Electronic Supplementary Material (ESI) for Lab on a Chip.

A Transparent Low Intensity Pulsed Ultrasound (LIPUS) Chip for High-throughput Cell Stimulation

*Haoyang Chen [a], Ninghao Zhu [a], Mohamed Osman [a], Ryan Biskowitz[a], Jinyun Liu[a], Shubham Khandare[a], Peter Butler[a], Pak Kin Wong[a,b] and Sri-Rajasekhar Kothapalli[a,c]**

[a] Department of Biomedical Engineering, The Pennsylvania State University, University Park, PA 16802, USA
[b] Department of Mechanical Engineering, The Pennsylvania State University, University Park, PA 16802, USA
[c] Penn State Cancer Institute, The Pennsylvania State University, Hershey, PA 17033, USA
* author to whom correspondence should be addressed.

Supplementary Figures

- **Figure S1. Dead assay staining after LIPUS cell stimulation using 3 Vpp, 25% duty cycle and 30 second duration**
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- **Video S1. COMSOL Simulation of the TUT surface vibration**
- **Video S2. On-chip cell stimulation when TUT is excited with 3 Vpp and 25% duty cycle for 30 seconds.**
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- **Video S4. On-chip cell stimulation when TUT is excited with5 Vpp and 50% duty cycle for 0.5 seconds.**

