Surface attached nanobubbles and nanoparticle decorated magnetic bubbles : stability, control, and dynamics

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**Surface Attached Nanobubbles and
Nanoparticle Decorated Magnetic Bubbles:
Stability, Control, and Dynamics**

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Summary

This thesis presents experimental studies on nanoscopic scales related to bubbles in liquids. Two objects are chosen, surface attached nanobubbles and microbubbles decorated with nanoparticles.

In Part I we investigated the dynamic of surface nanobubbles. Surface attached nanobubbles are remarkably stable against diffusion and live for days. Two difficulties hinder the research on nanobubbles: First, the currently available high-resolution technique atomic force microscopy has long scan duration. Second, the sample environment is susceptible to contamination which is difficult to distinguish from the gaseous nanobubbles.

To overcome these difficulties, we developed a technique to observe nanobubbles with an optical microscope. The nanobubbles adsorb a fluorescent dye and are visualized with total internal reflection fluorescent (TIRF) microscopy, which selectively excite the first 100 nm on the surface with an evanescent wave. This technique not only visualizes nanobubbles larger than 230 nm, but also resolves their subtle change in height. With this setup, we can record nanobubbles dynamic at up to 2000 frames per second to resolve fast dynamics which were previously inaccessible.

Firstly we visualized the nanobubbles nucleation during water-ethanol-water exchange. The recordings show that during the mixing of ethanol and water, nanobubbles nucleate in the water-ethanol mixture and dissolve in ethanol. They are only stable in the subsequently replaced water, which agrees with the prediction of gas supersaturation after exchange. Next we detect the existence of residual flow with fluorescent tracer particles. Particle tracking results show only Brownian motion in the absence of a net flow

predicted by theory.

Additionally, we studied the nanobubbles coalescence dynamics. Coalescence can occur between neighboring nanobubbles or with a triple contact line. Once two neighboring nanobubbles merge under external perturbation, they grow rapidly and their contact line remains pinned. The growth dynamics can be resolved by the change in TIRF signal. The diffusion model we proposed agrees with experimental results. On the other hand, when a receding contact line meets with nanobubbles, the nanobubbles collapse. Imaging these events allows us to distinguish gaseous objects from particles and droplets. We also found that the contact line motion cannot be explained with bulk hydrodynamics but needs to account for contact line motion on a molecular level.

In Part II of this thesis microbubbles coated with magnetic nanoparticles are studied. When ultrasonic driving is applied, the nanoparticles detach from the bubble's interface and are transported with the oscillatory flow field. Their trajectory can be modeled by balancing inertia and Stokes' drag. We tested the potential application in drug delivery by adding Doxorubicin capsules onto magnetic shell. We showed that the capsules can enter the cellular cytoplasm of cells located within a hydrogel.

Lastly, we built a magnetic bubble trap for magnetic bubbles. There, the buoyancy is balanced with a magnetic force from a feedback-controlled electromagnet. The trap can confine the magnetic bubbles within $13\mu\text{m}$ for a long periods of time. In addition, the bubble can be translated by the magnetic field.

Declaration of Authorship

I, Chan Chon U, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Publications during Candidature

1. C. U Chan & C.-D. Ohl, Total-Internal-Reflection-Fluorescence microscopy for the Study of Nanobubble Dynamics, *Physical Review Letters*, 109, 174501 (2012).
2. F. Li, C. U Chan & C.-D. Ohl, Yield strength of human erythrocyte membranes to impulsive stretching, *Biophysical Journal*, 105, 872 (2013).
3. C. U Chan*, L. Chen* & C.-D. Ohl, Collapse of surface nanobubbles. *Physical Review Letters*, 114, 114505 (2015)
(* Equal contribution)

Manuscripts under preparation:

1. C. U Chan, M. Arora & C.-D. Ohl, Coalescence and growth of surface attached nanobubbles, *submitted*.
2. Y. Gao*, C. U Chan*, Q. Gu, A. M. Alsema, D. Yeo, M. Arora, C.-D. Ohl, C. Xu, Controlled nanoparticle release from oscillating magnetic microbubbles. *In preparation*. (* Equal contribution)

Contributions by Others to the Thesis

Chapter 3.3 Manish Arora analysed the data and proposed the diffusion model.

Chapter 3.4 Longquan Chen contributed to the experiments and analysis.

Chapter 6 Yu Gao contributed to the experiment.

Chapter 6.1 Yu Gao and Chenjie Xu improved the fabrication protocol.

Chapter 6.2 Qiushi Gu contributed to the experiment.

Chapter 6.4 Yu Gao prepared the cell culture and functional magnetic bubbles.

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List of Acronyms

AFM	Atomic force microscope
AM	Amplitude modulation
AOM	Acousto-optical modulator
CCD	Charge-coupled device
CL	Contact line
CMOS	Complementary metal–oxide–semiconductor
DOX	Doxorubicin
EMCCD	Electron multiplying charge coupled device
HOPG	Highly ordered pyrolytic graphite
MMB	Magnetic microbubble
MR	Magnetic resonance
MSD	Mean square displacement
NP	Nanoparticle
PDMS	Polydimethylsiloxane
PID	Proportional-integral-derivative
PLGA	Poly(lactic-co-glycolic acid)
PVP	Poly(vinylpyrrolidone)
RKO	Colon carcinoma cell line
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscope
TIR	Total internal reflection
TIRF	Total internal reflection fluorescent

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Part I.

Optical Study of Surface Nanobubbles

Introduction

Surface nanobubbles are nano-scale gas bubbles attached to submerged solid surfaces. Research interest into surface nanobubbles has increased in recent years. The research is motivated by understanding the flow of liquids near surfaces for interfacial water technologies. Interestingly, surface nanobubbles show a rather long lifetime for which a full understanding is lacking, and they show a much larger (water-side) contact angle than expected [35, 48]. In this chapter, the lifetime of small spherical bubbles in the bulk of a liquid is briefly introduced (Chap 1.1). Then we present experimental techniques used to access nanobubble morphology (Chap 1.2) and the protocol for nanobubble nucleation (Chap 1.3). We also discuss the limitations of current measurement techniques and what would be needed to resolve the “nanobubble puzzle”. It is followed by a review of critiques discussing the existence of nanobubbles (Chap 1.4) and various theories to explain the stability of nanobubbles (Chap 1.5). At last we outline the importance of nanobubble studies (Chap 1.6).

1.1. Gas Bubbles Lifetime

Through liquid-gas interfaces gas molecules can diffuse, either gas molecules dissolved in the liquid enter the gas phase or molecules from the gas phase diffuse into the liquid. Empirical results show, the so-called Henry’s law, that the concentration of a dissolved

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gas in liquid c is proportional to the partial pressure* P of that gas in the gas phase [33]:

$$P = k_h c \quad , \quad (1.1.1)$$

where the constant k_h is Henry's constant. This constant depends on the nature of the gas and the liquid as well as on the temperature.

Epstein & Plesset [17] have solved in their classical work the diffusion equation with precise boundary conditions for sufficiently slow growth or shrinkage of the bubble. In 1997 a similar solution was redeveloped by Ljunggreen & Eriksson [28]. They focused on very small bubbles, where the static pressure is much smaller than the Laplace pressure, which is valid for bubbles with $1\mu\text{m}$ and below. Additionally, they ignore convective transport and assume validity of Henry's law. They obtain that the lifetime of the bubble is given by this simple expression:

$$t_l = \frac{k_h R_0^2}{3RTD} \quad , \quad (1.1.2)$$

where k_h is Henry's constant, $R = 8.314\text{ J/K}\cdot\text{mol}$ is the universal gas constant, D is the diffusion constant of gas molecules in liquid phase, T is the liquid temperature and R_0 is the initial bubble radius. Note that the bubble lifetime is proportional to the initial bubble surface area and independent of surface tension and static pressure. For nitrogen bubbles in water, we can insert $k_h = 1.56 \times 10^5\text{ J/mol}$, $D = 2 \times 10^{-9}\text{ m}^2/\text{s}$, $T = 293\text{ K}$ and obtain $t_l = 1.07 \times 10^{10} R_0^2\text{ sec}$. This implies for bubble radii of $1\mu\text{m}$, 100nm , 10nm the lifetime is 10ms , 0.1ms , $1\mu\text{s}$ respectively.

1.2. Observation of Surface Nanobubbles

Since 2000, nano-scale soft domains are detected by atomic force microscopy (AFM) on submerged hydrophobic surfaces [30]. These soft domains have later been proven to be

*If the gas phase consists of more than one type of gas, the partial pressure of each gas component P_i is defined as the total gas pressure P_{tot} times the molar fraction of that component K_i : $P_i = K_i P_{tot}$.

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filled with gas [47, 49]. Their morphology is a spherical-cap with typical dimension of $0.05\text{-}1\text{ }\mu\text{m}$ in diameter and $10\text{-}100\text{ nm}$ in height (Figure 1.2.1). Their radii of curvature is approximately 500 nm thus they should dissolve within 2.5 ms . Please note that we have so far not accounted for the specific shape of the bubble. In stark contrast to the predictions, they remain stable for days [49].

Figure 1.2.1.: Left: One of the first nanobubble images reported. The bubbles are imaged by AFM on a water-mica interface. Figure extracted from [30]. Right: Typical morphology of surface nanobubbles. The nanobubble is nucleated by depositing gas-saturated water on HOPG and imaged by AFM. Data acquired by Tan Beng Hau.

The advancement in AFM instrumentation and theory allows imaging in a liquid environment [19]. Amplitude modulation (AM, also called tapping) mode is usually used to measure the morphology of soft biological samples. There, a soft silicon nitride cantilever with spring constant less than 1 N/m is excited to oscillate near its resonance frequency (typically $5\text{-}20\text{ kHz}$ in water). When the cantilever tip approaches the sample surface, the oscillation amplitude is reduced due to tip-sample interaction. During the scan a piezoelectric element approaches or retracts the cantilever to maintain a previously fixed amplitude setpoint. The lower the setpoint value is on a scale of $0\text{-}100\%$ of the amplitude far from the surface, the higher is the energy dissipation. By monitoring the piezo displacement value, the height of the sample is mapped.

Most of the current surface nanobubbles studies are conducted in AM-AFM. To map

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the morphology of nanobubbles truthfully, it has to minimize the penetration and distortion on the gas domain by the AFM tip. This is achieved by a high setpoint value, typically between 90-95% [6]. Plasma treatment of AFM tips, which makes them hydrophilic, can also improve image quality [52]. During the last ten years, AFM was the only method that provides sufficient resolution to resolve the nanoscopic morphology of surface nanobubbles. Imaging in liquid environment is not trivial in other high resolution techniques (e.g. electron microscope [22]). The major drawback of AFM is the long scanning time of minutes for a single image. Some attempts have been reported to increase temporal resolution by reducing the scan area, e.g. scans along a single line [45]. Using other techniques allows to increase time resolution, yet with the loss of spatial resolutions. Examples are the collective measurements on a large area with IR spectroscopy [47, 49] or using a microbalance [26]. The latter technique is based on the vibrational frequency of a quartz crystal; the frequency depend on the mass attached, hence the technique can measure the reduced mass of gas phase. The latter two techniques still do not have sufficient spatial resolution to resolve the fast dynamical processes occurring during nucleation or under external perturbations. These processes are, however, crucial for the understanding the physics of surface nanobubbles.

This missing piece of nanobubble experimental technique, which has both the spatial and temporal resolution to study their dynamics motivated the development of an optical microscope technique to observe nanobubbles in “real-time”. An optical technique has been developed to visualize nanobubbles[23]. Their interference based technique utilizes a specially coated substrate. The substrate reflects a reference plane wave which interferes with the wave reflected from the gas-liquid interface of the nanobubbles. The limitation of this technique is that only microfabricated, microscopically flat substrates can be used, hence it cannot be generally applied to other common substrates such as glass. Also, the interference based imaging is sensitive to surface roughness and other contamination in the optical path. This motivated us to develop an alternative optical visualization technique [9].

1.3. Nucleation Methods

The most common protocol to create a stable population of surface nanobubbles on solid/liquid interfaces is the water-ethanol-water exchange process. There, water is replaced first by ethanol and then by water again [29]. The higher gas solubility in ethanol [3] and the exothermic mixing of ethanol and water lead to the release of dissolved gas from the water to ethanol during the first exchange. The subsequent replacement of the gas-rich ethanol with water is made responsible for the nanobubbles nucleation from the now supersaturated water [30]. This method can nucleate nanobubbles on both hydrophobic (Mica, HOPG) [43] and hydrophilic surfaces (glass, SiO_2) [53]. Besides gas concentration, liquid temperature also affects the nucleation of nanobubbles. This is reasonable as the saturation concentration is lower at elevated temperature. It is generally shown that nanobubble density increases when heating up liquids before applying water-ethanol-water exchange [43, 53]. For hydrophobic surfaces, nanobubbles nucleate when liquid with proper gas concentration and temperature is deposited [4, 32, 52]. This nucleation method does not require organic solvent exchange and hence the chance of contamination by dissolved polymers is reduced. Note that although it is reported that supersaturation is not necessary for nanobubble nucleation [35], their result may be influenced by contamination[†] as shown in [4]. It is interesting to determine, both theoretically and experimentally, how much gas concentration is required for nanobubbles to form.

1.4. Are Surface ‘Nanobubbles’ Really Bubbles?

Whether surface nanobubbles with long lifetime exist has been a controversial topic [10]. Some researchers suggest that the nanoscopic objects detected are not gaseous but solid or polymeric contaminant [18]. Indeed, recent experiments demonstrate that such nanobubble-like contaminant could be present [4]. Yet there is still ample of good exper-

[†]Private discussion with Prof. Detlef Lohse.

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imental support that objects detected with an AFM and other techniques are gaseous.

Firstly, gas bubbles are sensitive to the dissolved gas concentration. It is shown that nanobubbles dissolve when exposed to degassed liquid [51, 52]. Also the formation of nanobubbles is greatly inhibited in degassed liquids [52, 53]. The dependence on gas concentration demonstrates the mass transfer between nanobubbles and liquid.

Secondly, nanobubbles should consist of the same type of gas as the dissolved gas in liquid. This has been confirmed by detecting the IR signature of CO₂ gas with attenuated total reflection Fourier transform infrared spectroscopy [47, 49]. Moreover, the gas content in nanobubbles can exchange with the surrounding liquid, as demonstrated by flushing CO₂ saturated liquid on air nanobubbles [20]. Although this spectroscopic technique is a collective effect on an area much larger than individual nanobubbles, it does support the enrichment of gas molecule at the interface when nanobubbles are attached.

At last, if nanobubbles are gaseous, they should coalesce with another gaseous body when they are brought into contact. Interaction with a AFM tip show that they can indeed coalesce with each other [5, 36, 42, 43]. Yet the same behavior holds between droplets. A rational approach is to expose nanobubble-like objects to the interface of a gas phase, e.g. the atmosphere. This intuitive method inspires us to develop a technique to distinguish nanobubbles from contamination.

Previous as well as current research gives strong evidence that nanobubbles do exist. However, it is important to be aware of the possibility of contamination. In nanobubble experimentation it is of utmost importance to prevent contamination as well as have means to confirm the gaseous content through some control experiments.

1.5. Nanobubble Stability

Once the surface nanobubbles are formed, they remain fixed at their position for days, i.e. they do not dissolve [46, 49]. From the analysis in Chap. 1.1, the pressure difference across the bubble surface drives a net diffusion flux into the liquid phase. This can only

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be explained that the long-lifetime of these gas bubble is due to a hindered outflux of gas or that the gas outflux is compensated by an influx.

From the first view point of reduced gas flux, some authors suggested that the gas diffusion is limited by water insoluble materials adsorbed on the nanobubble surface [11, 12, 16]. It is deduced from the experimental observations that the liquid side contact angle of nanobubbles is always higher ($120^\circ - 170^\circ$) on a hydrophobic surface [35, 48]. Hence they interpreted this as a result of reduction in surface tension. This hypothesis has been later rejected by purposely introducing surfactant with the observation that and the nanobubbles remain intact [32, 48, 50].

