【Grant-in-Aid for Scientific Research (S)】

Deep-learning generation of high-resolution ultrasound images of cultured cells and systematic study of local mechanical stimulation on them by focused ultrasound and their responses

Principal Investigator	Osaka University, Graduate School of Engineering, Professor OGI Hirotsugu　　Researcher Number : 90252626
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Purpose and Background of the Research

●Outline of the Research

We will develop an original ultrasound cell-analysis system that can apply local mechanical stimulation to cultured cells using high-frequency focused ultrasound and establish the methodology to generate super-resolution ultrasound images by deep learning. We then aim to systematically explore cellular responses to noninvasive and precisely controlled local mechanical stimulation over a long period of time and contribute to elucidating the mechanism of cellular responses to mechanical stimulation. In particular, we will perform the selective-noninvasive mechanical stimulation to cell nucleus to investigate its effects on function, differentiation potential, and secretory substances of the cell. We will also explore the effects of mechanical stimulation on intracellular protein aggregation, a pathologically important phenomenon, and focus on the intracellular aggregation ability of the protein responsible for Parkinson's disease for diagnosis and drug-efficacy evaluation.

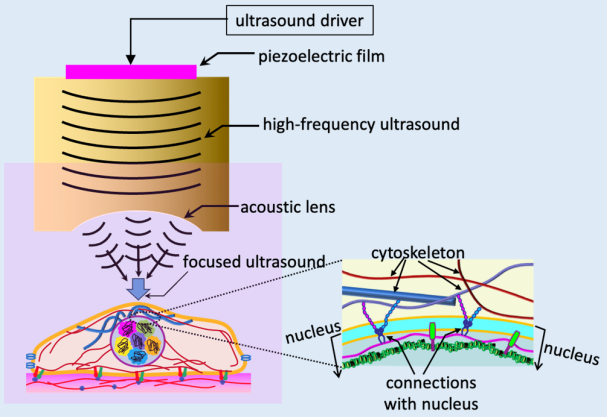

Figure 1. Schematic of the experiments conducted in this study. The effects of noninvasive, long-time, and label-free mechanical stimulation to local areas in cells, particularly to nucleus, will be studied.

We maintain our life activities while being subjected to various mechanical loads. Cells have adapted to these mechanical loads, and how they sense and use of the mechanical stimulation and reflect to their functions, is an essential question in life science. With this background, studies on cellular responses to various mechanical stimuli have been conducted. However, because of four problems, (i)invasiveness, (ii)macroscopic stimulation, (iii)lack of quantification, and (iv)experiments in non-culture environments, conventional mechanical stimulation techniques have not yet elucidated the essential mechanisms.

Among various existing technologies, only high-frequency focused ultrasound can solve all of the above issues. In this study, we aim to develop an ultrasound cell analysis system to make a significant contribution to the elucidation of the mechanism of cell responses to mechanical stimuli. In particular, we will conduct selective and noninvasive stimulation of a nucleus to quantitatively and systematically explore the effects of the mechanical nuclear stimulation on cell functions and intracellular protein aggregation ability. The mechanical stimulation of the nucleus has so far used highly invasive techniques such as extracting the nucleus from the cell, where the cell death cannot be avoided. In this study, we propose the selective and noninvasive stimulation of nucleus by matching the frequency of ultrasound used for stimulation to the mechanical resonance frequency of nuclear expansion and contraction.

Expected Research Achievements

In order to solve the above problems that have not been solved in mechanobiology field, we will develop the measurement system that can image cells in culture and apply various mechanical stimuli locally and over a long period of time (Figure 2). Ultrasound images are acquired by manipulating an acoustic probe using a short-pulse, low-power ultrasound. The probe is then moved to the point where mechanical stimulation is to be applied, and high power burst waves are input to apply mechanical stimulation. The power of the applied ultrasound can be precisely controlled electrically. An optical microscope system utilizing the scattered light detection method with oblique light incidence will be installed from the undersurface. However, since the light invasion cannot be avoided in the optical measurement, super-resolution of ultrasound images will be generated by using deep learning for a long-time continuous observation (Figure 3).

The measurement system developed in this study will be used to deepen our understanding of intracellular aggregation of proteins. The main cause of Parkinson's disease is the intracellular toxic aggregates of the corresponding protein. We will conduct intracellular protein aggregation experiments under various mechanical stimuli to investigate their effects on the aggregation reaction, which will contribute to diagnosis and drug discovery.

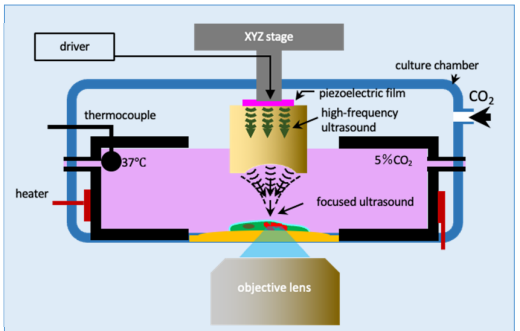

Figure 2. Schematic of the focused ultrasonic cell-analysis system to be developed.

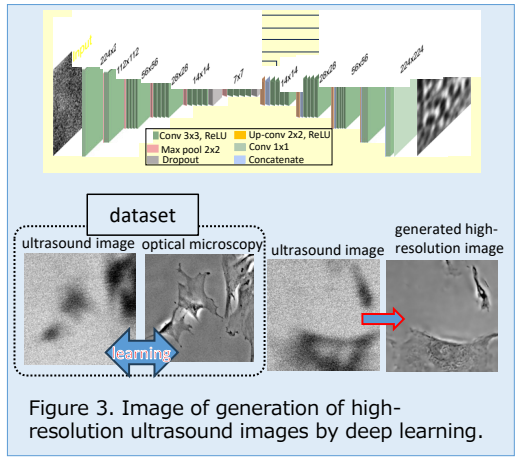

Figure 3. Image of generation of high-resolution ultrasound images by deep learning.

Homepage Address, etc.	http://www-qm.prec.eng.osaka-u.ac.jp