AN ABSTRACT OF THE THESIS OF

Ali Almaqwashi for the degree of Master of Science in Physics, presented on March 21, 2011
Title: Optical Trapping and Acoustical Probing of Ultrasound Contrast Agent Microbubbles Confined in Capillaries

Abstract approved:

_________________________________________________________________________________

David H. McIntyre

In an effort to develop an optical-acoustical understanding of ultrasound contrast agent microbubble dynamics in a micro-environment that resembles blood vessels, this thesis presents experimental work on optical trapping and acoustical probing of ultrasound contrast agent microbubbles confined in regenerated cellulose capillaries. First, we showed by acoustical means that the pressure threshold of an individual microbubble shell rupture increases significantly when confined in regenerated cellulose capillaries. We report that the shell rupture threshold in regenerated cellulose capillaries increased by at least 0.3 MPa from 0.8 MPa for unconfined microbubbles. Second, we achieved optical trapping and manipulation of ultrasound contrast agent microbubbles confined in capillaries using Hermite-Gaussian laser beams.
Optical Trapping and Acoustical Probing of Ultrasound Contrast Agent Microbubbles Confined in Capillaries

by
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Chair of the Department of Physics

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Author: Ali Almaqwashi
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CHAPTER ONE
INTRODUCTION
1.1 Introduction:

The advance of technology often relies on the outcome of physics studies, especially when dealing with a very diminutive scale in space and time. Micro-science and nanotechnology have become dominant research fields in physics with a wide range of applications. In particular, physicists are contributing significantly to the improvement of medical diagnosis and therapy. Such multidisciplinary medical research provides an opportunity to develop more effective and hybrid approaches that lead to better experimental solutions. This thesis presents an example of physicists wandering into medical research “territory” to offer some experimental techniques, but in return learning about a whole new medical research field such as ultrasonic targeted therapies.

Ultrasound targeted therapies rely on a gaseous micro-sized bubble to deliver a medical substance to targeted body tissues. These microbubbles have been used already in different medical applications such as enhancing ultrasound imaging. Known as ultrasound contrast agent (UCA) microbubbles, it is very essential to understand the physical properties that govern their dynamics. An important experimental question that I tried to tackle in this thesis is how UCA microbubble dynamics may change when they are confined in the blood vessels, in order to provide the necessary knowledge for the implementation of ultrasound targeted therapies.

Present techniques for studying UCA microbubbles confined in a micro-environment are limited because the microbubbles move in the fluid during the experiment and because of the impact of nearby surfaces. Optical tweezers can offer a
promising possibility to overcome such limitations because of its ability to precisely control the position. However, optical trapping of microbubbles inside a microenvironment that resembles blood vessels involves multiple challenges that we were able to overcome.

1.1.1 Overview:

First, the thesis introduction lays out briefly some optical trapping concepts related to UCA microbubble optical properties. Then chapter two introduces ultrasonic properties of UCA microbubbles and shows experimentally that their dynamics change in micro-confinement such as a microtube or a capillary. The last chapter provides the experiments that we conducted to trap UCA microbubbles. Furthermore, chapter three provides a detailed proposal for integrating an acoustical and optical setup. Then a thesis conclusion highlights the new findings of this work.
1.2 Optical Trapping:

1.2.1 Background:

A quarter century ago, the innovation of optical tweezers was introduced to the scientific community in 1986 by Ashkin and his colleagues in Bell laboratories. When a single Gaussian laser is sharply focused by an objective of high numerical aperture (N.A. 1.25, water immersion), Ashkin et al reported the first optical tweezing of dielectric particles with a refractive index \( n' = 1.5 \) to 3 and a radius of range 25nm to 10 \( \mu \)m in water (refractive index \( n = 1.33 \)) [1]. High-index micro/nanoparticles (\( \frac{n'}{n} > 1 \)) were found to be attracted to the higher intensity region of the laser beam. The particles were trapped in the highest beam intensity at the focus in a three dimensional trap [1]. Lateral trapping results from the transverse intensity gradient of the Gaussian beam. Axial trapping relies on focusing the beam with a high N.A. objective, so that the intensity along the optical axis has a large gradient toward the focal plane at the beam minimal waist (\( w_0 \)).

The optical trapping mechanism for microparticles can be interpreted by Newton’s third law and ray optics [2]. The high-index particle exerts a piconewton-order force to refract the light ray and therefore the particle experiences an equal and opposite force exerted by the beam. For a microbead displaced laterally from the beam (see figure 1.1ai), a ray of light refracted by the particle on the side away from the beam is less intense then a ray refracted near the center of the beam. The result is that the net transverse force of a pair of rays (from both sides) points toward the beam center and the
particle is pulled to the higher intensity at the center [3]. The net force exerted by the pair of rays points toward the beam focus because of the steep gradient along the optical axis. For a particle downstream of the focus against the beam propagation (see figure 1.1a(ii), it is important to note that the net force exerted by the refracted rays (the gradient force) pulling backward to the focus can overcome the net force exerted by reflected rays (the scattering force) pushing forward along the optical axis. The interplay between the gradient and the scattering forces causes the trapped particle at the beam focus to be shifted slightly forward from the focal plane as shown in figure 1.1b.

Once the particle is trapped at the laser beam focus, the trap restoring force in each dimension can be approximated for small displacements as an elastic spring response as shown in figure 1.1b where $k_x$ is called the trap stiffness in x axis, similarly $k_y$ and $k_z$ in y and z [4]. Furthermore, following the equipartition theorem:

$$\frac{1}{2} k_x \langle x^2 \rangle = \frac{1}{2} k_B T \quad (1.1)$$

The equipartition approximation is valid for small deviations from the optical trap equilibrium position. It becomes less valid as the displacements grow bigger, and where the actual trap potential represents more of a Gaussian potential rather than a parabolic potential [5].
Figure 1.1: a diagram for optical tweezers trapping of high-index microparticles, a) white arrows show the net force direction, black shows refraction forces, yellow arrows show reflection forces (scattering), dashed arrows are for beam rays, i) a particle displaced laterally so the refractive force toward the beam center is bigger, ii) a particle downstream of the optical propagation where the refractive forces overcome the scattering, b) a trapped microparticle at the focal plane but slightly shifted because of scattering, the trapping restoring force is approximated by a spring force.

1.2.1 Trapping Low-Index particles:

Low-index particles have a refractive index (n’) lower than the surrounding medium refractive index (n). For a high-index particle, a pair of collimated rays is refracted inward. However, for a low index particle, the pair of rays is refracted outward, as illustrated in figure 1.2. Hence, the force exerted by a Gaussian beam pushes the low-index particle away from the beam in contrast to pulling the high-index particle inward. Considering the force that is exerted by the beam in one dimension (say x axis), the high-index particle experiences a potential well with a minimum at the center of the Gaussian
beam (figure 1.2a). However, the low-index particle experiences a potential “hill” at the beam intensity maximum.

![Diagram](image)

**Figure 1.2**: a diagram describes the type of the particle and the resulting potential energy. 

a) High index particle illuminated by a Gaussian beam which leads to a potential well. b) Low index particle illuminated by a Gaussian beam, the particle experience a potential barrier.

The repulsive force that a Gaussian beam exerts on a low-index particle creates a potential barrier [6]. In one dimensional motion, two potential barriers can trap the particle in a potential valley rather than a potential well (see 1.3b). Similarly, low-index particles can be trapped by reshaping the laser beam to construct a three dimensional potential barrier, such as a donut-shaped beam forming an optical vortex (Figure 1.3a1).
The optical vortex is a dark area inside the transverse cross section of the beam intensity profile [6].