On the other hand, some authors looked into the possibility of a dynamical equilibrium between influx and outflux. In their theory, the gas influx is either driven by a gas enrichment layer near the triple contact line, which is sourced either from the hydrophobic attraction near the solid wall [8], or a recirculation flow from above the nanobubble [34]. The latter case is supported by a surprising experimental result: they performed an open loop feedback-disabled AFM scan on top of a nanobubble. An upthrust force is measured on top of the nanobubble which they explain with a jetting flow in upward direction impinging on the AFM tip. They explain this water jet as a result of a dilute gas effect. The Knudsen gas has a preferential velocity in the upward (normal to the substrate) direction and therefore imparts momentum on the liquid. As a result a liquid jet with velocity of a few meters per second is created which induces a re-circulating flow. The flow then carries back the dissolved gas to the contact line.

Yet Brownian particle tracking experiments do not detect such high speed flow [9, 15]. In addition, their experimental result could not be reproduced recently[‡].

Beside the above theories, there are more and recent theories which attribute the stability to contact line pinning. It is shown that the triple contact line of nanobubbles remains pinned at the solid interface during dissolution [27, 46]. When the pinned nanobubbles shrinks, their radii of curvature decreases and in turn the Young-Laplace pressure lowers.

[‡]Private discussion with Prof. Detlef Lohse.

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Taking into account the spherical cap geometry, the authors predict the life time to be of a few hours [46]. Meanwhile another theory extended the line pinning effect and considers the dissolved gas concentration in bulk liquid [40]. The authors argue that the initially supersaturated liquid equilibrates with the ambient air on a diffusive time scale. This time scale limits the concentration gradient to develop above the nanobubble and hence greatly reduces the diffusion flux across the nanobubble interface. This theory predicts nanobubble lifetime of the order of days. However, this theory has not been tested with experiment. A simple counter experiment by continuously flushing undersaturated liquid after nanobubbles have formed, should eliminate the diffusion limited “traffic jam” effect that hinders nanobubbles dissolution. Yet there is no conclusive result of their recent experiment with this approach [4].

In conclusion, there has been a number of theories proposed to explain the nanobubbles lifetime. All the theories have either been falsified or not fully supported with experiments. In my opinion, the contact line pinning effect is a general observation [27, 46] and likely contributes to the nanobubbles stability. More quantitative measurement on the morphological change during nucleation, dissolution or the evolution from a non-equilibrium state, would help us to understand the gas flux and thermodynamical equilibrium of this system.

1.6. Significance of Nanobubble Studies

Long-lived gas phases in-between solid/liquid interfaces a.k.a. surface nanobubbles will affect many interfacial phenomena. Even before the discovery of surface nanobubbles it was argued whether nanobubbles can exist at all on submerged solid interfaces [28, 31] and contribute to the observed long range attraction force between two hydrophobic surfaces. Gaseous surfaces affect the boundary condition and results in a fluidic slip [14, 21, 24, 39] reducing the skin friction. They modify surface wettability and hence the rupture of a wetting film [37]. They also affect hybridization and transportation

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along the surface [13, 25] and may promote nanoparticle removal from the surface [44]. Nanobubbles may serve as the nucleation sites for heterogeneous cavitation [1, 7], which affects the micro-boiling behavior [38]. In biological systems nanobubbles could block the interaction between biomolecules and substrates, influencing molecule absorption [41] and gating of protein pores [2]. In addition, this nanoscopic gas domain is a platform to test the validity of fundamental theories, e.g. Young–Laplace equation, that were established in macroscopic scale.

To facilitate engineering controls of the above processes, there are two main questions to be answered: how to detect the existence of nanobubbles? Is there a way to create/eliminate surface nanobubbles on-demand? Both questions require fundamental studies of their response to forces and the mechanism leading to their long life and shape.

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Visualization with Optical Microscopy

We have developed a technique to observe surface nanobubbles optically using the total internal reflection fluorescence (TIRF) excitation. The TIRF microscopy technique was invented by Daniel Axelrod in 1981 [1]. In TIRF microscopy the excitation beam selectively excites the first 100nm above a solid substrate. It results in images with high signal-to-noise ratio, which is beneficial to the study of surface nanobubbles occupying a similar height range. Here we first introduce the principle behind TIRF microscopy (Chap 2.1) and then describe the experimental setup for nanobubble studies (Chap 2.2). The effectiveness of visualizing nanobubbles with TIRF is presented (Chap 2.3). We conclude by comparing TIRF microscopy with other techniques on nanobubbles studies (Chap 2.4).

2.1. Basic Principles

2.1.1. Properties of evanescent waves

Total internal reflection (TIR) occurs when a ray of light strikes from the higher refractive index (n_I) medium to the lower (n_T) one at an angle larger than the critical angle $\theta_{tot} = \sin^{-1}(n_T/n_I)$. Although the incident light is entirely reflected, the continuity at the interface leads to the formation of an evanescent electric field. At the low refractive index side, this electric field strength E_0 decays exponentially away from the interface: $E(z) = E_0 \exp(-z/z_0)$. The distance at which the electric field strength drops to $1/e$ is

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called the penetration depth*:

$$z_0 = \frac{\lambda}{2\pi\sqrt{(n_I \sin \theta_I)^2 - n_T^2}} \quad , \quad (2.1.1)$$

where λ is the wavelength of incident light and θ_I is the incident angle.

Figure 2.1.1.: (Blue) Characteristic penetration depth as a function of incident angle (Eq. (2.1.4)), with system parameters as input. (Green) TIR critical angle.

The evanescent wave described above can be applied in fluorescence microscopy. The reflective index of a substrate (e.g. glass) is usually larger than that of the liquid sample (e.g. water). If the incident angle of the excitation beam is large enough, TIR occurs at the interface between the substrate and the sample. The excited volume is confined to a very thin layer of liquid within the evanescent wave. The intensity of excitation in the sample is

$$I(z) = \frac{I_0}{2} \exp\left(-\frac{2z}{z_0}\right) \quad . \quad (2.1.2)$$

The intensity at the interface, I_0 , depends on the polarization direction. Our incidence

*A detailed derivation is shown in appendix A.

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beam polarization is perpendicular to the TIR plane. In this case, I_0 is related to the electric field strength E_0 by [2]:

$$I_0 = E_0^2 \frac{4 \cos^2(\theta_I)}{1 - n_T/n_I} \quad . \quad (2.1.3)$$

Please note that once the incident angle is set, the parameters are fixed during the experiment and I_0 can be treated as a constant. Also, the penetration depth, z_0^I , does not depend on the polarization direction.

Comparing with Eq. 2.1.1, we obtain the penetration depth for the excitation intensity:

$$z_0^I = \frac{z_0}{2} = \frac{\lambda}{4\pi \sqrt{(n_I \sin \theta_I)^2 - n_T^2}} \quad . \quad (2.1.4)$$

With the parameters in our system as input, the penetration depth versus incident angle is plotted in Fig. 2.1.1. The penetration depth drops drastically to 250 nm within one degree above the critical angle. Below 100 nm the depth decreases gradually with increasing incidence angle. It approaches a minimum depth of 57.5 nm (for 532 nm laser) when the incidence angle approaches 90°. Since only the first 100 nm above the substrate are illuminated, the background fluorescence in epi-fluorescence is eliminated in TIRF microscopy (Fig. 2.1.2). Hence TIRF provides a high signal-to-noise fluorescence image of the solid/liquid interface volume.

2.1.2. Requirements for objective-based TIRF

To implement a TIRF setup in an inverted microscope, i.e. an objective-based TIRF, there are two requirements to be fulfilled. First, an objective lens with large enough numerical aperture is needed to achieve a maximum incidence angle above the critical angle. For an oil immersion objective, the minimum requirement is $NA = n \sin \theta_{tot} = 1.51 \sin 61^\circ = 1.32$. In our setup, an oil immersion objective (Olympus ApoN) with NA 1.49 is used. In the experiment we can achieve conveniently this angle of incidence; the

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Figure 2.1.2.: Fluorescence image of 200 nm fluorescent particles in (Left) conventional epi-fluorescence and (Right) TIRF microscopy. The background fluorescence is significantly suppressed in the latter case.

maximum possible angle θ has been determined with a prism to be about 74° . The corresponding penetration depth is estimated as 70 nm by Eq. (2.1.4).

The second requirement is that the incidence angle should be continuously adjustable while illuminating the same area during the transition from epi-fluorescence to TIRF. To understand how this can be achieved, we can reverse the optical path between the objective lens with a ray transfer matrix (also called ABCD matrix) [7, Chap 15]. This matrix calculus of rays is assuming geometrical optics. A schematic drawing is shown in Fig 2.1.3a. Here we approximate the objective lens with a thin convex lens. For each ray that passes through the lens, it intersects with the both focal plane f at the lateral position x_1, x_2 and with incidence angle θ_1, θ_2 , respectively. These two sets of parameters are related by:

$$\begin{bmatrix} x_2 \\ \theta_2 \end{bmatrix} = \begin{bmatrix} 1 & f \\ 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 \\ -1/f & 1 \end{bmatrix} \begin{bmatrix} 1 & f \\ 0 & 1 \end{bmatrix} \begin{bmatrix} x_1 \\ \theta_1 \end{bmatrix} = \begin{bmatrix} 0 & f \\ -1/f & 0 \end{bmatrix} \begin{bmatrix} x_1 \\ \theta_1 \end{bmatrix} . \quad (2.1.5)$$

Under this formulation, the above mentioned requirement, i.e. adjustable incidence

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angle on the same illumination area, is equivalent to increase θ_2 while keeping x_2 constant. According to Eq. (2.1.5) it can be easily achieved by keeping θ_1 constant for all rays and increasing x_1 . This implies shifting the focal point in lateral direction along the back focal plane(Fig. 2.1.3a). An easy approach to perform this action is shown in Fig. 2.1.3b. Here a laser beam is first alighted according to an epi-fluorescence setup. The collimated beam is focused by L_1 to the back aperture P_2 of objective lens L_2 and propagates along the optical axis. By the same principle, Eq. (2.1.5), shifting the focal spot laterally at P_2 is equivalent to tilting the propagating angle at the back focal plane P_1 . This approach is used in the experimental setup detailed below.

Figure 2.1.3.: (a) Schematic drawing to illustrate the excitation beam of conventional epi-fluorescence (green, center) and TIRF microscopy (purple, right) passing through a convex lens. A light ray is shown for each excitation beam, with green dotted line and purple solid line for epi-fluorescence and TIRF, respectively. Vertical dashed line illustrates the optical axis. (b) A two lenses system that can transit from epi-fluorescence to TIRF microscopy. Horizontal dashed line illustrates the optical axis. All variables are defined in main text.

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Figure 2.2.1.: (a) Sketch of the optical path for TIRF microscopy. Epi-fluorescence and TIRF microscopy excitations are available by rotating mirror M1. (b) Cross section of the PDMS channel to study the water-ethanol-water exchange. (c) Schematic of the flow line consisting of two syringes and syringe pumps, a T-connection, and a 5 cm feed to the microchannel. The microchannel is 5 mm long and 1 mm wide.

2.2. Experimental Setup

2.2.1. Basic TIRF setup to visualize nanobubbles

Figure 2.2.1a depicts the beam forming and injection into the microscope. We use a green DPSS CW laser ($40\mu\text{W}$, $\lambda = 532\text{ nm}$) expanded to 12 mm in diameter and steered into the side port of an inverted microscope (Olympus IX71). A mirror and a lens set the angle of incidence θ , the mirror (M1) steers and lens (L3) focuses the beam onto the back focal aperture of the microscope objective (Olympus ApoN 60x, NA 1.49). By adjusting the rotating mirror M1 located at the focal plane of L3, the incident angle θ can be varied while the illumination spot remains fixed. Due to the limited working distance of the high-NA microscope objective only thin glass plates (here a cover slip

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glass #1 with $140\text{ }\mu\text{m}$ thickness) can be used. The cameras we use give a typical pixel resolution of about 110 nm/pixel . Our lateral resolution is limit by the diffraction limit of the objective, which is about 230 nm (at 550 nm).

2.2.2. High speed TIRF setup

Similar to conventional fluorescent microscopy, the recording frame rate in TIRF microscopy is limited by the low fluorescent intensity. Increasing emission intensity by high intensity illumination speeds up photobleaching of the fluorescent molecules [5]. This results in fading of the fluorescent intensity within seconds. Here we attempt to overcome this limit with laser illumination of 1000 times higher power but during a short period of time simultaneous to the recording of the camera image only.

The experimental setup is depicted in Fig.2.2.2. Now a 2W continuous wave laser (Excel, Laser Quantum, UK) with low intensity fluctuation is used as a light source. The laser is fed [4] into an acousto-optical modulator (AOM) (AOMO 3080-125, Gooch & Housego, UK) powered with a home-built ultrasonic source (80 Mhz, 0.7 Watt, parts from Mini-Circuits, USA). The ultrasonic source is equipped with a high-isolation switch. When the acoustic wave is applied on the optical crystal (Tellurium Dioxide) of the AOM, sinusoidal density waves within the crystal result into a spatially periodic variation of the refraction index. This manifests as a Bragg grating; the incident beam is refracted [3, Chap 9]. Due to the much longer acoustic wavelength Λ than optical wavelength λ in the crystal, the first order Bragg angle θ_B , which is given by $\sin \theta_B = \lambda/2\Lambda$, is typically a few mrad. To maximize the intensity of the first order diffracted beam, the incident angle needs to be precisely adjusted to match the Bragg angle. The switching time between zero and first order beam is measured to be less than 100 ns. Exiting from the AOM, the zero order beam is blocked and the first order beam passes through a linearly polarized plate to filter the undeflected stray light. Moreover, due to the small aperture size of the AOM, a diffraction pattern exists in the output beam and results in a nonuniform beam profile. To avoid this for the TIRF illumination, the beam is focused on to a pinhole

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for spatial filtering before feeding into the microscope for TIRF illumination. The setup there after is the same as the basic setup described. The usage of the reflected beam from the TIRF objective for auto-focusing is explained next.

Figure 2.2.2.: Upper: Experimental setup of high speed TIRF excitation. Light green line indicates the optical path from the laser source to the side port of the microscope. Dark green line indicates the reflected beam from TIR and exits the side port. Lower: Schematic drawing of the optical setup and light path outside the microscope. The optical path in the microscope is the same as Fig 2.2.1.

2.2.3. Auto-focusing

When the flow channel is operated some pressure builds up which eventually flexes the cover slip glass mildly. Also during long recording period thermal drift in the microscope body is detectable. Therefore and as TIRF is sensitive to the sub 100 nm scale the dis-

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tance of the microscope objective to the object has to be adjusted during the experiment. The height adjustment is aided by the back reflection of the laser beam from TIR at the glass-liquid surface.

In an early setup, this reflection beam passes through an aperture and is detected with a photodiode. During the experiments the photodiode signal is kept constant by adjusting the stage manually and thus keeping the distance between objective and glass constant. In the high speed TIRF setup, the back reflected beam is sampled with a 2° wedge prism with one sided anti-reflection coating. This may prevent interference and distortion to the excitation beam. The sampled beam is guided to a lateral position sensitive detector (PDP90A, Thorlabs, USA), which converts the position of a light spot on the detector to two analog voltages. These are proportional to the two lateral distances from the center of the detector. The microscope objective is mounted on a piezoelectric nano-positioner (nanoMIPOS 400, Piezosystem jena, Netherlands). Its vertical position can be controlled by feeding an analog input to the positioner. The position detector signal is realtime processed with a PID feedback algorithm (detailed in Chap. 7.1.3) to adjust the analog input voltage of the objective nano-positioner. Once operating, the fluctuation of objective-glass distance are markedly reduced to 20 nm .