Supplementary Figures

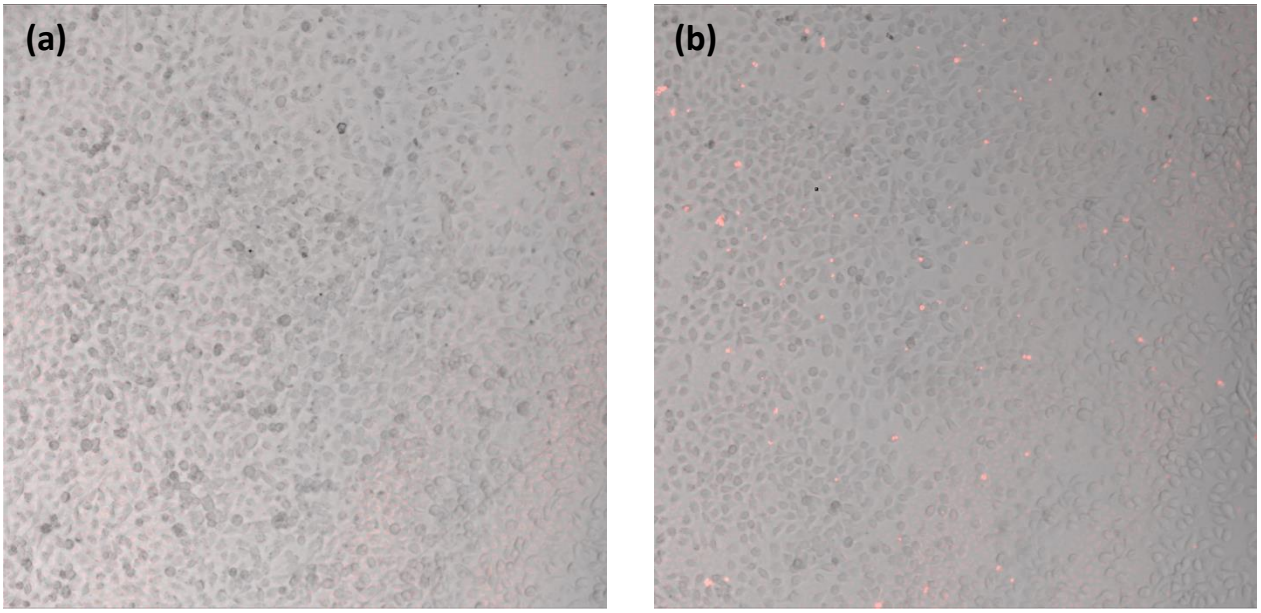


Figure S1. Dead assay staining after LIPUS cell stimulation using 3 Vpp, 25% duty cycle and 30 second duration. (a) This dead assay stain was conducted directly after the 30-second LIPUS stimulation of the cells with 3 V_{pp} amplitude from function generator, 20 dB gain power amplifier and 25% duty cycle. The dead assay fluorescence is overlaying the bright field image through the transparent ultrasound transducer, and red marks indicate the dead cells. The live cell ratio was calculated to be 99.87%. (b) This dead assay stain was conducted 48 hours after the 30-second LIPUS stimulation of the cells with 3 V_{pp} amplitude from function generator, 20 dB gain power amplifier and 