On the interaction of Waves carrying Light, Sound and small Particles

Wave-based methods for miniature laboratories and
fast optical sensing

Jorick van 't Oever

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Cover picture: The colors of the waves are based on the painting 'Prague Castle' by Wilma van 't Oever

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ON THE INTERACTION OF WAVES CARRYING LIGHT,
SOUND AND SMALL PARTICLES
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FAST OPTICAL SENSING

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Introduction to this thesis

As water covers 71 percent of the Earth's surface, one could conclude that the name of our planet is a misnomer. The Water Planet¹ would be an excellent alternative with Planet Rock coming in at second place. Water is absolutely vital for all life on Earth [1] and is also expected to be essential for any life we encounter elsewhere [1, 2]. Early humans, like other animals, used naturally occurring sources of water. The Neolithic Revolution (starting around 10,000 BC) in the Levant and Mesopotamia led to a transition from a lifestyle of hunting and gathering to agriculture and settlement, including large-scale modification of the natural water environment [3, 4].

Irrigation and deforestation allowed for a surplus production of food enabling large settlements and trade [3]. To produce irrigation and drinking water from ground water humans engineered kilometer long tunnel-like structures, called qanats, around 700 BC. The importance of drinking water quality was recognized early. The Greek physician Hippocrates recommended boiling and straining water by the use of a 'Hippocrates Sleeve' around 400 BC. A manuscript written in Sanskrit from 200 BC mentions that "It is good to keep water in copper vessels, expose it to sunlight, and filter it through charcoal." [5]. It would then take more than 2000 years before we understood that infectious diseases are caused by microorganisms such as bacteria [6, 7]. Until the end of the Middle Ages, there was no significant improvement in sanitation which, combined with dense population in cities, caused epidemic outbreaks of water-borne diseases [8].

The microbiological quality of drinking water is still a relevant issue in the modern age everywhere in the world [9]. In underdeveloped countries problems of low drinking water quality are often coupled with inadequate sanitation and hygiene. Drinking water sources are often not protected or separated from excremental waste water. In 2012, unsafe drinking water sources and bad sanitation led to an estimated 685,000 deaths caused by diarrhea [10]. These deaths are preventable by improving the water sources, sanitation and

1. Oceania would be nice too, but that is already claimed by Australia et alii.

personal hygiene [11]. Even though the sanitation is better in the developed world, drinking-water disease outbreaks still occur frequently [12]. For instance, such outbreaks occurred on average roughly once per month in the USA in the period from 2001 to 2006 [13]. In all cases worldwide, timely and accurate detection of pathogens in drinking water is essential for prevention and mitigation of outbreaks.

The detection of microbial pathogens in drinking water typically involves three steps: 1) concentration or enrichment, 2) purification and separation and 3) characterization by analysis [14]. Often the concentration of pathogens in drinking water is so low that an initial concentration step is needed to increase the concentration to a practical level. Typical concentration methods also concentrate non-pathogenic microbes and other water constituents. A subsequent purification and separation step then separates the target pathogens from other material. These two steps need to be repeated if the concentration or purity of the resulting concentrated sample is still too low for practical detection. The final analysis step detects and identifies the pathogens in the final sample.

The primary method for pathogen detection in the Netherlands is based on microbial culturing [15]. A sample of typically 100 ml of water is filtered through a membrane with pores smaller than the target pathogens, such that these are retained on the membrane. Then the membrane is placed onto a plate with culture medium which, for bacteria, typically consists of agarose gel and other nutrients. Additional chemicals can be added to specifically favor or disfavor growth of pathogens. If the medium is favorable, a single bacterium in the sample can be amplified exponentially into visible colonies. Culturing as method is not without downsides. Firstly, it takes between 16 and 44 hours for the amplification to be sufficient. The time between sampling and the actual detection of a microbiological hazard is therefore so long that the water might already have been consumed. Secondly, different pathogens require different conditions before commencing multiplication². In the case of endospores, both availability of correct nutrients and activation by heat is required before germination starts, making culturing of sporulated bacteria a specialized and time consuming technique. Some bacteria found in drinking water are nonculturable [17]. Lastly, culturing requires a microbiological laboratory, specialized personnel and material and transport of the samples to the lab, all adding to the detection time and cost. A decentralized system con-

-
2. Viruses require host cells. Sporulating bacteria can be in endospore form, a dormant state which protects the bacterium from heat, freezing, desiccation, cleaning chemicals and ultra-violet radiation. Bacterial spores can remain dormant for a very long time. Scientists revived *Bacillus sphericus* endospores from the guts of a fossilized bee, which was about 25 million years old [16].

taining many low-cost, automated mini-laboratories, placed at critical steps during drinking water production and delivery would be a great step forward.

By scaling down essential laboratory operations to the nano and micro-liter scale it becomes possible to build a Laboratory on a Chip (LoC), combining microfluidical, electronical and microelectromechanical systems. Such systems can have advantages over regular laboratory systems, depending on the application. Typical advantages are low cost due to mass production, usage of very small sample and reagent volumes, shorter analysis times due to short diffusion distances and small device footprint [18]. A LoC for early detection of pathogens for in-line testing (continuous sampling and analysis) of drinking water could consist of a multi-step process: concentration steps which increases the concentration of pathogens from the sampled volume, separation steps increasing the purity and a final analysis step for pathogen detection and identification. The concentration reduces analysis time being spent on analyzing clean water without pathogens, thereby increasing the available measurement time and improving the signal-to-noise ratio. The work described in this thesis focuses on a specific technology for the concentration steps. While research on separation, detection and analysis of waterborne pathogens is an interesting and active field [19,20], it did not become part of this thesis due to time constraints.

The main topic of this thesis is *acoustophoresis* as a concentration method for use in in-line LoC systems. The literal meaning of acoustophoresis is ‘the act of carrying by sound’. Particles in a fluid experience a force due to scattering of acoustic pressure waves, leading to particle trapping, guiding or concentration. From the perspective of pathogen concentration in drinking water, acoustophoresis is an interesting method compared with technologies primarily used in conventional labs, which are centrifugation, filtration and chemical precipitation [14]. Sedimentation by centrifugation is batch-based and hard to scale down to the microscale. Filtration using microporous membranes suffers from fouling and clogging, necessitating periodic maintenance and replacements. Chemical precipitation methods use added chemicals to induce aggregation of the target pathogens, easing concentration and detection. However, a broad spectrum of targets requires multiple types of chemicals, leading to increased chip complexity and again periodic maintenance (refilling) is required.

Acoustophoresis has a few properties which make it an interesting alternative for application in an in-line Lab-on-a-Chip for particle concentration from water. Firstly, depending on the geometry and other parameters, the acoustic force field can be effective in the whole volume of a microchannel, making it a bulk force. Some other methods are only active close to a surface (such as surface acoustic waves) or within a localized region (like with op-

tical or acoustic tweezers). Secondly, the force acting on suspended particles is carried by sound, which makes it a non-contact method. Often, the force field can be chosen such that the particles are concentrated towards the center of the channel. By keeping particles (such as bacteria) clear from the channel walls clogging and fouling is reduced. This is a clear advantage compared with methods with a relatively large contact area, such as filter-based methods and deterministic ratchets. In the latter method, suspended particles are repeatedly displaced into one direction by a periodic pattern of pillars placed in the channel. The third advantage of acoustophoresis is that it does not affect the viability of pathogens [21, 22] which can be important depending on the analysis method used [14].

Many other research topics emerged during my research into the application of acoustophoresis for particle concentration, all of which are to some degree related to the main topic. This thesis is divided into two parts to logically group the various subtopics.

In the first part I discuss acoustics and microfluidics related work. **Chapter 1** introduces the theoretical background of the physics of acoustic waves in microfluidic systems. First I discuss microfluidic flows, acoustic waves and resonances, acoustically induced flow called acoustic streaming, followed by a discussion of the forces on particles in an acoustic resonance.

In **Chapter 2** I dive into the topic of ultrasonic acoustophoresis of small plastic particles in water. After the discussion of the literature, I show experiments which reveal the lower size limit of acoustophoresis. In the last section I discuss numerical simulations which reveal a concentration mode using acoustic streaming which effectively lowers the size limit towards submicrometer-sized particles.

Chapter 3 is the last chapter of this part of the thesis. As I will show in the first chapter, mixing of liquids presents a challenge in microfluidic systems because the confinement forces flow to be laminar. As mixing is a useful function in a LoC, development of embeddable micromixers is important. In this chapter I show how a centimeter scale acoustic micromixer consisting of 3D microstructures is created using Direct Laser Writing. Pre-alignment is essential to achieve good balance between fabrication time, accuracy and structural stability when fabricating microstructures in an existing microchannel.

Most characterization methods for acoustic resonances in microfluidic systems are based on acoustophoretic measurement of suspended particles. In the first part of this thesis I will show that the strength of acoustophoretic concentration strongly reduces for small particles. Therefore the acoustophoretic characterization methods become unusable when using small particles or low particle concentrations.

In the second part of this thesis I focus on a particle-less, interferomet-

ric imaging technique which was developed for the measurement of acoustic resonances in microfluidic systems. Theoretical background is provided in **Chapter 4**, starting with a discussion on statistical optics and partial coherence, followed by the functioning of the Michelson interferometer and stroboscopic illumination.

Acoustic resonances are used in many LoC functions such as acoustophoresis and acoustic mixing. Measuring and visualizing the acoustic field inside a closed microchannel is useful for both research and engineering purposes but is challenging due to the closed system. In **Chapter 5** I present a stroboscopic imaging Michelson interferometer for imaging acoustic resonances in a water-filled microchannel. The method is relatively easy to apply and directly measures the standing pressure wave. The local acoustic pressure influences the local water density and thus also the refractive index. The acoustic field, both amplitude and phase, is determined by measuring the acoustically induced optical delay using stroboscopic, spatially incoherent illumination.

Finally, in **Chapter 6** I show a spectral version of same imaging technique applied to a different problem: measurement of micro-cracks in rail road tracks. The height profile of a rail surface is measured using a single optical pulse using a spectral white-light interferometer, which allows single shot detection of fatigue induced-microcracks. This method is comparable with Optical Coherence Tomography, but then applied to a non-transparent sample.

Part I

Acoustics & Microfluidics

1 | Theoretical background

The purpose of this chapter is to give the reader theoretical background of acoustophoresis in microchannels. First we discuss the governing equations, which express conservation of mass and momentum in a fluid. By nondimensionalization we show that microfluidic flow is typically laminar, which has important consequences for applications. Secondly we discuss thermoviscous acoustic theory, which is used to calculate acoustophoretic forces. Finally we look at how the acoustic fields and forces scale with excitation frequency and particle size.

1.1 Microfluidic flow in a Lab-on-a-Chip

Lab-on-a-chip (LoC) microfluidics is a developing field and has grown tremendously during the last decades [23]. Lab-on-a-Chip systems aim at integrating standard laboratory operations, such as particle separation [24], cell sorting [25], particle trapping [26], droplet manipulation [27], biological assays [28] and mixing [29] into a single microchip using very small sample and reagent volumes compared with traditional methods. Other attractive capabilities are: small footprints; short analysis times combined with high sensitivity, and low fabrication and operation costs [18]. The dimensions of the fluid channels in such a system are therefore typically on the order of tens of micrometers and the manipulation of fluids on this scale is called *microfluidics*. The scale of containment, in literature ranging from tens of nanometers up to millimeters, has pronounced effects on the way fluids behave. To see this we must first derive the Navier-Stokes equation. While this is standard textbook material, the derivation gives insight into underlying physics of microfluidic flow and is therefore a great starting point. We will use parts of the more detailed derivation given in the textbook on theoretical microfluidics written by Bruus [30].

The behavior of a fluid is governed by the continuity of mass, momentum and energy (the latter we will discuss in the next section). Conservation of mass implies that the rate of change of density ρ [kg m^{-3}] in a fixed, infinitesimal volume equals the influx of mass current density $\rho \mathbf{v}$,

$$\partial_t \rho = -\nabla \cdot [\rho \mathbf{v}] \quad (1.1)$$

with $\mathbf{v}$ [m s^{-1}] the velocity field. Likewise, conservation of momentum dictates that the rate of change of momentum equals the stress forces acting on the surface of the test volume and the influx of momentum current density $\rho \mathbf{v} \mathbf{v}$,

$$\partial_t (\rho \mathbf{v}) = -\nabla \cdot [\rho \mathbf{v} \mathbf{v} - \boldsymbol{\sigma}] \quad (1.2)$$

with $\boldsymbol{\sigma}$ the stress tensor, which for an isotropic and viscous fluid is given as

$$\boldsymbol{\sigma} = \boldsymbol{\tau} - p \mathbf{1} \quad (1.3a)$$

$$\boldsymbol{\tau} = \eta [\nabla \mathbf{v} + (\nabla \mathbf{v})^T] + \left[\eta^b - \frac{2}{3} \eta \right] (\nabla \cdot \mathbf{v}) \mathbf{1} \quad (1.3b)$$

with $\boldsymbol{\tau}$ the viscous stress tensor, $\mathbf{1}$ the unit tensor, superscript T indicating tensor transposition and p [Pa] the pressure. η [Pa s] and η^b [Pa s] are the dynamic and bulk viscosity of the fluid, measures for the resistance to shearing and uniform compression, respectively.

Next we shortly discuss some basic properties of tensors which are useful for manipulation and evaluation of some of the encountered equations. For simplicity we do this for two dimensions, extension to the three dimensional case is straightforward. A dyadic tensor, such as the the momentum current density, can be constructed from two column vectors $\mathbf{a}$ and $\mathbf{b}$ using the dyadic tensor product:

$$\mathbf{ab} = \mathbf{a} \otimes \mathbf{b} = \mathbf{ab}^T = \begin{pmatrix} a_x b_x & a_x b_y \\ a_y b_x & a_y b_y \end{pmatrix} \quad (1.4)$$

where the subscripts x, y indicate the x and y components respectively. The divergence of a dyadic tensor can be written in terms of its constituting vectors using the identity

$$\nabla \cdot (\mathbf{ab}) = \mathbf{a} \cdot \nabla \mathbf{b} + \mathbf{b}(\nabla \cdot \mathbf{a}). \quad (1.5)$$

The divergence of a scalar field φ times a tensor $\mathbf{A}$ is

$$\nabla \cdot (\varphi \mathbf{A}) = \nabla \varphi \cdot \mathbf{A} + \varphi \nabla \cdot \mathbf{A} \quad (1.6)$$

and the divergence of a symmetric tensor $\mathbf{S}$ can be written as a row-wise divergence

$$\operatorname{div} \mathbf{S} = \nabla \cdot \mathbf{S}^T = \nabla \cdot \mathbf{S} = \begin{pmatrix} \nabla \cdot [S_{xx}, S_{xy}] \\ \nabla \cdot [S_{yx}, S_{yy}] \end{pmatrix} = \begin{pmatrix} \partial_x S_{xx} + \partial_y S_{xy} \\ \partial_x S_{yx} + \partial_y S_{yy} \end{pmatrix} \quad (1.7)$$

with $\partial_{x,y,t}$ meaning the partial derivative with respect to the variable indicated.

We can simplify the momentum continuity equation (eqn. 1.2) a bit by expanding and using above identities, resulting in

$$(\partial_t \rho) \mathbf{v} + \rho \partial_t \mathbf{v} = -\mathbf{v} \nabla \cdot [\rho \mathbf{v}] - \rho \mathbf{v} \cdot \nabla \mathbf{v} - \nabla \cdot [p \mathbf{I}] + \nabla \cdot \boldsymbol{\tau}. \quad (1.8)$$

The first term on the left-hand side cancels with the first term on the right-hand side due to the conservation of mass (eqn. 1.1). Next we will assume a Newtonian fluid, for which the dynamic and bulk viscosities are assumed to be constant and are taken out from the spatial derivatives in the viscous stress tensor. Taking the divergence of the viscous stress tensor term-by-term results in

$$\nabla \cdot \boldsymbol{\tau} = \eta \nabla^2 \mathbf{v} + \eta \nabla (\nabla \cdot \mathbf{v}) + \eta^b \nabla (\nabla \cdot \mathbf{v}) - 2/3 \eta \nabla (\nabla \cdot \mathbf{v}) \quad (1.9)$$

and together with equation eqn. 1.8 we find the famous Navier-Stokes equation for a Newtonian fluid:

$$\rho [\partial_t \mathbf{v} + (\mathbf{v} \cdot \nabla) \mathbf{v}] = -\nabla p + \eta \nabla^2 \mathbf{v} + \left[\eta^b + 1/3 \eta \right] \nabla (\nabla \cdot \mathbf{v}). \quad (1.10)$$

On the left-hand side we recognize inertial terms containing the mass density times accelerations and on the right-hand side we have external and viscous (internal friction) force densities. We can gain some insight about the relative importance of these terms when we make the Navier-Stokes equation dimensionless using characteristic scales. This means we divide the spatial variables, time, velocity and pressure each by a characteristic scale which is typical for a particular system, resulting in dimensionless quantities indicated with a tilde. In this case we choose a characteristic length and velocity scale l_c and v_c , such that $x = l_c \tilde{x}$ and $\mathbf{v} = v_c \tilde{\mathbf{v}}$ and the scales for time and pressure become $t = l_c/v_c \tilde{t}$ and $p = \eta v_c/l_c \tilde{p}$. The dimensionless Navier-Stokes is found by inserting the scaled variables, leading to

$$\begin{aligned} \rho \left[\frac{v_c}{t_c} \partial_{\tilde{t}} \tilde{\mathbf{v}} + \frac{v_c^2}{l_c} (\tilde{\mathbf{v}} \cdot \tilde{\nabla}) \tilde{\mathbf{v}} \right] = \\ - \frac{p_c}{l_c} \tilde{\nabla} \tilde{p} + \frac{\eta v_c}{l_c^2} \tilde{\nabla}^2 \tilde{\mathbf{v}} + \left[\eta^b/\eta + 1/3 \right] \frac{\eta v_c}{l_c^2} \tilde{\nabla} (\tilde{\nabla} \cdot \tilde{\mathbf{v}}) \end{aligned} \quad (1.11)$$

which becomes

$$Re \left[\tilde{\partial}_t \tilde{\mathbf{v}} + \left(\tilde{\mathbf{v}} \cdot \tilde{\nabla} \right) \tilde{\mathbf{v}} \right] = -\tilde{\nabla} \tilde{p} + \tilde{\nabla}^2 \tilde{\mathbf{v}} + \left[\eta^b / \eta + 1/3 \right] \tilde{\nabla} \left(\tilde{\nabla} \cdot \tilde{\mathbf{v}} \right). \quad (1.12)$$

The numerical prefactor of each term on the right-hand side is of the order of unity, while the prefactor on the left is the dimensionless Reynolds number¹,

$$Re = \frac{\rho_l v_c}{\eta}. \quad (1.13)$$

We can see that in case of $Re \gg 1$ the inertial terms are dominant while for $Re \ll 1$ the viscous terms are most important. The characteristic length in a channel is the hydraulic diameter, which is defined as $D_H = 4A/P$ with A and P being the area of the cross-section and the wetted perimeter, respectively. For a typical microfluidic system consisting of a water-filled microchannel with a rectangular cross-section of $380 \mu\text{m} \times 160 \mu\text{m}$, $D_H = 2wh/(w+h)$. Combined with a typical flow speed of $100 \mu\text{m s}^{-1}$ the Reynolds number is $Re \approx 0.05 \ll 1$. This is an important result because it means that microfluidic flows typically are *laminar* flows, such that the fluid flows in parallel layers with very little disruption. This in turn has two mayor consequences: 1) a laminar flow provides a high amount of control over the flow and suspended microparticles and is therefore very suitable for certain Lab-on-a-Chip operations such as manipulation of suspended particles; and 2) as opposed to a turbulent flow there is no intrinsic way to make the fluid mix itself, which poses a challenge if one would like to implement a mixing function in a Lab-on-a-Chip.

1.2 Thermoviscous acoustics

In the previous section we derived the Navier-Stokes equation for a Newtonian fluid and we showed that, generally speaking, a microfluidic system operates in a laminar flow regime. Typically one can then simplify the problem by assuming a non-compressible fluid, i.e. the divergence of the velocity is always zero. However, in an acoustical problem we describe sound as a wave that propagates by compression and decompression of the medium and therefore the non-compressible approximation is not appropriate. Furthermore, for the thermoviscous acoustic problem which we will discuss shortly, we will also include the temperature dependence of the viscosities, making them spatially varying. The starting points for the acoustic theory are the

1. The Reynolds number Re should not be confused with the real part operator, $\text{Re}\{a + bi\} = a$.

continuity of mass (eqn. 1.1) and momentum (eqn. 1.2) equations. The typical acoustical system which we would like to describe is illustrated in figure 1.1. The geometry consists of a rectangular microchannel filled with water and an acoustic resonance is excited in the horizontal direction. Two acoustic forces act on suspended microparticles: an acoustic radiation force due to scattering of the acoustic wave from the particle and a streaming-induced drag force due to steady flow generated by the resonance. Particles become concentrated if the radiation force is dominant (figure 1.1a) and particles will follow the streaming rolls in case of a dominant streaming drag force (figure 1.1b). The forces depend on the acoustic fields which we will calculate using thermoviscous acoustic theory.

The thermoviscous acoustic description of a Newtonian fluid is governed by the continuity of mass, momentum and energy equations, combined with thermodynamic relations, i.e. the equations of state, relating the independent variables, temperature T [K] and pressure p to the dependent variables, mass density ρ , internal energy per unit mass ϵ [J kg⁻¹] and entropy per unit mass s [J K⁻¹ kg⁻¹]. The full derivation and additional details can be found in the cited paper by Muller and Bruus [31]. We will first discuss the governing equations for single acoustic resonances (excited at a single frequency). Next we will use a perturbation expansion of the variables around their equilibrium state, e.g. $T = T_0 + T_1 + T_2$ with T_0 the equilibrium state temperature and T_1 and T_2 the first-order and second-order terms which express small changes away from equilibrium. Using the perturbation expansion we find the first and second-order equations which can be solved to find the acoustic fields for a single resonance. Finally we show that the acoustic fields for single resonances can be added in a linear superposition to find the fields in case of multiple simultaneously excited modes (at different frequencies). The acoustic radiation force and Stokes drag force are then calculated from the fields.

Like before, the conservation of energy implies that the rate of change of the sum of the internal and kinetic energy density equals the inflow of power by stress forces working on the surface of the test volume, heat conduction power and energy current density,

$$\partial_t (\rho\epsilon + \frac{1}{2}\rho v^2) = -\nabla \cdot [-\mathbf{v} \cdot \boldsymbol{\sigma} - k_{th} \nabla T + \rho (\epsilon + \frac{1}{2}v^2) \mathbf{v}] \quad (1.14)$$

with k_{th} [W m⁻¹ K⁻¹] the thermal conductivity. It is preferable to express the dependent variables (ρ , ϵ and s) in terms of the independent variables (T and p) to simplify the equations. Direct substitution requires an equation of state of water, for which an analytical expression is only known for certain ranges of parameters. However, small changes in the dependent variables can be

(a) Microparticles are concentrated in the vertical center of the microchannel by the acoustic radiation force (black arrows). The value of the acoustic pressure p_1 at a certain time is illustrated with the black curve, showing a half-wavelength resonance. The pressure is high at the sides and has a node in the middle of the channel. The dashed curve shows the pressure half a period later.

(b) The acoustic resonance also leads to steady streaming flow, so-called Rayleigh-Schlichting streaming, which is excited in the viscous boundary layers (dashed arrows) at solid walls due to absorption of acoustic energy. The particles do not become concentrated.

Figure 1.1: Illustration of the cross-section of a microchannel filled with water and suspended small particles. The fundamental horizontal acoustic resonance is excited in the water. Depending on particle size and other variables, particles are either (a) concentrated into a vertical region due to the acoustic radiation force, or (b) the particles are following the streaming flow.

expressed as small changes in the independent variables using the following thermodynamic relations [31]:

$$Tds = C_p dT - \frac{\alpha_p T}{\rho} dp, \quad (1.15a)$$

$$\frac{1}{\rho} d\rho = \kappa_T dp - \alpha_p dT, \quad (1.15b)$$

$$\rho d\epsilon = (C_p \rho - \alpha_p p) dT + (\kappa_T p - \alpha_p T) dp, \quad (1.15c)$$

where the temperature and density are evaluated at the equilibrium state, i.e. $T = T_0$ and $\rho = \rho_0$. Equations 1.15 can be used to express all equations in terms of T and p . In the perturbation expansion the acoustic perturbations then enter through the small deviations from equilibrium, i.e. $dT = T - T_0$. The thermodynamic coefficients are the isobaric heat capacity per unit mass C_p [$\text{J kg}^{-1} \text{K}^{-1}$], isothermal compressibility κ_T [Pa^{-1}] and isobaric thermal expansion coefficient α_p [K^{-1}], which are defined as follows:

$$C_p = T \left(\frac{\partial s}{\partial T} \right)_p, \quad (1.16a)$$

$$\kappa_T = \frac{1}{\rho} \left(\frac{\partial \rho}{\partial p} \right)_T, \quad (1.16b)$$

$$\alpha_p = -\frac{1}{\rho} \left(\frac{\partial \rho}{\partial T} \right)_p. \quad (1.16c)$$

Finally, we express the temperature and density dependence of the shear viscosity η as

$$\eta(T, \rho) = \eta_0(T_0, \rho_0) + \left(\frac{\partial \eta}{\partial T} \right) (T - T_0) + \left(\frac{\partial \eta}{\partial \rho} \right) (\rho - \rho_0) \quad (1.17a)$$

and the temperature dependence of the bulk viscosity η^b is included as

$$\eta^b(T, \rho) = \eta_0^b(T_0, \rho_0) + \left(\frac{\partial \eta^b}{\partial T} \right) (T - T_0). \quad (1.18a)$$

The partial derivatives with respect to temperature and pressure are evaluated at the equilibrium temperature and pressure, respectively.

See table 1.1 for numerical values of the relevant parameters and coefficients.