2.2.4. Surface nanobubble nucleation

In our TIRF experiment the nanobubbles are nucleated by water-ethanol-water exchange discussed in Chap. 1.3. We perform the exchange in a microchannel for better flow control. Figure 2.2.1b sketches the side view of the beam reflecting from the glass-liquid interface. The liquid is transported in a microchannel with rectangular cross-section (1 mm width, $20\ \mu\text{m}$ height). The channel is fabricated with standard soft lithography technique, the patterned PDMS channel is bonded on a glass cover slip (Menzel-Glaser, Germany). The glass slides were cleaned with acetone, isopropyl alcohol and ethanol in an ultrasonic bath at 40°C for 20 min each before blow-dried with nitrogen and bonding. On the clean glass surface, we measured an equilibrium water contact angle $\theta_{eq} = 21.7 \pm 1.7^\circ$

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and an advancing water contact angle $\theta_{ad} = 39.4 \pm 1.0^\circ$. However, we did not obtain a receding contact angle θ_{re} since the contact line does not recede even when the water was almost completely withdrawn. $5 \mu\text{M}$ Rhodamine 6G fluorescent dye is dissolved both in the DI water (purification with Sartorius Arium 611vf, France) and in 99% ethanol (Riverbank Chemicals, Singapore). Both liquids are loaded into separate syringes. They are discharged in sequence using syringe pumps. Before the liquids reach the channel they flow through a T-junction which is about 5 cm away from the channel inlet.

2.3. Visualization of Nanobubbles

Figure 2.3.1a demonstrates a typical figure recorded after the water-ethanol-water exchange in TIRF mode. The surface lights up with disc shaped objects filling almost completely the surface of the glass cover slip. Before the exchange an unstructured dim light is recorded. Yet, after the exchange protocol strongly contrasted objects appear. We attribute these objects with surface nanobubbles. The typical contrast of the surface bubbles as defined by $(I_{max} - I_{min})/(I_{max} + I_{min})$ is approximately 0.5 and the signal to noise ratio is 3.

Figure 2.3.1b-d are enlarged views of the area indicated with a white square in Fig. 2.3.1a (scale bar length is 500 nm). For magnification they are re-sampled using linear splines. Figure 2.3.1b is just the enlarged part of Fig. 2.3.1a, while Fig. 2.3.1c and Fig. 2.3.1d compare the same view now in brightfield mode and in epi-fluorescence ($\theta = 0^\circ$), respectively. From this comparison it becomes clear that the TIRF microscopy provides a strong contrast for nanobubble visualization. Nanobubbles are not visible in the brightfield mode, and only some of the largest structures appear but weak in the epi-fluorescence mode.

What leads to the stark contrast of nanobubbles under TIR illumination? It is well known that Rhodamine is accumulating at liquid/gas interfaces. Zheng *et al.* [10] demonstrate that the fluorescence is about 60 times stronger from the water-air interfaces as compared to the bulk. Thus we explain the strong contrast of nanobubbles in TIRF

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Figure 2.3.1.: (a) Nanobubbles observed under TIRF microscopy. Scale bar is $5\mu\text{m}$. The square area is zoomed into for comparison of the different technique (b) TIRF microscopy (scale bar is 500 nm), (c) brightfield and (d) epi-fluorescence.

microscopy by the combination of adsorption of Rhodamine at the nanobubble interface and the short penetration depth of the evanescent wave exciting preferentially the liquid on the scale of the nanobubble height, i.e. about 100 nm .

2.4. Comparison with Other Detection Methods

The evanescent excitation layer of TIRF microscopy provides high contrast images of nanobubbles. Although the lateral resolution can only resolve nanobubbles larger than 200 nm , the much higher temporal resolution than AFM provides valuable benefit to study dynamical processes of nanobubbles. In addition, it can acquire a large sample population and detect nanobubbles over larger surface areas than conventional AFM. Since the imaging process is non-intrusive, the nanobubble system is not perturbed during imaging. The only concern is that the fluorescence molecules adsorbed on the bubble surface may alter the gas permeability or surface tension. Yet it is shown that addition of surfactant has little effect on the morphology and stability of nanobubbles [6, 8, 9].

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An additional limitation imposed by the high NA TIRF objective is that the transparent substrate should be thinner than 0.19 mm, the maximum working distance of the selected microscope objective. The narrow focal depth also requires a highly stable objective-sample distance.

2.5. Summary

In this chapter, we have demonstrated the effectiveness of employing TIRF microscopy to visualize surface nanobubbles. The principle and requirement for an objective-based TIRF setup is explained. The setup is optimized to allow high frame rate recording and objective-sample distance control. This nucleation process, a.k.a. water-ethanol-water exchange, is carried out in a microfluidic channel for better flow control. With a surface active fluorescent dye and the evanescent TIRF excitation, nanobubbles with stark contrast is observed. We will validate the gaseous nature of those objects in Chap. 3.4.

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Surface Nanobubble Dynamics*

In this chapter we apply the TIRF microscopy to investigate the dynamical processes related to nanobubbles and answer some of the outstanding questions. We first investigate how nanobubbles are nucleated during water-ethanol-water exchange (Chap 3.1). It is shown that not all nanobubbles are nucleated immediately after the exchange. It takes up to a few minutes before a stable population is reached. Next we turn to investigate the models that explain the prolonged lifetime of nanobubbles. We first test the circulating flow hypothesis [8, 27] by tracking the Brownian motion of nanospheres around nanobubbles (Chap 3.2). We found no trace of such re-circulating flow but only Brownian motion. This motivated us to develop an alternative model. We are inspired by studying the dynamics of nanobubble coalescence in two scenarios. In the first case, two nanobubbles coalesce and subsequently grow to an equilibrium height (Chap 3.3). We test the single nanobubble growth dynamic with classical diffusion laws. Based on this classical model we predict that pinned nanobubbles can naturally be stable against diffusion. In the second case, a receding contact line approaches nanobubbles. Once they are in contact, the nanobubble flattens and the contact line of nanobubble shrinks until it vanishes. (Chap 3.4). We demonstrate that the collapse behavior of nanobubbles is distinct from the behavior of nanoparticles and micro-droplets that do not show this

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3. Surface Nanobubble Dynamics

phenomenon. This addresses the recent concerns that “nanobubbles” detected in AFM are not gaseous.

3.1. Nucleation Dynamic

Water-ethanol-water exchange is commonly used to nucleate surface nanobubbles. AFM measurements showed that nanobubbles nucleate after the exchange process. Here we examine the dynamic during the exchange process with TIRF microscopy. It reveals that nanobubbles nucleate during the mixing of water and ethanol. Also we observed nanobubbles nucleation up to 15 minutes after the exchange process.

3.1.1. Experimental setup

The nanobubbles are identified with TIRF microscopy as described in the Chap 2.2.1. A cooled slow scan CCD camera (Sensicam QE, PCO, Germany) with a pixel size of $6.45 \times 6.45 \mu\text{m}^2$ is used for imaging. We have measured a pixel resolution of 108 nm/pixel. The diffraction limited resolution for the objective is about 230 nm (at 550 nm). The camera records a field of view of $55 \times 69 \mu\text{m}^2$ with a typical exposure time of 25-40 ms at 18 frames/s. This is sufficiently fast to resolve the nanobubble nucleation dynamics.

3.1.2. Nucleation during exchange

Figure 3.1.1 presents the different stages leading to a surface decorated with nanobubbles. For this experiment, both syringes holding water and ethanol are discharged one after another. Initially the feed line and the whole microchannel, see Fig. 2.2.1c, is filled with water. Then a fast flow (Ethanol) of $125 \mu\text{m}^2/\text{s}$ transports the water-ethanol interface from the T-junction to the inlet of the microchannel. Thereby we reduce diffusion at the T-junction liquid-liquid interface. Then a slow flow of $1 \mu\text{m}^2/\text{s}$ pushes the interface through the microchannel. The dynamics is presented in Fig. 3.1.1: at time $t = 0$ the channel is filled with water only. At time $t = 18 \text{ s}$ the water is partly replaced from the left

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Figure 3.1.1.: Snapshots of images obtained during water-ethanol-water exchange. The intensity is normalized for each frame. Upper row: Bubbles are formed when water is replaced by ethanol. These bubbles dissolved quickly. Lower row: Bubbles again nucleate when ethanol is replaced by water. Bubbles continue to nucleate after the exchange is finished. Scale bar is $10\ \mu\text{m}$.

with ethanol and quickly bright spots appear on the surface. These nanobubble, however, quickly dissolve and after 40 s all bubbles have dissolved. Then, we keep ethanol for about 15 minutes in the channel before starting the replacement. Again, for this exchange the water is pushed initially with a fast flow through the feed line followed by a slow flow through the microchannel. At $t = 16\text{ min } 32\text{ s}$ the water front arrives at the field of view and pushes out the ethanol to the right. At time $t = 16\text{ min } 51\text{ s}$ the microchannel is completely filled with water and the nanobubbles start to nucleate. 11 s later many more nanobubbles have nucleated, see last frame of Fig. 3.1.1.

3.1.3. Nucleation after exchange

Due to some unresolved experimental conditions, for instance gas concentration, nucleation may take several minutes before a steady nanobubble population is reached. The temporal development of nanobubble nucleation at a flow rate of $5\ \mu\text{m}^2/\text{s}$ is shown in Fig. 3.1.2.

At time $t = 2.5\text{ min}$ after water-ethanol-water exchange a few isolated nanobubbles

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Figure 3.1.2.: The channel is filled with water after water-ethanol-water exchange. Bubbles nucleate gradually over several minutes. The exchange flow is from left to right. The Scale bar is $10\,\mu\text{m}$.

appear. 2 minutes later the surface shows about $0.1\text{ bubbles}/\mu\text{m}^2$; another 2 minutes the surface density has quadrupled. The number of nucleation events is obtained by subtracting two adjacent frames and simple image processing routines which include thresholding on the intensity and the bubble size. The time dependent nanobubble density is then obtained by summing up the nucleation events and dividing it over the measurement area (Fig.3.1.3a).

The rise time of the nanobubble density, i.e. from 10% to 90% of the maximum bubble density, is about 240 s. An analysis of the nanobubble diameter is presented in Fig.3.1.3b, which is measured manually from 1000 nanobubbles. We understand that

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bubbles below the resolution limit of about 230 nm can't be resolved but detected. Thus, the distribution starts with these relative large diameter nanobubbles. Similar sizes with diameters of up to $1\text{ }\mu\text{m}$ have been observed on rough surfaces [7].

Figure 3.1.3.: The bubble density plotted as a function of time for the experiment shown in Figure 3.1.2. The triangles state the times where the snapshots in Figure 3.1.2 are taken. Inset figure provides size distribution of bubbles formed. Bins increase by the pixel size. The smallest bin is 2 pixels wide.

3.2. Residual Flow above Nanobubbles

The optical detection of nanobubbles allows to combine the technique with other microscope based observation techniques. Using small tracer particles we can probe if the Brownian motion of particles is affected near to nanobubbles. It has been proposed that nanobubbles are stabilized against dissolution through a re-circulating flow emanating from the apex of the bubble towards its three-phase contact line with a lateral dimension of at least the lateral size of the nanobubble [27] (see Chap.1.5 for details). We are probing this flow by tracking the motion of 200 nm diameter red fluorescent particles

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near and far from the nanobubbles.

3.2.1. Experimental setup

The nanobubbles are formed and identified with TIRF microscopy as described in the Chap.2.2.1. Areas on the surface with low density of nanobubbles are first chosen. We identify 18 nanobubbles in the field of view with a size distribution as shown in Fig.3.2.1. We photo bleach the nanobubbles by increasing the laser power to 150 mW to increase the contrast of the particles near to them and then slowly replace the water with DI water containing the nanoparticles. The particles are recorded at 21 frames/s ($dt \approx 48$ ms) with an exposure time of 1 ms and the tracks are obtained from image processing. To ensure that the photo bleaching does not affect the nanobubble we add, after the experiment, fresh water with the Rhodamine dye and detect the nanobubbles at the same location as prior to the photo bleaching.

Figure 3.2.1.: Size distribution of nanobubbles shown in Figure 3.2.3. They are all analysed in current study.

3.2.2. Image processing

The trajectories of the particles are obtained from a multi-step algorithm, consisting of pre-processing the images, particle detection, linking the trajectories, and validation.

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Pre-processing For image pre-processing a background and flat field image are generated from each recorded frame using a histogram based algorithm [10]. The first image with a sampling window slightly larger than particle diameter is used for background subtraction. The other image with a three times larger sampling window is normalized and used for flat field correction. Each frame is normalized by its mean value to compensate for some fluctuations of the laser energy.

Particle detection and connecting trajectories After the pre-processing, particles in each frame are identified by an intensity threshold combined with a threshold on the particle size. The particle centroids weighted by their intensity level are extracted. We use a home built algorithm to connect particle positions between frames to trajectories, i.e. particle tracking.

Doing so, each particle is connected to its nearest neighbor in the succeeding frame as long as it moves less than $4\mu\text{m}$. Since particles may move out of the narrow focal depth, they are allowed to disappear for up to 2 frames before the trajectory is ended. Only trajectories that have more than 4 detected particle positions are considered valid. The image processing above is automated using Matlab (The Mathworks Inc). Our algorithm sets an upper bound to the velocity of $80\mu\text{m/s}$.

Validation of the results We conducted frame-by-frame inspection to assure that only valid trajectories are detected. A second important check whether the trajectories are captured correctly is a comparison of their motion to Brownian Motion. We compute the mean square displacement (MSD), $\langle [\Delta r(t)]^2 \rangle$ over all 2000 captured trajectories. Figure 3.2.2 depicts the result: the MSD increases linearly in agreement with a diffusion limited process. We understand that the 3-dimensional diffusion process is hindered by the presence of the lower glass plate. This type of diffusion has been studied experimentally for example in [19]. There, a lateral diffusion constant (that means parallel to the glass surface) for 200 nm polystyrene spheres on top of a glass cover slip was estimated

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Figure 3.2.2.: Mean square displacement (MSD) of 200 nm diameter polystyrene particles in DI water. Please note the linear dependency of MSD for sufficiently small distances. The noise/deviation for larger distances of about $10\mu\text{m}$ is due to lower number of data points.

with $D_{\parallel} = 1.53 \mu\text{m}^2/\text{s}$. We find a similar value, i.e. our measurement predicts an in-plane diffusion constant of $D = \langle (\Delta r(t))^2 \rangle / 4t = 1.20 \mu\text{m}^2/\text{s}$ for our 200 nm diameter particles.

Figure 3.2.3.: A selection of the trajectories of the nanoparticles are overlaid onto a TIRF picture of the nanobubbles. The erratic motion from the particles very much resembles a Brownian motion.

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3.2.3. Result and discussion

The area of interest of each nanobubble is defined as a circle centered at the nanobubble with a radius up to half the distance to its nearest neighbor. Any trajectory that enters this area of interest is selected for velocity distribution measurement. Fig. 3.2.3 shows a reduced set of trajectories overlaid on the TIRF picture showing the nanobubbles. Some of the particles trajectories move over nanobubbles, while some seem to repel them. Yet, we do not find any drift flow near the nanobubble, which would be expected from a re-circulating flow from an upward jet.

We then analyze the differential speed of the particle

$$\Delta r / \Delta t(d) = \pm |(\mathbf{r}(t + \Delta t) - \mathbf{r}(t))| / \Delta t \quad , \quad (3.2.1)$$

as a function of the distance $d = |(\mathbf{r}(t + \Delta t) + \mathbf{r}(t))| / 2$, where $\mathbf{r}(t)$ is the distance vector to nanobubble center in each frame. The recirculating flow would lead to a preferential particle motion close to the surface towards the three phase contact line. Our spatial resolution of approx. 100 nm is able to detect drift velocities larger than $2 \mu\text{m/s}$, which is about 6 orders of magnitude smaller than the predicted jet velocity. We have analyzed 18 nanobubbles with altogether 2000 tracks. Fig. 3.2.4 analyzes the result from the particle tracking: We measure particle speeds of up to $30 \mu\text{m/s}$ independent of the distance. The averaged velocity is very close to zero; it is less than $+2 \mu\text{m/s}$ for all measured distances. A remaining drift flow is weaker than a few micrometers per second at a distance of 400 nm from the nanobubbles' center. The mean-square-displacement demonstrates that the particles follow Brownian Motion(Fig 3.2.2). We find no indication of a re-circulating flow close to the nanobubble. The same conclusion is reached by a similar follow up study [13].

