Imaging local acoustic pressure in microchannels

JORICK VAN'T OEVER,^{1,2,*} RAIMOND FRENTROP,¹ DANIEL WIJNPERLÉ,² HERMAN OFFERHAUS,¹
DIRK VAN DEN ENDE,² JENNIFER HEREK,¹ AND FRIEDER MUGELE²

¹University of Twente, Optical Sciences, P.O. Box 217, 7500 AE Enschede, The Netherlands

²University of Twente, Physics of Complex Fluids, P.O. Box 217, 7500 AE Enschede, The Netherlands

*Corresponding author: j.j.f.vantoever@utwente.nl

Received 18 May 2015; revised 18 June 2015; accepted 25 June 2015; posted 26 June 2015 (Doc. ID 241195); published 17 July 2015

A method for determining the spatially resolved acoustic field inside a water-filled microchannel is presented. The acoustic field, both amplitude and phase, is determined by measuring the change of the index of refraction of the water due to local pressure using stroboscopic illumination. 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. © 2015 Optical Society of America

OCIS codes: (120.5475) Pressure measurement; (100.3175) Interferometric imaging; (170.1065) Acousto-optics; (110.7170) Ultrasound.

<http://dx.doi.org/10.1364/AO.54.006482>

1. INTRODUCTION

Lab-on-a-chip (LoC) microfluidics is a developing field and has grown tremendously over the past decades [1,2]. Various laboratory functions, such as particle separation [3], cell sorting [4], particle trapping [5], droplet manipulation [6], biological assays [7], and mixing [8], are integrated into a single microchip using very small fluid volumes compared with traditional methods.

Following the trend toward the microscale, various acoustic techniques such as surface acoustic waves [9–11] (SAWs), acoustic bubbles [12], or acoustic actuation [13] have been developed for use in LoC technology. Likewise, the use of sound-based particle separation and trapping was explored [14–17].

Ultrasonic standing waves for particle manipulation have been integrated onto silicon/glass microchips by precise micro-fabrication techniques and various configurations have emerged on the silicon/glass platform [18–22]. To aid design and improve performance, physical models have been developed to describe the acoustic resonances [23–28], which predict the resonance frequency and pressure amplitude distributions well for thin chips [29].

Due to the high sensitivity of the acoustic resonances to 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 among different experiments and configurations. For measuring the acoustic amplitude, particle-based methods [30–32] 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 work, 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 the index of refraction of water due to acoustic pressure. Stroboscopic, incoherent illumination allows 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 the 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 [25,30]. 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 of the water (see Fig. 1). 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 the 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 of the water.

Fig. 1. 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 y -direction (p_1), being positive on one side, 0 at the node in the center, and negative on the other side. The arrowed paths illustrate the optical paths for the 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 Fig. 2).

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. The measured pressure distributions match the theory well.

In the first section, we give the theoretical basis of the pressure modes of the chip and the theory of acousto-optical interaction. After that the experimental setup and measurement method are discussed, followed by results and concluding remarks.

2. THEORETICAL BACKGROUND

A. Acoustic Resonances

The silicon/glass walls surrounding the water are assumed to be infinitely hard. The acoustic pressure $p = P - P_0$ in water in a lossless rectangular, hard-walled channel at resonance is given by

$$p = p_a \cos\left(\frac{n_x \pi}{l} x\right) \cos\left(\frac{n_y \pi}{w} y\right) \cos\left(\frac{n_z \pi}{h} z\right) \cos(2\pi f_{n_x, n_y, n_z} t), \quad (1)$$

with P the absolute pressure; P_0 the equilibrium pressure; p_a the pressure amplitude; n_j an integer indicating the mode order in the respective direction; and l , w , h , respectively, the length, width, and height of the channel [26]. The corresponding resonance frequencies are

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

with c the speed of sound in water. The modes relevant in this work are pure modes in the y -direction (n_x and n_z equal 0) and the modes are referred to as p_{n_y} . A cross section of the actual

chip and the acoustic pressure at resonance p_1 is illustrated in Fig. 1.

