Surface acoustic waves inside polystyrene microparticles through photoacoustic microscopy

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Abstract

We demonstrate surface acoustic waves inside polystyrene microspheres of different sizes experimentally through photoacoustic microscopy and validate the experimental result with simulation. A novel method for sample preparation of a lifted sample is also presented where the microparticles are suspended in agarose above the surface of the petridish. Another objective of this study was to investigate the results with different laser focus hitting on the microparticles and their impact on photoacoustic images and photoacoustic signals. An absorbing microsphere is excited with a pulsed laser of wavelength 532 nm and the photoacoustic signal is detected using a 40 MHz transducer. On analyzing the photoacoustic signals from microspheres, we find the signature of surface acoustic waves.

1. Introduction

A surface wave can be defined as a mechanical wave traveling along the interfaces between differing media. Surface waves are often found in nature in a variety of forms, for instance, seismic waves along the earth’s crust, water waves that follow along the air-water interface, and ultrasonic surface waves at different interfaces. In all these cases, a standard feature of surface acoustic waves (SAW) is that energy is localized near the surface, and the sound intensity decreases exponentially with propagation in the depth of the material[1, 2]. Lord Rayleigh in the 19th century was the first to explain these acoustic modes and their properties [3]. Later, the excitation of broadband SAW pulses by any absorbing material within the ultrasound region with focused short laser pulses was a big development in SAW research.[4-7].

Photoacoustic microscopy is a hybrid imaging technology based on photoacoustic effect having optical excitation and ultrasonic detection[8, 9]. The microparticles are excited optically by employing a pulsed laser, where the laser hits the sample; light is absorbed, which leads to heating. Heating further leads to thermoelastic expansion eventually giving rise to pressure waves[10]. Photoacoustic techniques have been used in finding several information ,including oxygen saturation[11], temperature[12], flow velocity[13-15], viscoelasticity[16] etc.
Surface acoustic waves has been detected by different microscopy techniques such as scanning tunnelling microscopy[17], interdigital transducers[18], adaptive interferometric detection[19]. SAW technology has many potential applications in several fields such as sensors[20], drop size controlling[21], geophysics[22], microfluidics[23], flow measurement, sensors[24].

In recent times, microparticles have been shown for several applications using the phenomenon of photoacoustics. Microparticles using photoacoustics have been shown to be utilized in image-guided cancer therapy[25]. Cells can be incubated with microparticles to know the intracellular temperature using changes in the photoacoustic amplitude[26]. Xiaoxiang Gao has shown that eigen vibration information of light-absorbing microstructures can be known from photoacoustic amplitudes enabling noncontact evaluation of elastic properties. A.B. Matsko[27] showed acoustic coupling between acoustic whispering gallery modes created by the surface acoustic waves. SAWs have been used as a method of finding acoustic properties of the near-surface layer using the property that SAW travels parallel to the surface[28].

This study aims to investigate the existence of surface acoustic waves inside microparticles both experimentally and theoretically using photoacoustic signals. This can be used in several potential applications such as coating the microparticles with bacteria to know different parameters like the size of bacteria. We report photoacoustic imaging of microparticles of different sizes having diameters of 20 microns, 40 microns, and 80 microns. We have also shown that the embedded microparticle samples in agarose gave a clear oscillation of signals in comparison to the settled microparticles. We demonstrate that focusing on different parts of the microspheres can affect the quality of the image and the strength of signals obtained. We present C-scan photoacoustic images of polystyrene microspheres. The photoacoustic images of microparticles having rings are also presented and discussed. A COMSOL modeling was done to show the generation of surface acoustic waves originating inside the microspheres. The simulation results align with the experimental results.