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Figure 3.2.4.: Upper: differential speed of the particle as a function of distance to nanobubble center. Lower: mean value of differential speed at each distance interval. The first interval centered at $0.43 \mu\text{m}$. Each interval width is $0.11 \mu\text{m}$.

3.3. Coalescence and Growth Dynamic

Previous works have already explored the effect of changes to the liquid environment on the stability of nanobubbles: For example, chemical changes like change in pH, salt concentration, and the effect of substrate electrostatic potential have been shown to affect the number density and size of the nanobubbles [21], while temperature and dissolved gas concentration [33, 34] also may affect their stability. By exposing nanobubbles to ultrasound, Brochie and Zhang [9] showed they can grow in size, while coalescence of nanobubbles by interaction with a scanning probe microscope tip has been reported in a number of studies [4, 29, 32, 33].

In this chapter, we report rare events of coalescence, i.e. the merging of two neighboring bubbles. The new bubble covers the convex hull of their “footprint”, with most of the three phase contact line remaining pinned. Interestingly, the newly formed bubble is not

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shape stable but grows in height within several 100 ms. This growth and the stability of the bubble can be accurately described by a classical diffusion theory using contact line pinning and Henry's law.

3.3.1. Experimental result

We first nucleate nanobubbles with water-ethanol-water exchange (see Chap. 2.2.4) and monitor with the TIRF microscopy setup detailed in Chap. 2.2.2. Once a stable nanobubble population has formed, we maintain the water flow rate at $200\text{ }\mu\text{L}/\text{min}$ (equivalent to an averaged velocity of 0.17 m/s). Interestingly, in rare events a sudden increase of the TIRF signal intensity occurs. There, two nanobubbles have merged into one with a greatly increased intensity. Figure 3.3.1a depicts such a merging event; the first frame marks two neighboring bubbles (B1 and B2) of nominal diameter of 209 nm and 313 nm, respectively, which merged in the successive frame into a considerably brighter object of 603 nm in diameter. Measurement uncertainty of the lateral size is estimated to be of the order of one pixel ($\sim 108\text{ nm}$). Note that the area of the merged nanobubble is larger than the original areas and it spans the convex hull of the previous nanobubbles. Interestingly, the triple contact line during the merging of the initial and final bubble remains fixed, except for the central area connecting both bubbles.

After merging a gradual decrease in brightness of the merged bubble is observed while the surrounding bubbles keep their contrast, see Fig. 3.3.1c. The temporal change of intensity is documented in Fig. 3.3.3b; there the averaged pixel intensity of the merged bubble area is plotted as a function of time (time of merging $t = 0$). The intensity increases to a higher brightness within the interframe time, followed by a slow decrease on a time scale of several hundred milliseconds.

What is the cause of this slowly reducing brightness? We can rule out photo bleaching as the intensity of the surrounding bubbles remain unchanged. The diffusion limited transport of dye molecules from the bulk onto the new surface of the bubble is considerably faster than the temporal resolution. Advection of the dye if present will further

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Figure 3.3.1.: (a) Rapid merging event of two individual bubbles B1 and B2 within less than 20 ms. Thereafter, the combined bubble is considerably brighter but slowly decreases in intensity within 400 ms. (b) Sketch of the side view of the nanobubble merger event, followed by slow vertical growth. (c) Consecutive images of the merged bubble as a function of time. The decrease in the intensity of the merged bubble while the surrounding nanobubbles remain unaffected.

reduce this re-equilibration time. Hence both diffusion and advection effect for the fluorescent dye can be neglected. Therefore, the height of the merged bubble must be changing with time: Initially, the bubble interface must be very close to the surface and over time the bubble increases in height, see Fig. 3.3.1b. In order to understand the changes in the height of the surface nanobubbles, the kinetics of gas diffusion from the medium into the bubble must be considered. In the following section, we model this diffusion of gas for a surface attached nanobubble and its contact line being pinned.

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Figure 3.3.2.: Sketch of the surface attached nanobubble system modeled in the present paper. The nanobubble is in spherical-cap shape with height h , lateral radius a and radius of curvature r_b . The system is kept at ambient pressure P_∞ . The gas concentration in the bulk liquid and outside the bubble surface are C_l and C_s , respectively. The difference in these two concentrations drives gas diffusion over a boundary layer thickness L .

3.3.2. Diffusion model for a pinned nanobubble

We consider a surface attached bubble of height h and lateral radius a (Fig. 3.3.2). The number of gas molecules inside the nanobubble, N , is given by:

$$N = V_b \rho_g = \frac{\pi}{6} (3a^2 + h^2) h \rho_g \quad , \quad (3.3.1)$$

where V_b is the volume of the bubble and, ρ_g the number density of the gas phase. A change of the number of gas molecules leads to a change in the volume and the density of the gas. Relating the density to the Laplace pressure and assuming an ideal gas law we obtain:

$$\frac{dN}{dt} = \left[\frac{\pi(3a^2 + h^2)h(a^2 - h^2)}{6(a^2 + h^2)^2} \frac{4\sigma}{RT} + \frac{\pi}{2} (a^2 + h^2) \rho_g \right] \frac{dh}{dt} \quad . \quad (3.3.2)$$

The lateral size a is assumed constant due to contact line pinning. Using Fick's 1st law of diffusion, the net flux of dissolved gas into the bubble is given by the product of concentration gradient, ∇C_s , surface area, A_b , and the diffusion coefficient D :

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$$\frac{dN}{dt} = -A_b D \nabla C_s \quad . \quad (3.3.3)$$

Thus we can solve for

$$\frac{dh}{dt} = - \frac{(a^2 + h^2)D}{\left[\frac{(3a^2 + h^2)h(a^2 - h^2)}{6(a^2 + h^2)^2} \frac{4\sigma}{RT} + \frac{1}{2}(a^2 + h^2)\rho_g \right]} \nabla C_s \quad . \quad (3.3.4)$$

To solve Eq. (3.3.4) we approximate the concentration gradient at the bubble wall interface as

$$\nabla C_s = \frac{C_s(t) - C_L}{L} \quad . \quad (3.3.5)$$

Here, C_s is the gas concentration just outside the nanobubble surface and C_L is a constant gas concentration in the liquid far away from the nanobubble. Though the value of C_L might change over time due to changes in experimental conditions, for the timescale and conditions involved in these experiments C_L can be treated as constant. An appropriate choice for the mass diffusion boundary layer thickness, L , is

$$L = \left(\frac{1}{\sqrt{\pi D t}} + \frac{1}{r_b} \right)^{-1} \quad . \quad (3.3.6)$$

This choice of boundary layer thickness is motivated by the fact, that after a step change (such as merger of two nanobubbles or abrupt change in curvature) the boundary layer needs to rebuild for the new surface concentration. Its scales as $\sqrt{\pi D t}$. After some growth, the boundary layer develops and for later times, assuming quasi-static growth of the nanobubble, the diffusion layer is given by the radius of curvature, r_b , of the bubble. This expression for the boundary layer thickness can be shown to be exact for the case of a quasistatic spherical bubble [14].

With the above approximation, Eq. (3.3.4) can be solved numerically using a standard Runge-Kutta scheme. To compare with experimental observations, we start with a flattened surface nanobubble, i.e. lateral size $a(t = 0) = 603 \text{ nm}$, initial height

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$h(t = 0) = 2 \text{ nm}$). This initial height of the merged bubble is chosen by applying mass conservation i.e. the merged bubble is formed by combining gas present in bubbles B1 and B2 spread over the footprint of merged bubbles[†]. For these numerical calculations, we further assume that the concentration of dissolved gas on the liquid side of the nanobubble interface is given by Henry's law, i.e.

$$C_s(t) = \frac{1}{k_H} \left(P_\infty + \frac{2\sigma}{r_b(t)} \right) \quad , \quad (3.3.7)$$

with $k_H = 1465 \text{ L} \cdot \text{atm/mol}$ as the Henry's law constant for air[‡] [25]. Since the dissolved gas concentration in the vicinity of the nanobubbles is not known, the numerical simulations are carried out at various concentrations, see Fig.3.3.3a. For undersaturated liquids, i.e. gas concentrations below 100% here, the nanobubbles dissolve but above this value the nanobubbles reach a stable height. This is in contrast to free gas bubbles, which either grow continuously or dissolve by gas diffusion. The effect of the dissolved gas concentration on the resulting height of a stable surface attached nanobubble is analyzed below.

As discussed in Chap.2.2.1, at a first approximation the intensity of the nanobubble images is proportional to the intensity of the illuminating evanescent wave. Because the experimental TIRF intensity $I(h)$ is a function of height and drops as $I(h) = I_0 \exp(-h/\lambda_{air})$ [17] we can compare the observed change in intensity with the model prediction[§]. The penetration depth of the evanescent wave in air, λ_{air} , is about 40 nm for our experimental setup. Figure 3.3.3b compares the experimental TIRF signal (dashed line) with the expected intensity for a variety of dissolved gas concentrations. The simulated TIRF intensities show similar reduction over time as seen in experiments for mild super-saturations in the range of about 115%. Here, both the time scale and magnitude

[†]Volume of bubbles B1 and B2 is estimated from equilibrium height corresponding to an iteratively estimated supersaturation of 115%

[‡]Here we assume air to be 80:20 mixture of oxygen and nitrogen. Hence the effective constant is calculated as $k_H = (1639 \cdot 0.8 + 769 \cdot 0.2) \text{ L} \cdot \text{atm/mol} = 1465 \text{ L} \cdot \text{atm/mol}$.

[§]This relationship is a stark simplification of the complex scattering near the three phase contact lines and therefore may only hold for sufficiently large bubbles.

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Figure 3.3.3.: (a) Temporal evolution of the height of simulated nanobubbles at various dissolved gas concentration. It is compared against the experimental result obtained in Figure 3.3.1c (black dotted line). (b) Simulated TIRF signal for growing nanobubble in comparison to experimentally observed change in TIRF signal (black dashed line).

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Figure 3.3.4.: Simulated (lines) and experimental (dots) TIRF intensity change is plotted as a function of the nanobubble’s lateral size for 110%, 120% and 130% dissolved gas concentration. These curves are compared against 5 experimental observations of bubble merging. All observations are in agreement with a mild super-saturation of 10%-30%.

of the observed TIRF intensity agrees with simulation results. Yet, to obtain this good agreement we had to reduce the surface area, A_b , by 8 times. We speculate that the gas transport is hindered by Rhodamine dye absorbed on the surface, effectively reducing the surface area.

The reduction of the surface area only affects the time scale of the dynamics but does not change the final height of the simulated nanobubbles. In the five merger events that we recorded, the growth dynamics agrees nicely with the model with moderate oversaturation levels between 110% and 130% in the liquid, see Fig. 3.3.4. Such oversaturation levels can be easily attained, considering that ethanol can hold much more gas than water [3]. We therefore can describe the growth of nanobubbles with a classical diffusion theory under the assumption of contact line pinning. The second interesting observation is that the bubbles grow to a limited final size. This is in contrast to free gas bubbles in

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the bulk, which either grow or dissolve indefinitely by gas diffusion [15]. We now discuss the stability of the nanobubble shape.

3.3.3. Stability of surface attached nanobubbles in supersaturated liquid

Considering surface nanobubble as a dynamical system governed by Eq. 3.3.3 its stable and unstable equilibrium height can be found by finding the zeros of the time derivative, i.e.

$$\frac{dN}{dt} = -\frac{A_b D}{L k_H} (P_\infty + \frac{2\sigma}{r_b} - k_H C_L) = 0 \quad . \quad (3.3.8)$$

It is convenient to define the supersaturation pressure $P_{ss} \equiv k_H C_l - P_\infty$ as a measure of the supersaturation of the liquid. It follows that

$$C_l = \frac{P_\infty + P_{ss}}{k_H} \quad , \quad (3.3.9)$$

and the equilibrium condition now reads

H

$$P_{ss} = \frac{2\sigma}{r_b} \quad . \quad (3.3.10)$$

P_{ss} is positive if the liquid is supersaturated and negative if the liquid is undersaturated. Hence a equilibrium radius only exists in supersaturated liquid. Note that Eq. 3.3.8 has no geometrical constrain and Eq. 3.3.10 is applicable to bulk bubbles. In the case of bulk bubbles, it can be easily shown that dN/dt has the same sign as the radius perturbation and thus the equilibrium radius is always unstable.

In the case of surface attached nanobubbles, there are additional constrains due to contact line pinning and spherical-cap geometry. By expressing r_b explicitly in terms of

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lateral contact radius a , we obtain a quadratic equation about h :

$$h^2 - \frac{4\sigma}{P_{ss}}h + a^2 = 0 \quad , \quad (3.3.11)$$

it becomes apparent that a surface attached nanobubble is in equilibrium with the surrounding liquid for two heights, h_1 and h_2 :

$$h_1 = \frac{2\sigma - \sqrt{4\sigma^2 - (P_{ss}a)^2}}{P_{ss}} \quad h_2 = \frac{2\sigma + \sqrt{4\sigma^2 - (P_{ss}a)^2}}{P_{ss}} \quad . \quad (3.3.12)$$

To analyze the stability of these two equilibrium heights, Eq. (3.3.8) can be rewritten as:

$$\frac{dN}{dt} = \frac{A_b D}{k_H L} \frac{P_{ss}}{(a^2 + h^2)} (h - h_1)(h - h_2) \quad . \quad (3.3.13)$$

Note that, since dN/dt has the same sign as dh/dt for a surface attached nanobubbles with pinned contact line (i.e. a fixed, see Eq. 3.3.2), the smaller of the two equilibrium heights, h_1 , is stable and h_2 is unstable. As illustrated in Fig. 3.3.5, for a given supersaturation, nanobubbles smaller in height h_1 and between height h_1 and h_2 will change their height by gaining or losing gas, respectively, till their height reaches height h_1 . While the bubbles larger than h_2 will grow indefinitely. Even though there is no equilibrium solution for undersaturated liquid (i.e. P_{ss} negative), as long as liquid is slightly supersaturated the equilibrium nanobubble height is stable. The smaller the supersaturation the smaller the stable equilibrium height. An alternative form of the equilibrium shape is in terms of the liquid side contact angle, θ_c , which can be obtained from the geometry:

$$\sin\theta_c = \frac{aP_{ss}}{2\sigma} \quad . \quad (3.3.14)$$

The equilibrium contact angle also imposes a maximal lateral size. As the left hand side of Eq. 3.3.14 is positive and smaller than 1 it follows that the lateral radius of the nanobubble $a < 2\sigma/P_{ss}$. Bubbles beyond this lateral radius grow unbounded as there

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is no solution in Eq.3.3.11 and $dN/dt > 0$ for all bubble heights. In ambient pressure ($P_{ss} = 1.0 \text{ atm}$), inserting the above estimated saturation of 116% ($P_{ss} = 0.16 \text{ atm}$) we obtain a maximum lateral nanobubble radius of $a = 8 \mu\text{m}$, and $a = 1.2$ for 200% saturation. These predictions give a reasonable size estimate, i.e. we have not observed bubbles larger than a few micrometers in the experiments reported here. The predicted contact angle of stable nanobubbles and maximum feasible lateral size are consistent with numerical results obtained by [20].

Figure 3.3.5.: Diagram using Eq. 3.3.13 to illustrate the stability of the two equilibrium heights. An initial height smaller than h_2 converge to a stable height h_1 , otherwise it grows unbounded and eventually detaches. The bubble shapes corresponding to the two equilibrium heights are shown, too.

3.3.4. Conclusions

Our experimental results show that most nanobubbles are stable even in a strong flow yet some may depin partly and coalesce. After coalescence the area of the merged bubble spans the convex hull of both initial bubbles. The bubble remains pinned yet the nanobubble height changes rapidly.

