventional culture methods or molecular based methods which consume few hours to days for the process [24]. The process of separation is also performed in subsequent steps, after mixing of reagent and sample and hence will be discussed in detail in this chapter.

2.2.1.3 Microfluidic mixing

Micromixer is a major component in the LOC system. The necessity comes from the slow reaction time caused by the low Reynolds number flow or the laminar flow. The only source of mixing is via diffusion, which might take a long time not acceptable for many applications and most importantly for an LOC device. Hence, there have to be some changes in the channel design or inclusion of an external agitation to realise fast mixing of liquids. In general, micromixers are classified into either passive or active mixers [25,26].

Passive Micromixers do not rely on external energy sources to cause mixing of fluid. Instead, diffusion or chaotic advection gives rise to mixing. The principle behind the fluid mixing involves modification in the channel geometry (shape) to split, stretch, fold and break the flow [27]. Since no more external actuation sources are required, the passive structures are simple, robust, stable and very easy for integration. The passive micromixers suffer from the disadvantage of using relatively longer mixing periods to achieve the same mixing efficiency as active mixers [28].

Active micromixers use external perturbations to actuate the fluids. The active mixers are classified based on the energy source that drives the mixing. As shown in Table 2.1 we summarise the various active mixing schemes for microfluidics.

Various mixers have been reported so far, however, mainly studied case by case. As many factors influence the mixing performance including the dimension, fluid diffusivity, inertia (Peclet number) and mixing quantification methods. Only when these mixers are compared under same or at least comparable conditions, we can make accurate conclusions. However, in general, the active mixing could be more efficient as it involves moving parts. However, the active mixers requires actuators and corresponding controllers, which could make the system more complicated and increase fabrication cost. In comparison, for passive mixing, the fluids must flow through

a certain length of the channel to get good mixing, and this may cause wastage of the precious liquid samples. The absence of moving parts and external controllers makes it simpler, cheaper and more reliable. From the operation point of view, passive mixing may be more suitable for continuous inline mixing; while active mixing can be used for both inline mixing and batch mixing (or mixing in static chambers).

Mechanism	Principle	Mixing time(ms)	Mixing-index	Reference
Acoustic/ Ultrasonic	Using acoustics to drive trapped microbubbles causing cavitation microstreaming	120	0.025	[29]
	Surface acoustic wave induced acoustic streaming	600	0.9	[30]
Electro hydrodynamic	Motion of charge carriers in the presence of an electrical field	100	0.2-0.5	[31–33]
Dielectrophoretic	Force is exerted on a dielectric particle subjected to a non-uniform electric field, gives rise to a chaotic trajectory and accelerates mixing		0.85	[34, 35]
Magnetohydrodynamic	A mutually perpendicular electric field and magnetic fields acting on a fluid generate Lorentz force to drive mixing.	1100	0.977	[36]
Optics (laser induced mixing)	Laser-induced formation of cavitation bubbles, which expands and collapse to generate mixing.	50	Capable of starting an enzyme-catalyzed reaction	[37]

Table 2.1: Various types of active micromixers

Among the various active mixing methods, acoustic-based mixers have reaped high importance due to their non-invasive nature, simple and inexpensive fabrication process and instrumentations [29, 38–40].

2.2.2 Separation

This stage becomes significant in two classes of applications: one for purifying a sample from the other contaminants or unwanted objects, and the other involving applications where the segregated particle needs to be analysed. This part finds widespread applications in the field of biological analysis, chemical synthesis and mineral processing [41–44, 44]. The most widely discussed application involves the separation of rare circulating tumour cells (CTCs) from blood for diagnostics [45, 46]. In the field of therapeutics, there is a significant interest towards using the separation methods to purify the blood (separating bacteria, CTCs, etc.) before transfusing to a donor [47]. In the realm of cell biology, it becomes necessary to separate cells of a particular type and study their behaviour [48]. Similar to the micromixing methods mentioned in the previous section, the separation technique is classified into fundamentally two approaches: active and passive. Additionally, there are a few variants, which combine the passive and active methods to achieve improved performance. Various review papers have discussed the different types of passive and active separation devices. The review paper by Wyatt et al. [49] and Lee et al. [50] discusses in detail various cell separation techniques. The thesis focuses on using a class of separation devices, which falls into the active separation category. The review papers from Gossett et al. [51], Sajeesh et al. [41] discusses elaborately on the different types of the passive separation device. A few of them includes hydrodynamic filtration based on size and shape, microscale filters depending on the size and deformability, another sorting method based on the dimension of the particle relative to the microchannel dimension involves the phenomenon involving the interplay of inertial and Dean forces. The passive device, in general, have a low throughput (less than 10 $\mu\text{l}/\text{min}$), lower separation resolution ($>5\text{ }\mu\text{m}$ inertial) and lower purity or efficiency of segregation (90 %). On the contrary, an active method of separation ensures a high separation efficiency (100 %) [52], higher resolution of isolation ($<0.4\text{ }\mu\text{m}$) [53] and high throughput

(400 $\mu\text{l}/\text{min}$) [54]. Table 2.2 shows a comparison of different active separation techniques for blood cells.

Among the different separation methods relying on their physical properties (label-free), the acoustic methods provide unique features. The acoustic separation of cells has little impact on its viability. It is an entirely label-free approach with no modifications needed on the medium in which the cells are cultured and separated. The Table 2.2 shows that acoustic-based method is the only active separation process that can differentiate cells based on size, density and compressibility, it becomes necessary to separate leukaemia cells from normal leukocytes with similar size but varying compressibility. Furthermore, the SAW devices used for separation are very compact (few square. centimetres) and uses lower power ($<1\text{W}$), which makes it favourable for an LOC.

2.2.3 Detection

In the following section, we review the various detection techniques utilised in the microfluidic devices. The subsequent discussion builds on the review papers from [61–65]. The detection techniques mainly include optical, electrochemical, magnetic, mass spectrometry and biosensors. At the end of this section, a new sensing modality namely photoacoustics (PA), extensively used in imaging applications is introduced with capabilities for multi-parameter detection- optical and mechanical properties of the analyte. Table 2.3 lists the various detection approaches with an emphasis on the sensing in a microfluidic channel.

Method	Principle	Separation criteria	Sample	Separation resolution (μm)	Throughput ($\mu\text{L}/\text{min}$)	Purity/efficiency	Reference
Acoustic	Acoustic radiation force due to a travelling or standing acoustic wave using SAW or ultrasound transducers	Size, density and compressibility	Cell (BAW)	>1	80	100%	[55]
			MCF-7 from WBC	0.2	2	80 %	[52]
			Polystyrene particles	0.2-0.4	4-6	80-90%	[53, 56]
Magnetic	Magnetophoresis enabled by permanent magnets or coils exerting force on cells labelled with magnetic particles	Size	CTCs from mouse blood		20	90%	[57]
	Magnetic force on particles due to difference in magnetic susceptibility to the bulk and magnetic field gradient	Using native magnetic susceptibility	isolating rare nucleated red blood cells from peripheral blood	Not based on size but separate paramagnetic from diamagnetic	216-450	95%	[54]
Dielectrophoresis (DEP)	Force exerting on a polarizable particle due to a non-uniform electric field. Particles need not be intrinsically charged	Polarizability, size	Liposomes	0.5 μm (particle size > 1 μm , due to lack of effective focussing)	6	90%	[58]
Optical	Formation of an optical lattice or arrays through multiple beam interference	Refractive index, size	Particles	<2	1500 particles/min	96%	[59]
	CCD-enabled detection followed by cell transportation by optical tweezers	Size and fluorescence	yeast cells and human embryonic stem cells	-	10 nL/min	90%	[60]

Table 2.2: Comparison of active separation techniques found in existing lab-on-a-chip systems

Method	Principle	Sensitivity	Advantage	Disadvantage	Ref
Fluorescence	The phenomenon of light emission by a molecule (fluorophore) after initial electronic excitation due to light absorption. The emitted light has a wavelength higher than the excitation wavelength	Detected 10-100 nM of Rhodamine 6G Detected 10 nM of Alexa Fluor 633 dye	Low detection limit High selectivity	Requires label (sample tagging)-Time consuming as it requires fluid handling, mixing and washing steps Fluorescent dyes are costly, limited shelf life and affected by pH which varies between samples Isolate leakage excitation light from the detector, calls for additional polarisation schemes to block	[66–69]
Chemiluminescence	A chemical reaction generates light	Detect hydrogen peroxide with LOD = 5 μ M	Excitation light source and emission filters are not required- Reduced background interference	Require highly sensitive detectors Require chemi-luminescent labels for detection	[70]
UV-Vis absorbance	Relies on the optical absorption or attenuation at a particular optical wavelength	Alexa Fluor 633 dye detected at 1 μ M BSA detected at 15 nM Coumarin dye with an LOD = 4.3 μ M	Label-free approach Instrumentation is simple	Decreased sensitivity due to decreased optical path length and hence reduced absorbance (by Beer-lambert law)	[68, 71, 72]
Electrochemical detection	Cyclic voltammetry is an amperometric technique. The voltage is varied, and the current induced is detected. The limiting current is proportional to analyte concentration by the Nernst equation	RNA detection in saliva = 0.4 fM	Wide dynamic range Low cost	Electrodes may get fouled due to by-products of the reactions Lower response time - time for the analyte to diffuse to the electrode surface	[73]
Continued on next page					

Method	Principle	Sensitivity	Advantage	Disadvantage	Ref
Mass spectrometry	Electrospray ionisation mass spectrometry (ESI-MS)- detects the molecular weight of macromolecules	Detection of cytochrome c (proteins) at 10 $\mu\text{g/mL}$	Detect samples that have a larger mass High accuracy in mass measurement	Samples must be purified before analysis and hence cannot be applied to analyse mixtures Contamination between tests High voltage requirement for ionisation (in KVolts)	[74]
Biosensor	It consists of a transducer that converts a biochemical signal into a readable electrical format A molecular recognition element on the transducer surface binds to the analyte to generate a sensor response	SPR attains superior sensitivity- detect ultralow-molecular-weight (244 Da) biomolecules at picomolar concentrations	high sensitivity Selectivity Label-free	Low throughput (flow rate is low) Optical alignment difficulties in SPR sensing Small penetration depth (evanescent) Large sensing area required	[75, 76]
		Surface acoustic wave sensors has a sensitivity of $6.7 \times 10^{-16} \text{ g/cm}^2/\text{Hz}$, which is sensitive to detect a single DNA base Capable to detect a DNA sequence at 1.8 femtomolar concentration		Difficulties to detect small molecular weight analytes Non-specific interactions Time-consuming and complex Surface treatment required	[77, 78]

Table 2.3: Comparison of the detection techniques found in existing lab-on-a-chip systems

Though biosensors (including SPR and SAW) possess high sensitivity, they lack the throughput for detecting rare analytes (e.g., circulating tumour cells such 1-100 per 1 mL of blood) and require tedious pre-treatments before detection. In this context, volumetric or bulk detection possess advantages. The conventional opti-

cal sensors (in Table 2.3), photonic crystal liquid sensors [79] and optomechanofluidics [80] operate directly on the bulk fluid. Recently there is much emphasis on a technique utilising laser-generated acoustic wave in both sensing and imaging applications. Photoacoustic (PA) is the physics of generating an acoustic wave due to absorption of a pulsed laser irradiation on a sample [81]. In the arena of biomedical imaging, the PA technique achieves much significance as it combines the advantages of strong optical contrast and high-resolution acoustic detection (reduced scattering compared to the light). The PA is also used for trace gas detection (in ppb levels) [82, 83]. The capability of spectroscopy with sensitive detection is highly beneficial for multi-analyte detection. PA has been applied recently for single cell detection. Also, the PA flow cytometry was used for malaria diagnosis with detection sensitivity thousand times better than existing tests [84]. Since the PA fundamentally works on the optical absorption principle, as mentioned in Table 2.3, it is a label-free method. In comparison to other optical detection techniques, PA is ideally, a background-free technique, since the signal is generated only by the absorbing analyte. On the other hand, conventional absorption detection is an indirect method, as it uses the reflected or transmitted signals. The transmitted signal is severely affected by low absorption coefficients, opaque samples and scatterers inside the sample. PA overcomes these limitations to form an optimum detector [85]. Similar to the fluorescence detection, the PA signal depends on the incident power. The PA signal is linearly proportional to the laser fluence (energy per unit area) and absorption coefficient of the sample [81, 86, 87]. Fig 2.1 shows the principle of PA signal generation, where the sample analyte absorbs laser excitation (nanosecond pulse width). The electromagnetic wave absorption generates heating in the sample. A ultrashort pulse duration prevents heat loss during the laser irradiation period. Thermal and stress confinement are satisfied by the nanosecond laser pulse. The thermal expansion converts into a longitudinal acoustic wave, detected using an ultrasound transducer.

Figure 2.1: Photoacoustic principle- generation of Photoacoustic (PA) signal due to a pulsed laser excitation [88].

2.3 Surface acoustic wave microfluidics

Surface acoustic wave (SAW) are nanomechanical waves ($\sim \text{nm}$ to $\text{\AA}$) propagating on a piezoelectric material [89–91]. The elastic wave that propagates on the surface of the piezoelectric material (like lithium niobate, quartz, etc.) is also known as Rayleigh waves. Unlike in bulk, at the surface, the longitudinal and transverse modes are coupled. Due to different displacements of the modes, the Rayleigh waves are elliptically polarised. Another important property of the surface waves is the evanescent behaviour of the waves and hence the particle displacements decay inside the substrate within one-two wavelengths. See Fig 2.2(a) for the acoustic displacement profile along the piezoelectric substrate. SAW is generated in a piezoelectric material through various methods including interdigital transducers (IDT), non-contact excitation using pulsed laser [92] and wedge-coupled ultrasound transducer [93]. Fig 2.2(b) shows the most commonly used method of SAW excitation using an inter-digital trans-

ducer (IDT) patterned the piezoelectric substrate. The IDT also acts as a narrow band receiver of the surface acoustic wave. The design specification of a SAW device involves finding the interdigital electrode (IDT) width for a certain frequency. As shown in Fig 2.2(c), The inter-finger spacing d is the design parameter for a specific frequency

$$d = v/4f \quad (\text{Eq. 2.1})$$

Here, v is the velocity of the surface acoustic wave and f is the frequency. However, the above design requires the exact value of the SAW velocity, which might change due to various second order effects such as bulk acoustic wave radiation, inter-finger reflection, etc. One of the standard design techniques to reduce the unwanted finger reflections is to make the use of a split-electrode ($\lambda/8$) design. Here, λ refers to the wavelength of the SAW. Hence, the IDT width d will be $\lambda/8$ instead of $\lambda/4$ [94]. SAW has been widely used for communication devices for the design of RF and IF filters for mobile phones, high-Q resonators and receivers for precision filtering. The ability to perform complex signal processing within a small footprint and mass-volume fabrication capabilities are prime factors contributing to the widespread use of SAW devices in communication applications [95,96]. Over the past decade, the surface acoustic wave has been receiving significant attention in the field of microfluidics [97–99]. Compared to conventional ultrasound or bulk acoustic waves (BAW), the concentration of SAW energy within the surface of the piezoelectric substrate causes higher efficient transfer of acoustic energy into the fluid at low power (<1 W). Also, the relatively (BAW and ultrasound) high frequency (MHz order) SAW is capable of generating high accelerations (10^8 m/s²) to drive a multitude of microfluidic applications. A difference in the acoustic wave velocity in the piezoelectric substrate (4000 m/s² for 128°YX lithium niobate, LiNbO₃) and fluid (1500 m/s²) loaded on the substrate causes wave to refract into the liquid. The longitudinal wave inside the fluid causes flow via a phenomenon known as acoustic streaming [99]. Yeo et al. [98] explain the existence of different forms of acoustic streaming. Based on the various length scale of the system the

Figure 2.2: Schematic of a surface acoustic wave device. (a) Displacement profile for a Rayleigh SAW propagation (elliptical motion on the surface). Displacement decays within two wavelengths. (b) A SAW delay line (two IDTs) with the wave propagation along the piezoelectric substrate (lithium niobate, LiNbO_3). (c) General configuration of the IDT to excite SAW. d denotes the electrode pitch.

acoustic streaming can be classified as Schilting streaming, Rayleigh streaming and Eckart streaming. Fig 2.3 shows a SAW wave generated from an IDT interacting with a droplet on the substrate. Fig 2.3(b) shows the wave refracting into the droplet following the Snells law of refraction. A Rayleigh wave on interacting with a droplet leaks energy into the fluid and hence a leaky SAW is generated on the substrate. For particle manipulation using SAW, either a standing SAW (SSAW) or travelling SAW (TSAW) are employed [53]. The use of SSAW to actuate particles is termed as acoustophoresis. In SSAW, two oppositely directed travelling SAW (from two IDTs) interfere to create a standing acoustic wave. The SSAW is manipulated to either trap [100] and move [101, 102] cells/particles at either the pressure nodes/antinodes of the SSAW. Four forces are acting on a particle: acoustic radiation force, viscous force (drag force), buoyant force and gravity. The last two of the forces are typically neglected, as the two

Figure 2.3: (a) Schematic of the SAW (not to scale) interacting with a droplet. (b) A cross-sectional view of a SAW refracting into the fluid and inducing acoustic streaming. The direction of the propagation of longitudinal wave inside the fluid is determined by the ratio of the velocity of sound in the piezoelectric substrate and fluid by the Snells law.

forces are equal in magnitude and opposite in direction. The acoustic radiation force (F_r) causes the particles to move to either the nodes/antinodes [103].

$$f_r = 4\pi\phi(\beta', \rho')ka^3E_s\sin(2kx) \quad (\text{Eq. 2.2})$$

where ϕ is the acoustic contrast factor depending on the compressibility (β') and density (ρ') of the fluid and particle, k the wave-number, a the radius of the particle, E_s the acoustic energy density of the standing wave and x is the distance from a pressure node along the acoustic wave propagation direction. The particle movement under the

acoustic field creates a viscous drag force (F_v) to oppose the motion, given by:

$$f_v = -6\pi\eta av_p \quad (\text{Eq. 2.3})$$

where η, v_p denotes the medium viscosity and particle velocity respectively. The force F_v , together with the acoustic force F_r determines how fast the particles travel to the nodes/antinodes. The acoustophoretic contrast factor (ϕ) in the radiation force equation determines if the particles aggregate at nodes ($\phi > 0$) or the antinodes ($\phi < 0$).

$$\phi(\beta', \rho') = \frac{1}{3} \left[\frac{5\rho' - 2}{2\rho' + 1} - \beta' \right] \quad (\text{Eq. 2.4})$$

$\rho' = \frac{\rho_p}{\rho_0}$ and $\beta' = \frac{\beta_p}{\beta_0}$ are the relative density and compressibility respectively for the particle (subscript, p) and fluid (subscript, 0) respectively. The acoustic radiation force generated by a plane progressive (TSAW) wave on a particle is given by [104]:

$$f_r = 4\pi\phi(\beta', \rho')k^4 a^6 E_p \quad (\text{Eq. 2.5})$$

Rearranging Eq. 2.2 and Eq. 2.5 as explained by Groschl [104], the $f_r \propto (ka)^3$ and $f_r \propto (ka)^6$ for the SSAW and TSAW respectively. The Eq. 2.2 and Eq. 2.5 are derived with the condition that (particle size < wavelength). Hence, it can be concluded that an SSAW generates larger acoustic radiation force than a TSAW.

2.3.1 SAW fluid actuation and manipulation

The SAW generated acoustic streaming, and acoustic radiation force is utilised for various applications in microfluidics to manipulate both fluid and particles. The TSAW is applied in both open and closed microfluidic channel to achieve fluid mixing, pumping (translation), jetting, particle concentration, and controlling nanoparticles. The standing wave formed by SSAW is used in applications including focussing

particles, separation based on acoustic properties (density, size and compressibility), patterning particles in a stagnant fluid using 2D and 3D tweezers and to align nano-materials. The review paper by Ding et al. [97] and Friend et al. [99] provides an exhaustive review of the various applications realised using SAW microfluidics. SAW techniques are suitable for such applications mainly because of the unique features of being compact, inexpensive [105], compatible with bio-relevant samples [101, 106], energy efficient (energy is concentrated only on the surface) and versatile in handling different fluids regardless of their electrical, magnetic or optical properties. Moreover, SAW provides contact-free manipulation of liquids. SAW is also coupled to disposable superstrate avoids contamination of the substrates and makes it reusable for multiple analysis.

