Master's Thesis
석사 학위논문

# Development of a high frequency ultrasound microbeam system for cancer cell manipulation and characterization

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Advisor : Professor Jae Youn Hwang

Co-advisor : Professor Hongsu Choi

by

Jin Man Park
Department of Information and Communication Engineering
DGIST

A thesis submitted to the faculty of DGIST in partial fulfillment of the requirements for the degree of Master of Science in the Department of Information and Communication Engineering. The study was conducted in accordance with Code of Research Ethics[1]

8. 1. 2016

Approved by

Professor 황 재 윤 ( Signature )
(Advisor)

Professor 최 홍 수 ( Signature )
(Co-Advisor)

[1] Declaration of Ethical Conduct in Research: I, as a graduate student of DGIST, hereby declare that I have not committed any acts that may damage the credibility of my research. These include, but are not limited to: falsification, thesis written by someone else, distortion of research findings or plagiarism. I affirm that my thesis contains honest conclusions based on my own careful research under the guidance of my thesis advisor.

# Development of a high frequency ultrasound microbeam system for cancer cell manipulation and characterization

Jinman Park

Accepted in partial fulfillment of the requirements for the degree of Master of Science.

8. 1. 2016

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박진만. Jinman Park. Development of a novel non-contact multimodality system for cell manipulation and characterization. Department of Information and communication. 2015. 30p. Advisors Prof. Hwang, Jae Youn, Prof. Co-Advisors Choi, Hong Su

## ABSTRACT

Recently, many high frequency ultrasound microbeam techniques such as acoustic tweezers, acoustic microscopy, and a single cell stimulator have been developed for various biomedical applications. In this thesis, I developed a high frequency ultrasound microbeam system with a 30MHz single element lithium niobate (LiNbO3) ultrasound transducer for cancer cell manipulation and characterization. A single-element LiNbO3 ultrasound transducer with a center frequency of 30 MHz and an f-number of ~ 0.7 was employed to form highly-focused ultrasound microbeams at focus for manipulation of a cell. Sine-bursts from a function generator were input to the transducer after amplification in a RF power amplifier of a home-built pulser-receiver for generation of the high frequency ultrasound microbeams. The ultrasound transducer was integrated to x-, y-, and z- linear motorized stages and then attached to an inverted fluorescence microscope to manipulate and characterize a cancer cell. The motorized stages were here controlled by a program developed for precise beam focusing to a target cell. Also, an electron multiplying charge coupled device was implemented to the microscope in order to perform live-cell fluorescence imaging for monitoring and characterizing of a target cell. To evaluate the performance of the high frequency ultrasound microbeam system I developed, the system was applied to trap a 10 μm polystyrene microbead in a highly-focused microbeam and the performance of acoustic trapping of the microbead in transparent and turbid media has been then compared. The results demonstrated that a 10 μm polystyrene microbead could be successfully trapped in the media by using the system. Interestingly, it was found that its trapping performance was degraded in the turbid media compared to the transparent

media. Furthermore, the system was employed as acoustic tweezers to manipulate and characterize a cancer cell for development of more useful biomedical applications. In particular, I investigated whether the degree of invasiveness of breast cancer cells with different phenotypes in suspension could be realized by quantification of morphological and calcium responses of cancer cells to acoustic trapping. The results showed that the highly-invasive breast cancer cell (MDA-MB-231) was likely to exhibit strong calcium responses at lower input voltages than the weakly-invasive breast cancer cell (MCF-7) during acoustic trapping as well as the highly-invasive breast cancer cell was largely deformed than the weakly-invasive breast cancer cell due to acoustic trapping at a certain input voltage. Altogether, these results suggested that breast cancer cells with different phenotypes in suspension might be discriminated by quantification of morphological and molecular responses of the cells to acoustic trapping using the high frequency ultrasound microbeam system I developed, thus demonstrating it potentials as a promising biophysical tool for cancer cell manipulation and characterization.

**Keywords**: High frequency ultrasound microbeam, acoustic tweezers, breast cancer cell, invasiveness, calcium imaging

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# I. INTRODUCTION

Cancer is known as a malignant tumor which has the potential to invade into other organs of the human body. In 2015, an estimated 1,658,370 cases of cancer has been diagnosed in United States. It showed ~ 35 % mortality in the patients with cancer. Among various types of cancer, breast cancer is the most frequent cancer in woman [1]. Its incidence rate is still increasing (Figure 1). In particular, one of the most devastating events in breast cancer patients is cancer metastasis since it can result in cancer recurrence in other organs, thus leading to death of patients with breast cancer [2]. In order to reduce the mortality of patients with breast cancer due to cancer recurrence and metastasis, it has been widely known that the early detection of cancer is highly important as well as the determination of invasion potentials of breast cancer at an early stage would be beneficial. Therefore, many researchers have made many efforts to develop new methods allowing the early detection of cancer and also determination of its invasiveness with high accuracy, but still remaining great challenges.

