# Polydimethylsiloxane microstructure-induced acoustic streaming for enhanced ultrasonic DNA fragmentation on a microfluidic chip

# 1. On-chip DNA fragmentation with a same on-chip microstructure for different transducer power / voltage values

Acoustic streaming is the main contributor to DNA fragmentation enhancement. Faster streaming velocity leads to higher tensile stress, thus a better stretching effect on molecules. DNA molecules are commonly coiled in solution and can be stretched by separation flow-induced tensile forces. DNA fragmentation improvement is achieved by the combined effect of streaming tensile stress and cavitation. The synergistic effect of tensile stress and ultrasonic cavitation causes DNA molecules to be stretched first and then cut by the jet formed by cavitation, leading to the observed enhancement of fragmentation performance in terms of speed and uniformity of small-size distribution.

In the main article, it has been demonstrated that the DNA fragmentation enhancement by triangular structure is significant compared to the others. Here we evaluate the on-chip DNA fragmentation performance with a same structure for different transducer power levels or corresponding voltage values. A triangular microstructure was chosen for this purpose for example. The simulated streaming stress distributions are shown in Fig. 1a, which contains both shear and tensile stress components. The red areas with a high-stress values contribute to the DNA fragmentation improvement, which occurs on a plane of symmetry in between two counter-rotating vortices, in particular close to stagnation points, where tensile stresses should be high while shear stresses are low. Rapid and strong convection causes strong streaming stress values up to ~0.06 kPa, ~0.11 kPa, ~0.16 kPa, and ~0.20 kPa corresponding to the conditions under four different applied voltages to the transducer, respectively. Fig. 1b shows the streaming velocity distributions corresponding to the four different applied voltages. The speed of the streaming flow exceeds ~0.04 m/s, ~0.07 m/s, ~0.11 m/s, and ~0.14 m/s in locations close to the triangle sharp edges, respectively.



**Fig. 1** Simulated results, including streaming stress distribution and streaming velocity. (a) Streaming stress distribution corresponding to the conditions under four different applied voltages to the transducer (50 V, 100 V, 150 V and 200 V, respectively). Peak values are situated between two adjacent vortices (shown in b). (b) Streaming velocity distribution, showing high-velocity zones on both sides of a microstructure (peak values are close to the sharp edges).

On-chip  $\lambda$  DNA fragmentation experimental results under different voltages are shown in Fig. 2, the DNA fragmentation speed increases with the input voltage. The fluorescent gel image of  $\lambda$  DNA fragment result is shown in Fig. 2a, and corresponding DNA fragmentation result distribution curves are shown in Fig. 2b. The DNA average length was sheared to ~3.0 kbp, ~1.5 kbp, ~800 bp and ~300 bp in 150 s, respectively (Fig. 2b). These results indicate that on-chip DNA fragmentation performance increases with voltage using the same structure (during the same period).



Fig. 2 On-chip DNA fragmentation results obtained with triangular structure under different powers. (a) Fluorescent gel images for  $\lambda$  DNA samples (48.5 kbp). Fragmentation has been carried out on-chip by applying ultrasound under different voltages during the same period (40 kHz, 150 s). (b) Corresponding DNA fragmentation result distribution curves.

#### 2. Videos 1-3 (Streaming flow)

ESI videos 1-3 show how fluorescent bacteria in the suspension enable tracing the streamlines of the induced flow patterns once the ultrasound is switched on (channel width 600  $\mu$ m), ESI videos 1-3 correspond to half-circular, rectangular, and triangular microstructures, respectively. The microstreaming flow patterns are nearly symmetrical with respect to the position of the structures and the axis of the main channel. Vortices are generated by each protruding structure with the center of rotations in the vicinity of the structure/main channel wall intersection on either side.

### 3. Video 4 (Cavitation)

ESI video 4 shows a bright field microscopy video of the cavitation phenomenon generated by ultrasonic actuation ( $\geq$  300V, 40 kHz). However, the cavitation phenomenon at this voltage is too

strong and the streaming flow is greatly influenced by irregular cavitation, which is not beneficial to DNA fragmentation.

## 4. Independent samples t-test1

An independent samples t-test is added to demonstrate the statistical significance of the on-chip enhancement by triangular structure compared to others and modified the description in the revised manuscript. Fig.3 below shows the t-test result of the on-chip DNA fragmentation got by triangular and rectangular structures (200V,150 s) as an example (significance level  $\alpha$ =0.05). As shown in the figure, T(2)=4.203, p = 0.014 <  $\alpha$ =0.05, which means there is a statistically significant difference between the enhancement by triangular structures and rectangular structures. Together with the smallest mean value got by triangular structures, the t-test results show that the enhancement by triangular structure is significant compared to the others.



Fig. 3 The t-test result of the on-chip DNA fragmentation got by triangular and rectangular structures (200V,150 s).

#### 5. Independent samples t-test2

An independent samples t-test is added to demonstrate the statistical significance of the on-chip results compared to the commercial system, calculated using IBM SPSS Statistics @ software. Fig.4 below shows the t-test result of the DNA fragmentation got by on-chip (200 V,150 s) and Covaris (100 W, 90 s) as an example (significance level  $\alpha$ =0.05). As shown in the figure, T(2)= -0.061, p = 0.954 >  $\alpha$ =0.05, which means there is no statistically significant difference between the two samples, it also indicates that the on-chip performance is comparable to the commercial system.



Fig. 4 The t-test result of the DNA fragmentation got by on-chip (200 V,150 s) and Covaris (100 W, 90 s).