Quantitative acoustophoresis:

A contact-free assay for the mechanical characterization of bioparticles

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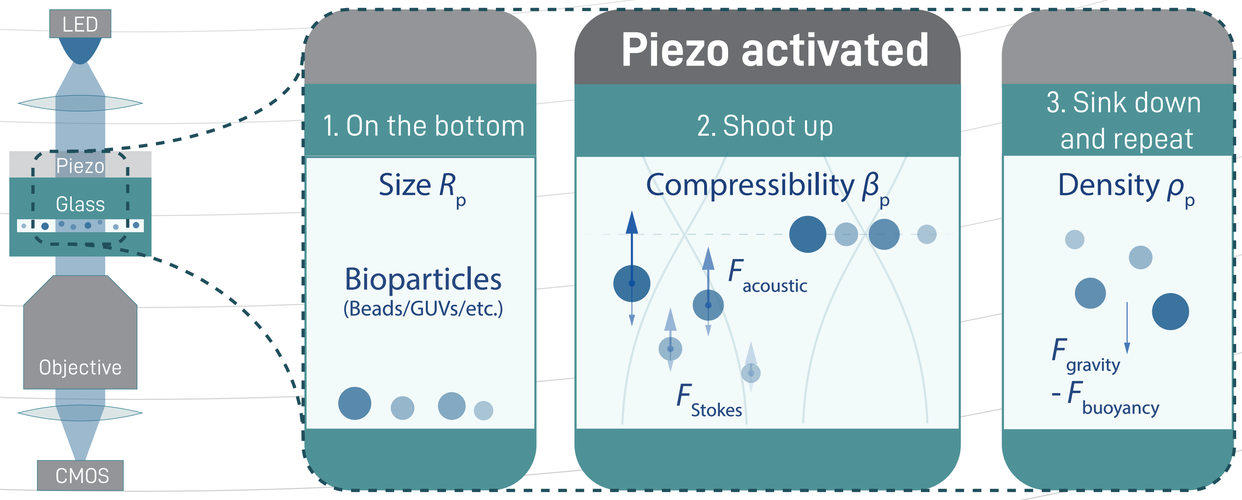
Introduction

In acoustophoresis, the acoustic field is used to exert forces on the micrometer-sized bioparticles directly. Their response depends heavily on their mechanical properties: volume, density, and compressibility, a measure of a relative volume change in response to a pressure change. Each of these properties can be measured independently during the experiment. This makes acoustophoresis a valuable biomechanical technique and a powerful tool for cytoskeletal studies. Here we demonstrate the method's validity and show how to use it for mechanical characterization of a diverse range of samples.

Unlike other techniques, like AFM and micropipette aspiration, this approach is label- and contact-free. Thus, any distortions introduced by the probes are absent in acoustophoresis.

Setup and procedure



Theory

A piezo element is attached to the top of the flow chamber. When the voltage is applied, an acoustic field is generated inside the channel. All the particles in the field region underneath the piezo experience an acoustic force[1-3] F_{ac}, which depends on the setup-related parameters: the field intensity E^*_{ac} and the applied voltage U; and on the sample properties: the volume V and the contrast factor φ. The latter is a function of the particle's and medium's densities ρ and compressibilities β.

$$F_{ac} = -\underbrace{E_{ac} k_{ac} \sin(2(k_{ac} z - \phi_{ac}))}_{\text{constant for different experiments}} U^2 \frac{4}{3}\pi R_p^3 \left(\frac{5\rho_p - 2\rho_m}{2\rho_p + \rho_m} - \frac{\beta_p}{\beta_m}\right) \propto U^2 V \varphi_p$$

If the contrast factor $\varphi < 0$, the particle is pushed away from the field node, if $\varphi > 0$, the particle is attracted to the node. The force is particularly large when the frequency is tuned to experience resonance due to the chip geometry.

During the shooting-up, the particle speed is recorded and the acoustic force F_{ac} is calculated using the force balance equation:

$$F_{ac} = F_{Stokes} + F_{grav} - F_{buoy} = 6\pi R_p \nu_p \eta_m \lambda(z_p) + \frac{4}{3}\pi R_p^3 (\rho_p - \rho_m) g$$

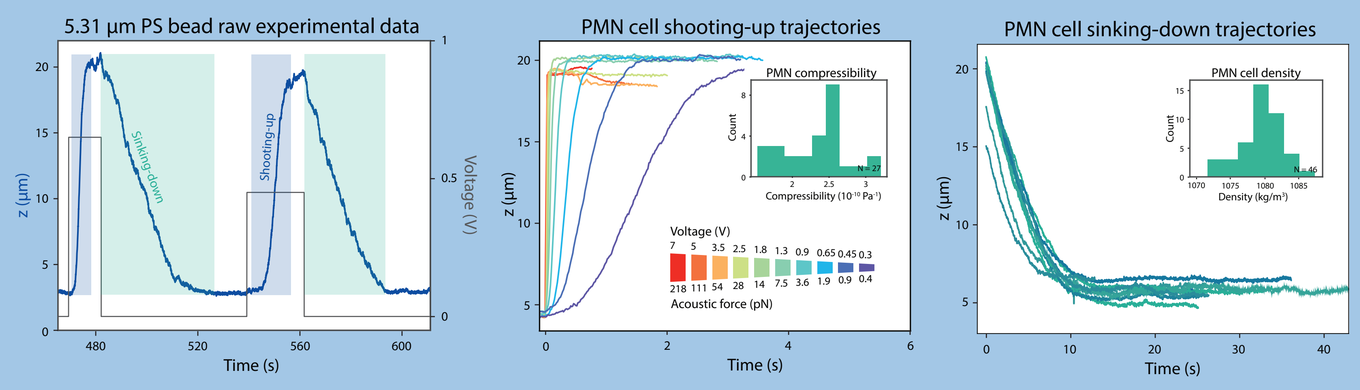
To increase the precision, the procedure is repeated at least five times at different voltages . The voltage-normalized acoustic force f_p (VNAC) is then compared to that of the calibration sample f_{cal} to determine the contrast factor φ_p and the compressibility of the bioparticle β_p.