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

$$E_a = p_a^2 / (4\rho_0 c^2), \quad (3)$$

$$E_a(f) = \frac{E_0}{\left[\frac{2Q}{f_0}(f - f_0)\right]^2 + 1}, \quad (4)$$

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

B. Acousto-optic Interaction

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 (LL) (or Clausius–Mossotti) equation, which relates the index of refraction n to the molecular polarizability α [33]:

$$\frac{n^2 - 1}{n^2 + 2} = 4\pi/3 \left(\frac{\rho N_A}{M} \right) \alpha. \quad (5)$$

Here ρ is the mass density, N_A is Avogadro's number, and M is the molar mass. The 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 [34].

Taking the derivative of Eq. (5) with respect to density yields

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

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 [35]. For most liquids Δ_0 is very close to 0 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% at 5.92 GPa and 673 K compared with that under standard conditions [36], showing that for our experimental conditions we may reasonably take Δ_0 equal to 0.

The adiabatic equation of state relates the pressure and density fluctuations in a fluid [37]:

$$p = P - P_0 = \beta s + 1/2\beta(\gamma - 1)s^2, \quad (7)$$

with β the adiabatic bulk modulus, $\gamma - 1$ the parameter of nonlinearity, condensation $s = (\rho - \rho_0)/\rho_0$, and ρ_0 the unperturbed density.

Combining Eq. (6) with the derivative of Eq. (7) with respect to density and using the thermodynamic definition $c^2 = \beta/\rho_0$, with c the speed of sound, yields

$$\frac{dn}{dp} = \frac{1}{\rho_0 c^2} \frac{(n^2 - 1)(n^2 + 2)}{6n(s + 1)(1 + 3/2(\gamma - 1)s)}. \quad (8)$$

Direct evaluation of Eq. (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 the index of refraction δn due to acoustic pressure p :

$$\delta n = \alpha_{ac} \cdot p + \epsilon_{ac} \cdot p^2. \quad (9)$$

Using the positive root of Eq. (7) for condensation as function of acoustic pressure and equilibrium parameters, $s(p) = (\sqrt{1 + 2(\gamma - 1)(\rho_0 c^2)^{-1}p} - 1)(\gamma - 1)^{-1}$, wavelength of light $\lambda = 870$ nm, water temperature of 30°C , $\rho_0 = 995.6$ kg/m³ [38], $n = 1.3264$ [39], $c = 1509$ m/s [40], and $\gamma = 6.2$ [41], we find the acousto-optic coefficients $\alpha_{ac} = 1.58 \times 10^{-10}$ Pa⁻¹ and $\epsilon_{ac} = -6.13 \times 10^{-19}$ Pa⁻². These values will be used in the remainder of the paper.

Other investigators derived a value of $\alpha_{ac} = 1.26 \times 10^{-10}$ Pa⁻¹ assuming a linear dependence of electric susceptibility on density [42].

3. EXPERIMENTAL SETUP

The setup is based on a white-light phase-shifting Michelson interferometer design as used by Shavrin *et al.* [43] (see Fig. 2). 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 limited coherence exists among different points on the surface. The emitting surface is imaged through the beam splitter onto both the reference mirror in the reference arm and the sample in the sample arm using the illumination lens. Because the beam splitter splits the field coherently, the two images are mutually pointwise coherent [44]. The images are re-imaged through the beam splitter and projected onto the EMCCD detector by the detection lens. The sample is aligned such that the images overlap on the sensor.

Fig. 2. Schematic illustration of the experimental setup, which consists of a fast light-emitting diode (LED), a microscope objective (OBJ) (M Plan SLWD 40× 0.40, Nikon), a mirror (M), an illumination lens (L_1) ($f = 100$ mm), a nonpolarizing 50:50 beam splitter cube (BSC), a reference mirror on an axial translation stage (M_{ref}), sample with a water-filled microchannel with an attached excitation piezo, a 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.

