On the time-resolved tracking of position and orientation of prolate spheroids in microflows

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Extended abstract

The separation of microparticles according to their shape defines a growing field with applications in solar energy storage, biomedicine and biofuel production (Behdani et al. (2018)). Besides passive methods, such as deterministic lateral displacement, the use of active methods offers a great flexibility and scalability. A very promising method is the active particle separation with acoustofluidic devices, e.g. using standing surface acoustic waves (sSAW) in microfluidic environments (Fan et al. (2022)). With these devices, particle separation is often based on the so-called acoustic radiation force (ARF), which scales with the volume and acoustic contrast of the particles. Since the ARF is determined by gradients in the acoustic fields including the wave scattered by the particle, a separation of elongated and spherical particles according to their shape is further conceivable. To investigate this potential separation mechanism, the dynamic behavior of suspended prolate polystyrene spheroids subjected to a standing acoustic pressure field in a microchannel originating from an sSAW is analyzed by optical measurement techniques based on particle tracking.

Methods

The fluorescent spheroids were suspended in a pressure-driven flow within a microchannel, which was attached centrally in between two interdigital transducers (IDTs) on a piezoelectric substrate of LiNbO\(_3\). In this setup, an sSAW can be generated in the microchannel to enable the active particle separation. During the experiments, the microfluidic device was positioned on an inverted microscope (Axio Observer 7, Zeiss GmbH) and the particles were illuminated by green laser light (\(\lambda = 532\) nm). Images were acquired by guiding the emitted light from the particles to a camera (imager sCMOS, LaVision GmbH). To detect the position \((x,y)\) and orientation \((\varphi, \theta)\) of the prolate spheroids, the raw images were processed in two steps. First, potential particle images were detected by applying a segmentation based on a global intensity threshold. To account for out-of-plane tilting, a second segmentation was performed with a local intensity threshold. For each segmented particle image, the in-plane coordinates \((x,y)\) and estimates for the in-plane orientation \(\varphi\) as well as the length of the major and minor axes \((a_{\text{max}}, a_{\text{min}})\) were determined. Second, the in-plane angle \(\varphi\) was specified more precisely by a least square fit of a rotated two-dimensional Gaussian intensity distribution to the particle image. After detection, a simple particle tracking was applied. Assuming that an individual particle is fully visible and focused in the \(xy\)-plane \((\theta = 90^\circ)\) at least once during the time-resolved tracking, the out-of-plane angle \(\theta\) was approximated by the length of the major axis.

Results

The image processing algorithm is validated based on synthetic images of prolate spheroids with well-known ground truth generated by MicroSIG (Rossi (2020)). A quantitative evaluation on the deviation of the detected in-plane coordinates \((x, y)\) and the in-plane angle \(\varphi\) from the ground truth is given in Fig. 1(a) and (b), respectively. The corresponding mean absolute errors \(MAE_{x,y}\) and \(MAE_{\varphi}\) reveal subpixel accuracy. According to Fig. 1(c), a higher uncertainty in the determination of the out-of-plane angle \(\theta\) is evident in region I and III. However, a reliable prediction of \(\theta\) with \(MAE_{\theta} = 2.42^\circ\) is achieved in region II for ground truth values of \(\theta_{\text{GT}} \in [18.6^\circ, 67^\circ]\). The typical tumbling motion of two selected prolate spheroids with comparable velocity measured in a pressure-driven microflow is depicted in Fig. 1(d)-(f). By evaluating their dynamical behavior, a lower frequency of rotation is observed for the particle with higher aspect ratio \(\Gamma = a_{\text{max}}/a_{\text{min}}\), which is in very good agreement to recent predictions by numerical simulations (Lauricella et al. (2022)). Furthermore, this particle is aligned in flow direction \((\varphi \rightarrow 180^\circ \vee \varphi \rightarrow 0^\circ)\) for a longer duration until performing a fast rotation. The results confirm the applicability of the algorithm to experimental data and enable a statistical analysis with increased sample size.
Conclusions and outlook In this study, an algorithm to detect the in-plane position and orientation of prolate spheroids was introduced and validated based on synthetic particle images. Its application to experimental data promise a deeper understanding of the dynamic behavior of prolate spheroids in a microchannel flow with and without active separation mechanism. For the latter, findings will be presented at the example of an sSAW field (Sachs et al. (2022)), demonstrating the applicability of the algorithm at active separation mechanisms and also revealing the influence of acoustic pressure fields on the motion of prolate particles.

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References


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