![Image of optical vortex and microbubble](image)

**Figure 1.3**: a) Picture 1 is a donut-shaped beam, Picture 2 shows a microbubble trapped inside the optical vortex. b) a diagram for the potential energy along the x axis.

Microbubbles (low-index gaseous micro-size bubbles with refractive index \(n'\sim1\) for air) can be trapped in optical vortices [7]. Figure 1.3a2 shows a trapped microbubble within a donut beam and figure 1.3b illustrates in one dimension the optical trapping of the microbubble in the optical vortex. The laser beam spot can be reshaped into an optical vortex by means of diffraction. The required beam diffraction can be caused by transmission or reflection from a surface specifically designed to lead the beam into interference pattern that produces the desired beam shape [8]. Such designed surfaces are called holograms and chapter three shows some examples in sections 3.2.1 and 3.3.2.
1.3 Ultrasound Contrast Agent Microbubbles:

1.3.1 Background:

Ultrasound contrast agent (UCA) microbubbles contain an inert gas encapsulated by a biocompatible shell as thin as 15 nanometers (e.g. Albumin shell in Optison™ microbubbles, see figure 1.4 and table 1.1) [9]. Ultrasound is widely employed in medical imaging, and UCA microbubbles offer contrast enhancement [10]. When injected in the bloodstream, these UCA microbubbles follow similar rheological behavior to red blood cells but are significantly more echogenic. UCA microbubbles respond differently to the applied medical ultrasound than organs, which enhances imaging contrast for disease detection and classification.

In an ultrasound field, UCA microbubble dynamics go from oscillation to destruction depending on the ultrasound parameters (e.g. pressure, frequency…etc). Conventionally, the mechanical index (MI) is used to measure the ultrasonic pulse strength by normalized units [11]. This index is used medically to evaluate the bio-effect of ultrasound. MI is obtained from the applied rarefractional pressure ($P_i$) in MPa units divided by the square root of the pulse center frequency ($f_c$) in MHz units then normalized to an ultrasonic pulse of $P_i=1$ MPa and $f_c=1$ MHz [12]. The US Food and Drug Administration (FDA) regulation for the upper limit of medical ultrasound use is $MI=1.9$ [13].

Based on the ultrasonic pulse MI, the microbubble acoustical response is categorized in three dynamical regions: symmetrical oscillations ($MI \leq 0.05$), nonlinear
oscillations (0.05 ≤ MI ≤ 0.3), and violent oscillations (MI > 0.3) that cause microbubble shell fragmentation or shell rupture [14]. In a weak ultrasound field (MI < 0.05), microbubbles oscillate symmetrically about the equilibrium radius as they expand and contract. As the ultrasonic field strength increases (MI > 0.05), the microbubbles become more resistant to the contraction than to the expansion [15], as shown in figure 1.5. This asymmetric oscillation produces higher harmonic frequencies, which provides a contrast in medical imaging compared to tissue response, as shown in figure 1.6, [10]. In a strong ultrasonic field (MI > 0.3), the UCA microbubble shell reaches unstable oscillation between a maximum and minimum radius leading to microbubble fragmentation or rupture.

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<td><strong>Mean Diameter</strong></td>
<td>3-4.5 μm</td>
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<tr>
<td><strong>Inert Gas</strong></td>
<td>Octafluoropropane C₃F₈</td>
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<tr>
<td><strong>Shell substance</strong></td>
<td>Human Albumin</td>
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<tr>
<td><strong>Film Thickness</strong></td>
<td>15 nm</td>
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<tr>
<td><strong>Surface Tension</strong></td>
<td>0.9 N/m</td>
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**Table 1.1**

**Figure 1.4**

**Table 1.1**: Facts for Optison™ Microbubbles, **Figure 1.4**: UCA microbubble.
**Figure 1.5**: Demonstration of microbubble response to compression and rarefaction of a one-cycle ultrasonic pulse. The microbubble equilibrium size is followed by contraction, then expansion. Thicker arrows represent stronger restoring forces.

**Figure 1.6**: Ultrasonic images of liver lesion; left/right is before/after injecting UCA microbubbles into the bloodstream [10].
The UCA microbubble symmetrical oscillation in a weak ultrasound field can be approximated as a simple harmonic oscillator [11] (neglecting the medium damping and the surface tension of encapsulated shell):

\[ m \ddot{x} + kx = F_{\text{driv}}(t) \quad (1.2) \]

where \( m \) is the effective mass, \( k \) is the system stiffness, \( F_{\text{driv}}(t) \) is the driving force (the applied ultrasonic pulse) and \( x \) is the radial displacement of the microbubble wall from the equilibrium radius \( R_o \). Equation 1.3 gives the resonance “natural” frequency (\( f_R \)) of the oscillating microbubble:

\[ f_R = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \quad (1.3) \]

The effective mass and stiffness values were derived [16] [17]:

\[ m = 4\pi R_o^3 \rho, \text{and} \quad k = 12\pi \gamma P_o R_o \]

where \( \rho \) is the density of the medium, \( \gamma \) is the heat capacity ratio \( (C_p/C_v) \) and \( P_o \) is the ambient pressure (~1 atm =100 kPa). The resonance frequency in this approximation is:

\[ f_R = \frac{1}{2\pi R_o} \sqrt{\frac{3\gamma P_o}{\rho}} \quad (1.4) \]

For air bubbles in water, equation 1.4 becomes:

\[ f_R \approx \frac{3 (ms^{-1})}{R_o} \quad (1.5) \]

Considering a microbubble of 3 \( \mu \)m diameter, the resonance frequency is roughly ~2 MHz. In medical applications, ultrasonic transducers of frequencies close to the UCA microbubble resonance frequency are used to improve the UCA microbubble response (maximizing the oscillation amplitude) [18].
Most of the medical applications of UCA microbubbles are still under extensive research. First, the optimization of the ultrasound imaging efficacy within the (FDA) safety range is an important concern [19]. Second, the dependence of microbubble oscillation on its intrinsic physical properties such as diameter, elasticity, viscosity …etc. in experimental conditions that resemble blood microcirculation is an active research field [20]. Third, UCA microbubbles offer a biocompatible carrier to deliver drugs to targeted tissues [21]. Fourth, fluorescent UCA microbubbles have been recently developed to enhance deep tissue optical imaging [22].

1.3.2 Ultrasound targeted therapy:

Applications of UCA microbubbles in medical imaging avoid strong ultrasonic fields (IM > 0.3) that lead to UCA microbubble destruction. In contrast, ultrasound targeted therapies rely on shell fragmentation [23]. Drug delivery utilizes microbubble shell rupture to unload cargo drugs at a specific medical target as shown in figure 1.7 [24]. The microbubbles reach the targeted tissue (such as a tumor) through the blood microcirculation. Exposing the targeted tissue to a proper ultrasonic pulse, microbubble shell rupture releases the cargo drugs. This therapy mechanism requires precise knowledge of minimum shell rupture threshold of UCA microbubbles.

Our objective is to determine the rarefractional pressure threshold for shell rupture of confined UCA microbubbles. The confinement environment aims to resemble similar oscillation conditions that UCA microbubbles experience inside blood microcirculation.
We will discuss in the next chapter the experimental methodology of detecting the shell rupture rarefactional pressure threshold for both unconfined and confined individual UCA microbubbles. This work provides a clearer acoustical understanding of UCA microbubble dynamics confined in a microtube or a capillary.