Interference of light returning from the sample and reference arms only 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 (OPD) 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 μm .

A. Microchannel Chip

The microchannel was fabricated using photolithography and deep reactive-ion etching on a $\langle 100 \rangle$ silicon wafer. Access holes of 1 mm diameter were made from the back 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 cm by 1.5 cm. The length l , width w , and depth h of the microchannel are 4 cm, 380 μm , and 155 μm , respectively. See Fig. 3 for the measurement region in the microchannel. The image is obtained by the EMCCD detector by placing the reference mirror such that no interference is visible, i.e., the OPD 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 μm .

The channel was completely filled with demineralized water (Milli-Q) through 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 the piezo-element attached was mounted into the sample arm of the setup.

Fig. 3. False-color top-view image of the microchannel. The red-colored regions indicate the silicon outside the microchannel and the dashed white rectangle indicates the measurement region.

B. 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 with 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 Fig. 1. To measure at the bottom of the channel, the sample position is manually adjusted such that the total optical path from the beam splitter to the interface and back (B) is equal (within the coherence length) to the optical path from the beam splitter 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 $-z$ direction until interference fringes are again observed. The reference mirror is kept at its center position (see Section 4) during the alignment procedure. This method gives a positioning precision better than the coherence length l_c .

C. 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).

4. ACOUSTO-OPTICAL MEASUREMENT METHOD

An acoustic resonance as described by Eq. (1) induces an instantaneous change in the 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 [45].

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 Fig. 4). By setting the time delay τ between the acoustic excitation signal and the pulse, the moment 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 a function of mirror position) can be recorded per pixel. The interferogram peaks at its maximum value when the OPD between the light returning from the

Fig. 4. Schematic illustration of the acousto-optic measurement method. For each mirror position z_m (see Fig. 2), 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 a 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.

sample and the reference arms is 0. An acoustically induced variation will result in a shift of the interferogram equal to the amount of change in the 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 z -direction ($n_z \neq 0$).

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.

A. Data Post-processing

For each pixel, four interferograms are recorded, one for each time delay. To find the relative shifts among 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

$$I_{\text{ref}} = c_1 + c_2 \cos(2\pi/c_3 \cdot z_m + c_4) \cdot \exp\left(\frac{(z_m - c_5)^2}{2c_6^2}\right), \quad (10)$$

with c_3 and c_6 the 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 of which may vary with the position within the measurement region. c_3 and c_6 are found by fitting Eq. (10) to the 100 interferograms with the strongest signal per scan and using an 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 substep accuracy using Fourier interpolation followed by sine fit of the peak.

The amplitudes of the acoustically induced variations in the index of refraction $\delta n(x, y)$ and phase $\varphi(x, y)$ can be calculated using the quadratures I and Q :

$$I = z_0 + z_1 - z_2 - z_3, \quad (11)$$

$$Q = z_0 - z_1 - z_2 + z_3, \quad (12)$$

$$\delta n = \sqrt{I^2 + Q^2}/2h, \quad (13)$$

$$\varphi = \tan^{-1}(Q/I), \quad (14)$$

with z_n the shift in the optical path length of the interferogram for a delay τ_n relative to the digital reference signal. The phase $\varphi(x, y)$ contains the relative timing of the periodic acoustic effect among different pixels.

The variation of the 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 a variation in the index of refraction due to pressure and the inverse of Eq. (9):

$$p(x, y) = \frac{-\alpha_{ac} + \sqrt{\alpha_{ac}^2 + 4\epsilon_{ac}\delta n \cos(2\pi\varphi)}}{2\epsilon_{ac}}. \quad (15)$$

Measurements are done at two interfaces: subscripts B and T indicate measurements done at the bottom and top of the channel, respectively (see Fig. 1). 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 the 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_y} or mixed modes such as $p_{n_x, n_y, 0}$ with low n_x can be regarded to be constant in the x -direction within the measurement area. In such cases, the average can be taken over the x -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 Eq. (15).