1.2.1 Perturbation expansions

It is not straight-forward to find an analytic solution to the total thermoviscous acoustic problem. The continuity equations (eqn. 1.1, 1.2 and 1.14) are

<i>General parameters</i>			
Ambient temperature	T_0	25	°C
Ambient pressure	p_{amb}	1.013×10^5	Pa
<i>Pure water</i>			
Mass density ^a	ρ_0	9.971×10^2	kg m ⁻³
Shear viscosity ^b	η_0	8.900×10^{-4}	Pa s
Bulk viscosity ^c	η_0^b	2.485×10^{-3}	Pa s
Heat capacity ^a	C_p	4.181×10^3	J kg ⁻¹ K ⁻¹
Heat capacity ratio ^a	γ	1.011	
Thermal expansion ^b	α_p	2.573×10^{-4}	K ⁻¹
Thermal conductivity ^d	k_{th}	6.065×10^{-1}	W m ⁻¹ K ⁻¹
Isothermal compressibility ^a	κ_T	4.525×10^{-10}	Pa ⁻¹
Speed of sound ^a	c_s	1.497×10^3	m s ⁻¹
Thermal sensitivity of η ^(b)	$\frac{1}{\eta_0} \frac{\partial \eta}{\partial T}$	-2.278×10^{-2}	K ⁻¹
Density sensitivity of η ^(a)	$\frac{1}{\eta_0} \frac{\partial \eta}{\partial \rho}$	-3.472×10^{-4}	kg ⁻¹ m ³
Thermal sensitivity of η ^{b (c)}	$\frac{1}{\eta_0^b} \frac{\partial \eta^b}{\partial T}$	-2.584×10^{-2}	K ⁻¹
<i>Polystyrene particles</i>			
Mass density [32]	ρ_{ps}	1.050×10^3	kg m ⁻³
Poisson's ratio [33]	σ_{ps}	0.35	
Heat capacity [34]	$C_{p,ps}$	1.22×10^3	J kg ⁻¹ K ⁻¹
Heat capacity ratio [35]	γ_{ps}	1.04	
Thermal expansion [35]	$\alpha_{p,ps}$	2.09×10^{-4}	K ⁻¹
Thermal conductivity [36]	$k_{th,ps}$	1.40×10^{-1}	W m ⁻¹ K ⁻¹
Isentropic compressibility ^e	$\kappa_{s,ps}$	2.38×10^{-10}	Pa ⁻¹
Speed of sound [37]	$c_{s,ps}$	2.350×10^3	m s ⁻¹
Transverse speed of sound ^f	$c_{t,ps}$	1.068×10^3	m s ⁻¹

^a From polynomial fit from ref. 31, based on data from ref. 38

^b From polynomial fit from ref. 31, based on data from ref. 39

^c From polynomial fit from ref. 31, based on data from ref. 40

^d From polynomial fit from ref. 31, based on data from ref. 41

^e Calculated as $\kappa_{s,ps} = \frac{3(1-\sigma_{ps})}{(1+\sigma_{ps})(\rho_{ps}c_{s,ps}^2)}$ from ref. 42

^f Calculated as $c_{t,ps} = \left[\frac{3}{4} \left(c_{s,ps}^2 - \frac{1}{\rho_{ps}\kappa_{s,ps}} \right) \right]^{1/2}$ from ref. 35

Table 1.1: Parameter values used for pure water and polystyrene micro-particles.

instead numerically solved using a perturbation expansion, which is a mathematical method for linearization of a nonlinear problem. Consider a partial differential equation $\mathcal{D}(g) = 0$ with $\mathcal{D}(g)$ a differential operator acting on a field g , which could be for example the velocity $\mathbf{v}$ or density ρ field. We would like to find the solution for our field g . The differential operator $\mathcal{D}$ may contain time and space derivatives and might even be nonlinear, so finding a solution directly is not straight-forward. By using a perturbation expansion we write the field as $g = g_0 + g_1 + g_2$, where g_0 is assumed to be the homogeneous (spatially constant), isotropic (the same in all directions) and quiescent (temporally constant) state. g_0 , g_1 and g_2 are respectively called the zeroth, first and second-order perturbation terms of g . Each higher perturbation term is assumed to be a small change with respect to the previous term. The expression for g is inserted into the differential equation and first all zeroth-order terms are retained and higher orders are discarded, leading to a solvable homogeneous differential equation. After finding the solution for g_0 the process is repeated for the first order, keeping only first-order terms. Some of the terms will be source terms containing the zeroth-order solution. Solving the first-order equations leads to the first-order solutions and the process is repeated for the second-order. The viscosities in terms of the perturbation series become

$$\eta = \eta_0 + \eta_1, \quad (1.19a)$$

$$\text{with } \eta_1 = \left(\frac{\partial \eta}{\partial T} \right)_{T=T_0} T_1 + \left(\frac{\partial \eta}{\partial \rho} \right)_{\rho=\rho_0} \rho_1, \quad (1.19b)$$

$$\eta^b = \eta_0^b + \eta_1^b, \quad (1.19c)$$

$$\text{with } \eta_1^b = \left(\frac{\partial \eta^b}{\partial T} \right)_{T=T_0} T_1. \quad (1.19d)$$

The zeroth-order field g_0 is often simple so we know it beforehand: in our case ρ_0 is the density before any acoustic perturbation and T_0 is the ambient temperature. There are two non-obvious choices here, namely the velocity and pressure. We will assume that there is no flow in the absence of the acoustic disturbance, $\mathbf{v}_0 = \mathbf{0}$. The zeroth-order pressure is not the ambient pressure in the fluid but rather it represents the cohesive energy density, a measure of binding energy between the molecules. When the fluid is acoustically perturbed the intermolecular bonds are stretched and compressed, so we are actually perturbing the intermolecular bond energy, and the unperturbed state is thus the density of the binding energy. For liquid water at 25 °C this is the enthalpy of vaporization $\Delta H_{vap} = 2442 \text{ kJ kg}^{-1}$ [43], leading to an energy density of 2.44 GJ m^{-3} or equivalently $p_0 = 2.44 \text{ GPa}$. Note that the pressure

only enters the governing equations through spatial derivatives and therefore the exact value of the constant p_0 is normally not of interest. However, for the validity of the perturbation expansion is it important that subsequent terms are much smaller than the previous (nonzero) terms. Typically used acoustic waves have an amplitude $p_1 \lesssim 10$ MPa and $p_1/p_0 \lesssim 4 \times 10^{-3}$.

1.2.2 First-order equations in frequency domain

The first-order equations are found by substituting the perturbation series $g = g_0 + g_1 + g_2$ for all fields and for the viscosities into the continuity equations, applying thermodynamic relations equations 1.15a to 1.15c to cast the equations in terms of the independent variables and keeping only first-order terms. The order of a term is indicated by the sum of the indices of the variables in a term, e.g. $\rho_0 v_1$ is a term of order 1.

We assume a harmonic time dependence for all first-order fields, i.e. $g_1(\mathbf{r}, t) = \text{Re}\{g_1(\mathbf{r})e^{-i\omega t}\}$. Note that generally $g_1(\mathbf{r})$ is complex, with its argument (i.e. phase) depending on the time delay with respect to the driving field. As all first-order fields have the same harmonic time dependence we transform into the frequency domain using the substitution $\partial_t \mapsto -i\omega$. The first-order mass, momentum and energy continuity equations become, respectively,

$$-i\omega\alpha_p T_1 + i\omega\kappa_T p_1 = \nabla \cdot \mathbf{v}_1 \quad (1.20)$$

$$-i\omega\rho_0 \mathbf{v}_1 = \nabla \cdot [\boldsymbol{\tau}_1 - p_1 \mathbf{1}] \quad (1.21)$$

$$-i\omega\rho_0 C_p T_1 + i\omega\alpha_p T_0 p_1 = k_{th} \nabla^2 T_1 \quad (1.22)$$

with $\boldsymbol{\tau}_1$ given as

$$\boldsymbol{\tau}_1 = \eta_0 \left[\nabla \mathbf{v}_1 + (\nabla \mathbf{v}_1)^T \right] + \left[\eta_0^b - \frac{2}{3}\eta_0 \right] (\nabla \cdot \mathbf{v}_1) \mathbf{1}. \quad (1.23)$$

The first-order equations are linear in first-order fields and therefore separate solutions calculated with different frequencies can be added linearly to obtain the solution in which these resonances are excited simultaneously. For example, the fields for two resonances at angular frequencies $\omega_1 = 2\pi f_1$ and $\omega_2 = 2\pi f_2$ are calculated separately, leading to solutions for the first-order velocity fields $\mathbf{v}_1^{\omega_1}(\mathbf{r}, t) = \text{Re}\{\mathbf{v}_1^{\omega_1}(\mathbf{r})e^{-i\omega_1 t}\}$ and $\mathbf{v}_1^{\omega_2}(\mathbf{r}, t) = \text{Re}\{\mathbf{v}_1^{\omega_2}(\mathbf{r})e^{-i\omega_2 t}\}$. The first-order velocity field resulting from simultaneous excitation of these two resonances then is simply

$$\mathbf{v}_1(\mathbf{r}, t) = \mathbf{v}_1^{\omega_1}(\mathbf{r}, t) + \mathbf{v}_1^{\omega_2}(\mathbf{r}, t). \quad (1.24)$$

1.2.3 Time-averaged second-order equations

The second-order equations are found by following the same steps as for the first-order equations while staying in the time domain and keeping only second-order terms. Because the first-order terms have a harmonic time dependence, the second-order terms which are products of two first-order variables, e.g. $\rho_1 \mathbf{v}_1$, introduce second-order terms which also have a harmonic time dependence but with either the sum or difference frequency of the two first-order terms. This can be shown using the fact that for a complex number z ,

$$\text{Re}\{z\} = \frac{1}{2}[z + z^*], \quad (1.25)$$

resulting in

$$\begin{aligned} \rho_1^{\omega_1}(\mathbf{r}, t) \mathbf{v}_1^{\omega_2}(\mathbf{r}, t) &= \text{Re}\{\rho_1^{\omega_1}(\mathbf{r})e^{-i\omega_1 t}\} \text{Re}\{\mathbf{v}_1^{\omega_2}(\mathbf{r})e^{-i\omega_2 t}\} \\ &= \frac{1}{2} \text{Re}\{\rho_1^{\omega_1} \mathbf{v}_1^{\omega_2} e^{-i(\omega_1 + \omega_2)t}\} + \frac{1}{2} \text{Re}\{\rho_1^{\omega_1} \mathbf{v}_1^{\omega_2*} e^{-i(\omega_1 - \omega_2)t}\} \end{aligned} \quad (1.26)$$

where we have dropped the explicit spatial dependence on the right hand side for brevity and where the asterisk $*$ denotes complex conjugation. Note that if ω_1 equals ω_2 , equation 1.26 reduces to the well known result of a steady (time independent) term and an unsteady, oscillating term with doubled frequency.

In typical acoustophoretic microfluidic experiments only the steady components are observed because the oscillating fields are too fast to be resolved and therefore we take the time average of the second-order equations, resulting in time-averaged second-order variables. The time-averaging operation is indicated as $\langle g \rangle$ and averages over one oscillation period. As all calculated second-order variables are inherently time-averaged they are indicated without the angled brackets. The time-averaged second-order continuity equations for mass and momentum become respectively

$$\nabla \cdot [\rho_0 \mathbf{v}_2 + \langle \rho_1 \mathbf{v}_1 \rangle] = 0 \quad (1.27)$$

$$\nabla \cdot [\boldsymbol{\tau}_2 - p_2 \mathbf{1} - \rho_0 \langle \mathbf{v}_1 \mathbf{v}_1 \rangle] = \mathbf{0} \quad (1.28)$$

with $\boldsymbol{\tau}_2$ given as

$$\begin{aligned} \boldsymbol{\tau}_2 &= \eta_0 [\nabla \mathbf{v}_2 + (\nabla \mathbf{v}_2)^T] + [\eta_0^b - \frac{2}{3}\eta_0] (\nabla \cdot \mathbf{v}_2) \mathbf{1} \\ &+ \langle \eta_1 [\nabla \mathbf{v}_1 + (\nabla \mathbf{v}_1)^T] \rangle + \langle [\eta_1^b - \frac{2}{3}\eta_1] (\nabla \cdot \mathbf{v}_1) \mathbf{1} \rangle. \end{aligned} \quad (1.29)$$

Note that these second-order equations do not depend on T_2 . The time-averaged second-order energy continuity equation (not shown here, see ref. 31) does only depend on T_2 and first-order terms and does not contribute

to other second-order terms. For calculation of the acoustic forces it is therefore sufficient to solve the time-averaged second-order continuity equations for mass and momentum only.

Terms containing first-order variables are called source terms. It is not trivial how the time-averaged source terms in the second-order equations should be evaluated in case of simultaneous excitation of modes at various frequencies. These multiple frequencies come into the equations through each first-order field as seen from equation 1.24.

In case of a single excitation frequency ω , the frequency of each first-order field is equal. From equation 1.26 it follows that the product of two first-order fields with the same frequency leads to a constant and an oscillating term at 2ω . Subsequent time averaging removes the 2ω components from the source terms, leaving only the constant components.

In case of multiple excitation frequencies, removing all non-stationary components by time averaging is only realistic if the lowest frequency components, the difference frequencies $|\omega_j - \omega_k|$ for $j \neq k$, are still much larger than the temporal resolution of typical experiments. This assumption is valid for the resonances considered in this paper. The source terms in the case of N simultaneous excitation frequencies can be written as

$$\begin{aligned} \langle \rho_1 \mathbf{v}_1 \rangle &= \left\langle \left(\sum_{j=1}^N \rho_1^{\omega_j}(\mathbf{r}, t) \right) \left(\sum_{j=1}^N \mathbf{v}_1^{\omega_j}(\mathbf{r}, t) \right) \right\rangle \\ &= \sum_{j=1}^N \sum_{k=1}^N \langle \rho_1^{\omega_j}(\mathbf{r}, t) \mathbf{v}_1^{\omega_k}(\mathbf{r}, t) \rangle. \end{aligned} \quad (1.30)$$

As seen from equation 1.26, products of unequal frequencies ($\omega_j \neq \omega_k$) do not contain stationary terms and are removed by the time averaging, resulting in

$$\begin{aligned} \langle \rho_1 \mathbf{v}_1 \rangle &= \sum_{j=1}^N \langle \rho_1^{\omega_j}(\mathbf{r}, t) \mathbf{v}_1^{\omega_j}(\mathbf{r}, t) \rangle \\ &= \frac{1}{2} \sum_{j=1}^N \text{Re} \{ \rho_1^{\omega_j}(\mathbf{r}) \mathbf{v}_1^{\omega_j*}(\mathbf{r}) \}. \end{aligned} \quad (1.31)$$

The same reasoning holds for all source terms. Note that the end result in equation 1.31 contains only time-independent terms. All source terms in the time-averaged second-order equations separate linearly into constant source terms for each excitation frequency. Therefore also the second-order equations separate linearly into equations for each excitation frequency. As with the first-order solutions, one is allowed to calculate separate solutions for

each excitation frequency and to linearly combine them up to time-averaged second order to find the solution in the case of simultaneous excitation.

Finally, for determination of the resonance frequencies we use the acoustic energy density in the fluid given as [44]

$$E_{ac}(\mathbf{r}) = \frac{1}{2}\kappa_s \langle p_1^2 \rangle + \frac{1}{2}\rho_0 \langle \mathbf{v}_1^2 \rangle \quad (1.32)$$

with $\kappa_s = \kappa_T/\gamma$ the isentropic compressibility. The two terms correspond to the potential and kinetic energy parts respectively.

1.3 Acoustophoretic forces

The time-averaged acoustic forces on a suspended microparticle in a fluid are the acoustic radiation force $\mathbf{F}_{rad}$ due to scattering of the acoustic waves from the particle and the Stokes drag force $\mathbf{F}_{drag}$ due to the steady acoustic streaming.

The acoustic radiation force is the time-averaged force on a suspended particle due to the scattering of the incident acoustic field. Multiple physical effects can contribute to the acoustic radiation force. If the compressibilities of the medium and the suspended particle differ, then an incident periodic acoustic field causes the particle to homogeneously compress relative to the medium, causing scattering of the incident acoustic field into monopole radiation. A difference in density likewise leads to a relative displacement, causing dipole scattering. The incident acoustic field also leads to a periodic temperature field and the periodic thermal difference between particle and medium also contributes to a monopole scattering component. Finally, when the particle size is comparable with the acoustic wavelength, particle resonances lead to periodic changes in shape causing multipole scattering.

The acoustic radiation force acting on a thermoelastic microparticle of radius a in a thermoviscous fluid, in an acoustic field with wavelength $\lambda \gg a$ is [35]

$$\mathbf{F}_{rad} = -\pi a^3 \left[\frac{2}{3}\kappa_T \langle c_M p_1 \nabla p_1 \rangle - \rho_0 \langle c_D \mathbf{v}_1 \cdot \nabla \mathbf{v}_1 \rangle \right] \quad (1.33)$$

with c_M and c_D the complex-valued monopole and dipole scattering coefficients respectively, which we write in shorthand as

$$c_M = \frac{c_{M1} + c_{M2}H}{1 + c_{M3}H} \text{ and } c_D = \frac{c_{D1}(1 - G)}{c_{D1} + 3(1 - G)} \quad (1.34)$$

with the parameters defined in appendix A. The subcoefficients c_{M1-3} , c_{D1} are functions of material properties only, while the functions H and G also depend on the particle radius and acoustic resonance frequency.

In order to compare the strength of the acoustic radiation force for different media and particle materials it is useful to make a few simplifications. In case of inviscid fluid and ignoring thermal effects, the functions H and G tend to zero (see appendix A and ref. 35) and the scattering coefficients simplify to

$$c_M = 1 - \tilde{\kappa}_s \quad (1.35)$$

$$c_D = \frac{2(\tilde{\rho}_0 - 1)}{2\tilde{\rho}_0 + 1}. \quad (1.36)$$

A tilde over a parameter indicates the ratio of particle parameter to fluid parameter, e.g. for polystyrene particles in water $\tilde{\kappa}_s = \frac{\kappa_{s,ps} \gamma}{\kappa_T}$ and $\tilde{\rho}_0 = \frac{\rho_{ps}}{\rho_0}$. We also assume an one dimensional resonance (a standing wave) between two coplanar walls placed as $x = 0$ and at $x = l$. The acoustic pressure is then given by $p_1(x) = p_a \cos(kx)$ with wave number $k = n\pi/l$, n mode number and p_a the acoustic amplitude. The acoustic radiation force simplifies to

$$F_{rad,1D} = 4\pi\Phi a^3 k E_{ac} \sin(2kx), \quad (1.37)$$

$$\Phi = \frac{1}{3}c_M + \frac{1}{2}c_D, \quad (1.38)$$

$$E_{ac} = \frac{\kappa_T}{4\gamma} p_a^2 \quad (1.39)$$

with Φ the acoustophoretic contrast factor (AC) and E_{ac} the acoustic energy density [45, 46]. The contrast factor can be used to compare the relative strength of the acoustic radiation force for various particles and media, see table 1.2. For particles like solid beads, bacteria and bacterial spores, the acoustic contrast factor typically is positive and the force will push the particles towards the nodes of acoustic resonance (at minimum pressure amplitude, see figure 1.1a). The acoustic radiation factor can be negative, as is the case for small gas bubbles in water. In that case, the radiation force pushes the bubbles towards the anti-nodes of the acoustic resonance.

Acoustic resonances can lead to the development of a steady flow due to dissipation of the acoustic energy in the viscous boundary layer on walls. This phenomenon will be discussed in more detail in the next chapter. A suspended microparticle will experience a drag force in such a steady flow. The time-averaged Stokes drag force $\mathbf{F}_{drag}$ acting on a spherical particle of radius a moving with a velocity $\mathbf{u}$ in an acoustic streaming flow with velocity $\mathbf{v}_2$ due to a single acoustic resonance is given by

$$\mathbf{F}_{drag} = 6\pi\eta_0 a (\mathbf{v}_2 - \mathbf{u}). \quad (1.40)$$

valid for particles sufficiently far from the walls [49, 50]. Both forces are time-averaged second-order quantities. As discussed in the previous section, the forces in case of simultaneous excitation of multiple resonances can be found by linear addition of the forces calculated for single resonances.

Particle type	Medium	Particle density ρ [kg m ⁻³]	Particle compressibility κ_s [Pa ⁻¹]	AC Φ
Polystyrene bead ^a	Water	1.050×10^3	2.38×10^{-10}	+0.17
Silica bead ^b	Water	2.648×10^3	2.80×10^{-10}	+0.39
Silica bead ^b	Glycerol	2.648×10^3	2.80×10^{-10}	+0.14
Bacillus subtilis spores ^c	Water	1.14×10^3	1.10×10^{-10}	+0.30

^a See table 1.1.

^b Values from ref. 43.

^c Compressibility estimated as $\kappa_s = \frac{3(1-2\nu)}{E}$ with Young's modulus $E = 13.6$ GPa [47] and an estimated Poisson's ratio $\nu = 0.25$. Density from ref. 48.

Table 1.2: Acoustophoretic contrast factor for various particles and media combinations.

1.4 Scaling of the fields and forces

For experiments, engineering and applications of acoustophoresis it is useful to approximately know the acoustic fields and forces scale with particle size and frequency. From the scaling one can estimate the working regime of acoustic devices, such as particle concentrators and also learn how to improve them.

We consider the order of magnitude of the acoustic power input and power dissipation [31]. In the numerical calculations presented in the next chapter, the acoustic resonances are driven by a oscillating boundary condition on the first-order velocity v_1 at the vertical walls, which mimics oscillating walls. The amplitude of this forced oscillation is $v_{bc} = d_0 \cdot \omega$, where the $d_0 = 0.1$ nm is the displacement amplitude of the walls. The displacement amplitude is directly related to the amplitude of an oscillating excitation piezo element in experiments.

The acoustic cavity formed by the left and right walls is pumped by incoming energy current densities at the left and right walls given by $\langle p_1 v_{bc} \rangle$, going through the side surface areas hl and the magnitude of the incoming power becomes $P_{in} \sim 2(hl)(\frac{1}{2}p_1^a v_{bc})$. l is the length of the microchannel and the factor 1/2 comes from the time average (equation 1.26). Neglecting viscosity in the first-order mass conservation (eqn. 1.21) and ignoring numerical preterms such as i leads to the estimation of the magnitude of the first-order pressure $p_1^a \sim \rho_0 \omega / k |v_1| = \rho_0 c_s v_1^a$ with $k = \omega / c_s$ the acoustic wavenumber. The incoming power becomes $P_{in} \sim hl \rho_0 c_s v_1^a v_{bc}$. Most of the power dissipation occurs in the viscous boundary layers. The magnitude of the dissipated

power can be estimated as [31] $P_{out} \sim \frac{1}{2} \frac{w l \eta_0}{\delta_s} (v_1^a)^2$ with $\delta_s = \sqrt{\frac{2\eta_0}{\rho_0 \omega}}$ being the length scale of the viscous boundary layer. The incoming and dissipated powers are equal in steady state, leading to the magnitude of the first-order velocity,

$$v_1^a \sim 4 \frac{c_s d_0 h}{w} \sqrt{\pi \rho_0 f / \eta_0}. \quad (1.41)$$

Likewise we find the frequency dependence of the first-order fields to be $v_1^a, p_1^a, T_1^a \propto \sqrt{f}$. From the second-order mass conservation (eqn. 1.27) and momentum conservation (eqn. 1.28) ignoring viscosity we find $v_2^a, p_2^a \propto f$. Using the definition (eqn. 1.32) we find the dependence for the acoustic energy density to be $E_{ac} \propto f$. For the resonance linewidth Δf and the quality factor we use the equivalent definition

$$Q = 2\pi \frac{\text{Energy stored in resonator}}{\text{Energy dissipated per cycle}} \propto \omega \frac{E_{ac}}{P_{out}} \quad (1.42)$$

and the frequency dependencies become $Q, \Delta f \propto \sqrt{f}$. Typical values of these parameters for polystyrene particles in water are: acoustic frequency $f \approx 2$ MHz, $v_1 \approx 0.7$ m s⁻¹, $p_1 \approx 1$ MPa, $T_1 \approx 15$ mK, $v_2 \approx 100$ μ m s⁻¹, $p_2 \approx 100$ Pa and $E_{ac} = 100$ J m⁻³. In the next chapter we will explore the dependency on particle size and frequency with experiments and numerical calculations.

For the forces we find the dependence on frequency and particle size to be $\mathbf{F}_{rad} \propto a^3 \cdot f^2$, $\mathbf{F}_{drag} \propto a \cdot f$ and the ratio of radiation to drag force scales as $R_{r/d} \propto a^2 \cdot f$. The transition from radiation force to drag force-dominated behavior occurs at the critical point where the forces are balanced.² The critical particle diameter is approximately 1.8 μ m for polystyrene (PS) particles in water at an acoustic frequency $f \approx 2$ MHz (see chapter 2). As an example, consider that for a certain application we need concentration of PS particles of 900 nm in diameter. All else being equal, we can estimate from the scaling of the force ratio that as the particle size halves, the acoustic frequency needs to increase with a factor of 4 or more. Both the radiation and drag force scale in the same manner with input power, so input power cannot be used as a knob to balance the forces.

2. Particles smaller than the critical size are in the drag force-dominated regime which prevents strong concentration. Larger particles are in the radiation force dominated regime and can be concentrated using acoustophoresis. See figure 1.1.