	Electrohydrodynamic	Dielectrophoretic	Magnetohydrodynamic	Acoustic(SAW)
Working fluid	Dielectric fluids	Wide range depends on ionic density and pH	Only on electrolyte solution	Any fluids
Approximate size(mm ³)	10-250	100-1000	Depends on required magnetic field	5-10
Operating voltage (V)	40-600	40-1500	Low voltage (~1V) but also require to generate magnetic field	30-40
Reusable	No	No	No	Yes
Microfluidic applications	Continuous	Continuous	Continuous	Both droplet and continuous microfluidics are supported
Effects on fluid	Joule heating	Joule heating	Joule heating	Pulsed excitation-reduces fluid heating

Table 2.4: Advantages of SAW microfluidics

Table 2.4 shows the comparison of various active microfluidic actuation techniques. Hence we can conclude that the acoustic wave technology is a good solution for a lab-on-chip platform (smaller size, lower power, absence of physical contact with the liquid and ability to work with most of the fluids).

Chapter 3

Acoustofluidic mixing: The study of an acoustically coupled multi-layered microfluidic platform on SAW substrate

As described in Chapter 1, one of the advantages about SAW microfluidics is its possibility to be reusable. However, existing literature shows that the coupling to superstrates involves losses with roughly 50% transmission efficiency [107]. Hence, it becomes essential to optimise the device design to reduce the dissipative losses through the coupling layer and superstrates. Optimisation of the reusable microfluidic platform on surface acoustic wave (SAW) requires a clear understanding of the various factors that affects the acoustic energy transmission to the fluid in the microchannel. This chapter reports the characterization and analysis of the reusable SAW microfluidic platform. The acoustic wave was coupled through a liquid layer to a disposable superstrate. Using the microfluidic mixing phenomenon we characterised the acoustic

energy transfer through various layers. We conducted experiments and developed new analytical models to relate the influence of the input voltage, frequency and coupling layer thickness to the mixing efficiency. The anti-symmetric higher order Lamb waves generated on thin glass plate makes compressional waves in the liquid to induce fluid motion. The acoustic energy delivered to the fluid increased as the square of the applied voltage and saturated at 50 V. The frequency response demonstrated a higher acoustic energy transmission for the 100MHz compared to the 50MHz. By detailed numerical modelling, we show that the existence of second order Lamb wave mode (A2) in the 100 MHz device relative to the first order mode (A1) existing in the 50 MHz improves the transmission efficiency. Power transmitted through the coupling layer displayed a sinusoidal dependence on the normalised thickness of the layer. Finally, we measured the temperature rise and calculated the variance in sound speed, and this is analysed to confirm the validity of the developed models. The contents of this chapter is based on our work given in [108].

3.1 Introduction

A recent review discussed the growing interest in using the disposable superstrates on the expensive piezoelectric substrates [97]. SAW coupling to a glass superstrate using water as a couplant was reported previously [107]. Disposable glass superstrate was also used to demonstrate droplet actuation using slanted finger interdigital transducer (IDT) [109].

Rapid mixing on liquid droplets using SAW as the primary force was demonstrated earlier by different groups [110–112]. The quantitative study relating the efficiency of the chaotic mixing process due to SAW for variations in the fluid viscosity and input power was also performed in a microfluidic well [113]. The effect of SAW was used as a mixer in polydimethylsiloxane (PDMS) microchannel bonded to the lithium niobate (LiNbO_3) substrate [114]. They had analysed the effect of the channel orienta-

tion on mixing efficiency. At electrical signals of 35 volts and a frequency of 9.6 MHz, a mixing efficiency of 94% was obtained. Sritharan et al. [115] reported on using SAW for mixing at low Reynolds number flow. They made use of a SAW device at 146 MHz and observed the mixing behaviour for water and beads through a Y shaped channel. Tan et al. [116] introduced channels by etching the LiNbO_3 substrate and studied the mixing behaviour for different frequencies. They observed that when the channel length is larger than the wavelength of the sound at a particular frequency, a uniform to mixing flow transition occurs. The two ends of the microchannel reflect the SAW wave and hence form a transverse standing wave across the channel width. Luong et al. [117] proposed the use of a focussed interdigital transducer (F-IDT) to improve mixing. The F-IDT gives a mixing efficiency of 90% compared to the 10% efficiency obtained with a parallel IDT for a voltage of 80 V with Peclet number of 74400 and flow rate of $167 \mu\text{L}/\text{min}$. This improvement in efficiency is because the F-IDT focusses the wave to a small area. However, the temperature of the substrate increases at the focal point due to wave focusing. Johansson et al. [118] used polydimethylsiloxane (PDMS) channels as a superstrate for particle manipulations in continuous-flow microfluidic operation, and performed a detailed evaluation of the acoustic wave coupling mechanisms and distribution of acoustic nodes in the fluid relative to the channel walls for effective particle manipulations. A qualitative visualisation of the effects of PDMS microchannel in the acoustic waves within the fluid is performed using finite element methods (FEM). In our work, we use mixing phenomenon to characterise the acoustic energy transfer through the various layers.

The reusable platform consists of microchannel created on a polydimethylsiloxane (PDMS) bonded to a disposable superstrate (glass), and acoustically coupled to the piezoelectric substrate. The fluid motion in the microchannel is caused due to the higher order antisymmetric Lamb wave modes generated on the glass superstrate, which acts as a thin plate [107]. The antisymmetric mode with prominent transverse vibrations is coupled to the fluid in the microchannel causing fluid motion. This chapter

focuses to characterise SAW transmission in the liquid channel in a direct manner, which is through mixing. We established a novel mechanism for characterising the SAW energy transmission in fluidic channels, which is essential for all the SAW microfluidics design, using a mixing structure. The main contribution of the work comes from the discussion of the effect of thickness of coupling layer and frequency on the mixing efficiency in a Y-shaped microchannel and development of analytical models. The analytical models that are developed in this chapter can be used to optimise the power transmission coefficient and hence increase mixing efficiency. The platform can be extended to other SAW-based microfluidic applications. The decoupling of the expensive piezoelectric substrate with the microfluidic device enables lower cost and disposability. This makes the platform an ideal fit for the development of point of care clinical diagnostics [119], screening or analytical tests [120].

3.2 Materials and methods

3.2.1 SAW device design and simulation

The anisotropic piezoelectric substrate used was a LiNbO_3 wafer with the crystal cut rotated 128° around the Y-axis. A double-electrode transducer was used to reduce the inter-finger reflection and improve its performance [94]. Table 3.1 shows the design specifications of the IDT for the frequencies of 50 MHz and 100 MHz.

Parameter	Value	
	50 MHz	100 MHz
IDT pitch ($\lambda/8$)- μm	9.7	4.765
Number of IDT pairs	50	50
IDT thickness- μm	0.15	0.15
Aperture-mm	4	3

Table 3.1: SAW design parameters

Figure 3.1: Fabricated SAW devices at frequency of 50 MHz. The yellow regions show the gold pads and the fine lines show the IDTs

3.2.1.1 SAW device fabrication

The fabrication process of the SAW device begins with a layer of aluminium patterned (thickness of 300 nm) on the 128° rotated Y-cut LiNbO₃ substrate using a positive photoresist to form the IDT. After patterning and stripping of the photoresist, the gold of thickness 1.5 μm was then sputtered to create the metal pads for the contacts. To protect the IDT from oxidation, an additional layer of AZ-4620 photoresist was deposited by evaporation, for a thickness of 0.8 μm , except in the SAW propagation area. The final fabricated device is shown in Fig 3.1.

3.2.1.2 SAW device characterization

The fabricated device was bonded to a printed circuit board (pcb). The centre frequency of the fabricated device was characterized using the E5061B ENA Series Network Analyzer. Maximum acoustic power was generated from the SAW device at this frequency. The tolerance of the IDT width was 2% and 4% for the devices working

Figure 3.2: Measured S11 using the network analyser for the two devices at frequencies (a) 50.5 MHz and (b) 103 MHz. The devices were designed for 50 MHz and 100 MHz respectively.

at frequencies of 50 MHz and 100 MHz respectively. This could be induced by the photomask fabrication and photolithographic process, which in turn caused variance to the centre frequency. Fig 3.2 shows the S11 (reflection coefficient) for the fabricated devices, which was measured to be -20dB at 50 MHz and -8dB at 100 MHz.

3.2.2 Micromixer design

Fig 3.3 shows the schematic of the reusable micromixing platform. The SAW was transmitted from the piezoelectric substrate through a coupling layer of water and a glass superstrate into the fluid flowing through the microchannel. A Y-shaped microchannel made of PDMS enclosed the liquid. A reservoir was specially designed to hold the water couplant.

Figure 3.3: Reusable SAW mixing schematic. The acoustic wave generated on lithium niobate (LiNbO_3) was transmitted through a coupling layer of water into the microchannel made of PDMS bonded to glass. The mixing channel has a width of 1.15 mm and a height of $200\mu\text{m}$.

3.3 Experimental setup

3.3.1 Electrical setup

The electrical signal was generated from a pulsed RF power amplifier, as a high voltage ($>30\text{ V}$) was required for mixing to take place. The pulsed RF input was generated using the Tektronix AFG3252 arbitrary function generator. A pulsed RF signal with a period of T_p of 5 ms and T_{ON} (duration for which the signal is turned on) of 1 ms was generated. The output signal from the function generator with an amplitude range of 100-200 mV was amplified by a pulsed power amplifier (TOMCO) of 60 dB gain. The output of the pulsed power amplifier provided the input to the SAW device.

3.3.2 Coupling layer

Water forms the coupling layer in our experiments. Other coupling layers like the UV cured epoxy (NOA61) and ultrasonic gel were also used. For reusing the SAW device for different tests, water was chosen as the coupling layer. To control the water layer thickness, a U-shaped reservoir made out of PDMS was used, which is opened in the front as shown in Fig 3.4. The opening in the front prevents further attenuation of SAW by the PDMS. A fixed volume of liquid is introduced by a pipette, which helps to control the water layer length. The thickness of the coupling layer is equal to the thickness of the PDMS reservoir block (~ 1 mm thick).

Figure 3.4: A block of PDMS functions as a reservoir to control the coupling layer thickness

3.3.3 Placement of the microfluidic channel

Fig 3.5 shows the Y-shaped microchannel made on PDMS and bonded to glass. The channel is placed on top of the substrate via the coupling layer. The coupling layer length in the X direction (see Fig 3.4 for axis direction) is controlled by the placement of the microchannel, which was approximately 1.5 mm for the study. The layout and the dimensions of the microfluidic channel that was used for the experiment is shown in Fig 3.6. PDMS attenuates the acoustic wave, hence the PDMS was trimmed to reduce the attenuation.

Figure 3.5: Microfluidic Y channel placed on top of the substrate via coupling layer

3.3.4 Microfluidic setup

The mixing effect due to the SAW disturbance was visualised using CMOS video camera (INFINITY1). Food dyes of red and green colours were introduced into

the microchannel at a flow rate of 1 $\mu\text{L}/\text{min}$. The flow rate was controlled by syringe pumps (NE-1600, New Era, USA).

Figure 3.6: Y shaped microchannel on PDMS. The edge facing the IDT is trimmed to reduce the SAW attenuation

3.3.5 Mixing efficiency calculation

The mixing efficiency(σ) was calculated by extracting the pixel intensities I_{unmix} before mixing, I_i obtained with the acoustic wave induced mixing and I_{mix} after complete mixing [122] as follows:

$$\sigma = 1 - \sqrt{\frac{1}{N} \sum_{i=1}^N \sigma_i^2} \quad (\text{Eq. 3.1})$$

where $\sigma_i = \frac{I_i - I_{\text{mix}}}{I_{\text{unmix}} - I_{\text{mix}}}$ and N is the total number of pixels. The value of σ varies from zero for non-mixing to one for complete mixing. Please note that during the mixing test, the experimental setup (mixing platform and the channel placement) remain unchanged. By the laminar nature of the flow, the two liquids initially flowed separately without significant mixing as seen in Fig 3.7(a). Upon initializing the SAW, mixing oc-

curred between the two streams of fluid, as shown in Fig 3.7(b). The two dyes were then homogenously mixed externally and flowed through the microchannel as shown in Fig 3.7(c), this was representative of the completely mixed state. The region of interest (ROI) was selected to be within the SAW aperture region, shown in Fig 3.7(a).

Figure 3.7: Images of the SAW mixing experiment (a) unmixed liquids at the beginning of the experiment, (b) fluids mixed using SAW (c) Homogeneously mixed liquids where the two dyes were mixed externally to form the homogeneously mixed solution.

3.4 Results and discussion

To calculate the efficiency using Eq. 3.1, the pixel values (corresponding to the intensity) was extracted using the ImageJ software [123]. An average of 20,000 pixels (N in Eq. 3.1) in the ROI was used to calculate the efficiency.

3.4.1 Transient behaviour of mixing

Fig 3.8 shows the transient response of the mixing phenomena. The increase in mixing efficiency can be observed immediately, before saturating at a value dependent on the voltage level. The experimental result demonstrated that the mixing be-

Figure 3.8: Transient mixing phenomena. The mixing achieved saturation within 1.5 s.

haviour saturated within a time of 1.5 seconds. 70% efficiency at a voltage input of 45 V was observed in 1.5 s. Hence, in this work we extract the frame at 1.5 s to calculate the acoustic wave induced mixing efficiency.

3.4.2 Effect of voltage on mixing

To examine further the influence of voltage on mixing, the voltage was varied from 20 V to 60 V. The mixing efficiency was calculated at each voltage step. Fig 3.9 shows the mixing efficiency with different voltage for the device working at 50 MHz. Maximum efficiency of 80% was attained with an input voltage of 50 V. The mixing efficiency graph demonstrated two trends with increasing voltages. At lower voltages, the mixing efficiency increased with voltage rapidly following a second order polynomial response. At higher voltages (around 50 V), the mixing efficiency saturated. The interaction of the acoustic wave with fluid generates acoustic streaming [124]. As the wave is applied in a direction perpendicular to the liquid flow direction, acoustic streaming forces a mass movement in an orthogonal direction of flow and thus rapidly induces mixing. Acoustic streaming is a nonlinear second-order phenomenon. As reported by Nguyen and White [125], the acoustic streaming velocity is proportional to the square of the applied voltage. Mixing efficiency, which is proportional to the acoustic streaming velocity, thus increases with the square of the voltage. The experiment observation obtained is in line with this trend. At higher voltages, the nonlinearity of the acoustic field in the fluid causes the saturation of the acoustic streaming velocity [126, 127]. In this study, the mixing efficiency saturated beyond an applied voltage (50 V) which supports prior research findings. Due to saturation in the level of mixing, the input power need not be increased beyond 50 V. Any further increase would reduce the power conversion efficiency

3.4.3 Effect of coupling layer thickness on mixing efficiency

The acoustic mixing efficiency is dependent on the thickness of the coupling layer. The effect of varying thickness on the efficiency was examined experimentally. The experimental observation in 3.11 shows that a larger coupling layer thickness gives rise to a higher mixing efficiency. The acoustic impedance of the coupling layer

Figure 3.9: Mixing efficiency as a function of voltage. The error bars show the mixing efficiency variation from the mean value. The solid line shows a second order polynomial fit for voltages less than 50 V and further saturates at higher voltages.

prevents total reflection of the incoming wave from the LiNbO₃ substrate. The wave diffracts into the water coupling layer from the SAW device at an angle of 22.1°. However, on passing through the coupling layer of a certain thickness (L), the wave gets attenuated [128]. Fig 3.10 shows the wave amplitude through the various layers to determine the power transmission coefficient (T_r). The power transmission coefficient can be calculated from [129]:

$$T_r = 4r_{13}/(r_{13} + 1)^2 [1 - (r_{23}^2 - 1)(r_{12}^2 - 1)(\sin K_2 L)^2 / (r_{13} + 1)^2] \quad (\text{Eq. 3.2})$$

with $r_{ij} = \rho_j c_j / \rho_i c_i$, where ρc represents the characteristic acoustic impedance with c and ρ denoting the wave velocity and density respectively in the medium. L denotes the coupling layer thickness. The parameter of Eq. 3.2, other than the sinusoidal function remains unchanged for the experiment. The power transmission coefficient (T_r) is directly proportional to the square of the sinusoidal function. For the operating fre-

Figure 3.10: Wave transmission through the various layers. A_i and B_i represent the transmitted and reflected wave amplitude respectively at each layer. The terms in red denote the incident (A_1) and transmitted waves (A_2, A_3). The reflected waves (B_1, B_2) are represented in green. The exponential part shows the phase dependence of the wave on the acoustic wave frequency (ω) and wavenumber (k).

quency of 50 MHz, the square of sine function gives values of 8.6×10^{-8} , 0.71 and 0.75 for the coupling layers of thicknesses 0.6 mm, 0.7 mm and 0.8 mm respectively. Thus, T_r increases for thicknesses in the order of 0.8 mm, 0.7 mm and 0.6 mm. The experimental results shown in Fig 3.11 thus confirms the theoretical model. An optimum coupling layer thickness can be designed to reduce the attenuation.

