Chapter 9 |

Outlook

Abstract

During the course of a PhD, many research ideas are pursued that do not become successful or completed projects. Even though some of these are not developed enough to become chapters in this thesis, they are still promising directions for future research. In this final chapter, I outline a couple of those projects.

9.1 Air-filled microspheres

In this thesis, we have used AFS to apply forces on polystyrene microspheres. Polystyrene microspheres are advantageous because of their commercially availability in a wide range of sizes, their mono-disperse size distribution and the possibility to couple them to various chemical labels. As shown in **Error! Reference source not found.a**, different materials have different acoustic properties and polystyrene does not have the strongest response to acoustic fields. While we have optimized our chip configuration in Error! Reference source not found., we have never optimized the microsphere material in order to get the highest efficiencies. A higher efficiency in force with the same size microsphere means, of course, that higher forces can be reached. Another possibility is to reduce microsphere size, which results in a faster response time and a higher localization accuracy of the system (**section** Error! Reference source not found. and Error! Reference source not found.).

To quantify how strong material responds to the acoustic field, the acoustic contrast factor (Φ) is used[151]:

$$\Phi = \frac{\rho_p + 2/3(\rho_p - \rho_m)}{2\rho_p + \rho_m} - \frac{1}{3}\frac{\rho_m {c_m}^2}{\rho_p {c_p}^2} \quad (9.1)$$

With ρ_p and ρ_m being the densities, and c_p and c_m being the speed of sound of the particle and the medium, respectively. A polystyrene particle has an acoustic contrast factor of 0.22, denser and stiffer material like glass 0.54 (**Table 9.1**). Since the acoustic radiance force scales with the volume of the object (**equation** Error! Reference source not found.), the decrease in particle size for a glass microsphere that can be used with AFS is limited.

Inspired by the field of ultrasound imaging, we have been testing acoustic contrast agents[152]. These are air-filled particles, often made from lipids, with a very high acoustic contrast factor, due to the high difference in density of air compared to water (**Table 9.1**). Note that the contrast factor is negative, meaning that these particles are pushed to the anti-node of the acoustic field. However, the compressibility of air is also very high, as a result, these particles generate a strong acoustic field around them that interacts with other particle or the surface, called secondary Bjerknes forces[153]. These secondary effects could interfere with our measurement. For this reason, we have set out a search to find air-filled particle with a low compressibility. Dmitry Grishenkov (Department of Medical engineering, the Royal Institute of Technology, Sweden) has provided us with air-filled polymer-shelled microspheres[154]. These microspheres have an average size of 2 µm in