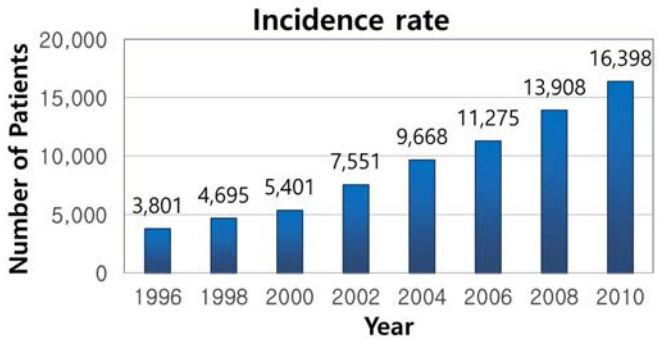


**Figure 1 Incidence rate of Breast**

Currently, various tools and techniques have been developed to determine cancer invasiveness since the determination of invasion potential of cancer would be helpful to select treatment aggressiveness. In previous studies, it was shown that atomic force microscopic techniques were capable of measuring stiffness of cancer cells. The studies reported that the stiffness of cancer cells was proportional to their invasiveness

[3, 4]. Also, by applying fluid shear force to a cancer cell, calcium responses of the cancer cell to the external force [5-13] have been monitored in order to identify signaling pathways in cancer progression [14]. Recently, ultrasonic techniques based on high frequency ultrasound have been developed and applied to determine invasiveness of a cancer cell [15]

The high frequency ultrasound has been considered as a new frontier for ultrasonic imaging techniques since the higher frequency ultrasound yields improved better spatial resolution by sacrificing the depth of penetration [16]. Moreover, it has been utilized as a new biomedical tool based on high-frequency ultrasound microbeams, defined as a micro-sized ultrasound beam formed by a highly-focused ultrasound transducer at high frequency over ~ 15 MHz, for cell manipulation and characterization [15, 17-20]. Note that if high frequency ultrasound at 200 MHz is highly focused with an ultrasound transducer with an f-number of 1.15, the ultrasound microbeam with the lateral diameter of ~ 9 μm, which is a comparable size with the size of a single cell, can be achieved at focus [18, 21].

The high-frequency ultrasound microbeam techniques have been so far utilized for various biomedical applications. For examples, the high frequency ultrasound microbeam was utilized for acoustic microscopy with high resolution to map local acoustic impedances of a cell [22-24]. The study demonstrated that it enables to measure cytoplasmic acoustic impedances of a living cell. Also, a high frequency ultrasound microbeams was utilized to trap a tiny biological samples for manipulations and characterizations of the biological samples [15, 17, 18, 25, 26]. A single-beam acoustic trapping technique with a 193 MHz press-focused lithium niobate ($LiNbO_3$) transducer was employed to study mechanical properties of a breast cancer cell. In the study, a 5 μm fibronectin-coated polystyrene microbead acoustically trapped was attached to a target cell and was then pulled with acoustic tweezers in order to measure elastic property of

the cell [18]. Moreover, the high frequency ultrasound microbeams were applied to stimulate a cell as a mechanotransduction tool [15, 19]. High frequency ultrasound microbeams at 200 MHz elicited calcium elevation in human umbilical vein endothelial cells. In the study, it was found that the calcium responses of the cells to the ultrasound microbeam stimulation have good agreements with the previous outcomes obtained by other methods [7, 9, 12, 14, 27-29]. Furthermore, the high frequency ultrasound microbeams at 200 MHz were applied to breast cancer cells in order to investigate its potential as a useful tool to determine their invasiveness by quantification of calcium responses of breast cancer cells to the high frequency ultrasound microbeam stimulation. In the study, it was found that highly invasive cancer cells exhibited more significant calcium elevation than weakly invasive cancer cells due to high frequency ultrasound microbeam stimulation. As shown in these previous studies, the high frequency ultrasound microbeam techniques have been shown to be a very promising tool for cell characterization and manipulation. However, the systems have been optimized to manipulate a cultured cell. It may not be suited for manipulate a cell in suspension and characterize their mechanical and molecular properties of the suspended cell. Note that the suspended state of a cell is more relevant to practical applications of cellular biophysics to medicine [30]. Therefore, to achieve better manipulation and characterization of a cell in suspension, particularly a breast cancer cell of my main interest, it may need to develop a new type of a high frequency ultrasound microbeam system suited for the specific application.

In this thesis, I therefore built a high frequency ultrasound microbeam system capable of manipulating and characterizing a cancer cell in suspension such as a breast cancer cell. In particular, the system developed was applied to trap a breast cancer cell in suspension and determine its invasion potential by quantification of changes in intracellular calcium level and morphology of the cells due to acoustic trapping. For

generation of high frequency ultrasound microbeams in the system, a 30 MHz Lithium niobate single element ultrasound transducer with f-number of 0.7 was fabricated and attached to 3-axis motorized stages integrated with an IX73 inverted fluorescence microscope (Olympus, Japan). A pulser-receiver including a RF power amplifier was developed to drive the transducer as well as focus the transducer onto a target cell. The high-frequency ultrasound system was then evaluated by performing acoustic trapping of a fluorescence microbead in turbid and transparent media. After the system evaluation, it was applied to trap breast cancer cells such as highly- (MDA-MB-231) and weakly- (MCF7) invasive cells[31] and the calcium and morphological responses of the cancer cells to acoustic trapping were then quantitatively measured at different acoustic intensities, followed by examination of the cell viability. Altogether, these results demonstrated the potentials of the high frequency ultrasound microbeam system to manipulate and characterize a cancer cell with different phenotypes.

