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Evaluation of a high-throughput acoustic particle sorter

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Introduction

The goal of this independent study was to determine whether the standing pressure field that drives fluid transport from a resonant ultrasonic jet/droplet generator could preferentially retain (or trap) suspended particles during fluid ejection. Meacham et al. have reported thorough characterization of the physics underlying the droplet generator (e.g., resonant operation and pressure field focusing at frequencies corresponding to the sample reservoir geometry, as well as phenomena that predict whether ejection occurs as discrete droplets or continuous jets) using a variety of computational and experimental visualization methods [1-3]. These results suggest that at particular resonant frequencies of a fluid-filled reservoir a standing pressure field develops and is focused within pyramidal nozzles of the device, leading to fluid ejection from microscopic orifices.

Significant research efforts have explored use of various particle characteristics (size, density, compressibility, magnetic susceptibility, etc.) to separate microparticles and cells from heterogeneous mixtures. Among available separation techniques, acoustophoretic methods provide high biocompatibility due to the gentle noncontact nature of the applied forces. For example, it is well established that acoustic radiation forces cause suspended particles to migrate to the nodes (pressure minima) or antinodes (pressure maxima) of an applied standing acoustic pressure field. In addition to field properties (amplitude and frequency), particle size/shape and material density and compressibility relative to the suspending medium dictate the amplitude and direction (toward/away from zero-pressure nodes) of these forces.

Here, I explore use of the standing acoustic pressure field that drives ultrasonic droplet generation as a means of separation of cell-sized particles. Working with M. Kim (Meacham lab PhD student), I first confirmed ejection of water from device orifices for a range of chamber heights (corresponding to different available frequencies of operation). After identifying suitable operating modes, the sample chamber was loaded with suspensions of 5 and/or 10 μm polystyrene (PS) beads, and particle concentration was measured before and after treatment to determine retention (enrichment capability) based on particle size. Though results were inconclusive, data suggested that 5 μm beads were less likely to be retained in the device versus 10 μm beads. Further exploration of the parameters governing separation in the ultrasonic droplet generator is needed before such a device can be applied to more complicated biological mixtures (e.g., diluted whole blood).

Procedures and Apparatus

The device concept is shown in Fig. 1. Briefly, a sample reservoir is sandwiched between an ultrasonic resonator (piezoelectric transducer with/without an acoustic coupling layer of aluminum) and an array of pyramidal horns wet etched into silicon. When the reservoir is driven at a longitudinal resonant frequency (i.e., pressure wave oriented parallel to the ejection direction; nodal field lines located perpendicular to the ejection direction), a high pressure gradient is established near the tips of the nozzles. This pressure gradient propels fluid from the orifices, and for a mixture of suspended particles, a fraction of the particles is carried along with the fluid due to viscous drag. In the realized device, the ejection rate (and thus overall flow rate) is not 'on' or 'off', and the degree to which nozzles are ejecting has a significant impact on resultant flow drag and particle retention. Therefore, initial experiments used water only to identify optimal ejection frequencies evaluated based on quality of ejection. Ejection was qualitatively assessed using a three-level system: not spraying, misting and robust ejection. For each reservoir height, the first 3–4 device resonances were typically found (see Fig. 2), though only a subset of these exhibited robust ejection. Available device assemblies included polycarbonate reservoirs varying in height from 0.9 to 1.3 mm with resonances covering an almost continuous range of frequencies from 500 kHz to 2.5 MHz. Transducer temperature was monitored during experiments to ensure operation at reasonable temperatures (<50°C). The piezoelectric transducer temperature can increase rapidly when operating at a non-optimal frequency.