2 | Acoustophoresis of small particles

Acoustophoresis is the manipulation of particles by sound and is used in Lab-on-a-chip systems to concentrate or separate suspended matter from a liquid. For many applications separation of submicrometer particles is of great interest, but the application of standing-wave acoustophoresis for such small particles is limited due to the increasing effect of acoustic streaming for diminishing particle size. In the first part of this chapter we show experimentally that the lower particle size of separation of polystyrene beads from water, in a typical geometry, is around 1 μm diameter using an ultrasonic resonance around 2 MHz. In the second part of this chapter we numerically investigate the transition from the acoustic radiation force-dominated regime into the streaming-dominated regime using numerical particle tracing. We find that using higher-order modes and by simultaneous excitation of two modes the particle size limit can be lowered to 100 nm to 700 nm, depending on the required concentration enhancement.[†]

2.1 Introduction

One of the operations in a Lab-on-a-Chip is the manipulation of suspended microparticles (such as bacteria, fat droplets, plastic microbeads or human cells) in a bulk fluid (like water, milk or blood) [18]. A popular method is ultrasonic acoustophoresis which uses sound waves for the particle manipulation. Over the last decade there have been significant developments in the theory, implementation and application of ultrasonic acoustophoresis in Lab-on-a-Chip systems [52–55]. The processing of submicrometer particles

[†]. Parts of this chapter are published in ref. 51.

(with a diameter smaller than a micrometer) is of great interest for applications in microbiological analysis, food and drinking water quality analysis and biomedicine. However, the application of acoustophoretic focusing of submicrometer particles is limited due to acoustic streaming flow [56, 57], which typically becomes the dominant effect for particles smaller than a few micrometer. Accordingly, there is an ongoing interest in engineering streaming flow patterns which allow focusing of smaller particles.

Significant theoretical and experimental work has been done on the fundamental, half-wavelength ($\lambda/2$) resonance in a rectangular geometry by Bruus and coworkers [31, 32, 45, 53, 58]. The fundamental resonance often provides an optimal geometry with low complexity for acoustic focusing. It generates a single concentration node in the center of the channel for acoustically hard particles (with a positive acoustic contrast factor), preventing clogging or adsorption on the walls and allows straightforward separation of the concentrated stream using a trifurcation at the end of the channel. The lower size limit of such systems for polystyrene (PS) beads in water is around 1 μm for a resonance frequency around 2 MHz (corresponding to a rectangular microchannel of approximately 375 μm wide). A higher operation frequency increases the strength of the acoustic radiation force relative to the Stokes drag force, and subsequently lowers the size limit, but also leads to a smaller width w of the channel due to the resonance condition for the fundamental resonance, $w = n \cdot \lambda/2$ with mode number n equal to one. To compensate for the lower throughput one could use multiple parallel channels [59].

Higher-order modes at higher frequencies allow combination of a wider channel with a stronger radiation force compared with the fundamental resonance [60]. Nilsson et al. used the first harmonic mode ($2\lambda/2$) at 1.96 MHz to achieve focusability of 5 μm diameter polyamide beads in water, as the acoustic radiation force at fundamental resonance was too weak [61]. They also showed operation at the second harmonic ($3\lambda/2$) and third harmonic ($4\lambda/2$) modes but did not measure the performance of these modes as this would require a more complex chip design for separation using the increased number of concentration nodes. Most other research done on higher-order modes work in the limit of dominant acoustic radiation force, i.e. above the lower size limit. Grenvall et al. used the first and second-harmonic mode to separate acoustically hard and soft components and cells from raw milk [62, 63]. They show that the fundamental resonance causes severe clogging because the soft lipids are concentrated in the two anti-nodes close to the walls where they adhere and accumulate. The fundamental and the two harmonic modes are all excited around 2 MHz and were implemented by using various channel widths. The higher-order modes provide an improved geometry for separation of hard and soft particles. However, because the excitation frequency is

the same for all modes, there is no relative increase of the strength of the acoustic radiation force by using a higher frequency. Kothapalli et al. also used the first-harmonic mode for simultaneous concentration of polymer beads and polymer-shelled microbubbles [64].

There is some literature on acoustophoresis using temporally and spatially combined modes. Switching between modes can be used to separate beads with different sizes [52, 65]. Liu et al. separate 5 and 10 μm PS beads into two parallel streams by switching between the fundamental and second harmonic ($3\lambda/2$) based on the dependence of particle concentration speed on particle size [66]. The same effect is used in free flow acoustophoresis using the fundamental resonance [67]. Cho et al. sweep back and forth between modes $n = 4$ to $n = 7$ by changing the excitation frequency from 1.8 MHz to 3.1 MHz, sweeping 10 μm PS particles towards one side of the channel.

Other researchers combine resonances in two dimensions to increase control over the concentration process. Grenvall et al. show two-dimensional particle focusing with two orthogonal fundamental resonances at 2 and 5.3 MHz for microchip impedance spectroscopy of 3, 5 and 7 μm PS beads and red blood cells [68]. In other work they combined two orthogonal fundamental resonances to improve particle sorting, but these modes were on different location on chip, i.e. spatially separated [69]. Leibacher and coworkers used a two-dimensional (2D) mode at 870 kHz to study acoustophoresis of hollow and core-shell particles [70].

Antfolk and coworkers reported on focusing 500 nm diameter polystyrene beads and *E. coli* bacteria in water using a 2D half-wavelength mode in a square microchannel [71]. The numerical analysis suggests that, even though the Stokes drag force due to the acoustic streaming is the dominant force, focusing is achieved because the acoustic radiation force causes slight displacements from the otherwise closed streamlines, causing the particles to spiral inwards. This observation provides a path towards lowering the size limit of acoustophoresis.

In this chapter we will first show acoustophoretic experiments with PS microparticles in water, showing the fundamental and higher-order resonances which can be used for particle concentration from water. We also show that the fundamental resonance becomes ineffective at particle concentration for smaller particles. In the next part we discuss numerical simulations showing the transition from radiation-dominated to streaming-dominated acoustophoresis, which suggests an alternative geometry for submicrometer particle concentration using higher-order modes.

2.2 Acoustophoretic experiments

In these experiments we flow water with a low concentration of suspended micrometer-sized particles through a microchannel. A piezo element on the microchip containing the microchannel is forced to oscillate using an electric signal. An ultrasonic, acoustic standing wave is excited along the width of the channel, perpendicular to the flow direction, when the excitation frequency f of the piezo signal matches the resonance frequency which is approximately given by the resonance condition

$$n \cdot \frac{\lambda}{2} = w, \text{ or equivalently } f = \frac{n \cdot c_s}{2w} \quad (2.1)$$

with n the mode number, λ the acoustic wavelength, w the width of microchannel and c_s the speed of sound in water. The resonance condition expresses the fact that at resonance n half-wavelengths fit in the acoustic resonator. Equation 2.1 is approximate because it does not take into account acoustic losses, which shifts the exact resonance frequency down slightly.

In the results we first show particle focusing for modes $n = 1$ to $n = 6$ and next we show the decreasing efficiency of particle focusing using the fundamental mode ($n = 1$) for decreasing particle size.

2.2.1 Experimental details

Microchip fabrication

The microchannel was fabricated in a silicon-glass chip using photolithography and Deep Reactive-Ion Etching (DRIE) on a $\langle 100 \rangle$ silicon wafer. Access holes of 1 mm diameter were made from the backside in the same way as the channels. The channel wafer is anodically bonded to a 500 μm thick borofloat glass wafer and then diced into glass-silicon microchannel chips measuring 6 by 1.5 cm, see figure 3.1. The length l , width w and depth h of the microchannel are 4 cm, 380 μm and 155 μm respectively. A piezo element measuring 12.7x12.7x1 mm (PZ 27-302, Ferroperm) was attached to the back of the chip using cyanoacrylate glue. The connectors for tubing are made by gluing pipette tips (VWR 0.5-20 μL) with UV-glue (Dynmax OP-24B) to the access holes, followed by 300 s curing in a UV-oven (Spectrolinker XL-1500) and strengthened with a small additional layer of 2-component glue (Araldite).

Polystyrene fluorescent particles

Fluorescent polystyrene microparticles (PS-FluoRed, microParticles GmbH) with 0.5, 1, 2 and 4 μm diameter were put into separate 0.01% w/v suspensions

with milli-Q water. The polystyrene particles are dyed with red fluorophore (Macrolex Fluorescence Red G) with a peak optical absorption at 535 nm and emission at 584 nm.

Setup

The suspension of particles is placed in a plastic syringe in a syringe pump (PhD 2000, Harvard Apparatus) and the syringe is connected to one of the access holes of the microchip using Teflon tubing (Tygon, inner diameter 2.3 mm). Suspension flowing from the other access hole is directed into a waste beaker using a piece of tubing. The microchip is placed glass-side down on top of an inverted microscope (Nikon TE2000) equipped with a 10x objective. The microscope has a built-in white-light mercury lamp and a filter wheel. A fluorescence filter set consisting of an excitation filter (Semrock D540/25), dichroic mirror (Semrock DM565) and emission light filter (Semrock D605/55) ensures that the suspended microparticles in the microchannel are excited and that the fluorescence emission is directed towards the output port of the microscope. An EMCCD camera (Andor Luca S) is connected to this port recording the intensity distribution of the fluorescent light originating from the microparticles flowing in the microchannel. The camera is aligned such the microchannel is oriented horizontally such that the flow flows from left to right. The piezo element on the microchip is excited electrically using an excitation signal generated using a function generator (Rigol DG4162) and amplified using a RF amplifier (E&I 2200L).

Method & data processing

A solution with microparticles (containing particles with a single size) is continuously flowed through the microchannel by setting a flow rate of 0.01 mL min^{-1} . For the multi-mode results the piezo excitation signal amplitude was varied for each mode, while the amplitude was constant at 50 mVpp at the function generator for the frequency sweeping experiments. This leads to a peak signal amplitude $U \approx 10 \text{ Vpp}$ at the piezo element. The microchannel was imaged at the center of the chip.

The resonance frequency for finding the fundamental and higher-order modes is found by manually adjusting the excitation frequency and observing the particle positions as they flow by. For the efficiency measurements we use a frequency sweep with the following method. The excitation frequency is increased in steps of 10 kHz. After changing the frequency we wait 15 s so that the suspension in the microchannel is completely refreshed. Subsequently a frame is recorded with an exposure time of 1 s.

Figure 2.1: First to sixth-order resonances (a-f) in a glass-silicon microchannel creating a standing wave in the x direction. Water containing $4\ \mu\text{m}$ diameter polystyrene beads is flowing from left to right through the microchannel. The figures show the fluorescence emitted by the particles using an exposure time of 1 s for each measurement. Particles are forced into the nodes of the resonance by the acoustic radiation force.

The exposure time is the same for all experiments and is chosen to be much longer than the time needed for a particle to cross the field of view (which is approximately $1.5 \times w$ in each direction). Each recorded frame contains multiple horizontal paths from particles which passed by. The projection of the frame onto the vertical x axis is thus a measure of the average particle positions in the x direction. If the excitation frequency matches a resonance frequency, an acoustic resonance will build up in the microchannel and the particles will be pushed towards the node of standing waves. This method has the advantage that it combines a large field-of-view with sensitivity for particles smaller than the optical resolution.

A measurement set for the frequency sweep consists of all frames taken at subsequent frequency steps for one particle size. Each frame from a set is processed as follows: 1) The average of the first and last frame of the set is subtracted from the frame to remove stationary particles (which are stuck at the walls, bottom or glass); 2) The frame is projected onto the vertical axis by summing of all horizontal pixels for each vertical row; 3) Negative values of the summed intensity occur when particles become stuck during the experiment and step 1 thus creates a negative artifact for frames before this moment. The artifact is removed by setting the negative values to zero.

2.2.2 Experimental results & discussion

Particle focusing using fundamental and higher-order modes

The results for acoustophoresis of $4 \mu\text{m}$ diameter particles for modes 1 to 6 is shown in figure 2.1. The particle movement generates horizontal lines indicating the particles are trapped in the nodes of the standing waves in x direction. The number and position of the nodes matches with the resonance condition discussed above. The frequency f is systematically lower than the ideal resonance frequency using $c_s = 1497 \text{ m s}^{-1}$ and $w = 380 \mu\text{m}$ and deviates on average 1.4%. As the frequency is found manually this adds to the error. A small part of the deviation can be explained by acoustic losses. Also a lower water temperature than 25°C , which is used for the value of the speed of sound, could also lower the resonance frequencies. Finally the width of the channel could also be slightly larger than measured after fabrication.

In the measurements for $n = 1$ to 4 (figures 2.1a to 2.1d) we see also a difference in intensity between the nodes. This could either be due to a different number of particles in each node during the measurement period or due to inhomogeneous illumination of the channel. The background and the nodes in figures 2.1e and 2.1f are more homogeneous thus inhomogeneous illumination is unlikely. The acoustic field between the measurement zone and

Figure 2.2: Average particle position along the x axis as function of excitation frequency f around the fundamental resonance for 0.5, 1, 2, and 4 μm diameter polystyrene particles (a-d). The radiation force becomes weaker compared with the streaming-induced drag force with decreasing particle size, leading to widening of the particle distribution.

the inlet might not be symmetric and might disturb the spatial distribution of the particles before the particles are trapped in the nodes. We also see a fairly strong background in the images which is due to adhered fluorescent microparticles on the microchannel sidewalls, bottom and glass. We compensate for this background in the frequency sweep measurement method as discussed above.

Particle concentration as function of particle size

The effect on particle size on the concentration efficiency is shown in figure 2.2. Each figure shows the average particle position along the x axis as function of excitation frequency, which is swept around the ideal resonance frequency for the fundamental mode around 1.97 MHz. For the largest particles with 4 μm diameter we see a narrow concentration of particles at the node in the middle of the channel for a excitation frequency between 1.85 and 2.08 MHz (figure 2.2d). For the smaller particles with 1 and 2 μm diameter the distribution becomes wider as the particle size decreases (figures 2.2b and 2.2c), indicating an increasing strength of the streaming-induced drag force. For the smallest particle size no concentration is obtained as indicated by the channel wide distributions. The average full-width at half-maximum of the distributions measured for 1 μm diameter particles is estimated as $0.4 \times w$ and therefore we can reasonably conclude that this particle size is the lower size limit for acoustophoresis of polystyrene particles in this geometry (mode order and frequency).

In addition to the general trend of widening particle distributions for lower particle sizes we can also see variation of the distribution as function of frequency. As noted above, the 4 μm particles show a distribution with a relatively constant width over the frequency span of 1.85 and 2.08 MHz (fig. 2.2d), in contrast with the 1 μm particle distributions which shows broad but separate resonances, e.g. at $f = 1.9$ MHz and 1.98 MHz (fig. 2.2b). The broad single resonance suggested by the 4 μm particle distribution could be attributed to the piezo element which is also resonant in this frequency range. The broad piezo resonance then causes a forced acoustic oscillation in the microchannel when the frequency does not match the channel resonance exactly. The resulting acoustic radiation force is then still sufficiently strong to focus the particles into the node in the middle of the channel over the whole frequency range. For the 1 μm particles the influence of the streaming increases and the focusing is only strong enough at channel resonances. If the acoustic cavity is extended to also include the thick silicon walls, then other modes than the ideal ‘water’-modes are possible [72,73]. Extended modes combined with different thicknesses of the silicon walls (between the water and air) might also

explain the ‘wiggling’ of the node position over the frequency range as seen in figure 2.2c and 2.2d. A measurement with higher frequency resolution and possibly with longer integration time would lead to stronger conclusions.

The 0.5 μm particle distribution (fig. 2.2a) looks irregular due to the small particles used, resulting in a low signal-to-noise ratio which makes a broad distribution hard to resolve. A very broad distribution is seen for frequencies under 1.85 MHz, indicating particles have no preferred position. At the end of the frequency range, at 2.03 MHz and up, no difference from the background is measurable, i.e. absolutely no focusing of particles is occurring for this frequency range. In the middle between 1.85 and 2.03 MHz some structure is seen, which is probably due to streaming-induced mixing causing particles to flow in and out from the focal plane. It can be concluded that no focusing in the middle of the channel occurs for this particle size.

2.2.3 Conclusion

We used a simple optical method to measure microparticle acoustophoresis in a microchannel in a glass-silicon microchip. 4 μm diameter polystyrene beads could be concentrated into the nodes of the first six acoustic resonances, indicating operation in the radiation-dominated regime. We also observe a lower particle size limit around 1 μm beads for the fundamental resonance, which indicates the transition to the streaming-dominated regime. This transition will be the topic of the numerical calculations in the next section.

2.3 Numerical study of streaming-assisted acoustophoresis

A suspension with microparticles can be concentrated by using radiation-dominated acoustophoresis. In a standard geometry, the particles are concentrated into the vertical node of the acoustic standing wave using the fundamental resonance, creating a vertical sheet of concentrated particles. The concentrate and the depleted carrier liquid can then be separated by a trifurcation (three-way splitter) at the end of the microchannel. In the previous section we have seen that the lower particle limit is around one micrometer diameter for polystyrene beads in water at a resonance frequency of 2 MHz.

In this section we will study the transition from radiation-dominated to streaming-dominated acoustophoresis using numerical calculations. The transition is marked by the streaming-induced radiation force becoming the force which predominantly dictates particle movements. We will show that the combined effect of both forces leads to a different geometry for particle

concentration with a lower particle size limit than in the radiation-dominated regime. The method requires the use of the higher-order resonances discussed in the experiment section. We will discuss both the case of single mode excitation as the case of simultaneous double excitation of two different modes.

First the acoustic fields are calculated by solving the first and second-order perturbation terms of the governing equations as discussed in chapter 1 and the acoustophoretic forces are calculated from the fields. Using the forces we perform particle tracing simulations which mimic acoustophoretic experiments in typical silicon-glass microchips. Finally we give an analysis of the results.

2.3.1 Numerical implementation

The thermoviscous acoustic model as discussed in chapter 1 is implemented using Finite Element Method (FEM) in the software package COMSOL Multiphysics (version 4.4, COMSOL Inc.) and is based on a previously published model by Muller and Bruus [74]. Their model uses the adiabatic approximation for improved simulation speed and lowered complexity. We adapted the model to the thermoviscous case described in the previous sections, used different boundary conditions and optimized the mesh for higher-order modes. The equations for the first and second-order fields are cast in weak form [75] and then implemented in COMSOL using the Weak Form PDE-module. The FEM model is controlled from MATLAB (version R2013a, The Mathworks Inc.), such as setting parameters and evaluating the results, for increased flexibility. After calculation the fields are exported to MATLAB, after which the acoustophoretic forces are calculated and particle tracing is performed. MATLAB can be coupled to COMSOL using the ‘LiveLink’-feature, which can be started by the running the command

```
C:\Program Files\COMSOL44\bin\win64\comsolserver.exe matlab
```

which should start the COMSOL Server in the background and MATLAB as a front-end client¹. This allows direct interaction with the numerical model from MATLAB.

We study the first five resonances in a water-filled microchannel with rectangular cross section with width $w = 380 \mu\text{m}$ and height $h = 160 \mu\text{m}$ in which the acoustic cavity is formed by the two vertical walls at $x = \pm w/2$, see figure 2.3. A mode with mode number n is excited by setting the angular excitation frequency ω equal to the resonance frequency ω_n , which in case of a lossless cavity means fulfilling the resonance condition $n\lambda/2 = w$ or equivalently $\omega_n = \pi n \cdot c_s/w$. Our model is not lossless due to the viscosity

1. The location of the comsolserver binary depends on your specific installation.

Figure 2.3: (a) Schematic illustration of the cross-section of the numerically studied microchannel with width $w = 380 \mu\text{m}$ and height $h = 160 \mu\text{m}$. The numerical domain Ω contains the upper half of the microchannel due to symmetry of the fields and thus consists of three walls and a symmetry boundary: a hard upper wall, two oscillating vertical side walls and a symmetry boundary at $y = 0$. The hatched region indicates the upper right quadrant. (b) Illustration of the optimized mesh used for discretization of the domain. The green region on the left is indicated in the right upper corner in figure (a) and shows the mesh in the bulk and the finer mesh at the walls. A zoom-in of the fine boundary mesh in the upper right corner of the domain is shown in orange on the right.

and the exact resonance frequencies are determined using a frequency sweep. All variable values used for the calculations are shown in table 1.1.

Governing equations and boundary conditions

The walls are assumed to be infinitely hard, to have an infinite heat conduction and to impose a no-slip condition for the velocity. The resonances are excited through a boundary condition for the first-order velocity $\mathbf{v}_1$ on the vertical walls imposing an oscillation with amplitude $v_{bc} = \omega \cdot d_0$ with displacement $d_0 = 0.1$ nm. Optimal coupling to the symmetric (even n) and asymmetric (odd n) modes is ensured by taking their symmetry into account at the boundary conditions, see equation 2.2e. The symmetry boundary in first order is given by a zero orthogonal velocity component (v_{1y}) and zero orthogonal derivative of the parallel velocity component ($\partial_y v_{1x}$). In second order the same conditions are applied to the mass flow instead of the velocity. Lastly, to select a solution for the second-order pressure we set the spatial average of p_2 to zero using a Lagrange multiplier.

The first and second-order fields are calculated using equations 1.20-1.22 and 1.27-1.28 respectively, using the boundary conditions

$$T = T_0 \text{ on all walls,} \quad (2.2a)$$

$$\mathbf{v} = \mathbf{v}_1 = \mathbf{v}_2 = \mathbf{0} \text{ on top wall,} \quad (2.2b)$$

$$\mathbf{v}_{1y} = \mathbf{v}_2 = \mathbf{0} \text{ on side walls,} \quad (2.2c)$$

$$\mathbf{v}_{1x} = v_{bc} \exp(-i\omega t) \text{ on left wall,} \quad (2.2d)$$

$$\mathbf{v}_{1x} = \begin{cases} v_{bc} \exp(-i\omega t) & n \text{ odd} \\ -v_{bc} \exp(-i\omega t) & n \text{ even} \end{cases} \text{ on right wall,} \quad (2.2e)$$

$$\mathbf{v}_{1y} = 0 \text{ on symmetry boundary,} \quad (2.2f)$$

$$\partial_y \mathbf{v}_{1x} = 0 \text{ on symmetry boundary,} \quad (2.2g)$$

$$\mathbf{v}_{2y} = 0 \text{ on symmetry boundary,} \quad (2.2h)$$

$$\partial_y(\rho_1 \mathbf{v}_{1x} + \rho_0 \mathbf{v}_{2x}) = 0 \text{ on symmetry boundary.} \quad (2.2i)$$

In ref. 31 and 74 a nonzero $\mathbf{v}_2$ boundary condition for the actuated walls was used to ensure a zero mass flow through the walls by compensating for the second-order term $\rho_1 \mathbf{v}_1$, while the zero boundary condition as above was used in ref. 32 and 58. The difference between the non-zero or zero boundary condition stems from interpreting the velocity at the actuated wall either as a Lagrangian or an Eulerian quantity, respectively. In the first case, one takes into account the fact that the wall on which the boundary condition is applied is moving. The resulting non-zero $\mathbf{v}_2$ flow is a few orders of magnitude smal-

ler than the streaming velocity in the channel and therefore the difference with our choice is negligible.

Numerical mesh and convergence

The numerical domain is discretized using rectangular mesh elements and is divided into two regions; a boundary region close to the walls with a width w_b and a coarser inner domain, as illustrated in figure 2.3b. The inner domain is divided into N_x and N_y elements, which set the number of mesh cells in the x and y direction, respectively. The boundary region has a finer mesh compared with the inner domain to properly resolve the viscous boundary layer generating the acoustic streaming. To ensure that the viscous boundary layer is properly resolved for all modes, we set $w_b = 10 \cdot \delta_s^{\omega_1} = 3.6 \mu\text{m}$ with $\delta_s^{\omega_1} = \sqrt{2\eta_0/(\rho_0\omega_1)}$ as the length scale of the boundary layer in case of the fundamental resonance. The boundary region is split into N_b slices running parallel to the walls. The thickness of the slices follows a geometric sequence with a ratio R_b , being finest close to the wall. The slices are split in the direction parallel to the walls using the size of the mesh cells nearby in the inner region. This leads to strongly elongated rectangular mesh elements close to the walls.

To balance calculation accuracy with simulation cost (calculation time and

Figure 2.4: Mesh convergence C of the optimized mesh. The dashed line indicates a relative convergence of 1×10^{-3} .

memory requirements) a metric is needed for the accuracy of a solution g as function of mesh parameters. The relative convergence parameter $C(g)$ as defined in ref. 32 is given as

$$C(g) = \sqrt{\frac{\int (g - g_{\text{ref}})^2 dx dy}{\int g_{\text{ref}}^2 dx dy}} \quad (2.3)$$

which gives the relative convergence of the solution g with respect to the reference solution g_{ref} . The free mesh parameters are varied such that convergence measured as $C(g)$ is better than 1×10^{-3} for all fields and for all modes while keeping the number of domain elements as low as possible. We calculate the reference solution g_{ref} on a finer mesh which has the free mesh parameters N_x, N_y and N_b doubled in value compared with the optimized mesh for g .

The optimized mesh has parameters $N_x = 71$, $N_y = 6$, $N_b = 5$ and $R_b = 9$ leading to mesh elements in the inner region measuring $5.5 \times 12.5 \mu\text{m}$ and the finest mesh elements in the upper corners measuring $0.19 \mu\text{m} \times 0.19 \mu\text{m}$, see figure 2.3b. The relative convergence of this mesh for the modes is shown in figure 2.4. All fields are well under the convergence criterion $C(g) < 1 \times 10^{-3}$. Higher-order modes are more demanding compared with the fundamental mode due to a stronger amplitude of the spatial derivatives, and likewise so for the second-order fields compared with first-order. The most demanding is x -component of the second-order velocity, which has a value of 8.6×10^{-4} for the $n = 5$ mode. The optimized mesh is used for all calculations.