# II. METHODS

## 2.1 High Frequency Ultrasound Microbeam System

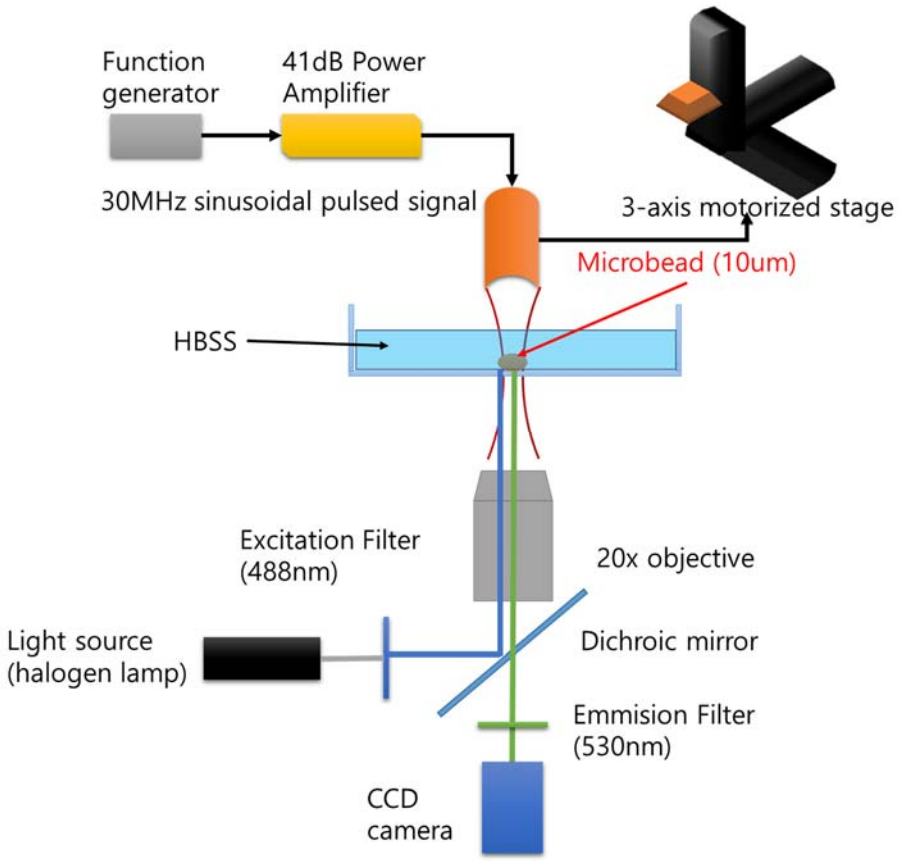


**Figure 2 High Frequency Ultrasound Microbeam System**

A high frequency ultrasound microbeam system was consisted of a 30MHz signal generator (DC to 2.025GHz signal generator, Stanford Research Systems, Model SG382), a 41dB amplifier (Customized high frequency amplifier), a 30MHz lithium-niobate single element ultrasonic transducer attached to a 3-axis motorized stage with a f-number of 0.7 for a tightly focused high frequency ultrasound beam. Research fluorescence Inverted Microscope (Olympus, IX 73), Light source (Halogen lamp), and CCD camera (Photometrics Cascade 512B) were utilized to monitor a trapped cell by the system. The signal from the signal generator was amplified by a 41dB power amplifier. The amplified signal is inserted into a single

element ultrasonic transducer which was attached to a 3-axis motorized stage. The focus point was adjusted precisely by a 3-axis motorized stage with 1 um resolution. This adjustment was stopped when the ultrasound echo signal was maximized while moving the transducer in z-axis. The tilt angle of ultrasound transducer was also important to performance of the high frequency ultrasound microbeam system. The tilt angle was also adjusted until the ultrasound echo signal is maximized.

## i. Ultrasound Transducer

A lithium-niobate single element transducer with f-number of 0.7 in order to generate a tight focus ultrasound beam was prepared for the high frequency ultrasound microbeam system. The blue line of the graph shown in Figure 4 indicated impulse response and the red line of the graph showed frequency response from the transducer.

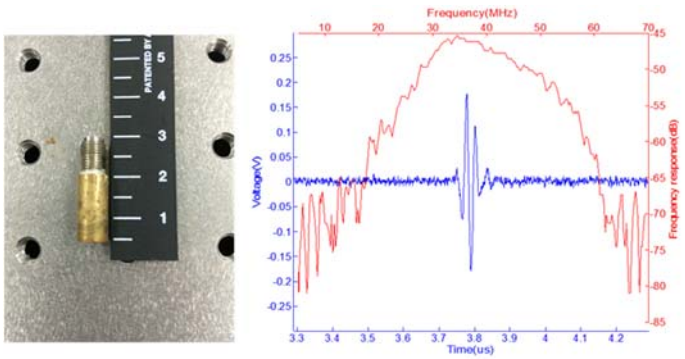


**Figure 3 (a) A 30MHz Lithium-niobate single transducer (b) Impulse response of a trnasducer**

This transducer could generate the 30MHz high frequency ultrasound microbeam to the tightly focused point. The transducer was fabricated with press focused structure at a matching layer in order to support tight focus for the high frequency ultrasound microbeam system.

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## ii. Signal generator

In order to trap the cell, the pulse modulated 30MHz sinusoidal signals were generated by signal generator [18] with 500 cycles of a signal in 1ms period. The pulse duration was determined as 16.6us to achieve 500 cycles of the sinusoidal signal wave. Figure 5 exhibited the schematic of the pulse modulated 30MHz sinusoidal signals. The power of the 30MHz sinusoidal signal was adjusted from 0mVpp to 300mVpp in