Figure 3.11: Mixing efficiency variation with coupling layer thickness

3.4.4 Effect of frequency on mixing

The SAW devices designed for frequencies of 50 MHz and 100 MHz were used to study the effect of frequency on the mixing efficiency. A coupling layer thickness of 1 mm was used for the experiment. As observed experimentally and shown in Fig 3.12, the SAW device working at 100 MHz has a higher mixing efficiency than 50 MHz at lower voltages. However, at higher voltages, the mixing efficiency reaches a maximum as mentioned in the effect of voltage, and both the devices deliver nearly equal mixing efficiency. The analysis for the effect of frequency is twofold: SAW power (P_s) is generated on the substrate at different frequencies and delivered into the fluid through the various transmission layers. The power produced by the transducer is given by [90]:

$$P_s = G_a |V|^2 / 2 \quad (\text{Eq. 3.3})$$

where G_a is the IDT conductance and V is the applied electrical potential. As the IDT is bidirectional, only half of the wave power is delivered into the microfluidic chip. G_a

Figure 3.12: Mixing efficiency obtained for SAW devices at 50 MHz and 100 MHz.

is proportional to the frequency and the aperture of the IDT. The power generated by the transducer for the two frequencies is:

$$P_s(50MHz) = 0.533P_s(100MHz) \quad (\text{Eq. 3.4})$$

Hence, the acoustic wave power generated by the 100 MHz SAW device is approximately twice the power delivered by the 50 MHz SAW transducer. The acoustic wave generated passes through the various layers before it reaches the microchannel as shown in Fig 3.13. As the wave passes through the thin water layer, it undergoes an attenuation of 0.55 dB and 2.3 dB for the 50 MHz and 100 MHz devices respectively. The longitudinal wave generated in the fluid reaches the water-glass (bottom) interface at an incident angle (θ_w) of 22.1° . The compressional waves from water excite Lamb waves in the thin glass plate of thickness 0.15 mm. As discussed previously by Hodgson et al. [107], higher order antisymmetric waves are generated on the glass plate. The dispersion curve for the glass [130, 131] shows that at a frequency of 50

MHz A_1 mode is excited. The 100 MHz incident acoustic wave excites the A_1 , S_1 and A_2 Lamb wave modes. For the excited modes, the phase velocity of the normal wave is less than the speed of the longitudinal waves c (5960 m/s) and close to the transverse velocity b (3200 m/s) on the plates. The displacements for mode A_n in this case are [132] given below. (Refer to Appendix A for details on the derivation of Eq. 3.5):

$$\begin{aligned} u_1 &\cong (n + \frac{1}{2}) \frac{\pi}{h} A \left\{ -\sin \left[(2n + 1) \pi \frac{x_2}{2h} \right] + 2(-1)^n \frac{\sinh(\chi k x_2)}{\sinh(\chi k h)} \right\} \\ u_2 &\cong i K A \left\{ \cos \left[(2n + 1) \pi \frac{x_2}{2h} \right] + (2n + 1)(-1)^n \frac{\pi \chi}{k h} \frac{\chi k x_2}{\chi h} \right\} \end{aligned} \quad (\text{Eq. 3.5})$$

where u_1 and u_2 are the longitudinal and vertical (transverse) components of the mechanical displacement. The plate thickness along the x_2 direction is equal to $2h$ and n represents the mode number. Computation of the transverse component of displacement (u_2) on the surface of the plate ($x_2=h$) shows that the magnitude of second order mode (A_2) is about three times the magnitude of the first order mode (A_1). The second order mode excited in the 100MHz excitation generates larger displacement, consequently improves mixing efficiency. The sound wave transmitted through the glass plate placed between two liquid media, can also be analysed quantitatively by the Eq. 3.6 obtained from Brekhovskikh [133]. (Refer to Appendix B for the derivation of Eq. 3.6).

$$\begin{aligned} W &= 2N / [2M + i(M^2 - N^2 - 1)] \\ M &= (Z_2/Z_1) \cos^2 2\gamma_2 \cot P + (Z_{2t}/Z_1) \sin^2 2\gamma_2 \cot Q \\ N &= Z_2 \cos^2(2\gamma_2) / Z_1 \sin P + Z_{2t} \sin^2(2\gamma_2) / Z_1 \sin Q \\ P &= k_2(h/2) \cos \theta_2 \\ Q &= \kappa(h/2) \cos \gamma_2 \end{aligned} \quad (\text{Eq. 3.6})$$

where K_2 , κ_2 are the wavenumbers of the acoustic wave for the longitudinal and transverse component respectively in glass; Z_2, Z_{2t}, Z_1 denotes the acoustic impedance for the longitudinal, transverse wave in glass, and the compressional wave in water re-

spectively; θ_2, γ_2 are the angle of incidence for the longitudinal and transverse wave in glass. The longitudinal wave in glass undergoes total internal reflection and the wave only resides on the surface. The angle of incidence of the longitudinal wave is represented in the complex form. We set $\theta_2 = \pi/2 + i\zeta$, with $\zeta=0.9579$, using this $\sin\theta = \cosh\zeta$, $\cos\theta = -i\sinh\zeta$. The transmission coefficient (power- $|W^2|$) for the 50MHz and 100MHz acoustic wave is 0.155 (-8dB) and 0.2916 (-5.35dB) respectively. The quantitative results align with the qualitative results obtained above, with the 100MHz excitation conveying larger power to the fluid above compared to the 50MHz acoustic wave excitation. From the reflection coefficient of the device shown in Fig 3.2, the 50 MHz and 100 MHz devices have a reflection coefficient of 0.794% and 15% respectively. Thus, the transmission coefficients for the two devices are -0.034 dB and -0.71 dB. There is an additional reduction of power by -3.01 dB due to the bidirectional nature of the IDTs. Hence, summing up the various losses, the total coupling attenuation of power was 11.594 dB and 11.37 dB for the 50 MHz and 100 MHz devices respectively. The transmitted power is equal to the generated power by the SAW device minus the attenuated power through various layers. Since the generated power is almost 2 times higher in the 100 MHz device when compared to the 50 MHz device, the acoustic power delivered to the fluid is two times higher in the SAW device at 100 MHz when compared to the 50 MHz. Hence the mixing efficiency is higher when excited by a 100 MHz SAW device. Thus, the numerical computation shows that the power transmitted by the 100 MHz device was higher than the 50 MHz device.

3.4.5 Acoustic heating

Temperature rise changes the acoustic impedance of various layers and can hinder the model presented in Sec 3.4.3 for calculating the power transmission coefficient. To justify the validity of the model, we performed the following experiment to measure the temperature. The temperature was recorded at the LiNbO₃ surface for a time-period of 20 seconds using a Fluke DT-610B thermometer. The observation

Figure 3.13: Acoustic Wave transmission through the various layers, showing the waves and its refracting angles at each layer. Medium 1, 2, 3 and 4 are the piezo-electric lithium niobate substrate, water coupling layer, glass and fluid within the microchannel respectively. $P_{l-water}$ represents the longitudinal wave in water, at an incident angle of 22.1° ; $P_{t-glass}$ is the transverse wave in the glass with a transmission angle of 53.4° , the longitudinal component undergoes total internal reflection and will have the character of surface waves.

points (P1, P2 and P3) were chosen along the centre line of the SAW aperture as shown in Fig 3.14. Different Voltages (25V, 35V and 45V) were also applied for the experiment. Fig 3.15 shows the experimental results for 20 s. It indicates that for 20 s, the temperature rises only from 24.35°C to 39.05°C (corresponding to 45 V and position P1). The mixing efficiency in our experiment was calculated at 1.5 s. The temperature rises by a maximum of 0.8°C (24.35°C to 25.15°C) for the period of 1.5 s. Considering the dependence of the velocity of sound in water [134] and density [135] as a function of temperature, a 0.16% change in the acoustic impedance is obtained. This temperature rise is considered negligible and is unlikely to cause a substantial amount of shift in the acoustic impedance. Hence, the Kinsler approximation model discussed in sec 3.4.3 is appropriate. The temperature increase is also not substantial to cause any heating of the liquid.

Figure 3.14: Positions along the SAW device for measuring the temperature. Three points were chosen along the centre line of the SAW aperture at distance of 1 mm (P1), 5 mm (P2) and 13 mm (P3) from the IDT

3.5 Concluding remarks

In this chapter, we report the design, fabrication and characterization of a reusable microfluidic platform using SAW device. The Y-shaped microchannel was made from PDMS and bonded to a glass superstrate. It was coupled to the SAW, generated on the LiNbO_3 substrate using water as an acoustic coupling layer. The platform was characterised using microfluidic mixing phenomenon. Two food-dye solutions were introduced into the microchannel and mixing efficiency was calculated. Mixing efficiency of 80% was observed within 1.5 s for an input signal of 50 V. Experiment was conducted with varying coupling layer thickness to find the optimal thickness for maximum acoustic energy transmission. A SAW device at 50 MHz gives rise to greater than 80 % mixing efficiency for a coupling layer of 0.8 mm thickness. An analytical model formulated to predict the behaviour for different thicknesses substantiates the experimental findings. We then compared the mixing efficiency for two devices at

Figure 3.15: The temperature measured for a time period of 20 sec with different voltages (25V, 55V and 45V) at various positions as indicated in Fig 3.14. The inset shows the magnified view of the graph for a time period of 2 sec

frequencies of 50 MHz and 100 MHz. The experimental result shows that the power transmission was higher for the 100 MHz as compared to the 50 MHz, mainly due to the existence of higher order Lamb wave modes with larger displacement amplitudes. The results obtained provide useful design guidance for the selection of voltages, frequencies and the thickness of couplant layers for improving the acoustic energy transfer for various microfluidic manipulations.

Chapter 4

Photoacoustic induced surface acoustic wave (SAW PA) sensor for sensing analytes in a microfluidic channel

In this chapter, we report a novel sensor utilising the photoacoustic induced surface acoustic wave (SAW-PA) to detect the optomechanical properties of analytes inside a microfluidic channel. The longitudinal wave generated inside the microfluidic channel is mode-converted to a surface acoustic wave (SAW) and detected by the interdigital transducer (IDT). We present an analytical model to explain the mode conversion process and its attainable efficiency. We then demonstrate by experiments the usage of the sensor to investigate the optical and acoustical properties of standard dye solutions and gold nanoparticles. Subsequently, we elucidate the response mechanism of the sensor using detailed numerical FEM simulation. The simulation study has shown that the microfluidic channel on the substrate acts as an acoustic resonator with mul-

multiple eigenmodes. PA signals generated inside the microfluidic channel resonates at these modes. The displacement profile on the piezoelectric surface confirmed that the pressure mode propagating parallel to the piezoelectric substrate has the highest mode conversion efficiency (longitudinal wave to SAW). This mode is sensitive to the microchannel dimensions. The study has shown that a SAW device matched to this mode /frequency could be used for improving the sensitivity of the sensor. These numerical observations supported with an experimental verification has led to an understanding of the SAW-PA sensor.

4.1 Introduction

SAW sensors have been used to determine the mechanical properties including viscosity and density of the fluids flowing through a channel between the electrodes [136, 137]. On the other hand, optofluidic sensors have been explored to characterise the optical properties of the liquid samples [138–141] including absorptivity, refractive index and even mechanical properties of the fluids such as viscosity [142, 143]. However, the measurement of the fluid viscosity requires introducing particles into the fluid as a probe and involves complex calculations. To achieve development of a comprehensive and concurrent (optical and mechanical properties) characterization pathway for diversified analytic samples, a sensor incorporating smart integration of light and sound is highly desirable. Recently, the development of new optical imaging techniques based on photoacoustic (PA) effect such as PA imaging and its combination techniques have been used extensively for sensing and imaging applications [144–147], especially in biomedicine [148, 149]. Details of the PA sensing technique was discussed in Chapter 2. Although PA sensing and imaging have demonstrated promising results in both anatomical and functional imaging of small animals in vivo, its applications in a compact microfluidic environment is rarely explored. This could be attributed to the inherent limitation of integrating the bulky ultrasound

transducer on a small analytical microchip. In this chapter, we present the design and fabrication of a SAW-PA device, which performs PA sensing with a Rayleigh SAW device. The analyte sample for the investigation was loaded into a microfluidic channel made of polydimethylsiloxane (PDMS). The nanosecond pulsed laser focused on the fluid sample in the microchannel triggers PA waves, which couples into a Rayleigh SAW on the piezoelectric substrate for detection. This platform enables characterization of liquid samples by measuring both its optical and mechanical properties simultaneously. Furthermore, the usage of SAW as the receiver of the PA signal in this new design enables a couplant-free (water-free) PA signal sensing at a low cost and compact pathway. In this work, we first demonstrated the usage of the SAW-PA sensor to detect the optomechanical properties of organic dyes and plasmonic nanoparticles (NP), which represents an optically homogeneous and optically heterogeneous medium respectively for generating PA. The multitude of applications possible due to nanoparticles in scientific fields like medicine, pharmacy, nano and biotechnology, electronics, photonics and environmental science [150] highlights the importance of detecting NP and determining its morphology using a lab-on-chip device. The primary goal of the numerical modelling was to determine the frequency of the PA excited SAW and use the information in the design of the interdigital transducer (IDT), for increasing the sensitivity of the sensor. Besides the frequency determination, the chapter aims to elucidate: 1) The need of a narrow-band device to detect a wideband PA signal. 2) How do we efficiently convert a longitudinal acoustic wave to SAW without additional wedge structures as discussed in the previous chapter. Various groups [86, 87] performed work about the analytical and numerical models for PA generation in liquid. According to our knowledge, the study on the mode-conversion of PA generated longitudinal waves in fluids into SAW on piezoelectric substrates has not been done elsewhere. However, some early works were performed to study the laser-generated surface acoustic waves [151, 152] on solid materials. In this section, we prove that the laser-generated longitudinal wave in the microfluidic channel undergoes resonance at

various modes. The frequency which excites surface waves at the highest amplitude is the pressure mode in the channel which propagates in a direction parallel to the piezoelectric surface. The derivation of the model is based on the coupling of multiple physics interfaces in COMSOL Multiphysics 4.4. The thermoacoustic module was used to solve for the acoustic degree of freedom inside the fluid, pressure propagation in the polydimethylsiloxane (PDMS) was modelled using pressure acoustics and the piezoelectric interface comprising of solid mechanics and electrostatics simulates the SAW propagation and electrical voltage from the IDT, respectively. The accuracy of the frequency predicted by the model has been validated by performing an experiment on the sensitivity of the SAW-PA sensor to IDTs at different centre frequencies. The contents of this chapter is based on our work published in [153, 154]

4.2 Materials and Methods

4.2.1 SAW device design and fabrication

SAW device was designed and fabricated on a 128°YX lithium niobate (LiNbO_3) substrate with split-finger inter-digital transducer (IDT). Table 4.1 and Fig 4.1 provide the detailed dimensions of the SAW IDT and the frequency response (S_{11}) of the SAW device (measured using E5061B ENA Series Network Analyzer) respectively. The resonant frequency of the device is at 12.9 MHz.

Parameter	Values
IDT width ($\lambda/8$ in μm)	37.5
Number of IDT pairs	25
IDT thickness (nm)	150
Aperture (mm)	4

Table 4.1: SAW-IDT design parameters

Figure 4.1: Frequency response (S11) of the SAW device measured using the network analyser. The resonant frequency of the device is 12.9 MHz.

4.2.2 Design of microfluidic channel

A Polydimethylsiloxane (PDMS) microfluidic channel of width 1.15 mm, depth 150 μm and length 15 mm was fabricated by soft lithography with through-holes punched for both inlet and outlet. The PDMS was trimmed along the width (sidewall thickness- T_s of 3 mm as shown in Fig 4.2) to reduce the PDMS attenuation of the acoustic wave. The PDMS was bonded to the substrate via oxygen plasma treatment (Inspiraz Technology). The PDMS and the LiNbO_3 substrate were cleaned and exposed to oxygen plasma for 2 min. The PDMS microchannel was then aligned and bonded to the SAW substrate. The device was kept in an oven maintained at a temperature of 80 $^{\circ}\text{C}$ to strengthen the bond.

4.2.3 Reagent and solutions

We have selected aqueous solutions of two commonly used food colouring dyes and gold nanoparticles as plasmonic materials for analysis. The dyes, erythro-sine (red) and brilliant blue were purchased from SigmaAldrich Singapore. Spherical

Figure 4.2: Schematic illustration of the PDMS microfluidic channel on the SAW substrate, where T_s denotes the side wall thickness

gold nanoparticles (GNPs) and gold nanorods (GNRs) were obtained from Nanopartz Inc., USA. All the solutions were prepared with millipore deionized (DI) water. UV/vis absorption spectra of the dyes and NPs were obtained using a Shimadzu UV-3600 UV-vis-NIR spectrometer.

4.2.4 Experimental setup

A nano-second pulsed laser light of 1 mJ pulse energy was focused on the microchannel within the aperture of the SAW device, to induce PA signal. The laser-induced PA signal within the fluid in the microchannel was converted to surface acoustic waves in the piezoelectric ($128^\circ\text{YX LiNbO}_3$) substrate. The surface acoustic wave propagating on the substrate was detected by the IDT. Fig 4.3 shows the schematic representation of the device. Fig 4.4 illustrates the experimental configuration consisting of the optical interrogation units, surface acoustic wave device and the electrical measurement units in SAW-PA device. An optical parametric oscillator (OPO) pulsed laser with tunable wavelength (410-2100 nm), 7 ns pulse-width, and 1 mJ pulse energy, was employed as the light source. After attenuation by the neutral density (ND) filter and focusing by the condenser lens, the light was illuminated on the microfluidic channel filled with liquid sample. A pre-amplifier (Panametrics 5077PR) with 59 dB gain was used to amplify the surface wave from the IDT, followed by data acquisition

from an oscilloscope (WaveSurfer from LeCroy). Fig 4.5(a) shows individual components including split-finger aluminium metal IDT of thickness 150 nm, which was deposited on the $128^\circ\text{YX LiNbO}_3$. Metal pad of thickness 500 nm was used to connect the IDT to the external electrical circuits. Fig 4.5(b) shows the vertical alignment of fluidic compartment made of PDMS bonded to the piezoelectric substrate.

Figure 4.3: Schematic representation of the SAW-PA device. The fluid sample in the microfluidic channel absorbs the pulsed laser and generates longitudinal acoustic waves, which is mode-converted into SAW on the piezoelectric substrate-liquid interface. The SAW is detected using the IDT on the LiNbO_3 substrate

Figure 4.4: The experimental setup for PA measurement using the proposed SAW-PA sensor. OPO: optical parametric oscillator; ND: neutral density; ConL: condenser lens; IDT: interdigital transducer

Figure 4.5: The experimental setup for PA measurement using the proposed SAW-PA sensor. (a) Photographs of individual components integrated in the SAW-PA device. (b) Photograph showing side view of SAW-PA sensor with the bonded PDMS microfluidic channel

4.2.5 Sensor theory and data processing

4.2.5.1 Analytical study of the PA to SAW mode conversion process

The generated PA is converted to leaky SAW beneath the microchannel, which is then converted into a SAW at the edge of the microchannel. The principle of the mode conversion process involved in exciting a SAW is shown in Fig 4.6. The conversion from leaky wave to surface wave will be 100 percent efficient, if we assume that the substrate fields of the leaky and surface wave are similar [93]. The generated PA signal inside the liquid is a longitudinal wave. The conversion process is equivalent to the excitation of the Rayleigh wave by a wedge transducer as mentioned

in a previous publication [155]. A Rayleigh wave is excited on the substrate if it satisfies the condition that c_R , the Rayleigh wave velocity in the substrate (3979 m/s) [90], should be greater than c_w , the longitudinal wave velocity in the liquid (1500 m/s in water). This condition is satisfied in our configuration. All the longitudinal waves inclined

Figure 4.6: Schematic representation of the mode conversion process in generating a Rayleigh surface acoustic wave excitation triggered by the photoacoustic waves. P_i , P_r and θ_i refer to the incident pressure, reflected pressure and angle of incidence respectively

at an angle θ_i greater than the critical angle defined by: $\sin\theta_i = c_w/c_R$, satisfies the criteria to excite the Rayleigh wave. The efficiency of the conversion process from the longitudinal to the leaky surface wave is calculated analytically by the equation:

$$\eta_0 = 2[1 - \exp(-\alpha L)]^2 / \alpha L \quad (\text{Eq. 4.1})$$

where α denotes the leak rate or attenuation per unit length of the surface acoustic wave under the microchannel, which is a function of the frequency of the wave, acoustic impedance in the liquid and the ratio of mode displacement to the power flow. L denotes the aperture or the length on the substrate where the longitudinal wave

strikes. Fraser et al. [155] plots the efficiency with respect to the factor αL and shows that a conversion efficiency of 81% is obtained in the mode conversion process. High conversion efficiency of 81% percent proves the significance of using the SAW as a detector of the longitudinal PA signal.

4.2.5.2 Data processing

The electrical signal received from the SAW device was post-processed to extract the sample generated PA signal from the background. Fig 4.7(a) shows the signal obtained at the IDT due to the laser absorption in the dye for a wavelength of 530 nm. Erythrosine (red) dye was used as the fluidic sample. Laser excitation generates background acoustic signals from the PDMS microfluidic channel. Two control experiments were performed. The first experiment involved detecting the SAW-PA signals produced by the PDMS microchannel (without containing any fluid in it) and the second experiment was conducted with deionized (DI) water as the sample fluid in the microchannel. The transient signal obtained from this control experiment was subtracted from the signal of interest generated by the dye in the microchannel. At 530 nm, the background signal remains relatively weak compared to the signal generated by the dye as shown in Fig 4.7(d). From experiments we observed that the influence of the PDMS was the maximum in the blue spectral region. Fig 4.8 shows the background signal generated by the PDMS microfluidic channel for different optical wavelengths. The power spectral density in Fig 4.7(c) of the time domain signal (0-45 μs) obtained from the dye has a dominant frequency component at 12.9 MHz corresponding to the SAW resonant frequency. Fig 4.9 shows the PSD spectra of the transient signals obtained for the Erythrosine dye from 2-4 μs (red curve) and 6-8 μs (black curve). The spectra show a dominant frequency component at 12.9 MHz, which again coincides with the SAW resonant frequency.

Figure 4.7: Photoacoustic signal output obtained at the IDT: (a) Transient signal generated at the IDT from the absorption of light at wavelength 530 nm from PDMS microchannel (black curve), DI water (red curve) and Erythrosine dye (blue curve) in the microchannel. (b) Shows the zoomed signal from 6-8 μs . Signal from the dye is strong compared to the background signal from PDMS due to the high optical absorption of the dye compared to water and PDMS. (c) Results of the power spectral density (PSD) analysis for entire duration of the transient signal with peak magnitude at the SAW center frequency of 12.9 MHz (d) Signal amplitude for the three different conditions at 530 nm wavelength.