Particle tracing

After calculation of the first and second-order fields we can calculate the forces on a suspended polystyrene microparticle in water for each mode using equations 1.33 and 1.40 and calculate the total force $\mathbf{F}_{\text{total}}$ for a particle of radius a by linear superposition

$$\mathbf{F}_{\text{total}}(a, \omega) = \sum_{j=1}^5 c_j \left(\mathbf{F}_{\text{rad}}^{\omega_j} + \mathbf{F}_{\text{drag}}^{\omega_j} \right) \quad (2.4)$$

with $c_j \geq 0$ the contribution from the mode with mode number j , having angular resonance frequency ω_j . The contributions c_j relate directly to the piezo excitation voltage in experiments. The acoustophoretic particle tracks are calculated by solving Newton's equation of motion

$$m \frac{d\mathbf{u}}{dt} = \mathbf{F}_{\text{total}} \quad (2.5)$$

Figure 2.5: Average acoustic energy density E_{ac} as function of normalized excitation frequency $(f - f_{res})/\Delta f$ for the fundamental mode $n = 1$ up to the fourth harmonic $n = 5$. The dots are the result of the numerical calculation and the solid lines are Lorentzian fits to the data points. The arrow indicates the direction of increasing mode number.

with m the mass of the particle and assuming point-like particles. We neglect other forces like gravity, buoyancy, particle-particle and particle-fluid interactions. The calculation is performed in MATLAB for improved speed using the following steps: 1) the radiation force and streaming flow fields are imported from COMSOL, 2) the total force field is calculated, and 3) the equation of motion, resulting in 2 coupled ODEs, is solved using the built-in ODE15s-solver with variable time stepping. The tracks are calculated for 304 particle starting positions homogeneously dispersed over the calculation domain (1 position per $10 \mu\text{m}^2$) and for particle diameters $2a$ equal to 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500 and 2000 nm respectively. The particle velocity at $t = 0$ is set to zero and a sticky boundary condition is used on the walls, i.e. the position is fixed when touching the wall. Each particle track is calculated from $t = 0$ to $t = 60$ seconds.

Computational requirements

All fields use third-order basis functions (cubic Lagrange functions) for discretization on the mesh except the velocity fields v_1 and v_2 which use fourth-

order functions (quartic Lagrange functions). The optimized mesh consists of 891 domain elements and 287 boundary elements, which combined with the basis functions leads to 86333 degrees of freedom per mode. Simultaneous calculation of the first and second-order fields of the five modes takes 69 seconds and requires 5.6 GB of RAM. The simulation time for the particle tracing depends on the acoustophoretic forces and thus on mode and particle size, as the solver adjusts to smaller time steps in case of a larger force and particle acceleration. In case of single-mode excitation the average calculation time is 6.1 seconds for the $n = 1$ mode increasing to 66.7 seconds for $n = 5$. Combinations of modes require a calculation time of the same order of magnitude. Calculations were performed on a desktop computer with two Intel Xeon E5-2650 CPUs, 64 GB RAM and 64 bit Windows 7.

2.3.2 Results

Resonance analysis

The acoustic resonance frequency f_{res} for the fundamental mode and up to the fourth harmonic is determined by calculating the spatially averaged acoustic energy density in the fluid (eqn. 1.32) for a range of excitation frequencies. We perform excitation frequency sweeps around the ideal resonance frequencies $f_{geo} = n \cdot c_s / (2w)$. The exact resonance frequency f_{res} , the full width Δf at half maximum and the acoustic energy density at resonance $E_{ac,res}$ are determined using a least-squares fit using a Lorentzian lineshape. The resonance quality factor is calculated using $Q = f_{res} / \Delta f$.

The result of the frequency sweep is shown in table 2.1 and in figure 2.5. We use the superscript ^a to indicate the amplitude of the field which we take to be the maximum value of the field in the fluid. Figure 2.5 shows the average acoustic energy density in the fluid is plotted versus the scaled frequency $(f - f_{res}) / \Delta f$ for comparison of the modes. All modes display the typical

n	f_{res} [MHz]	f_{geo} [MHz]	Q	Δf [kHz]	$E_{ac,res}$ [J m ⁻³]	v_1^a [m s ⁻¹]	p_1^a [MPa]	T_1^a [mK]	v_2^a [mm s ⁻¹]	p_2^a [Pa]
1	1.96701(1)	1.969	416	4.72(9)	106.(1)	0.6989	0.9741	17.92	0.1054	106.3
2	3.93538(6)	3.939	579	6.79(6)	205.(6)	0.9729	1.357	24.97	0.2049	206.3
3	5.90398(7)	5.908	695	8.49(8)	295.(9)	1.168	1.629	29.98	0.2926	297.4
4	7.87270(4)	7.877	783	10.0(5)	375.(9)	1.317	1.838	33.82	0.3689	378.5
5	9.84149(6)	9.847	890	11.5(5)	445.(4)	1.436	2.003	36.85	0.4339	449.3

Table 2.1: Resonance parameters found by excitation frequency sweep and maximum values of first-order and second-order fields at resonance.

Figure 2.6: Spatial dependence of the first-order fields evaluated on the line $y = 0$. Each field is scaled for comparison with its respective maximum on the line $y = 0$.

Lorentzian lineshape with a resonance frequency slightly below the purely geometrically expected value due to damping in the viscous boundary layers.

The acoustic energy density at resonance increases approximately linearly with increasing mode number, as expected from the scaling discussed in section 1.4. The amplitudes of the first-order velocity v_1^a agrees with the theoretical scaling (eqn. 1.41) within a 6% margin. The frequency dependencies of the other variables also agree with their respective theoretical scaling. We also note that $g_2/g_1 < 10^{-3}$ such that the perturbation expansion is valid.

Resulting fields with single-mode excitation

The first-order pressure p_1 , velocity v_1 and temperature T_1 are almost constant over the height of the channel but vary harmonically in the x direction. In figure 2.6 the value of fields is shown along the horizontal center line $y = 0$, where each field is scaled for comparison to the maximum value along the line. For the first-order velocity we show the dominant x -component as $v_{1x} \gg v_{1y}$ for all modes. The velocity goes to zero at the walls while the pressure and temperature are maximum, consistent with the hard-wall boundary condition. All first-order fields meet the half-wavelength resonance condition $n\lambda/2 = w$, with the pressure and temperature shifted by $\pi/2$ with respect

Figure 2.7: Time-averaged second-order velocity v_2 (arrows) and its magnitude [color scale ranging from 0 (dark) to 75% of the maximum v_2^a (light), see table 2.1] for modes $n = 1$ to 5 in the upper right quadrant of the microchannel (see figure 2.3). The dashed line for modes $n = 3, 4, 5$ is placed $\lambda/4$ away from the upper wall.

to the velocity. Compared with the fundamental resonance, first-order fields of the higher order modes are spatial multiples with higher amplitudes. The second-order pressure p_2 is also almost constant over the height of the channel and also varies harmonically in the x direction, but with a doubled period compared with the first-order pressure (not shown). The spatial dependence and amplitudes found for the first and second-order fields are consistent with experiments [21, 45, 50, 58, 76, 77].

The streaming velocity v_2 for the modes $n = 1$ to 5 is shown in figure 2.7. Only the upper-right quadrant of the rectangular microchannel is shown for visibility of the details. The streaming velocity is mirror symmetric around the lines $x = 0$ and $y = 0$, i.e. the streaming flow shown for $n = 1$ represents the typical pattern of four flow rolls (one per quadrant). The streaming flow has a more complex spatial dependence than the previously discussed fields. It is driven from the viscous boundary layer at the upper wall due to the source term $\langle \rho_1 v_1 \rangle$ in the second-order mass continuity equation (eqn. 1.27), thereby driving bulk rolls in the rest of the channel. The number of driving regions per quadrant is equal to the mode number n with the driving direction alternating, resulting in n counter rotating neighboring rolls with a horizontal extension of $\lambda/(2n)$. The centers of the rolls are located at $x = w/(2n) \cdot (j - 1/2)$ with $j = 1, \dots, n$. The alternating rotation direction also leads to vertical flow between the rolls which is alternating in direction. The rolls extend $\lambda/4$ vertically given enough space, which is the case for modes $n = 4$ and 5, and partially for $n = 3$, as indicated with the dashed lines placed a quarter wavelength away from the upper wall. This creates a relatively streaming-free region in the center of the microchannel, akin to the effect of increasing the aspect ratio [32] h/w . The bulk rolls for a particular mode are all very similar in structure. The rolls closest to the vertical wall (the most right roll in each quadrant) are influenced by the no-slip condition on the wall, slowing these rolls down and thereby pushing the rolls slightly towards the driving region. This effect is more pronounced for higher order modes.

Particle tracing with single-mode excitation

The particle tracks obtained with particle tracing can be compared with experimentally obtained tracks as verification of the simulation results but also to test the efficiency of different excitation modes for acoustophoretic concentration of particles. In case of single mode excitation we excite only one mode for the calculation of the forces in equation 2.4: for excitation with mode number n we set $c_n = 1$ and the other coefficients equal to zero. We first show the results for the $n = 2$ mode to explain the mechanism of streaming-

assisted acoustophoretic concentration in the case of single-mode excitation, after which we show results for the all five resonances.

The time-averaged radiation force $\mathbf{F}_{rad}$ for the $n = 2$ mode in the upper-half of the microchannel is shown in figure 2.8. The radiation force is nearly constant over the height of the channel and is spatially doubled compared with the force generated by fundamental mode [32]. If all other forces can be neglected, which is the case in the radiation-dominated regime, particles are pushed into two vertical regions located at $x = \pm\lambda/4$. In typical acoustophoretic concentrators, the vertical sheets containing a high concentration of particles are then separated from the rest of the flow by vertical splitting of the microchannel.

However, in streaming-dominated acoustophoresis the particles are not concentrated into a region but instead follow the rotating streaming flow. To study the transition from radiation-dominated to the streaming-dominated acoustophoresis we study particle tracing of PS particles with a diameter $2a = 1 \mu\text{m}$ using the first harmonic mode ($n = 2$) at $f = 3.9 \text{ MHz}$, which is in the intermittent regime. In the particle tracing we include the radiation force shown in figure 2.8 and the streaming-induced drag force due to the streaming field shown in figure 2.7 for $n = 2$. The results are shown in figure 2.9, showing the initial particle positions and the positions and tracks at three subsequent times after particle release at $t = 0$. At $t = 0.42 \text{ s}$ we see the development of two regions with different particle movement. A streaming flow dominated region is visible close to the top horizontal wall where the particles follow the rotating streaming flow. In the rest of the channel the drag force and the acoustic radiation force constructively combine to push particles into vertical sheets located at $x = \pm\lambda/4$. At time $t = 1.02 \text{ s}$ most particles from the bulk of the channel are concentrated into the vertical sheets.

Figure 2.8: Time-averaged radiation force $\mathbf{F}_{rad}$ (arrows) and its magnitude [color scale ranging from 0 (dark) to 94.4 pN (light)] for mode $n = 2$ in the upper half of the microchannel (see figure 2.3).

(a) Initial particle positions at $t = 0$ s, $CE = 0.06$

(b) Particle tracks at $t = 0.42$ s, $CE = 0.09$

(c) Particle tracks at $t = 1.02$ s, $CE = 0.19$

(d) Particle tracks at $t = 2.70$ s, $CE = 0.72$

Figure 2.9: Calculated particle tracks for a PS particle with diameter $2a = 1$ μm using $n = 2$ mode excitation at time (a) $t = 0$ s, (b) $t = 0.42$ s, (c) $t = 1.02$ s and (d) $t = 2.70$ s. The particle positions are indicated with a dot while the tracks are indicated with lines [color scale indicating local particle velocity magnitude ranging from 0 (dark) to $160 \mu\text{m s}^{-1}$ (light) for all figures].

The particles are then conveyed upwards towards the streaming-dominated region due to the streaming flow which has an upward component. After time $t = 2.70$ s all particles are in the streaming-dominated region where they will stay and circulate.

In this intermittent region we see a combination of particle behavior from streaming-dominated and radiation-dominated acoustophoresis. Particles relatively far from the streaming source initially gather into the vertical sheets, but are then dragged towards the horizontal walls. This mechanism is also observed in experiments [58]. For $2a = 1 \mu\text{m}$ particles in the $n = 2$ mode we see that all particles from the bulk finally end up in the horizontal streaming regions (in both upper and lower half of the channel), an effect we call streaming-assisted acoustophoretic concentration.

For definition of a metric for the concentration enhancement we vertically divide the microchannel into three regions, mimicking a vertical microchannel trifurcation: a depleted region centered around $y = 0$ with a vertical thickness h_{dep} and two concentration regions extending from $y = \pm h_{\text{dep}}/2$ to $\pm h/2$, respectively. h_{dep} is calculated from the vertical particle position distribution such that the center region contains 5% of the particles and the other regions at the top and bottom contain 95% of the particles together. We then define the relative concentration enhancement $CE = h_{\text{dep}}/h$, such that $CE \approx 0$ when the particles are located over the full extent of the channel (in that case $h_{\text{dep}} \approx 0$) while $CE = 1$ when 95 % of the particles are concentrated in thin horizontal layers at the horizontal walls (in that case the center of the channel is almost depleted and $h_{\text{dep}} = h$). The particle density in the concentration layers is then a factor $0.95/(1 - CE)$ higher compared with a homogeneous distribution of particles in the channel. Using this definition we calculate $CE = 0.06, 0.09, 0.19$ and 0.72 at times $t = 0, 0.42, 1.02$ and 2.70 s for the $2a = 1 \mu\text{m}$ particles using the $n = 2$ mode, showing the time development of the concentration of particles in the horizontal streaming layer.

In figure 2.10 we show the concentration enhancements for modes $n = 1$ to $n = 5$ calculated from particle tracing of PS particles with diameters from 50 nm up to $2 \mu\text{m}$. The values are calculated as the average over the period $t = 50$ to 60 s and the shaded area has a thickness equal to twice the standard deviation during that period.

The same trend is visible for each mode. Large particles are first concentrated into vertical nodes due to the dominant radiation force, but end up at the horizontal walls due to the conveyor mechanism explained earlier. This leads to the horizontal plateau at the right side of the graph. Smaller particles are also concentrated but into a layer whose thickness increases with decreasing particle size. For very small particles no concentration occurs and particle motion is fully described by rotating streaming flow. The transition from al-

Figure 2.10: Concentration enhancement calculated from particle tracks using single mode excitation for modes $n = 1$ to $n = 5$ for PS particle diameters from 50 nm up to 2 μm . The values are calculated as the average over the period $t = 50$ to 60 s and the shaded area has a thickness equal to twice the standard deviation during that period.

most complete concentration at the topsides ($CE \approx 1$) to concentration in a more extended boundary layer occurs when the magnitude of the streaming-induced drag force becomes of the same order as the radiation force.

In section 1.4 we showed that the ratio of radiation force to drag force scales as $R_{r/d} \propto a^2 \cdot f$. Using this scaling and the data shown in figure 2.10 we estimate the critical particle diameters for mode $n = 1$ to $n = 5$ to be approximately 1.8, 1.3, 1.1, 0.9 and 0.8 μm respectively. Furthermore, the slope of CE as function of particle diameter for particles smaller than the critical particle size can be estimated from $R_{r/d}$ which suggests a slope of $a \cdot f$. The quadratic dependence on particle size is observed for $n = 1$ but not for the other modes. However the approximately linear dependence of the slope on frequency is clearly visible and thus the force ratio can be used as a simple but useful indicator for scale.

As an estimation of concentration speed we also calculate the time at which the concentration enhancement reaches a value of $CE_{\min}$ for the first time during $t = 0$ and $t = 60$ s, with $CE_{\min}$ being the minimum value of CE during $t = 50$ and $t = 60$ s. The average time over all modes and particle

sizes is 12.4 s, with a minimum time of 0.6 s and a maximum time of 46.8 s. To compare with classical acoustophoresis experiments we estimate the time required for 3 μm diameter PS particles in the $n = 1$ mode to all gather into the vertical sheet at $x = 0$, which takes approximately 6 s. Concentration of sub-micrometer particles using streaming-assisted acoustophoresis using these modes is thus expected to be up to a factor 8 slower in experiments compared with classic acoustophoresis with small particles in the micrometer range.

Particle tracing with double-mode excitation

In this section we study the concentration of submicrometer particles using simultaneous excitation of two modes. We calculate the acoustophoretic forces using equation 2.4 with two coefficients c_j equal to 1 and the rest equal to zero, perform particle tracing with the same parameters as in the previous section and calculate the concentration enhancement for various mode combinations as function of particle size.

The results for five excitation combinations are shown in figure 2.11 where we omitted the largest particle sizes for clarity of the graph. Generally the same trends are visible as in the case of single mode excitation, except for the combined $n = 1$ and $n = 5$ (indicated as 1 + 5) mode which shows zero concentration enhancement for all particle diameters except 50 and 100 nm. The same effect but with varying cut-off size also occurs for other double excitations modes not shown in this graph (modes 1 + 4, 2 + 3, 2 + 5, 3 + 5 and 4 + 5). CE is zero because more than 5 percent of the particles are concentrated in the channel center at the line $y = 0$ instead of at the horizontal walls. This can be explained as follows. For single mode excitation, the drag force in the regions between the streaming rolls only has a vertical component whose direction alternates between towards and away from the channel center (see section 2.3.2). The radiation force pushes particles into the regions with upwards streaming drag and out from regions with downward streaming drag in the upper half of the channel (compare figures 2.7 and 2.8 for $n = 2$). In case of combined modes the radiation force and streaming drag force are not necessarily aligned in the same manner. For example, the radiation force contribution from $n = 5$ in the 1+5 combination pushes particles towards the vertical line $x \approx 2w/5 = 152 \mu\text{m}$, while the $n = 5$ contribution to the streaming drag force is very small in the microchannel center. The contribution from the $n = 1$ mode is the opposite: the radiation force is much smaller and neglectable due to the lower frequency but the streaming for $n = 1$ extends over the full height of the channel, with a downward component at the vertical plane at $x \approx 2w/5 = 152 \mu\text{m}$. The combination of forces leads to a fraction of particles being concentrated at the positions $(x, y) = (\pm 2w/5, 0)$,

Figure 2.11: Concentration enhancement calculated from particle tracks in the case of simultaneous excitation of two modes for PS particles with diameters ranging from 50 nm up to 900 nm. Note the different particle size range compared with figure 2.10. The values are calculated as the average over the period $t = 50$ to 60 s and the shaded area has a thickness equal to twice the standard deviation during that period.

The calculated particle tracks at time $t = 12.0$ s and the time evolution of the concentration enhancement of the circled combination is shown in figure 2.12a.

while the rest is concentrated in the streaming rolls close to the horizontal walls. This effect is a combination of geometry and force scaling (dependence on a , f) and might be used for particle size selective concentration through proper choice of mode strengths (c_j).

The other modes shown in figure 2.11 perform better than their single mode counterparts in concentrating particles at the horizontal walls. The $1+2$ excitation shows better concentration (higher CE) for all particles sizes compared with the $n = 1$ and $n = 2$ modes. The $1+3$, $2+4$ and $3+4$ combinations concentrate particles smaller than 600 nm better than the best single mode, which is $n = 5$. As a general trend we observe that combined modes with higher frequencies concentrate better but the scaling is not clear as in the case of the single modes. We focus on the result for 100 nm PS particles with the $2+4$ mode combination, (indicated with a circle in figure 2.11). The

 (a) Calculated particle tracks at time $t = 12.0$ s.

(b) Concentration enhancement as function of time.

Figure 2.12: Particle tracks and concentration enhancement vs. time for PS particles with diameter $2a = 100$ nm with simultaneous excitation of the $n = 2$ and $n = 4$ modes. (a) Calculated particle tracks at time $t = 12.0$ s. The particle positions are indicated with a dot while the tracks are indicated with lines [color scale indicating local particle velocity magnitude ranging from 0 (dark) to $344 \mu\text{m s}^{-1}$ (light)]. The dotted line is placed at $y = CE_{t=60\text{ s}} \cdot h$ and indicates that 95% of the particles in the upper half of the channel are located above this line at time $t = 60$ s. (b) Concentration enhancement as function of time.

calculated particle tracks at time $t = 12.0$ s are shown in figure 2.12a and the time evolution of the concentration enhancement (or equivalently the vertical particle distribution) is shown in figure 2.12b. From the particle tracks a streaming flow pattern is visible with four rolls per quadrant, like the $n = 4$ mode. The addition of the $n = 2$ modes leads to unequally sized rolls with two large rolls on the sides and two small rolls in the middle of each quadrant, which matches the superposition of the streaming patterns for $n = 2$

and $n = 4$ shown in figure 2.7. The rolls are also displaced compared with the roll positions for $n = 4$: the four inner rolls between $x = \pm w/4$ are approximately $5\text{ }\mu\text{m}$ closer to $x = 0$, while the outer rolls are displaced the same amount to the outside. Most particles are trapped in the streaming flow of the large rolls and slowly spiral inwards as time progresses, as shown in figure 2.12b. A possible explanation for the spiraling could be an asymmetry in the radiation force caused by the slight displacements of the rolls and their non-circular shape. In case of single modes the radiation force is symmetric around the roll center and aligned with the symmetrically shaped streaming flows. The radiation force on the particle during one period of orbiting the roll center then leads to an average zero particle displacement. The roll displacements relative to the radiation force field and the asymmetric shape of the rolls caused by mode combination might introduce asymmetry, resulting in a small inward-pointing displacement every turn and subsequently causing the inward spiraling. The concentration by inward-spiraling is also observed using combined modes $1 + 2$, $1 + 3$ and $3 + 4$ and this explains the improved concentration compared with single mode excitation. The inward-spiraling for these modes can likewise be explained by the asymmetry reasoning above. Further study should lead to a more precise description and explanation of this effect.

2.3.3 Discussion

The results obtained do not include particle-particle or particle-fluid interactions so are valid only for low concentrations of particles. The accuracy of the particle tracing is limited by the assumption of point-like particles, especially in the streaming source regions close to the horizontal walls which contains large spatial gradients in the streaming velocity. This leads to a high sensitivity of the resulting particle trace on the particle position prior to entering the streaming source region. This effect can be seen when comparing figure 2.9c to figure 2.9d where the particle positions are symmetric around $x = 0$ at $t = 1.02\text{ s}$, but some are asymmetric at $t = 2.7\text{ s}$ shortly after going the source region. In figure 2.12a we also see a small asymmetry in the fraction of particles adhering to the wall. Additionally we see a small left-right asymmetry for particle tracks in regions with very low velocity like in the depleted regions of 2.12a, which might be due to the assumed boundary conditions for the second-order velocity. Overall we expect a small error in the quantitative results due to these assumptions but no effect on the qualitative results.

This also suggests a few improvements for follow-up studies. The effect of Brownian motion becomes increasingly important for submicrometer sized particles and thus could be included for improved modeling accuracy

of particle concentration. Another interesting route would be to include resonance modes which are spatially orthogonal to the modes described in this paper. Combining orthogonal modes is straightforward when the modes have a sufficiently large frequency difference because then the resulting first-order and time-averaged second-order fields can be linearly combined. Orthogonal modes at the same frequency are experimentally indistinguishable (the relative phase between the modes is ill-defined, but important [71]) so the use of frequency-separated modes simplifies experimental realization. Experimental verification of the second-order boundary condition would improve the accuracy of simulations, as well as including a particle wall distance-correction to the drag force. Generally the drag force is underestimated by equation 1.40 when the particle is close to the walls (within tens of micrometers [49]). Finally, an analytic study could improve the understanding of the role of asymmetry in spiraling particle concentration using simultaneous excitation.

Experimental verification of the results found is important and we note that the geometry of the streaming-assisted concentration method is different from usual experiments. The method shown concentrates particles in horizontal layers close to the walls instead of concentration of particles into vertical sheets, which is the case for classic acoustophoretic systems. However, by rotation of the geometry described in this paper by 90 degrees, i.e. by exciting vertical resonances, one can directly use the regular microchannel geometries and fabrication methods [52]. Particles are then concentrated towards the vertical side walls and can be extracted using the side outlets while the depleted fluid is extracted using the center outlet in standard trifurcation geometries [78, 79]. Combined with recirculation techniques we expect that enrichment of very small submicrometer particles is possible with high recovery [80].

2.4 Conclusion

In the first part of this chapter we gave a short overview of the literature regarding theoretical and experimental studies of acoustophoresis using fundamental and higher-order resonances. Little research has been done on acoustophoresis using simultaneous excitation of multiple higher-order modes in the acoustic streaming dominated limit.

After that we showed acoustophoretic experiments of the concentration of suspended polystyrene particles in water. The fundamental and first five harmonic modes of a rectangular microchannel in a glass-silicon microchip were used. The concentration of the particles was visualized by a simple op-

tical method based on fluorescence. 4 μm diameter polystyrene beads could be concentrated into the nodes of the first six acoustic resonances, indicating operation in the radiation-dominated regime. We also observed a lower particle size limit around 1 μm beads for the fundamental resonance, which indicates the transition to the streaming-dominated regime.

In the last section we numerically solved the thermoviscous acoustic problem in a water-filled rectangular microchannel for the first five horizontal acoustic resonances using a perturbation series. The first and second-order fields are obtained with good convergence and are consistent with experimental results from literature. The acoustic radiation force and streaming drag force are calculated from the fields for single modes and for combinations of simultaneously excited modes. The calculated force fields are used to perform particle tracing on small PS beads which closely mimics particle acoustophoretic experiments. We show that submicrometer sized PS particles are concentrated in streaming layers due to the limited vertical extent of the streaming flow combined with a stronger radiation force at higher frequencies. This leads to a significantly lower particle size limit for PS particles in water using higher-order resonances.

Simultaneous excitation of two modes can lead to improved concentration of submicrometer particles in the streaming layer but also in the channel center, depending on mode geometry and scaling of the forces. We observe concentration of particles as small as 100 nm by spiraling into the streaming rolls and offer an asymmetry argument as explanation. With this paper we set a next step towards engineering of streaming patterns for acoustophoretic concentration of submicrometer particles.