5. RESULTS

A. Acoustic Pressure Distributions at Resonance

The measured instantaneous local pressures $p(x, y)$ for the modes p_1 , p_2 , and p_3 are shown in Fig. 5. On the left, the pressure distributions as measured on the bottom of the channel p_B and on the right the x -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 Eq. (15) 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 (2.1 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 with the surrounding pixels, which is especially apparent in Fig. 5(b). 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% for $n_y = 3$. This could be partially explained by a lower temperature used in experiment resulting in a lower speed of sound and resonance frequency.

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

The wavelengths of the resonances λ_{n_y} and the pressure amplitudes p_a are found by fitting a cosine to $\bar{p}_B$. Confidence bounds at 95% are included. The results from the fit are $\lambda_1 = 764 \pm 5.3 \mu\text{m}$, $\lambda_2 = 374 \pm 3.3 \mu\text{m}$, and $\lambda_3 = 265 \pm 1.3 \mu\text{m}$, and the corresponding values for p_a are 9.55 ± 0.20 MPa, 154 ± 2.1 kPa, and 196 ± 2.5 kPa. The acoustic pressures show a clear sinusoidal variation in the y -direction as indicated by the small confidence bounds and are almost constant in the x -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 0.5–1.9 Vpp for the fundamental resonance, which would correspond to over 16 MPa at 50 Vpp, which we used for the p_1 mode [30]. 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 and Aider obtained a pressure amplitude of 110 kPa with 5 Vpp excitation [31] and Lakämper *et al.* measured 53–160 kPa at 10 Vpp [32], values comparable to the ones we obtained.

The measurements done at the top of the channel are shown as $\bar{p}_T$ on the right in Fig. 5. Because the top interface is smooth, all pixels show a good signal and the uncertainty is lower than those of the measurements done at the channel bottom.

The acoustic variation in the OPD, shown as in-water equivalent pressure $\bar{p}_T$, is much smaller than the acoustic variation in the 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 antinodes 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 cannot 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 the 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 x -direction (57 values), the sensitivity is approximately 5 kPa as seen from the fit confidence bounds.

B. Pressure Mode Spectroscopy

By measuring pressure distributions at a range of frequencies and plotting $|\bar{p}_B|$ as a function of excitation frequency f , spectrograms of acoustic resonances can be made. The result for frequency ranges around the resonances in Section 5.A is shown in Fig. 6. The spectrograms give an overview of the pressure resonances present in the system. Relative peak amplitudes and the spatial distribution over the width of the channel

Fig. 6. Mode spectrograms: x -averaged pressure amplitude distributions $|\bar{p}_B|$ as functions of excitation frequency f in the vicinity of the resonances p_1 , p_2 , and p_3 , which are around $f = 1.93$ MHz, $f = 3.83$ MHz, and $f = 5.67$ MHz, respectively.

can be compared. The n_y 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 distribution of the pressure amplitude over the width of the channel and does not include the phase $\varphi(x, y)$. The phase depends on the total time delay between signal generation at the function generator and the 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, the response of the electronic equipment, the mode coupling between the excitation piezo and the acoustic mode, etc. Therefore, using $\bar{p}_B$ as a function of frequency would result in a very chaotic map, which defeats the purpose of giving an overview of the acoustic modes available in the microchannel.

To estimate the influence of glass modes on $|\bar{p}_B|$, measurements at the top layer have also been done. Within each of the three ranges displayed in Fig. 6, the highest in-water equivalent pressures $\bar{p}_T$ are 1.7 MPa at 1.93 MHz [as shown in Fig. 5(a)], 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.A are clearly visible at $f = 1.93$ MHz, $f = 3.84$ MHz, and $f = 5.68$ MHz, respectively. Mixed modes are also visible. These tend to have a lower peak amplitude and are lossier (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 [25].