25% duty cycle. The dead assay fluorescence is overlaying the bright field image through the transparent ultrasound transducer, and red marks indicate the dead cells. The live cell ratio was calculated to be 93.02%.

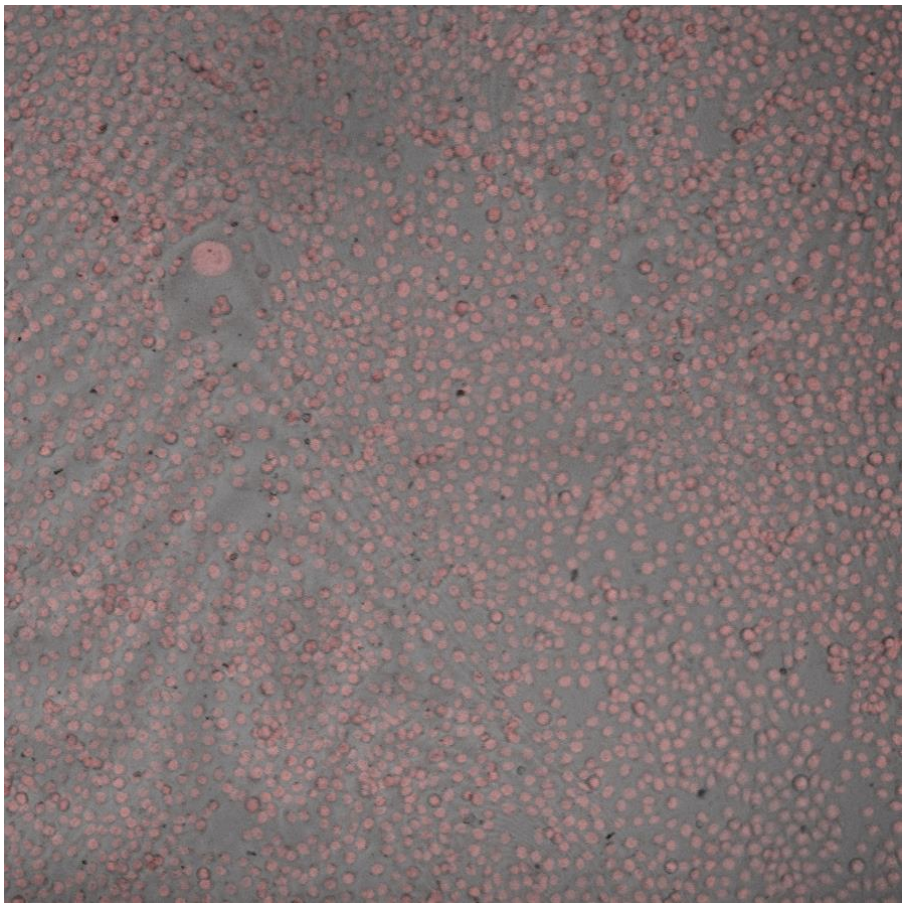


Figure S2. Dead assay staining after stimulating with 7.5 V_{pp} for 30 second duration and 50% duty cycle. This dead assay stain was conducted one hour after the 30-second stimulation of the cells with 7.5 V_{pp} amplitude from function generator, 20 dB power amplifier, and 50% duty cycle. The dead assay fluorescence is overlaying the bright field image through the transparent ultrasound transducer, and red marks indicate the dead cells. The live cell ratio was calculated to be less than 1%.

