SINGLE LIVING CELL MANIPULATION AND MICORHEOLOGICAL STUDY WITH LASER-INDUCED CAVITATION BUBBLES
Z. G. Li1, P. A. Quinto-Su2, J. B. Zhang3, C. D. Ohi2 and A.Q. Liu1*
1School of Electrical & Electronic Engineering, Nanyang Technological University, SINGAPORE 639798
2School of Physical and Mathematical Sciences, Nanyang Technological University, SINGAPORE 639798
3Data Storage Institute, A*STAR, SINGAPORE 117608

ABSTRACT
In this paper, a new method for measuring the pure mechanical responses of single living cells is proposed using laser-induced cavitation bubbles. An optical system with a high speed Charge Coupled Device (CCD) camera is built to generate cavitation bubbles in a chamber with sample fluid and capture the ultra fast process of the deformation of the living cells. A Madin-Darby canine kidney (MDCK) in cochineal color food dye solution is squeezed by the pressure field generated by a pair of cavitation bubbles and the deformation process is recorded and analyzed. This method has great potential applications in cell manipulations and measurement of rheological response of single living cell.

KEYWORDS: Single living cell, Microdroplet, Micorheological study, Laser-induced cavitation bubbles

INTRODUCTION
The mechanical forces can be sensed and converted into biological response by living cells, and the biological signals are also known to affect the mechanical properties of living cells. Studies into the mechanics of single cells are a rapidly growing research field during the past decade with significant implications for biotechnology and human health. Several investigative tools have been proposed to study the rheological behavior of living cells. One common method is atomic force microscopy (AFM) [1, 2]. In AFM, a local deformation on the cell surface is generated by a sharp tip at the free end of a flexible cantilever. The applied force can be estimated by calibrating the resulting deflection of the cantilever tip. Another typical method is micropipette aspiration [2, 3]. In the micropipette aspiration, a micropipette is applied to suck a cell and the recorded deformation of the cell is used to estimate the elastic response of the cell. However, for these long time scale methods, cells have the ability to recognize the mechanical environment and adjust their behaviors. The pure rheological responses can not be measured using these kind of long time scale method. Recent developments of the formation of the laser-induced cavitation bubbles [4] provide a new chance to solve this problem. In this paper, a non-contact method by applying a pulsed pressure field generated by the laser-induced cavitation bubbles is proposed for ultra-short time pure rheological response measurement. This method also provides the potential to measure the rheological responses of multiple cells.

THEORETICAL ANALYSIS
Figure 1 shows the schematic of the principle of a living cell manipulated and squeezed by a pair of laser-induced cavitation bubbles. The pair of laser-induced cavitation bubbles is generated using an optical system in a chamber full of fluids with living cells. Between the pair of bubbles, a pressure field is formed since the expansion of bubbles. The pressure field is applied to a living cell and the cell is squeezed by the pressure. The pressure values between the two bubbles along x-axial direction have a Gaussian distribution and reach its maximum on the y-axial at \( x = 0 \) and the cell is squeezed along x-axial direction and elongated along the y-axial direction. Once the bubbles collapse, the induced pressure field disappears and the deformed cell may resume its original shape. If the bubble is larger, the boundaries of the two bubbles will change from circular shape to semicircular shape since two bubbles are deformed by each other. The neighboring boundaries of the two bubbles will change to two parallel lines.

Figure 1: The schematic of the stretching of the cell in a pulsed pressure field. The pressure field is generated by a pair of laser-induced cavitation bubbles. The cell is stretched in the pressure field and elongated along the y-axial direction.
EXPERIMENTAL SETUP

Figure 2 shows the experimental setup for the cavitation bubble-induced droplets coalescence [4]. A bubble is created by a single pulse from an Nd:YAG laser at the wavelength of 532 nm with duration of 6 ns. The linear polarization of the laser beam is rotated by a half-wave plate, since the phase modulation provided by the reflective spatial light modulator (SLM) depends on the polarization state of the incident light. The beam is then expanded by a telescope formed by L1 and L2 to fill the active area of the SLM. The lens L3 (f = 250 mm) collimates and images the hologram into the back aperture of a microscope objective (20×, NA = 0.75, Olympus, Singapore) that is integrated within an inverted microscope platform (IX-71, Olympus, Singapore). The illumination propagates through the dichroic mirror of the microscope and pictures the bubbles within the droplets on the sensor of a high-speed CCD camera (SA-1, Photron, USA) at 100,000 frames per second. A notch filter is used to block the 532-nm laser light from reaching the camera. The sample of a Madin-Darby Canine Kidney (MDCK) cell is prepared using a liquid gap filled with cochineal color food dye due to its biocompatibility. The chamber is a sandwiching spacer using two #1 microscope slides and the size of the chamber is 20 × 20 mm² laterally with a height of 15 µm.

Figure 3 shows the micrographs of a pair of laser-induced cavitation bubbles. The radius of the bubbles increases with increasing laser energy. Once the laser energy reaches 120 mJ, the neighboring boundaries of the two bubbles deformed into two parallel lines and a parallel pressure field between the bubbles is formed.

The relationship between the radius of the laser-induced cavitation bubble and the laser energy is shown in Fig. 4. It is seen that the radius of the bubble increase linearly with the increase the energy of the laser. When the laser energy is increased from 42 mJ to 138 mJ, the radius of the bubble grows linearly from 43 µm to 94 µm with a growth rate of 0.58 µm/mJ.

EXPERIMENTAL RESULTS AND DISCUSSIONS

Figure 5 shows the deformation process of a living MDCK cell between a pair of laser-induced cavitation bubbles. The living MDCK cell is located at the center between two bubbles. The boundaries of the two bubbles change from circular shape to semicircular shape with the bubbles expanding as shown in Fig. 5(a) since two bubbles are deformed by each other. The squeezed cell is elongated in the y-direction from 12 µm to 17 µm as shown in Fig. 5(b) to (e). Then, the cell is released and the y-direction length shrinks to 14 µm as shown in Fig. 5(e) to (h). For the pair of cavitation bubbles, their radii reach maximum at 20 µs and then begin to contract and collapse. The pair of cavitation bubbles totally disappeared at 50 µs.
The time revolution of the stretch ratio of the cell along y-axial direction is depicted in Figure 4. The stretch ratio slowly is increased from 8% at 40 µs and peaks at 60 µs to 42%. After the peak, the cell is released and finally the stretch ratio decreases to 8%. From the Fig. 6, it is seen that the cell begin to be stretched at 40 µs and up to the longest at 60 µs. At the same time, the radii of the pair of bubble reach maximum at 20 µs and the bubbles totally disappeared at 50 µs. There exist a delay between the bubble evolution and the cell deformation, and the delay time is about 10 µs.

CONCLUSIONS

In conclusion, a new method for measuring the ultra-short time pure mechanical response of single living cell is developed. An optical system is built to generate a pair of laser-induced cavitation bubbles in a chamber setup with cochineal color food dye fluid and record the ultra-fast process. A pressure field is formed between the pair of cavitation bubbles. An MDCK cell at the center of this pressure filed is squeezed and deformed and the stretch ratio is up to 42%. This technique may allow a non-invasive way of measuring the pure mechanical response of cells and other biological materials.

REFERENCES


CONTACT
*A. Q. Liu, Tel: +65-6790 4336; Fax: +65-6793 3318; Email: eaqliu@ntu.edu.sg