**2 Materials and Methods**

2.1 ACOUSTOFLUIDIC DEVICE

2.1.1 Fabrication AND MODELLING

The acoustofluidic device is made by integrating a piezoelectric transducer with a polydimethylsiloxane (PDMS) microchannel sealed with a glass reflector layer. The surface displacement transducer was created by milling a 0.1 mm-deep indent into the backside of a 30×20 mm2 piezoelectric transducer (*Pz-26, Parker Meggitt, UK*), determining the trapping region. The tilted shape of this indentation and its milling files were designed and generated using AutoCAD and Fusion 360 (*Autodesk Inc, CA, US*). For the milling of both the SDT and the mould, a computerized numerical control (CNC) milling machine (*Modela MDX-40A 3D Milling Machine, Roland DGA, CA, US*) was used.

The transducer was then integrated with the PDMS microchannel. This was done through standard soft lithography. The polymethyl methacrylate mold was milled from a 0.5-mm PMMA substrate and placed in a petri dish. PDMS was mixed in a 10:1 ratio of monomer to curing agent (*SYLGARD 184, Dow Corning, MC, US*) and was then poured into the mould, where the SDT had already been placed. This assembly was degassed for 45 minutes and then cured at 60ºC for 90 minutes. After fluidic connection were done with a 20 ga syringe (*Instech Laboratories Inc., PA, US*), the microchannel was sealed with a glass reflector layer. This was done by using an oxygen plasma treatment to activate the surface of a 75×38×1 mm3 borosilicate microscope slide (*Corning Inc, NY, US*) and of the PDMS slab, which were then bonded together through direct contact.

2.1.2 DEVICE Modelling

Similarly to previous work, two theoretical models were combined to predict the operation of the device. A 1D model based on an acoustic transmission line was used to determine the main axial resonances generated by the various material layers of the transducer. Then, COMSOL Multiphysics (*COMSOL 6.1, COMSOL Multiphysics, Sweden*) was used to find the adequate lateral dimensions of the SDT-protrusion to maximize the lateral resonances of the system through an eigenmode simulation. This work allowed a sweep of various eigenmodes and lateral indent dimensions that informed the selection of an ideal acoustic field node distribution. The frequency that generated this acoustic field was then confirmed by an impedance sweep (*Z-Check 16777k,**Analog Instruments*) to determine the SDT’s admittance peaks. Based on this, the frequency of 2.020 MHz was selected to provide a stable and well-defined grid.

2.1.2 Trapping area tilt modelling

The algorithm used for prediction the flow line interactions with the clusters was programmed using Python. This was done by creating a 7x7 grid based on the COMSOL Multiphysics simulations and was overlayed with X flow lines to represent the fluid. For particles at the nanoscale in laminar flow, they are assumed to follow the flow lines. The scoring system was constructed using the acoustic pressure decay with distance function from COMSOL Multiphysics and was normalized from 1-10. This system was used to sweep all possible angles of the grid for their performance in terms of how the flow lines interact more or less with the acoustic cluster field.

2.2 Experimental

2.2.1 Setup and samples

The experimental setup used with the EchoTilt was composed of a signal generator (*DS345, Stanford Research Systems, CA, US*), a 4× current amplifier (*ADA4870ARR-EBZ, Analog Devices, MA, US*), a syringe pump (*PHD Ultra, Harvard Apparatus, MA, US) and a* fluorescence microscope (*Axiovert 135M, Carl Zeiss AG, Germany*). In terms of fluorescence acquisition, the exposure time used was XXXX ms, the gain was XXX at a magnification of 10×. The fluidic connections were made with plastic tubing (*BTPE-60, Instech Laboratories Inc., PA, US*), metallic connectors (*SC20/15, Prime Bioscience, Singapore*) and plastic syringes (*Plastipak, BD Bioscience, NJ, US*).

The nanoparticle suspensions were created by diluting a stock of polystyrene nanoparticles. The ratio used was 1/100 mL (100× dilution). This method was followed for every particle size, including 500 nm ( ), 200 nm ( ), 100 nm ( ) and 50 nm ( ). The silica solution was created by mixing 1 wt% detergent (Tween-20) with 10 µm silica particles (*Sigma-Aldrich, Switzerland*) in deionized water. To prime the chip, deionized water was used. To clean the chip between experiments, 50% ethanol was used.

2.2.2 Experimental procedure

This work relied on the silica-enhanced seed particle method. For this, a high concentration silica solution is flowed through the channel and then the acoustic field is activated. This creates the grid of silica clusters, which is then washed with MQ water at 15 mL/min to remove the excess silica from the device. Finally, the nanoplastic sample is loaded into the device at the flow rate relevant for the experiment which results in the capture of the nanoparticles within the silica clusters. An image was taken every time point with the fluorescence microscope. This data was processed using ImageJ and graphed with GraphPad (*Graphpad Software Inc, US*).