2. Methods and Materials

The microparticles samples were prepared inside the agarose matrix in two ways—the first method where microparticles are settled in petridish and agarose covering from the top and the second one where microparticles are floating in agarose, which gives the microparticles more degrees of freedom to vibrate. It was observed that microparticles that are floating and boundaryless are giving clear resonances/oscillation of signals in comparison to unlifted samples. This can be attributed to
microparticles being able to vibrate in all possible dimensions when it is floating. The steps for preparation of agarose were kept similar in both samples

2.1 Preparation of agarose

0.3 gram of agarose powder (Sigma Aldrich, Type I, low EEO) is dissolved with 30 mL of distilled water and poured into a beaker. The solution is then heated on a heat plate at a temperature of around 80°C with a magnetic stirrer to stir the solution. The agarose solution beaker is slowly heated for around half an hour to avoid the loss of too much water due to evaporation. The molten agarose solution can then be poured either into a mould or directly on the petridish.

2.2 Settled Sample

The molten solution of agarose was poured onto a rectangular mould configuration. The liquid agarose on the rectangular mould was kept for around 30 minutes to solidify. The solid agarose was cut with a razor blade. However, in this case, firstly 3 μL of microparticles was kept onto polymer petridish. The solidified layer of agarose was then kept on top of microparticles as shown in figure (2) and then left to dry.

2.3 Embedded Sample

A volume of 500 mL of molten agarose was poured onto the petridish. The petridish having agarose was left to solidify for around half an hour. An amount of 3 μL of microparticles is then mixed with molten agarose kept in the molten agarose. A drop of molten agarose mixed with microparticles is then poured upon the solidified agarose in petridish. The whole sample was then heated below the melting point of the agarose so that the microparticles are embedded into the agarose matrix and at the same time lifted from the bottom of the petridish too. This protocol was repeated similarly for microparticles of all sizes i.e. 20 μm, 40 μm, and 80 μm. The goal of
making this sample was to embed the microparticles inside the agarose matrix so that they are floating inside the agarose and are boundaryless. It was observed that the photoacoustic signals from this embedded sample gave clear oscillation/ringing of signals in comparison to the earlier settled sample. This can be attributed to the vibration of microparticles in more degrees of freedom.

3. Experimental setup

To investigate the photoacoustic response from the microparticles, an experimental setup was built up around an inverted microscope (Leica DMi8) with additional optical and acoustic components as shown in figure (2). A high precision scanning stage (ASI MS-2000) shown in figure 2 (a) was used on the microscope to utilize 2D photoacoustic images of the microparticle samples using mechanical raster or serpentine scanning. The control of stages was provided by a LabVIEW program. This LabVIEW program assured synchronization with the optical and acoustic components, the needed communication, and data transfer with the time-critical digitizing implemented in FPGA hardware (National Instruments). An optical camera (sCMOS, Andor Zyla) was also used in wide-field reflection mode to provide optical images of the investigated samples.

The optical components are shown below the scanning stage in figure 2 (c) where all are assembled on an optical to minimize effects from mechanical vibrations. These components consist of a 532 nm Q-switched mode-locked pulsed laser (Elforlight SPOT-20-200-532) that yields pulses with widths down to 1.6 ns, repetition rates up to 10 kHz, and energies up to 20 μJ. An optical isolator was placed immediately after the laser to avoid unwanted back reflection into the laser cavity and allow one-way transmission of laser light. The laser pulse was also spatially filtered using a 50-μm diameter pinhole to remove different aberrations and clean up the laser beam. After filtering, the beam was brought into the inverted microscope through an infinity-port and focussed by a Leica objective lens (NA: 0.3, depth of field: 10μm, magnification-10X)

The acoustic parts of the setup which are located above the scanning stage include a focused ultrasound transducer (Olympus) with a specified center frequency of 50 MHz and f-number of 2. To obtained overlapping field-of-view for the optical and acoustic fields, the ultrasound transducer and the laser were coaxially and confocally aligned before imaging as illustrated in figure 2 (b). Further on, to obtain good signal integrity, the signal from the transducer was amplified and low-pass filtered with a customized pre-amplifier and then digitized with a high-speed 12-bit digitizer (NI-5772) at a 400 Ms/s sampling rate.
Figure (2). (a) camera view of our setup during the scan with petridish mounted on the scanning platform. (b) schematic view for the generation of the photoacoustic wave by optical excitation (c) Simplified PAM experimental setup. OI: optical isolator, BS: beam splitter, ND filter: neutral density filter, M: mirror kept at an angle of 45°, PD: photodiode, OL: objective lens. TL: tube lens, OC: optical camera