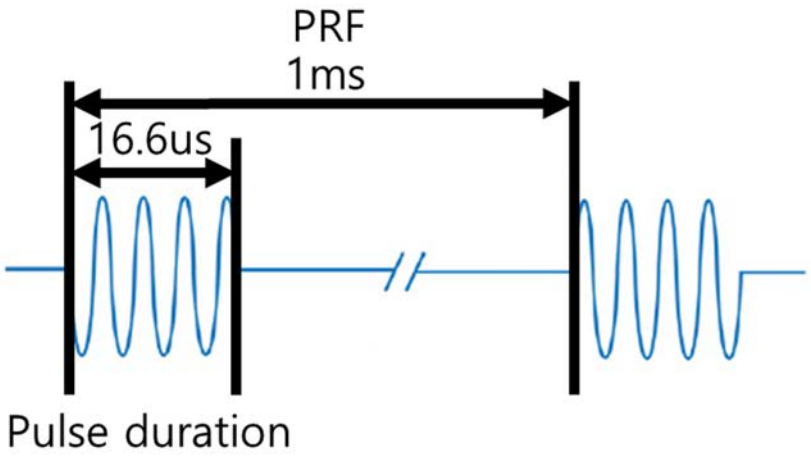


**Figure 4 Pulse modulation timing diagram**

this paper. The power of this signal was amplified by a 41dB amplifier. Finally, this signal has amplified power range from 0Vpp to 32.2Vpp.

## iii. Power Amplifier

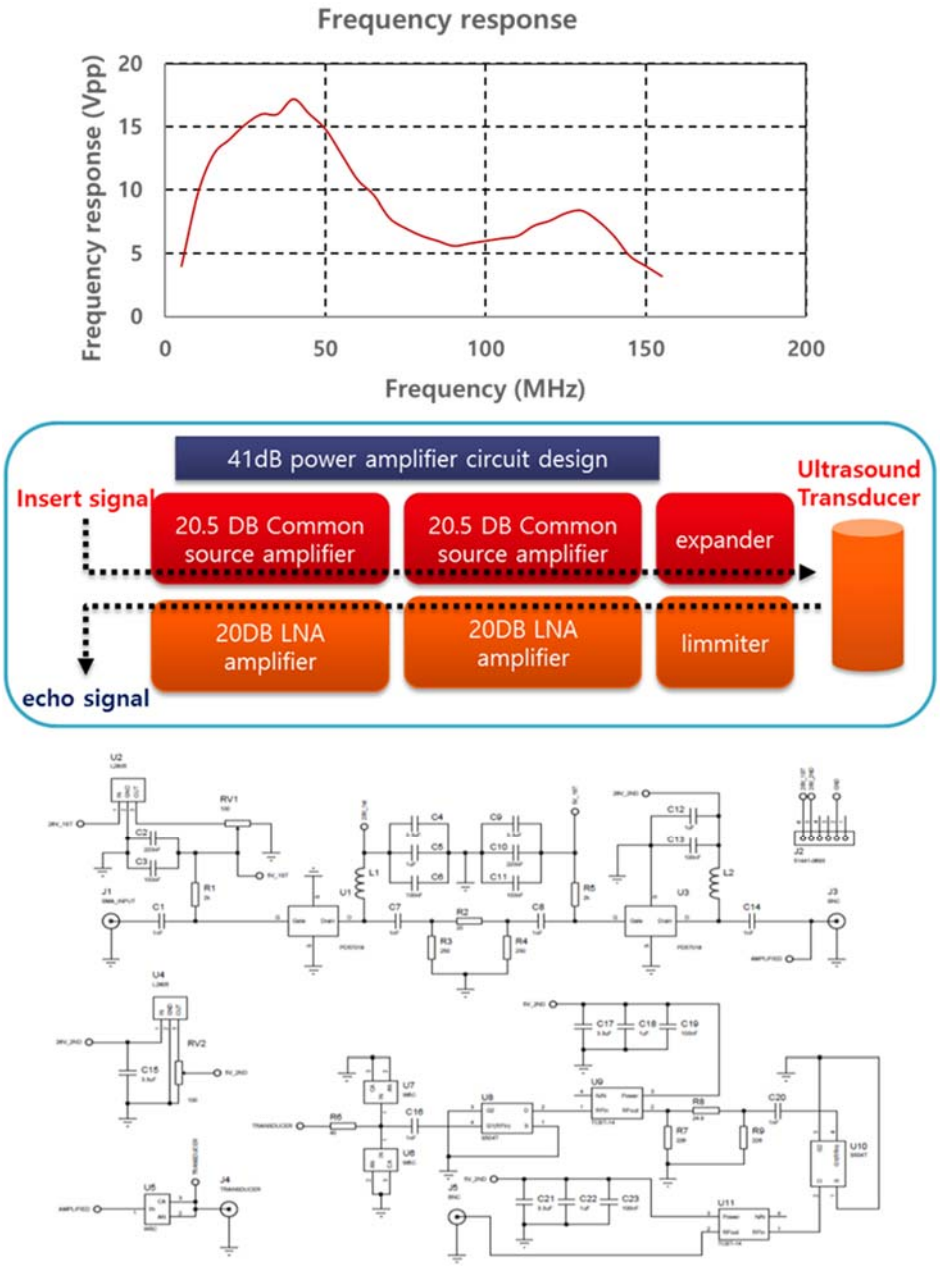


**Figure 5 a 41dB amplifier circuit frequency response, block diagram, and reversed circuit**

A 41dB high frequency amplifier was designed to amplify a 30MHz signal from a signal generator. The amplified signal was inserted into ultrasound transducer and generate 30MHz ultrasound. This circuit utilized 28V main voltage and continuously consume current 0.35A. The bias voltage was adjusted by variable resistors at a common source amplifier stage. Each bias voltage is determined as 3.3V.