4.2.5.3 Analytical modelling

The microfluidic channel was approximated as a thin slab (width of 1.15 mm and thickness of 150 μm). The pressure waveform was approximated as a Heaviside

Figure 4.8: Background SAW-PA signal intensity generated by the PDMS microfluidic channel for different optical excitation wavelengths

step function from $-d/2$ to $+d/2$ where d denotes the channel thickness. The pressure at the bottom of the channel, which is converted to SAW, is obtained by using the following [87]:

$$p(z, t) = \frac{1}{2}p_0(z - v_{st}) + \frac{1}{2}p_0(z + v_{st}) \quad (\text{Eq. 4.2})$$

The acoustic pressure at $z (= -d/2)$ is a rectangular pulse of width d/v_s , with a thickness (d) c. a. $150 \mu m$ and acoustic velocity (v_s) of 1500 m/s in water generates a pulse of width $0.1 \mu s$. The corresponding frequency spectrum is a sinc function with the first minima at 10 MHz . Deconvolution of the SAW-PA signal from the SAW transfer function results in a low pass signal with a -40 dB response at 10 MHz which represents the main lobe of the expected PA frequency spectrum. Hence, the generated PA spectrum agrees with the theoretical analysis. The repeatability of the signal was confirmed

Figure 4.9: Results of the power spectral density (PSD) analysis for specific durations (2-4 μs and 6-8 μs) of the transient signal displaying the dominant frequency component at 12.9 MHz

by using Pearsons linear correlation coefficients on multiple sets of transient data as shown in Fig 4.10. To illustrate the reproducibility of the transient data shown in Figure 4.7(a), we take a set of time domain data obtained for erythrosine dye solutions of various concentrations (75, 50 and 25 % volume concentrations each) and also calculated the cross-correlation between the transient signals to highlight the robustness of the signal. The repeatability can be estimated by correlation analysis [156]. The Pearsons linear correlation coefficients for the transient signals obtained for the signals s1 and s2 in Figure 4.10 is 0.816, and for signals s2 and s3 is 0.904. A value greater than +0.7 indicates very high correlation between signals. Hence, the transient data is repeatable

Figure 4.10: Repeatability of the data: Transient signals obtained for various Erythro-sine dye concentrations: a) 25%, b) 50% and c) 75%.

4.3 Experimental Results and discussion

4.3.1 SAW-PA signal from standard dye solutions

4.3.1.1 Dye absorption spectroscopy using SAW-PA device

The UV/Vis absorption spectroscopy measurement was performed on the erythrosine (red) and the brilliant blue dyes prior to performing the SAW-PA device characterization. UV/Vis absorption spectra presented in Fig 4.11(a) for the erythrosine (6×10^{-6} M) and brilliant blue (6×10^{-6} M) dyes show characteristic absorption max-

imums at 510 nm and 620 nm respectively. During our first set of experiments with the SAW-PA device, the dyes were introduced into the microchannel and the entire device was kept in a shock-free assembly during the measurement. The pulsed-laser repetition rate was set to a low value of 1 Hz to prevent increase in temperature and subsequent heating of sample in the microchannel. The acoustic signal obtained was further post-processed using MATLAB (Matrix Laboratory Programming Language). A band-pass filter with the center frequency of 12.9 MHz and a 6-dB bandwidth of 4 MHz filters the other signal frequencies. The maximum peak-peak amplitude for each optical wavelength excitation was subtracted from the background signal amplitude obtained for the same wavelength. The signal was further normalised against the laser intensities. In Fig 4.11(b) the red symbols shows the signal obtained from erythrosine loaded in the SAW-PA device for laser wavelength excitations varying in the 450 nm to 650 nm spectral range. The measurements were repeated three times

Figure 4.11: (a) UV/Vis absorption spectrum of erythrosine and brilliant blue (b) Measured SAW-PA signal intensity from the erythrosine and brilliant blue dyes at different laser wavelengths.

to confirm repeatability and the variation was less than 10%. Measured peaks for the erythrosine and the brilliant blue dye were observed at 500 nm and 620 nm respec-

tively, which was in good agreement with the absorption maxima of the dye found in Fig 4.11(a). It must be also noted that a 10 nm blue shift for the mean value of the measured red dye spectra against the expected peak could be attributed to variations in the laser intensity and laser focusing at different wavelengths.

4.3.1.2 Effect of concentration on the SAW-PA signal

Further experiments were conducted using the SAW-PA device to evaluate the PA signal generated on the SAW device for different concentrations of the dye. Erythrosine dye was used as the sample liquid. The dye was diluted with DI water at room temperature (23 °C) to prepare various volume concentrations ranging from 0 to 100%. The laser light at a fixed wavelength of 530 nm with pulsed excitation was used to illuminate the sample. Fig 4.12 shows the magnitude of the signal received by the SAW-PA device for different concentrations of the erythrosine dye. The amplitude of the PA signal depends on the optical absorption coefficient (μ_a) and the path length (l). For $\mu_a L \ll 1$, the PA signal is directly proportional to the optical absorption coefficient and hence the SAW-PA signal increases with concentrations. However, at higher concentrations the optical penetration depth becomes smaller than the path length, causing saturation of the signal. A previous research [157] has reported the PA amplitude of the form mentioned in Eq. 4.3 for variation in the optical absorption coefficient, which is proportional to the concentration.

$$PA(z, \mu_a) = \mu_a F_0 K_1 \exp(-\mu_a z) \quad (\text{Eq. 4.3})$$

where μ_a is the optical absorption coefficient of the medium which is the product of concentration and molar extinction coefficient, F_0 is the optical energy received per unit area; k_1 is the PA conversion factor, and z denotes the depth of the microchannel. An exponential fitting function of the form mentioned in Eq. 4.4 is used to correlate with

Figure 4.12: Signal received by the SAW-PA device for different concentration of the dye (expressed in volume percentage). An exponential function of the form mentioned in Eq. 4.4 was used to fit the experimental data. Error bars are presented to confirm the repeatability of the photoacoustic signal received on the SAW-PA device. The model equation fits to the experimental results with an R-square value of 0.985.

Eq. 4.3 and incorporate the saturation of the PA signal.

$$Y = Y_0 + A_1 \exp(-x/t_1) \quad (\text{Eq. 4.4})$$

where Y_0 , A_1 and t_1 are the unknown parameters. x denotes the concentration of the solution. Y_0 indicates the saturation voltage level due to increasing concentration. $A_1 \exp(-x/t_1)/t_1$ depicts the sensitivity and t_1 denote the concentration at which the signal saturates. After fitting the model to the experimental data, the obtained values for the unknowns are $Y_0 = 2.04$, $A_1 = -2.123$ and $t_1 = 80.625$. The goodness of fit has an R-square value of 0.985. The SAW-PA amplitude reaches close to saturation (e^{-1}) at a concentration of 80.625%. The dye with various concentrations was analysed five times to confirm the repeatability. Each reading was averaged over 200 sweeps to improve the signal-noise ratio. As shown in Fig 4.12, a maximum standard deviation c.a 4.8% for 75% volume concentration was obtained, which indicates good repeatability

in the current SAW-PA device.

4.3.1.3 Effect of viscosity on the SAW-PA signal

The ability of our platform to detect the mechanical parameters of a liquid sample was confirmed by sensing its viscosity. For this study, erythrosine dye-glycerol (EG) solutions of varying concentrations were prepared at room temperature (23 °C) and used as samples for the viscosity tests. The effect of viscosity was analysed by comparing the received SAW-PA signal from the EG solution with the erythrosine dye-water (EW) solution of similar dye concentrations. The laser light at a fixed wavelength of 530 nm with pulsed excitation was used to illuminate the sample. Glycerol added to the dye induces optical clearing effects to reduce scattering and therefore enhanced the SAW-PA signal [158]. Fig 4.13 shows the percentage increase in the SAW-PA signal amplitude of the EG solution with respect to the EW solution for various dye concentrations. At 50% dye concentration, a 65% increase in the signal intensity was observed for the EG solution. This result demonstrates a clear enhancement in the SAW-PA signal amplitudes due to increasing viscosity of the fluids. At higher dye concentrations, the viscosity of the EG solution decreases and becomes comparable to the EW solution, which reduces the relative magnitude of the SAW-PA signal intensity.

4.3.2 SAW-PA signals from gold nanoparticles

Metal nanoparticles (NPs) with high plasmon absorption in the visible and the near-infrared wavelengths provide enhanced capabilities for the specific detection of analytes for biosensing applications. The ability of the SAW-PA device to detect plasmonic absorption of gold nanoparticles (GNPs) and gold nanorods (GNRs) was investigated by introducing GNPs and GNRs (stabilised with CTAB surfactant) dispersed in water into the microchannel. The plasmonic absorption and subsequent

Figure 4.13: Percentage increase in the SAW-PA signal intensity of the erythrosine dye-glycerol with respect to the erythrosine dye-water solutions for varying dye concentrations

nonradiative relaxation dominated PA generation for optically heterogeneous nanostructures differ fundamentally from the PA signals generated by the optically homogeneous dye solutions. A pulsed laser with wavelengths 480-600 nm was used for spectroscopic characterization of the nanoparticles. The signal received from the SAW-PA device was averaged over 300 sweeps. Water in the PDMS microchannel was used as the reference solution to which the GNP induced SAW-PA signal was compared. Fig 4.14(a) shows the transient SAW-PA signal from 12 nm GNP solution (2 mg mL^{-1}) compared to water as a reference, at an excitation wavelength of 520 nm. As shown in Fig 4.14(a), an apparent enhancement in the SAW-PA signal was observed for the GNPs in the microchannel. We further measured the spectroscopic response of the GNPs. Fig 4.14(b) shows the UV/Vis absorption spectrum of GNPs (2 mg mL^{-1} , c. a. $6 \times 10^{12} \text{ particles mL}^{-1}$) in aqueous solution, with an absorption maximum at 520 nm. The SAW-PA signal (normalised to laser intensity) shown in Fig 4.14(c) follows the absorption profile from 500 nm and higher wavelengths, with an absorption peak at 520 nm. The deviations at lower wavelengths (less than 500 nm) were also reported

in a previous work, which was attributed to scattering effects [159].

Figure 4.14: (a) SAW-PA signals from 12 nm GNPs and water in the microfluidic channel for an excitation wavelength of 520 nm. (b) UV/Vis absorption of GNP (2 mg mL⁻¹) (c) SAW-PA signal intensity of GNPs (2 mg mL⁻¹) for different laser wavelengths

4.3.2.1 Influence of gold nanoparticle sizes

Spherical GNPs of various sizes (10 nm-50 nm in diameter) were diluted in water to obtain identical extinction values (0.7 cm⁻¹) at an excitation wavelength of 532 nm. Fig 4.15 shows that the SAW-PA signal for different sizes of the GNPs at an excitation wavelength of 532 nm increases as a third-order polynomial function of the NP diameter. The standard deviation was calculated from five repeated measurements. The NPs generate nonlinear PA signals at a threshold fluence of 300 mJ cm⁻² [160].

Beyond the threshold fluence, the temperature of the NPs quickly increases to the boiling point of the surrounding water and generates nanobubbles. Previous researchers have proposed analytical models for the bubble generation and dependence of the NP diameter on PA signal amplitude. Accordingly, the PA signal amplitude increases with NP sizes [161]. The threshold for the nonlinearity in the dye is higher than the nanoparticle. For the Erythrosine dye with an optical absorption coefficient of 100 cm^{-1} , the temperature rise is c.a 0.5°C . Hence, the nonlinear behaviour in dye is less profound than in the NPs.

Figure 4.15: Normalized SAW-PA response for different GNP sizes at optical wavelength of 532 nm. The fitting curve represents a third order polynomial function.

4.3.2.2 Sensing the transverse and longitudinal plasmon modes of gold nanorods

The efficacy of the SAW-PA for biosensing applications can be further emphasised by sensing the plasmon resonance modes of the gold nanorods (GNRs) at visible and near-infrared regions. The longitudinal plasmon bands are extremely sen-

sitive to changes in the dielectric properties of the surrounding medium, improving the detection signal-noise ratio [162]. GNR of dimension 10 nm $\times$ 85 nm, with the transverse and longitudinal plasmon resonance modes at 512 nm and 1064 nm respectively were used for the study. Fig 4.16 illustrates the normalized SAW-PA response from

Figure 4.16: SAW-PA response of the gold nanorods (10 nm $\times$ 85 nm) at 532 nm and 1064 nm for different concentrations.

various concentrations of GNR solutions at optical excitation wavelengths of 532 nm and 1064 nm. At each concentration, mean and standard deviations were obtained with five repeated measurements. The significant increase in the SAW-PA signal at 1064 nm compared to the amplitude at 532 nm conforms to the longitudinal resonance of the GNRs.

4.3.2.3 Influence of shape of nanoparticles

The SAW-PA response of the GNP and GNR was compared by selecting three different nanoparticle solutions at a concentration of 2 mg mL^{-1} . The samples consisted of 40 nm (diameter) gold nanosphere, GNRs of dimension $10 \text{ nm} \times 85 \text{ nm}$ (GNR1) and $10 \text{ nm} \times 40 \text{ nm}$ (GNR2) -diameter $\times$ length. Fig 4.17 shows the TEM images of the nanoparticles with corresponding UV-Vis absorption spectra. The GNP

Figure 4.17: UV-vis absorption spectra and TEM images of the gold nanostructures. (a),(b) and (c) represents the UV-vis absorption spectrum of the 10 nm x 85 nm gold nanorod, 10 nm x 40 nm gold nanorod and 40 nm gold nanoparticle respectively with the TEM images of the corresponding nanostructures.

has a peak plasmon resonance at 527 nm. The two GNR's selected has a transverse plasmon absorption peak at 512 nm and 528 nm and corresponding longitudinal peaks at 1064 nm and 758 nm. Fig 4.18 shows the experimental results for the SAW-PA response for the three nanoparticle solutions. From the graphs, at 800 nm and 1064 nm optical wavelengths, the GNR2 and GNR1 have the maximum signal amplitudes respectively. GNR2 with an effective radius of 21.5 and aspect ratio of 3.4 has a per micron optical absorption at 800 nm of ca. $400 \mu\text{m}^{-1}$ and the optical absorption of GNP at 532 nm is $75 \mu\text{m}^{-1}$ (ca.) [163]. This explains the reason for larger signal amplitude at 800 nm for GNR2 compared to GNP at 532 nm. GNRs with high aspect ratio and smaller effective radius increase the optical absorption leading to improved SAW-PA response. The lower response for the GNR1, which has an effective radius of 25 nm and an aspect ratio of 5.48 at 1064 nm, is attributed to the increased contribution of scattering to the absorption, which increases nonlinearly with effective radius. The variation in morphology of the nanoparticle shifts the peak absorption wavelength, which is determined by measuring the optical wavelength at which the SAW-PA response is the maximum as shown in Fig 4.18.

4.4 FEM modelling of the SAW-PA sensor

4.4.1 Theoretical analysis

According to PA theory, a laser pulse duration shorter than the acoustic and thermal confinement time is considered as a source of delta heating [87]. The corresponding PA has a wideband spectrum. Strohm et al. [164] performed FEM studies for determining PA frequency response from micron sized particles with different boundary conditions. In the SAW-PA sensor, the PA source is surrounded by the PDMS microfluidic channel and piezoelectric substrate. The reflections from the boundary modify the PA frequency spectrum. The PA generated inside the microfluidic channel

Figure 4.18: Normalized SAW-PA response of 40 nm gold nanoparticle (GNP-532), 10 nm $\times$ 40 nm gold nanorod (GNP-800) and 10 nm $\times$ 85 nm gold nanorods (GNP-1064) measured at three optical wavelengths-532 nm, 800 nm and 1064 nm.

acts as a resonator, with resonance along X and Y-direction. The mode with an integer number of half-wavelengths generated in the X-direction within the PDMS microfluidic channel (as shown in Fig 4.19(b)) is ideal for SAW excitation, due to the absence of bulk wave losses caused by wave refraction into the piezoelectric substrate.

4.4.2 Finite element mode

Fig 4.19(a) shows the 2D FEM geometry used to simulate the SAW displacements on the LiNbO_3 substrate under PA excitation. Thermoacoustic module in COMSOL implements fully linearized compressible Navier-Stokes, continuity, and energy equations to obtain the acoustic pressure variations p , fluid velocity variations and acoustic temperature variations T . The input for the simulation is a constant heat source Q (in frequency domain represents a wideband heat source) which generates an acoustic pressure wave. In case of the photoacoustics (PA), an incident laser pulse

Figure 4.19: (a) 2D sketch of the SAW-PA sensor consisting of LiNbO_3 substrate and fluid filled PDMS microfluidic channel. The center region of the fluid is the laser induced heat source. Note that the figure is not drawn to scale.(b) FEM computational domain with the boundaries. An acoustic mode (in yellow) with wave propagation along X-direction is the optimal mode for SAW excitation.(c) FE mesh of the SAW-PA sensor

generates heat. The heat Q acts as the source term for the energy equation and solves for the temperature (T). Depending on the material properties such as thermal coefficient of volume expansion (β) and isothermal compressibility (κ), the tempera-

ture change (T) is converted to PA pressure (p), as shown by the Eq. 4.5 below.

$$\frac{dV}{V} = -\kappa p + \beta T \quad (\text{Eq. 4.5})$$

In this model, the nanosecond pulsed laser (1 ns) was approximated as an impulse excitation. This assumption explains the reason for applying a constant value as the heat source input for the thermoacoustics physics model. The heat source input of 1 W/m² was used for the thermoacoustic model. In this model, water was used as the fluid domain. The acoustic properties of water match closely to the dye used in the experiment. The default values specified in COMSOL for the material properties (water, PDMS) are applied in the simulation. The thermoacoustic properties for water calculated at 273⁰C is given in Table 4.2. The piezoelectric properties of 128°YX LiNbO₃ were selected from the work done by Kannan [165]. Air-PDMS boundary was modelled as a sound soft boundary because the impedance condition follows $Z_{\text{air}} \ll Z_{\text{PDMS}}$ [166, 167]. Fig 4.19(b) shows the computational domain with the boundary conditions and domain interfaces. Three half-sinuousoidal waves (in yellow) represents an acoustic mode (0,3) with wave propagation along the X direction. Perfectly matched layers (PML) were used to model an infinte extent of the substrate. The laser excitation domain was represented by a rectangular region in the centre of the fluid domain with a width of 100 µm. To keep the simulation computationally efficient, the thermoacoustic model for the fluid was coupled to pressure acoustics domain for the PDMS. A custom mesh with maximum element size equal to wavelength/8 (corresponding to a frequency of 100 MHz) was used to mesh the acoustic domain. The solid domain close to the IDT was modelled with a maximum element size of wavelength/5 [165]. Mesh refinement study was performed to confirm the accuracy of the selected meshes for the simulation.

Parameter	Value
Coefficient of thermal expansion	$70 \times 10^{-6} \text{ [1/K]}$
Specific heat at constant pressure, C_P	4216.6 J/(kgK)
Ratio of specific heats, γ	1
Density, ρ	1003.9 kg/m^3
Speed of sound v_s	1403 m/s

Table 4.2: Thermoacoustic properties of water at 273^0C

4.4.2.1 Analysis of resonance modes

The PDMS microfluidic channel acts as a resonance cavity for the laser-generated acoustic wave. Multiple acoustic modes exist within the rectangular cavity. The resonance frequencies of a cavity with height (H) and width (W), is given by [168].

$$f_j = \frac{c}{2} \sqrt{\left(\frac{l}{H}\right)^2 + \left(\frac{m}{W}\right)^2} \quad (\text{Eq. 4.6})$$

where, l and m represent the acoustic modes along the height and width of the cavity respectively. The resonant medium consists of PDMS and water. Frequency domain study was performed to determine the different acoustic modes and obtain the corresponding SAW displacements. Fig 4.20 shows the numerical simulation results obtained for a microfluidic channel of dimension $500 \mu\text{m}$ (width) x $350 \mu\text{m}$ (height). The PDMS wall had a sidewall thickness (T_s) of 1 mm. Fig 4.20(a) presents the frequency response of the displacement (refer to Fig 4.19(b) for the axis representation) along the Y-axis at a point on the surface of the piezoelectric substrate outside the channel area. The transverse displacement has a maximum amplitude at a frequency of 13.4 MHz; the corresponding pressure profile is shown in Fig 4.20(b). The colour plot confirms that an incident pressure profile parallel to the substrate is generated. This mode favours the highest conversion efficiency (longitudinal to surface wave) without the usage of an external wedge element. Fig 4.20(c) represents the Y-axis displacement, which confirms the SAW excitation.