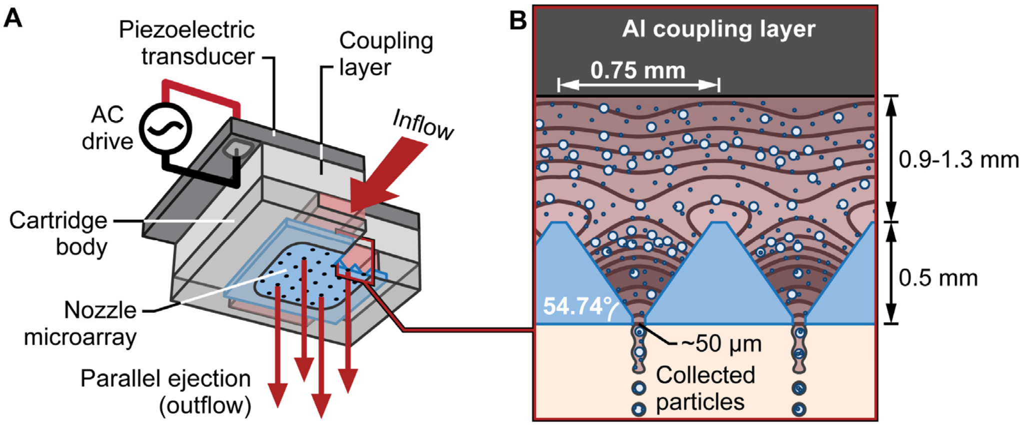


Figure 1. Micro machined ultrasonic droplet generator: ***A*** *schematic of device assembly and* ***B*** *concept for particle retention at nodes of a resonant acoustic pressure field that drives droplet ejection.*

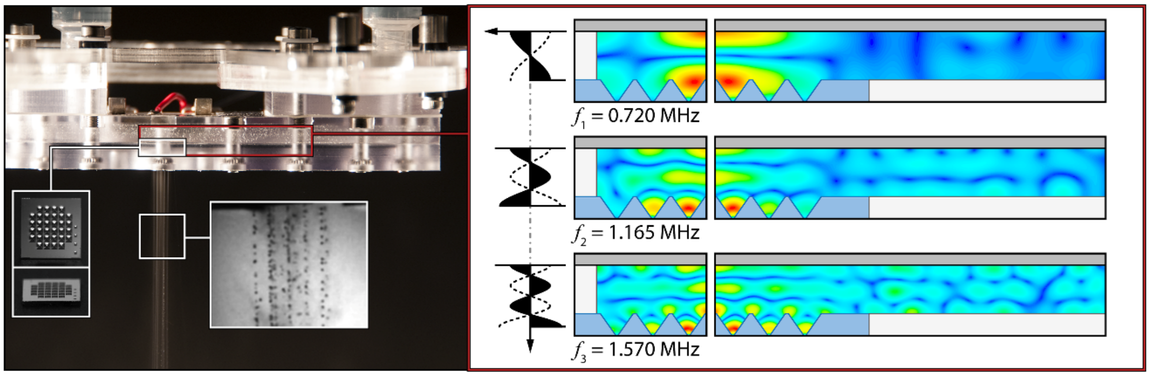


Figure 2. Device operation: spraying from a 32-orifice nozzle array (including high-speed imaging of droplet breakup) and resonant operation illustrating the first three half-wave resonances corresponding to fluid ejection from a 1.0 mm height reservoir.

In addition to drag induced by the bulk flow of sample from the nozzle orifices, particles in the standing acoustic pressure field are acted upon by the primary acoustic radiation force F_{ac}

$$F_{ac} = -\frac{4\pi}{3}a^3\nabla\left[f_1\frac{1}{2}\kappa_o\langle p_{\text{in}}^2\rangle - f_2\frac{3}{4}\rho_o\langle v_{\text{in}}^2\rangle\right],$$

$$f_1(\tilde{\kappa}) = 1 - \tilde{\kappa} \text{ with } \tilde{\kappa} = \frac{\kappa_p}{\kappa_o} \text{ and } f_2(\tilde{\rho}) = \frac{2(\tilde{\rho}-1)}{2\tilde{\rho}+1} \text{ with } \tilde{\rho} = \frac{\rho_p}{\rho_o},$$

where F_{ac} scales with particle volume (radius cubed, $\sim a^3$), field parameters (p_{in} and v_{in}), and parameters f_1 and f_2, which are functions of the particle compressibility κ_p and density ρ_p, respectively. Importantly, the expression for F_{ac} indicates that the magnitude of the force has a strong dependence on particle size with larger particles experiencing much larger trapping forces than smaller particles.