## iv. 3-axis motorized stage

The motorized stage (SIGMA KOKI, SHO-304GS) was used to control the position of ultrasound transducer. This product had 1um resolution to control the position which was capable to adjust focus point. The motorized stage was controlled by LabView program through serial communication. This Serial communication was initialized with 9600 bps communication speed. The communication protocol for a motorized stage was clarified in SHOT-304GS in Figure 7.

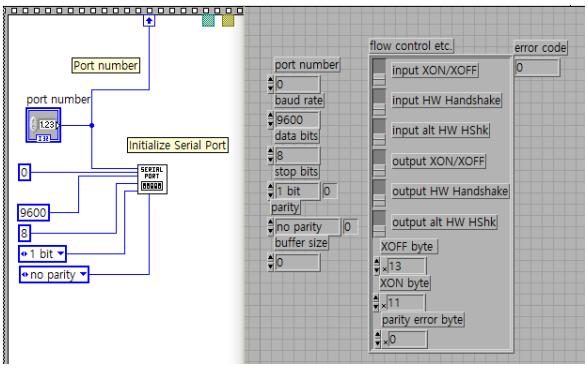


**Figure 6 Serial initialization for motorized stage**

One of the protocol to control the motor was M-command. This command was consisted of M: (M command), 1(axis number), +(motor direction), and P1000 (moving distance 1000um). After to send this M-command, SHOT-304GS return OK ACK signal. Finally, the G-command was sent to the motorized stage and the PC received OK again. Finally, the motor was moved to target position.

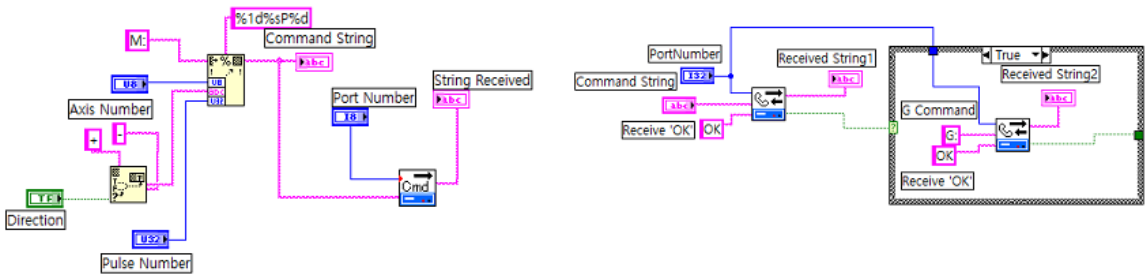


**Figure 7 M command and G command program by LabView**

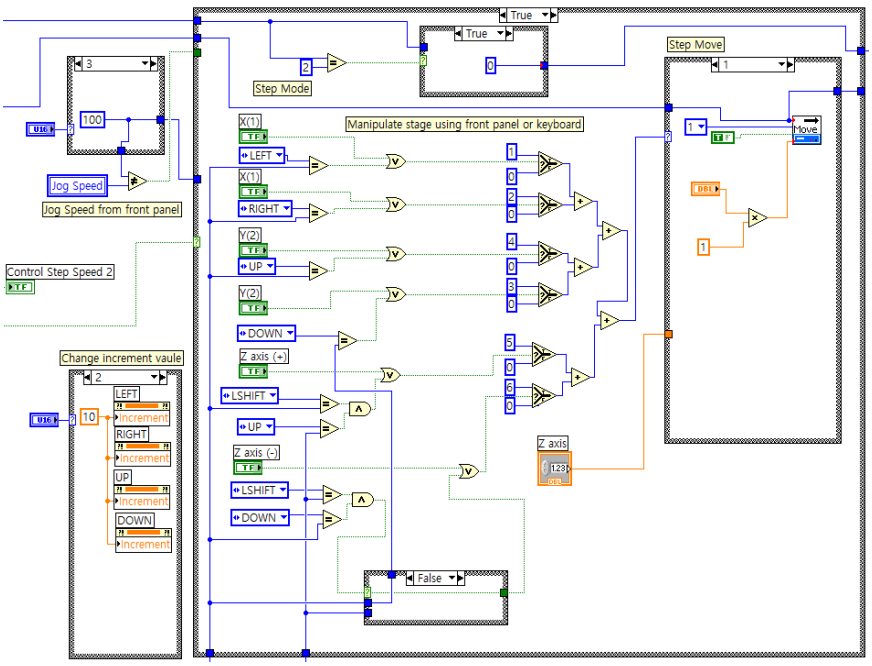


**Figure 8 Motorized stage controller program**

The motorized stage controller included key input event handlers. This motorized stage controller program provided the interface between key input and 3-axis motorized stage. The distance of the movement when insert a key input could be adjusted manually. This motorized stage was used for an ultrasound transducer focus adjustment by controlling axial direction. After trapping an object, a motorized stage controled lateral direction in order to make a movement of a trapped object.