Figure 4.20: (a) Frequency spectra of the transverse displacement (Y-axis) on the surface of LiNbO_3 at a point outside the microfluidic channel. Response at 13.4 MHz, corresponding to the frequency at which maximum displacement occurs: (b) Color plot of acoustic pressure inside the microfluidic channel (c) Color plot of the Y-axis displacement on LiNbO_3 .

4.4.2.2 Effect of microchannel dimensions

Considering the numerical complexity required for solving a large geometry, a smaller geometry was considered for the study with a PDMS side wall thickness of $100\ \mu\text{m}$. Fig 4.21(a) displays the frequency spectra for the Y-component of displacement at a point on the LiNbO_3 surface using a PDMS microfluidic which follows the dimensions of Model A represented in Table 4.3. Fig 4.21(b) shows that at the frequency of peak displacement (7.6 MHz), the resonance mode is parallel to the X-axis.

	Channel height (H)	Channel width(W)
Model A	350 μm	250 μm
Model B	500 μm	250 μm
Model C	350 μm	500 μm

Table 4.3: Dimensions of the microfluidic channel

Figure 4.21: Frequency spectra of the transverse displacement (Y-axis) on the surface of LiNbO_3 with a microfluidic channel of dimension shown in: (a) Model A, (d) Model C of Table 4.3. Color plots of the acoustic pressure in the microfluidic channel of dimensions: (b) Model A, (c) Model B and (e) Model C.

Varying height of the microchannel Fig 4.21(c) depicts the pressure profile for the microfluidic channel of Model B at a frequency of 7.6 MHz. For Model B, the width remains the same as Model A, but the height is increased. Eq. 4.6 shows that for modes propagating parallel to the X-axis ($l=0$), the resonant frequency will not vary with the height of the channel. The simulation results of Fig 4.21(c) prove that the zero-order modes parallel to the X-axis will remain unaffected by changes in channel height.

Varying width of the microchannel The numerical simulation confirms that the variation in the width of the microfluidic channel affects the cavity modes. The maximum displacement for Model C (different width and equal height as Model A) is obtained at a frequency of 14.6 MHz (Fig 4.21(d)), with a higher order mode parallel to the X-axis as shown in Fig 4.21(e). The study confirms that the resonant frequency can be tuned by varying the channel width and the PDMS side wall thickness (compare the side-wall thickness in Fig 4.20 and Fig 4.21).

4.4.3 Experimental measurement

4.4.3.1 Experimental platform

Experiment was conducted to assess the influence of appropriate acoustic frequency on the sensitivity of the SAW-PA sensor. As shown in Fig 4.22, it consists of a nanosecond pulsed laser of 1 mJ pulse energy focussed on the fluid contained in the PDMS microfluidic channel. The pulsed laser at a wavelength of 532 nm was illuminated at a repetition rate of 10 Hz. The laser was focused into a spot-size of 100 μm in the channel. Fig 4.22(b) shows the schematic of the microfluidic channel used in the experiment. The PDMS was trimmed along the width at the area overlapping the IDT. The PDMS side wall thickness (T_s) is 1mm. The channel region has a width (W) of 500 μm and depth (H) of 350 μm . The dimensions of the microfluidic channel used in the experiment follows the simulation shown in Fig 4.20. Carbon black ink

is used as the sample fluid for the PA generation owing to its high optical absorption over a wideband. For this study, two-port SAW delay lines with resonant frequencies at 10 MHz and 13 MHz were used. Both the devices had 20 pairs of electrodes with a split-electrode configuration. The device was characterised using the E5061B ENA Series Network Analyzer. Fig 4.23(a) shows the measured S_{11} for the two devices. The signal obtained from the SAW device was amplified using a pre-amplifier (Pana-metrics 5077PR) with 49 dB gain, followed by data acquisition using an oscilloscope (wavesurfer from Lecroy) which was triggered by the laser illumination.

Figure 4.22: (a) The experimental setup for measuring the SAW-PA signal. C1: collimating lens; BS: beam splitter; M1: mirror; C2: objective lens. The SAW device with the microfluidic chip is zoomed to show the physical device. (b) Schematic of the microfluidic channel. For illustrating the channel, the top part of the microfluidic chip is separated. Note that the channel has been trimmed. Cross-section along the dotted line is shown, T_s indicates the PDMS side wall thickness; W and H represent the width and height of the microfluidic channel respectively.

4.4.3.2 Frequency response of the SAW-PA sensor

The accuracy of the numerical simulation was verified by comparing the sensitivity of the SAW-PA signals to different IDT frequency. According to the simulation results shown in Fig 4.20, the maximum surface displacement occurs at a frequency of 13.4 MHz. Hence selecting the SAW device with centre frequency at 13 MHz is expected to give better sensitivity compared to the device at 10 MHz. Fig 4.23(b) shows the output voltage obtained from the two SAW devices. It is clearly observed from the voltage amplitudes that the SAW device at 13 MHz has a higher sensitivity to the PA signal generated inside the microfluidic channel. The output signal was averaged over 20 sweeps and normalised to the laser intensity measured using a photodiode.

Figure 4.23: (a) Measured S11 of the SAW devices. The resonant frequency of the devices is 13 MHz (Dev13M) and 10 MHz (Dev10M). (b) Transient SAW-PA signals obtained from the SAW devices. Note that the signal from the Dev13M is shifted vertically by 2 V to improve the clarity.

4.4.4 Sensitivity calculation

For the simulation model shown in Fig 4.20, the simulation results shows that for a heat input of 1W/m^3 , the output voltage obtained is 4×10^{-14} V. We consider a reference squaraine dye with applications in detecting analytes such as aminothiols in blood [169]. The optical absorption coefficient ($\alpha = \epsilon C$) for the squaraine dye with molar extinction coefficient (ϵ) of $10^5 \text{ M}^{-1}\text{cm}^{-1}$ and a concentration (C) of 1 nM is 10^{-4}cm^{-1} . The heat generated per unit volume (Q) by a pulsed laser of 1 ns (t_s) duration is given by:

$$Q = \alpha F / t_s \quad (\text{Eq. 4.7})$$

where F is the fluence of the laser (=Energy / irradiated area). For a laser energy 1 mJ and laser spot size of $100 \mu\text{m}$ (diameter), the heat generated = $1.27 \times 10^{10} \text{ W/m}^3$. On multiplying the heat (Q) with the output voltage generated by the heat input of 1 W/m^3 , an electrical potential of $508 \mu\text{V}$ is obtained. We have verified that our model is linear, with the heat input linearly proportional to the electrical potential. Thus, the device has a sensitivity of $508 \mu\text{V/nM}$ at 13.4 MHz. The use of a narrow band SAW-IDT designed to the 13.4 MHz reduces noise and hence becomes a very sensitive detection method.

4.5 Conclusion

In conclusion, we demonstrate a novel integrated SAW-PA sensor platform for a couplant-free, simultaneous optical and mechanical characterization of fluids flowing through a microchannel. The PA generated longitudinal acoustic wave is mode converted into Rayleigh SAW at the interface of the liquid-LiNbO₃ substrate. By exploiting the piezoelectric substrate as the medium for acoustic wave propagation, the requirement of a coupling layer (water) for the ultrasound transducer was eliminated in this platform. Using analytical modelling, we show that a high mode conversion efficiency of 80 percent is possible by optimal designs of the SAW-PA device. The optical charac-

terization capability of the SAW-PA device was demonstrated by performing the optical absorption spectroscopy and measuring the concentration of the fluid sample in the microchannel using two standard dye solutions. We further investigated the effects of viscosity on the SAW-PA signal to exhibit the ability of the platform to detect mechanical parameters of the fluid, which is otherwise difficult to detect using standard optical protocols. The sensor was also successfully applied to study the SAW-PA response of plasmonic nanoparticles by selecting various sizes of GNPs and GNRs as a model system. The sensitivity to the nanoparticles size and shape was also illustrated. In future, the SAW-PA platform can also be applied for bio/chemical sensing by detecting the shift in optical absorption spectra, which is induced by the receptor-analyte binding. Further, we implemented a finite element model for the SAW-PA sensor using COMSOL multiphysics 4.4. The PDMS microfluidic channel behaves as a rectangular resonator with multiple modes along the width and height of the channel. The modelling results show that maximum mode conversion efficiency (longitudinal to surface acoustic wave) is obtained at a frequency in which the pressure is directed parallel to the piezoelectric substrate. SAW devices designed at this frequency will have the highest sensitivity to the PA signal inside the microfluidic channel. The resonant behaviour of the PA signal in a microfluidic channel makes the high Q SAW device potentially an optimal sensor for detecting PA waves induced in a microfluidic channel. In conclusion, we show that the wideband PA signal is converted to a narrowband signal due to resonance inside the microfluidic channel and existence of modes parallel to the substrate, satisfies the condition for maximum acoustic coupling to SAW and alleviates a wedge structure

Chapter 5

Integrated microfluidic system using SAW for real-time separation and detection of particles on a single substrate

In this chapter, we introduce a microfluidic system seamlessly integrating separation, concentration, and detection of particles on a single piezoelectric (128° YX LiNbO₃) chip. The existing capabilities of the standing surface acoustic waves (SSAW) for high-efficiency separation of particles and cells is utilised along with a single IDT based particle aggregation to preconcentrate the sample before sensitive detection. The detection scheme involves sensing the surface acoustic waves (SAW) generated by the optically induced mechanical resonance of the photoacoustic(PA) wave produced by the particles inside the microfluidic cavity. As a proof of concept study, the developed integrated microfluidic system was applied to separate 3- and 10- μm -diameter polystyrene particles with an efficiency of separation above 95 %. The separated 10

μm particles were directed into the sensing chamber, where a single SAW was used to concentrate the particles before real-time quantitative detection. The sensor presented a reliable ($R^2 = 0.98$) detection of the $10\ \mu\text{m}$ particles down to 7 particles in $10\ \mu\text{L}$ of the sample volume in 15 minutes.

5.1 Introduction

Emerging technologies utilising micro and nanoscale engineering have profound applications in the health and point of care theranostics. Therefore, developing such low-cost diagnostic devices which follow a rapid and sensitive detection protocol receives prime attention. Currently available rapid diagnostic tests (RDTs) which possess cheaper and quick response time are limited mainly by the low detection limit (for example 100 parasites/L) and lack of quantification [170]. On the other hand, sensitive and selective detection methods such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) require expensive equipment and trained professionals to perform the tests. In this context, integrated microfluidic systems combining microfluidic actuation with various sensing technologies to realise point-of-care (POC) devices is essential [171–173]. In addition to the benefits of miniaturisation, such devices offer low power consumption profile with higher sensitivity. Kong et al. [173] demonstrated the use of deformability separation techniques to enrich infected red blood cells and magnetic resonance relaxometry sensing for early malarial detection at a level of 0.0005 %. Similarly, Tokel et al. [171] demonstrated the merging of microfluidics with plasmonic sensing approaches to realise a label-free detection platform. Thus, among other parameters, sample enrichment and detection are vital steps to accomplish an efficient integrated microfluidic system.

In the field of microfluidics, surface acoustic wave (SAW) technology has been receiving much attention over the past several years [97, 99, 174, 175]. The nanometer scale vibrations excited by metal electrodes on piezoelectric substrate

(128° YX LiNbO₃) couples to the fluid and perform various microfluidic manipulations. Exposing a droplet to an asymmetric SAW distribution causes rapid (in few seconds) particle/cell concentration within it. The interaction of acoustic streaming and acoustic radiation forces causes the particles to aggregate within a droplet [176, 177]. The technique was utilised by performing nanoparticle aggregation within the solution to aid surface-enhanced Raman scattering (SERS) detection [178]. Furthermore, the primary acoustic radiation force (ARF) exerted by the standing surface acoustic wave (SSAW) was utilised for high-efficiency separation of particles and cells. Reported results [52, 100] demonstrated segregation of particles with 97-99 % efficiency and rare cancer cells from normal WBCs with a recovery rate over 83% [45]. The separation performance can be further improved by optimising the flow rate, input power and channel dimensions. Though many research works were focussed on separating rare clinical samples from the bulk using SAW, the downstream detection still relies on microscopes and cell counters like haemocytometers which have been performed off-chip [52]. Sample loss while transferring between successive stages is the risk associated with such schemes [179]. In this scenario, a microfluidic platform integrating the high-efficiency SAW separation and real-time quantitative detection is seldom reported, which could be useful for segregate and quantitatively detect bio-relevant substrates.

In this Chapter, we present a SAW based integrated microfluidic platform for sample separation, concentration and detection. In this platform, a mixture of two different particle sizes is first introduced into a SAW separation stage. Particles with larger size are separated and transported by the flow into the outlet chamber for detection. The response time and sensitivity of the sensor were improved by utilising the SAW to induce particle concentration in the microfluidic reservoir. Further, the pulsed laser was irradiated on the concentrated particles for detection. The sensing principle relies on our recently developed optically induced acoustic resonant sensing platform: photoacoustic (PA) wave induced SAW (SAW-PA). [153] The integrated platform was

used to demonstrate the separation of polystyrene beads of two different sizes 3- and 10 μm -diameter. The separated 10 μm particles inside the microfluidic cavity were detected using the SAW-PA sensor. The sensor detected 7-200 particles inside a 10 μL of fluid in 15 mins.

5.2 Materials and methods

5.2.1 Device design and operation

A schematic of the integrated microfluidic platform is shown in Fig 5.1. The integrated device is structurally divided into three parts. The first part is a piezoelectric substrate (128° YX LiNbO₃, lithium niobate) which encompasses the metal electrodes (IDT, interdigital transducers) for the actuation and sensing. The second part consists of a PDMS microfluidic channel. The main channel is 200 μm high and 200 μm wide. The PDMS channel is bonded to the substrate using oxygen plasma. The third part consists of a 532 nm, nanosecond (1nsec) pulsed laser that operates at a pulse repetition frequency of 10 Hz. The laser irradiates on the separated particles to excite the SAW-PA, which is detected using the sensing IDTs. The pulsed laser illuminates the sensing chamber from the top with a laser fluence of 31 mJ/cm^2 for 200 μm spot size. An optical microscope was used to visualise the particles inside the sensing chamber and a translation stage allowed the device to be moved to the site for the laser irradiation. The pulsed laser was split into two outputs using an optical fibre and focussed into the active(G) and reference(E) sensing cavities. The laser was targeted to the concentrated particles using a mechanical scanner. The SAW device was held on a platform and translated in the horizontal direction by using two DC servo motors (KDC101, Thorlabs) for the X and Y directions. The step size of each scan points was set to 50 μm . Two stages of low noise amplifier (ZFL-1000LN+) were cascaded to obtain a gain of 40 dB gain. Each output from the IDTs was connected to the amplifier,

followed by data acquisition using an oscilloscope (WaveSurfer from Lecroy).

Figure 5.1: (a) Schematic diagram of the SAW-PA integrated microfluidic device. The particles are injected through the centre inlet. The sheath flow through the outer inlets focuses the particles in the main channel. The particles of two different sizes (3- and 10 μm) are separated using the Tilted-angle standing surface acoustic wave (taSSAW) generated by interdigital transducer (IDTs)-A. The larger particles flow downstream into the sensing reservoir-G while the smaller particle moves to the waste reservoir-F. E-denotes the reference cavity, which is filled with DI water. The IDT- B concentrates the particles (10 μm) inside the sensing cavity. Further, the pulsed laser irradiation on the sensing and reference reservoir generates a photoacoustic (PA) signal, which resonates inside the polydimethylsiloxane (PDMS) reservoir and mode converts into a SAW. The IDTs- C(active) and D (reference) detects the PA-induced SAW. (b) The IDTs inclined at an angle (15°) to the direction of fluid flow, used to separate the particles (red and green coloured spheres inside the channel represent the large and small sized particles respectively). (c) The IDTs produces SAW that interacts with the particles inside the reservoir and concentrates them. (d) The laser irradiates on the particles to generate a SAW, which is detected by the IDT

5.2.1.1 Experimental setup for the sensing stage

The experimental setup for the sensor is illustrated in Fig 5.2. The pulsed laser is set to the external trigger mode. A personal computer (PC) provides a trigger signal to the pulsed laser and the DC servo motors (controlling the mechanical scanning stage). The laser synchronisation signal triggers the oscilloscope to acquire the SAW-PA signal obtained after amplification. The raw data received from the oscilloscope is stored on the PC. The device is translated in the horizontal direction by the scanner to the laser scanning region for sensing. A combination of a collimator lens (C1), neutral density filter (NDF) and a condenser lens (Con1) is used to focus the light into the optic fiber. A microscope working in the reflection mode is used to observe the particles inside the reservoir.

Figure 5.2: Schematic of the experimental system used for SAW-PA sensing of particles

5.2.2 SAW device fabrication

The fabrication of SAW device involves photolithography, metal deposition and lift-off process. The lithium niobate wafer was first treated in a hexamethyldisilazane (HMDS) oven to promote photoresist (PR) adhesion. A layer of PR (AZ9260, Microchem) was spin-coated on the wafer, placed in a hot-plate (for two mins) and then exposed to a UV light source to develop the pattern. The patterned wafer is dipped in a PR developer solution (AZ400K). A double layer of metal (Cr/Au, 20 nm/200 nm) was deposited on the wafer using magnetron sputtering. Subsequently, the wafer was dipped in acetone for lift-off and forms the IDT.

5.2.3 SAW device characterization

The frequency response of the fabricated SAW IDTs is measured using the E5061B ENA Series network analyser. The S-parameters obtained for the different sets of IDTs in the integrated device are shown in Fig 5.3. Fig 5.3 - (a) and (b) shows the S11 measurement obtained for a single port of the IDT used for separation and concentration respectively. Fig 5.3(c) and 5.3(d) shows the S11, S12 and S22 measurement for one pair of sensing IDTs designed for 10 MHz and 235 MHz centre frequencies

5.2.4 Functionality of the integrated device

A single SAW substrate incorporates the actuation and sensing functionalities. Functionally; the SAW device is divided into three stages.

5.2.4.1 Design of the separation stage

The first part consists of a Tilted-angle standing surface acoustic wave (taS-SAW) [52] for high-efficiency separation of particles based on their difference in size.

Figure 5.3: S-parameter obtained for different SAW devices. (a-b) corresponds to the S11 parameter of the SAW device used for separation and concentration stage respectively. (c-d) shows the reflection coefficients (S11, S22) and insertion loss (s12) of the devices for sensing at 10 MHz and 235 MHz respectively.

The IDTs had ten pairs of electrodes with constant electrode spacing and finger-overlap of $40\text{ }\mu\text{m}$ and 2.5 mm respectively. The SAW propagates in an orthogonal direction to the usually preferred X-propagation direction of the substrate as shown by the IDT-A in Fig 5.1(a). The sound speed in this direction is slightly lower and equals to 3680 m s^{-1} [180]. According to the design, the fundamental frequency for SAW generation exists at 46 MHz which is observed in the S11 plot (see Fig 5.3(a)). However, at 81.7 MHz there exists a stronger resonance peak. This frequency is selected for the separation experiment. Coherent AC signals which were generated by an RF signal generator (Tektronix, AFG 3252) and amplified by a power amplifier (Minicircuits,

ZHL-100W-GAN+) were applied to the IDTs to form a standing surface acoustic wave (SSAW) within the microfluidic channel. The input power was set to 24 dBm. The IDTs were oriented at an angle of 15° to the flow direction through the main channel. A schematic of the separation stage is shown in Fig 5.1(b). Particles of size 10- and $3\text{ }\mu\text{m}$ were introduced into the central inlet, and they were converged towards the centre of the main channel by the exterior sheath flows. The particles were introduced into the main channel at a flow rate of $\sim 1.2\text{ mm/s}$. Continuous flow was generated by connecting the inlet to syringes driven by NE-1000 syringe pumps.