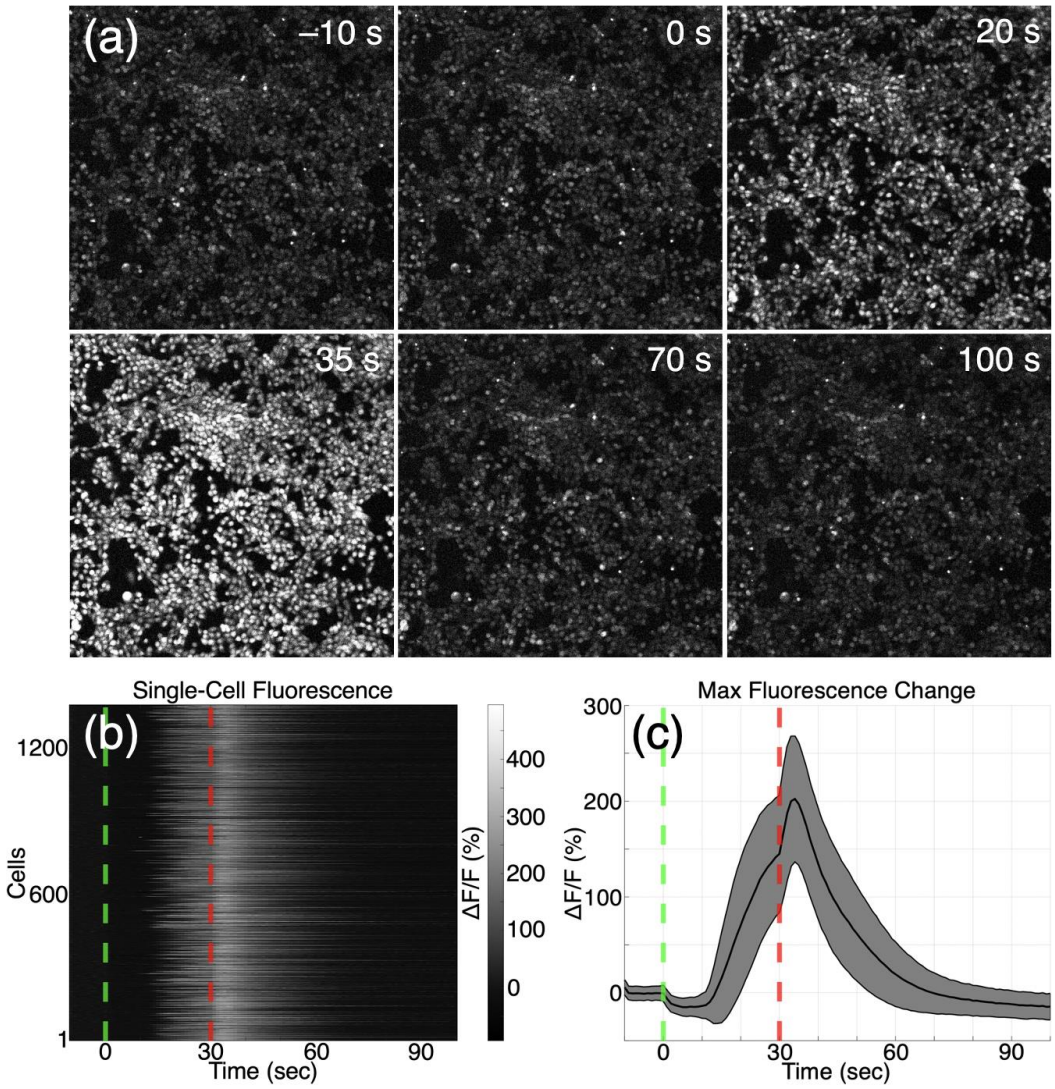


Figure S3. Single-cell analysis for stimulation with 5 V_{pp} pulsed-ultrasound cell stimulation for 30 second duration and 50% duty cycle. (a) Time-lapsed confocal microscopy images of calcium fluorescence for the plated bladder cancer cells. The ultrasound stimulation was turned on at t = 0 seconds and turned off at t = 30 seconds with 5 V_{pp} amplitude from function generator, 20 dB power amplifier, and 50% duty cycle. (b) The single-cell fluorescence spectrogram depicts the relative intensities of each cell over 110 seconds of imaging. The green and red lines indicate the beginning and the end of the stimulation, respectively. (c) The averaged single-cell relative fluorescence is plotted against elapsed time. The solid line represents the averaged fluorescence change and shaded region represents the standard deviation of the intensities for each frame.