## 2.2 Single Beam Acoustic Trapping Mechanisms

The acoustic trapping effect was elicited by the differences of the acoustic power in acoustic beam. In general, the focused ultrasound microbeam had a Gaussian formed power porfile in lateral direction [32]. An object which was in the acoustic beam refracted the acoustic beam path which exerted the surface of an object. These refracted beam generated the scattering force to the acoustic tweezers focal plane [33]. Also the trapped cell was pressed by the scattering force concurrently. The acoustic tweezers could ideally

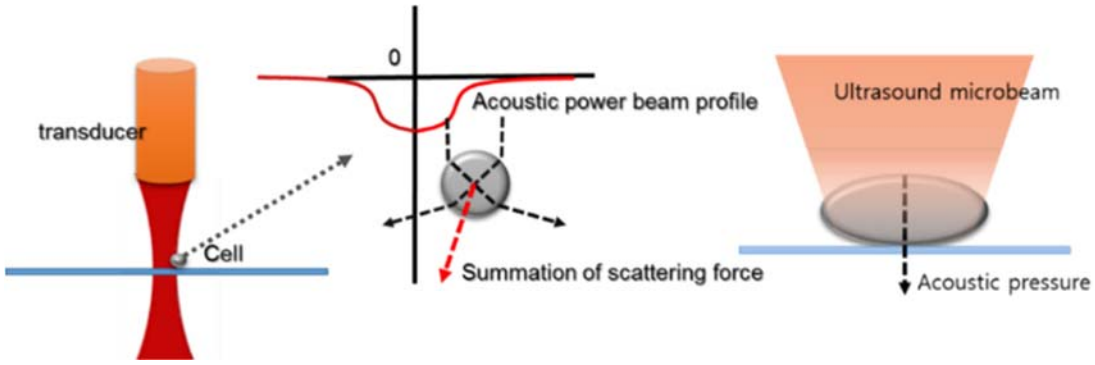


**Figure 9 Principle of Acoustic Trapping**

stimulate a cell since the acoustic tweezers trapped and pressed a cell simultaneously in non-contact condition. The Figure 10 exhibited the direction of the integrated scattering forces. The direction of the integrated scattering force pointed to focal plane. By using these phenomenon, we monitored the morphological deformation and molecular reaction by mechanical stimulation in a cell.

## 2.3 Cell Preparation

### i. Cell Selection

The cell MDA-MB-231 and MCF7 cells were selected as a highly invasive cell and a weakly invasive cell respectively. MDA-MB-231 was expected that MDA-MB-231 cell has a low Young's modulus rather than MCF7 cell has. Each cell demand similar solutions which is compounded with DMEM 500mL, FBS 50mL, and penicillin 10mL in order to culture that cancer cells. The cells are cultured in $CO_2$ incubator (Panasonic, MCO-18AC-PK) with temperature 37°celcius degree $CO_2$ 5% density.

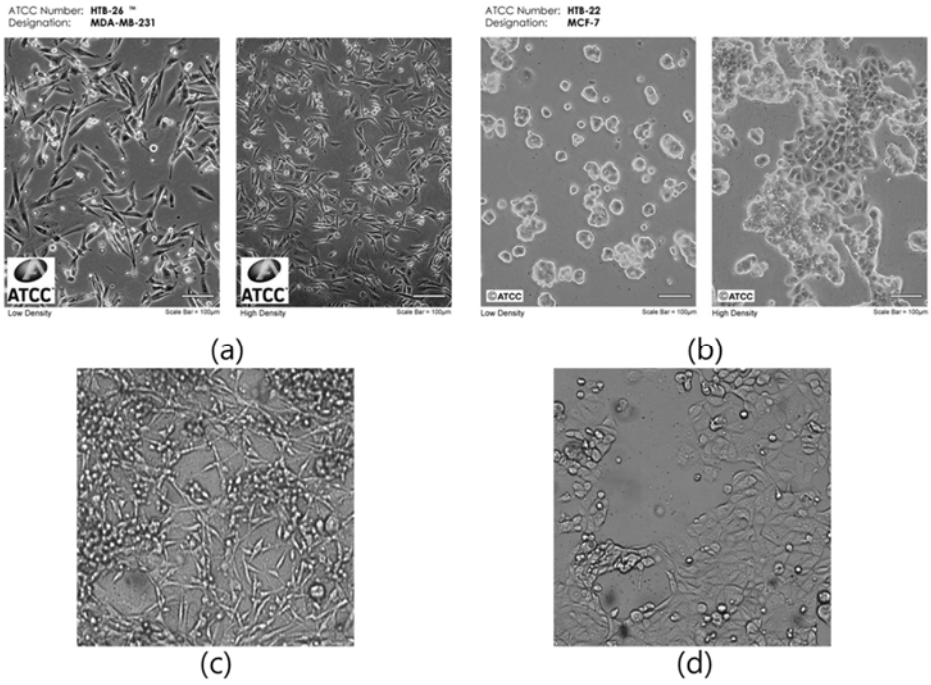


**Figure 10 (a) Ideal MDA-MB-231 cultured cell (ATCC) (b) Ideal MCF7 cultured cell (ATCC) (c) Cultured MDA-MB-231 cell (d) Cultured MCF7 cell**

The cells were cultured in $CO_2$ Cell incubator. After growing the cells, we marked the calcium fluorescence calcium indicator using HBSS 2mL, Pluronic F-127, and 1mM Fluo-4 solution compounded with Fluo-4 and 45.58uL DMSO (Figure 13).