A classical gas diffusion model for the surface attached nanobubble agrees with the experimental findings that the new bubble grows up to a unique height given by the gas supersaturation and the lateral size. The timescale of this growth can be modelled with a reduced surface area participating in the gas diffusion. This is expected as the dye molecules necessary for the observation adsorb at the interface thus effectively reduce the surface area.

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This model supports that, due to contact line pinning, surface attached nanobubbles can attain a stable shape, which is in contrast to bubbles in bulk liquid. In supersaturated liquid, a surface nanobubble with lateral size smaller than $\sim 10\mu\text{m}$ and initial height smaller than a critical height will approach an equilibrium shape within one second. If either one of these dimensions is larger than the critical size, it will grow continuously.

In summary, a classical model is sufficient to describe the dynamics of nanobubble growth to a stable size. The model is based on a constant surface tension coefficient, Laplace's equation, and Henry's law applied to the mildly curved gas-liquid interface. Yet, gas supersaturation and pinning of the contact line are required for stable bubbles, in agreement with most experiments reported.

3.4. Collapse of Surface Nanobubbles

In accordance with discussions in Chap. 1.4, nanobubble research is hindered by lacking a good protocol to generate nanobubbles reliably between labs and to prove that their content is really gaseous. In this Chapter, we present a technique which helps to accomplish this goal. We comparatively study the interaction of receding contact lines (CLs) with surface nanobubbles, polymeric droplets, and solid particles. The latter two represent two kinds of potential surface contamination which may interfere with the identification of nanobubbles. Interestingly, surface nanobubbles spontaneously collapse once the receding CL touches them, while a fast receding process and a pinning-depinning process is observed on polymeric droplets and on solid particles, respectively.

3.4.1. Experimental setup

Nanobubbles, polymeric droplets and solid particles are first prepared on the glass coverslip before the receding CLs passes by. The nanobubbles nucleation and microscopy techniques follow the description in Chap. 2.2.4 and 2.2.2, respectively.

Polymeric droplets were deposited by electrospraying [16] PDMS onto a clean glass

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slide placed on top of a metallic and grounded plate. A blunt steel needle with an inner diameter of $250\text{ }\mu\text{m}$ was filled with a PDMS monomer (Slygard 184, Dow Corning) and placed at a distance of 1.2 mm above the glass slide. Microscopic PDMS droplets were ejected and deposited on the glass slide by applying a positive voltage with an amplitude of 12 kV for 200 ms on the needle. The resulting sizes of the deposited PDMS droplets vary largely yet some of the small droplets have radii down to $2.0\text{ }\mu\text{m}$.

To simulate solid contamination, monodisperse polystyrene particles labelled with red-fluorescent dye (Fluoro-Max, Thermo Scientific) with $0.5\text{ }\mu\text{m}$ -radii were used. For a homogeneous deposition of the particles, clean glass slides were first immersed into and then pulled out of aqueous particle suspension ($10^{-6}\text{ wt\%}$) at a speed of about 0.5 mm/s , which resulted in a particle density of around $100\text{ particles/mm}^2$.

The receding speed of the contact line over the surface nanobubbles was controlled by slowly pumping air into the microchannel (Fig.3.4.1). For PDMS droplets and polystyrene particles, the receding speed was monitored by evaporating $4\text{ }\mu\text{L}$ water droplets deposited on them. The receding CL over the surface nanobubbles and the PDMS droplets were observed using an EMCCD camera (iXon3, Andor, UK) with $10\text{-}46\text{ fps}$ under TIRF microscopy. The pixel resolution is 119 nm/pixel and the diffraction limited resolution is about 230 nm . To capture the fast dynamics of the shrinking of nanobubbles, a high-speed camera (SA-X2, Photron, Japan) with a lower pixel resolution of 300 nm/pixel was used. We have optimized the laser energy and exposure time to maximize the fluorescent intensity without detectable photobleaching. Under this condition the maximum frame rate we can record is 2000 fps . The slow dynamics of the receding CL over individual particles was imaged with a standard CMOS camera (UI-1640LE, IDS GmbH, Germany). There the recording rate is 19 fps and the pixel resolution is 110 nm/pixel .

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Figure 3.4.1.: Schematics of the TIRF microscopy used to observe receding CL over surface nanobubbles and PDMS droplets.

3.4.2. Traction with receding contact lines

Gaseous bodies

After water-ethanol-water exchange (see Chap.2.2.4) bright objects are observed on the glass coverslip with TIRF microscopy(Fig.3.4.2a). The right frames of Fig. 3.4.2a display the dynamical process of interaction of a receding CL traveling from the left to the right across one of the bright objects: The speed of the CL is $\sim 0.52 \mu\text{m/s}$ before it touches the bright disc with a radius of $0.45 \mu\text{m}$; the 4 frames are selected from a movie recorded at 46 fps. At the moment the CL reaches the object, it shrinks abruptly and its brightness changes, which is already visible at $t = 22 \text{ ms}$. Please note that we cannot infer from the brightness of the pixels the height of the bubble due to motion blurring. At $t = 44 \text{ ms}$, the disc shrinks to a radius of $\sim 0.15 \mu\text{m}$ (marked with dotted circle in Fig.3.4.2a) and disconnects from the CL. Only once the CL touches the object again (after about 1 s) it shrinks and vanishes within one frame. Similar multi-step collapse processes were observed for larger objects, while a single collapse process was observed only for small objects.

The shrinkage and disappearance of the object in combination with the particular dynamics of the CL indicates that we are observing a shrinking surface attached bubble

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with an initial height of several tens of nanometers. Next we demonstrate that other objects such as polymeric droplets and solid particles result in prominently different CL dynamics.

Figure 3.4.2.: Snapshots of a CL receding over (a) surface nanobubbles, (b) a PDMS droplet (b), and (c) a colloidal particle. The CL receding speed in (a) to (c) is $0.52 \mu\text{m/s}$, $0.60 \mu\text{m/s}$ and $0.40 \mu\text{m/s}$, respectively. The lengths of the scale bars are in (a) $1 \mu\text{m}$ and in (b) and (c) $2 \mu\text{m}$. (d)-(f) Sketch of the collapse dynamics of a surface nanobubble seen from the side.

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Hydrophobic droplets

The first frame of Fig. 3.4.2b depicts a $2\text{ }\mu\text{m}$ -radius PDMS droplet submerged in water with the contact line at some distance to its right. The fluorescent dye from the water has diffused into the PDMS droplets giving a similar appearance as surface nanobubbles under TIRF microscopy. In Fig. 3.4.2b top to bottom the CL moves with an initial speed of $\sim 0.60\text{ }\mu\text{m/s}$ towards the left. The hydrophobic droplet on a hydrophilic glass surface leads to a local decrease of the interfacial energy and changes the local surface wettability [11, 12]. When the CL is far away from the droplet, its receding speed is almost uniform everywhere. However, at a distance of $\sim 1.0\text{ }\mu\text{m}$ from the droplet's edge, the CL region closest recedes faster than other regions (marked with an arrow in Fig. 3.4.2b). This suggests that a hydrophobic precursor film exists around the PDMS droplet [11, 12]. Once the CL near the droplet meets the droplet's ridge, the CL traverses quickly the droplet, here within 0.1 s (see $t = 1.5\text{ s}$ to 1.6 s in Fig. 3.4.2b). Then, the CL passes the droplet and remains fixed until the outer slowly moving sections catch up with it, see $t = 7\text{ s}$ in Fig. 3.4.2b.

Solid particles

The receding process of a CL towards a $0.5\text{ }\mu\text{m}$ -radius solid particle at a speed of $0.40\text{ }\mu\text{m/s}$ with selected frames is shown in Fig. 3.4.2c. Again a uniform receding CL is also observed far from the particle. However, once it reaches the particle, the CL near the particle is anchored due to the adhesive pinning force, see $t = 5\text{ s}$ in Fig. 3.4.2c. As a result, the retreating speed is slowed down and the CL is deformed. With further receding of the CL, the region close to the particle becomes highly bend ($t = 11\text{ s}$ in Fig. 3.4.2c), which in turn results in an increase of the capillary force towards the receding direction of the CL. Once this capillary force is larger than the pinning force, the CL depins from the particle. Eventually, a uniformly receding CL is recovered at $t = 17\text{ s}$ in Fig. 3.4.2c.

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Comparison

As described above, a fast jump and a pinning-depinning behaviour of the CL are observed when contact lines recede over a polymeric droplet and a solid particle, respectively. In both cases, the objects remain fixed when the CL passes by in Fig. 3.4.2b-c (although we are aware of the possibility of detachment and transport of particles by the moving CLs). In contrast, the nanobubbles in Fig. 3.4.2a exhibit typical properties of gaseous bodies being exposed to the ambient atmosphere: shrinking and complete removal [12]. These distinct phenomena could be used to identify surface nanobubbles.

3.4.3. Dynamics of nanobubble shrinkage

To study the process of nanobubble shrinkage in greater detail, we utilize a high-speed camera. The rather weak fluorescent signal limits the framing rate to a maximum of 2000fps. Unfortunately, due to the larger pixel size at the same magnification, each nanobubble is now captured with only a few pixels. Although we do not have the spatial resolution to trace the instantaneous lateral radius, we can study the temporal dynamics of the collapse by analyzing the averaged pixel intensity. Fig. 3.4.3 shows the average pixel intensity I as a function of time for selected nanobubbles with an initial lateral radius R_l of $0.38 - 0.56 \mu\text{m}$. I is about constant when the CL is away from the surface nanobubble. However, once the CL meets the bubble, the surface nanobubble shrinks and thus its intensity decreases. A zoom into the intensity curves during collapse (inset of Fig. 3.4.3) revealed that the shrinking is very fast in the beginning before it gradually approaches a new equilibrium radius. For larger bubbles we observe multi-step collapse, while smaller bubbles only shrink once.

What drives the collapse of the surface nanobubble? Figure 3.4.2d-f sketches a plausible dynamics. Due to the Laplace pressure [12], the gas pressure inside the nanobubble is higher than the pressure in the gas phase behind the contact line. This pressure difference can be quantified assuming the shape of the nanobubble being a spherical cap

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Figure 3.4.3.: Averaged pixel intensity I of nanobubbles as a function of time t . The inset shows the change of I during the rapid shrinkage of a nanobubble with $R_l = 0.38 \mu\text{m}$. The arrows mark the time the collapse process starts.

with a radius R_c with $\Delta P = 2\sigma/R_c$, where σ is the surface tension. When the CL approaches the surface nanobubble, the liquid film separating the bubble and ambient atmosphere thins and eventually ruptures (Fig 3.4.2d). As a result, the gas can escape through and the pressure inside and outside the bubble equilibrates (Fig. 3.4.2e). Since the nanoscopic contact angle of the surface nanobubble, θ (water side), is much larger than the equilibrium contact angle [31, 28], θ_{eq} , the interfacial tensions σ , σ_{LS} , σ_{VS} at the liquid-solid-vapour CL of nanobubble are not balanced (Fig. 3.4.2e). In this non-equilibrium state, a net capillary force (per unit length), $\sigma(\cos\theta_{eq} - \cos\theta)$, acts on the CL of the nanobubble, which leads to the shrinkage of the nanobubble and the movement of the nanobubble CL. With ongoing shrinking, the contact angle decreases, the capillary force decreases, and thus the shrinking speed should decrease. This may be indeed observed in the experiment as a slow decay of signal in Fig. 3.4.3, yet we cannot rule out other processes such as gas diffusion or diffusion of the fluorescent dye which may lead to a

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similar signal. If the nanobubble size is small, the air inside the bubble can be completely squeezed out and the bubble vanishes. However, if the bubble size is sufficiently large, the hole connecting the bubble and atmosphere can be closed again as the bubble region close to CL shrinks backwards, leaving a daughter bubble (Fig. 3.4.2f). This phenomenon has some similarity with the bubble entrapment during drop impact on solid surfaces [2].

3.4.4. Contact line motion during collapse

We now estimate a time scale for the nanobubble collapse, T_c , in the present experiments from the decrease of the spatially averaged intensity signal by fitting the experimental data with a smooth step function:

$$I(t) = I_0 + \frac{\Delta I}{2} [1 + \tanh(\frac{2(t - t_0)}{T_c})] \quad , \quad (3.4.1)$$

where the fitting parameters I_0 is the initial intensity, ΔI is the intensity change and t_0 is the time of transition. As mentioned previously, most bubbles show a multi-step collapse, thus many of them shrink to a smaller radius. Therefore, we plot the time T_c from above fit as a function of the relative lateral radius change, ΔR_l in Fig. 3.4.4. The collapse time T_c is of few milliseconds and increases linearly with the bubble size: $T_c = \Delta R_l / u_c$, where u_c is equivalent to a speed of this collapse process. A linear regression of the data results in $u_c \approx 110 \mu\text{m/s}$. This collapse speed should be related to the speed of the moving nanobubble contact line. In hydrodynamics, the contact line dynamics is described by the type of forces resisting the capillary force: If the inertial force is dominant, the contact line speed scales as $u_i \sim \sqrt{\sigma/\rho L}$ [12]; if viscous force is dominant, the contact line speed scales as $u_v \sim \sigma/\mu$ [11, 12], where ρ is the density of the liquid, L is the characteristic length and μ being the bulk liquid viscosity. However, we find that both u_i and u_v are 5-6 orders of magnitude larger than the collapse speed obtained from Fig. 3.4.4. Therefore, bulk hydrodynamic processes cannot explain the collapse dynamics of the surface nanobubbles.

Figure 3.4.4.: Collapse time T_c as a function of the relative lateral radius change ΔR_l . The solid line is a linear fit, i.e. $T_c = \Delta R_l / u_c$. The linear regression obtains a slope of $u_c \approx 110 \mu\text{m/s}$.

This inspired us to compare the speed scale observed with a microscopic model: the molecular kinetic theory [30]. Here, the contact line movement is described by the displacement of liquid molecules in the vicinity of the microscopic contact line [5, 6]. While the contact line moves on a solid surface, the unbalanced capillary force per unit length, $\sigma(\cos \theta_{eq} - \cos \theta)$, drives the liquid molecules to hop over the energy barrier of wetting which originated from the viscous friction among liquid molecules, and the adhesion between the solid and the liquid. The contact line speed is influenced by the heterogeneity of the solid surface (physical or chemical defects) [18], phase change (condensation or evaporation) near the contact line [1, 22, 23] and molecular slippage on the liquid-solid interface [24, 26], and takes the form [5, 6, 30]:

$$u_m = k \frac{\sigma}{\mu} (\cos \theta_{eq} - \cos \theta) \quad , \quad (3.4.2)$$

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where k is a non-dimensional parameter determined by the factors affecting contact line movement mentioned before; k has a value of the order of 10^{-6} for water on a smooth surface [22, 23]. Unfortunately, we do not know θ and cannot give a dynamic model of the motion, yet, we can estimate it as of $(\cos\theta_{eq} - \cos\theta) \sim O(1)$, thus we obtain $u_m \sim 73 \mu\text{m/s}$, which agrees with the order of magnitude with the collapse speed measured, i.e. $110 \mu\text{m/s}$.

3.4.5. Summary

In summary, we reported different dynamics of a receding contact line over surface nanobubbles, polymeric droplets and solid particles. First, we found that surface nanobubbles show gaseous properties when they are exposed to atmosphere: they shrink and vanish. In contrast, a fast receding and pinning-depinning processes was observed for polymeric droplets and solid particles, respectively. These findings may offer robust criteria to distinguish between surface nanobubbles from liquid/solid contamination in nanobubble research. Second, we found that the collapse of surface nanobubbles is much slower than bulk hydrodynamic scaling would predict. The collapse speed is in agreement with the molecular kinetic theory for contact line dynamics.

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Conclusion and Outlook for Part I

4.1. Conclusion

We have developed and applied an optical technique to observe nanobubble dynamics. So far, it is only possible to observe these fast nanobubble dynamics under TIRF microscopy. TIRF microscopy not only provides strongly contrasted images for nanobubbles larger than 230 nm, but also detects subtle changes in their height. Studying nanobubbles in video frame rate reveals some new insights that have previously been inaccessible with AFM.