$$f_{ac} = F_{ac}/U^2 \qquad \varphi_p = \frac{R_p^3}{R_{cal}^3}\frac{1}{\varphi_{cal}}\frac{f_{cal}}{f_p} \qquad \beta_p = \left(\frac{5\rho_p - 2\rho_m}{2\rho_p + \rho_m} - \varphi_p\right)\beta_m$$

Results

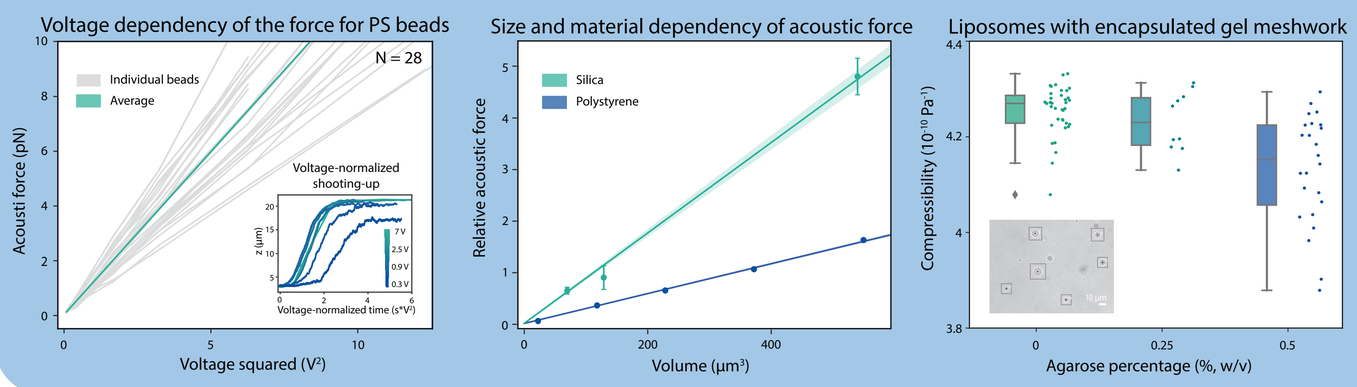
1. No prior information about the sample is needed

The size of the bioparticles is measured by a camera, their density is determined from the sinking-down slope and their compressibility is calculated via the fit of the shooting-up trajectories. Thus, even samples with unknown properties, such as neutrophil cells (PMN), can be characterized mechanically.



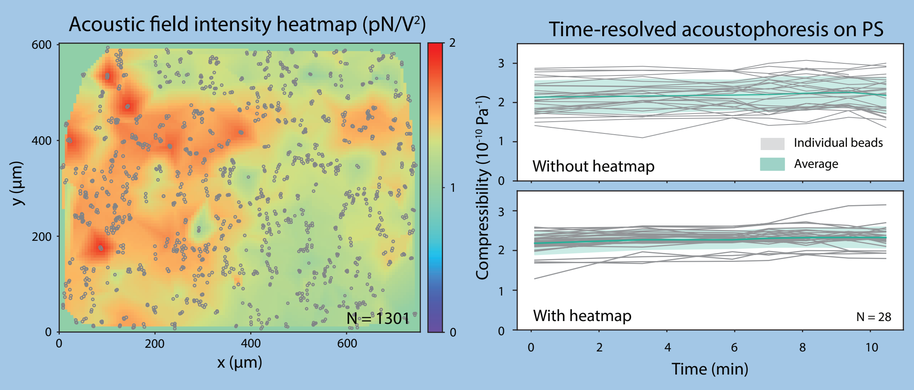
2. Shooting up response depends on the mechanics of the sample

Dependency of F_{ac} on the voltage-squared, particle volume and the contrast factor (density and compressibility) of the sample was shown. Moreover, the compressibility of liposomes (produced with Ref. 4) with cytoskeleton-mimicking gel meshwork was compared to show its application for artificial cells.



3. Mechanical changes can be tracked over time

By mapping the acoustic field intensity in X and Y, the method's precision can be increased further to resolve the mechanical properties of individual bioparticles and track their changes over time.



Outlook

Acoustophoresis is a powerful method to characterize bioparticles and monitor their mechanical changes over time. Its relative simplicity of use (commercial AFS setups), fast experimental routine (15 min), and large statistics in a single measurement (up to 150 particles) make it a valuable approach in the fields of cell mechanics, synthetic biology, and material science.

For this project, we will further develop acoustophoresis's sensitivity and time-resolved applications in cytoskeletal studies, including the assessment of the cytoskeletal reconstitution in artificial cells.

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