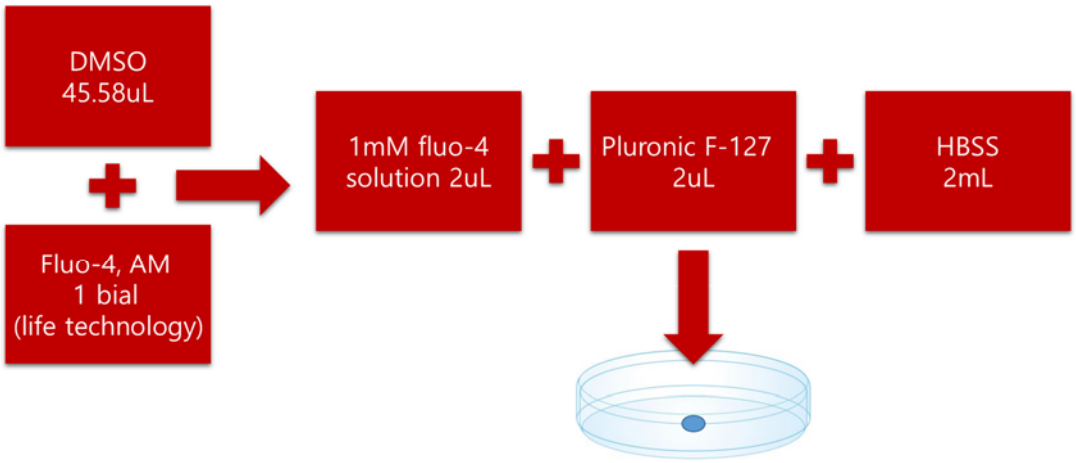


**Figure 11 Sequence of fluorescence calcium labeling**

## 2.4 Area deformation quantification method

The deformation rates of the breast cancer cell was proportional to the metastatic potential of the breast cancer cell. Also, this deformation rate was related to calcium elevation. In order to measure the deformation, we developed the area measurement algorithm.

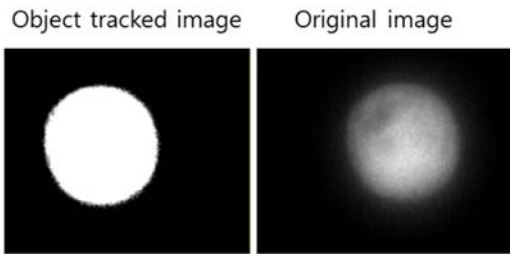


**Figure 12 Tracked object using ImageJ**

Firstly, the object was tracked by the ImageJ which was the open source image processing program in Figure 13. Then, we found the center of object by calculating the mean value of the position index of the object pixels. The contour of object is detected by canny edge detection algorithm.

$$x_{center} = E(x_{edge}),\ y_{center} = E(y_{edge})$$

$$r(n) = \sqrt{\left(x(n)_{edge} - x_{center}\right)^2 + \left(y(n)_{edge} - y_{center}\right)^2}$$

$$\theta(n) = tan^{-1}\left(\frac{y(n)_{edge} - y_{center}}{x(n)_{edge} - x_{center}}\right)$$

$$d\theta(n) = \theta(n+1) - \theta(n)$$

$$S = \int_0^N \frac{r(n)^2}{2} d\theta(n) \quad N: number\ of\ edge\ pixels$$

Finally, the area S was calculated by integrating the area of the sectors. This equation could reduce the pixel errors of a trapped object. The CCD camera with 512×512 resolution was used to monitor the object. The images acquired by CCD camera had very low resolution to measure the area deformation. In order to minimize error of the area of the object, we employed the integration method for area measuring. When we simply counted the number of pixels in an object, there were a lot of area fluctuations even though we monitored the microbeads. However, we could stabilize the area of the microbeads by using this integration method. Therefore, we thought that this integration method could suggest more reliable results rather than pixel counting method.

# III. RESULTS

## 3.1 Evaluation of a high frequency ultrasound microbeam system

### i. Acoustic trapping performance in clear media

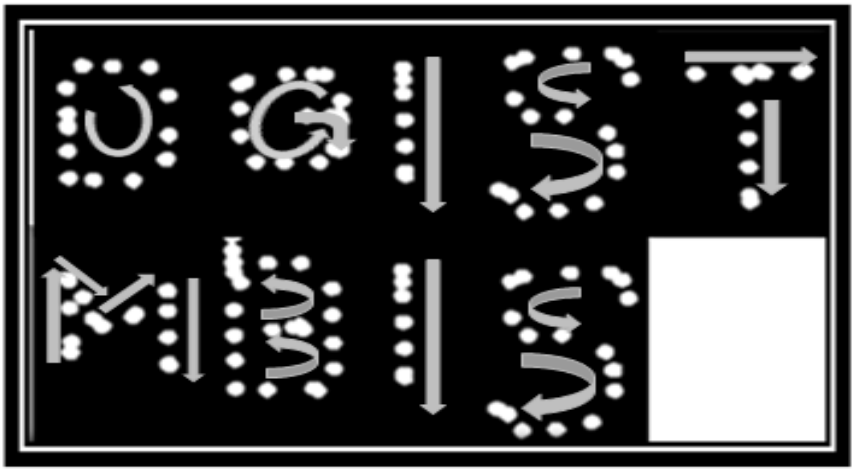

**Figure 13 The overlay image of trapped particle movements**

We performed the acoustic trapping of a fluorescence microbead (life technologies, F8836). We wrote the word “DGIST”, and “MBIS” using the high frequency ultrasound microbeam system in Figure 15 which was the overlay images of the particle movement. We inserted 140mVpp to the 41dB amplifier and 16.3Vpp signal was inserted to ultrasound transducer. Fluorescence microbeads were excited with 488nm light. These microbeads were monitored using 530nm optical bandpass filter.

In order to trap the fluorescence microbead, we supplied 16.3Vpp to the ultrasound transducer with 500cycle of sinusoidal signal. This pulsed signal was repeated every 1ms. The tilt of ultrasound transducer was adjusted to the position which maximizes echo signal from the dish.