4. COMSOL simulation

In order to get a deeper understanding of the experimental results, a numerical modeling simulation was done where a cross-section of the experimental setup was simulated. The numerical model was done to understand the incident light, interaction of light with microparticles, and detection of the acoustic waves generated. The photoacoustic experiment performed on microparticles of different sizes was implemented in COMSOL Multiphysics software, which uses the finite element method (FEM) for computation. This was a simulation of the experimental setup where the laser beam hits the microparticle in an inverted microscope setup in transmission mode. The laser beam was modeled as a Gaussian pulse and it was assumed that the incident laser light intensity follows a Gaussian distribution with respect to distance from the origin when it enters another medium such as in our case microparticles, it remains Gaussian with a few modifications in the parameters of Gaussian beam. The levitation distance of microparticles was assumed constant as 200 μ above the bottom layer of agarose. We assumed agarose to behave like water
since the acoustic impedance of agarose is close to water. To model the absorption of laser light by microparticles, beer-lambert’s law was used.

The way to implement this in COMSOL is to solve the heat equations in the model and to relate them to the elastic wave equation. The optically induced initial pressure wave distribution is related to the heating produced by the deposited laser energy by equation (1)

\[ p = \Gamma \mu F \]  

the Grüneisen parameter (a constant), \( \mu \) is the absorption coefficient of sample and \( F \) is the fluence of the laser beam. Some of the laser light gets reflected at the dielectric interface, so the intensity of light at the surface of the material is reduced to 0.95 of the incident intensities. This condition is implemented with a Dirichlet boundary condition. Zero flux boundary condition was applied to the face opposite to the incident face which can be physically interpreted as meaning that any light reaching that boundary will leave the domain. Most other boundaries were chosen to be default thermal insulation, fit for implementing the symmetry of the temperature field. According to this law, if the light hits a semi-transparent material, absorption of energy will take place assuming monochromatic and collimated light. The law can be described in its differential form as to

\[ \frac{\partial I}{\partial z} = \alpha(T)I \]  

where \( \alpha(T) \) is the absorption coefficient, which is dependent on the temperature of the material. However, we assumed it to be temperature independent since the temperature increase was small in our case.

The heat diffusion equation tells us about the temperature variation in material over time. It can be expressed as to

\[ \rho C_p \frac{\partial T}{\partial t} = \nabla (k \nabla T) + Q \]  

where \( \rho \) is the mass density, \( C_p \) is the heat capacity with constant pressure, \( k \) denotes the thermal conductivity and \( Q = \alpha(T)I \) represents the external heat source which equals to (laser in this case). Due to thermal confinement, the diffusion from heat flux term is set to zero which gives

\[ \rho C_p \frac{\partial T}{\partial t} = Q \]  

The implementation of the heat equation was done by the coefficient form PDE. This time-dependent partial differential equation was solved for intensity calculations which are as follows

\[ e_s \frac{\partial^2 I_n}{\partial t^2} + d_s \frac{\partial I_n}{\partial t} + \nabla \cdot (-c \nabla I_n - \alpha I_n + \gamma) + \beta \nabla I_n + a I_n = f \]  
\text{equ.(5)}

Since this equation is linear and stationary, the initial values do not affect the solution for the intensity variable.

The time-dependent equation, which was solved in COMSOL for temperature measurements, is

\[ e_s \frac{\partial^2 T}{\partial t^2} + d_s \frac{\partial T}{\partial t} + \nabla \cdot (-c \nabla T - \alpha T + \gamma) + \beta \nabla T + a T = f \]  
\text{equ.(6)}

where \( e_s \) is the mass coefficients, \( d_s \) is the damping coefficient, \( c \) is the diffusion coefficient, \( \alpha \) is the conservative flux convection coefficient, \( \beta \) is the convection coefficient, \( a \) is the absorption coefficient, \( \gamma \) is the conservative flux source term and \( f \) is the source term. The momentum equation was solved for a linear elastic material

\[ \rho \frac{\partial^2 u}{\partial t^2} = \nabla (s) + F_v \]  
\text{equ.(7)}

The temperature change is affecting the elastic equation by a tensor relation between stress and strain in equation (8) which holds for an isotropic, linear elastic material.