A notable exception is the relatively strong mode $f = 3.5$ MHz, which cannot be explained, as all modes represented by Eq. (1) satisfy the hard-wall condition (antinodes at the boundary). The pressure distribution of this mode has nodes at the boundary and one antinode in the middle. No notable signal is measured at this frequency at the top of the channel.

1. Determination of Resonance Quality

For experiments and design purposes, it is important to know the resonance quality of an acoustic resonance, as measured by

Fig. 7. Measured energy density $E_a = \bar{p}_a^2 / (4\rho_0 c^2)$ and a Lorentzian fit as a function of frequency for the p_1 mode.

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 Eq. (3) and is plotted as a function of frequency f (see Fig. 7). A Lorentzian function defined by Eq. (4) is fitted to the data using the least-square 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.

6. CONCLUDING REMARKS

Using a stroboscopic interferometric technique, we have 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 OPD due to change in the index of refraction due to the local acoustic pressure. The mode profiles at resonance for three measured resonances closely resemble the theoretical model. Fields measured at a range of excitation frequencies can be combined into mode spectrograms showing which modes are present at which

frequency. From the measurements the local pressure amplitude, acoustic energy density, and Q -factor of modes can also 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 affected most significantly 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 [18,46].

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 those used in shaped acoustic fields [11], acoustophoresis [46], and even piezoprintheads. Due to the spatial selectivity, the local pressure around micro-objects can be measured and novel configurations in acoustic microfluidics can be investigated.

Funding. Foundation for Fundamental Research on Matter (FOM); Wetsus, European Centre of Excellence for Sustainable Water Technology.

Acknowledgment. This work is part of the research program of the Foundation for Fundamental Research on Matter, which is part of the Netherlands Organization for Scientific Research. This work was performed in the cooperation framework of Wetsus, European Centre of Excellence for Sustainable Water Technology (www.wetusus.nl). Wetsus is funded by the Dutch Ministry of Economic Affairs and Ministry of Infrastructure and Environment. The authors would like to thank the participants of the research theme Sensing for the fruitful discussions and their financial support.