5.2.4.2 Design of the particle concentration stage

The taSSAW separated particles migrated towards the sensing and the waste outlets. The larger particles collect in the sensing outlet. The flow was cut-off once the sensing outlet was filled with $\sim 10\text{ }\mu\text{L}$ of sample volume. Before sensing, the particles were concentrated inside the sensing chamber using a single SAW IDT, which forms the second part of the integrated device. Fig 5.1(c) shows the schematic representation of the particle concentration in a fluid filled cavity using a single SAW IDT. The IDT used for aggregation consists of ten pairs of electrode with a pitch (p) and width of $199\text{ }\mu\text{m}$ and 4 mm respectively. The SAW propagates in an orthogonal direction to the usually preferred X-propagation direction of the substrate as shown by the IDT-B in Fig 5.1(a). The SAW device was excited at a resonant frequency of 15.8 MHz (refer Fig 5.3(b) for the s_{11} response of the IDT). The IDT was excited with a driving stage similar to that used for the taSSAW. Following the theoretical explanation published in work by Destgeer et al. [177], a standing surface acoustic wave (SSAW) is critical for particle concentration. The SAW attenuation length (x_s) $>$ fluid size (radius, r_d) should be satisfied for attaining particle concentration. This condition ensures that the travelling wave from the IDT reflects back from a boundary before it attenuates. The back-propagating wave interferes with the forward travelling wave to generate an SSAW. Following the design criteria mentioned above (refer to Fig 5.4 for the dimensions and design of the

open-chamber), fluid was filled in an open-chamber surrounded by polydimethylsiloxane (PDMS). The PDMS microfluidic channel had an air-cavity surrounding the fluid in the chamber. The cavity acts as a boundary and ensured that the surface waves gets reflected back before it traverses a distance more than or equal to the attenuation length (x_s). The attenuation length on the substrate at it interacts with the fluid is given by $x_s = \rho_s c_s \lambda_s / \rho_f c_f$ where $\rho_s = 4630 \text{ kg m}^{-3}$ and $\rho_f = 998 \text{ kg m}^{-3}$ are the densities of the substrate (LiNbO_3) and water respectively; $c_s = 3680 \text{ m s}^{-1}$ and $c_f = 1480 \text{ m s}^{-1}$ are the sound speeds in the substrate and water respectively and λ_s is the wavelength of the acoustic wave in the substrate. At a frequency of 15.8 MHz, the attenuation length (x_s) = 2.7 mm. The cavity was designed such that the surface waves encountered a soft boundary condition (PDMS-air interface) at a distance of 2.6 mm. The equivalent r_d is 1.3 mm. The wave reflects before travelling its attenuation length, and hence a standing wave is generated in the fluid-filled open cavity to concentrate the particles. Additionally, the k -factor defined by $k = \pi d_p / \lambda_f$, where d_p is the diameter of the particle and λ_f is the acoustic wavelength in the fluid, has a value of 0.3. The condition that $k < 1$, to create particle concentration in the centre of the fluid is also satisfied. Hence, the design of the open-cavity satisfies the two conditions that cause particle concentration in the centre of the sessile droplet. The validity of the design criteria mentioned above is further confirmed by performing the particle concentration experiment with the microfluidic channel having an open cavity of a larger diameter (5 mm). The acoustic attenuation length was less than the cavity dimension. Following the theory explained above, the particles should not concentrate.

Microfluidic cavity design for SAW concentration: The cavity aims to meet the conditions for generating standing wave to cause particle concentration. Fig 5.4 depicts the channel design with the sensing and reference cavity. The cavity design ensures that the travelling surface acoustic wave (TSAW) reflects from a boundary at a distance (2.6 mm) less than its attenuation length (3.2 mm), thus forming a standing

acoustic wave inside the sensing cavity

Figure 5.4: The design of the microfluidic channel. The boxes represent air-cavity (not filled with PDMS), only one of them is highlighted. The travelling surface acoustic wave(TSAW) from the concentrator IDT travels in the direction shown by the arrow (blue in colour). The TSAW reflects from the cavity of size 2.6 mm, which is at a distance less than the SAW attenuation length for a frequency of 15.8 MHz (3.2 mm). The sensing cavity filled with the fluid has a diameter of 2 mm.

5.2.4.3 Design and working principle of the sensing stage

The sensing constitutes the last stage of the integrated device. It consists of two pairs of IDTs: one for the sensing the particles (indicated by IDTs-C in the Fig 5.1(a)) and the other as a reference (denoted by IDTs-D). The reference cavity is filled with DI water (see symbol-E in Fig 5.1(a)). The signal from the active-IDT is calibrated with the reference-IDT to account for variation in the laser intensity and other substrate changes including temperature effects. The SAW-PA sensor consists of ten pairs of split-finger IDTs with a uniform electrode width and spacing of $49.75 \mu\text{m}$ (Refer Fig 5.3

for the s-parameters of the sensing IDTs). The first and the most important criteria for designing the SAW-PA sensor is in selecting the optimum working frequency. The details about the significance of choosing the correct frequency were discussed in our previous work [154]. According to the theory of photoacoustics (PA), the laser excitation on a spherical particle gives rise to a wideband bipolar pressure wave with the centre frequency (f) provided by [86, 87].

$$f = \frac{v_s}{2R_s} \quad (\text{Eq. 5.1})$$

where v_s denotes the speed of sound in the polystyrene particle (2350 m s^{-1}) [181] and R_s is the radius of the particle. For a particle of size $10 \mu\text{m}$ (diameter), the centre frequency is calculated to be 235 MHz. From PA theory, it appears that designing a SAW transducer at 235 MHz is more sensitive. However, our previous work [154] signifies that the PA signal generated inside the microfluidic channel undergoes acoustic resonance and mode converts into a SAW. We performed a 2D numerical simulation using COMSOL Multiphysics 4.4 to determine the SAW-PA resonant frequencies (refer section 5.3.3.1 for details on the numerical simulation model). The frequency response study results revealed that the resonant frequencies were dominated at low frequencies. All frequencies above 50 MHz were strongly attenuated, explaining that the PA centre frequency was down-converted to a much lower frequency by the acoustic resonance within the microfluidic channel. To further validate the accuracy of the numerical simulation model, we fabricated sensors with a centre frequency of 235- and 10- MHz and used it to sense an equal number of particles in the cavity (see Fig 5.5 for the transient SAW-PA signals from the sensors). The high-frequency SAW device (centre frequency of 235 MHz) consists of ten pairs of IDTs with an IDT-pitch of $8.46 \mu\text{m}$ (diameter). The experimental results confirm that the SAW IDT at 10 MHz was more sensitive than the 235 MHz SAW IDT to detect the SAW-PA signals from the ten particles. The peak amplitude of the 10 MHz device is ten times larger than the 235 MHz SAW device. We developed a model to establish the relationship between

Figure 5.5: SAW-PA signals obtained from two SAW devices with a centre frequency of 10 MHz and 235 MHz, for the same concentration of 10 μm (diameter) particles.

the measured SAW-PA signal and the particle count. The SAW-PA amplitude can be expressed as:

$$A_{SAW-PA} = K_{SAW-PA}^1 N_p + K_{SAW-PA}^2 + n_A \quad (\text{Eq. 5.2})$$

$$K_{SAW-PA}^1 = \Gamma_p \eta_{thp} \mu_{ap} F K_{PA-SAW} K_{IDT} K_{amp} \quad (\text{Eq. 5.3})$$

$$K_{SAW-PA}^2 = \Gamma_f \eta_{thf} \mu_{af} F K_{PA-SAW} K_{IDT} K_{amp} \quad (\text{Eq. 5.4})$$

where K_{SAW-PA}^1 and K_{SAW-PA}^2 is a constant value, which denotes the SAW-PA output signal from a single particle and background (including the water and substrate) respectively. It includes the Grueneisen parameter (Γ), heat conversion percentage (η_{th}), absorption cross-section (μ_a), optical fluence (F), longitudinal wave generated by PA inside the fluid to surface acoustic wave conversion efficiency (K_{PA-SAW}), acoustic

to electrical conversion efficiency of the IDT (K_{IDT}) and amplifier gain (K_{amp}). The subscript p and f denotes the particle and the fluid respectively. N_p is the number of particles. The power spectral density (PSD) of a signal $y(t)$ can be expressed as:

$$E\{y^2(t)\} = \frac{1}{2\pi} \int_{-\infty}^{+\infty} S_{yy}(j\omega) d\omega \quad (\text{Eq. 5.5})$$

where $E()$ is the mean and $S_{yy}(j\omega)$ is the power spectral density of $y(t)$. Let the signal $y(t)$ be represented as in (Eq. 5.2):

$$\begin{aligned} E\{y^2(t)\} = & (K_{SAW-PA}^1 N_p)^2 + (K_{SAW-PA}^2)^2 + \\ & E(n_A^2) + 2K_{SAW-PA}^1 N_p K_{SAW-PA}^2 + 2K_{SAW-PA}^1 N_p E(n_A) \\ & + 2K_{SAW-PA}^2 N_p E(n_A) \quad (\text{Eq. 5.6}) \end{aligned}$$

where n_A is the generated output noise.

$$E\{y^2(t)\} = (K_{SAW-PA}^1)^2 N_p^2 + (2K_{SAW-PA}^1 K_{SAW-PA}^2) N_p + (K_{SAW-PA}^2)^2 + E(n_A^2) \quad (\text{Eq. 5.7})$$

From Eq. 5.5 and Eq. 5.7, the PSD has a quadratic relation with the number of particles (N_p). The validity of the linear scaling of the SAW-PA amplitude with the number of particles (N_p) exists only if the SAW-PA signals generated from each particle undergoes constructive addition. The source of the SAW signal originates from the PA generated longitudinal wave impinging on the substrate. We can consider each point on the substrate below the sensing cavity as a source of SAW excitation. If the particles are separated from the substrate at a vertical distance, h_p , the signals would undergo linear addition by particle count. Eq. 5.8 shows the distance at which a transition from near field to far field occurs.

$$h_p > \frac{D^2}{4\lambda} \quad (\text{Eq. 5.8})$$

D is the diameter of the source. Only the particles irradiated by the laser generates the SAW-PA signal. Hence we consider the maximum value of D as the laser spot size. λ denotes the acoustic wavelength ($=v_s/f_0$), v_s is the sound speed in water and f_0 is the centre frequency of the SAW device. Satisfying Eq. 5.8 for the particle separation from the substrate ensures that the particles are located in the far-field. Hence, each particle would constructively add the signals at any point on the substrate. For a SAW IDT at centre frequency 10 MHz (f_0) and a laser spot size of 200 μm (D), the transition distance equals 68 μm . Due to the SAW concentration effects, the particles are aggregated at a height (h_p) near the fluid-air interface inside the sensing cavity (~ 2 mm). The concentration ensures that the condition mentioned in Eq. 5.8 is satisfied. The PA signals generated by the particles residing in the far-field will, therefore, undergo constructive addition. The linear scaling of the SAW-PA signal amplitude with increasing number of particles described in Eq. 5.2 is, therefore, valid

5.2.5 Materials

Ten- and three-micron diameter black coloured polystyrene beads (polyscience), with a concentration of $\sim 4.55 \times 10^7$ and 1.68×10^9 particles/mL (2.5 % solids-suspension) were used to characterise the SAW integrated platform. The black dye has a broad absorption spectrum across the visible light wavelength, with an absorbance value of ~ 0.7 .

5.3 Results and discussion

5.3.1 Separation of particles

The taSSAW approach demonstrated separation efficiency of 100% and > 95% for the 10- and 3 μm beads respectively. The separation of particles can be traced from the stacked images in Fig 5.6. The large particles (10 μm) were collected

in the upper outlet cavity (sensing reservoir), while the flow transported the smaller particle ($3\text{ }\mu\text{m}$) to the lower outlet (waste reservoir). The ratio of the flow rate through the inlets was maintained in the ratio of 10:1:2. The upper inlet had the largest flow rate to prevent particles from flowing into the sensing outlet without turning on the SAW. The particle flow through the centre inlet has the lowest rate. The flow-rates

Figure 5.6: Separation process for the 10- and $3\text{ }\mu\text{m}$ diameter polystyrene beads in the outlet region of the microfluidic channel. (a) Shows the particle flowing to the waste reservoir when the SAW is turned OFF and (d) when the SAW is turned ON, the $10\text{ }\mu\text{m}$ particles are pushed to the sensing outlet by the SAW acoustic radiation force. (b and d) shows the zoomed-in view of the waste outlet and the sensing outlet, respectively.

ensured that the particle flow was sandwiched between the two outer sheath flows, closer towards the bottom outlet. It was observed that increasing the power above

25 dBm would push the 10 μm particles into the upper part of the main channel and remain trapped. On turning off the SAW input, the trapped particles will continue with the flow into the sensing outlet. Further optimisations on the flow rate and the input SAW could achieve 100% separation efficiency for both the particle sizes.

5.3.2 Concentration of particles

The syringe pump was stopped when the separated particles filled the upper outlet. The cavity was filled to a volume of approximately 10 μL in less than 10 mins. Fig 5.7 presents screen shots of the 10 μm particles before and after the SAW exposure. As shown in Fig 5.7(a), particles are randomly positioned inside the cavity, even at various heights, before the SAW exposure. By exposing the suspension of 10 μm particles in water to a 15.8 MHz SAW at input amplitude of 26 dBm, the particles tend to aggregate, though not at the centre. The condition $K < 1$ and $x_s > r_d$ (where r_d in this case denotes one-half the sum of the cavity diameter and the width of the PDMS, 1.3 mm and $x_s=3.2$ mm) are satisfied in our design. According to the theory developed by Destgeer et al. [177], a droplet with particle satisfying the above two conditions will cause the particles to concentrate in the centre of the droplet. The shift in the location of the concentrated particles from the centre of the cavity could be due to the acoustic streaming force (ASF) pattern generated inside a PDMS cavity being different from a sessile droplet. Fig 5.7(b-d) shows that a small (<10) to large (200) number of particles are concentrated using the SAW. Image analysis software (ImageJ, NIH) was used to count the larger number of particles(>100). A SAW exposure causes the particles to aggregate closer to the periphery of the droplet. The concentration process was relatively slow compared to the time taken for aggregation within a sessile droplet (few sec [177]). The concentration-time for an input power of 26 dBm was 40-50 secs. The longer response time can be attributed to the weaker response of the IDT at the operating frequency (see Fig 5.3 for the S11, which is -4 dB). For the subsequent sensing stage, the concentration attained is sufficient. The aggregation

Figure 5.7: Ten micron (diameter) particles at different levels of concentration inside the sensing cavity. Screenshots of the particle-laden open-cavity before (a) and after (b-d) SAW exposure for a sufficient period to obtain the particles concentration at the required location.

Figure 5.8: Particles of size $10\ \mu\text{m}$ placed inside an open cavity (surrounded by PDMS) of size 5 mm exposed to a 15.8 MHz SAW. Particles do not concentrate, unlike a smaller sized cavity (2 mm) as shown in the figure before ($t=0\ \text{s}$) and after ($t=2.6\ \text{s}$) SAW exposure

helps to satisfy the two condition required: 1) the particles has to remain at a far-field distance. The particle aggregates at a distance closer to the free surface ($\sim 2\ \text{mm}$). 2) The mechanical scanner operates at 1 Hz, the response time of the sensing stage can be minimised by reducing the scanning area. Particles are concentrated within half of the cavity as shown in Fig 5.7(b-d). During the measurement, only half of the

cavity containing the particles are scanned. While concentration is achieved using a $10\text{ }\mu\text{m}$ particle inside a cavity of diameter 2 mm, the particles fail to aggregate within the cavity of 5 mm diameter. The particles circulate under the acoustic streaming forces inside the fluid. The particle arrangement before and after SAW exposure in a 5 mm sized cavity is shown in Fig 5.8. SAW exposure at 15.8 MHz for 2.6 s at 26 dBm causes streaming inside the cavity, but the particles do not aggregate. The attenuation length at 15.8 MHz is smaller than the dimension of the cavity, and as a result, there is no standing wave generated to create an aggregation of particles. Optimisation of the SAW frequency and cavity dimensions will reduce the concentration-time further.

5.3.3 Sensing of particles

5.3.3.1 Numerical simulation

Two-dimensional (2-D) frequency domain simulation is performed using COM-SOL Multiphysics. The numerical simulation is carried out with two objectives: (a) To investigate the relationship between the number of particles (N_p) and the amplitude of the piezoelectric surface displacement. (b) Study the SAW-PA frequency response of the particle to design the IDT for the sensor. The SAW concentration stage aggregates the particles in a horizontal 2-D plane (X-Z plane) at a certain height (h_t) from the substrate. We utilise this functionality to simplify the numerical model. In the simulation, particles are arranged along the X-direction (see Fig 5.9 for the axis direction) in a linear array. We assume that the particles arranged in the Z-direction focused at a far-field distance from the substrate will linearly contribute to the acoustic displacements with N_p . The assumptions simplify the simulation to a 2-D model (X-Y plane). Fig 5.9 shows the simulation geometry. It consists of a PDMS microfluidic channel with an open-cavity of width $w_1 = 2\text{ mm}$ and height $h_1 = 3\text{ mm}$. The PDMS channel is trimmed at the bottom with a thickness $w_2 = 300\text{ }\mu\text{m}$. The trimming ensures that the cavity length for concentrating the particles as shown in Fig 5.4 is 2.6 mm.

The PDMS at the upper section extends for a longer length. The infinite domain of the PDMS is modelled using a perfectly matched layer (PML) which causes the wave to pass through without reflection from the sides. The PML is represented at the ends of the PDMS and piezoelectric domain. The polystyrene particles are arranged in a linear array at a height $h_t = 2$ mm from the substrate surface.

Thermoacoustics, Frequency domain physics was used to solve the pressure generated by the input heat from the polystyrene particles. Each polystyrene particle represented a heat source of 1 W/m^3 . The top boundary of the water filled domain is modelled as a wall with normal impedance boundary condition and adiabatic thermal condition. The impedance offered by air is set to 420 Pa s/m [182]. The pressure from the thermoacoustic domain is coupled to the pressure in the PDMS through the thermoacoustic-pressure acoustics boundary condition. Further, the pressure is coupled to the piezoelectric domain by the Multiphysics coupling nodes of the thermoacoustic/pressure acoustics- structure interaction. A soft boundary condition is used at the PDMS boundary to model the PDMS-air boundary. Fig 5.10(a) gives the result of the frequency domain simulation. The colour plots in the PDMS /fluid and piezoelectric domain shows the pressure and acoustic displacement respectively at 10 MHz for 10 particles arranged at a height (h_t) of 2 mm (in Y-direction). The fluid is filled in a cavity of height 3 mm which is matched to the cavity dimension used in the experiment. In the simulation, the particles are arranged linearly in the X-direction at a height $h_t = 2$ mm. Fig 5.10(b) shows the frequency response of the acoustic displacement at a point (close to the IDT) on the LiNbO_3 substrate. The magnitude of the acoustic displacements drops for frequencies above 50 MHz, which could be attributed to the mechanical resonances inside the PDMS cavity that attenuates the high-frequency modes. The acoustic displacement has the largest magnitude at the 9 MHz frequency. Further, the effect of particle count (N_p) on the SAW displacement was studied. The sum of the frequency response from 9-11 MHz is calculated as the sensing parameter, as it matches with the bandwidth of the IDT designed for the sensor. Fig 5.10(c) shows

Figure 5.9: 2D Geometry used for the simulation with the corresponding domains. The fluid channel of width $w_1=2$ mm and height $h_1=3$ mm used for the simulation. It consists of a PDMS chip (grey), water (green) with polystyrene beads (black), piezoelectric transducer (blue, Lithium niobate, LiNbO_3) surrounded by air. The polystyrene beads are placed at a height, h_t (2 mm) from the surface of the piezoelectric material. Perfectly matched layers (PML) are placed at the ends of the piezoelectric and PDMS domains to avoid reflections from the ends. An impedance boundary condition (red dotted line) at the water-air interface and soft boundary condition at the PDMS-air interface (continuous blue line) represents the boundaries in the model. The figure is not drawn to scale.

that a linear relationship exists between N_p and the sensing parameter. From Eq. 5.2 a linear relationship between particle count and SAW displacement is expected, which aligns the theory and the numerical simulation.