\[
\begin{bmatrix}
\sigma_{11} \\
\sigma_{22} \\
\sigma_{33} \\
\sigma_{23} \\
\sigma_{13} \\
\sigma_{12}
\end{bmatrix} = \frac{E}{(1+v)(1-2v)} \begin{bmatrix}
1 - v & v & 0 & 0 & 0 & 0 \\
v & 1 - v & v & 0 & 0 & 0 \\
v & v & 1 - v & 0 & 0 & 0 \\
0 & 0 & 0 & \frac{(1-2v)}{2} & 0 & 0 \\
0 & 0 & 0 & 0 & \frac{(1-2v)}{2} & 0 \\
0 & 0 & 0 & 0 & 0 & \frac{(1-2v)}{2}
\end{bmatrix} \begin{bmatrix}
\varepsilon_{11} \\
\varepsilon_{22} \\
\varepsilon_{33} \\
\varepsilon_{23} \\
\varepsilon_{13} \\
\varepsilon_{12}
\end{bmatrix} - \alpha \Delta T \begin{bmatrix}
1 \\
1 \\
1 \\
1 \\
1 \\
1
\end{bmatrix} \text{equ.(8)}
\]

where \( E \) represents Young’s modulus, \( v \) is Poisson’s ratio, \( \alpha \) is the coefficient of thermal expansion and \( \Delta T \) is the increase in temperature[29].

In the tables given below, we present a summary of the important parameters used in our model. Table 1 contains the details about the parameters related to the material and elastic properties of polystyrene microparticles used in the model. Table 2 possesses information about the parameters of the laser beam used.

Table 1.

| Parameter                     | Value     |
|-------------------------------|-----------|
| Absorption coefficient        | 1/100     |
| Levitation distance of microparticle | 200 μm   |
| Young’s Modulus               | 3.5×10⁹ Pa|
An ultrasonic transducer was implemented in the model to receive the PA signals, which consisted of a piezoelectric polymer and a backing material. A thin film of Polyvinylidene Fluoride (PVDF) was implemented as a piezoelectric material like the transducer used in experiments. The model was solved in COMSOL using the transient response using a finite-size custom mesh.

It was observed that by changing the pulse width resulted in a different frequency spectrum for the photoacoustic signal which can also be proven experimentally by a slight change in the focus. This confirms that the pulse width of the laser beam changes after it interacts with the microparticle and inside the microparticles. This simulation method can also be used to calculate the speed of longitudinal and shear waves inside the microparticle. The speed of the longitudinal wave and shear wave calculated here from the model is approximately 2310 m/s and 1110 m/s. The absorption coefficient has a role to play as well because the photoacoustic signal is proportional to the absorption properties of the microparticle. It was observed in the modeling that the amplitude of the signal decreased with low absorption coefficients. The elastic property of microspheres such as density, Young’s modulus, Poisson’s ratio of polystyrene microspheres also affected the photoacoustic signals.

5. Results and Discussions

The photoacoustic imaging of microparticles having sizes 20 μm, 40 μm, and 80 μm (microParticles, GmbH) was done. The microparticles absorbing the laser light at 532 nm gave a good absorption-based image contrast. The applied dye responsible for absorption on these polystyrene microparticles was Sudan red-type. This dye is distributed uniformly throughout the microparticle’s volume in a homogeneous way. These polystyrene microparticles were produced along emulsion polymerization routes.