3 Direct laser writing of a large streaming-based micromixer

Direct Laser Writing (DLW) is a 3D-capable fabrication technique based on two photon polymerization of resist. We explore the use of DLW for the fabrication of a centimeter-scale acoustic micromixer, which consists of many cuboid micropillars. A prefabricated microfluidic chip is filled with resist and the laser writing of structures is performed through the glass cover of the chip. This ‘in-chip’-method provides good attachment of fabricated structures to both top and bottom of the microchannel, which is harder to achieve when fabricating structures in an open channel. Characterization of the structures using Confocal Laser Scanning Microscopy suggests that small initial misalignment limits the spatial extent of in-chip DLW. Therefore a trade-off must be made between writing time, accurate structure placement and initial alignment.[†]

3.1 Introduction

In the previous chapter we have seen that acoustic streaming limits the use of acoustophoresis for submicrometer particles using the classic rectangular microchannel geometry and using the fundamental resonance at 2 MHz. The original thought behind the work described in this chapter is to create a structure inside the microchannel which partially obstructs the streaming flow, thereby lowering the streaming velocity and thus lowering the streaming-induced drag force. The structure should be made from acoustically trans-

[†]. Parts of this chapter are published in ref. 81.

parent material to prevent scattering of the acoustic standing wave, i.e. the material should have the same specific acoustic impedance as the fluid in the microchannel. The specific acoustic impedance z_0 is the product of the mass density ρ times the speed of sound c_s and resins exist with acoustic impedances close to water [82]. The structures should also be robust and therefore should have good adherence into the microchannel. In-chip Direct Laser Writing is a fabrication technique suitable for this goal. However, only two specific acrylate-based resists were available for the use with DLW, with unknown acoustic properties. The fabricated structures proved to be unsuitable for streaming suppression as the mismatch of impedance leads to induced streaming at the structure walls itself, making the structure useful as a streaming-based micromixer instead.

There has been a considerable development in the field of mixing in microfluidic devices over the last decade [29, 83, 84]. Mixing, passive or active, is part of many laboratory methods and therefore adapting it to the micro and nano scale is important for Lab-on-a-Chip (LoC) and micro scale Total Analysis Systems (μ TAS) [85]. Fluidics at this scale are typically dominated by viscous forces and turbulent mixing is therefore hard to achieve.

One class of active mixers is ultrasonic mixers, which use acoustic fields at ultrasonic frequencies. Typically, these mixers either excite a flow at the sidewalls of a microfluidic chamber [86–88] or excite a flow inside the chamber using a bubble [89–91]. In case of homogeneous mixing, the chamber size is limited by the spatial extend of the induced flows, which subsequently limits the flow rate. Bubbles can be placed inside the volume and therefore allow higher throughput compared with sidewall induced flows, enabling fast mixing [92]. However, bubble-based micromixers also show serious challenges [88, 93] with regard to stability. The excitation frequency must match the geometric resonance frequency closely [94] and therefore it is dependent on flow rate [92] and is influenced by contamination and dissolving over time.

The mixer shown in this chapter operates by locally exciting flow around pillars embedded inside the microchannel, providing the main advantage of bubble-based mixing without the mentioned drawbacks. This method allows, in principle, for a larger channel cross section and effective mixing dispersed over that cross section, enabling high flow rates combined with improved stability compared with bubble mixers. To compensate for lower induced mixing flow speed, many micropillars in a row are used. The use of in-chip Direct Laser Writing is explored as a fabrication technique for the mixing structure, which requires accurate and repeatable pillar placement and shape over an extensive distance.

Direct Laser Writing is a flexible fabrication method for small batches of arbitrarily shaped microstructures. Using two photon polymerization only

the resist in the focal volume is cured. By displacing the focus with respect to the substrate arbitrarily shaped 3D structures can be created. The height of freestanding structures is limited by their structural strength.

The maximum fabricated size in previously reported work is limited to hundreds of micrometers [95, 96]. In this work we explore a strategy for in-chip DLW fabrication of microstructures on a centimeter scale. An important aspect in this work is the choice of resist, enabling a faster and simpler workflow [96]. The resist has a low viscosity, making it suitable for straightforward filling, development and cleaning by flowing chemicals directly through the microsystem. The resist also does not require pre- or post-exposure baking. This, combined with the improved handling, enables a 10x faster preparation and development process (2.5 hours vs. 26 hours) compared to earlier methods [97]. Confocal Laser Scanning Microscopy (CLSM) is used to non-destructively characterize the in-chip fabricated structures [98]. As opposed to Scanning Electron Microscopy (SEM), CLSM only requires optical access to the sample. Previously, DLW has been used to fabricate a wide variety of structures, such as nanochannels [99], “overpass”-structures for microfluidics [100], gold substrates for surface enhanced Raman scattering [101] (SERS) and nanoshells [102].

Integration into larger structures, as needed for microfluidics [18], is not straightforward. Due to the difference in scale, DLW cannot easily be used to fabricate both the structures and the channels since that would take unacceptably long. As such microstructures fabricated by DLW are usually fabricated separately from the channels, requiring fine control of structure size during fabrication and accurate alignment during assembly. The same is true for strategies where the structure is partially embedded into a channel which is subsequently covered with a glass or polydimethylsiloxane (PDMS) layer [103]. Examples of other in-chip fabrication techniques are 3D patterning using laminar flows [104], patterning of surfaces using microchannel networks [28] and structured elastomeric membranes [98]. However, these techniques do not offer the flexibility of arbitrary shapes as is the case with DLW.

In-chip DLW enables the direct integration of various functional structures into existing microchannels. Resist is flowed into the existing channels and structures are directly written and anchored into place. This ensures a good fit and reduces the amount of chemicals required. In-chip DLW was first used for enzymatic 3D microreactors by Iosin et al. [105]. Amato et al. fabricated a porous filter into an existing microchannel [97] and Serra et al. fabricated nematic liquid crystal cells [95]. Wu et al. demonstrated fabrication of both channels and microstructures by combined DLW and direct femtosecond-laser writing [106].

DLW allows rapid prototyping of 3D-microstructures without the need

Figure 3.1: Photograph (left) and cross-sectional sketch (right) of the mixing device (not to scale). The chip consists of a microchannel etched into silicon (grey), sealed with borofloat glass (blue). Access holes are etched into the backside of the chip on which tubing is connected (orange). The mixing structure fabricated using DLW (green) consists of two parallel rows of square pillars, both rows extending over a region of 1.2 cm. The acoustic mixing is excited using a piezo-element which is placed on top of the chip (illustrated in purple in both photograph and sketch). See figure 3.3 for a cross-sectional sketch in the xz -direction.

of masks for each variation. The work presented in this chapter provides the basis for more complicated structures, including structures which require full 3D capability, fabricated with DLW with an extensive length. At the same time in-chip DLW provides good bonding of structures which is important for robustness of the micromixer and mitigates the problem of alignment during assembly. In the next section we will first explain the in-chip DLW fabrication process, after which we characterize the structure in terms of geometry and strength. We show the limits of this fabrication method for long microstructures and finally we show acoustic mixing around the micropillars.

3.2 Experimental

3.2.1 Fabrication

The DLW system (Photonic Professional, Nanoscribe) uses a femtosecond pulsed laser at 780 nm wavelength at 80 MHz repetition rate. The system contains a Zeiss Axiovert inverted microscope with an autofocus / interface finding system and charge-coupled device (CCD) camera. The sample is moved using a positioning system consisting of a motorized stage and piezo stage. The motorized stage is used for coarse movements and has a lateral range of 100 mm in both directions and position accuracy better than 1 μm . The piezo stage is used for the laser writing and has a range of 300x300x300 μm^3 .

An objective with higher numerical aperture (NA) produces a smaller focal spot and higher resolution but also a smaller polymerized volume. For writing large structures a lower NA objective and increased laser power reduces writing time at the expense of resolution. However, the aspect ratio of the focal volume scales inversely with the NA, so that a lower NA translates into a more elongated focal volume. For in-chip laser writing also the objective working distance is important, as this limits the maximum axial size of the structures.

The objective used is a Zeiss LD Plan-Neofluar 63x with a numerical aperture of 0.75 and a working distance of 1.7 mm, resulting in a lateral and axial DLW resolution of 560 nm and 5.5 μm respectively. The resist is IP-L, a negative tone acrylate based resist (IP-L, Nanoscribe). IP-L does not require pre- or post-baking and has a low viscosity (0.8 Pa s), such that direct injection into microchannels is possible.

The microchannel used for in-chip DLW was fabricated using photolithography and deep Deep Reactive-Ion Etching (DRIE) on a $\langle 100 \rangle$ silicon wafer. Access holes of 1 mm diameter were made from the backside in the same way as the channels. The channel wafer is anodically bonded to a 500 μm thick borofloat glass wafer and then diced into glass-silicon microchannel chips measuring 6 by 1.5 cm, see figure 3.1. The length L_c , width w_c and depth h of the microchannel are 4 cm, 373 μm and 155 μm respectively.

The microchannel is filled with resist by connecting a tube to one of the access holes and manually filling the channel using a syringe. The tube is then removed and the chip is placed glass-down into the sample holder in the DLW system.

Sample tilt and rotation correction is performed using the interface finder and CCD. The writing of structures is performed from the silicon towards the glass (see figure 3.3), to prevent an aberrated structure due to diffraction from previously polymerized resist.

Large structures are written in sections using the piezo stage, then the motorized stage is used to move the sample to the beginning of the next section.

After writing the sample is removed from the sample holder and tube connectors are connected to the access holes. Using a syringe pump (PHD 4400, Harvard Apparatus), first 20 mL propylene glycol monomethyl ether acetate (RER600, Fujifilm) developer is flowed through the channel at a flow rate of 20 mL h⁻¹, followed by 20 mL isopropanol at 20 mL h⁻¹. A gentle flow of air is applied over the access holes until all isopropanol has evaporated, which takes less than 30 seconds.

3.2.2 Characterization

Structural analysis

For the 3D analysis of the structures we use Confocal Laser Scanning Microscopy (CLSM). The channel is filled with index matching oil (Nikon immersion oil, $n = 1.5$) to limit image deterioration by diffraction. We use a home-build CLSM based on a Nikon TE300 inverted microscope, with a 25 mW, 488 nm laser (iFlex-Mustang 488, QiOptiq), spinning disk-based confocal scanner (UltraView ERS, Perkin Elmer) and camera (C4742-95-12ERG, Hamamatsu). The axial z-position of the objective (S Plan Fluor ELWD 60X NA 0.7, Nikon) was scanned using an objective piezo scanner (PIFOC P-720, Physik Instrumente) with a range of 100 μm . Only the emitted autofluorescence from the structure was transmitted to the camera using a low-pass emission filter with a cut-off wavelength of 525 nm. For the overview image in Fig. 3.4 a regular fluorescence microscope (TE2000, Nikon) was used with a lower magnification objective (S Plan Fluor ELWD 45X NA 0.45, Nikon).

Using the autofluorescence of the polymerized resist only the structures are visualized, providing background-free 3D imaging. Although the fluorescence is used to our advantage, in other experiments the autofluorescence can be severely interfering (i.e. biofluorescence experiments). The autofluorescence excitation-emission map of cured IP-L resist was measured (F900 fluorometer, Edinburgh Instruments) and is shown in Fig. 3.2.

Acoustic mixing

To visualize the acoustic mixing we use the same CLSM. The channel is filled with 80%/20% by weight glycerol/water mixture, in which an 18% volume fraction of fluorescent silica tracer particles is suspended. The mixture is index matched to the silica particles. The spheres have an outer diameter of $2r = 1 \mu\text{m}$ and a core of 0.5 μm diameter filled with fluorescein isothiocyanate (FITC) fluorophore. The fundamental acoustic resonance of the channel at $f = 1.92 \text{ MHz}$, corresponding to a standing wave along the channel width, is excited using a piezo plate (PZ 26-302, Ferroperm) connected to an amplifier and function generator. The piezo plate is attached on top of the chip using a drop of paraffin oil as coupling liquid.

Assuming a plane wave resonance, the magnitudes of the acoustic radi-

Figure 3.2: Autofluorescence map of polymerized IP-L resist. A strong fluorescence emission band around 510 nm can be observed for excitation wavelengths from 520 nm down to 300 nm. The strong signal at emission wavelengths from 600 to 700 nm is a grating artifact.

ation force and the Stokes drag are [107]

$$F_{rad.} = 4\pi\Phi kr^3 E_{ac} \sin(2kx) \quad (3.1)$$

$$\Phi = \frac{1}{3} \left[\frac{5\frac{\rho_p}{\rho_m} - 2}{2\frac{\rho_p}{\rho_m} + 1} - \frac{k_p}{k_m} \right] \quad (3.2)$$

$$k = \frac{2\pi f}{c_m} = 2\pi f \sqrt{\rho_m k_m} \quad (3.3)$$

$$F_{drag} = 6\pi\eta r v_p \quad (3.4)$$

where Φ is the acoustic contrast factor, E_{ac} is the acoustic energy density, k is the wavenumber of the sound wave, x is the position along the width of the channel, c_m is the speed of sound in the mixture, $\rho_p = 2648 \text{ kg m}^{-3}$ and $\rho_m = 1208 \text{ kg m}^{-3}$ are the densities of the particles and the mixture, $k_p = 280 \text{ TPa}^{-1}$, $k_m = 255 \text{ TPa}^{-1}$ are the respective compressibilities, $\eta = 60 \text{ mPa s}$ is the viscosity of the mixture and v_p is the particle speed (values from Ref. 43, 108). Using $E_{ac} = 100 \text{ J/m}^3$, a particle speed of $30 \text{ } \mu\text{m s}^{-1}$ and evaluating the radiation force at it's peak position $x = w_c/4$ gives a ratio of

Figure 3.3: A cross-sectional sketch of the microchannel with the designed structure. The chip consists of a microchannel with width $w_c = 373 \mu\text{m}$ and height $h = 155 \mu\text{m}$. The structure (green) consists of two rows of square pillars, each extending over the full height of the channel and having a width of $w_p = 20 \mu\text{m}$. The rows are placed at a quarter and three quarters of the channel width. Each pillar is written using the focused laser beam (red) with the starting position of the focus designed to be at an anchoring depth $d_a = 7.5 \mu\text{m}$ inside the silicon. The progression of writing is from the silicon towards the glass as indicated by the arrow.

forces $F_{\text{drag}}/F_{\text{rad.}} \approx 85$. Therefore the silica particles are small enough to be used as tracers since the acoustic radiation force is negligible compared with the Stokes drag.

3.3 Results and discussion

3.3.1 Fabrication

The micromixer consists of two parallel rows of pillars, placed at one quarter and at three quarters of the channel width (see Fig. 3.3). Each pillar has a designed square cross-section of $20 \mu\text{m}$ by $20 \mu\text{m}$ and extends over the full depth of the channel. The writing volume extends into the glass and silicon by $7.5 \mu\text{m}$, ensuring proper anchoring. Two versions of the structure have been fabricated using different center-to-center spacings (Λ). The first version with $\Lambda_1 = 30 \mu\text{m}$ is used for characterization, the second version with $\Lambda_2 = 70 \mu\text{m}$ is used for the acoustic mixing. Both structures have a total length of 1.2 cm .

We used a distance between written lines and planes of 400 nm and 2 μm respectively, writing at a speed of 1 mm s^{-1} parallel with the channel. Writing is started at an anchoring depth of approximately 7.5 μm into the silicon. The rows are written using the piezo stage in sections of 9 subsequent pillars for Λ_1 and 4 subsequent pillars for Λ_2 , alternating between the top and bottom row after each section and moving to the next section using the motorized stage. Writing time per pillar is 295 seconds and the total time is approximately 66 hours and 29 hours for Λ_1 and Λ_2 respectively. The average power at the sample is estimated to be 70 mW.

3.3.2 Characterization

The confocal measurements for the structural analysis were done using steps of 100 μm in the z-direction using the objective scanner, 100 ms integration time per slice and 25 mW of laser power.

With exception of the last 1.1 mm, the fabricated structure matches the design closely with some minor deviations which are discussed in the sections below. The last segment shows strong irregularities, which will be investigated in Sec. 3.3.2.

Pillar placement

A typical section is shown in Fig. 3.4. The average side-to-wall distance between the top row and nearest wall is 84.0 μm and for the bottom row this distance is 81.2 μm . The designed value is 82.3 μm , so the structure is offset from the center of the channel by approximately 2 μm . The average angle between the rows and the walls is 6 mdeg. Corrected for the angle, the standard deviation in distance to the wall is 1.4 μm , which can be explained by the accuracy of the motorized stage. Within rows of nine consecutively written pillars (i.e. for structures written without the use of the motorized stage), the standard deviation in side-to-wall distance is on average 0.3 μm . reflecting the accuracy of the piezo stage using a relatively high writing speed.

The horizontal placement of the pillars is less accurate, reflecting in an average side-to-side separation of 7.1 ± 1.1 μm . Again, between consecutively written pillars the variation is smaller with 0.8 μm . The significant deviation from the designed separation of 10 μm is mostly explained by the consistently wider pillars, leaving less space in between. The variation in the width is 0.7 μm , explaining most of the variation between consecutively written pillars. The additional variation is caused by the motorized stage, but also by the writing direction: because of the relatively high speed the piezo positioning in this direction is less accurate.

Figure 3.4: Fluorescence image of part of the double row structure with $\Lambda = 30 \mu\text{m}$. Visible are four consecutively written pillar sections, with small jumps between sections by the motorized stage visible. The lines (red) indicate the microchannel walls.

Pillar shapes and cross sections

At the glass-pillar interface, the pillars have an average width in direction parallel to the channel of $22.5 \pm 0.7 \mu\text{m}$ and a width normal to the channel of $21.5 \pm 0.6 \mu\text{m}$. The pillars are not perfectly square and also slightly larger than the designed value of $20 \mu\text{m}$. In both the parallel and normal direction, most of the extra width is due to non-zero lateral size of the DLW focal volume. In the parallel direction, additional width comes from the fact that this is the laser writing direction. Given the small variations in the width, both effects could be corrected for in the design.

All pillars extend over the full depth of the channel. The change in cross section over the full height is less than $3 \mu\text{m}$ in both widths compared with the values at the glass interface. The displacement with respect to the glass surface is less than $4 \mu\text{m}$, meaning that the sidewall angle of the pillars is less than 1.5° .

Structural strength

The fabricated pillars are sufficiently strong for microfluidic applications, such as a ultrasonic micromixer. Forces due to developer and solvent flow, interfacial tension due to evaporation of the solvent and the force due to the ultrasonic pressure resonance have no influence on the structure.

The Reynolds number for the flow is

$$Re = \frac{\rho \cdot \bar{v} \cdot D_H}{\mu} \quad (3.5)$$

with ρ the density and μ the dynamic viscosity of the fluid, $\bar{v}$ the average flow speed and D_H the hydraulic diameter. For our case of a completely filled channel with rectangular cross section, D_H equals $2h \cdot w / (h + w)$. The average flow speed is the flow rate divided by the cross section and equals 97 mm s^{-1} . The Reynolds number for the developer and solvent flows are 1.7 and 0.8 respectively, sufficiently low to treat the flows as laminar.

Due to the presence of the walls, the drag on a single cylinder F_D does not depend on the Reynolds number, but on geometry only and has to be calculated using a wall correction factor $\lambda = F_D / (\mu \cdot v_{\max})$, with $v_{\max}$ the maximum flow speed in the channel [109, 110]. For a single circular cylinder in our geometry, $\lambda \approx 4$ and the drag force is estimated to be $F_D = 1 \text{ mN}$ [111]. Estimation of the drag via shear stresses, using a laminar flow profile and no-slip yields the same order of magnitude. Comparing this estimation with our case of square cylinders placed in a row shows that this value should be taken as a lower estimate, since both a square cross section as placing pillars closely in a row increase the drag [112, 113].

Based on the curvatures of the solvent surface surrounding the pillars during evaporation, we estimate a maximum Laplace pressure of -0.8 kPa inside the solvent, corresponding with a peak inward faced capillary force on the outermost pillar of approximately $3 \text{ }\mu\text{N}$.

Fabrication defects

The last segment of the structure covers the last 1.1 mm of the double row structure. Here the structure shows strong deviations from the designed characteristics, potentially limiting the scale on which we can use in-chip DLW. Some pillars are misplaced towards neighboring pillars, sometimes even touching completely. Towards the end of the section, some pillars are missing, implying that the flow forces during development were potentially the cause. CLSM measurements of pillars close to the beginning of the second segment are shown in Fig. 3.5 and 3.6.

Figure 3.5: Cross section of three pillars by CLSM taken at 128 μm from the glass interface, as indicated by the line in Fig. 3.6.

Figure 3.5 shows the cross section of three pillars at 128 μm from the glass interface. The cross sections of the pillars are intact, showing that pillars extend at least to this depth. The left pillar is wider in the direction parallel to the channel than expected, suggesting that the pillar is bent. The side-to-side distance between the first pillar and second is around 16 μm and 8.5 μm between the second and third, again indicating a writing error or bending in the first pillar. The right pillar is cut-off due to optical reflections and seems narrower than in reality.

A cross section along the height of the pillar is shown in Fig. 3.6. The pillar visible in the top-left is indeed bent at an angle of 5° and seems partially detached from the silicon interface. To the left is another pillar partially visible, showing the same problem.

This suggests that the sample tilt remaining after the tilt correction is approximately 0.04°, resulting in a slowly diminishing anchoring depth over the length of the rows and causing only partial anchoring in the last segment. The forces during the development phase, as discussed earlier, then cause bending or complete displacement of pillars if the adhesion at the glass is insufficient. In the most extreme case pillars are removed altogether. This shows the importance of correct tilt and rotation correction for large-scale microstructures.

Fabrication time

The total fabrication time is long when compared to other rapid prototyping techniques, although those do not provide the same flexible 3D fabrication

Figure 3.6: Cross section of three pillars by CLSM. The line indicates the position of the cross section shown in Fig. 3.5. The silicon and glass interface is at the top and bottom of the figure respectively.

capability. A possibility to speed up the process is to increase the line and plane separation, which was set to 400 nm and 2 μm respectively. Considering the DLW focal spot size, the plane separation could have been increased to 4 μm , diminishing the writing time by almost 50%. Further more, a strategy can be employed where only the walls of the structure are written, trapping liquid resist inside that can be cured with an additional illumination step [102].

3.3.3 Acoustic mixing

Figure 3.7: Streaming induced flow around micropillars visualized by fluorescent tracer particles using CLSM. The black line at the bottom is a measurement artifact, not the channel wall. The piezo excitation frequency is 1.92 MHz.

The confocal measurements were done at $10\text{ }\mu\text{m}$ from the glass/channel interface, 100 ms integration time per frame and 25 mW of laser power. The structure has a center-to-center pillar spacing Λ of $70\text{ }\mu\text{m}$ and is in all other aspects the same as the structure characterized in Sec. 3.3.2. The piezo excitation voltage is 16 V_{pp} and the excitation frequency is 1.92 MHz.

The local acoustic field, composed of the fundamental channel resonance and the locally scattered fields, induces Rayleigh-Schlichting streaming at the pillar walls [57, 114]. The streaming is visualized by fluorescent tracer particles around the pillars (see Video 3.7). The asymmetry of the streaming is likely to be caused by the slow bulk flow going to the right. The speed of the induced flow u_{ac} is approximately $30\text{ }\mu\text{m s}^{-1}$ and leads to rigorous mixing of the suspension in the vicinity of the pillars. To give an estimate of mixing performance, we assume a mixing flow around each pillar of u_{ac} and a flow distance required for mixing of $w_c/2$ (because of two rows). The structure would then require a mixing time of approximately $t = 6$ seconds. Since pillars over a length of $l = 12\text{ mm}$ contribute to the mixing, this leads to an estimated flow rate of $7\text{ }\mu\text{L min}^{-1}$ which is comparable to other acoustic mixers [87, 115, 116] but not as fast as the fastest bubble mixer [92].

3.4 Conclusion and Outlook

We have demonstrated the fabrication of a 1.22 cm long micropillar micromixer using in-chip Direct Laser Writing. Most of the structure matches the designed double row very well. The largest source of deviation is caused by the motorized stage. Pillars which were consecutively written are accurately placed with micrometer precision. The fabricated pillars show good bonding with both the glass and silicon and are strong enough to withstand forces in the order of millinewtons.

The intrinsic fluorescence of the acrylic-based resist allows for straightforward optical characterization. Both fluorescence microscopy as well as confocal laser scanning microscopy was employed to successfully assess the fabricated structure.

We have demonstrated the proof-of-principle of a novel type of ultrasonic micromixer, based on locally inducing flows on micropillars embedded inside the microchannel. The local streaming flow speed in the vicinity of the pillars is $30 \mu\text{m s}^{-1}$, which would yield an estimated flow rate of approximately $7 \mu\text{L min}^{-1}$ with homogeneous mixing. The fabrication technique explored is able to precisely fabricate a long mixing structure, which is important for high flow rate mixing. This combined with further research on the effect of pillar placement and 2D/3D shape on the induced streaming flow and mixing efficiency will likely lead to improvement of the micropillar-based micromixer, which combines robustness and stability with higher throughput flows.

Part II

Optical Techniques

Introduction to Part II

In part I we learned about the theory, experiments and applications of acoustics in microfluidic systems, especially focusing on acoustophoresis which is driven by the acoustic radiation force. For submerged particles in an acoustic resonator, I have shown that the effectiveness of acoustophoretic particle concentration depends directly, amongst other parameters, on the acoustic resonance strength. The experimental characterization of an acoustic resonance is typically done using a particle-based method.

However, the relative strength of the acoustophoretic effect diminishes with particle size due to the different scaling of the acoustic forces involved. When studying this transition from the acoustic radiation force-dominated regime into the acoustic streaming regime, then particle-based resonance characterization is not feasible and an independent, alternative method is required.

In the next part of this thesis I will discuss an interferometric imaging technique which is especially developed for particle-less characterization of acoustic resonances in microfluidic systems. An acoustic wave is a compression wave, modulating both the pressure and density of the medium. The modulated density of the fluid also leads to modulation of the refractive index. By measuring this refractive modulation at various positions in a microchannel it is possible to image acoustic resonances.

The imaging of acoustic resonances is shown in Chapter 5 after introduction of the theoretical background. In the final chapter I show a spin-off of the same optical method, but applied to a different problem: fast measurement of micro-cracks in railroad tracks. Instead of a varying refractive index, changes in optical path occur due to height differences on the track. The white-light interferometric method allows single shot detection of micro-cracks.