REFERENCES

- G. M. Whitesides, "The origins and the future of microfluidics," *Nature* **442**, 368–373 (2006).
- D. Mark, S. Haeberle, G. Roth, F. Von Stetten, and R. Zengerle, "Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications," *Chem. Soc. Rev.* **39**, 1153–1182 (2010).
- A. Bhagat, H. Bow, H. Hou, S. Tan, J. Han, and C. Lim, "Microfluidics for cell separation," *Med. Biol. Eng. Comput.* **48**, 999–1014 (2010).
- C. Wyatt Shields IV, C. Reyes, and G. López, "Microfluidic cell sorting: a review of the advances in the separation of cells from debulking to rare cell isolation," *Lab Chip* **15**, 1230–1249 (2015).
- J. Nilsson, M. Evander, B. Hammarström, and T. Laurell, "Review of cell and particle trapping in microfluidic systems," *Anal. Chim. Acta* **649**, 141–157 (2009).
- H. Gu, C. U. Murade, M. H. G. Duits, and F. Mugele, "A microfluidic platform for on-demand formation and merging of microdroplets using electric control," *Biomed. Microfluidics* **5**, 011101 (2011).
- E. Delamarche, D. Juncker, and H. Schmid, "Microfluidics for processing surfaces and miniaturizing biological assays," *Adv. Mater.* **17**, 2911–2933 (2005).
- C.-Y. Lee, C.-L. Chang, Y.-N. Wang, and L.-M. Fu, "Microfluidic mixing: a review," *Int. J. Mol. Sci.* **12**, 3263–3287 (2011).
- X. Ding, P. Li, S.-C. S. Lin, Z. S. Stratton, N. Nama, F. Guo, D. Slotcavage, X. Mao, J. Shi, F. Costanzo, and T. J. Huang, "Surface acoustic wave microfluidics," *Lab Chip* **13**, 3626–3649 (2013).
- Z. Wang and J. Zhe, "Recent advances in particle and droplet manipulation for lab-on-a-chip devices based on surface acoustic waves," *Lab Chip* **11**, 1280–1285 (2011).
- J. Reboud, Y. Bourquin, R. Wilson, G. S. Pall, M. Jiwaji, A. R. Pitt, A. Graham, A. P. Waters, and J. M. Cooper, "Shaping acoustic fields as a toolset for microfluidic manipulations in diagnostic technologies," *Proc. Natl. Acad. Sci. USA* **109**, 15162–15167 (2012).
- Y. Chen and S. Lee, "Manipulation of biological objects using acoustic bubbles: a review," *Integr. Comp. Biol.* **54**, 959–968 (2014).
- T. Xu, F. Soto, W. Gao, R. Dong, V. Garcia-Gradilla, E. Magaña, X. Zhang, and J. Wang, "Reversible swarming and separation of self-propelled chemically powered nanomotors under acoustic fields," *J. Am. Chem. Soc.* **137**, 2163–2166 (2015).
- K. Yasuda, S.-I. Umemura, and K. Takeda, "Concentration and fractionation of small particles in liquid by ultrasound," *Jpn. J. Appl. Phys.* **34**, 2715 (1995).
- J. J. Hawkes and W. T. Coakley, "A continuous flow ultrasonic cell-filtering method," *Enzyme Microb. Technol.* **19**, 57–62 (1996).
- J. J. Hawkes and W. T. Coakley, "Force field particle filter, combining ultrasound standing waves and laminar flow," *Sens. Actuators B* **75**, 213–222 (2001).
- H. Nilsson, M. Wiklund, T. Johansson, H. M. Hertz, and S. Nilsson, "Microparticles for selective protein determination in capillary electrophoresis," *Electrophoresis* **22**, 2384–2390 (2001).
- J. Svennebring, O. Manneberg, P. Skafte-Pedersen, H. Bruus, and M. Wiklund, "Selective bioparticle retention and characterization in a chip-integrated confocal ultrasonic cavity," *Biotechnol. Bioeng.* **103**, 323–328 (2009).
- N. R. Harris, M. Hill, S. Beeby, Y. Shen, N. M. White, J. J. Hawkes, and W. T. Coakley, "A silicon microfluidic ultrasonic separator," *Sens. Actuators B* **95**, 425–434 (2003).
- M. Bengtsson and T. Laurell, "Ultrasonic agitation in microchannels," *Anal. Bioanal. Chem.* **378**, 1716–1721 (2004).
- J. J. Hawkes, R. W. Barber, D. R. Emerson, and W. T. Coakley, "Continuous cell washing and mixing driven by an ultrasound standing wave within a microfluidic channel," *Lab Chip* **4**, 446–452 (2004).
- O. Manneberg, S. Melker Hagsäter, J. Svennebring, H. M. Hertz, J. P. Kutter, H. Bruus, and M. Wiklund, "Spatial confinement of ultrasonic force fields in microfluidic channels," *Ultrasonics* **49**, 112–119 (2009).
- M. Hill and R. J. K. Wood, "Modelling in the design of a flow-through ultrasonic separator," *Ultrasonics* **38**, 662–665 (2000).
- M. Hill, "The selection of layer thicknesses to control acoustic radiation force profiles in layered resonators," *J. Acoust. Soc. Am.* **114**, 2654–2661 (2003).
- R. Barnkob and H. Bruus, "Acoustofluidics: theory and simulation of radiation forces at ultrasound resonances in microfluidic devices," *Proc. Meet. Acoust.* **6**, 020001 (2009).
- H. Bruus, "Acoustofluidics 2: perturbation theory and ultrasound resonance modes," *Lab Chip* **12**, 20–28 (2012).
- A. Lenshof, M. Evander, T. Laurell, and J. Nilsson, "Acoustofluidics 5: building microfluidic acoustic resonators," *Lab Chip* **12**, 684–695 (2012).
- P. Hahn, O. Schwab, and J. Dual, "Modeling and optimization of acoustofluidic micro-devices," *Lab Chip* **14**, 3937–3948 (2014).
- S. M. Hagsäter, T. G. Jensen, H. Bruus, and J. P. Kutter, "Acoustic resonances in microfluidic chips: full-image micro-PIV experiments and numerical simulations," *Lab Chip* **7**, 1336–1344 (2007).
- R. Barnkob, P. Augustsson, T. Laurell, and H. Bruus, "Measuring the local pressure amplitude in microchannel acoustophoresis," *Lab Chip* **10**, 563–570 (2010).
- O. Dron and J.-L. Aider, "Acoustic energy measurement for a standing acoustic wave in a micro-channel," *Europhys. Lett.* **97**, 44011 (2012).
- S. Lakämper, A. Lamprecht, I. A. T. Schaap, and J. Dual, "Direct 2D measurement of time-averaged forces and pressure amplitudes in acoustophoretic devices using optical trapping," *Lab Chip* **15**, 290–300 (2015).
- B. J. Hoenders, "The painful derivation of the refractive index from microscopical considerations," in *Proceedings of Light-Activated Tissue Regeneration and Therapy Conference*, R. Waynant and