Figure 1.7: Drug Delivery Scheme for using UCA Microbubbles in ultrasound targeted therapies, the microbubbles reach the targeted tissue through the blood vessels then unload cargo drugs because of shell rupture after ultrasonic exposure [24].
CHAPTER TWO

Acoustical Probing of UCA Microbubbles

Confined in Capillaries
2.1 Unconfined UCA microbubbles:

2.1.1 Background:

Acoustical probing of the cavitation acoustic pressure threshold was previously reported [25-28]. In these studies, several criteria were implemented to detect the shell rupture threshold of UCA microbubbles. However, only one of the previous acoustical criteria is backed by optical observation. Ammi et al reported that when shell rupture of a single microbubble is optically observed, the acoustical probing system detects first a signal from the microbubble during the ultrasonic excitation and then there is a post-excitation signal that arrives 1-5 µs later [29-30]. However, the post-excitation signal is always absent when the microbubble only oscillates in the ultrasonic field and no shell fragmentation occurs [14].

These optical observations validate an acoustical criterion for shell rupture criterion called the inertial cavitation criterion (or post-excitation emissions criterion) which links shell rupture events and post-excitation emission detected by a passive cavitation detector (PCD) after 1-5 µs from the principle response signal [31]. We used the post-excitation criterion in our experiments to determine the minimal shell rupture threshold for unconfined and confined UCA microbubbles.
2.1.2 Passive Cavitation Detector setup:

In a PCD detection system; a transmitter provides ultrasonic excitation with a narrowband frequency near the resonance frequency of UCA microbubbles (~2.25 MHz). Then the microbubble emission is detected by a broadband receiver (typically centered at 15 MHz) as shown in figure 2.1. We used focused transducers for our PCD system and the ultrasonic focal volumes were determined for each transducer by a calibrated hydrophone needle. Attached to an automated positioning system, the needle hydrophone scans the focal volume of a transducer providing the peak-to-peak ultrasonic pressure measurements (see figure 2.2). The PCD volume is the shared focal volume between the two aligned transducers where the ultrasonic field is greater than half the maximum peak-to-peak pressure (i.e. -3dB cross section volume). We prepare our UCA microbubble solution so that we have an average concentration of a single microbubble per PCD volume inside a degassed water tank.

![Figure 2.1: Passive cavitation detector PCD, a transmitter provides ultrasound excitation at frequency 2.25 MHz, and a receiver of 15 MHz broadband frequency. The PCD system is in a degassed water tank.](image-url)
Figure 2.2: Measurements for XY scan of the peak-to-peak pressure of ultrasonic field for 2.25 MHz focused transducer, X is the acoustical axis. The maximum peak-to-peak pressure value is 2.1 MPa.

2.1.3 Post-Excitation Emission:

Here we detail an experimental example of detecting shell rupture for unconfined microbubbles using the post-excitation emission criterion. An individual microbubble was excited by a one-cycle pulse of rarefactual pressure 2.2 MPa and of frequency 2.25 MHz (MI=1.7). Figure 2.3 shows a signal detected by the 15 MHz broadband receiver. The principal response of the microbubble arrived first, and then a post-excitation emission followed 1 µs later. The Time-Frequency spectrogram in figure 2.3 shows that the post-excitation emission is significantly above the background noise. The post-excitation emission occurs after a shell rupture event, which results in the formation of free gas microbubbles.
Figure 2.3: Signal from PCD setup; detected by 15-MHz broadband receiver after exciting with 2.25-MHz focused transducer applying incident rarefractional pressure of 2.5 MPa, one cycle pulse and pulse repetition frequency (PRF) of 500 Hz. Lower: Corresponding Time-Frequency spectrogram. The zero time is arbitrary.

In the case of microbubble oscillation without a shell rupture event, only the principal response during excitation is detected. Figure 2.4 demonstrates the contrast between the shell rupture signal of a microbubble and the oscillation-only signal of another microbubble. Both were excited by a one-cycle pulse of rarefractional pressure 1.8 MPa and of frequency 2.25 MHz (MI=1.2), the upper signal is for the ruptured microbubble and the lower signal is for the oscillating microbubble. While the principal response is detected in both signals, the post-excitation emission is only detected in the
upper signal. The red dash lines in figure 2.4 define the positive and negative limits of the background noise.

**Figure 2.4:** Two different signals detected by 15-MHz broadband receiver after excitation by 2.25-MHz focused transducer applying incident rarefactional pressure of 1.8 MPa, one cycle pulse and PRF of 500 Hz. The upper signal shows a ruptured microbubble where the principal response is followed by the post-excitation emission after ~ 1.5 µs. The lower signal shows an oscillating microbubble where the principal response is only detected. The red dash lines define the positive and negative limits of the background noise.
In our preliminary experiments on unconfined microbubbles, we detected shell rupture events after excitation by a minimum applied rarefactional pressure of 0.8 MPa (one-cycle pulse, frequency 2.25 MHz, MI= 0.5). Figure 2.5 shows the received signal including 10 µs prior to the microbubble principal response and 10 µs after the post-excitation emission. The positive and negative limits of the background noise are indicated by red dash lines which distinguish the microbubble principal response and post-excitation emission from the rest of the signal. The inset in figure 2.5 is the Time-Frequency spectrogram for a portion of the signal (5 µs to 15 µs) which differentiates the frequency range and intensity between the microbubble signal and the background. For the post-excitation emission, the signal-to-noise ratio (SNR) is 9.5 dB [$A_{signal} = 7.5$ mV and $A_{noise} = 2.5$ mV, where $A$ is the root mean square (RMS) amplitude].

**Figure 2.5:** A signal detected after excitation by incident rarefactional pressure of 0.8 MPa (one cycle pulse, frequency 2.25 MHz and PRF of 500 Hz). The red dash lines show the positive and negative limits of the background noise. The principal response is followed by the post-excitation emission after ~2.5 µs. The inset is the Time-Frequency spectrogram for a portion of the signal from 5 µs to 15 µs.
Figure 2.6: Reported rarefational pressure thresholds for different applied frequencies (0.9 MHz, 2.8 MHz and 4.6 MHz) and different ultrasonic pulse cycles (3, 5 and 7) [31]. The red dashed line marks the minimum rarefational pressure 0.8 MPa (one-cycle pulse and applied frequency 2.25 MHz) at which shell rupture is detected in our experiments.

The rarefational pressure threshold was previously reported for unconfined UCA microbubbles based on the post-excitation criterion [31]. In that study, the shell rupture threshold increased with frequency (0.9 MHz, 2.8 MHz and 4.6 MHz) and decreased with the number of cycles (3, 5 and 7) as shown in figure 2.6 [31]. For an ultrasonic pulse of frequency 2.5 MHz, the reported rarefational pressure threshold is in the range 0.7- 0.9 MPa [31]. The red dashed line in figure 2.6 marks the minimum rarefational pressure at which we detected shell rupture after excitation with one-cycle pulse from a 2.25 MHz transmitter. For our experimental setup, the shell rupture threshold of unconfined microbubbles is at most ~0.8 MPa which is a reference value for later experiments when we investigate shell rupture threshold for microbubbles confined in capillaries.
2.2 Confined UCA microbubbles:

The oscillation of UCA microbubbles in an ultrasonic field varies significantly with the distance from nearby surfaces [32]. Since microbubbles are confined in the blood vessels when injected for medical imaging, similar micro-confinement should be examined. The blood microcirculation can be imitated experimentally by confinement in microtubes. There is no previous investigation for shell rupture threshold in capillaries using post-excitation criterion. Moreover, it is important that the micro-confinement has mechanical properties that resemble the blood vessels. Some studies used silicon microtubes although they differ from human capillaries [33-34]. The mechanical tensile failure strength of human capillaries ($\sigma_t$) ranges from 0.5 MPa to 5 MPa while silicon microtubes have $\sigma_t = 8.4$ MPa [34]. However, a regenerated cellulose (RC) hollow fiber has $\sigma_t = 1.3$ MPa which makes it a better candidate for microbubble confinement.