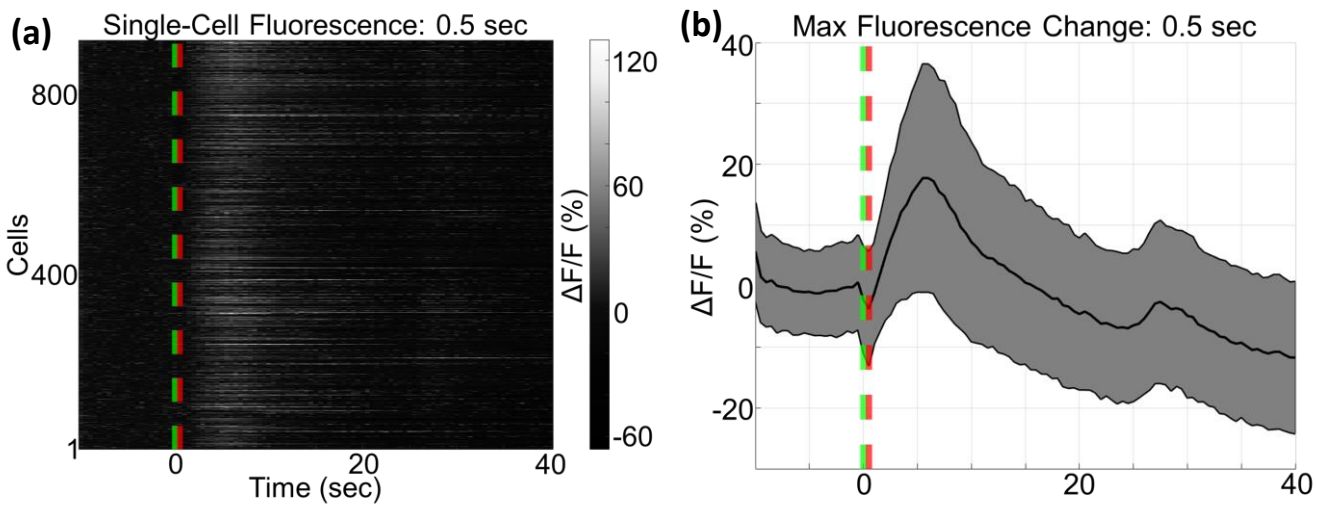
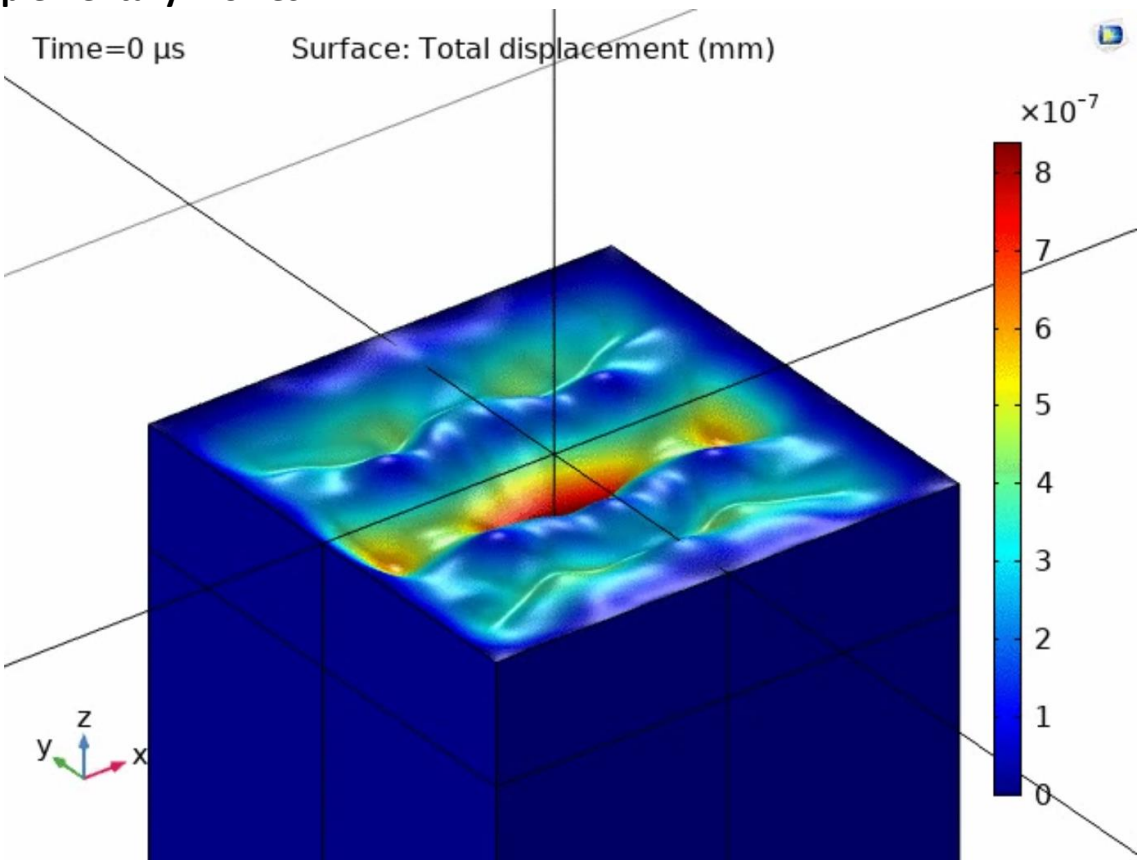