5.3.3.2 Experimental results

The laser beam of spot size $200\text{ }\mu\text{m}$ was focussed into the concentrated particles for sensing. The alignment of the laser focus into the particles was achieved by mechanical scanning. The scanner was moved in the horizontal plane at a step size of $50\text{ }\mu\text{m}$. At each scanning point, the pulsed laser at the repetition rate of 10 Hz was irradiated. Signals were averaged for ten cycles. After SAW actuation, a concentration of 1-200 particles per $10\text{ }\mu\text{L}$ of the solution was aggregated within one-half of the PDMS

Figure 5.10: Numerical simulation results of particle detection using photoacoustic induced surface acoustic wave. (a) Colour plots of the 2-D simulation illustrating the acoustic pressure in the water-PDMS domain and y-axis displacement in the LiNbO₃ substrate, at 9 MHz for ten particles located at a height (h_t) of 1 mm. (b) The frequency response of the displacement amplitude at a point on the surface of the substrate from 1 MHz-300 MHz, with peak amplitude at 9 MHz. The inset shows zoomed in frequency response from 1 MHz- 50 MHz. (c) Linear response of the acoustic displacement to the number of particles ($R^2=0.99985$).

cavity. Hence, the scanning area is limited to half of the cavity size, equal to an area of $1500\mu\text{m} \times 750\mu\text{m}$, with 450 scanning points. The total response time is the sum of the scan time and signal averaging time. For a mechanical scanner of rate 1 Hz and signal averaging time of 1 sec for each point, the total response time is 15 mins. To demonstrate quantitative measurement of particle counts, we prepared solutions with different concentrations. A $10\mu\text{m}$ diameter particle with a stock concentration of

$\sim 4.55 \times 10^7$ particles per millilitre (2.5 % solids-suspension) was selected. The solution was diluted to prepare an initial concentration of ~ 200 particles per $10 \mu\text{L}$. The solution was further serially diluted by two times to obtain concentrations of 100, 50, 25, and 10 particles per $10 \mu\text{L}$.

5.3.3.3 Signal processing

The use of reference SAW calibrates the variation of laser energy and other substrate variations. The reference SAW alleviates the requirement of a photodiode to calibrate fluence variation. The signal obtained from the sensing SAW is normalised by the variations in the reference SAW signal. The SAW sensor IDT is designed with a centre frequency of 10 MHz with a bandwidth of 10 %. The sum of the power spectral density of the signal from 9-11 MHz is selected as the sensing parameter. The sensed parameter obtained from each scanned point is interpolated using MATLAB interpolation function (interp2). The interpolation is used to get a continuous range of values of the sensing parameter from the spatially discrete signals obtained from the mechanical scanning. The maximum value obtained from the interpolation function is selected as the quantitative metric for various concentrations. The scanning step size of $50 \mu\text{m}$ for a laser spot size of $200 \mu\text{m}$ satisfies the Nyquist sampling rate condition for interpolation. Fig 5.11 shows the experimental results for different particle counts. Fig 5.11(a) shows the transient waveform obtained from a single IDT for $10 \mu\text{m}$ particles at a concentration of c.a 155 particles per $10 \mu\text{L}$. The voltage waveforms were captured at the two sensing IDTs (IDT1 and IDT2). The sensor demonstrated a quadratic response ($R^2 = 0.98$) between the sum of power spectral density (PSD) in the 9-11 MHz (SAW-PA sensing parameter) and the particle concentration (varying from ~ 7 -200 particles per $10 \mu\text{L}$ range). The sensing parameter was normalised by the PSD calculated from the reference cavity. Hence, the variations in temperature and laser fluence are calibrated. The experimental response fits the analytical model presented in Eq. 5.7. The target fitting function was $Y = K_1 N_p^2 + K_2 N_p + K_3$, where K_1, K_2 and K_3

Figure 5.11: SAW-PA signal from 10 μm (diameter) polystyrene beads at different concentrations. (a) Voltage waveform for ~ 155 particles in 10 μL detected using the SAW-PA sensor. The inset shows the corresponding power spectral density. (b) In-band spectral power (9-11 MHz) for various particle counts at the two IDTs. The spectral power followed a second-order polynomial $Y = K_1 N_p^2 + K_2 N_p + K_3$, with R^2 value of 0.9828 and 0.9789 for IDT1 and IDT2 respectively.

are unknown constants. The actual particle count was obtained from the CCD camera images: by manually counting for particles at low concentration and using particle counting functionality in ImageJ for larger concentrations (~ 200 particles). For particles at the larger concentration shown in Fig 5.7(d), the concentration area was bigger than the spot size of $200\text{ }\mu\text{m}$. Fitting the output signal to the fitting curve shown in Fig 5.11, we obtain a particle count equal $\sim 155(\pm 3)$, where the deviation is obtained at IDT1 and IDT2, respectively. The calculated value was less than the total particle count of 194 (using ImageJ). The maximum number of particles fitting a circle of size $200\text{ }\mu\text{m}$ (using least square fitting) was found to be c.a 152 which is close to the particle count obtained by fitting the SAW-PA sensing parameter to the quadratic response curve. The response time of the sensor is limited currently by the mechanical scanner (1 Hz) and laser pulse repetition rate (10 Hz). An optical scanning mirror (in KHz, Thorlabs) and laser with higher repetition rate (100 Hz) can reduce the response time to less than a minute.

5.4 Conclusion

We have developed a SAW-PA integrated microfluidic system that combined standing surface acoustic wave based separation, particle concentration and sensing on a single LiNbO_3 substrate and demonstrated real-time quantitative detection of polystyrene beads. Though SSAW methods can achieve high separation efficiency ($\sim 100\%$), the downstream detection is currently performed off-chip cell counters and haemocytometers, which risks the possibility of sample loss while transferring. We demonstrate that a pulsed laser illuminating the separated particles can generate a photoacoustic induced SAW (SAW-PA), which can be analysed to perform a label-free quantitative detection of the sample in a few mins with a sensitivity of $\sim 7\text{-}200$ particles per $10\text{ }\mu\text{L}$ of the sample solution. The system can be extended to label free detection of cells from whole blood and other body fluids such as urine. A single cell level

sensitivity is possible by using optimising the SAW design and microfluidic channel dimensions to match the acoustic resonance inside the microfluidic channel.

Chapter 6

Conclusions and Future Work

In this chapter, we provide a summary of the work carried in this thesis and outline the significant contributions made. The chapter and dissertation will conclude with potential future works that could arise from these findings.

6.1 Conclusions

In this thesis, we commenced with an overview of lab-on-a-chip (LOC) systems and discussed an important microfluidic platform based on surface acoustic wave (SAW) devices. The capability to perform actuation and sensing makes the SAW device a potential candidate for realising an LOC. To explore the field of SAW in microfluidics, we first looked into the use of SAW as an actuator, where we conducted a comprehensive experimental and theoretical work related to the acoustic energy transmission to a fluid in a reusable microfluidic channel. Followingly, we proposed and demonstrated using extensive experimental and theoretical study, a novel sensor for characterising optical and acoustical properties of dyes and nanoparticles in a microfluidic channel. The sensor relies on detecting the SAW generated by photoa-

coustics (PA) in a microchannel. Later, by performing a detailed numerical modelling using finite element method (FEM) we unravelled the existence of optically induced acoustic resonance inside the microfluidic channel and efficient mode conversion of a longitudinal wave into SAW at certain frequencies dependent on the microchannels dimensions. Finally, we demonstrate an integrated microfluidic platform, which combines the microfluidic actuation (separation, concentration) and sensing (detection of particles) on a single lithium niobate substrate. The main contribution of this thesis are as follows:

- (i) Compared to other areas studied on SAW, less research effort has been concentrated on using a disposable superstrate for SAW microfluidic applications. The first complete study of SAW coupling to fluids in the microfluidic channel on disposable superstrate was revealed. We studied the influence of input voltage, frequency and coupling layer thickness on the acoustic energy induced mixing efficiency. The main significance of this work comes from the development of analytical models which can be used to optimise the acoustic energy transmission to the fluid and improve the mixing efficiency.
- (ii) We propose for the first time a SAW-PA device which detects the PA generated in a microfluidic channel with a SAW device. The existence of optoacoustic resonance inside the microfluidic channel converts the wideband PA into a narrow band signal, justifying the reason for using SAW (high-Q) as a sensitive detector. This label-free sensing platform facilitates the characterization of liquid samples by measuring both its optical and mechanical properties simultaneously. Furthermore, the usage of SAW as the receiver of the PA signal in this new design enables a couplant-free (water-free) PA signal sensing in a low cost and compact pathway.
- (iii) We propose a SAW-PA integrated microfluidic platform which can perform a label-free quantitative detection of the separated particles on the same piezo-

electric substrate. The sensor exhibited detection of the 10 μm particles down to 7 particles in 10 μL of the sample volume in 15 minutes. To the best of our knowledge, this is for the first time that a fully integrated microfluidic system using SAW is exhibited.

6.2 Future work

(i) Application of the integrated platform for detecting rare cells.

Though separation of rare circulating tumour cells was performed using a taS-SAW device, its applicability as a diagnostic device is limited due to the lack of an integrated downstream detection. In this scenario, the SAW-PA integrated microfluidic system is an excellent choice. This work requires optimisation of sensor to detect down to a single cell, as the rare CTCs are present in a concentration of ~ 100 cells/mL. Further, to achieve the throughput (process one mL of sample) for rare CTC detection, requires lowering the sensors response time to ~ 1 min for 10 μL of solution. The various possibilities could include; using optical scanning mirrors, high pulse repetition rate or by precisely controlling the position of cells in the channel using SAW based tweezers, which can even discard the use of scanning system.

(ii) Portable SAW-PA sensor:

The portability of the SAW-PA sensor is limited due to the requirement of having a bulky solid-state pulsed laser. In this work, we are exploring the use of laser diodes which are relatively simple, compact and inexpensive. However, the limitation with laser diodes is its limited pulse energy (for nanosecond time duration) of several microjoules. The sensitivity of the SAW device has to be improved to detect such weak PA-induced SAW signals.

(iii) Low concentration detection of analytes.

In Section 4.4.4 we calculated the sensitivity of the SAW-PA sensor to detect a squaraine dye to $508 \mu\text{V}/\text{nM}$. In this work, we could explore the limit of detection of the SAW-PA sensor. The lower response time (< 1 min) owing to lack of surface treatment and biofunctionalization along with high sensitivity (nM) could pave way to state-of-art performance improvement [76].

Appendix A

Lamb waves in a plate

Here we derive an equation for the displacement of a lamb wave excited in an isotropic plate shown in Fig A.1, following the derivation provided in [132].

The displacement of a material can be considered to be the sum of a scalar ϕ and vector potentials ψ , given by

$$u = \nabla\phi + \nabla \times \psi \quad (\text{Eq. A.1})$$

where the component $\nabla\phi$ and $\nabla \times \psi$ represents the longitudinal and the transverse components of the displacements. From vector identities, the curl of a gradient of a

Figure A.1: Isotropic Plate

scalar field $\nabla \times \nabla \phi$ and the divergence of the curl of any vector field $\nabla \cdot \nabla \times \psi$ is zero. Hence, $\nabla \phi$ should act in the direction of the wave propagation, forming a longitudinal wave. While, $\nabla \times \psi$ should act orthogonal to the propagation direction constiuting a transverse wave. The potentials satisfy the following wave equations.

$$\begin{aligned}\nabla^2 \phi - \frac{1}{V_L^2} \frac{\partial^2 \phi}{\partial t^2} &= 0 \\ \nabla^2 \psi - \frac{1}{V_T^2} \frac{\partial^2 \psi}{\partial t^2} &= 0\end{aligned}\tag{Eq. A.2}$$

where V_L and V_T denotes the phase velocities of the longitudinal and transverse waves respectively. The lamb wave is considered to be propagating in the x_1 direction, and considering there is no diffraction or variation along the direction of x_3 , with $\partial_3 \equiv \frac{\partial}{\partial x_3} = 0$. For the sinusoidal propagation, $\frac{\partial}{\partial x_1} = \partial_1 = -ik$. From Eq. A.1, we can write the displacement components as:

$$\begin{aligned}u_1 &= \partial_1 \phi + \partial_2 \psi_3 = -ik\phi + \partial_2 \psi_3 \\ u_2 &= \partial_2 \phi - \partial_1 \psi_3 = \partial_2 \phi + ik\psi_3\end{aligned}\tag{Eq. A.3}$$

Defining p and q as

$$\begin{aligned}p^2 &= \frac{\omega^2}{V_L^2} - k^2 \\ q^2 &= \frac{\omega^2}{V_T^2} - k^2\end{aligned}\tag{Eq. A.4}$$

The Laplacian operator is given by:

$$\nabla^2 = \frac{\partial^2}{\partial x_1^2} + \frac{\partial^2}{\partial x_2^2} = -k^2 + \frac{\partial^2}{\partial x_2^2}\tag{Eq. A.5}$$

Using Eq. A.2, Eq. A.4 and Eq. A.5, the functions ϕ and $\psi \equiv \psi_3$, satisfies the eqn:

$$\begin{aligned}\delta_2^2 \phi + p^2 \phi &= 0 \\ \delta_2^2 \psi + q^2 \psi &= 0\end{aligned}\tag{Eq. A.6}$$

A.1 Derivation of stress in an isotropic solid

For an isotropic solid, the elastic tensor c_{ijkl} can be represented by two independent constants referred to as Lamé constants λ and μ by the following equation:

$$c_{ijkl} = \lambda \delta_{ij} \delta_{kl} + \mu (\delta_{ik} \delta_{jl} + \delta_{il} \delta_{jk}) \quad (\text{Eq. A.7})$$

Since the tensors T_{ij} and S_{kl} representing the stress and strain tensors are symmetric, the number of independent elastic constants reduces from 81 to 36. Assigning value of 1 to 6 for the pairs (ij) and (kl) , we get the following relations:

$$\begin{aligned} c_{11} &= c_{22} = c_{33} = \lambda + 2\mu \\ c_{12} &= c_{23} = c_{13} = \lambda \\ c_{44} &= c_{55} = c_{66} = \mu = (c_{11} - c_{12})/2 \end{aligned} \quad (\text{Eq. A.8})$$

Using Hooke's law in an isotropic solid, the normal stress (T_{11}, T_{22}, T_{33}) can be written as:

$$T_{ii} = c_{iikl} S_{kl} \quad (\text{Eq. A.9})$$

substituting Eq. A.7 in Eq. A.9 with $j=i$, we get

$$T_{ii} = \lambda (S_{11} + S_{22} + S_{33}) + 2\mu S_{ii} \quad (\text{Eq. A.10})$$

and the tangential stress T_{ij} , with $i \neq j$ is given by:

$$\begin{aligned} T_{ij} &= \mu (\delta_{ik} \delta_{jl} + \delta_{il} \delta_{jk}) S_{kl} \\ T_{ij} &= 2\mu S_{ij}, i \neq j \end{aligned} \quad (\text{Eq. A.11})$$

From Eq. A.10 and Eq. A.11, we can define the stress tensor as:

$$T_{ij} = \lambda S \delta_{ij} + 2\mu S_{ij} \quad (\text{Eq. A.12})$$

where $S = S_{11} + S_{22} + S_{33}$ is the dilation. Since $S_{33} = 0$, the dilation is :

$$S = S_{11} + S_{22} = \delta_1 u_1 + \delta_2 u_2 = \nabla^2 \phi \quad (\text{Eq. A.13})$$

A.2 Derivation of mechanical displacement components of Lamb wave

The solution of Eq. A.6 should satisfy the stress free conditions, hence $T_{22} = T_2 = 0$ and $T_{12} = T_6 = 0$ at $x_2 = \pm h$. The normal stress $T_2 = T_{22}$ is defined by using equation Eq. A.12 and first of Eq. A.8.

$$\begin{aligned} T_2 = T_{22} &= c_{11}S - 2c_{66}S_{11} \\ &= c_{11}\nabla^2 \phi - 2c_{66}\partial_1 u_1 \\ T_2 &= c_{11}\nabla^2 \phi + 2c_{66}[k^2 \phi + ik\partial_2 \psi] \end{aligned} \quad (\text{Eq. A.14})$$

The last step of Eq. A.14 follows from Eq. A.3. Using Eq. A.2, Eq. A.5, Eq. A.6, we find

$$\rho\omega^2 = (k^2 + q^2)c_{66} \quad (\text{Eq. A.15})$$

From Eq. A.2:

$$c_{11}\nabla^2 \phi = -\rho\omega^2 \phi \quad (\text{Eq. A.16})$$

Substituting Eq. A.16 in Eq. A.14

$$T_2 = -\rho\omega^2 \phi + 2c_{66}[k^2 \phi + ik\partial_2 \psi] \quad (\text{Eq. A.17})$$

Further using Eq. A.15 in Eq. A.17,

$$T_2 = c_{66}[(k^2 - q^2)\phi + 2ik\partial_2 \psi] \quad (\text{Eq. A.18})$$

Next we define the tangential stress $T_6 = T_{12}$. From Eq. A.11,

$$\begin{aligned} T_6 &= 2c_{66}S_{12} \\ &= c_{66}[\partial_2 u_1 + \partial_1 u_2] = c_{66}[\partial_2^2 \psi - 2ik\partial_2 \phi + k^2 \psi] \end{aligned} \quad (\text{Eq. A.19})$$

Using Eq. A.6 in Eq. A.19, we get

$$T_6 = c_{66}[(k^2 - q^2)\psi - 2ik\partial_2 \phi] \quad (\text{Eq. A.20})$$

The stress free conditions $T_2 = T_6 = 0$ at $x_2 = \pm h$ are satisfied only if the stresses T_2 and T_6 are even or odd functions of x_2 . Hence, we define the solution for the potential functions as:

$$\begin{aligned} \phi &= B\cos(px_2 + \alpha) \\ \psi &= A\sin(qx_2 + \alpha) \end{aligned} \quad (\text{Eq. A.21})$$

Using Eq. A.21 in Eq. A.3, gives the mechanical displacements

$$\begin{aligned} u_1 &= -ikB\cos(px_2 + \alpha) + qA\cos(qx_2 + \alpha) \\ u_2 &= -pB\sin(px_2 + \alpha) + ikA\sin(qx_2 + \alpha) \end{aligned} \quad (\text{Eq. A.22})$$

From Eq. A.22, we note that there are two types of lamb waves corresponding to:

- (i) Symmetric mode for $\alpha = 0$, here we note from Eq. A.22 that the longitudinal component u_1 is an even function of x_2 , while the transverse component u_2 is an odd function.
- (ii) Antisymmetric mode for $\alpha = \pi/2$, where the longitudinal and the transverse components are odd and even functions of x_2 respectively.