2.2.1 Experimental setup:

We used a passive cavitation detector aligned in a degassed water tank with a RC capillary vertically crossing the PCD volume ($V_{PCD} \sim 0.086 \text{ mm}^3$) as shown in figure (2.7, B). The transmitter and receiver (focused 2.25 MHz and 15 MHz, Olympus) were calibrated with a 75 $\mu$m-diameter needle hydrophone (Precision Acoustics). The capillary (RC hollow fiber dialysis, Spectrum) has an inner/outer diameter of 200/280 $\mu$m. The ends of the capillary were connected and sealed into 1-mm diameter tubes and the upper tube was connected to a manual micro-pumper. The capillary exposure volume $V_C$
(as shown in figure 2.7 C) was estimated to be $\sim 0.88 \times 10^{-2}$ mm$^3$ and a proper microbubble solution was prepared to have an average concentration of an individual microbubble per $V_C$ ($\sim 1$ microbubble$/10^2$ mm$^3$). Before injecting the microbubbles, echo signals from a water-filled RC capillary were recorded for all applied ultrasonic rarefactional pressures (an example signal is shown in figure 2.8).

**Figure 2.7:** A) Left/Right is a scheme/Picture of the experimental setup. B) Left to right respectively: the inner diameter of RC capillary (200 $\mu$m), the capillary crossing vertically PCD volume and the alignment of the transmitter-receiver with 60 degree angle separation. C) The UCA microbubble solution was prepared to have a single microbubble per the capillary volume that crosses PCD volume.
2.2.2 Detecting In-capillary Shell Rupture:

Before injecting the microbubbles, echo signals from a water-filled RC capillary were recorded for the applied ultrasonic rarefational pressures. Recording the reflected signal from the capillary provides a detected background signal. This allows us to clearly identify microbubble emission in the total detected signal after injecting microbubbles into the capillary. The gray-line signal (figure 2.8, left) is reflected from a capillary with no microbubbles (applied pulse: 4-cycles and incident rarefational pressure $P_i=2.2$ MPa). The detected signal after injecting the microbubbles is represented by the dark line (figure 2.8, right). The gray-line signal masks out the capillary reflection in the later signal to distinguish the microbubble signal (figure 2.9). The principal response occurs during the excitation and can be seen in figure 2.9 within the microtube echo. The post excitation emission arrives after excitation (outside the capillary response) indicating a shell rupture event. Similarly, figure 2.10 shows a ruptured microbubble after excitation with a 2-cycle pulse of $P_i=2.2$ MPa.

![Figure 2.8:](image)

**Figure 2.8:** Left is the reflected signal from water-filled RC capillary before injecting the microbubbles. Right is the signal from water-filled RC capillary after injecting the microbubbles. The applied pulse is 4-cycle, $P_i=2.2$ MPa, 2.25 MHz and PRF=100 Hz.
Figure 2.9: In-capillary probing of UCA microbubble shell rupture. Two superimposed signals after excitation by an ultrasonic pulse of 4-cycle, $P_i=2.2$ MPa, frequency 2.25-MHz and PRF=100 Hz. The gray line is the reflected signal from the RC capillary before injecting the microbubbles. The dark line is for in-capillary microbubble emission. The microbubble principal response occurred during excitation then the post excitation emission followed $\sim 2\ \mu$s later (outside the capillary echo).

Figure 2.10: In-capillary probing of UCA microbubble shell rupture after excitation by an ultrasonic pulse of 2-cycle, $P_i=2.2$ MPa, frequency 2.25-MHz and PRF=100 Hz. The microbubble principal response occurred within the capillary echo then the post excitation emission followed $\sim 1\ \mu$s later.
The duration of the capillary response (due to wall reflection) was ~3 µs for the 4-cycle pulse and ~2 µs long for the 2-cycle pulse. The microbubble principal response in our observations occurred within the last microsecond of the capillary response. In the case of a shell rupture event, the post-excitation emission occurred outside the capillary response (1-5 µs after the principal response). For this reason, it is possible to identify the post-excitation emission even though the microbubble principal response is not resolved within the capillary wall reflection. While figure 2.11 shows an oscillating microbubble where the principal response is barely identified, figure 2.12 shows a ruptured microbubble where the post-excitation emission is clearly identified but the principal response is not detected.

**Figure 2.11:** In-capillary probing of oscillating UCA microbubble (applied pulse: 4-cycle, $P_i=2.2$ MPa, frequency 2.25-MHz and PRF=100 Hz). Only a weak microbubble principal response is detected.
In-capillary probing of UCA microbubble shell rupture after excitation by an ultrasonic pulse of 4-cycle, $P_i=2.2$ MPa, frequency 2.25-MHz and PRF=100 Hz. The post-excitation emission is detected but the microbubble principal response is not resolved within the capillary wall reflection.

2.2.3 Results and discussion:

The applied rarefractional pressure was varied from 0.5-2.5 MPa using 2-4 cycle ultrasonic pulses (frequency 2.25-MHz and PRF=100 Hz). No post-excitation emission was detected for applied pressure in the range 0.5-1.08 MPa. Above 1.08 MPa, post-excitation emission was observed for 4-cycle ultrasonic pulses, but the first post-excitation emission detection for 2-cycle pulse was at 1.35 MPa. Figure 2.13 (A, B) shows two signals for two different shell rupture events after excitation with 2-cycle and 4-cycle pulses and applied rarefractional pressure of 1.35 MPa. The post-excitation emission followed the capillary response but the principal response is only identified for the 4-cycle pulse (figure 2.13 B). The absence of post-excitation emission in the applied
pressure range 0.8-1.08 MPa indicates that the shell rupture threshold for UCA microbubbles inside the RC capillary is larger than our measured threshold pressure for unconfined microbubbles (0.8 MPa) by at least ~0.3 MPa. Further investigation is needed to precisely determine the threshold in the rarefactive pressure range 1.1-1.35 MPa.

Figure 2.13: A) Microbubble shell rupture (applied pulse: 4-cycle, $P_i=2.2$ MPa, frequency 2.25-MHz and PRF=100 Hz), the post-excitation emission followed the capillary echo but the principal response is not resolved within the RC capillary echo. B) Microbubble shell rupture (applied pulse: 4-cycle, $P_i=2.2$ MPa, frequency 2.25-MHz and PRF=100 Hz), the post-excitation emission occurred ~1.5 μs after the principal response.
The increase in the rupture pressure threshold for a confined microbubble can be linked to a decrease in the effective in-capillary rarefational pressure $P_{\text{eff}}$. Although a water-filled RC capillary is expected to be highly transparent, the possible reduction in $P_{\text{eff}}$ because of reflection from the capillary wall reflection was experimentally investigated. We wanted to evaluate quantitatively how much the wall reflection contributes to the increase in the pressure threshold. The percentage of the ultrasonic pulse that is reflected by the capillary can be evaluated by measuring the transmitted pulse power. Considering that the ultrasonic focus is at $(0,Y_o,Z_o)$, the hydrophone needle scanned a $2\times2$ mm$^2$ plane centered at $(0.8 \text{ mm},Y_o,Z_o)$ as shown in figure 2.14. The peak-to-peak pressure (P) scanning measurements for the specified plane were obtained with the presence of the water-filled RC capillary in the ultrasonic focus. The measurements were repeated without the capillary.