To explore the relationship between particle size and collection (complement of retention) rate, various bead solutions were run through the ultrasonic droplet generator and particle concentrations of the stock and collected samples were compared. Beads were suspended in phosphate-buffered saline (PBS) with 5 μm beads at approximately double the concentration of the 10 μm beads. Bead concentrations were measured by counting particles in the grid of a hemocytometer. Beads on edges were only counted on the top and left edges to avoid repetition. C_1 identifies concentration of the stock solution before ejection. C_2 is the concentration of collected sample. If d and h are the side length and height of a grid square volume, then the collection rate R_{coll} is found as follows:

$$C_1 = \frac{N_{\text{pre}}}{d^2h} \text{ and } C_2 = \frac{N_{\text{post}}}{d^2h}$$

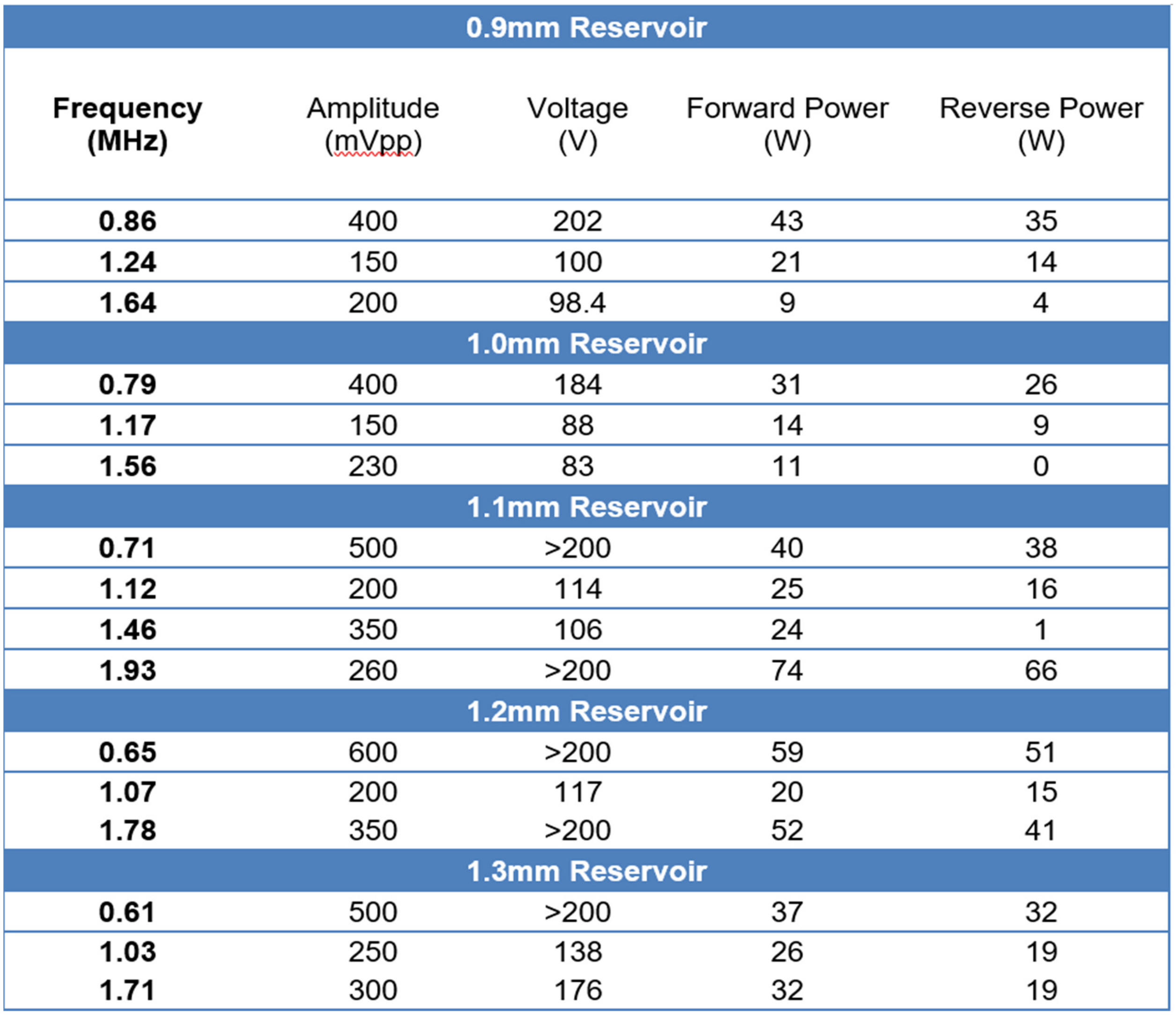
$$R_{\text{coll}} = \frac{C_2}{C_1} = \left(\frac{N_{\text{post}}}{d^2h}\right)\Big/\left(\frac{N_{\text{pre}}}{d^2h}\right) = N_{\text{post}}/N_{\text{pre}}$$

