SUPPLEMENTAL 1. Calculation of attenuation of ultrasound intensity passing through the 1mm thick polystyrene layer of the cell culture plate.

Attenuation by reflection (Cobbold, 2007)[41]:

Assume initial intensity is 5 W/cm^2. The acoustic impedance of polystyrene is assumed as 2.42 Mrayls (Selfridge, 1985)[42] and the acoustic impedance of water is assumed as 1.48 Mrayls, and the incident angle is assumed to be 0.

The intensity reflection coefficient $R = ((Z_2\cos\theta_i - Z_1\cos\theta_t)/ (Z_2\cos\theta_i + Z_1\cos\theta_t))^2 = ((2.42 - 1.48)/(2.42 + 1.48))^2 = 0.058$

Reflected intensity $I_R = I_0 \times R = 5 \times 0.058 = 0.29$

Remaining total intensity = $I_T = I0 - IR = 5 - 0.29 = 4.71$ W/cm^2.

~94% intensity remained after reflection.

Attenuation by transmission (Cobbold, 2007)[41]:

$I_x = I_0 e^{(-2\alpha x)}$

Taking the attenuation coefficient α = 0.4dB/cm at 1MHz for polystyrene (Selfridge, 1985)[42]

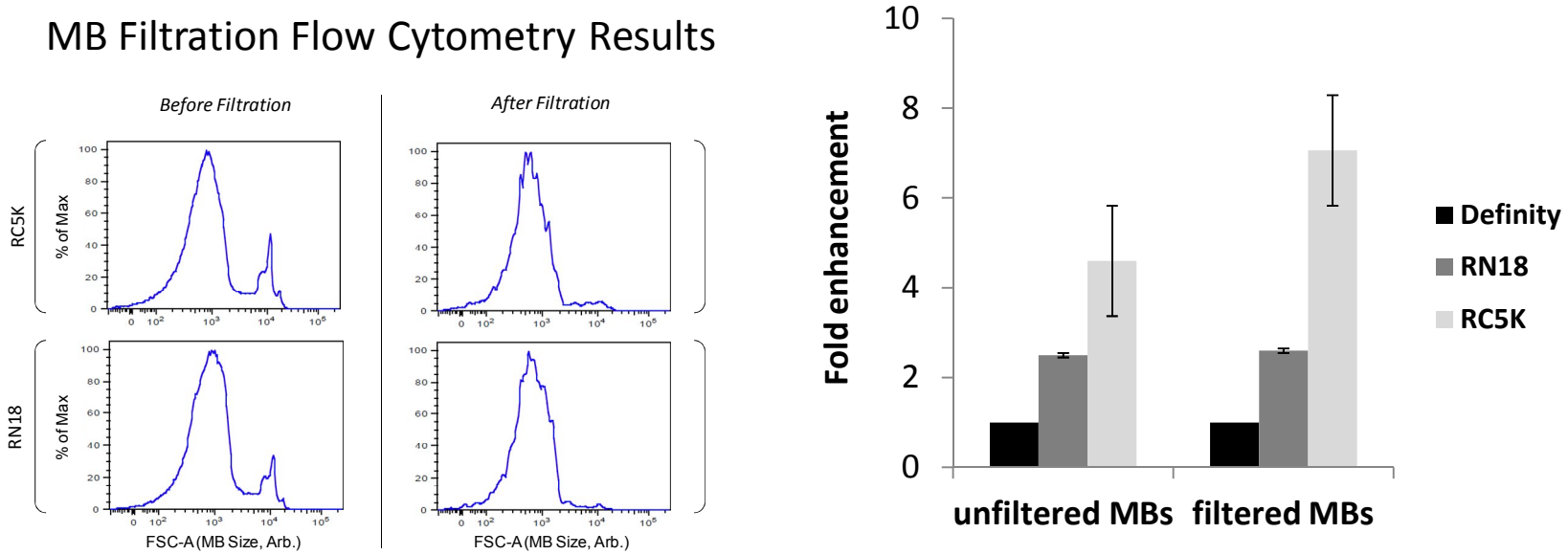
$I_{0.1cm} = I_0 e^{(-2\alpha x)} = 4.71 * e^{(-2x0.4x0.1)} = 4.35$ W/cm^2

~92% intensity remained after transmission.