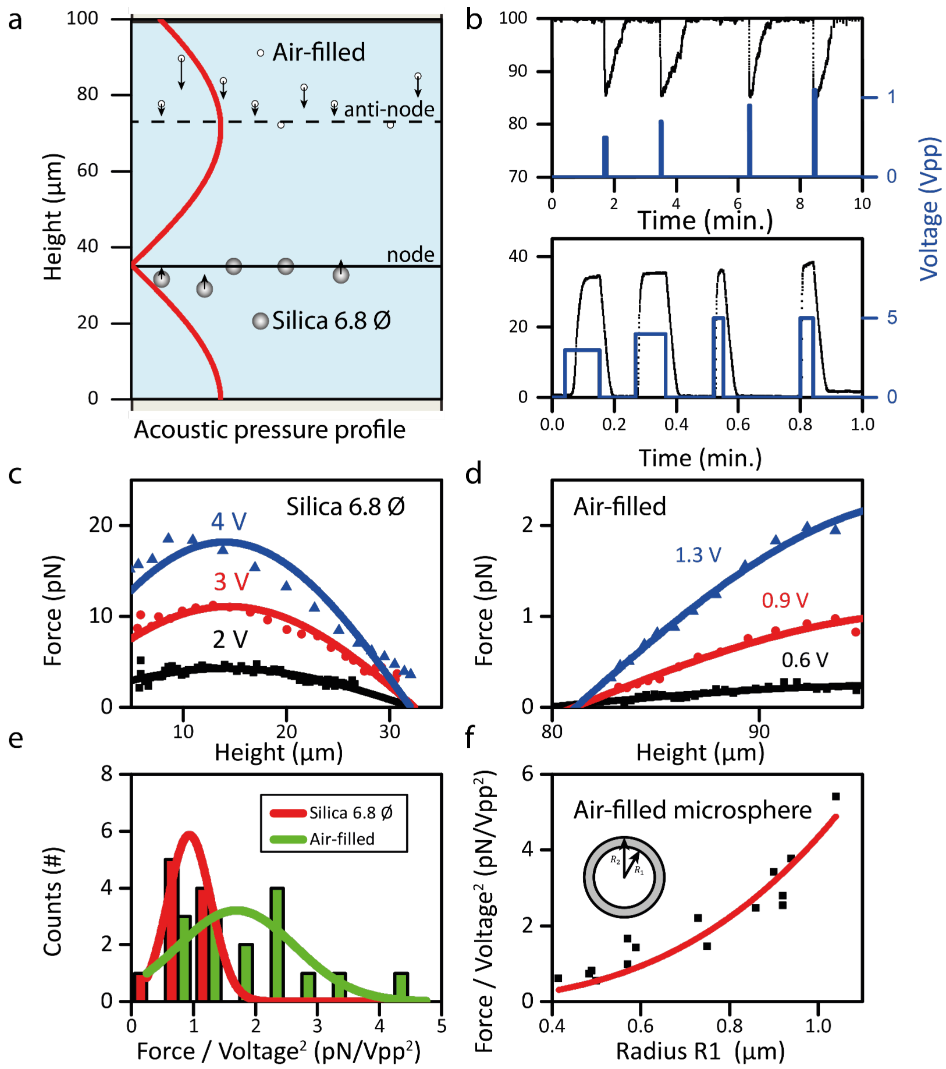

Figure 9.1 | Calibration of acoustic forces acting on air-filled microspheres

(**a**) Graph showing a cross section of the flow cell. The acoustic pressure profile (red line) is plotted of the 10.3 MHz resonance frequency, using the bottom force chip described in Error! Reference source not found.. Silica and air-filled microspheres are pushed toward the node and the anti-node, respectively. (**b**) Graphs showing the height location of the silica (bottom) and air-filled (top) microspheres over time, while applying different voltages. (**c**-**d**) Measured (dots) and fitted (line) force profile for the silica (**c**) and the air-filled (**d**) microspheres plotted for three different voltages. (**e**) Distribution of the calibrated force/voltage^2 relation of the air-filled and the silica beads. Distributions are fitted with a Gaussian function, yielding a peak at 0.94 ± 0.01 and 1.7 ± 0.2 pN V^{-2}, and a width of 0.69 ± 0.03 and 1.8 ± 0.5 pN V^{-2} for the silica and air-filled microspheres, respectively. (**f**) Calibrated force/voltage^2 relation plotted versus the measured microspheres size and fitted with a third power function. Fit yields 4.3 ± 0.2 pN V^{-2} μm^{-1}. (fit values ± s.e.m. for **c**-**f**)

diameter, the shell is approximately 300 nm thick, made of poly-vinyl alcohol (PIV) and the reported acoustic contrast factor is -60.7[154]. To test if these particles are suitable for AFS experiments, we calibrate their response in the AFS setup.