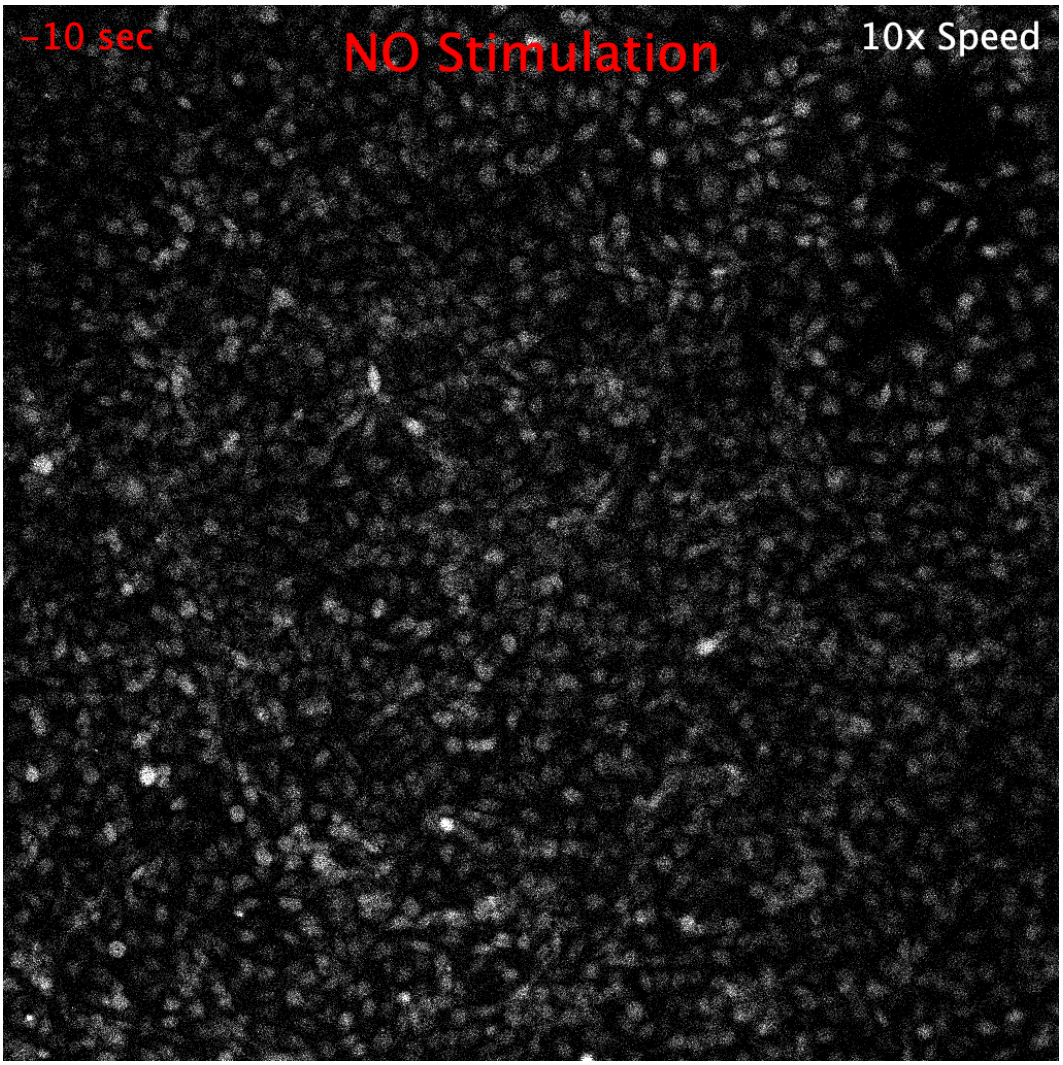