4 | Theoretical background

This chapter serves as a theoretical introduction to the next chapters where we discuss interferometric measurements of acoustic waves and surfaces. First we discuss a simple model for acoustic resonances in microchannels. Next we show how acoustic resonances interact with light by showing how the pressure modulates the refractive index. Lastly we discuss the theory of partially coherent light and its application to interferometric measurements.

4.1 Simplified acoustic resonances

The acoustic resonances in this part of the thesis are treated in the adiabatic limit, leading to some simplification of the theory compared with chapter 1. The acoustic pressure $p = P - P_{\text{atm}}$ in water in a rectangular, hard-walled channel at resonance can be approximated by [75]

$$p = p_a \cos\left(\frac{n_x \pi}{w} x\right) \cos\left(\frac{n_y \pi}{h} y\right) \cos\left(\frac{n_z \pi}{l} z\right) \cos(2\pi f_{n_x, n_y, n_z} t) \quad (4.1)$$

with P absolute pressure, P_{atm} the equilibrium pressure, p_a acoustic pressure amplitude, n_j an integer indicating the mode order in the respective direction and l, w, h length, width and height of the microchannel respectively. The corresponding resonance frequencies are

$$f_{n_x, n_y, n_z} = \frac{c_s}{2} \sqrt{\frac{n_x^2}{w^2} + \frac{n_y^2}{h^2} + \frac{n_z^2}{l^2}} \quad (4.2)$$

with c_s the speed of sound in water. Pure modes are considered to be modes with a nonzero mode number in one dimension and zero in the others, e.g. the fundamental ‘width’-mode has $n_x = 1$ and n_y and n_z equal to zero. We will mostly use the pure ‘width’-modes and these modes are referred to as

p_{n_x} . The acoustic energy density E_a at resonance follows a Lorentzian line shape as function of frequency around the resonance frequency f_0 :

$$E_a = p_a^2 / (4\rho_0 c^2) \quad (4.3)$$

$$E_a(f) = \frac{E_0}{\left[\frac{2Q}{f_0} (f - f_0) \right]^2 + 1} \quad (4.4)$$

with E_0 the energy density at f_0 , ρ_0 the density at equilibrium and Q being the quality factor related to the energy dissipation. The full width at half maximum (FWHM) Δf equals f_0/Q .

4.2 Acoustic modulation of refractive index

Compression of a transparent optical medium typically increases the optical index of refraction, i.e. the light propagates slower. As we will see below, the dominant mechanism is that compression leads to a higher mass density and the optical wave thus interacts with more matter. The interaction strength between the acoustic pressure and index of refraction is expressed by the adiabatic acousto-optic coefficient $\alpha_{ac} = \partial n / \partial p$. The starting point of derivation is the Lorentz-Lorenz (or Clausius-Mosotti) equation, which relates the index of refraction n to the molecular polarizability α [117]:

$$\frac{n^2 - 1}{n^2 + 2} = 4\pi/3 \left(\frac{\rho N_A}{M} \right) \alpha. \quad (4.5)$$

Here ρ the mass density, N_A Avogadro's number and M the molar mass. The Lorenz-Lorenz (LL) equation assumes a homogenous, isotropic medium in which all interactions are dipolar in nature. From first principles it can be shown that the LL equation is valid for water [118]. Taking the derivative of formula 4.5 with respect to density yields

$$\rho \frac{dn}{d\rho} = \frac{(n^2 - 1)(n^2 + 2)}{6n} (1 - \Delta_0) \quad (4.6)$$

with Δ_0 defined as $-(\rho/\alpha)(d\alpha/d\rho)$ and the term on the left hand side known as the elasto-optic coefficient [119]. For most liquids Δ_0 is very close to zero, because the intermolecular distance is large such that compression of the medium has no influence on the molecular charge distribution and thus also not on the molecular polarizability. High pressure measurements of water show a modest change in α of about 4 percent at 5.92 GPa and 673 K compared with standard conditions [120], showing that for our experimental conditions we

may reasonably take Δ_0 equal to zero. The adiabatic equation of state relates the pressure and density fluctuations in a fluid [44]:

$$p = P - P_0 = \beta s + 1/2\beta(\gamma - 1)s^2 \quad (4.7)$$

with β the adiabatic bulk modulus, $\gamma - 1$ the parameter of nonlinearity, the condensation $s = (\rho - \rho_0)/\rho_0$ and ρ_0 the unperturbed density. Combining formula 4.6 with the derivative of formula 4.7 with respect to density and using the thermodynamic definition $c_s^2 = \beta/\rho_0$ with c the speed of sound yields

$$\frac{dn}{dp} = \frac{1}{\rho_0 c_s^2} \frac{(n^2 - 1)(n^2 + 2)}{6n(s + 1)(1 + 3/2(\gamma - 1)s)}. \quad (4.8)$$

Direct evaluation of equation 4.8 at $p = 0$ gives the acousto-optic coefficient α_{ac} , while taking the derivative with respect to p and subsequent evaluation at $p = 0$ gives the quadratic acousto-optic coefficient ϵ_{ac} . Together we get the change in index of refraction δn due to the acoustic pressure p

$$\delta n = \alpha_{ac} \cdot p + \epsilon_{ac} \cdot p^2. \quad (4.9)$$

Using the positive root of formula 4.7 for the condensation as function of acoustic pressure and equilibrium parameters,

$$s(p) = \left(\sqrt{1 + 2(\gamma - 1)(\rho_0 c^2)^{-1}p} - 1 \right) (\gamma - 1)^{-1}, \quad (4.10)$$

wavelength of light $\lambda = 870$ nm, water temperature of 30 °C, $\rho_0 = 995.6$ kg/m³ [121], $n = 1.3264$ [122], $c = 1509$ m/s [123], $\gamma = 6.2$ [124], we find the acousto-optic coefficient $\alpha_{ac} = 1.58 \times 10^{-10}$ Pa⁻¹ and $\epsilon_{ac} = -6.13 \times 10^{-19}$ Pa⁻². We will use these values in the next chapter. Other investigators derived a value $\alpha_{ac} = 1.26 \times 10^{-10}$ Pa⁻¹ assuming a linear dependence of electric susceptibility on density [125].

4.3 Partial coherence of light

Light we encounter in the real, physical world is neither fully coherent nor incoherent. Coherent light is associated with periodic properties and is therefore perfectly predictable. For instance, consider the case of the monochromatic plane wave, described using the complex wavefunction $U(\mathbf{r}, t)$,

$$U(\mathbf{r}, t) = A \exp[-i\mathbf{k} \cdot \mathbf{r}] \exp[i2\pi\nu t] \quad (4.11)$$

with A indicating the amplitude, $\mathbf{k} = (k_x, k_y, k_z)$ the wavevector describing the direction and ν the frequency of the electromagnetic radiation. The magnitude of the wavevector equals the wavenumber $k = 2\pi/\lambda$, with $\lambda = c/\nu$ the wavelength and c the speed of light in the medium. The complex representation is convenient for manipulation during calculations. In the end the electric field is found by taking the real part of the resulting complex wavefunction:

$$\begin{aligned} u(\mathbf{r}, t) &= \text{Re}(U) = \frac{1}{2} [U(\mathbf{r}, t) + U^*(\mathbf{r}, t)] \\ &= A \cos(\mathbf{k} \cdot \mathbf{r} - 2\pi\nu t). \end{aligned} \quad (4.12)$$

The electric field of the monochromatic plane wave is periodic in both space and time, as seen from equation 4.12. Therefore the electric field is exactly known at any time and at any place.

Now consider the light from a real light source, for instance the sun, a candle or light emitting diode (LED). Light is generated in random events from a large ensemble of emitters (ie. atoms, molecules or electron-hole pairs), at random positions and times, creating unpredictable fluctuations. The resulting electromagnetic fields are therefore not totally predictable and cannot be described completely by a deterministic form as in case of the plane wave. To describe real light we need to introduce a few statistical measures. In the next sections we summarize the treatment of statistical optics by Saleh and Teich [126].

An important measure is the intensity, or irradiance, because this is the property most detectors measure. It corresponds to the energy carried by the light per unit area and per unit time. The average optical intensity

$$I(\mathbf{r}, t) = \langle |U(\mathbf{r}, t)|^2 \rangle \quad (4.13)$$

is taken as an ensemble average over many fluctuating fields generated under the same conditions. If the average optical intensity is independent of time, then U and $|U|^2$ fluctuate randomly but the average optical intensity is stationary. The average can then be taken over time instead:

$$I(\mathbf{r}) = \lim_{T \rightarrow \infty} \frac{1}{2T} \int_{-T}^T \langle |U(\mathbf{r}, t)|^2 \rangle dt. \quad (4.14)$$

For practical measurements, one can take the averaging period T to be shorter than infinity, but long enough such that the average is still statistically stationary during that period. This is the case for the experiments described in this thesis.

4.3.1 Temporal coherence

Partially coherent light is locally coherent but lacks long range order. So it would be useful to have a measure of coherence, i.e. how fast does the local order disappear. The autocorrelation function of $U(\mathbf{r}, t)$ at a single point $\mathbf{r}$ with a time delay τ is called the temporal coherence function and is given by

$$G(\tau) = \langle U^*(t)U(t + \tau) \rangle \quad (4.15)$$

where average is to be taken as the time average discussed above. $G(\tau)$ measures how much the field U and a time-shifted version of itself change in unison, as function of time delay. Note that $G(0) = I(\mathbf{r})$ so that G contains information about the average intensity as well as the temporal extent of the coherence. For a pure measure of coherence we divide by the average intensity to get the complex degree of temporal coherence $g(\tau)$:

$$g(\tau) = \frac{G(\tau)}{G(0)} = \frac{\langle U^*(t)U(t + \tau) \rangle}{\langle U^*(t)U(t) \rangle}. \quad (4.16)$$

$g(\tau)$ is a nice, well behaving measure of temporal coherence as its absolute value $|g(\tau)|$ attains values between 0, meaning no coherence, and 1, meaning full coherence.

In case of a physical light source $|g(\tau)|$ will decrease from 1 for $\tau = 0$ towards zero for increasing τ . The value of τ for which $|g|$ drops to $1/e$ is called the coherence time τ_c ¹. During a period shorter than τ_c , the field behaves proximately sinusoidally, but at times earlier or later than τ_c it is not possible to predict either amplitude or phase. The coherence time corresponds with a coherence length

$$l_c = c \cdot \tau_c \quad (4.17)$$

which is the distance over which the traveling light behaves approximately sinusoidally. Note that the coherence length l_c tells you nothing about coherence in the direction perpendicular to the direction of travel, such as on a screen on which light impinges. The lateral coherence, or *spatial coherence*, will be touched upon in section 4.3.3.

The monochromatic plane wave defined by formula 4.11 has a complex degree of temporal coherence

$$g(\tau) = \exp(i2\pi\nu\tau) \quad (4.18)$$

with $|g(\tau)| = 1$ independent of τ and a coherence time τ_c and length l_c equal to infinity, exhibiting perfect temporal coherence.

1. The coherence time is a measure for the width of $|g(\tau)|$. Many other measures of width are possible, such as the value of τ for which $|g|$ drops to $1/2$, or the power-equivalent width $\tau_c = \int_{-\infty}^{\infty} |g(\tau)|^2 d\tau$.

4.3.2 Power spectral density

The temporal coherence function of the field $G(\tau)$ is related to the power spectral density $S(\nu)$ of the light via the Wiener-Khinchin theorem; they are in fact a Fourier transform pair:

$$S(\nu) = \int_{-\infty}^{\infty} G(\tau) \exp(-i2\pi\nu\tau) d\tau \quad (4.19)$$

$$\begin{array}{c} \Downarrow \mathcal{F} \\ G(\tau) = \int_{-\infty}^{\infty} S(\nu) \exp(i2\pi\nu\tau) d\nu. \end{array} \quad (4.20)$$

The power spectral density, or loosely ‘spectrum’, measures how much intensity a single frequency, or color, is carrying. This relation is used in numerous experimental techniques where either the time-delayed autocorrelation $G(\tau)$ or the spectrally resolved power density $S(\nu)$ is easier to measure and then the relation is used to calculate the other. Interesting examples range from spectrally resolved imaging in space [127] and magnetic resonance spectroscopy [128] to terahertz-spectroscopy [129], mass spectroscopy [130] and resolving the structure of proteins [131].

The width of the spectrum $\Delta\nu$ is inversely proportional to the coherence time τ_c . A broader spectrum has a larger bandwidth and thus supports faster changes, leading to a shorter coherence time and coherence length. The monochromatic plane wave consists of only a single frequency and therefore has an infinitely small spectrum and, as we saw before, an infinite coherence length. Sunlight has a broad spectrum and a short coherence length, on the order of 500 nm, while a single-mode Helium-Neon laser has a coherence length of 300 m. This also means one can increase the temporal coherence by limiting the spectrum, e.g. by using a spectral filter.

4.3.3 Spatial coherence

Previous discussion limited the description of partial coherence to a single position in space. The effects of spatial coherence can be included by using the cross-correlation of the field at two positions $\mathbf{r}_1$ and $\mathbf{r}_2$ with a time delay τ . The result is the mutual coherence function $G(\mathbf{r}_1, \mathbf{r}_2, \tau)$

$$G(\mathbf{r}_1, \mathbf{r}_2, \tau) = \langle U^*(\mathbf{r}_1, t) U(\mathbf{r}_2, t + \tau) \rangle \quad (4.21)$$

and the normalized form, the complex degree of coherence

$$g(\mathbf{r}_1, \mathbf{r}_2, \tau) = \frac{G(\mathbf{r}_1, \mathbf{r}_2, \tau)}{\sqrt{G(\mathbf{r}_1, \mathbf{r}_1, 0)G(\mathbf{r}_2, \mathbf{r}_2, 0)}} = \frac{G(\mathbf{r}_1, \mathbf{r}_2, \tau)}{\sqrt{I(\mathbf{r}_1)I(\mathbf{r}_2)}}. \quad (4.22)$$

The absolute value of $g(\mathbf{r}_1, \mathbf{r}_2, \tau)$ assumes values between and including 0 and 1 and contains information about both spatial and temporal coherence.

To get a measure of spatial coherence only, we set τ to zero and we end up with the normalized mutual intensity $g(\mathbf{r}_1, \mathbf{r}_2)$

$$g(\mathbf{r}_1, \mathbf{r}_2) = \frac{G(\mathbf{r}_1, \mathbf{r}_2, 0)}{\sqrt{I(\mathbf{r}_1)I(\mathbf{r}_2)}} \quad (4.23)$$

whose absolute value is again bounded by 0 and 1 to give a measure of spatial coherence. The spatial coherence of light at a point $\mathbf{r}_1$ is given by $|g(\mathbf{r}_1, \mathbf{r}_2)|$ as function of the distance $|\mathbf{r}_1 - \mathbf{r}_2|$. The area for which $|g(\mathbf{r}_1, \mathbf{r}_2)| > 1/e$ is called the coherence area and the corresponding radius ρ_c of the inscribed circle is called the coherence distance.²

4.3.4 Propagation of partially coherent light

Imagine a partially coherent light source such as an candle. Light is originating from two different points $\mathbf{r}_a$ and $\mathbf{r}_b$ in the flame. Although the light has the same spectrum (color), the electric fields are completely uncorrelated because they originate from different excited molecules decaying at different times. The field at each point is coherent with itself, as seen from equation 4.18. For simplicity, I assume at this point that the light is spatially incoherent,

$$|g(\mathbf{r}_1, \mathbf{r}_2)| = \sigma \delta(|\mathbf{r}_1 - \mathbf{r}_2|) \quad (4.24)$$

with δ being the Dirac delta function and $\sigma = \int g(\mathbf{r}) d\mathbf{r}$ being the area under $g(\mathbf{r})$.

When incoherent light propagates through an optical system with an impulse response function $h(\mathbf{r}, \mathbf{r}')$, the mutual intensity $G(\mathbf{r}_1, \mathbf{r}_2, 0)$ becomes

$$G_2(\mathbf{r}_1, \mathbf{r}_2, 0) = \sigma \int h^*(\mathbf{r}_1; \mathbf{r}) h(\mathbf{r}_2; \mathbf{r}) I_1(\mathbf{r}) d\mathbf{r} \quad (4.25)$$

with $I_1(\mathbf{r})$ the input intensity distribution and primed positions indication positions in the input plane. Equation 4.25 shows that spatial coherence is

2. Different choices for the threshold value are possible, like in the case of the temporal coherence time.

gained after propagation. Propagation through free space can be described by the impulse response function

$$h(\mathbf{r}; \mathbf{r}') = h_0 \exp\left\{-i\pi \frac{x^2 + y^2}{\lambda d}\right\} \exp\left\{i2\pi \frac{xx' + yy'}{\lambda d}\right\} \quad (4.26)$$

with d the distance between the input and output planes, $\mathbf{r} = (x, y, d)$ and $\mathbf{r}' = (x', y', 0)$ coordinates of points in the output and input planes, respectively and $h_0 = (i/\lambda d) \exp(-i2\pi d/\lambda)$. Equation 4.26 uses the Fraunhofer approximation and therefore is valid for large distances d . By substitution of eqn. 4.26 into eqn. 4.25 we find that the mutual intensity after a large distance of free propagation becomes

$$|G_2(\mathbf{r}_1, \mathbf{r}_2, 0)| = \sigma |h_0|^2 \left| \iint \exp\left\{i \frac{2\pi}{\lambda d} [(x_2 - x_1)x + (y_2 - y_1)y]\right\} I(x, y) dx dy \right| \quad (4.27)$$

and for the normalized mutual intensity we get

$$|g_2(\mathbf{r}_1, \mathbf{r}_2, 0)| = \left| \mathbb{I}_1 \left(\frac{\mathbf{r}_2 - \mathbf{r}_1}{\lambda d} \right) \right| / \mathbb{I}_1(\mathbf{0}) \quad (4.28)$$

with $\mathbb{I}_1(\nu_x, \nu_y) = \mathcal{F}(I_1(\mathbf{r}))$ being the Fourier transform of the input intensity distribution. If the area of the source is small, then the Fourier transform will be wide and therefore the spatial coherence area at the output plane will also be large. Assuming a spherically shaped candle flame with a diameter a of 1 cm, then the coherence distance ρ_c at 100 meter distance is $\rho_c = 1.22 \frac{\lambda d}{a} \approx 7$ mm. Note that we started with completely incoherent light which gained coherence by traversing free space.

4.4 Wave interference with partial coherence

When two partially coherent waves with complex wavefunctions U_1 and U_2 are combined and measured, we measure the average intensity I

$$\begin{aligned} I &= \langle |U_1 + U_2|^2 \rangle = \langle |U_1|^2 \rangle + \langle |U_2|^2 \rangle + \langle |U_1^* U_2| \rangle + \langle |U_1 U_2^*| \rangle \\ &= I_1 + I_2 + \text{Re}(G_{12}) \\ &= I_1 + I_2 + 2\sqrt{I_1 I_2} \text{Re}(g_{12}) \\ &= I_1 + I_2 + 2\sqrt{I_1 I_2} |g_{12}| \cos \varphi \end{aligned} \quad (4.29)$$

with $G_{12} = \langle |U_1^* U_2| \rangle$ the statistical average, $g_{12} = G_{12}/\sqrt{I_1 I_2}$ the normalized average and $\varphi = \arg \{g_{12}\}$. The exact form of the statistical average

Figure 4.1: A scanning Michelson interferometer, consisting of a beam splitter (BS), a mirror in the sample arm (M_{sample}) and another in the reference arm (M_{ref}). The position z_m of the reference mirror can be changed, such that the optical path length difference between the two arms is changed by twice that amount.

depends on how the two waves are related to each other and how they are combined.

In a Michelson interferometer, the light wave originating from a light source is divided by a beam splitter into two arms, at the end of each the light is reflected back through the beam splitter where it is combined into the output port, see figure 4.1. The division and recombination of incoming and outgoing waves, respectively, is done while preserving a fixed phase relation between the waves. An ideal 50:50 beam splitter separates an incoming wave into two coherent copies, each carrying away half of the incoming intensity.

A scanning mirror in one of the arms induces an optical delay $\tau = 2z_m/c$ by making the optical path larger or smaller with the amount z_m . When light from the two arms is combined at the output of the interferometer, the light is added to a time-shifted version of itself. The complex wavefunction of a partially coherent plane wave traveling in the z direction is

$$U(\mathbf{r}, t) = a(t - z/c) \exp(i2\pi\nu(t - z/c)) \quad (4.30)$$

with $a(t)$ a random fluctuating amplitude. Substituting the wavefunction into

equation 4.21 gives the mutual coherence function

$$G(\mathbf{r}_1, \mathbf{r}_2, \tau) = g_a(\tau - (z_2 - z_1)/c) \exp(i2\pi\nu(\tau - (z_2 - z_1)/c)) \quad (4.31)$$

with z_1 and z_2 the z -component of $\mathbf{r}_1$ and $\mathbf{r}_2$ respectively and $g_a(\tau) = \langle a^*(t)a(t + \tau) \rangle$. The complex degree of temporal coherence $g(\tau)$ becomes

$$g(\tau) = g_a(\tau) \exp(i2\pi\nu\tau) \quad (4.32)$$

and the intensity on the output screen is given by eqn. 4.29:

$$I = 2I_0 \{1 + |g_a(\tau)| \cos(2\pi\nu\tau + \phi_a(\tau))\}. \quad (4.33)$$

By recording I as function of τ (by shifting the mirror) one obtains an interferogram as seen in figure 5.4. The relevant length scale is the coherence length $l_c = c\tau_c$ which is inversely related to the width of the spectrum of the light. The interferogram $I(\tau)$ peaks around the point $\tau = 0$, which is when both interferometer arms have the same length ($z_m = 0$). When the optical delay is smaller than the coherence length fringes will be visible, with decreasing visibility for increasing delay. When the path length difference is larger than the optical coherence length, no fringes are visible anymore and the intensity becomes constant.

5 | Imaging acoustic modes with white-light interferometry

In this chapter we present a technique for imaging acoustic resonances in a water-filled microchannel using a stroboscopic Michelson interferometer. The local acoustic pressure influences the local water density and thus also the refractive index. The acoustic field, both amplitude and phase, is determined by measuring the acoustically induced optical delay using stroboscopic illumination. The pressure distributions are measured for the fundamental pressure resonance in the water and two higher harmonic modes. By combining measurement at a range of excitation frequencies a frequency map of modes is made, from which the spectral line width and Q-factor of individual resonances can be obtained.[†]

5.1 Introduction

Following the trend towards the microscale, various acoustic techniques such as surface acoustic waves [132–134] (SAW), acoustic bubbles [135] or acoustic actuation [136] have been developed for use in Lab-on-a-Chip (LoC) technology. Likewise the use of sound-based particle separation and trapping was explored [137–140].

Ultrasonic standing waves for particle manipulation have been integrated onto silicon/glass microchips by precise microfabrication techniques and various configurations have emerged on the silicon/glass platform [141–145]. To aid design and improve performance physical models have been de-

[†]. Parts of this chapter are published in ref. 77.

veloped to describe the acoustic resonances [73, 75, 79, 146–148] which predict the resonance frequency and pressure amplitude distributions well for thin chips [149, 150].

Due to the high sensitivity of the acoustic resonances on resonator geometry, chip geometry, ultrasonic transducer or SAW generator, acoustic coupling and other experimental factors, it is generally hard to predict and measure the exact acoustic mode and resonance strength, hindering direct comparison between different experiments and configurations. For measuring the acoustic amplitude, particle based methods [45, 76, 151] provide high sensitivity but require sufficiently large particles (typically 5 μm in diameter or larger) to be present in the region of interest. As such the acoustic amplitude cannot be measured in experiments without large particles or in regions where no particles can be placed. As the acoustic force exerted on the particles depends on the pressure squared, only the amplitude of the pressure can be measured and full information of the modes, including the acoustic phase, cannot be obtained.

In this chapter we present a novel and particle-less method for measuring the local acoustic field inside a microchannel. The method is based on optically measuring the local change in index of refraction of water due to the acoustic pressure. Stroboscopic, incoherent illumination allows for measuring both the local acoustic amplitude and phase of the pressure field. By combining measurements from a range of excitation frequencies, mode spectrograms can be made giving an overview of modes present. The resonance strength and frequency of a mode can be determined from the spectral line obtained from the spectrogram.

We study pressure resonances in a silicon/glass chip containing a microchannel filled with water, which is used as a platform for acoustophoresis [45, 73]. Our method is based on light traveling through the glass and water, reflecting at the bottom of the channel and returning along the same path, thereby probing the pressure induced variation of index of refraction δn in the water (see figure 5.2). Any acoustic density variations in the glass layer will also contribute to the measured variation. This contribution from the glass is determined separately by measuring light reflected from the glass/water interface and is found to be relatively small (up to 20%) compared with the acoustic variation due to the pressure the water.

We spatially resolve the acoustic pressure field, both amplitude and phase, of the fundamental resonance and two higher harmonic modes using a stroboscopic illumination scheme. The acoustic amplitudes measured are 9.55 ± 0.20 MPa, 154 ± 2.1 kPa and 196 ± 2.5 kPa respectively. The measured pressure distributions match the theory well. First we discuss the

experimental setup and measurement method, followed by results and concluding remarks.

5.2 Experimental setup

The setup is based on a white-light phase-shifting Michelson interferometer design as used by Shavrin et al. [152], see figure 5.1. A Light Emitting Diode (LED) with a center wavelength of $\lambda = 870$ nm and a spectral FWHM of $\Delta\lambda = 50$ nm is used.

The emission from the LED surface is highly multimode so that only a limited coherence exists between different points on the surface. The emitting surface is imaged through the beamsplitter onto both the reference mirror in the reference arm and the sample in the sample arm using the illumination lens. Because the beamsplitter splits the field coherently, the two images are mutually point-wise coherent [153]. The images are re-imaged through the beamsplitter and projected onto the EMCCD detector by the detection lens. The sample is aligned such that the images overlap on the sensor.