We observe that these air-filled microspheres are floating upwards to the top of the fluidic chamber, in contrast to other microsphere that are heavier than water and sink to the bottom. To calibrate the force applied to these microspheres, we used the method explained in **section** Error! Reference source not found., where the microspheres are forced from the surface to the acoustic node. To calculated the acoustic contrast factor, we compare the acoustic response of the air-filled microspheres to microspheres with a well-known acoustic contrast factor (silica microspheres with 6.8 µm in diameter). To make a correct comparison, we calibrate both microspheres with the same resonance frequency in the same chip. To this end, we use a resonance frequency that pushes the air-filled microspheres downwards to the acoustic anti-node and the silica microspheres upwards to the acoustic node (**Figure 9.1a**). We observe that the air-filled and the silica microspheres can be forced in a controlled fashion to the acoustic anti-node and node, respectively (**Figure 9.1b**). The force on the microspheres at each height location is determined from the velocity with which they move from the surface to the node (or anti-node), as explained in **section** Error! Reference source not found.. The force profiles for different applied voltages are fitted with sine functions (**Figure 9.1c** and **d**) and the force/voltage^2 ratio is calculated for a population of silica and air-filled microspheres (**Figure 9.1e**).

We notice a large spread in the force/voltage^2 ratio for the air-filled microspheres. Therefore, we use the upward velocity of the air-filled microspheres to calibrate each individual radius. To this end, we set out a force balance of all the forces experienced by the microsphere: the buoyance (F_b), gravitation (F_g) and the stokes drag force (F_{Stokes}) and solved it for the velocity:

$$F_b + F_g + F_{Stokes} = 0 \tag{9.2}$$

$$F_g = V\rho g = \frac{4}{3}\pi g\left(\left({R_2}^3 - {R_1}^3\right)\rho_{PVA} + {R_1}^3\rho_{air}\right) \tag{9.3}$$

$$F_{Stokes} = -6\pi\eta R_2 v \tag{9.4}$$

$$v = \frac{2g}{9\eta R_2}\left(\left({R_2}^3 - (R_2 - d)^3\right)\rho_{PVA} + (R_2 - d)^3\rho_{air} - {R_2}^3\rho_{water}\right) \tag{9.5}$$

Where V represents the volume of the microsphere, ρ the density, R_1 and R_2 the inner and the outer radius of the microsphere, respectively, η the viscosity of the medium and d the shell thickness (R_2 - R_1 = 300 nm). Using equation 9.5, the outer shell radius could be extracted from the floating velocity. Since the acoustic force scales with the volume of the particle, the force/voltage^2 ratio is plotted against the inner radius and fitted with a third power function (**Figure 9.1f**). When we extrapolate this function to the radius of the silica microspheres (3.4 µm), we find that the air-filled microspheres experience 170 ± 14 fold higher force than the silica ones, but in the opposite direction. As a result, we find that the acoustic contrast factor is -170 ± 14 · 0.54 = - 92 ± 7 compared to polystyrene microspheres (the microspheres material we typically use). The increase in force is about 400-fold, which means that we can use at least 7 times smaller microspheres and still reach the same force.

If those air-filled microspheres could be used in combination with AFS, it would provide an enhancement in the response time of the system and localization accuracy (see **section** Error! Reference source not found. and Error! Reference source not found.). However, they are at the moment not commercially available. Furthermore, we have not managed yet to chemically couple them to a biological sample and, lastly, the AFS chip is optimized to apply forces on particles with a positive acoustic contrast factor. These issues have to be overcome in order to make the air-filled microspheres suitable for experiments.

	Density ($Kg\ m^{-3}$)	*Speed of sound ($m\ s^{-1}$)*	*Acoustic contrast factor*
Water	1000	1482	-
Polystyrene	1050	2350	0.22
Glass	2230	5674	0.54
Air	1	332	-6662.
Cell	1100	1500	0.07

Table 9.1 | Acoustic contrast factor (Φ) for different materials.
Acoustic contrast factor calculated using ***equation 9.****1. Values for the density and speeds of sound are taken from Mikkel Settnes and Henrik Bruus*[18]*. Note, that there many different kind of cells and that the acoustic contrast factor can vary between cell types*[155]*.*

Another potential application of these microspheres could be to chemically modify them in such a way that they bind to specific parts inside a cell. When an acoustic field is applied, these microspheres generate force inside a living cell that can be controlled with the applied acoustic field. Because of their ability to generate large forces, even when the particles are small, forces can be applied at specific locations in the cell.

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