Figure (3) shows the photoacoustic signals from microparticles of different sizes in the time domain. The PAM signals arising from the microparticles have a short period and high intensity, which corresponds to the rapid thermoelastic expansion of

| Property               | Value         |
|------------------------|---------------|
| Poisson’s ratio        | 0.35          |
| Density                | 1050 kg/m³    |
| Thermal conductivity   | 0.035         |

Table 2

| Property       | Value       |
|----------------|-------------|
| Pulse width    | 5 μm        |
| Pulse duration | 6 ns        |
| Laser intensity| 1e6 W/m²    |
a microsphere induced by short-pulsed laser illumination. Previous studies have shown that this high-intensity signal coming from microparticles can be related to the optical absorption coefficient [16] and the size of the absorbing particles [30-32]. It also reveals that after the laser illumination, a periodic surface wave with a gradually decaying magnitude exists. After analyzing the time series, we observe that the waves after the peak intensity are not random but repeat after a certain interval of time which indicates that the microparticles vibrate even after narrow laser illumination. We observe that for here microparticles there is a ringing pattern that is representative of surface waves inside the microparticles.

Figure (3). (a) Time-domain photoacoustic signal from 20 μm particle (b) schematic diagram of petridish having microparticles in agarose relating to the origin of signals in part (a). (c) Time-domain photoacoustic signal from 40 μm particle (d) Time-domain photoacoustic signal from 80 μm particle

Figure (4) represents the simulated COMSOL generated PA signals and experimentally generated PA signals in frequency domain side by side. We see a similarity in the results generated in almost all the cases. The peak Eigen frequency in the case of the 80-micron sample is lying almost at the same location in both the cases as evident in 4(a) and (b). We also observe a good similarity in the trend and shape of the frequency spectrum in this case.
We observe that the simulation holds in agreement with the experimental results for microparticles of larger sizes such as 80 μm. It was seen that the laser light just passes through the particle in microparticles having smaller diameters and in this case, surface acoustic waves are not generated as evident from 4(e) and (f). Therefore, the experimental signals do not match very well with the simulation results.

We also performed experiments by focussing on different parts of the microparticle in the vertical z-direction. This was done by translating the scanning platform in the z-direction (vertically upward or downward). Figure (5) represented below demonstrates the effect of the change in a laser focus on the photoacoustic signal in the frequency domain. It is evident that focussing inside the microparticle has a different effect on the quality of the PAM image and the intensity of signals received.
This shows that the photoacoustic spectrum is sensitive to changes in laser beam diameter and with the shift in its laser focus.

Figure (5). Photoacoustic signals received at different focus. (a) At z = 0. (b) At z = -5 μm below (c) At z = -10 μm below (d) At z = -15 μm below (e) At z = -20 μm below (f) At z = +5 μm above (g) At z = +10 μm above (h) At z = +15 μm above (i) At z = 20 μm above

Figure (6). Photoacoustic image (a) 20 μm (b) 40 μm (c) 80 μm. The pixel size in 20 μm particle is 1 μm and in the 40 and 80 μm is 10 μm.

The photoacoustic image as shown in figure (6) of our samples of sizes 20, 40, and 80 μm was generated by 2-D raster scanning using the experimental set-up shown in figure(2). The image was reconstructed by mapping the pixels to an image where each pixel corresponds to a time series. The rings present in the photoacoustic image
of microparticles can be possibly attributed to possible resonance scattering inside the microparticles.

6. Conclusion

We have shown the photoacoustic image of microparticles of different sizes and simulated the photoacoustic signals. Surface acoustic waves are experimentally detected inside polystyrene microspheres through optical excitation and ultrasonic detection i.e., photoacoustic microscopy, and later proven true with COMSOL simulation. The experimentally generated signals and the simulated signals match largely in terms of their peak Eigen frequency. We show a novel way of sample preparation where the majority of microparticles are lifted and almost lie in the same plane. We also demonstrate the results by shifting the laser focus i.e. on the surface and inside the microparticle both experimentally and in simulation and a change in the frequency spectrum is observed with the shift.

The theoretical analysis and experimental measurements can also reveal the eigenvalues from the frequency spectrum which can further tell about the Young’s Modulus and Poisson’s ratio of these microparticles. A further possible application of this work can be to coat the surface of microparticles with bacteria and determine the thickness of bacteria with the help of a shift in the time series obtained.

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