Interference of light returning from the sample and reference arms only

Figure 5.1: Schematic illustration of the experimental setup, which consists of a fast Light Emitting Diode LED, microscope objective OBJ (M Plan SLWD 40x 0.40, Nikon), mirror M, illumination lens L_I ($f = 100$ mm), non-polarizing 50:50 beamsplitter cube BSC, reference mirror on an axial translation stage M_{ref} , sample with water-filled microchannel with attached excitation piezo, detection lens L_D ($f = 75$ mm) and an EMCCD camera. The light path illustrates the path originating from one point on the light emitting area of the LED.

occurs if the two fields are both spatially and temporally coherent. The first requirement is fulfilled by overlapping the projected sample and reference images onto the detector by rotating the sample. The second condition is satisfied by changing the axial sample position such that the optical path length difference between the sample and reference light paths is within the temporal coherence length l_c . The temporal coherence length l_c of the light source is approximately $15\text{ }\mu\text{m}$.

5.2.1 Microchannel chip

The microchannel was fabricated using photolithography and Deep Reactive-Ion Etching (DRIE) on a $\langle 100 \rangle$ silicon wafer. Access holes of 1 mm diameter were made from the backside in the same way as the channels. The channel wafer is anodically bonded to a $500\text{ }\mu\text{m}$ thick borofloat glass wafer and then diced into glass-silicon microchannel chips measuring 6 by 1.5 cm . The length l , width w and depth h of the microchannel are 4 cm , $380\text{ }\mu\text{m}$ and $155\text{ }\mu\text{m}$ respectively. A cross section of the chip and the acoustic pressure at resonance p_1 is illustrated in figure 5.2. See figure 5.3 for the measurement region in the microchannel. The image is obtained by the EMCCD detector by placing

Figure 5.2: Schematic illustration of the cross-section of the microchannel. The half-wavelength line in the water illustrates the instantaneous acoustic pressure for the fundamental mode in the x -direction (p_1), being positive on one side, zero at the node in the center and negative on the other side. The arrowed paths illustrate optical paths for light reflecting on the bottom (B) and top (T) of the water-filled channel. The excitation piezo is placed on the back of the silicon chip (see figure 5.1).

Figure 5.3: False color top-view image of the microchannel. Red colored regions indicate the silicon outside of the microchannel and the dashed white rectangle indicates the measurement region.

the reference mirror such that no interference is visible, i.e. the optical path length difference between the two arms is larger than the temporal coherence length for all pixels. The length scale is calibrated using the channel width as reference, which was measured during fabrication using an optical inspection microscope with an accuracy of approximately $1\ \mu\text{m}$.

The channel was completely filled with demineralized water (Milli-Q) using the access holes which were then sealed with scotch tape. A piezo-element for ultrasonic excitation was attached on the silicon side of the chip using a thin layer of cyano-acrylate glue. The width is chosen such that the fundamental acoustic resonance p_1 is present around 2 MHz, which is close to the resonance of the excitation piezo-element. The chip with piezo-element attached was mounted into the sample arm of the setup.

5.2.2 Optical alignment

The sample is aligned by changing its axial position and orientation using a six-axis sample holder, such that after alignment interference fringes can be seen by the camera at the interface of interest. Two interfaces are measured: the water-silicon interface at the bottom of the channel and the interface at the top of the channel, which is a glass-water interface in the channel and a glass-silicon interface outside. Two possible optical paths are illustrated

in figure 5.2. To measure at bottom of the channel, the sample position is manually adjusted such that the total optical path from the beamsplitter to the interface and back (B) is equal (within the coherence length) to the optical path from the beamsplitter to the reference mirror and back. To measure at the top of the channel, the sample is first aligned to the bottom and then moved $h/n \approx 117 \mu\text{m}$, with n the refractive index of water, in the $-y$ direction until again interference fringes are observed. The reference mirror is kept at its center position (see section 5.3) during the alignment procedure. This method gives a positioning precision better than the coherence length l_c .

5.2.3 Electronics

The excitation signal for the sample piezo-element (Pz26 26302, Ferroperm) and the pulse signal for the LED (ELD-870f-515-2, Roithner Laser) are generated by a double function generator (DG 4162, Rigol). The piezo excitation signal amplitude is set to 30 mV for frequencies from 1.7 to 2.1 MHz and 50 mV otherwise before amplification by an amplifier (2200L, E&I) with a fixed power gain of 53 dB. This results in a peak amplitude at the piezo of approximately 50 Vpp around the piezo resonance at 2 MHz. The pulse signal is amplified and added to a bias using a homebuilt amplifier. The axial translation stage for the reference mirror is based on a piezo actuator (PXY80D12, Piezosystem Jena), controlled by a homebuilt position sensor and feedback electronics to ensure linear dependence of position on control voltage. The function generator, control voltage, triggering and readout of the camera (Ixon DV887, Andor) are controlled by a PC using a data acquisition card (USB-6212, National Instruments) and a homebuilt control program (Labview 2011, National Instruments).

5.3 Acousto-optical measurement method

An acoustic resonance as described by formula 4.1 induces an instantaneous change in index of refraction, which results in an instantaneous change in the optical path length of light traversing the water. Using stroboscopic illumination and an interferometric reference it is possible to selectively measure this periodic change, analogous to lock-in detection [154].

In this experiment we use short pulses (18 ns FWHM) of light with a repetition rate equal to the acoustic excitation frequency f . During every acoustic period one short snapshot of the instantaneous optical path length is recorded, which is integrated over for many periods (see figure 5.4). By setting the time delay τ between the acoustic excitation signal and the pulse, the moment

Figure 5.4: Schematic illustration of the acousto-optic measurement method. For each mirror position z_m (see figure 5.1), four frames are recorded, each with a different time delay τ of the light pulses with respect to the excitation of the pressure wave. The time delays differ by exactly a quarter of the period of the excitation signal. After scanning the mirror, four interferograms (pixel intensity as function of mirror position) are available for each pixel $\{x, y\}$, differing only by τ , the moment the light probed the acoustic perturbation. The relative shift of the interferograms is a direct measure of the local acoustic pressure. The shifts are extracted by finding the peak location after cross-correlation with a digital reference.

of measurement during the acoustic period can be chosen. In our experiments we measure at four delays spaced a quarter period, $\tau_n = n \cdot T/4 = n/(4f)$, $n \in \{0, 1, 2, 3\}$.

The axial position of the reference mirror z_m can be changed using a piezo actuator. By scanning the mirror position an interferogram (light intensity as function of mirror position) can be recorded per pixel. The interferogram peaks at its maximum value when the optical path length difference (OPD) between light returning from the sample and the reference arm is zero. An acoustically induced variation will result in a shift of the interferogram equal to the amount of change in optical path length in the sample. By optically probing four times per acoustic period, both amplitude and phase of the pressure field can be measured. The measured amplitude is the average over the height of the channel h and is therefore unable to measure resonances in the y -direction (resonances with $n_y \neq 0$).

At each mirror position four frames are recorded, one for each delay τ using an integration time of 30 ms each. The mirror is moved in steps of 20 nm over a range of 40 μm for a full scan. To ensure that the mirror is stationary after moving, a stabilization period of 100 ms is used after each step before recording the frames. For the frequency mapping scans the excitation frequency f is varied with steps of 2.5 kHz.

Data post-processing

For each pixel four interferograms are recorded, one for each time delay. To find the relative shifts between them, each interferogram is first cross-correlated with a digital reference signal using a Fast Fourier Transform-based cross-correlation. The cross-correlation allows the full trace to contribute to the shift calculation instead of only the region around the maximum of the interferogram, thereby improving the signal-to-noise ratio. The reference is chosen differently for each pixel to accommodate local variations but it is the same for each of the four interferograms.

The reference signal is described as an explicit version of equation 4.29

$$I_{ref} = c_1 + c_2 \cos(2\pi/c_3 \cdot z_m + c_4) \cdot \exp\left\{\left(\frac{(z_m - c_5)^2}{2c_6^2}\right)\right\} \quad (5.1)$$

with c_3 and c_6 coefficients related to the center wavelength of the light source λ and its spectral width $\Delta\lambda$ respectively. These are assumed to be constant for all pixels. The other coefficients represent the amount of light returned from the sample, local sample topography, amount of stray light and the acoustically induced shifts, all which may vary with the position within measurement region. c_3 and c_6 are found by fitting equation 5.1 to

the 100 interferograms with the strongest signal per scan and using average value for the reference. The other coefficients are chosen such that the reference approximately matches the signal in terms of amplitude and shift. The shifts are then found from the position of the maximum in the resulting cross-correlation, which is extracted with sub-step accuracy using Fourier interpolation followed by sine-fit of the peak.

The amplitude of acoustically induced variation in index of refraction $\delta n(x, z)$ and phase $\varphi(x, z)$ can be calculated using the quadratures I and Q

$$I = z_0 + z_1 - z_2 - z_3 \quad (5.2)$$

$$Q = z_0 - z_1 - z_2 + z_3 \quad (5.3)$$

$$\delta n = \sqrt{I^2 + Q^2}/2h \quad (5.4)$$

$$\varphi = \tan^{-1}(Q/I) \quad (5.5)$$

with z_n the shift in optical path length of the interferogram for delay τ_n relative to the digital reference signal. The phase $\varphi(x, z)$ contains the relative timing of the periodic acoustic effect between different pixels. The variation of OPD is assumed to be smaller than $\lambda/2$ and all results larger than this value are rejected to suppress wrongly detected peak positions.

Finally the instantaneous acoustic pressure p is calculated using $\delta n \cdot \cos 2\pi\varphi$ as variation in index of refraction due to pressure and the inverse of equation 4.8:

$$p(x, z) = \frac{-\alpha_{ac} + \sqrt{\alpha_{ac}^2 + 4\epsilon_{ac}\delta n \cos(2\pi\varphi)}}{2\epsilon_{ac}} \quad (5.6)$$

Measurements are done at two interfaces: subscripts B and T indicate measurements done at the bottom and the top of the channel, respectively (see figure 5.2). The first contains acoustic effects from the water as well as from the glass layer, and the latter only contains effects from the glass. The measured change in index of refraction δn of both measurements is expressed in acoustic pressure to be able to directly compare the two measurements and p_T should thus be regarded as the in-water equivalent pressure.

Pure y-modes like p_{n_x} or mixed modes such as $p_{n_x,0,n_z}$ with low n_z can be regarded to be constant in the z -direction within the measurement area. In such cases the average can be taken over the z -direction to show the mode profile while the standard deviation calculated over the same data is an indication of the lower limit in sensitivity. Averages are indicated as $\bar{p}$ and are

calculated by averaging the quadratures and then following the rest of the calculation as above. The standard deviation σ is calculated over the pressure from equation 5.6.

5.4 Results

5.4.1 Acoustic pressure distributions at resonance

The measured instantaneous local pressure $p(x, z)$ for the modes p_1 , p_2 and p_3 are shown in figure 5.5. On the left the pressure distributions as measured on the bottom of the channel p_B and on the right the z -averaged values $\bar{p}_B$ (in red) and $\bar{p}_T$ (in gray) are shown. In each case, a phase is added to φ in equation 5.6 such that the pressure is shown at the peak moment in time.

In the pressure distributions some pixels are missing values (shown as black). As the bottom of the microchannel is slightly rough (approximately 2 μm RMS), light scatters strongly and at certain locations insufficient light returns from the sample resulting in unreliable values. This limits the spatial resolution to approximately 25 μm in highly scattering regions. In sufficiently smooth regions the resolution is equal to the optical resolution which is 5 μm . Some pixels show a phase difference of π compared to the surrounding pixels, which is especially apparent in figure 5.5b. This is not physical but an artifact due to faulty phase assignment. This could possibly be corrected by using an improved algorithm with a priori knowledge, such as setting a limit for the spatial variation of the pressure.

The resonance frequencies found are lower than expected from theory, up to 5 percent for $n_y = 3$. This could be explained by a lower temperature used in experiment resulting in a lower speed of sound and resonance frequency but also due to acoustic losses which lower the resonance frequency.

The wavelengths of the resonances λ_{n_x} and the pressure amplitudes p_a are found by fitting a cosine to $\bar{p}_B$. Confidence bounds at 95 percent are included. The results from the fit are $\lambda_1 = 764 \pm 5.3 \mu\text{m}$, $\lambda_2 = 374 \pm 3.3 \mu\text{m}$, $\lambda_3 = 265 \pm 1.3 \mu\text{m}$ and the corresponding values for p_a are $9.55 \pm 0.20 \text{ MPa}$, $154 \pm 2.1 \text{ kPa}$ and $196 \pm 2.5 \text{ kPa}$. The acoustic pressures show a clear sinusoidal variation in the x -direction as indicated by the small confidence bounds and are almost constant in the z -direction.

The uncertainty in the pressure is given by the standard deviation as indicated by the shaded area in the graph. The acoustic wavelengths found match the expected wavelengths within 2σ .

The acoustic amplitudes are comparable with values found by other studies. Using a comparable geometry, Barnkob et al. measured amplitudes of 80-660 kPa using excitation voltages of 0.5 to 1.9 Vpp for the fundamental

Figure 5.5: Pressure distribution $p_B(x, z)$ (left) and the z-averages $\bar{p}_B$ (right in red) and $\bar{p}_T$ (right in gray) at different excitation frequencies
 (a) $f = 1.9275$
 (b) $f = 3.8275$ and
 (c) $f = 5.6675$ MHz. Each pixel represents a region of 5 by 5 μm . The width of the shaded area equals 2σ .

resonance, which would correspond to over 16 MPa at 50 Vpp which we used for the p_1 mode [45]. The excitation voltage for p_2 and p_3 is much lower at approximately 15 Vpp due to a lower piezo efficiency at the corresponding frequencies. Dron et al. obtained a pressure amplitude of 110 kPa with 5 Vpp excitation [76] and Lakämper et al. measured 53-160 kPa at 10 Vpp [151], values comparable with the ones we obtained.

The measurements done at the top of the channel are shown as $\bar{p}_T$ on the right of figures 5.5. Because the top interface is smooth, all pixels show good signal and the uncertainty is lower than measurements done at the channel bottom. The acoustic variation in OPD, shown as in-water equivalent pressure $\bar{p}_T$, is much smaller than the acoustic variation in OPD due to the pressure resonances $\bar{p}_B$. The shape of $\bar{p}_T$ is asymmetric. This is explained by an acoustic resonance in the glass layer with a much larger wavelength than the width of the channel. As such only part of the wavelength is seen and results in an asymmetric pressure distribution without clear nodes or anti-nodes within the field of view.

An important result is that the acousto-optical signal originating from the glass layer p_T is much smaller than p_B . Light traveling through the glass and water picks up acoustic signals from both media and because the signals add harmonically, one can not distinguish between the two without knowing the relative phase between the two contributions. However, since p_T is much smaller than p_B we can be certain that the most significant part of the signal in p_B is in fact from pressure mode in the water and not from the glass.

The pressure sensitivity of this method is limited by camera noise. Without spatial averaging the sensitivity is approximately 50 kPa as given by the standard deviation in $\bar{p}_B$, with averaging over the z -direction (57 values) the sensitivity is approximately 5 kPa as seen from the fit confidence bounds.

5.4.2 Pressure mode spectroscopy

By measuring pressure distributions at a range of frequencies and plotting $|\bar{p}_B|$ as function of excitation frequency f , spectrograms of acoustic resonances can be made. The result for frequency ranges around the resonances of section 5.4.1 is shown in figure 5.6. Spectrograms give an overview on the pressure resonances present in the system. Relative peak amplitudes and spatial distribution over the width of the channel can be compared. The n_x order of the modes can be read from the number of nodes and the resonance quality (Q factor) of resonances can be estimated from the spectral width of a resonance line.

The absolute value $|\bar{p}_B|$ is used instead of $\bar{p}_B$, displaying the spatial dis-

Figure 5.6: Mode spectrograms: z-averaged pressure amplitude distributions $|\bar{p}_B|$ as function of excitation frequency f in the vicinity of the resonances p_1 , p_2 and p_3 (top to bottom), which are around $f = 1.93$ MHz, $f = 3.83$ MHz and $f = 5.67$ MHz respectively.

tribution of the pressure amplitude over the width of the channel and does not include the phase $\varphi(x, z)$. The phase depends on the total time delay between signal generation at the function generator and excitation of the acoustic mode. As such it does not only depend on the acoustic resonance alone, but also on resonances in the excitation piezo, response of the electronic equipment, mode coupling between the excitation piezo and the acoustic mode etc. Therefore, using $\bar{p}_B$ as function of frequency would result in a very chaotic map which defeats the purpose of giving an overview of acoustic modes available in the microchannel.

To estimate the influence of glass-modes on $|\bar{p}_B|$, also measurements at the top layer have been done. Within each of the three ranges displayed in figure 5.6, the highest in-water equivalent pressures $\bar{p}_T$ are 1.7 Mpa at 1.93 MHz (as shown in figure 5.5a), 56 kPa at 3.54 MHz and 68 kPa at 5.86 MHz. Glass-modes therefore have no significant effect and the pressure distributions shown are due to acousto-optic effects in the water.

The pure modes p_1 , p_2 and p_3 as studied in section 5.4.1 are clearly visible at $f = 1.93$, $f = 3.84$ and $f = 5.68$ MHz. Mixed modes are also visible. These tend to have a lower peak amplitude and are more lossy (spectrally broader). These can partially be explained by the theory as being mixed higher order modes. In other cases it might be needed to include acoustic resonances in the surrounding silicon chip [73].

A notable exception is the relatively strong mode $f = 3.5$ MHz which cannot be explained, as all modes represented by equation 4.1 satisfy the hard-wall condition (anti-nodes at the boundary). The pressure distribution of this mode has nodes at the boundary and one anti-node in the middle. No notable signal is measured at this frequency at the top of the channel.

Determination of resonance quality

For experiments and design purposes it is important to know the resonance quality of an acoustic resonance, as measured by the Q-factor. As an example, we will calculate the resonance quality of the p_1 mode using the measured spectrogram.

The peak amplitude p_a , calculated by averaging $\pi|\bar{p}_B|/2$ over the width of the channel, is expressed as acoustic energy density using equation 4.3 and plotted as function of frequency f , see figure 5.7. A Lorentzian function defined by equation 4.4 is fitted to the data using the least-squares method. The fitted values are: $f_0 = 1.9276$ MHz, peak acoustic energy density $E_0 = 10.6$ kJ/m³ and a Q-factor of 139. The corresponding spectral line width Δf equals $f_0/Q = 13.9$ kHz.

Figure 5.7: Measured energy density $E_a = p_a^2 / (4\rho_0 c^2)$ and a Lorentzian fit as function of frequency for the p_1 mode.

5.5 Concluding remarks

Using a stroboscopic interferometric technique we determined the acoustic field, both pressure amplitude and phase, in a water-filled microchannel for various excitation frequencies. The field was determined by measuring the optical path length difference due to the change in index of refraction due to the local acoustic pressure. The mode profiles at resonance for three measured resonances closely resemble the theoretical model. The measured fields measured at a range of excitation frequencies can be combined into mode spectrograms, which show which modes are present at which frequency. From the measurements also the local pressure amplitude, acoustic energy density and Q-factor of modes can be determined.

The influence of acoustic modes in the glass top layer could be investigated separately by virtue of the incoherence of the light used. We have shown that the optical path length of light traversing the glass and water is most significantly affected by the acoustic resonances in the water, compared with acoustic effects in the glass. This provides a basis for particle-free optical resonance tracking. In principle the optical tracking could be done for various frequencies simultaneously, which is of interest for multifrequency acoustophoresis experiments [141, 155].

Our method has a sensitivity of approximately 50 kPa without and 5 kPa with spatial averaging over the field of view and can measure pressures well

over 9.5 MPa without the use of particles. This opens up the possibility of in-situ optical characterization of acoustic modes on the microscale, which is important for a variety of systems such as used in shaped acoustic fields [134], acoustophoresis [155] and even piezo printheads. Due to the spatial selectivity, the local pressure around micro-objects can be measured and novel configurations in acoustic microfluidics can be investigated.

6 Spectral white-light interferometer for fast line profiling

A concern in railway quality is the occurrence of micro-cracks in railhead surfaces, due to rolling-contact fatigue. When these cracks are superficial, no more than about a millimeter in depth, the railhead lifetime can be increased by grinding or polishing. However, the deeper the cracks are, the sooner they will grow beyond a depth that can be polished away. We present a new technique for the detection of cracks in railheads, which is able to detect cracks of 20-100 μm depth optically, by way of broadband, spatially incoherent Optical Coherence Tomography (OCT).[†]

6.1 Introduction

In the previous chapter we used a scanning Michelson interferometer combined with an stroboscopic, partially coherent light source to measure the refractive index modulation by acoustic resonances in microchannels. One of the downsides of the technique is the measurement speed which is limited by the scanning of the reference mirror. In essence, each position of the reference mirror selects a different part of the optical spectrum to be used in the measurement. Combining measurements using many different spectra then allows calculation of the optical delay at different colors within the spectrum, which is directly related to the change in refractive index. Equivalently one could, in principle, perform separate measurements with a monochromatic light source with varying wavelength and, like before, combine the optical

[†]. Parts of this chapter are published in ref. 156.

phase as function of wavelength to calculate the optical delay. Based on this idea we implemented a non-scanning version of the technique from the previous chapter. Instead of a scanning monochromatic source we use, like before, a broadband, partially coherent light source and we separate the colors after the interferometer using an imaging monochromator. This eliminates the need for scanning at the expense of one spatial measurement dimension. This setup allows measurement of the optical optical path length along a line on the sample (instead of an area) using a single-frame measurement. In this chapter we explore the use of this technique for the detection of micro-cracks in railroad tracks, which requires both high speed and high depth resolution.

There are currently various combinations of ultrasonic and magnetic rail inspection techniques in use (PEC, ACFM) [157]. When integrated into an inspection train these techniques can measure cracks with depths of 2 mm and deeper at speeds exceeding 70 km/h [158]. Cracks less than 2 mm deep go undetected.

Ringsberg describes the progression of an rolling contact fatigue (RCF) induced crack in three phases [159]. Phase I involves the initiation of a crack on the rail surface due to shear stress, phase II is described as transient crack growth and phase III as tensile and/or shear-driven crack growth. Crack initiation is defined to be complete when phase I is over and the crack begins to grow further into the interior of the rail, usually at an oblique angle to the surface. At this point the surface crack is no more than 500 μm deep, below the detection limits of current non-destructive techniques (NDT). Ringsberg also calculates the estimated number of wheel passages needed to initiate an RCF crack for a specific situation, as did Wickramasinghe et al. [160]. Depending on the exact mass, speed and frequency of passing trains crack initiation could be complete between 2 months to over a year after initial use of the track. It is at this point that phase II begins, during which the crack grows to a size that is large enough to be detected by traditional methods. In another article Ringsberg describes a model to estimate the rate of crack growth after initiation [161]. It is estimated that a 400 μm deep crack will grow at a rate of 7 nm per cycle, that is, per wheel passage. This means it will take about $2 \cdot 10^5$ cycles for such a crack to grow by 1.5 mm, into a size measurable by eddy current based techniques that are currently in use. This corresponds to about 4 to 5 additional months of operational time before cracks become detectable. If the surface cracks could already be detected during the earliest stages of initiation this would add at least 7 months to monitor crack progression and plan maintenance for crack mitigation.

Preventive grinding of rail is already common practice on many networks, however without extensive knowledge of the location and level of the worst damage it is difficult to plan efficiently where to grind next and which sections

of track can be left alone for a while longer. As a grinding cycle removes no more than about 300 μm of a rail surface [162], any damage larger than this will remain as a starting point for crack growth, again reducing the useful life of the railhead. Thus a method for the more timely detection of RCF-induced surface cracking is needed, to enable more informed maintenance decisions.

6.2 Experimental details

6.2.1 Setup

The system is a broadband spatially incoherent optical coherence tomography (OCT) setup. It is a combination of a wide-field Michelson interferometer, an imaging spectrograph and a camera, as shown in figure 6.1. The light sources used are a JET 660-05 power LED (Light Emitting Diode, Roithner Laser, Austria) for initial alignment and resolution measurements, and a more powerful CT-120 power LED (Luminus, USA) for the final measurements performed on rail. The JET LED has an integrated collimation lens while an external lens

Figure 6.1: Schematic representation of the optical setup. A vertical line on the surface of the object is interferometrically imaged onto the entrance of the imaging spectrograph, which spectrally disperses the image horizontally.

(L_1 , $f = 40$ mm) was used with the CT-120 LED. Light from the LED is divided by a 50/50 beam splitter cube (BS) into the sample arm and the reference arm. It is then imaged onto the sample and the reference mirror (M_{ref}) by a pair of identical objectives (L_2 and L_3) with 2.5x magnification and 0.25 NA. The objectives image the sample and reference mirror surfaces onto the vertical entrance slit of the imaging spectrometer (IS, Newport 74100 with 1200 line/mm blazed grating). The image can also be diverted to a CCD camera (CAM, UEye UI-122xLE-m) using the removable mirror (RM) to take a microscope image of the measurement region. The imaging spectrograph images the entrance slit onto the CCD camera (Basler Ace acA3800-14um), while spectrally dispersing the line along the horizontal axis, thus yielding a spectrum for each position on the entrance slit.

Images from the sample and reference surfaces are spatially coherent as light is coherently divided and recombined by the beam splitter. These images are superimposed on the entrance slit, which slices out a thin vertical line, discarding the rest of the image. Each point along this line corresponds with one position on the sample and on the reference mirror. By measuring and analyzing the spectrum at each point we can calculate the height profile along the line on the sample. The spectra at all points are captured by the CCD in a single frame, allowing single-shot measurement of the height profile along a line on the sample.

The intensity on the CCD at the output of the spectrograph can be described as

$$I(x, \lambda) = S(\lambda) \left(c(x) + a(x) \cos \left(\frac{2\pi D(x)}{\lambda} \right) \right), \quad (6.1)$$

with $S(\lambda)$ being the spectrum of the light source, x the position along the line, $c(x)$ an offset related to the relative intensities returning from the sample and reference arm, $a(x)$ the amplitude of the oscillation and $D = 2h(x)$ the optical path length difference (OPD), with $h(x)$ being the height profile on the sample relative to the flat reference mirror [126].