**Figure 2.14**: Scheme for evaluating the transmitted pulse power with/without the presence of RC capillary. The peak-to-peak pressure measurements were collected by a needle hydrophone for a plane at 0.8 mm from the ultrasonic focus.
Figure 2.15 shows two peak-to-peak pressure scans of the described area before/after removing the RC capillary from the ultrasonic focus. The total transmitted power is proportional to $P^2$ [35-36] and was quantified for each scan. The evaluation yielded that the transmitted ultrasonic power through the RC capillary is at most 10% less than the “capillary-free” ultrasonic field. This indicates that the reflection by RC capillary walls may only explain partially the observed increase in the threshold pressure.

Figure 2.15: Left/Right peak-to-peak pressure scan measurements with/without the presence of RC capillary. The scans are for YZ plane at 0.8 mm from the focus. The peak-to-peak pressure maximum value is 2.1 MPa.

The increase in shell rupture threshold because of the confinement can also be linked to the extreme microbubble oscillations prior to shell rupture as the maximum radius reaches 4-8 times the equilibrium radius [30]. Assuming a microbubble
equilibrium radius of \(~3\mu m\) centered in a \(100\mu m\)-radius RC capillary, the microbubble can expand to 20\% of the capillary radius for incident ultrasonic pressure \(P_i\) that is close to the threshold pressure as illustrated in figure 2.16. This significant microbubble-to-capillary radius ratio is expected to induce a pressure by the compressed in-capillary fluid. As a result, the effective rarefractional pressure is reduced and the microbubble expansion is suppressed. This effect has been reported and verified by optical observations for PMMA capillaries. High speed camera measurements showed that the maximum expansion of microbubbles is reduced proportional to the decrease in PMMA capillary diameter [37]. Based on the Plesset and Mitchell criterion for shell rupture, the microbubbles have to reach a maximum radius that exceeds 10 times the minimum radius [38].

Figure 2.16: Illustration of the reduction in the effective rarefactional pressure because of the confinement. Under acoustical excitation by low applied pressure, the microbubble size is relatively small. Under acoustical excitation by high applied pressure, the microbubble radius reaches expansion of at least 20\% of the capillary radius when the applied pressure is close to the shell rupture threshold. White arrows represent forces by the capillary fluid.
2.2.4 Conclusion:

The acoustic pressure threshold was investigated for UCA microbubble destruction inside regenerated cellulose capillaries. The microbubble solution was prepared to achieve single microbubble measurements. Excitation was with 2-4 cycle ultrasonic pulses by 2.25 MHz transducer. The shell rupture threshold of UCA microbubbles confined in a RC capillary was found to be larger than unconfined microbubbles by at least 0.3 MPa. This is the first experimental investigation of shell rupture threshold in capillaries using post-excitation criterion. The loss in the applied ultrasonic power due to the capillary wall reflection was quantitatively examined. The transmitted ultrasonic pulse power was 10% less than the applied ultrasonic pulse. The confinement suppression of the in-capillary maximum expansion of the microbubbles is expected to contribute to the pressure threshold increase but the effective rarefactional pressure inside the capillary is yet to be measured.
CHAPTER THREE
Optical Trapping of UCA Microbubbles
Confined in Capillaries
3.1 Background:

Present experimental techniques to study the dynamics of individual ultrasound contrast agent (UCA) microbubbles are limited because of factors related to the natural motion of microbubbles, e.g. fluid flow, buoyant forces, Brownian motion and attractive forces to other objects. Furthermore, the response of UCA microbubbles to acoustical excitation depends on their distance from nearby walls [32]. These effects are challenging when confinement of microbubbles in capillaries is experimentally introduced to resemble the blood micro-circulation. Optical tweezers offers the capability to overcome these limitations by controlling the motion of individual UCA microbubbles and properly positioning them inside capillaries. Optical trapping also enables the introduction and manipulation of other objects within the microbubble environment and the precise measurement of their interaction forces.

Combining optical tweezers with an acoustical setup to probe confined microbubbles involves some challenges because of the optical properties of UCA microbubbles, the capillary confinement and instrumental limitations. First, Hermite-Gaussian (HG) laser beams typically used in conventional optical tweezers can’t trap the gaseous microbubbles. Second, focusing the laser beam through a microtube wall requires consideration of refraction index matching and surface curvature. Third, additional constraints are imposed on the optical tweezers components to ensure that the ultrasound focal field is undisturbed. The following three sections detail experiments
which were conducted in an effort to develop an optical tweezers setup that can be successfully integrated into a proposed opto-acoustical setup.

### 3.2 Optical Trapping of Microbubbles:

Microbubbles are mostly composed of gas with an index of refraction near 1 while water is \((n=1.33)\), so microbubbles are pushed away from the laser focus. The solution is to create a laser beam with a minimum of intensity in the center rather than a maximum [7]. A doughnut-shaped beam can be made with a spatial light modulator (SLM) implementing a fork-like computer generated (CG) hologram (Figure 3.1a). By illuminating the SLM with a Hermite-Gaussian laser beam of transverse mode TEM\(_{0,0}\) the reflected beam is converted to a Laguerre-Gaussian (LG) of transverse mode TEM\(_{m=0,c\neq0}\). Figure 3.1 shows HG beam, the converting hologram and the resulting LG beam TEM\(_{0,1}\). The radius of the donut beam at the focus is theoretically proportional to \(\sqrt{\ell}\) [39]. However, a rather linear proportionality empirically was reported [40].

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**Figure: 3.1**: a) Producing TEM\(_{0,1}\) LG beam using CG hologram implemented by SLM in inverted optical tweezers setup, b) up to down: HG laser beam at focus plane of the objective (100X), the fork-like CG hologram, the donut-shape TEM\(_{0,1}\) LG beam. The scale bar is 1 μm.
3.2.1 Exploratory experiments:

Optical vortices capable of trapping microbubbles have been previously demonstrated with both inverted and upright optical tweezers [41]. In several exploratory experiments using inverted optical tweezers, I’ve been able to trap UCA microbubbles (Optison™) of ~2μm in diameter. First, a HG beam of transverse mode TEM\(_{0,0}\) and wavelength λ~850 nm was converted to a LG beam by a SLM (Holoeye Inc. LC-R 2500) integrated into an inverted optical tweezers setup (see Figure 3.2). An oil immersion objective is used with high numerical aperture (N.A. 1.25) (oil immersion 100X, Edmund Optics).

**Figure 3.2:** Spatial light moderator (SLM) integrated into inverted optical tweezers setup, T1 and T2; two telescopes to adjust the laser beam size, first to cover the SLM screen then to fit into the objective. Mirrors (M) and lenses (L) directing the beam to SLM, the objective and CCD camera: M1, (M2, L1, DM3-dichroic mirror) and (DM4, L2, L3) respectively.
An optical vortex beam of $\ell=10$ was formed at the objective focal plane as shown in Figure 3.3. This optical vortex beam was able to trap a UCA microbubble of size equal to the ring radius. Furthermore, Figure 3.4 shows a microbubble of $\sim 2\mu$m in diameter that was trapped. After trapping the microbubble inside the optical vortex, the translation stage was moved with increasing automated speeds. The optical trap strength is typically evaluated by measuring the speed at which the microbubble escapes the optical trap. This motion caused the surroundings (water + microbubbles) to move in the objective focal plane while the microbubble was held in the optical tweezers trap as illustrated in Fig.3.4.

