Supplemental material: Optical feedback control loop for the precise and robust acoustic focusing of cells, micro- and nanoparticles

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1 Python code for the feedback control loop

Folder feedback-control-loop-main.zip contains the entire code for the feedback control loop (FCL). The FCL consists of 3 .py functions. main.py is the code that needs to be run to start the FCL and is where all variables should be set. image_manipulation.py is where all the images functions are written while fuctions.py contains the code for all the other functions that the FCL needs to operate. The code can also be accessed via Gitlab and is freely usable and modifiable. The authors would gladly discuss any augmentations and offer tech support.

2 Experimental data

The Figures, Matlab_Files, and Videos folders contain various subfolders for each experiment that was run with different particle diameters (5 micrometers 5um, 1 micrometer 1um, 600 nanometers 600nm and 450 nanometers¹ 450nm) and SaOs-2 cells SaOs.

The folder Figures contains all the plots of the linewidths (LW) at a given flow rate and particle type. As an example of how to find the saved data: the file .../Figures/1um/15_20220316-151030.png is the plot of the linewidth for a flow rate of $15 \,\mu L \,min^{-1}$ (indicated by the 15 before __ in file name). After __ the date and time on which the analysis of the videos took place is noted. Analogously, the other figures can be found.

The Matlab-Files contains all the .mat files for each individual video analysis. As an example, the file .../MatlabFiles/1um/15.mat contains the LW variables for a 1 μ m particle at a flow rate of 15 μ L min⁻¹. Analogously, the other .mat files can be loaded into Matlab program and read.

The Videos folder contains all the trimmed videos that were recorded for a given experiment. The videos were trimmed to analyse each flow rate individually. All videos were recorded with a frame rate of 60, while the frame rate of the image capture can be read at the bottom right hand corner of the video itself. As an example, ...Videos/1um/15_1PS.mp4 is a video at a flow rate of $15 \,\mu L \,min^{-1}$ of a 1 μm particle. The image capture frame rate was FPS: 20.34. Analogously, the conditions of the other videos can be read.

3 Experimental data readout

Folder MatlabCode contains all the MATLAB code that was used for the calculations and plots of the experiments. Typically, video_postproc_1_5.m is the first file that should be run with the correct folder directories. This calculates the LW data from the separate videos and creates MATLAB variables

 $^{^{1}}$ The image quality was deemed to low to include the readout of this experiment in the results of the paper, but focusing can be seen in the raw video footage.

with the LW and individual LW plots. These files can be found in the directory .../Experiments/1um /Results_Step_1_Linewidth_Calculation and analogous folders for the different particle diameters and SaOs-2 cells. lw_post_process_1_5.m creates plots with the LW at different flow rates. lw_all_plots.m is another code that plots all the data points in a MATLAB figure for the different flows. plotPhase2 plots the figures for the phase 2 of the FCL with the data that the FCL saves during the iterations. data_management_2.m can be used to organize the data. This MATLAB code was however not used.