In Chap. 3.1 the nanobubbles nucleation dynamics was visualized. Results from AFM measurements [6] show that nanobubbles are not observed until the last step of water-ethanol-water exchange. Our optical observations reveal dynamics that are too fast to be detected with an AFM. During water-ethanol-water exchange, we observed that nanobubbles already nucleate during the mixing of water and ethanol. In other words, both water-to-ethanol and ethanol-to-water exchange nucleates nanobubbles. However, nanobubbles dissolve in ethanol and only remain stable in water. These results imply that the mixing of ethanol and water leads to the release of dissolved gas to nucleate nanobubbles. Nanobubbles dissolve in ethanol due to the higher gas solubility. After the subsequent exchange the water becomes supersaturated. This important condition we think is necessary for stable nanobubbles.

To test the prediction of a fast recirculating flow above and near the nanobubbles

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[7], we combined TIRF microscopy with fluorescent particle tracking in Chap. 3.2. Our system measures particle speeds of up to $30\text{ }\mu\text{m/s}$, yet the averaged velocity is very close to zero. At a distance of 400 nm from the nanobubbles' center, the remaining drift flow is weaker than a few micrometers per second. Hence, we conclude that there is no residual flow near to nanobubbles. This result is later confirmed by a similar study [4].

We observed coalescence of neighboring nanobubbles subjected to external perturbation (Chap. 3.3). Such coalescence events show interesting dynamics: the new bubble covers the convex hull of the “footprint” of both bubbles before merging, which indicates a strong pinning force on the nanobubble contact line. Then the bubble grows in height within several 100 ms, during which the contact line remains pinned. The single bubble growth dynamics is compared against a simple diffusion model using only conventional assumptions, i.e. assuming validity of Henry's law. The model shows good agreement to the experimental observations. The time scale can be captured with a smaller diffusion constant of gas than the bulk value. This model predicts stable surface nanobubbles in supersaturated liquids and relies only on contact line pinning.

In Chap. 3.4 we studied another type of coalescence event. There, we observed surface nanobubbles' collapse when they meet with a receding contact line. We showed that the abrupt change in shape and size is distinct from that of a contact line meeting with a polymeric droplet or a solid hydrophobic particle. This method provides a simple yet rather direct proof of the gaseous nature of nanobubbles. With high speed recording up to 2000 fps, we study the brightness of the gaseous object during its fast shrinkage. Considering resistance from viscosity or inertia alone predicts much faster contact line motion speed. Alternatively, by employing a molecular kinetic model which takes into account the molecular scale boundary condition, the predicted contact line speed can be very well captured.

In this thesis, we add to the knowledge of surface nanobubbles quasistatics and their dynamics. We reveal fast dynamical processes once sufficiently perturbed, i.e. formation, coalescence, and shrinkage. This is important for understanding the nucleation processes

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as well as their response to external perturbation. We expect more experiments that are previously performed on microbubbles, e.g. acoustic excitation, can be extended to surface nanobubbles.

4.2. Suggestion for Future Work

Comparing with AFM, optical microscopy can image nanobubbles at much faster frame rates non-invasively. Consequently, TIRF microscopy has a higher flexibility to combine it with other experimental techniques which would be difficult with AFM. For example, we have combined TIRF microscopy with particle tracking in Chap 3.2. Other techniques are still to be explored; for instance, Borkent *et al.* [2] correlated cavitation inception sites [3] with presence of surface attached nanobubbles. They need to rely on statistical averages, as the microscopic image and the AFM image were taken at different locations, resolutions, and not simultaneously. *In situ* comparison however could be achieved using TIRF microscopy together with a shock wave generated with a pulsed laser, e.g. as demonstrated before [1]. We can then measure the cavitation inception threshold of the nanobubbles.

Our TIRF system's exposure time is determined by the gate time of the AOM, which is around 100 ns (see Chap. 2.2.2). Together with the ability to detect minor changes in height, we expect that we could detect periodic changes of nanobubbles height due to oscillation under acoustic driving. This allows to explore the size dependent resonance frequency of nanobubbles, which has not been investigated before.

At last, we highlight that a setup combining AFM and TIRF microscopy [5] has complementary advantages for nanobubbles studies. Optical observations can detect the locations for nanobubbles within a large area, while AFM can measure the high resolution morphology. Moreover, the AFM cantilever can be used to manipulate nanobubbles, with the fast response of the nanobubbles monitored by TIRF microscope. This is a useful technique to access the stability of nanobubbles under perturbation. We can also

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measure the magnitude of the pinning force required to depin the nanobubbles. These two results will quantify how strong the nanobubbles pin and reveal the role of pinning for their stability.

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Part II.

Nanoparticle-Decorated Magnetic Bubbles

Introduction

Colloidal particles often spontaneously adsorb on an interface between two phases. The driving force is the minimization of total surface energy, provided the surface energy (per unit area) between the two phases σ_{12} is larger than the difference between the solid-liquid energy of the two fluids $|\sigma_{1p} - \sigma_{2p}|$. In two phase liquid-liquid system, the self-assembly of the colloidal particles at the interface can stabilize emulsions effectively by resisting coalescence [2]. This so-called Pickering emulsion is used to fabricate liquid-in-liquid capsules called colloidosomes [3]. Their selective permeability and responsive dissolution to external stimuli is useful for the controlled release from a carrier [14]. The idea of Pickering emulsion can be extended to bubbles and foams by self-assembly of colloids at gas-liquid interfaces [4, 6, 10, 15]. Here the adsorbed particles not only prevents coalescence but also stabilize the bubbles against gas diffusion. Apparently the close packing of particles deforms the interface and practically diminishes the Laplace pressure [1].

Since the colloidal-particles-stabilized microbubbles have good stability, they are widely used as contrast agents for ultrasonic imaging [5]. Ultrasonic imaging is a very well-adopted diagnostic technique [9]. However, even with the enhancement of contrast agents, their contrast is still far less than other diagnostic techniques such as magnetic resonance (MR) imaging or X-ray [11]. This limitation inspires the incorporation of an MR contrast agent, magnetic nanoparticles, onto the microbubbles.

Such magnetic microbubbles (MMBs) have complementary advantage of both mag-

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netic NPs and microbubbles in biomedical imaging. The introduction of magnetic NPs endows MMBs with magnetic properties, i.e. they can be steered by a magnetic field [12] and enhance contrast in MR imaging [18]. Contrast-enhanced ultrasonography allows realtime and cost-effective diagnosis, while MR imaging provides high spatial resolution and better contrast in soft tissue [7].

Some of the pioneer works utilize MMBs for localized drug/gene delivery. Vlaskou *et al.* [16] proposed the first generation of MMBs. In their work, the MMBs are stabilized by a liposphere structure: perfluorobutane gas is entrapped in soybean oil, which contains magnetic nanoparticles; a lipid layer is then applied to stabilize the oil droplets and to carry lipophilic drugs. Similar methods are later employed by Yang *et al.* [17]. There, they replaced the lipid coating with a polymer-oil-gas double emulsion and encapsulated the drug in a form of nanoparticles. They have also demonstrated the ultrasound-mediated drug delivery to *in vitro* cell cultures.

The major drawback of all the methods discussed above is that the localized delivery is triggered by abrupt disruption/collapse of microbubbles upon applying a high-amplitude ultrasound. However, such rapid collapse might also damage surrounding tissues such as the endothelium [8] and cell membrane [13]. Also, the amount of released drug is uncontrollable. It motivated us to improve the controlled release techniques of MMBs. This requires a new fabrication protocol to incorporate magnetic nanoparticles onto the microbubbles.

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Controlled Nanoparticle Release from Oscillating Magnetic Microbubbles*

In this chapter we propose a solution to the challenge discussed in Chap. 5 by introducing an MMB fabrication protocol that, combining resonance driving, allows controlled and moderate release of NPs from MMBs. In our system, NPs can be continuously ejected from the MMBs without disruption. This can prevent cavitation damage, which may be prevalent in other techniques.

This chapter is organized as the following: We first detail our fabrication protocol, then we report the release and transport of nanoparticles (NPs) cargo from magnetic microbubbles (MMBs). In Chap. 6.2 the MMBs stimulated by ultrasound at their resonance frequencies oscillate in volume and surface modes. Above a threshold NPs from the MMB shell are released and can travel hundreds of micrometers away from the surface of bubble. In Chap. 6.3 we study the migration of NPs from MMBs in detail. We propose a force balance model in oscillatory flow to calculate the NPs velocity. More interestingly, this phenomenon is observed not only from MMBs but from the functional MMBs that contain both magnetic NPs and non-magnetic NPs (such as polymeric NPs or silica NPs). To test the viability to apply this technique to biomedical application, in Chap. 6.4 we deliver drug-containing NPs across the hydrogel barrier to target the cancer cells.

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6.1. Fabrication Protocol

Figure 6.1.1.: Characterization of magnetic microbubbles (MMBs). (a) Schematic illustration of a MMB composed of an air core and a shell of Fe₃O₄ NPs (50 nm). (b) Scanning electron microscope (SEM) image of the shell of MMBs. (c) Dark-field image of MMBs. (d) Schematic illustration of fluorescent MMBs composing an air core and a shell of Fe₃O₄ NPs (50 nm) and SiO₂ NPs (500 nm). (e) SEM image of the shell of fluorescent MMBs. (f) Fluorescence image of fluorescent MMBs. (g) MMBs float on the top of water surface and (h) are attracted by a magnet. (i) Mean diameters of MMBs prepared with different concentrations of Fe₃O₄/SiO₂ NPs and different time of homogenization (speed: 8000 rpm).

MMBs are prepared by emulsifying a solution containing anionic surfactants (i.e. sodium dodecyl sulfate (SDS)) and cationic magnetic nanoparticles (NPs) (i.e. 50 nm poly(vinylpyrrolidone) (PVP) coated iron oxide (Fe₃O₄) NPs). Magnetic NPs (~ 50 nm, Sigma-Aldrich) were first dispersed in deionized (DI) water to form a 2 mg/mL stock

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solution and ultrasound for 20 minutes before use. Then a mixture contains 400 μL of stock solution of Fe_3O_4 NPs, 150 μL of 10 mM SDS ($>99\%$, Fisher Scientific), and 150 μL of DI water were agitated either by moderately shaking (~ 160 oscillations per minute) for 1 minute or homogenization for 0.5 to 5 minutes at various stirring speeds. After stirring, MMBs were left overnight before being rinsed with PBS for 3 times before use.

Analysis with scanning electron microscope (SEM) revealed that magnetic NPs assembled on the surface and formed a dense shell (Fig. 6.1.1b). This magnetic shell not only allows MMBs to respond to magnetic manipulation (Fig. 6.1.1g&h), but also stabilize the MMBs against dissolution in five different buffers for at least ten days. When being stored in water, they are even intact for one year. The interaction of magnetic NPs and surfactant (i.e. SDS) increases the viscoelasticity of the interface and lowers the surface tension [1].

The size of MMBs can be controlled through either the concentration of magnetic NPs or the stirring speed and time. For example, MMBs size are between 100 to 200 μm when being made by moderately shaking (~ 160 oscillations per minute) for 1 minute (Fig. 6.1.1c). Using a homogenizer (D160, DS-160/5 probe, Scilogex, USA) with stirring speeds around 20000 rpm, the size decreases to 2 μm (Fig. 6.1.2). We believe that the bubble fragmentation in the high shear flow is responsible for the size decrement. Under a fixed speed (i.e. 8000 rpm with homogenizer) and magnetic NP concentration (2 mg/mL), a longer emulsification time can also reduce the size from 10 to 5 μm (Fig. 6.1.1i). If the synthesis is carried at a reduced pressure of 0.1 atm, the size of MMBs can be further reduced to ~ 450 nm. Interestingly, we observed that a higher concentration of magnetic NPs during the preparation increased the MMBs' size (Fig. 6.1.1i), which might be due to the thickening of their shell.

We can fabricate functional MMBs (Fig. 6.1.1d) by replacing magnetic NPs with a mixture of magnetic NPs and other types of cationic NPs encapsulating functional molecules. For example, fluorescent MMBs are obtained by mixing 500 nm fluorescent silica (SiO_2) NPs (encapsulating Tetramethyl-Rhodamine) with 50 nm Fe_3O_4 NPs during the prepa-

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Figure 6.1.2.: The size change of magnetic microbubbles (MMBs) corresponding to the change of stirring speeds. (a) The size distribution of the MMBs prepared at different homogenization speeds. (b) The comparison of the mean diameters of the MMBs prepared at different homogenization speeds. During the synthesis, magnetic nanoparticles were kept at 2 mg/mL. The preparation time was 2 minutes. The sizes of MBBs were from the measurement of at least 200 MMBs.

ration (Fig. 6.1.1e&f). As shown in Fig. 6.1.1e, SiO_2 NPs are embedded within the Fe_3O_4 NPs.

Similar to MMBs, the size of fluorescent MMBs can be controlled either through the concentration of NPs or the stirring speed and time. For example, with the fixed NP concentrations (2 mg/mL Fe_3O_4 NPs and 0.2 mg/mL SiO_2 NPs) and stirring speed (8000 rpm), fluorescent MMBs decreased from 15 to 5 μm by increasing the homogenization time from 1 to 5 minutes (Fig. 6.1.1i, 6.1.3). Elemental analysis showed that regardless of the stirring speed and time, the ratio of silicon to iron in fluorescent MMBs was between 0.009 and 0.031, which is comparable with the seeding ratio of silicon to iron (i.e. 0.027). This indicates that the ratio of two types of NPs on fluorescent MMBs is decided by the seeding density of NPs.

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Figure 6.1.3.: The size change of magnetic microbubbles (MMBs) corresponding to the change of homogenization time. During the synthesis, Fe_3O_4 NPs were kept at 2 mg/mL while the homogenization time was (a) 0.5; (b) 1.0; (c) 2.0; (d) 3.0; and (e) 5.0 minutes. The sizes of MMBs were from the measurement of at least 200 MMBs.

6.2. Controlled Release of Nanoparticles

Once the MMBs were exposed to a sufficiently strong resonant ultrasound field, they started to oscillate, despite a dense shell of magnetic NPs assembled on their surface. Under moderate acoustic driving pressure, the MMBs oscillated in a spherical mode near their resonance frequency [8], while under higher driving pressure high order shape oscillation modes were observed. If the driving pressure was high enough, some of the NPs were released and traveled away from the bubble, i.e. they were transported by the flow field generated by the oscillating bubble.

To assist a quantitative measurement of this process, we confined the motion of MMBs susceptible to buoyancy and acoustic forces [4]. This was achieved by letting the MMBs float in a microfluidic channel with a width of 1 mm and a height of 0.2 mm. The

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Figure 6.2.1.: Controlled particle release from magnetic microbubbles (MMBs). (a) Schematic graph of apparatus used for investigation of MMB oscillation and particle release from a single oscillating MMB. (b) Plot of resonance frequency vs. radius of MMBs. (c) Fluorescence images of a fluorescent MMB under the volume mode oscillation and ejected fluorescent SiO_2 particles at different times. (Scale bar: 50 μm) (d) A plot of the distance of released NPs from the fluorescent MMB center vs. time. (e) The simulation of the particle velocity vs. distance to the MMB center for particles with different diameters.

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channel was made through standard soft lithography and bonded to a glass substrate. The acoustic field was generated with a piezoelectric transducer glued to the substrate (Fig. 6.2.1a) [7]. Bright-field images and fluorescence images were captured with an attached high speed camera or CCD camera, respectively.

To find the resonance frequency of MMBs, we swept the driving frequency from a certain range (from 10 kHz to 1 MHz) at a low setting of the amplifier. For a driving frequency ≤ 100 kHz, the bubble radius was extracted from images taken with the high speed camera at 100,000 frames/s. The bubble contour is then fitted to a circle and its radius extracted [8]. The resonance frequency of MMBs at a certain size was determined from the maximum changes of radius of these bubbles during this sweep. For driving frequencies > 100 kHz, the radius is not detectable due to motion blur. However, we observed NPs detachment and microstreaming flow at a narrow range of frequency. This reflects that the oscillation amplitude and the resonance frequency is chosen as the central frequency in that range.