- D. B. Tata, eds., Vol. 12 in Lecture Notes in Electrical Engineering (Springer, 2008), pp. 297–305.
34. M. Salanne, R. Vuilleumier, P. A. Madden, C. Simon, P. Turq, and B. Guillot, "Polarizabilities of individual molecules and ions in liquids from first principles," *J. Phys.* **20**, 494207 (2008).
35. D. Pinnow, "Guide lines for the selection of acoustooptic materials," *IEEE J. Quantum Electron.* **6**, 223–238 (1970).
36. C. Sanchez-Valle, D. Mantegazzi, J. D. Bass, and E. Reusser, "Equation of state, refractive index and polarizability of compressed water to 7 GPa and 673 K," *J. Chem. Phys.* **138**, 054505 (2013).
37. L. E. Kinsler, *Fundamentals of Acoustics*, 4th ed. (Wiley, 2000).
38. "CRC Handbook of Chemistry and Physics, 92nd ed., 2011–2012," *J. Am. Chem. Soc.* **133**, 13766 (2011).
39. A. H. Harvey, J. S. Gallagher, and J. S. Levelt Sengers, "Revised formulation for the refractive index of water and steam as a function of wavelength, temperature and density," *J. Phys. Chem. Ref. Data* **27**, 761–767 (1998).
40. N. Bilaniuk and G. S. K. Wong, "Speed of sound in pure water as a function of temperature," *J. Acoust. Soc. Am.* **93**, 1609–1612 (1993).
41. R. T. Beyer, "Parameter of nonlinearity in fluids," *J. Acoust. Soc. Am.* **32**, 719–721 (1960).
42. T. Pitts, A. Sagers, and J. Greenleaf, "Optical phase contrast measurement of ultrasonic fields," *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **48**, 1686–1694 (2001).
43. I. Shavrin, L. Lipiäinen, K. Kokkonen, S. Novotny, M. Kaivola, and H. Ludvigsen, "Stroboscopic white-light interferometry of vibrating microstructures," *Opt. Express* **21**, 16901–16907 (2013).
44. A. F. Fercher, C. K. Hitzberger, M. Sticker, E. Moreno-Barriuso, R. Leitgeb, W. Drexler, and H. Sattmann, "A thermal light source technique for optical coherence tomography," *Opt. Commun.* **185**, 57–64 (2000).
45. T. Spirig, P. Seitz, O. Vietze, and F. Heitger, "The lock-in CCD-two-dimensional synchronous detection of light," *IEEE J. Quantum Electron.* **31**, 1705–1708 (1995).
46. O. Manneberg, B. Vanherberghen, B. Önfelt, and M. Wiklund, "Flow-free transport of cells in microchannels by frequency-modulated ultrasound," *Lab Chip* **9**, 833–837 (2009).