Next we derive the boundary conditions $T_2 = T_6 = 0$ on $x_2 = h$. Using Eq. A.21 on Eq. A.18 and Eq. A.20 results in the following equation.

$$\begin{aligned}(k^2 - q^2)B\cos(ph + \alpha) + 2ikqA\cos(qh + \alpha) &= 0 \\ (k^2 - q^2)A\sin(qh + \alpha) + 2ikpB\sin(ph + \alpha) &= 0\end{aligned}\tag{Eq. A.23}$$

For compatability, the determinant of the coefficient in Eq. A.23 is set to zero,

$$(k^2 - q^2)^2 \cos(ph + \alpha)\sin(qh + \alpha) + 4k^2pq\sin(ph + \alpha)\cos(qh + \alpha) = 0 \tag{Eq. A.24}$$

By replacing B in Eq. A.22 from Eq. A.23, the modified equation becomes

$$\begin{aligned}u_1 &= qA\left[\cos(qx_2 + \alpha) - \frac{2k^2}{k^2 - q^2} \frac{\cos(qh + \alpha)}{\cos(ph + \alpha)} \cos(px_2 + \alpha)\right] \\ u_2 &= ikA\left[\sin(qx_2 + \alpha) + \frac{2pq}{k^2 - q^2} \frac{\cos(qh + \alpha)}{\cos(ph + \alpha)} \sin(px_2 + \alpha)\right]\end{aligned}\tag{Eq. A.25}$$

Rewriting Eq. A.4 by replace k by the phase velocity $V = \omega/k$, we get

$$\begin{aligned}p^2 &= \omega^2\left(\frac{1}{V_L^2} - \frac{1}{V^2}\right) \\ q^2 &= \omega^2\left(\frac{1}{V_T^2} - \frac{1}{V^2}\right)\end{aligned}\tag{Eq. A.26}$$

In case of a glass plate (thickness of 0.15 mm) excited at frequency of 50 MHz and 100 MHz, the lamb wave excited is in the high frequency region where $kh \gg 1$. Higher order antisymmetric modes are generated in the glass at these frequencies. For the A2 mode generated at 100 MHz, the phase velocity (V) is such that $V_T < V < V_L$ and hence from Eq. A.26, $p = ik\chi$. The displacements for the antisymmetric mode are derived in the following. From Eq. A.24 for $\alpha = \pi/2$, we get

$$(k^2 - q^2)^2 \tan ph = -4k^2pq \tan qh \tag{Eq. A.27}$$

with $p = ik\chi$,

$$k \left(1 - \frac{q^2}{k^2}\right)^2 \tanh \chi h k = -4\chi q \tanh q h \quad (\text{Eq. A.28})$$

Since V approaches close to V_T , $q \rightarrow 0$, hence for a large value of $k \ 10^5$, $q_n h \rightarrow (2n + 1)\pi/2$ where $n=0,1,2$ etc. Substituting the value of $q_n h$ in Eq. A.25 gives the displacement for the mode A_n as:

$$\begin{aligned} u_1 &\cong (n + \frac{1}{2}) \frac{\pi}{h} A \left\{ -\sin \left[(2n + 1) \pi \frac{x_2}{2h} \right] + 2(-1)^n \frac{\sinh(\chi k x_2)}{\sinh(\chi k h)} \right\} \\ u_2 &\cong iKA \left\{ \cos \left[(2n + 1) \pi \frac{x_2}{2h} \right] + (2n + 1)(-1)^n \frac{\pi \chi}{kh} \frac{\chi k x_2}{\chi h} \right\} \end{aligned} \quad (\text{Eq. A.29})$$

Appendix B

Transmission coefficient of a sound wave from an arbitrary number of layers

Fig B.1 shows the sound wave incident from layer $n+1$ with an incident angle of θ_{n+1} and unit amplitude. The wave undergoes reflection and the transmitted wave is converted to both longitudinal and transverse waves. In the lower space denoted by *Layer1*, in the present case case refers to water, hence there is no transverse waves in *Layer1*. The waves contains a common factor $e^{i(\xi x - \omega t)}$ where,

$$\xi = k_{n+1} \sin \theta_{n+1} = k_i \sin \theta_i = \kappa_i \sin \gamma_i, i = 1, 2, n. \quad (\text{Eq. B.1})$$

First, we consider the layer n and temporarily set the boundary of layer n and $n-1$ as the origin of the coordinates. The expression for the potentials of the longitudinal and

Figure B.1: Sound wave transmission through arbitrary number of layers

transverse waves as discussed in A are:

$$\begin{aligned}\phi &= \phi' e^{i\alpha z} + \phi'' e^{-i\alpha x}, \alpha \equiv (k^2 - \xi^2)^{1/2} \\ \psi &= \psi' e^{i\beta z} + \psi'' e^{-i\beta x}, \beta \equiv (\kappa^2 - \xi^2)^{1/2}\end{aligned}\tag{Eq. B.2}$$

The displacement can be expanded into various components using Eq. A.1 as:

$$\begin{aligned}u_x &= \partial_x \phi - \partial_z \psi \\ u_y &= 0 \\ u_z &= \partial_z \phi + \partial_x \psi\end{aligned}\tag{Eq. B.3}$$

Similarly, using Eq. A.12 we can derive the normal stress ($T_{zz} = Z_z$ and shear stress $T_{xz} = Z_x$ and $T_{yz} = Z_y$).

$$\begin{aligned} Z_z &= \lambda(\partial_x u_x + \partial_z u_z) + 2\mu\partial_z u_z \\ Z_x &= \mu(\partial_x u_z + \partial_z u_x) \\ Z_y &= \mu(\partial_z u_y) \end{aligned} \quad (\text{Eq. B.4})$$

The quantities u_x, u_z, Z_z, Z_x at the upper boundary of the layer n at $z = d$ is denoted by $u_x^{(n)}, u_z^{(n)}, Z_x^{(n)}, Z_z^{(n)}$. Using Eq. B.3 and Eq. B.4 gives:

$$\begin{aligned} u_x^{(n)} &= i\xi\cos P(\phi' + \phi'') + \xi\sin P(\phi' - \phi'') + i\xi\cos Q(\psi' - \psi'') - \xi\sin Q(\psi' + \psi'') \\ &\quad \text{where, } P = \alpha d, Q = \beta d \end{aligned} \quad (\text{Eq. B.5})$$

Similarly expanding for the quantities $u_z^{(n)}, Z_x^{(n)}, Z_z^{(n)}$ and rearranging coefficients, we get the relation

$$\begin{aligned} u_z^{(n)} &= -\alpha\sin P(\phi' + \phi'') - i\alpha\cos P(\phi' - \phi'') + \xi\sin Q(\psi' - \psi'') + i\xi\cos Q(\psi' + \psi'') \\ Z_z^{(n)} &= -(\lambda k^2 + 2\mu\alpha^2)\cos P(\phi' + \phi'') + i(\lambda k^2 + 2\mu\alpha^2)\sin P(\phi' - \phi'') + 2\mu\xi\beta\cos Q(\psi' - \psi'') \\ &\quad - 2i\mu\xi\beta\sin Q(\psi' + \psi'') \\ Z_x^{(n)} &= 2i\mu\alpha\xi\sin P(\phi' + \phi'') + 2\mu\alpha\xi\cos P(\phi' - \phi'') + i\mu(\xi^2 - \beta^2)\sin Q(\psi' - \psi'') + \\ &\quad \mu(\beta^2 - \xi^2)\cos Q(\psi' + \psi'') \end{aligned} \quad (\text{Eq. B.6})$$

The displacement at the lower boundary of layer n is obtained by setting $P=Q=0$.

$$\begin{aligned} u_x^{(n-1)} &= i\xi(\phi' + \phi'') + i\xi(\psi' - \psi'') \\ u_z^{(n-1)} &= -i\alpha(\phi' - \phi'') + i\xi(\psi' + \psi'') \\ Z_z^{(n-1)} &= -(\lambda k^2 + 2\mu\alpha^2)(\phi' + \phi'') + 2\mu\xi\beta(\psi' - \psi'') \\ Z_x^{(n-1)} &= 2\mu\alpha\xi(\phi' - \phi'') + \mu(\beta^2 - \xi^2)(\psi' + \psi'') \end{aligned} \quad (\text{Eq. B.7})$$

Combining Eq. B.5, Eq. B.7 and Eq. B.7, we get

$$\begin{bmatrix} u_x^{(n)} \\ u_z^{(n)} \\ Z_z^{(n)} \\ Z_x^{(n)} \end{bmatrix} = \begin{bmatrix} a_{11} & a_{12} & a_{13} & a_{14} \\ a_{21} & a_{22} & a_{23} & a_{24} \\ a_{31} & a_{32} & a_{33} & a_{34} \\ a_{41} & a_{42} & a_{43} & a_{44} \end{bmatrix} \begin{bmatrix} u_x^{(n-1)} \\ u_z^{(n-1)} \\ Z_z^{(n-1)} \\ Z_x^{(n-1)} \end{bmatrix} \quad (\text{Eq. B.8})$$

where the elements of a_{ik} (i,k =1,2,3,4) are given by

$$\begin{aligned} a_{11} &= 2\sin^2\gamma\cos P + \cos 2\gamma\cos Q \\ a_{12} &= i(\tan\theta\cos 2\gamma\sin P - \sin 2\gamma\sin Q) \\ a_{13} &= i\sin\theta(\cos Q - \cos P)/\omega\rho c \\ a_{14} &= (\tan\theta\sin\gamma\sin P + \cos\gamma\sin Q)/\omega\rho b \\ a_{21} &= i(2\cot\theta\sin^2\gamma\sin P - \tan\gamma\cos 2\gamma\sin Q) \\ a_{22} &= \cos 2\gamma\cos P + 2\sin^2\gamma\cos Q \\ a_{23} &= (\cos\theta\sin P + \tan\gamma\sin\theta\sin Q)/\omega\rho c \\ a_{24} &= i\sin\gamma(\cos Q - \cos P)/\omega\rho b \\ a_{31} &= -2i\omega\rho b\sin\gamma\cos 2\gamma(\cos Q - \cos P) \\ a_{32} &= -\omega\rho[(c\cos^2 2\gamma/\cos\theta)\sin P + 4b\cos\gamma\sin^2\gamma\sin Q] \\ a_{33} &= \cos 2\gamma\cos P + 2\sin^2\gamma\cos Q \\ a_{34} &= i(\cos 2\gamma\tan\theta\sin P - \sin 2\gamma\sin Q) \\ a_{41} &= -\omega\rho b^2[(4/c)\cos\theta\sin^2\gamma\sin P + (\cos^2 2\gamma/b\cos\gamma)\sin Q] \\ a_{42} &= -2i\omega\rho b^2\sin\theta\cos 2\gamma(\cos Q - \cos P)/c \\ a_{43} &= ib^2[(\sin 2\theta/c^2)\sin P - (\cos 2\gamma/b^2)\tan\gamma\sin Q] \\ a_{44} &= 2\sin^2\gamma\cos P + \cos 2\gamma\cos Q \end{aligned} \quad (\text{Eq. B.9})$$

Following the methods of successive application of Eq. B.8 we can write the result in the form:

$$\begin{bmatrix} u_x^{(n)} \\ u_z^{(n)} \\ Z_z^{(n)} \\ Z_x^{(n)} \end{bmatrix} = \begin{bmatrix} A_{11} & A_{12} & A_{13} & A_{14} \\ A_{21} & A_{22} & A_{23} & A_{24} \\ A_{31} & A_{32} & A_{33} & A_{34} \\ A_{41} & A_{42} & A_{43} & A_{44} \end{bmatrix} \begin{bmatrix} u_x^{(1)} \\ u_z^{(1)} \\ Z_z^{(1)} \\ Z_x^{(1)} \end{bmatrix} \quad (\text{Eq. B.10})$$

where the matrix A in Eq. B.10 is the product of matrices of a i.e, $A = a^{(n-1)}..a^{(3)}a^{(2)}$. We represent the sum of layer thicknesses in B.1 by $H = (d_2 + d_3 + ...d_n)$. Considering only presence of longitudinal wave in the layer $n+1$ constituted by the liquid, the total field can be represented as

$$\phi_{n+1} = e^{-i\alpha_{n+1}(z-H)} + V e^{i\alpha_{n+1}(z-H)} \quad (\text{Eq. B.11})$$

where $\alpha_{n+1} = k_{n+1} \cos \theta_{n+1}$ and V is the reflection coefficient. Considering that at the lower space, i.e at $n=1$, the medium is constituted by liquid, the potential consists of longitudinal waves represented as

$$\phi_1 = W e^{-ik_1 z \cos \theta_1} \quad (\text{Eq. B.12})$$

where W represents the transmission coefficient of the longitudinal wave in medium 1. The coefficients V and W can be found from the conditions on the boundaries of media n and $n+1$, where

$$z = H, u_z^{(n+1)} = u_z^{(n)}, Z_z^{(n+1)} = Z_z^n, Z_x^{(n+1)} = Z_x^n = 0 \quad (\text{Eq. B.13})$$

Substituting the value of $u_z^{(n)}$, $Z_z^{(n)}$ and $Z_x^{(n)}$ from Eq. B.10, we get

$$\begin{aligned} u_z^{(n+1)} &= A_{21}u_x^{(1)} + A_{22}u_z^{(1)} + A_{23}Z_z^{(1)} + A_{24}Z_x^{(1)} \\ Z_z^{(n+1)} &= A_{31}u_x^{(1)} + A_{32}u_z^{(1)} + A_{33}Z_z^{(1)} + A_{34}Z_x^{(1)} \\ 0 &= A_{41}u_x^{(1)} + A_{42}u_z^{(1)} + A_{43}Z_z^{(1)} + A_{44}Z_x^{(1)} \end{aligned} \quad (\text{Eq. B.14})$$

where the values of $u_z^{(n+1)}$ and $Z_z^{(n+1)}$ are taken from $z = H$, while $u_x^{(1)}, \dots, Z_x^{(1)}$ from $z = 0$. Replacing $u_x^{(1)}$ in the values of $u_z^{(n+1)}$ and $Z_z^{(n+1)}$ by substituting for the value of $u_x^{(1)}$ from last of Eq. B.14, we then have

$$\begin{aligned} u_z^{(n+1)} &= M_{22}u_z^{(1)} + M_{23}Z_z^{(1)} + M_{24}Z_x^{(1)} \\ Z_z^{(n+1)} &= M_{32}u_z^{(1)} + M_{33}Z_z^{(1)} + M_{34}Z_x^{(1)} \end{aligned} \quad (\text{Eq. B.15})$$

where, $M_{ik} = A_{ik} - A_{i1}A_{4k}/A_{41}$, $i, k = 2, 3, 4$. Next we obtain the expression for the various quantities at $z=H$ and $z=0$. At $z=H$, we obtain $u_z^{(n+1)}$ and $Z_z^{(n+1)}$ by using Eq. B.3, Eq. B.4 and substituting the value of ϕ_{n+1} from Eq. B.11.

$$\begin{aligned} u_z^{(n+1)} &= i\alpha_{n+1}(V - 1) \\ Z_z^{(n+1)} &= -\omega^2\rho_{n+1}(1 + V) \end{aligned} \quad (\text{Eq. B.16})$$

Similarly at $z=0$, we use the form of ϕ_1 in Eq. B.12 to derive the following values:

$$\begin{aligned} u_z^{(1)} &= i(-\alpha_1 W) \\ u_x^{(1)} &= i(\xi W) \\ Z_z^{(1)} &= -\omega^2\rho_1 W \\ Z_x^{(1)} &= 0 \end{aligned} \quad (\text{Eq. B.17})$$

From Eq. B.16 we find that the reflection coefficient V can be represented as:

$$V = \frac{Z_{in} - Z_{n+1}}{Z_{in} + Z_{n+1}} \quad (\text{Eq. B.18})$$

where

$$Z_{in} = \frac{iZ_z^{(n+1)}}{\omega u_z^{(n+1)}}, \quad Z_{n+1} = \rho_{n+1}c_{n+1}/\cos(\theta_{n+1}) \quad (\text{Eq. B.19})$$

By using Eq. B.17 in Eq. B.15 and substituting in Eq. B.19, the input impedance of the set of layers Z_{in} is written as:

$$Z_{in} = (i/\omega) [(M_{32} - i\omega Z_1 M_{33}) / (M_{22} - i\omega Z_1 M_{23})] \quad (\text{Eq. B.20})$$

We next derive the equations for the transmission coefficient W . Using the first equation of Eq. B.14, and substituting for the value of $u_x^{(1)}, u_z^{(1)}, Z_z^{(1)}, Z_x^{(1)}$, we get the following:

$$W = \frac{i\alpha_{n+1}(V - 1)}{i\xi A_{21} - i\alpha_1 A_{22} - \omega^2 \rho_1 A_{23}} \quad (\text{Eq. B.21})$$

Replacing the value of V from Eq. B.18, gives

$$W = \left(\frac{2\alpha_1 Z_{n+1}}{Z_{in} + Z_{n+1}} \right) \left(\frac{1}{-\xi A_{21} + \alpha_1 A_{22} - i\omega^2 \rho_1 A_{23}} \right) \quad (\text{Eq. B.22})$$

On applying the value of Z_{in} from Eq. B.20 in Eq. B.22 and rearranging we get the following:

$$\frac{\rho_1}{\rho_{n+1}} W = \frac{-2i\omega Z_1 \alpha_1 [M_{22} - i\omega Z_1 M_{23}]}{[(M_{32} - i\omega Z_1 M_{33}) - (i\omega M_{22} + Z_1 \omega^2 M_{23}) Z_{n+1}] [-\xi A_{21} + \alpha_1 A_{22} - i\omega^2 \rho_1 A_{23}]} \quad (\text{Eq. B.23})$$

where $Z_1 = \omega \rho_1 / \alpha_1$ On substituting the value of M_{ik} in the term $\alpha_1 [M_{22} - i\omega Z_1 M_{23}]$ of Eq. B.23 in terms of the variable A as discussed earlier, we can prove that $\alpha_1 [M_{22} - i\omega Z_1 M_{23}] = [-\xi A_{21} + \alpha_1 A_{22} - i\omega^2 \rho_1 A_{23}]$. Using these findings, the transmission coefficient W is defined as:

$$\frac{\rho_1}{\rho_{n+1}} W = \frac{-2i\omega Z_1}{(M_{32} - i\omega Z_1 M_{33}) - (i\omega M_{22} + Z_1 \omega^2 M_{23}) Z_{n+1}} \quad (\text{Eq. B.24})$$

We write the quantities in Eq. B.24 in the form:

$$\omega M_{23} = 1/Z_1 N, \quad M_{22} + M_{33} = 2M/N, \quad M_{32} = \omega Z_1 (M^2 - N^2)/N \quad (\text{Eq. B.25})$$

where

$$\begin{aligned} M &= (Z_2/Z_1) \cos^2 2\gamma_2 \cot P + (Z_{2t}/Z_1) \sin^2 2\gamma_2 \cot Q \\ N &= Z_2 \cos^2(2\gamma_2)/Z_1 \sin P + Z_{2t} \sin^2(2\gamma_2)/Z_1 \sin Q \\ P &= k_2(h/2) \cos \theta_2 \\ Q &= \kappa(h/2) \cos \gamma_2 \end{aligned} \quad (\text{Eq. B.26})$$

Thus, the transmission coefficient is written in the form:

$$W = 2N/[2M + i(M^2 - N^2 - 1)] \quad (\text{Eq. B.27})$$

Publication

Conference Papers

- (i) R. Kishor, Y. Zheng, W. Zhenfeng, and T. T. Lim, "FEM modelling of a SAW microfluidic sensor based on the photoacoustic effect" accepted at *IEEE Int. Ultrason. Symp.*, Tours, France, 2016.
- (ii) R. Kishor and Y. Zheng, "Surface acoustic wave RF sensing and actuation for lab-on-a-chip platforms", accepted at *IEEE MTT-S International Microwave Workshop Series on Advanced Materials and Processes for RF and THz Applications (IMWS-AMP)*, China, 2016.

Journal Papers

- (i) R. Kishor, Y. P. Seah, Y. J. Zheng, H. M. Xia, Z. F. Wang, H. J. Lu, et al., "Characterization of an acoustically coupled multilayered microfluidic platform on SAW substrate using mixing phenomena," *Sensors and Actuators A: Physical*, vol. 233, pp. 360-367, 2015
- (ii) R. Kishor, F. Gao, S. Sreejith, X. Feng, Y. P. Seah, Z. Wang, et al., "Photoacoustic induced surface acoustic wave sensor for concurrent opto-mechanical microflu-

idic sensing of dyes and plasmonic nanoparticles," *RSC Advance*, vol. 6, pp. 50238-50244, 2016.

- (iii) R. Kishor, Ma Z.C., S. Sreejith, Y.P. Seah, Ai Y, Z.F. Wang, Lim T.T. and Y.J. Zheng, Integrated microfluidic system for real-time separation and detection of particles on a single piezoelectric substrate, *Sensors and Actuators B:Chemical*, (under revision).
- (iv) R. Kishor, Y.P. Seah, Z.F. Wang, Lim T.T. and Y.J. Zheng, A compact separation and sensing platform for microparticles using othogonal surface acoustic wave devices, *Lab on a chip*, (to be submitted).
- (v) R. Kishor,S. Sreejith, Y.P. Seah, Z.F. Wang, Lim T.T. and Y.J. Zheng, A SAW-PA integrated microfluidic sensing platform for detecting circulating tumor cells, (In preparation).

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