**Figure 3.3:** Optical vortex, $\ell=10$, with a diameter roughly $\sim 2\mu$m.

**Figure 3.4 L&R:** Images of trapped microbubble in optical tweezers trap. The trapped microbubble remains at the center of the images while the surroundings move in the direction of arrow.
For the 2μm-microbubble shown in Figure 3.4, the trap escape velocity in the focal plane was $v_e \sim 1\mu m/s$ using laser power of 35 mW. This trap escape velocity demonstrates a stable optical trapping in the focal plane in contrast to the trapping force along the optical axis ($z$) which was found to be weaker. Our results are consistent with previous reports using inverted optical tweezers [32]. The buoyancy force makes the microbubbles populate the upper end of the sample chamber further away from the objective optimum working distance in inverted optical tweezers setup. Typically, previous experiments studying microbubbles used an upright microscope. Yet, both vertical optical trapping setups (inverted and upright) don’t provide a uniform distribution of microbubble population in the capillary. The possible optical configuration that provides optimum working distance and a uniform distribution is a horizontal optical tweezers setup which will be discussed later. Moreover, microbubbles with bigger diameters demand higher LG order $\ell$, but low-cost commercial SLMs have limited capability in HG-to-LG conversion. Alternatively, multiple HG laser spots can provide a stable trapping and manipulation which will be demonstrated in the following section.

3.3 Optical Trapping in Capillaries:

Typically in optical tweezing, the laser beam is focused inside a standard planar sample chamber. The chamber is made of a cover slip and a slide separated by sticky tape (see figure 3.5 upper). The chamber is filled with a solution containing the trapped particles and the chamber height is 100 to 140 μm. In case micro-confinement is needed, the typical approach is to fabricate a planar microchannel as shown in figure (3.5 lower) [42].
Confinement within a curved surface, such as a capillary, is preferred for certain applications such as resembling blood microcirculation confinement of microbubbles. The geometry of nearby surfaces can significantly impact the acoustical oscillations of microbubbles [32]. Capillaries also provide an opportunity for accessing the sample from 360 degrees which allows integration of different probing systems such as illumination by another laser beam [42] or an acoustical setup.

**Figure: 3.5:** Upper: a diagram for the standard sample chamber of height $120 \, \mu m$ typically.

Lower: a planar microchannel fabricated on a slide.
Only a few previous studies were found on optical trapping inside capillaries which we will call “in-capillary trapping”. In-capillary trapping of 1 µm microbeads limited to the very bottom of glass microtubes (20µm-500µm) was previously reported [43]. In these reports, three approaches were used for in-capillary trapping. First, water-filled glass capillaries were placed above a glass slide with immersion oil matching the refractive indices as shown in figure 3.6.A. Second, immersion oil was placed directly between the objective and the glass capillary. Third, no immersion oil was placed on the capillary and instead a dry objective was used [42, 44]. To compensate for the curvature effect on the laser beam, calculated CG holograms were applied using phase-only SLM with the maximum efficiency commercially available (Hamamatsu, 40%). Only optical trapping of microbeads inside glass capillaries was reported and no in-capillary trapping of microbubbles was found in the literature.

We designed our capillary cell chamber differently to minimize undesired optical effects related to the capillary that were not avoided in previous reports [42]. The main effect of capillary surface curvature can be analyzed by paraxial invariant. Following the treatment of Cojoc et al we define a reference planar surface at the capillary bottom wall curvature as shown figure 3.6.A [42]. Because of the capillary curvature, equation 3.1 illustrates that the in-capillary optical trapping position (z’) from the planar surface is different relative to the standard chamber trapping position (z). The change in optical trapping position depends on the value of C(z, n, n’) as shown in equation 3.2. This value is less than one when n’ < n which always makes the in-capillary trapping position closer
to the capillary bottom wall than the standard trapping position (figure 3.7). However, $C(z, n, n')$ is equal to unity if $n=n'$ which is what we aimed for (i.e. refractive indexes completely match).

$$\frac{n' - n}{R} = \frac{n'}{z'} - \frac{n}{z} \quad \Rightarrow \quad z' = z * C(z, n, n') \quad (3.1)$$

$$C(z, n, n') = \frac{n' R}{n R + z(n' - n)} \quad (3.2)$$

For water-filled capillaries, refractive index matching can be achieved by immersing the capillary in a water-filled chamber as shown in figure 3.6.B. Moreover,

Figure 3.6: A) Glass microtube mediated by immersion oil. Refraction index is $n/n'$ for oil/water and in-capillary trapping position $z'$ is shortened from standard position $z$. B) Water-filled RC microdialysis capillary in a water-filled sample chamber resulting in $n=n'$ and $z - z'$. Dash lines represent planar reference for curvature and the optical axis.
there are other factors that were ignored in previous designs such as arbitrary edges between oil and glass and in-capillary internal reflection. Such factors are also avoided when water is used for index matching. The homogeneity between the solution inside the capillary and the surrounding water is maximized by using microdialysis capillaries (such as regenerated cellulose RC capillary) where water can diffuse through semi-permeable walls in contrast to a glass capillary. The physical properties of different capillaries were discussed in chapter two which showed also that RC cellulose capillaries have better resemblance of blood vessels.

3.3.1 The Capillary Cell Chamber:

After testing several sample designs, figure 3.7.A shows the design that has been used in our experiments. The capillary goes through a chamber ~360 µm in height (figure 3.7.B). The capillary outer/inner radius is 140/100 µm. This chamber is made of a cover slip, a standard glass slide and sticky tape setting the height of the chamber (figure 3.7.B). The capillary is fixed at both ends to the slide and connected and sealed into 1 mm-diameter tubes. A syringe or microinjector is used to control the liquid flow in the capillary. The chamber is filled with water by using another microtube that injects the liquid into the chamber through unsealed access in the sticky tape. This unsealed access allows refilling water to recycle the chamber for multiple experiments. The planar cover slip is in contact with the objective (N.A. 1.25, 100X, Edmund Optics).
Figure 3.7: A, both ends of RC capillary are fixed to a slide and connected to 1 mm-diameter tubes. Fluid is injected manually by syringe or micro-injector; RC capillary is sandwiched in the chamber between the slide and a cover slip. B, RC capillary inside water-filled chamber chamber of height 360 µm.
3.3.2 In-Capillary microbead trapping:

In order to evaluate the optical trapping capability inside our capillary cell chamber, a calibration was performed. We compared the optical trapping in the new design with the optical trapping of standard particles (2 µm polymer microbeads) inside the standard sample chamber. An inverted optical tweezers setup was used similar to figure 3.2 (detailed in section 3.2) with the exception of replacing the spatial light modulator by a mirror. For trapping individual microbeads no light modulation is needed for the laser beam.