As an example, a simulated height profile and the corresponding image on the CCD can be seen in figure 6.2. It is calculated using formula 6.1 using S and a equal to one, c set to zero and an observable spectral range of 16.8 nm, which is comparable with the spectral width of the light used in the experiments. The figure shows how different heights h encode into different periodicities and phase shifts. Light from a LED is spatially incoherent and therefore crosstalk between spectra recorded for adjacent positions is very low [163].

Figure 6.2: An example (a) height profile and (b) associated output spectrum from the setup. The differences between planar, angled, rising and receding surfaces can clearly be distinguished.

The theoretical lateral resolution, based on the NA of the objectives, is $4.5 \mu\text{m}$ [164]. In typical OCT, the depth resolution Δh depends on the spectral width of the light source as

$$\Delta h = \frac{2 \ln(2) \lambda_0^2}{\pi \Delta \lambda}, \quad (6.2)$$

with λ_0 being the central wavelength and $\Delta \lambda$ the full width at half maximum intensity (FWHM) [165]. However, in our case we have only a single reflection coming from the sample. The depth resolution is therefore limited only by the error in the determination of the fringe frequency and phase. Two important factors are the pixel signal-to-noise ratio and fringe visibility, which depends on sample reflectivity. The length of the imaged line is 1.4 mm and is currently limited by the CCD chip dimensions.

6.2.2 Data processing

Each frame is processed line by line along the spatial axis (for each position x). The fringe pattern for each position is first normalized to the spectrum by dividing by an 8th order polynomial fit. Next, the offset c and the amplitude a are determined from the dc-component and largest non-dc-component of the Fourier transform of the normalized pattern, respectively. Lastly, the OPD is determined by comparing the normalized pattern to formula 6.1 using a

range of trial values for D (in 1 micrometer steps) and $S(\lambda) = 1$. The value of D that produces the smallest absolute difference with the fringe pattern is chosen as the best fit. The fringe visibility, a measure of signal strength, is then determined as the ratio of a to c . If the visibility falls below 1% the fit is no longer deemed reliable and the extracted OPD value is not used. Finally the height profile is calculated as $h(x) = D(x)/2$.

6.3 Results

6.3.1 Determination of lateral and depth resolution

To determine the lateral resolution, we image a test sample with reflective lines and determine the smallest line width which is still properly resolved, corresponding to the spatial cut-off frequency of the modulation transfer function of the optical system [164]. The test sample consists of a lithography mask containing arrays of reflective chromium lines on a glass substrate. The width of the lines is different for each array and varies logarithmically from 1 μm to 50 μm in 20 steps. The side-to-side line separation is equal to the line width, creating a square periodic pattern with 50% duty cycle. The test sample is placed in the sample arm of the interferometer, the reference arm is blocked and for each periodic pattern a line image is recorded using the CCD behind the spectrograph. The visibility of the periodic patterns is calculated using $(I_{max} - I_{min}) / (I_{min} + I_{max})$ with I_{max} and I_{min} the measured intensity from a reflective and non-reflective part of the pattern, respectively. The lateral resolution is taken to be the smallest line width with a visibility larger than $1/e$ (approximately 37%) of the maximum visibility. The measurement results are shown in figure 6.3. The maximum visibility is approximately 17.5 % and the lateral resolution found is 8 μm .

To determine the depth resolution a purely reflective part of the calibration sample was measured while placed at an angle 1.83° relative to the reference mirror, leading to an increasing OPD along the line. The height profile was measured and a linear function was fitted using the least squares method. Both the profile and the profile minus the linear fit h^* is shown in figure 6.4. The standard deviation of the h^* profile is $\sigma = 0.94 \mu\text{m}$ and the depth resolution calculated as 2σ equals 1.9 μm .

6.3.2 Height profiles from a coin and rail

To test the functionality of the instrument, first a 20 cent euro coin was measured (see figure 6.5). The measurements were compared to reference measurements obtained on the same sample with a commercially available Bruker

Contour GT-I white light interferometer (WLI). The profile is taken along the height step between the sea on the left, just north of Scandinavia, and the plateau to the right of it. A microscope image of the region is imaged by placing the removable mirror into the light path and using the auxiliary camera. The white dashed line indicates the approximate region imaged onto the entrance slit of the spectrograph after removal of mirror RM. The resulting fringe pattern is shown in figure 6.5d. The measured profile together with the reference are shown in figure 6.5c. A data gap is present between 600 and 800 μm , corresponding to an approximately 10 μm deep groove and the vertical face of the step. In both regions insufficient light returns from the sample to form detectable fringes. The measured step height is approximately 20 μm , agreeing with the reference. The difference, mostly notable on the right side of the step, can be due to slightly different regions measured by the OCT system and the reference system. The standard deviation of the linearized datapoints is 4.4 μm . The exposure time was 31 ms while the reference measurement took 30 seconds.

Finally proof of principle measurements were carried out on the surface of a piece of used 70 mm wide railhead provided by Strukton Rail, The Netherlands. The surface of the rail contains a large number of micro-cracks in phase I of formation, thus with expected depths of no more than 500 μm . A photograph of the rail is shown in figure 6.6a, with the micro-crack of interest indicated by an ellipse. The microscope image is shown in figure 6.6b with a white dashed line indicating the approximate measurement line, correspond-

Figure 6.3: Determination of lateral resolution based on periodic pattern visibility. The dashed line is placed at $1/e$ of the maximum value.

Figure 6.4: Determination of depth resolution. Height profile of the calibration sample angled at 1.83° is seen above, and under the same profile minus a linear fit.

ing to imaging a line parallel to the direction of train motion. The measured fringe pattern is shown in figure 6.6d. The visibility of the fringes is lower compared to the previous measurements due to the lower reflectivity of the rail. The measured profile and the reference are shown together in 6.6c. Very

Figure 6.5: Measurement of a 20 µm height step on the common face of a 20 cent euro coin. (a) The line profile is taken along the small black as indicated in the photograph of the coin. (b) A microscope image of the step. The profile is measured approximately along the white dashed line. (c) Measured profile with the setup (blue dots, 31 ms exposure time) and reference system (orange line, 30 s measurement time). (d) Fringe pattern corresponding to the measured profile.

Figure 6.6: Measurement of a Phase-I RCF-induced micro-crack in a rail. (a) The profile is taken over the width of the crack indicated with the ellipse in the photo. (b) A microscope image of the micro-crack. The profile is measured approximately along the white dashed line. (c) Measured profile with the setup (blue dots, 11.8 ms exposure time) and reference system (orange line, 30 s measurement time). (d) Fringe pattern corresponding to the measured profile.

rough and nearly vertical patches are not resolved, leading to gaps in the data. However, the depth of the crack is clearly resolved, measuring $26\text{ }\mu\text{m}$ with a deviation of approximately $7\text{ }\mu\text{m}$ compared to the reference. This is again attributed to slightly different measurement positions along the micro-crack for the two measurements. The standard deviation of the linearized profile is $3.6\text{ }\mu\text{m}$. The exposure time was 11.8 ms while the reference measurement again took 30 seconds .

6.4 Discussion and outlook

As can be seen in the height profiles in figures 6.5 and 6.6 the number of points with a sufficient fringe visibility to extract a meaningful value for the height decreases as the sample becomes less reflective. No data points are rejected using a flat, highly reflective surface as with the calibration sample. For the coin a lot of data points were lost at the edge of the step and inside a shallow crack of approximately $10\text{ }\mu\text{m}$ deep. For the rail there is a loss of data points throughout, especially on the vertical sides of the crack. For all line profiles the minimum visibility was set to 1% , as any fit to the fringe pattern below that percentage was no longer reliable, resulting in jumps of several hundreds of μm at a time. Even so, a clear signal was seen from the micro-crack in figure 6.6, leading to a successful single-frame measurement of a phase I micro-crack in laboratory conditions. A different profile recovery algorithm could be implemented to improve robustness against low reflectivity [166].

A number of parameters need significant improvement before feasible integration of the device into an inspection train. First of all, the measurement time for a single profile needs to be decreased by several orders. To profile a rail while avoiding motion blur, the measurement time should be shorter than half the spatial resolution of the system divided by the speed of the inspection train. This corresponds to a measurement time of $0.28\text{ }\mu\text{s}$ using the current lateral resolution of $8\text{ }\mu\text{m}$ and a speed of 50 km h^{-1} . Depending on the type of cracks of interest a lower resolution can be used, relaxing the requirement on measurement time.

The required measurement times can be achieved either by replacing the CCD camera with a high speed camera or using a relatively slow camera as in this work combined with a high intensity pulsed light source. The rail profile was measured with a constant optical power of 16.5 mW at the rail, which can be improved significantly. High power LEDs [167, 168] or gas discharge lamps [169–171] would be suitable candidates for both constant or pulsed illumination. The optical power at the sample was distributed into a circular spot of approximately 5 mm in diameter, so the optical intensity delivered to

the measurement line can also be increased by using line-shaped illumination. There might also be a more optimal wavelength range with increased rail reflectivity compared to the red light used in this work.

We note that the reflectivity of rail might be higher for tracks in operation compared with the rail sample used in this work, as it was oxidized due to not being in operation. Additionally, micro-cracks in operational tracks might be filled with water, which would have two effects. First, the depth of a measured micro-crack would be overestimated by the ratio of the refractive index of water to the index of air which is approximately 33 percent. This effect can be compensated for by measuring at multiple times and taking the environmental conditions into account. The second effect is a slight reduction of light transmitted into and from the micro-crack due to reflection at the air-water interface. The strength of this effect is estimated to be two percent and can therefore be ignored.

Residual stresses (tensile or compressive) might be present in the bulk of the rail but do not influence the shape of the micro-cracks at the time of measurement. Residual stress does however influence the growth rate of the micro-cracks [172]. Therefore it might be possible to estimate the influence of residual stresses in the tracks from the measured development of micro-cracks over time.

Finally, as the system can only inspect surface damage it would ideally be used in tandem with established methods which can also detect cracking in the interior of the material to get a full picture of the state of rail. In a practical application the imaged line could be chosen either perpendicular to the direction of train motion, or parallel to it. As the surface cracks tend to be perpendicular to the direction of motion it is more likely to have a crack in a shot when imaging parallel to the direction of motion. Currently the profile line length is 1.4 mm which can be improved tenfold to 14 mm using a typical, commercially available imaging spectrograph and a camera with matching chip size. The measurement repetition rate is typically limited by the frame read-out rate of the camera. Using a typical rate of 20 frames per second, a 14 mm profile line length and an inspection train speed of 50 km/h, we can estimate the track coverage (ratio of measured track line length to total track length) to be around two percent. This can be improved by using a faster camera but typically this method leads to under sampling of the measured track. The development of micro-cracks can be estimated using statistical analysis on multiple time-separated measurement series gathered on the same track.

6.5 Conclusion

In this chapter we provide a proof of principle for an optical rail inspection technique two orders of magnitude more sensitive to micro-crack depth compared to existing techniques. The ability to optically detect a 26 μm deep rolling contact fatigue induced micro-crack in a single shot was successfully demonstrated with a two-sigma depth resolution of 7 μm . In a practical application it is desirable to decrease the measurement time by several orders of magnitude, the means for which are explained briefly in the discussion above.

Summary

The first topic of this thesis is an exploration of the use of acoustic waves to concentrate small particles from water. Continuous in-line methods which achieve this are of great interest for the application in Lab-on-Chips, which aims to offer laboratory functionality in a chip with small footprint and cost. Acoustophoresis is a interesting candidate with potentially high reliability and availability.

The balance between two acoustophoretic forces determines the suspended particle behavior: the acoustic radiation force, caused by scattering of the acoustic wave from the particle, and the steady acoustic streaming caused by viscous dissipation of the acoustic resonance close to the channel walls. The former can be used to concentrate particles into the nodes or anti-nodes of the resonance and the latter causes mixing of the particles into the medium via the Stokes drag force. The acoustic radiation force scales with the volume of the suspended particle and with the acoustic frequency squared, while the streaming induced Stokes drag-force scales with the particle radius and frequency. Therefore the streaming becomes dominant for very small particles. We have shown both experimentally and by numerical simulations that the cut-off size for concentration of polystyrene particles in water is around 1.5 μm in diameter. Smaller particles are not concentrated but are instead following the acoustic streaming flow.

Using numerical calculations a different mode of concentration is observed during the transition from radiation-force to drag-force dominated behavior. Instead of particle concentration into the nodes of the acoustic resonance we observe concentration into regions close to the walls. The numerical calculations show concentration of polystyrene particles down to 100 nm in diameter in the streaming region. The particles slowly spiral towards the concentration maximum, indicating that both the streaming-induced drag force and the radiation force contribute. Simultaneous excitation of multiple harmonic modes allows more complicated force fields which can improve or degrade the concentration performance. This work is a next step towards engin-

earing of streaming patterns for acoustophoretic concentration of submicrometer particles. Experimental verification and inclusion of diffusion effects are the next steps towards application of this method, which can be used to concentrate even smaller pathogens from sampled drinking water.

Another important function in a Lab-on-a-Chip is mixing, which needs to be induced actively due the inherent laminarity of microfluidic flow. Acoustic streaming can be used to purposefully induce local mixing flows. A centimeter-scale acoustic micromixer is fabricated using Direct Laser Writing (DLW), consisting of many cuboid micropillars. A prefabricated microfluidic chip is filled with resist and the laser writing of structures is performed through the glass cover of the chip. This 'in-chip'-method provides good attachment of fabricated structures to both top and bottom of the microchannel, which is harder to achieve when fabricating structures in an open channel. Characterization of the structures using Confocal Laser Scanning Microscopy suggests that small initial misalignment limits the spatial extent of in-chip DLW. Therefore a trade-off must be made between writing time, accurate structure placement and initial alignment. An acoustic mixing flow of $30 \mu\text{m s}^{-1}$ around the cuboid pillars was observed. The fabrication method allows for additive manufacturing of micromixers into existing (transparent) microfluidic systems.

Both the acoustic mixing and concentration function strongly depend on acoustic resonances. The amplitude and pressure distribution are very sensitive to changes in material properties (such as increase of particle concentration) and temperature. Existing resonance characterization methods depend on concentration of suspended particles and therefore cannot be used in the acoustic streaming-dominated regime or with low particle concentrations. Therefore we developed an optical method to directly measure the acoustic mode inside a microchannel using stroboscopic white-light interferometry. The acoustic field, both amplitude and phase, is determined by measuring the acoustically induced optical delay using stroboscopic illumination. The pressure distributions are measured for the fundamental pressure resonance in the water and two higher harmonic modes. By combining measurement at a range of excitation frequencies a frequency map of modes is made, from which the spectral line width and Q-factor of individual resonances can be obtained. The optical characterization method can be used to improve the design of acoustic microresonators in microfluidic systems.

The white-light interferometric imaging technique can also be applied to other problems. As a spin-off we used this method to measure micro-cracks in railroad tracks. The speed of measurement in the original application is limited by the scanning time of the mirror which is needed to build-up the interferograms. The need for scanning is eliminated by replacing the scanning

mirror with an imaging spectrograph, increasing the measurement speed significantly. We used this setup to measure the height profile of a railroad track containing a 26 μm deep micro-crack in a single shot of 11.8 ms. However, for practical application it is desirable to decrease the measurement time by several orders of magnitude, the means for which are explained briefly.

Samenvatting

Een gedistribueerd netwerk bestaande uit kleine, geautomatiseerde mini-laboratoria (zogenaamde 'Labs-on-a-Chip') kan zorgen voor een continue en goedkope controle van drinkwaterkwaliteit. In elk mini-lab is eerst een concentratiestap nodig waarbij mogelijke microbiologische pathogenen gescheiden worden van het drinkwater. Na deze stap kan het concentraat geanalyseerd worden op de aanwezigheid van pathogenen. Het eerste onderwerp in deze thesis is een onderzoek naar het toepassen van akoestische golven voor de concentratiestap. Manipulatie van deeltjes met akoestische golven wordt in het Engels 'acoustophoresis' genoemd.

Er zijn twee krachten die een belangrijke rol spelen: de akoestische concentratiekracht en de wrijvingskracht van het medium op een deeltje. Een deeltje in een akoestische staande golf ondergaat zowel periodieke compressie als een periodieke translatie, waardoor het deeltje zelf ook een akoestische golf uitstuurt. Dit leidt tot de akoestische concentratiekracht, dat een deeltje typisch naar een knoop in het akoestische veld duwt. De akoestische staande golf wordt ook geabsorbeerd aan de wanden dat door een niet-lineair effect leidt tot een constante stroming. Deeltjes zullen vanwege de Stokes wrijvingskracht de neiging hebben om deze stroming, genaamd 'streaming', te volgen. De concentratiekracht schaal met het deeltjesvolume en de akoestische frequentie in het kwadraat, de wrijvingskracht vanwege de streaming schaal met de deeltjesradius en met de akoestische frequentie. De stroming zal dus het dominante effect zijn voor hele kleine deeltjes. Uit experimenten en numerieke berekeningen hebben we laten zien dat voor polystyreendeeltjes in water, bij een akoestische frequentie van ongeveer 2 MHz, de ondergrens ongeveer 1.5 μm in diameter is. Kleinere deeltjes kunnen niet geconcentreerd worden omdat de stromingswrijving sterker is dan de concentratiekracht.

Wanneer de akoestische concentratiekracht dominant is zullen deeltjes zich typisch concentreren in de knopen van de akoestische staande golf. Uit numerieke berekeningen blijkt dat rond het omslagpunt van

concentratiekracht-gedomineerd naar stromings-gedomineerd ook nog een andere manier van concentreren mogelijk is. Hierbij werken beiden krachten samen en volgen de deeltjes een spiraalvormig pad richting een gebied dicht bij de muren van de akoestische resonator waar ze uiteindelijk geconcentreerd worden. Volgens de berekeningen moet deze methode werken voor polystyreendeeltjes van 100 nm in diameter en groter, bij een frequentie van 2 MHz. Het effect kan worden versterkt of worden verzwakt door het combineren van meerdere akoestische resonantie frequenties. Na experimentele verificatie van deze methode kan het gebruikt worden voor concentratie van nog kleinere pathogenen in water in mini-laboratoria.

Naast concentratie zijn er nog vele andere functies die noodzakelijk zijn voor een functionerend mini-lab, zoals bijvoorbeeld verwarmen en mixen. Vanwege de schaal waarop vloeistoffen in een mini-lab ingesloten zijn, neigen ze naar sterk laminair gedrag waardoor snel mixen op een actieve manier moet worden gedaan. Hiervoor kan akoestische streaming kan worden toegepast om lokaal een mengende stroming op te wekken. We hebben laten zien hoe een akoestische micro-mixer kan worden gefabriceerd in een bestaande microfluidische chip door middel van Direct Laser Writing. De chip wordt gevuld met lichtgevoelig resist en door middel van een laser kunnen lokaal microstructuren geschreven worden. De micro-pilaren hebben een goede hechting dankzij de fabricage in de bestaande chip. De maximale grootte van een structuur is bij deze techniek gelimiteerd door de uitlijning, waardoor een balans gevonden moet worden tussen fabricage tijd, initiële uitlijning en nauwkeurige plaatsing. Akoestische streaming met een snelheid van $30 \mu\text{m s}^{-1}$ is gemeten rond de micropilaren.

Zowel akoestische concentratie als akoestisch mixen hebben een akoestische resonantie nodig voor optimale werking. Akoestische resonanties zijn echter gevoelig voor verandering van materiaal (bijvoorbeeld veranderende concentratie van deeltjes in water) en temperatuur (vanwege de temperatuursafhankelijkheid van de golfsnelheid). De huidige karakteriseringsmethoden voor microfluidische systemen zijn gebaseerd op het meten van de akoestische concentratiekracht op deeltjes in suspensie, waardoor deze methodes niet geschikt zijn bij lage deeltjesconcentratie of bij kleine deeltjes. Daarom hebben wij een optische methode ontwikkeld dat zonder deeltjes in het medium de akoestische staande golf kan meten. Door middel van een afbeeldende interferometer met een breedbandige, stroboscopische lichtbron kan de modulatie van de dichtheid van het medium door de akoestische staande golf worden gemeten. Het akoestische veld van de fundamentele resonantie en twee hogere harmonische resonanties werden gemeten. Met deze optische meetmethode kan het ontwerp van akoestische microresonators worden verbeterd.

In feite is de optische meetmethode geschikt om snel kleine verschillen in het optische pad te meten. Als spin-off hebben we het toegepast op een ander probleem, namelijk het snel meten van het hoogteprofiel van treinrails. In treinrails ontstaan na verloop van tijd kleine haarscheurtjes. Indien deze tijdig worden verwijderd door polijsten van de rails kunnen ze uitgroeien tot scheuren, waardoor duurdere onderhoudsmethoden moeten worden toegepast. Tijdige detectie is dus van belang. Een aanpassing aan de originele optische methode stelt ons in staat om een haarscheur van 26 μm diep te meten met een meettijd van 11.8 ms. Voor de praktische toepassing van deze techniek, bijvoorbeeld als sensor onder een onderhoudstrein, moet de meet-snelheid nog flink verhoogd worden. Mogelijkheden hiervoor zijn genoemd in de conclusie.

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A | Scattering coefficients of the acoustic radiation force

The theory is taken from Karlsen and Bruus, ref. 35, and is included here for completeness. See table 1.1 for the used parameters and their numerical values. The monopole scattering coefficient including viscous and thermal effects for a particle with radius a much smaller than the acoustic wavelength is written in shorthand as

$$c_M = \frac{c_{M1} + c_{M2}H}{1 + c_{M3}H} \quad (\text{A.1a})$$

with

$$c_{M1} = 1 - \tilde{\kappa}_s \quad (\text{A.1b})$$

$$c_{M2} = 3(\gamma - 1) \left[(1 - \xi)(1 - \chi\xi) - \frac{4}{3}\chi\tilde{\rho}_0\xi\tilde{\kappa}_s \left(\frac{c_{t,ps}}{c_s} \right)^2 \left(1 - \frac{\xi}{\tilde{\kappa}_s} \right) \right] \quad (\text{A.1c})$$

$$c_{M3} = 4(\gamma - 1)\chi\tilde{\rho}_0 \left(\xi \frac{c_{t,ps}}{c_s} \right)^2 \quad (\text{A.1d})$$

$$H(a, \omega) = \frac{1}{(k^T a)^2} \left[\frac{1}{1 - ik^T a} - \frac{1}{\tilde{k}_{th}} \frac{\tan(k_{ps}^T a)}{\tan(k_{ps}^T a) - k_{ps}^T a} \right]^{-1} \quad (\text{A.1e})$$

with a tilde over a parameter indicating the ratio of particle parameter to fluid parameter, e.g. $\tilde{\rho}_0 = \rho_{ps}/\rho_0$, and with the following definitions:

$$\xi = \frac{\tilde{\alpha}_s}{\tilde{\rho}_0 \tilde{c}_p}, \quad \chi = 1 - \frac{4}{3} \left(\frac{c_{t,ps}}{c_{s,ps}} \right)^2 \quad (\text{A.1f})$$

$$k^T = \frac{1+i}{\delta_T} \left[1 + \frac{i}{2}(\gamma-1)(\Gamma_s - \Gamma_T) \right] \quad (\text{A.1g})$$

$$\Gamma_s = \frac{1}{2}(\eta_0^b/\eta_0 + \frac{4}{3})(\omega\delta_s/c_s)^2, \quad \Gamma_T = \frac{1}{2}(\omega\delta_T/c_s)^2 \quad (\text{A.1h})$$

$$\delta_s = \sqrt{\frac{2\eta_0}{\rho_0\omega}}, \quad \delta_T = \sqrt{\frac{2k_{th}}{\rho_0 C_p \omega}} \quad (\text{A.1i})$$

$$k_{ps}^T = \frac{1+i}{\delta_{T,ps}} \frac{1}{\sqrt{1-X}} \left[1 + \frac{i}{8} \frac{\gamma_{ps}^2 \Gamma_{T,ps}}{1-X} \right] \quad (\text{A.1j})$$

$$X = (1 - \gamma_{ps})(1 - \chi) \quad (\text{A.1k})$$

$$\delta_{T,ps} = \sqrt{\frac{2k_{th,ps}}{\rho_{ps} C_{p,ps} \omega}}, \quad \Gamma_{T,ps} = \frac{1}{2}(\delta_{T,ps} \omega / c_{s,ps})^2. \quad (\text{A.1l})$$

The dipole scattering coefficient including viscous and thermal effects is written as

$$c_D = \frac{c_{D1}(1-G)}{c_{D1} + 3(1-G)} \quad (\text{A.2a})$$

with the following subcoefficient and function G

$$c_{D1} = 2(\tilde{\rho}_0 - 1) \quad (\text{A.2b})$$

$$G(a, \omega) = \frac{3}{k^s a} \left(\frac{1}{k^s a} - i \right) \quad (\text{A.2c})$$

$$k^s = \frac{1+i}{\delta_s}. \quad (\text{A.2d})$$

δ_s and δ_T represent the widths of the viscous and thermal boundary layers in the fluid, surrounding the particle, respectively.

In case of an inviscid fluid and ignoring thermal differences, then the widths of the boundary layers tend to zero, functions H and G become zero and the scattering coefficients reduce to

$$c_M = 1 - \tilde{\kappa}_s \quad (\text{A.3})$$

$$c_D = \frac{2(\tilde{\rho}_0 - 1)}{2\tilde{\rho}_0 + 1}. \quad (\text{A.4})$$

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The main theme of this thesis is waves: sound waves for trapping, guiding or mixing suspended particles, and light waves for making things visible.

Sound waves can be used to concentrate small particles, such as beads or bacteria, from water. This is useful for applications where strict quality control is required, like controlling pathogens in drinking water.

This thesis reports on sound-based particle concentration and mixing in microchannels, and light-based interferometry which can be used to make these sound waves visible. The interferometric method used can also be used for other applications, such as measuring microscopic cracks in railroad tracks.