Total intensity remained after attenuation by transmission and reflection:

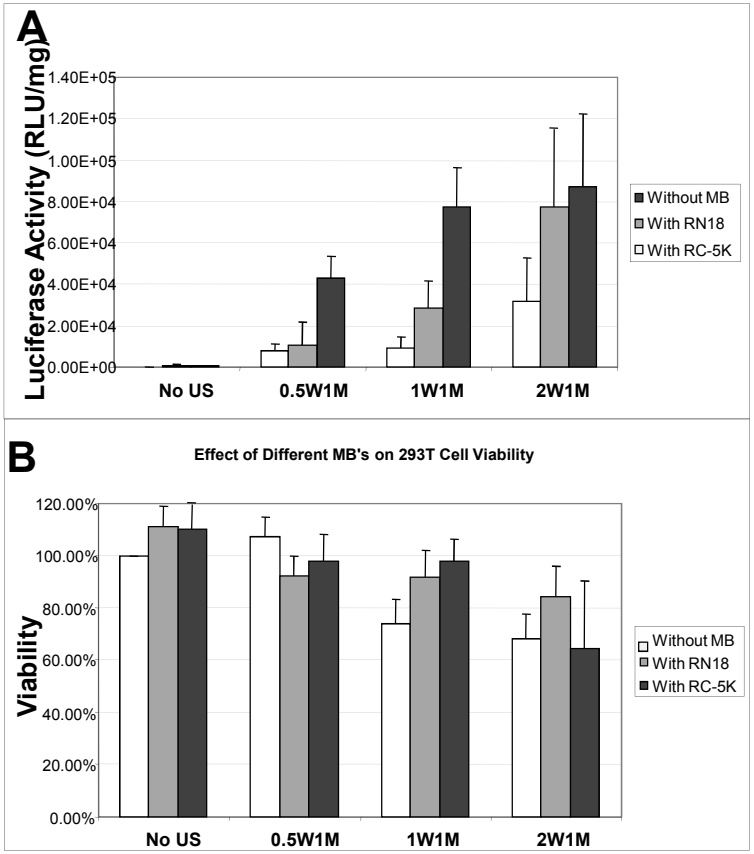
From the calculation, total intensity remained is 4.35 W/cm^2 from initial intensity of 5W/cm^2.
Thus, ~87% ultrasound intensity is remained.

SUPPLEMENTAL 2. Comparison of enhancement of ultrasound-mediated transfection efficiency using unfiltered and filtered MBs



Supplemental 2. Comparison of enhancement of ultrasound-mediated transfection efficiency using unfiltered and filtered MBs. 293T cells were transfected with pGL4 pre-incubated with unfiltered or filtered Definity, RN18, and RC5K MBs for 1 min, and exposed to US (1 MHz, 2 W/cm^2, 20% duty cycle, 100 Hz PRF) for 3 mins. Filtered MBs were obtained by filtering the MBs through syringe filters with 5µm pore size membrane (Millipore). Left panel, Flow analysis of microbubbles before and after filtration. Right panel, No significant difference in the fold enhancement of transfection efficiency was observed between unfiltered and filtered MBs.

SUPPLEMENTAL 3. Minimal enhancement was obtained for in vitro MB/US transfection of 293 T cells using “non-inverted” setup



Supplemental 3. Minimal enhancement was obtained for in vitro MB/US transfection of 293 T cells using non-inverted setup. 293T cells were transfected by pGL4 luciferase reporter construct without MBs or with neutral (RN18) or cationic (RC-5K) MBs and exposed to US (1 MHz, 0.5-2 W/cm^2, 20% duty cycle, 100 Hz PRF) for 1 min. The negative control(No US) was treated with the plasmid without ultrasound. (A) Luciferase activity, (B) viability of 293 T cells 48 hours after transfection. Data are presented as mean±STD (n=4), and * and ** were comparison between cells treated with the same ultrasound parameters. *means $P<0.05$ and ** means $P<0.01$.