Figure S4. Single-cell analysis for 5 V_{pp} pulsed-ultrasound cell stimulation with 0.5-second duration and 50% duty cycle. (a) The single-cell fluorescence spectrogram depicts the relative intensities of each cell over the first 50 seconds of imaging for the 0.5-second ultrasound stimulation experiment with 5 V_{pp} amplitude from function generator, 20 dB gain power amplifier, and 50% duty cycle. The green and red lines indicate the beginning and the end of the stimulation, respectively. (b) The averaged single cell relative fluorescence is plotted against elapsed time. The solid line represents the averaged fluorescence change and shaded region represents the standard deviation of the intensities for each frame.

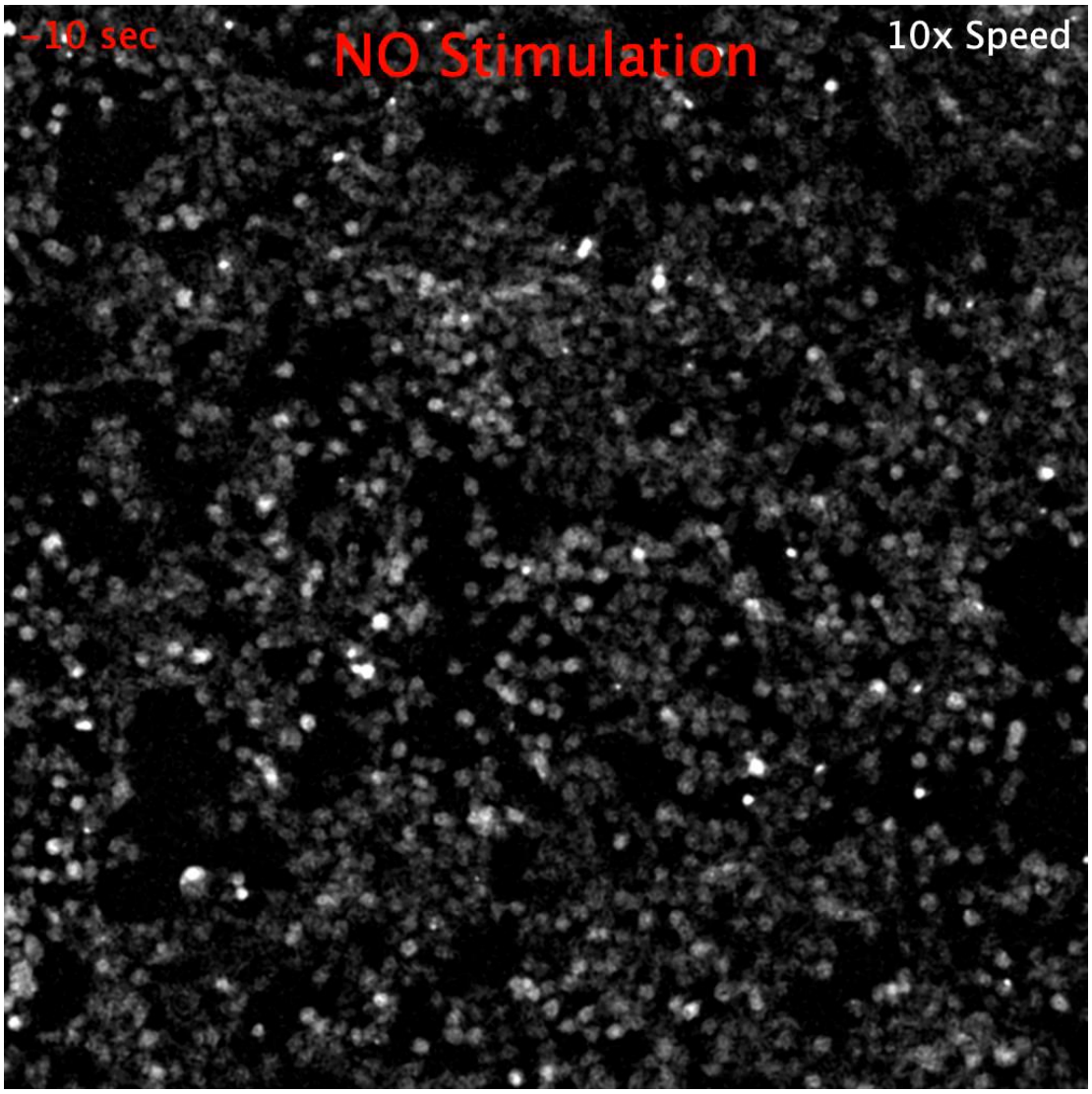
Supplementary Movies



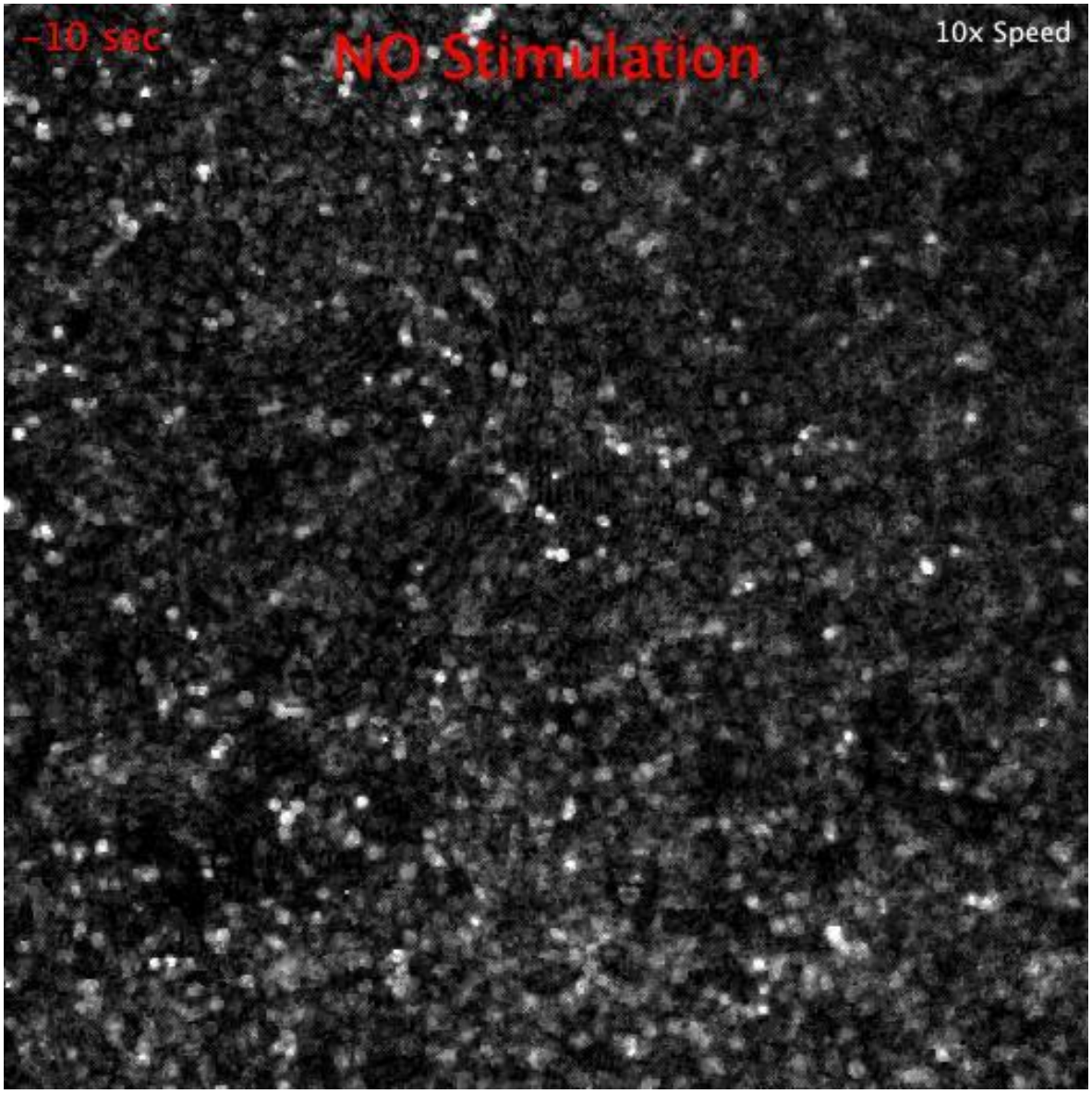
Video S1. COMSOL Simulation of the TUT surface vibration. The COMSOL simulation demonstrates that the mechanical vibration of the TUT is dominated by the quasi-longitudinal mode in the direction of the propagation of the wave.



Video S2. On-chip cell stimulation when TUT is excited with 3 Vpp and 25% duty cycle for 30 seconds. This video corresponds to **Fig. 4 of the main manuscript**. The 110 frames were captured at 1 frame per second during the experiment. The first 10 frames are baseline, the following 30 frames are during stimulation, and the remaining frames are post-stimulation. The movie is played at 10x speed.



Video S3. On-chip cell stimulation when TUT excited with 5 Vpp and 50% duty cycle for 30 seconds. This video corresponds to **Supplementary Fig. 2**. The 110 frames were captured at 1 frame per second during the experiment. The first 10 frames are baseline, the following 30 frames are during stimulation, and the remaining frames are post-stimulation. The movie is played at 10x speed.



Video S4. On-chip cell stimulation when TUT excited with 5 Vpp and 50% duty cycle for 0.5 seconds. This video corresponds to **Supplementary Fig. 4**. The 220 frames were captured at 2 frames per second for the 110-second duration of the experiment. The first 20 frames are baseline, the next frame is during stimulation, and the remaining frames are post-stimulation. The movie is played at 10x speed.