By examining MMBs ranging from 6.5 to $135\text{ }\mu\text{m}$ in radius (Fig. 6.2.1b), we found that small MMBs with radii $< 25\text{ }\mu\text{m}$ showed the characteristic response very similar to microbubbles without NP coating, whose resonance frequency is inversely proportional to the radius (Minnaert frequency) [6].[†] However, the resonance frequencies of MMBs with larger radii ($> 35\text{ }\mu\text{m}$) stabilized around 50 kHz, which might be caused by the confinement in the microfluidic channel (Fig. 6.2.1b).

Next we exposed a single MMB to an acoustic field at its resonance frequency and increased the acoustic pressure steadily. The response of the bubble switched from a radial volume oscillation to a shape oscillation (Fig. 6.2.2). At sufficiently low amplitudes (volume mode) the NPs were continuously detached from the microbubble's surface, traveling radially away from the bubble. Pronounced shedding of NPs was observed once the non-radial surface mode oscillations set in (Fig. 6.2.3). The detached NPs followed

[†]A comprehensive discussion on the resonance frequency of microbubbles can be found in [5] Chap 3.2 and 3.4.

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vortical flow lines known as microstreaming and detached as aggregates (Fig. 6.2.4). Once most of the NPs were removed from the bubble surface, the bubble shrank and dissolved (in absence of driving). The surface mode oscillations also lead to resonance frequency change and broadening. A good description of this kind of Mathieu resonances is given in the recent review by Kurz and Lauterborn [4].

Figure 6.2.2.: The response of the magnetic microbubble (MMB) switched from a volume mode oscillation to a shape mode oscillation. A single MMB oscillated by a volume mode at low amplitude of 34 Vpp (top). A 11% increase of radius of the MMB was shown (white arrow). The MMB exhibited a shape mode oscillation at amplitude of 60 Vpp (middle), in which a slight shape change was observed. The intensity of the shape mode increased when the amplitude increased to 68 Vpp (bottom). (Scale bar: 25 μm)

The NP shedding phenomenon allows MMBs as controlled release carriers that we demonstrate next using MMBs coated with fluorescent NPs. At a low driving pressure, a continuous efflux of fluorescent SiO_2 NPs was observed (Fig. 6.2.1c&d). At high driving pressure we observed a strong expulsion of fluorescent NPs and formation of a toroidal vortex (Fig. 6.2.5)), in which the spreading of NPs covered a distance of up to 400 μm . The fluorescent MMBs dissolved after the acoustic driving was switched off.

6.3. Nanoparticle Transport in Oscillatory Flow

The aim of localized delivery is to maximize the released dose at the targeted sites while avoiding potentially harmful deposition on the neighbor. It requires not only a

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Figure 6.2.3.: Continuous detachment of NPs in volume mode oscillation. (Left) A MMB ($150\mu\text{m}$ diameter) located near PDMS wall is driven by low amplitude (34 Vpp) ultrasound. Scale bar: $50\mu\text{m}$. (Right) Selected frames in the region of interest depict a continuous stream of NPs, which are detached from MMB and transported away by the flow field. Scale bar: $20\mu\text{m}$.

controllable release but also a predictable spreading distance by the oscillating bubbles. For reliable application it would be important to understand the effect of the outer parameters, such as the driving amplitude and size of the particles on the resulting trajectory of the particles. We therefore utilize a force balance model [2] to calculate the motion of the particles during the release. The balanced forces are liquid and particle inertia and a Stokes drag force. It reads as:

$$\frac{du_p}{dt} = \frac{\rho_l}{\rho_l C_A + \rho_p} \frac{Du_l}{Dt} - \frac{18\mu}{d^2(\rho_l C_A + \rho_p)}(u_p - u_l) \quad , \quad (6.3.1)$$

where ρ_l and ρ_p is the density of water and particle, u_l and u_p is the velocity of liquid and particle, d is the diameter of particle, R is the radius of bubble, r is the distance to the center of the MMB, dR/dt is the velocity of the bubble surface during the oscillation, and $C_A = 0.5$ is the added mass coefficient for a spherical particle [3]. We account for the 2D confinement by modeling the liquid velocity to drop with $1/r$. By mass conservation the liquid velocity is:

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Figure 6.2.4.: NPs release in surface mode oscillation. A MMB ($77\text{ }\mu\text{m}$ diameter) oscillates in surface mode 5 when high amplitude ultrasound of 320 Vpp is applied. NPs are detached and carried by the circulating microstreaming flow field. Note that the driving frequency (100 kHz) is a multiple of recording frame rate (25 kfps), hence the oscillation cycle is always in phase. Inter-frame time in each row: 0.24 ms , 0.24 ms , 0.48 ms , 0.96 ms . Scale bar: $50\text{ }\mu\text{m}$

$$u_l = \frac{R}{r} \frac{dR}{dt} \quad . \quad (6.3.2)$$

At low driving we assume a harmonic oscillation of the bubble radius $R = R_0(1 + \epsilon \cos(\omega t))$, where R_0 is the rest radius of the MMB, $\epsilon = 10\%$ is the amplitude of oscillation, and ω is the angular frequency of driving.

This model was compared with a recorded trajectory of a $1.4\text{ }\mu\text{m}$ fluorescent parti-

6. Controlled Nanoparticle Release from Oscillating Magnetic Microbubbles

Figure 6.2.5.: Shape mode oscillation of fluorescent magnetic microbubbles (MMBs). By applying the resonance frequency with an amplitude of 100 Vpp, fluorescent MMBs oscillated in a shape mode and a vortex ring was generated around the MMB. After releasing NPs, the fluorescent MMB dissolved. Figures from left to right indicate selected fluorescent images at different time points.

cle released from a $65\text{ }\mu\text{m}$ fluorescent MMB (Fig. 6.2.1c). Experimentally, we exposed the fluorescent MMB with moderate acoustic driving so that it oscillated in the volume mode. Then we depict the displacement of the particles against the time exposed to the oscillating bubble (Fig. 6.2.1d), which allowed us to derive the velocity of the fluorescent particles vs. the distance to the bubble center. As shown in Fig. 6.2.1e, the experimentally obtained velocity as a function of distance agrees nicely with the simulation. The simulation provided plots of the particle velocity vs. position (distance to the MMB center) for different particle sizes, which showed that the smaller particles tend to move away from the MMB with a slower speed.

6.4. Controlled Drug Release and Delivery

As mentioned in Chap 5, the current utilization of MMBs for drug delivery suffers from cavitation damage under high amplitude driving. The controlled and moderate release of NPs using the present MMB approach might provide a solution to this challenge. As a proof-of-concept, we prepared a type of functional MMBs with 50 nm Fe_3O_4 NPs and $2\text{ }\mu\text{m}$ PLGA particles containing a chemotherapeutic drug, Doxorubicin (Dox-PLGA MMBs). SEM images showed the PLGA particles were coated with Fe_3O_4 NPs and packed together to form the shell (Fig 6.4.1). To facilitate the visualization of the release of PLGA particles with fluorescent microscope, the size of Dox-PLGA MMBs was

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controlled around $100\text{ }\mu\text{m}$.

Figure 6.4.1.: Scanning electron microscope (SEM) characterization of Doxorubicin-containing poly(lactic-co-glycolic acid) particles (Dox-PLGA) and Dox-PLGA magnetic microbubbles (MMBs). (a) Dox-PLGA particles. (b) the shell of Dox-PLGA MMBs. (c) a zoom-in image of the shell of Dox-PLGA MMBs, in which Dox-PLGA particles ($2\text{ }\mu\text{m}$) are indicated with black arrows and iron oxide nanoparticles (50 nm) are labelled with red arrows.

Next, the stability of Dox-PLGA MMBs under oscillation was examined in the cell medium after being localized on the bottom of the petri dish with a magnet. As expected, Dox-PLGA MMBs firstly ejected particles with the MMB collapse ratio of only 8.5 % when driven at their resonance frequency with a moderate amplitude. When colorectal cancer cells (i.e. RKO cells) were cultured on the petri-dish during the experiment, they were labeled with the detached Dox-PLGA particles that were found inside the cellular cytoplasm (Fig. 6.4.2a&b) and significantly decreased the viability of cancer cells in 24 hours post-experiment (Fig. 6.4.2c). The cytotoxicity clearly came from the Dox-PLGA particles, as the viability of cancer cells were only slightly decreased when MMBs were used. More excitingly, when ultrasound was not applied during the experiment, the detachment of Dox-PLGA particles from microbubbles were neglectable and did not show any influence over the viability of cells (Figs. 6.4.2c & 6.4.3).

We also examined the penetration of NPs into a hydrogel. RKO cells were cultured within the alginate hydrogel ($\sim 3\text{ mm}$ thick). Then Dox-PLGA MMBs were localized on the hydrogel with a magnet before the ultrasound at the resonant frequency of Dox-PLGA MMBs was introduced to induce the oscillation (Fig. 6.4.4a). Within one minute, Dox-PLGA particles were found inside the hydrogel layer and some particles even entered

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Figure 6.4.2.: Controlled particle release from Dox-PLGA magnetic microbubbles (MMBs) to cancer cells 2D culture. (a) Bright field (top) and fluorescence (bottom) images of RKO cells untreated (left) and treated with oscillating Dox-PLGA MMBs (right), followed by a 24 hours incubation. Scale bar: 100 μm . (b) Confocal scanning microscopy of RKO cells treated with oscillating Dox-PLGA MMBs, in which Hoechst 33342 stains the nuclear, DiO stains the membrane, and red color represents Dox-PLGA particles. Scale bar: 10 μm . (c) Viability of RKO cancer cells treated with different methods, followed by a 24 hours incubation.

the cellular cytoplasm of cells within hydrogel (Fig. 6.4.4b&c). In comparison, neither free Doxorubicin nor Dox-PLGA particles (equivalent concentration of doxorubicin) could penetrate the hydrogel layer and label the RKO cells during the same incubation time (Fig. 6.4.4d-f).

6.5. Conclusion

In this Chapter, we demonstrated that NPs can be continuously ejected from the MMBs shell without the disruption of the bubble or other typical phenomena observed in the cavitation regime by resonance driving. The NPs cargo, both the magnetic and functional non-magnetic NPs, experience the same detachment and transport process. The size and

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Figure 6.4.3.: Dox-PLGA MMBs release by non-collapsing oscillation of microbubble to colorectal cancer cell line. (a-e) Bright field and (f-j) fluorescence images of RKO cancer cells treated with different methods, followed by a 24 hours incubation. (scale bar: $200\mu\text{m}$). (a) and (f) untreated RKO cells. (b) and (g) RKO cells treated with ultrasound. (c) and (h) RKO cells after treated with oscillating MMBs. (d) and (i) RKO cells after treated with oscillating Dox-PLGA MMBs. (e) and (h) RKO cells after treated with Dox-PLGA MMBs but without applying ultrasound.

composition of MMBs can be controlled through the ambient pressure, concentration of particles, and the stirring speed during preparation. The detachment and migration of NPs from the MMBs are dependent on the bubble's resonance frequency and pressure amplitude. The migration velocity is in agreement with a force-balance model. As a proof of concept drug-containing NPs are delivered across the hydrogel barrier to target the cancer cells that are hard to reach for free NPs. The ability to remotely control the release of NPs from MMBs poses opportunities for targeted drug delivery through/into tissues which are hard to diffuse or penetrate.

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Figure 6.4.4.: Controlled particle release from Dox-PLGA magnetic microbubbles (MMBs) to cancer cells cultured in hydrogel. (a) Schematic illustration of the controlled release of Dox-PLGA particles from Dox-PLGA MMBs. The released particles could penetrate into the alginate hydrogel and target the RKO cells. (b) Confocal scanning microscopy and (c) 3D projection of the RKO cells in the hydrogel after treated with oscillating Dox-PLGA MMBs. (d) RKO cells in hydrogel before any treatment. (e) RKO cells in hydrogel treated with free Doxorubicin for 15 minutes. (f) RKO cells in hydrogel treated with Dox-PLGA particles for 15 minutes. The green color comes from the DiO staining of the membrane, and red color represents Dox-PLGA particles.

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Magnetic Bubble Trap

As we demonstrated in the previous chapter, magnetic microbubbles (MMBs) have a wide variety of potential applications. By precisely controlling the location of MMBs, we can employ MMBs as manipulators or nanoparticle cargoes on local target. In this chapter we will explore the possibility to confine and stabilize a single MMB in three-dimensional space.

The trapping of a single microbubble is commonly used in single bubble sonoluminescence [4]. There, the buoyancy force of microbubbles is balanced by the time-averaged spatial gradient of the pressure field, the so-called primary Bjerknes force [3]. However, in order to trap a microbubble in a strong sound field, the parameter space of the pressure field that can be applied to small bubbles is limited [1]. There is some early work on the effect of a magnetic field on the bubble dynamics in water, e.g. [6]. However this is limited and manifests as an additional driving term only for very high magnetic fields due to low magnetic susceptibility in water.

In our magnetic trap it is possible to stabilize the MMBs solely by applying a diverging magnetic field and some feedback for controlling the force. Sufficiently strong magnetic forces to trap the paramagnetic nanoparticles coating on MMBs are already achieved with a home-built electromagnet. To our best knowledge, such 3D magnetic trap design has not been reported before. We start by discussing the setup required for a stable three dimensional confinement. Then we will access the performance of the trap and the influence from temperature change.

7.1. Experimental Setup

7.1.1. Magnetic force

The magnetic force acting on the paramagnetic spherical shell submerged in a non-magnetic medium is given by [2]:

$$\vec{F}_{mag} = \frac{\chi_b}{\mu_0} (\vec{B} \cdot \nabla) \vec{B} \quad , \quad (7.1.1)$$

where χ_b is the magnetic susceptibility of a spherical shell, μ_0 is the magnetic permeability in vacuum and $\vec{B}$ is the magnetic field strength. Decomposing $\vec{F}_{mag}$ into cylindrical coordinates, for each direction $\hat{i}$ the force component is:

$$F_{mag,i} = \frac{\chi_b}{\mu_0} (B_r \frac{\partial}{\partial r} + B_\phi \frac{1}{r} \frac{\partial}{\partial \phi} + B_z \frac{\partial}{\partial z}) B_i \quad . \quad (7.1.2)$$

In our magnetic trap, the magnetic field is generated by a home built electromagnet (Fig. 7.1.1). There, #10AWG Copper wire is coiled around an iron nail and fixed by epoxy. The function of the nail is to shape the magnetic field to a diverging field with maximum field strength at the tip.

For small objects as MMBs, we can consider them as point objects exposed to this diverging magnetic field $\vec{B}(r, z)$ from below. Note that $\vec{B}$ is axisymmetric around the vertical coil axis $\hat{z}$. On each plane tangential to coil axis, the in-plane field strength decays radially away from coil axis. From Eq. (7.1.2) the in-plane magnetic force components are:

$$B_\phi = 0 \Rightarrow F_{mag,\phi} = 0 \quad , \quad (7.1.3)$$

$$F_{mag,r} = \frac{\chi_b}{\mu_0} (B_r \frac{\partial}{\partial r} + B_z \frac{\partial}{\partial z}) B_r < 0 \quad . \quad (7.1.4)$$

7. Magnetic Bubble Trap

Figure 7.1.1.: Experimental setup: magnetic coil and imaging system. The width of the cuvette is 1cm.