It is important to notice that the height of the standard chamber is about half the height of our capillary chamber calibration (see figure 3.8). Experimentally we found that the furthest possible trapping position in the standard chamber corresponds to ~60 µm inside the capillary from the bottom wall. A solution of microbeads was injected manually by a syringe and then after a while the flow was slowed. The microbeads are mainly subject to Brownian motion and a microbead was optically trapped. Figure 3.9 shows trapping and manipulation of the 2 µm microbead at ~60 µm above the capillary bottom wall. First, a maximum trapping power was applied at ~40 mW then the power was gradually reduced. The minimal trapping power was ~1 mW when the microbead left the optical trap due to Brownian motion. This result is very comparable to the minimum optical trapping power previously measured for optical trapping inside the standard chamber for 2 µm microbead. This indicates that our capillary cell chamber has good optical trapping conditions similar to the standard chamber at least up to ~60 µm.
Figure 3.8: The maximum trapping position in a standard chamber compared to the capillary chamber is about 60 µm from the capillary lower wall. The distance between the cover slip is about 20 µm.

Figure 3.9: a&b) 2 µm undyed microbead trapped inside RC capillary and moved in both direction in the focal plane. The trapping was ~60 µm above the capillary bottom wall. Patches from different time frames are distinguished by black dash lines.
3.3.3 In-Capillary Microbubble trapping:

**Experimental Setup:**

After calibrating the new cell chamber with microbeads in similar trapping conditions, stable optical trapping and manipulation at the very top of the capillary was achieved for microbubbles. Only a HG laser beam was used to avoid the power loss that is associated with HG-to-LG conversion. The inverted optical tweezers is integrated to a simple low-cost SLM (SDE1024, Cambridge Correlators Ltd) in an optical setup similar to figure 3.2. This SLM has a phase shift range less than $2\pi$ which reduces the efficiency of the first order diffraction power and produces additional unwanted beam spots near the first order beam array [45]. Yet, with this limited SLM capability and despite increasing beam scattering near the upper capillary wall, the UCA microbubble was trapped and manipulated in the focal plane. The trap escape velocity was at least 5 times the trap escape velocity that we previously measured using a LG beam in a standard cell chamber.

A diluted solution of microbubbles (Optison™ 0.2 ml, water 5 ml) was injected manually by a syringe. Then, a sawtooth grating hologram (see figure 3.10) was implemented on the SLM to form a simple laser beam configuration, a line of three laser the focal spots (zeroth and first order beams). Figure (3.11.a) shows the laser beam configuration at focal plane -2 μm beneath the upper capillary wall where most of microbubble population is located.
**Microbubble Trapping and manipulation:**

The spacing between the beam spots is adjusted by the distance $\Lambda$ between the fringes in the hologram as shown in figure 3.10. The distance between the beams was set to $\sim 2 \, \mu m$. The line of beam spots is arranged against the capillary flow (top-to-bottom as indicated with a white arrow, see figure 3.11b) and a microbubble of proper size was stopped and held between two beam spots as shown in the unfiltered/filtered image in figure (3.11.b/c).

The microbubble was stopped while approaching with a speed of $\sim 10 \, \mu m/s$. This speed is 10 times greater than the trap escape velocity we previously measured for a microbubble with similar diameter trapped with a LG laser beam of similar laser power ($\sim 35 \, mW$) (see section 3.2.1). Furthermore, figure 3.11 (d. 1-6) shows the microbubble colliding with another microbubble in the flow, forming a double microbubble before moving with the flow while the microbubble remained fixed. This demonstrates utilizing such a simple laser configuration to hold and examine microbubbles against a flow or possibly ultrasonic pressure direction.
Figure 3.11: Illustration of minimal HG laser beam configuration required to trap microbubbles in a capillary, a) Three laser beam spots: the zeroth order and the two first order, distance can be adjusted to the preferable microbubble diameter. b) a microbubble is held between the zeroth order beam and the first order beam on the right against more than 10 µm/s flow speed. c) Filter is used for better visualization; the laser beam location is indicated by red circles. d) Detailing a collision event between the fixed microbubble and another microbubble forming double-microbubble which last for 1.07 s, black arrows show direction of motion for the second microbubble before and after collision.

Since the line of beam spots is only a trap against the flow direction (or rather a holder) we employed more HG beam spots to construct a square optical trap. A square of beam spots can be shaped by superimposing two grating holograms in single hologram
implemented on the SLM as shown in figure 3.12. As a result, a pair of square configurations is formed by eight HG beam spots as shown in figure (3.13a). The additional spots, in red circles, have less intensity (inherently from SLM). The spacing and location of the square configuration sides is adjustable by CG holograms which enables performing a dynamic trapping maneuver as detailed in figure (3.13 b 1-3). First, when a microbubble appears into the monitor screen, three laser spots of the square trap form a V-shape with a proper size to hold the microbubble against the flow. Then, the laser spots are arranged dynamically to enclose the microbubble inside the trap.

Figure 3.12: Left and middle are two sawtooth grating holograms with 90 degree shift between their fringes. Right is the resulting hologram resulted from superposing the two grating holograms which generates a square beam configuration.

Alternatively, the square trap is set with a trap entrance that permits a microbubble with a selected diameter (~3µm) (the distance between the circle and triangle in figure 3.13.c 1). The trap entrance is the square configuration side that is against the capillary flow. Microbubbles bigger than the selected diameter were pushed away by the trap entrance while smaller microbubbles slipped from the laser beam
configuration as shown in figure 3.13.c 1-2. Later, a microbubble 3 \( \mu \text{m} \) approaching with speed of -5\( \mu \text{m/s} \) was forced into the optical trap. Figure (3.13.d) shows three successive frames superimposed to detail the trajectory of the microbubble as it moves into the trap. The trapped microbubble was manipulated in the focal plane by moving the optical trap configuration (manually by adjusting Mirror (M 2) of the inverted tweezers setup shown in figure 3.2).

Figure 3.13: Stable trapping and manipulation of microbubbles using four HG laser beam spots; a) The trapping configuration at the capillary bottom wall, red circles around inherently weaker laser beam spots. b. 1-3) demonstration of dynamic trapping maneuver especially useful for microbubbles with big diameters, 1. The microbubble is held with three laser beams of suitable size, 2-3. While holding the microbubble against the flow, the laser beam configuration is adjusted to enclose the microbubble. c) 1. Big microbubble is pushed out by the trap entrance (triangle corner and the circle), 2. Two small microbubbles sharing the square configuration trap before slipping out d) Trapping of a microbubble with a proper size (size is set by triangle corner and the circle). f) Microbubble manipulation in the focal plane. e.) showing fluctuation of the microbubble within the trap.
Figure 3.13.f shows a full maneuver where the trapped microbubble was moved in the black arrows directions. The microbubble was slightly fluctuating (∼±0.2µm) along the square configuration trap diagonal (see figure 3.13.e). The optical trap escape velocity is greater than ∼5µm/s (the speed by which the microbubble entered the trap configuration) which indicates trapping strength that is at least five times greater than the LG beam trapping for microbubble about the same size using the similar inverted optical tweezers setup.

