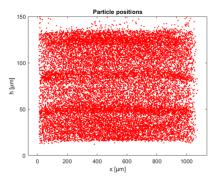
## **Supplementary Materials**

Figure S1 shows the processing steps to determine the local particle distribution, which is visualized in Figure 9 in the manuscript. APTV is a single-camera technique allowing to extend a planar image-based 2D-PTV approach to a 3D tracking approach with the help of astigmatic particle images. In the first step, a real-time image is recorded with the CCD camera from the top view. Using a predefined calibration curve, an exact height position in the microchannel (h-position) can be determined depending on the fluorescence of each particle. After this step, the counted particles can be positioned in the channel (Step 3). In a further processing step, the local particle distribution can be determined for all the particles at the respective height.



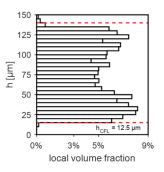
**Step 1**: A real-time image captured by the CCD camera in APTV measurement



**Step 3**: Particle positions after APTV analysis of h-x plane where x is a part of the channel length

 $a_{x} = 41 \text{ pix}$   $a_{y} = 62 \text{ pix}$   $h = 16 \mu\text{m}$ (a)  $a_{x} = 56 \text{ pix}$   $a_{y} = 43 \text{ pix}$   $h = 35 \mu\text{m}$ (b)  $a_{x} = 77 \text{ pix}$   $a_{y} = 34 \text{ pix}$   $h = 56 \mu\text{m}$ (c)  $a_{x} [\text{pix}]$ (c)  $a_{x} = 77 \text{ pix}$ (c)  $a_{x} = 77 \text{ p$ 

**Step 2**: A calibration curve and the individual particle images with different aspect ratios as an example



**Step 4**: Particle distribution in the microchannel obtained from the analysis of all particle positions

**Figure S1.** Visualization of the single steps for the analysis of the local particle distribution in the microchannel by APTV.

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