Hence the in plane magnetic force always attracts the MMBs towards the coil axis. This satisfies the condition for a bubble trap in that plane. Along the vertical axis the MMBs experiences buoyancy and a downward magnetic force. Note that the magnetic field gradient in axial direction is much larger than in the radial direction, which can be neglected:

$$F_z = \rho_w g V + \frac{\chi_b}{\mu_0} B_z \frac{\partial}{\partial z} B_z \quad , \quad (7.1.5)$$

where ρ_w is the water density V is bubble volume and g is the gravitational acceleration. To stabilize the MMBs in vertical direction, we need to adjust the current applied to the coil such that the downward magnetic force balances buoyancy. The vertical magnetic field strength in the region above the nail tip is mapped with a gaussmeter (GM04, Hirst Magnetic Instr.). Through repeated measurements for different currents and finding the maximum value in each horizontal plane, we can approximate the vertical magnetic field strength $B_z(I, z)$ along the axis as a function of I (Fig. 7.1.2). The following fitting

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functions give a good agreement with the measurement:

$$B_z(I, z) = 54.6\sqrt{I} \exp[-z/(4 \times 10^4)] \text{ mT} \quad . \quad (7.1.6)$$

A stable trap requires a restoring force in each direction to counter small perturbations from the equilibrium position. In the horizontal direction $\partial F_{mag,r}/\partial r < 0$, hence MMBs are always confined to the coil axis. However, in vertical direction $\partial F_{mag,z}/\partial z > 0$ and hence the trap is unconditionally unstable; even if the magnetic force is adjusted to balance buoyancy at certain height, a small perturbation in vertical direction will drive it away from equilibrium. To achieve a stable trapping in vertical direction, a feedback control loop is required to adjust the magnetic force dynamically.

Figure 7.1.2.: Measured magnetic field strength B_z as a function of applied current I and distance from the coil tip z . B_z is the vertical component of the magnetic field from the coil, along the coil axis. The location of the nail tip is defined as $z = 0$ mm.

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7.1.2. Feedback loop

Figure 7.1.3 depicts the schematic drawing of the bubble trap setup with a feedback loop. A single MMB is transferred with a micro pipette to a plastic cuvette (1cm x 1cm width) containing DI water. The electromagnet below the cuvette generates the magnetic field required to stabilize the MMB. The magnitude of the control current is determined from the position of the MMB, i.e. by tracking its dynamics. This is achieved by acquiring realtime images with a CCD camera (unbranded webcam) fed into Matlab's data acquisition toolbox (Mathwork). After realtime image processing the averaged position of the MMB is determined in each frame. This value is passed to a control algorithm to determine the control current required to stabilize the MMB (the algorithm will be detailed in Chap 7.1.3). An analog voltage is then sent through a data acquisition card (National Instrument) to the DC power supply (Gen20-76, TDK-Lambda). This completes the feedback cycle. The feedback cycle runs at 20-30 fps and is fast enough to stabilize the MMB, as shown in Chap 7.2.1.

The feedback rate is mainly limited by the data transfer rate. The exact frame rate of the webcam is known but not fixed. A second CCD camera (Pixelfly QE, PCO) and a long working distance objective (10x, Mitutoyo) are used to capture the MMB along a direction perpendicular to the webcam. This provides higher spatial and temporal resolution. Either brightfield or reflection images are taken. Figure 7.1.4 shows the experimental setup without the second camera. Note that a computer fan is used for cooling the electromagnetic coil. The optical table is air flowed and it has been taken care that the instruments do not cause significant vibration.

7.1.3. PID controller algorithm

The term PID controller refers to the commonly used closed-loop controller based on the Proportional, Integral and Derivative of the error between measured and desired value. Comparing to an open-loop system, a closed-loop system has the advantage of

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Figure 7.1.3.: Schematic drawing of the feedback loop

Figure 7.1.4.: Experimental setup of the magnetic trap. The cuvette shown in the picture is empty. During experiment it is filled with DI water with a single MMB.

noise rejection, either from measurement uncertainties or due to external disturbances. Without knowing the underlying dynamical response of the system to an external input,

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a PID can minimize setpoint errors and stabilize output.

To formulate our PID algorithm, let the time dependent error to be $e(t)$. The general output of the PID controller is given by:

$$u(t) = K_p e(t) + K_i \int_0^t e(\tau) d\tau + K_d \frac{d}{dt} e(t) \quad . \quad (7.1.7)$$

Parameter	Rise time	Overshoot	Settling time
K_p	Decrease	Increase	Small change
K_i	Decrease	Increase	Increase
K_d	Minor decrease	Minor decrease	Minor decrease
Parameter	Steady-state error	Stability	
K_p	Decrease	Degrade	
K_i	Decrease significantly	Degrade	
K_d	No effect in principle	Improves if K_d is small	

Table 7.1.: The effect of increasing parameters in a PID loop. The effect of decreasing is the opposite.

The three constants K_p, K_i, K_d determine the response of the system to the error $e(t)$. During the design of the PID controller these constants are tuned to optimize the performance of the controller. The effects of increasing the parameter are summarized in the table 7.1. In our case, the feedback loop is discrete and limited by the frame rate. The output current in the i^{th} image $I(i)$ is calculated according to:

$$I(i) = I_0 + K_p e(i) + K_i \sum_{I=1}^i e(I) + K_d \frac{e(i) - e(i-1)}{t(i) - t(i-1)} \quad , \quad (7.1.8)$$

where I_0 is the best guess of the current for the setpoint, $e(i)$ is the distance between the setpoint and bubble center in the image captured at time $t(i)$. A discrete controller has a delay to real object motion and hence has a slower response time compared to an ideal continuous controller. As a result, we need to maximize stability by increasing rise and

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settling time. Usually the bubble is first held at the bottom and we trap it at a location very close to the bottom. Then the bubble is slowly moved towards the center of the liquid. The response of the bubble is found to be sensitive to the parameters, which has to be optimized manually. We have employed Ziegler–Nichols [5] tuning method to determine an initial guess of the PID constants.

7.2. Result and Discussion

7.2.1. Bubble stabilization

Figure 7.2.1 illustrates the image sequence acquired for the feedback loop. After realtime image processing the bubble center height (green line) and the distance to setpoint (red line) are measured. It demonstrates that a MMB is trapped after being released from the bottom of the cuvette. The corresponding position and output current are shown in Fig 7.2.2a. The output current shows a similar trend as the bubble position due to the proportional term. In the case when there is a rapid change, the differentiation term dominates and slows down the response. The integral corrects the steady state error on the long term. Once the MMB is trapped its position is very stable (Fig 7.2.2b). The typical fluctuation are below the pixel resolution of $4.4\mu\text{m}/\text{pixel}$. The trap is shown to be stable for up to 16 minutes. For practical reasons the experiment has been stopped after 16 minutes, yet could have continued for longer time.

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Figure 7.2.1.: Image sequence captured during feedback loop. It shows the MMB being released from the bottom and approaching the setpoint height (red line). The green line is the bubble center height from image processing. The image sequence runs from left to right, and top to bottom. The MMB diameter is $175 \pm 5 \mu\text{m}$.

Figure 7.2.2.: (a) Detected bubble center height (blue circle) and the corresponding output current (red cross) of the image sequence in Fig 7.2.1. (b) Detected bubble center height (blue circle) and the corresponding output current (red cross) of a MMB stabilized in the bubble trap.

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7.2.2. Step displacement

After the MMB being trapped, it is also stable to a step change to a setpoint height. Figure 7.2.3A shows the MMB response to a step change of ± 5 pixels every 9 seconds. The error arose from the step change causes an overshoot but eventually the MMB is trapped again. In another case of 1 pixel in every 4 seconds (Fig 7.2.3B), the bubble can be stabilized in 4 to 5 seconds. The MMB remains stable during this process. Translation in the horizontal direction is also possible, simply by moving the electromagnet. This demonstrates that our bubble trap is robust in translating MMBs to a desired location in 3D space. The range it can cover is limited by the diverging magnetic force that drops exponentially. When MMBs are far from the coil, higher applied current is required to maintain the same magnetic force.

Figure 7.2.3.: Detected bubble center height (blue circle) and the corresponding output current (red cross) of a MMB responses to step change of setpoint height. (a) $22 \mu\text{m}$ in every 9 seconds (b) $4.4 \mu\text{m}$ in every 4 seconds.

7.2.3. Stability in long trapping time

To test the long-time stability of the trap we have trapped the bubble at one place for 16 minutes before we manually stopped our measurement (Fig 7.2.4). The bubble shows fluctuation of less than 3 pixels or $13.2 \mu\text{m}$. It also shows that the mean trapping current increases slightly with time. In another test we purposely switch off the cooling

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fan which resulted in heating the water in the cuvette by the coil below (Fig 7.2.5). The temperature was measured with a thermocouple placed into the liquid at 1 minute intervals. It shows that after the system is stabilized the trapping current is following the temperature increase. This may be explained by the isothermal expansion of the MMB.

From ideal gas law the gas volume V and temperature T are proportional. When the bubble is stabilized, buoyancy and magnetic force are balanced. From Eq. (7.1.6) the vertical magnetic field strength, at a given distance to coil tip z_0 , is related to current by $B(I, z_0) \propto \sqrt{I}$. Together with Eq. (7.1.5) we can determine the magnetic force and current relation:

$$F_{mag,z} \propto |B_z| \frac{\partial |B|}{\partial z} \propto I \quad . \quad (7.2.1)$$

The condition for a stationary MMB in the trap is that the downward magnetic force balances buoyancy. Hence the current required to keep the MMB stationary, in response to temperature change, is obtained by:

$$I \propto F_{mag,z} = -F_{buoyancy} \propto V \propto T \quad . \quad (7.2.2)$$

This proportionality is shown in the inset of Fig 7.2.5 by plotting I against T . If the proportionality holds the trend should follow a straight line, which is the case at the later stage. The deviation at the initial stage is presumably due to the time required for liquid temperature to reach equilibrium.

7.3. Conclusion

In conclusion, we have realized a magnetic bubble trap for MMBs. The balance between vertical magnetic force and buoyancy is unstable. To maintain a stable confinement, a feedback loop is required to dynamically adjust the applied current. Our magnetic trap shows robust confinement in three-dimension over long period of time. The typical

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Figure 7.2.4.: The bubble position and corresponding output trapping current to hold the bubble in place for 16 minutes.

Figure 7.2.5.: The output current (blue) to stabilize a MMB in trap when the water temperature (red) is raising. Note that the MMB is translated to the bulk liquid during the first minute. Inset: Current vs. temperature (blue) to illustrate the proportionality of 7.2.2. The red line shows a linear fit to the latter part of the curve.

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fluctuations are about $10\text{ }\mu\text{m}$ and limited by pixel resolution. In long trapping time the applied current is sensitive to temperature change in liquid.

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Conclusion for Part II

In this chapter, we proposed and characterized a MMB system that allows controlled NPs release without rupturing the bubbles.

We started by developing a fabrication protocol based on emulsification of magnetic NPs dispersion. The product size distribution can be controlled by adjusting the concentration of magnetic NPs and the stirring speed and time. The resultant diameter ranges between ~ 500 nm and ~ 200 μ m. MMBs can be functionalized by incorporating NP capsules on the magnetic shell. With this protocol we have fabricated MMBs with fluorescent and drug encapsulated NPs.

Despite a dense shell of NPs coating on their surface, these MMBs maintain their flexibility and oscillate under acoustic driving. When the oscillation amplitude of MMBs is high enough, the NPs detach from their shell. The detached NPs are then transported away by the oscillatory flow field induced by the MMBs. We model their trajectory by considering the Stokes' drag acting on NPs. A numerical solver calculates the net motion of NPs and shows good agreement with experimental results.

This detachment process can be employed as a controlled release technique. With low amplitude driving at the resonance frequency of MMBs, continuous streams of NPs are detached without rupturing the MMBs. This is in contrast to other drug delivery systems, at which drug release is triggered by rupturing microbubbles with high amplitude ultrasound. Their rapid collapse usually is associated with damage to surrounding tissues. By driving the oscillation at resonance frequency, the ultrasound amplitude required for

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drug release is much lower. As such the cell damage can be minimized.

As a proof of concept, NPs containing a chemotherapeutic drug, Doxorubicin (Dox), is coated on MMBs. By applying ultrasound the NPs detached and penetrated into hydrogel to reach the colorectal cancer cells (RKO) beneath. This penetration of NPs is not possible by diffusion alone. The Dox-PLGA NPs were found inside the cellular cytoplasm and significantly decreased the viability of cancer cells.

Finally, we realized a magnetic trap for MMBs. A feedback loop with real time image process controls the current applied to an electromagnet. As such the buoyancy is balanced by the magnetic force and any perturbation is compensated by the feedback loop. Once the MMBs are trapped they can be stabilized for long periods of time with position fluctuation of $\sim 10\ \mu\text{m}$. Translating the trapped bubble is also possible. This setup is a prototype for precise manipulation of MMBs in three dimensional space. Together with their acoustic response and controlled-release ability, they can be developed as a targeted sonoporation toolbox for *in vitro* cultured cells in future works.

APPENDIX

Derivation of Evanescent Wave Penetration Depth

Refraction of light at the interface of two materials with different optical density is governed by Snell's law: $\sin \theta_T = \frac{n_I}{n_T} \sin \theta_I$, where n is the refractive index and θ is the angle to norm, I and T indicate the incident and transmitted light, respectively. In the case when $n_I > n_T$, total internal reflection (TIR) occurs when $\theta_I > \theta_{cri}$, where $\frac{n_I}{n_T} \sin \theta_{cri} = 1 = \sin 90^\circ$. Hence in TIR $\sin \theta_T > 1$ and there is no real solution for θ_T .

We can however consider this problem using electromagnetic waves. The transmitted wave can be described as a plane wave

$$E_T(\vec{r}, t) = E_0 e^{i(\vec{k}_T \cdot \vec{r} - \omega t)} \quad , \quad (\text{A.0.1})$$

where the wave vector $\vec{k}_T$ can be decomposed parallel ($\hat{x}$) and normal ($\hat{z}$) to the interface:

$$\vec{k}_T = k_T \sin(\theta_T) \hat{x} + k_T \cos(\theta_T) \hat{z} \quad . \quad (\text{A.0.2})$$

We shall regard the exponential function in Eq. (A.0.1) as power series. Then we can express $\cos \theta_T$ as an imaginary number

$$\cos \theta_T = \sqrt{1 - \sin^2 \theta_T} = i \sqrt{\sin^2 \theta_T - 1} \quad . \quad (\text{A.0.3})$$

A. Derivation of Evanescent Wave Penetration Depth

Hence we can obtain the wave vector and wave function as

$$\vec{k}_T = k_T \sin(\theta_T) \hat{x} + k_T i \sqrt{\sin^2 \theta_T - 1} \hat{z} \quad \text{and} \quad (\text{A.0.4})$$

$$E_T(\vec{r}, t) = E_0 e^{-z k_T \sqrt{\sin^2 \theta_T - 1}} e^{i(x k_T \sin \theta_T - \omega t)} \quad . \quad (\text{A.0.5})$$

This expression can be rewritten in terms of θ_I, n_1, n_2 as

$$E_T(\vec{r}, t) = E_0 e^{-\kappa z} e^{i(k_x x - \omega t)} \quad , \quad (\text{A.0.6})$$

where $\kappa = \frac{\omega}{c} \sqrt{(n_I \sin \theta_I)^2 - n_T^2}$ and $k_x = \frac{\omega n_I}{c} \sin(\theta_I)$.

This solution shows that even in TIR, electromagnetic wave exists in the low optical density region. This wave propagates in the direction of the phase velocity $\vec{v}_p = \frac{\omega}{|\vec{k}|} \frac{\vec{k}}{|\vec{k}|}$. From the expression of k_x and $k_z = 0$, we have $v_p^x = \frac{n_I}{c} \sin(\theta_I)$ and $v_p^z = 0$. The direction of the Poynting vector follows the group velocity $v_g = \frac{\partial \omega}{\partial \vec{k}} \frac{\partial \vec{k}}{\partial \vec{k}}$ which yields $v_g^x = \frac{n_I}{c} \sin(\theta_I) = v_p^x$ and $v_g^z = 0$. Hence the light outside the TIR region propagates non-dispersively along the interface. Normal to the interface there is no energy transfer and the intensity decays exponentially with distance from the interface. This profile is govern by the characteristic penetration depth[1]

$$z_0 = \frac{1}{\kappa} = \frac{\lambda}{2\pi \sqrt{(n_I \sin \theta_I)^2 - n_T^2}} \quad , \quad (\text{A.0.7})$$

which depends on the system parameters λ, n_I, n_T , and the angle of incidence θ_I .

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