Measured particle concentration is influenced by bead settling from solution so samples were vortexed before each trial. The stock concentration of 10 μm beads was around 120 per grid and that of the 5 μm beads was approximately 220 per grid.

Results

As mentioned above, five different reservoirs with heights ranging from 0.9 mm to 1.3 mm were evaluated. For each test case, I recorded function generator amplitude, and forward and reverse power of the power amplifier. All operating conditions are reported in Table 1. Of note, Table 1 indicates that operating frequencies of less than 1 MHz require higher voltage amplitudes, which is logical as the natural longitudinal resonance of the 1.5 mm thick piezoelectric transducer is approximately 1.4 MHz. As operation moves away from this resonance, the impedance of the piezoelectric increases requiring higher voltage to achieve appreciable displacements.

Table 1. Summary of experimental data for ejection from various ultrasonic droplet generators.



Frequency (MHz)	Amplitude (mVpp)	Voltage (V)	Forward Power (W)	Reverse Power (W)
0.9mm Reservoir				
0.86	400	202	43	35
1.24	150	100	21	14
1.64	200	98.4	9	4
1.0mm Reservoir				
0.79	400	184	31	26
1.17	150	88	14	9
1.56	230	83	11	0
1.1mm Reservoir				
0.71	500	>200	40	38
1.12	200	114	25	16
1.46	350	106	24	1
1.93	260	>200	74	66
1.2mm Reservoir				
0.65	600	>200	59	51
1.07	200	117	20	15
1.78	350	>200	52	41
1.3mm Reservoir				
0.61	500	>200	37	32
1.03	250	138	26	19
1.71	300	176	32	19

Table 2 and Fig. 3 summarize collection data obtained using the above listed systems. Very little can be gleaned from the recorded data as all cases exhibit collection efficiency of 55 to 73 percent. At 1.17 MHz, a slight separation was observed between the 10 µm bead collection (~65%) and that for 5 µm (73%), though that was the only set of data for 5 µm beads that was valid. No clear dependence on frequency of operating is seen.

Instability of the ejection was a significant hindrance to obtaining repeatable data during these experiments. Often, it was not possible to achieve robust ejection, which lowers drag forces on suspended particles (as drag is directly related to the number of firing nozzles) and favors retention in the device. In addition, the solution concentration was not easily assessed due to measurement errors or perhaps settling issues with samples resting between experiments. Both issues need to be addressed before additional experiments are able to better characterize the retention capabilities of the ultrasonic droplet generator.