**Results and discussion:**

The trapping and manipulation of microbubbles inside RC capillaries with an inverted optical tweezers setup is demonstrated in figure 3.13 utilizing a square configuration of HG laser beam spots. The advantage of using a HG beam instead of the conversion to LG mode is preserving beam power and providing the optical trap with the maximum power outcome with less beam modulation. As a result, the trap escape velocity is improved significantly at least by a factor of five. It is important, however, to understand the dimensions and the limitation of the optical trap profile. This can be roughly analyzed by evaluating the Gaussian beam waist function:

$$w(z) = w_o \left[1 + \left(\frac{z}{z_R}\right)^2\right]^{1/2}, \quad z_R = \frac{\pi w_o^2}{\lambda} \quad (3.3.3)$$

where $w_o$ is the minimal focal beam waist at $z=0$ and $z_R$ is the Rayleigh range where the beam waist is less than $\sqrt{2} w_o$. Experimentally, we estimated that the laser beam minimal
spot size at focal plane is approximately equal to one wavelength $2w_o \sim \lambda$. This gives roughly $w_o \sim 0.5\mu m$ and $z_R \sim \pi/4 \mu m$. For a microbubble of diameter ($\sim 3\mu m$) trapped in a square beam configuration, figure 3.14 shows a microbubble packed closely between two of the trap four beam spots. By evaluating the beam waist at $z \sim \pm1.5\mu m$ above and below the focal plane, we can see that the beam waist is doubled $w \sim 2w_o$. While the distance between the two beam spots is $\sim 3\mu m$ (the microbubble diameter), the two beam separation is narrowed to $\sim 2\mu m$ above and below the microbubble. The optical trap consists of repulsive forces symmetrically distributed around the microbubble; maximized in the focal plane and with a gradient along the optical axis. This provides strong optical trapping and manipulation in the focal plane as demonstrated in figure 3.13.f, but the trapping along the optical axis is yet to be thoroughly examined. So, far we have not had a microbubble packed closely between the trap HG beam spots to effectively test the trapping along the optical axis. Moreover, the optical trapping for microbubbles along the optical axis is typically weakened in an inverted optical tweezers setup (see section 3.2) [32].

The trapping force along the optical axis can be maximized with a three-dimensional (3D) configuration of HG laser beam spots. We propose minimally a five HG beam spot configuration forming a trigonal bipyramid (two triangular pyramids sharing the same base) around the microbubble as shown in figure (3.14.b). The microbubble in such a configuration is trapped along the optical axis by two HG beam
spots above and below the trapping center. A three-dimensional arrangement of HG beam spots was demonstrated in previous reports for trapping microbeads in 3D arrays [41,46].

**Figure 3.14:** Two diagrams of microbubble trapping with HG laser beam, a) In-capillary trapping of microbubble beneath the capillary upper wall, two from the four trapping spots are represented, the beam waist is doubled at \( z \sim \pm 1.5 \mu m \) narrowing the optical trap above and below the microbubble. b) Proposed 3D trapping configuration of microbubbles with the minimal HG laser beam spots and maximum trapping strength.

### 3.3.4 Integrating opticoustical setup:

In prior reports, the oscillation of unconfined UCA microbubbles in a weak ultrasonic field with rarefactional pressures of 150-200 kPa (<< threshold pressure) was optically investigated using optical tweezers combined with a high speed camera but without acoustical probing [32, 47-49]. Yet, calibration of the applied pressures and the objective working distance from the ultrasound focus were not discussed. It is essential in
integrating optical tweezers to an acoustical setup to avoid disturbing the ultrasonic field which taints the microbubble acoustical response.

The scan measurements of peak-to-peak pressure of an ultrasonic field provide a necessary parameter for integrating an optical objective to the acoustical setup. Figure (3.15) shows a scan along the acoustical axis by a calibrated needle hydrophone measuring the peak-to-peak ultrasonic pressure at the focus of 2.25 MHz transducer. Assuming that the optical axis is chosen to cross the acoustical focus perpendicularly, objectives of working distances (1 mm, 1.5 mm) are subjected to at least (25%, 10%) of the maximum peak-to-peak ultrasonic pressure at the focus. This emphasizes the need for a longer working distance objective to preserve the ultrasonic field conditions after the setup integration.

![Image](image.png)

**Figure 3.15:** Measurements for XY scan of the peak-to-peak pressure of ultrasonic field for 2.25 MHz focused transducer, X is the acoustical axis. The maximum peak-to-peak pressure value is 2.1 MPa.
Figure 3.16: Scheme combining acoustical excitation to optical tweezers with minimum ultrasonic exposure to the objective and maximum trapping forces in the acoustical propagation. Actual dimensions of commercially available objective is indicated.

Optically, however, the objective should be a water immersion objective with a high N.A to produce reliable trapping. The longest working distance objective that is commercially available offers 2.5 mm separation between the objective and the acoustical focus (CFI Plan 100X W, N.A. 1.1, W.D. 2.5 mm, Nikon). The ultrasonic pressure at such distance is reduced significantly to ~2%/4% of the maximum without/with RC capillary placed at the focus. The effect of this minor background exposure can be further evaluated by profiling the acoustical field in the presence of a specific objective. Figure (3.16) illustrates combining ultrasonic excitation with the optical objective. The optical and acoustical axes are perpendicular to minimize the objective background exposure while maximizing the optical trapping forces along the ultrasonic propagation.

The integrated acoustical probing is a passive cavitation system (detailed in the acoustical introduction) where two transducers are used; a transmitter for ultrasonic excitation and a receiver detecting microbubble signals. The acoustical plane (defined by the acoustical axes of both transducers) can be superimposed onto the objective focal
plane to provide complete access to the ultrasonic focus in the opticoustical setup as it is schematically shown in figure (3.17). The RC capillary is vertical for a uniform distribution of the microbubbles and to neutralize the buoyancy forces by controlling the liquid flow. This proposed configuration of optical-acoustical integration requires rather a horizontal optical tweezers setup. We have built a horizontal optical tweezers setup which is depicted in figure 3.18. So far, the liquid flow was controlled with a microinjector to effectively suspend particles and the optical trapping is yet to be optimized and calibrated.

Figure 3.17: i) integrating optical and acoustical setup in a water-filled container, water immersed, long work distance objective and condenser are horizontally assembled to provide optical trapping inside a vertical capillary, acoustical excitement and probing by two transducers, the acoustical plane is perpendicular to both optical axis and the vertical capillary. ii) In the acoustical plane, 90 degree angle between transmitter and receiver to minimize exposure-probing cross section volume to capillary.
Figure 3.18: Up) Horizontal optical tweezers setup, T1 and T2 to adjust the laser beam size for SLM then into the objective. Lens (L) and mirrors M1, M2 and DM3 directing the beam to the objective. Down) The setup picture.

3.5 Conclusion:

Optical trapping inside regenerated cellulose capillaries was investigated using an inverted optical tweezers setup. The trapping calibration with microbeads indicated that a designed capillary cell chamber preserved the trapping conditions of a standard chamber. The in-capillary trapping and manipulation of microbubbles is reported using a square configuration of Hermit-Gaussian beams. The optical trapping strength, measured by the escape velocity, was improved at least by five times in comparison to our previous
trapping of microbubbles with typically used Laguerre-Gaussian beam. Furthermore, the integration of optical and acoustical setup was discussed and a proposed scheme was evaluated.
CHAPTER FOUR

THESIS CONCLUSION
4.1 Thesis Conclusion:

Finally, this thesis showed that UCA microbubble dynamics change significantly when confined in capillaries. An investigation of the rarefactional pressure threshold was conducted using in-capillary shell rupture acoustical detection. This thesis also examined optical trapping of UCA microbubbles inside capillaries in order to develop an optical-acoustical combined setup that provides position-controlled acoustical probing of UCA microbubbles. A detailed proposal for this experimental setup is discussed. This thesis newly reports the following: first, we report that the ultrasound rarefactional pressure threshold for UCA microbubbles confined in regenerated cellulose capillaries increased by at least 0.3 MPa from 0.8 MPa which we measured previously for unconfined microbubbles. Second, this thesis demonstrated a calibrated optical trapping inside regenerated cellulose capillaries with good trapping conditions. Third, we achieved optical trapping and manipulation of UCA microbubbles inside capillaries.
References