Table 2. Collection rate (retention complement) data.

Reservoir	Orifice	Freq	Particle	# Trials	Pre 1	Post 1	Percent 1	Pre 2	Post 2	Percent 2	Pre 3	Post 3	Percent 3	Pre 4	Post 4	Percent 4	Avg Pct
1	65	1.17	5um PS		228.5	166.75	72.97593										
1	65	1.17	10um PS		115.25	76	65.9436	120.875	80.25	66.3909	118.75	74.5	62.73684	150.75	98.875	65.58872	65.16502
1.1	45	1.13	10um PS	4	104.75	68.875	65.75179	100.5	64.625	64.30348	99.5	64.875	65.20101	94.75	62.5	65.96306	65.30483
1.2	45	1.07	10um PS	4	119.75	65.125	54.38413	125.75	73.375	58.3499	111.25	60.875	54.7191	107	68.125	63.66822	57.78034
1.3	45	1.03	10um PS	4	109.5	74.75	68.26484	144.375	101.875	70.56277	137.375	91	66.24204	104.25	74.75	71.70264	69.19307

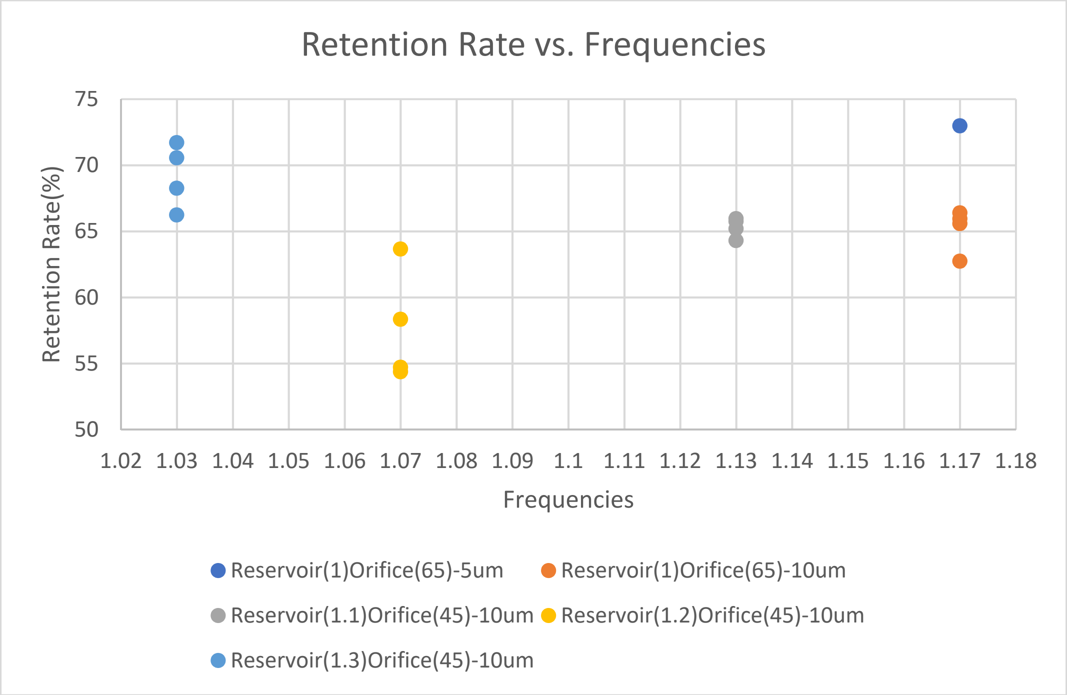


Figure 3. Collection rates for devices operating across a small frequency range.

Future Work

In addition to solving problems with concentration measurement and consistency of operation, additional experiments including 20 μm beads are needed to better assess the relationship between retention rate and particle size. Since temperature effects are important to ejection, temperature measurement and perhaps some form of temperature control would improve experimental outcomes.

Acknowledgement

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