## ii. Acoustic trapping performance in turbid media

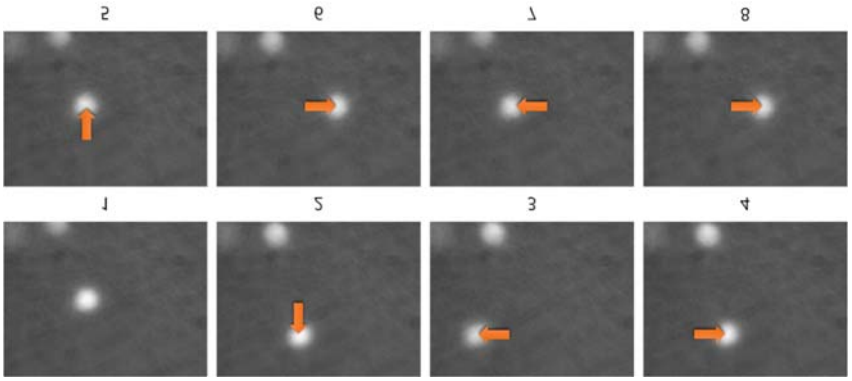

**Figure 14 Trapped MDA-MB-231 cell in turbid media**

One of advantages of the acoustic tweezers was that acoustic trapping could be used in turbid media. We prepared the intralipid (Intralipid 20% emulsion) 221mL, HBSS 2mL. When acoustic trapping of the microbead was performed in the turbid media, we could monitor the attenuated power of ultrasound microbeams.

**Table 1 microbead reaction in turbid media**

| Clear media (Vpp) | Reaction time (ms) | Speed (um/s) | Turbid media (Vpp) | Reaction time (ms) | Speed (um/s) |
|---|---|---|---|---|---|
| 5.92 | 190 | 53 | 5.92 | inf | 0 |
| 8.2 | 120 | 83 | 8.2 | inf | 0 |
| 10.48 | 80 | 125 | 10.48 | inf | 0 |
| 14.1 | 50 | 200 | 14.1 | inf | 0 |
| 15.2 | 40 | 250 | 15.2 | inf | 0 |
| 17.4 | 30 | 333 | 17.4 | inf | 0 |
| 19.4 | 30 | 333 | 19.4 | 100 | 100 |
| 21.2 | 20 | 500 | 21.2 | 80 | 125 |
| 23.8 | 20 | 500 | 23.8 | 60 | 167 |
| 25.8 | 20 | 500 | 25.8 | 50 | 200 |
| 27.7 | 20 | 500 | 27.7 | 50 | 200 |
| 29.6 | 20 | 500 | 29.6 | 50 | 200 |

| | | | | | |
|---|---|---|---|---|---|
| 31.4 | 10 | 1000 | 31.4 | 30 | 333 |
| 32.9 | 10 | 1000 | 32.9 | 20 | 500 |

Table 1 was the result of microbead reaction in turbid media and clear medial respectively. Parmar [34] adjusted attenuation coefficient by controlling the concentration of intralipid. The attenuation of the ultrasound was proportional to frequency of sound and the intralipid concentration. Here, we could monitor the degradation of the acoustic trapping performance.

## 3.2 Cell deformation by acoustic trapping

The MDA-MB-231 cell, MCF7 cell, and microbeads were pressed by acoustic pressure. The power of acoustic pressure was adjusted by controlling input voltage to the ultrasound transducer. The input voltage range was from 0Vpp to 32,2Vpp from a 41dB power amplifier. Each target is used 10 times to statistically measure the deformability of the cell and microbead. MDA-MB-231 and MCF7 cell areas

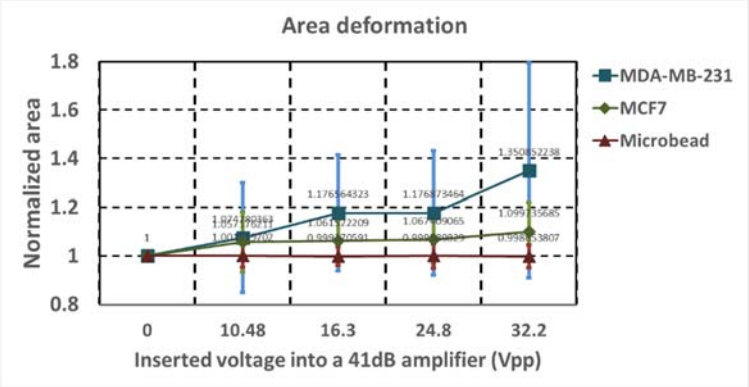


**Figure 15 The deformation of the MDA-MB-231 cell, MCF7 cell, and microbead**

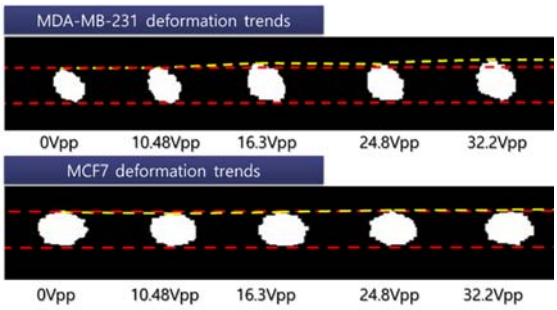


**Figure 16 MDA-MB-231 and MCF7 cell deformation trends**

were slightly increased following the increased acoustic pressure power in Figure 18. Figure 19 exhibited the comparison of the cell under from 0Vpp to 32.2Vpp respectively. The normalized deformation rates were 1.35, 1.1, and 1 on MDA-MB-231, MCF7 cell, and microbead respectively. In this experiment, we could prove the highly invasive cell MDA-MB-231 had a high deformation rate than weakly invasive cell MCF7.

## 3.3 Calcium elevations in cancer cells due to acoustic trapping

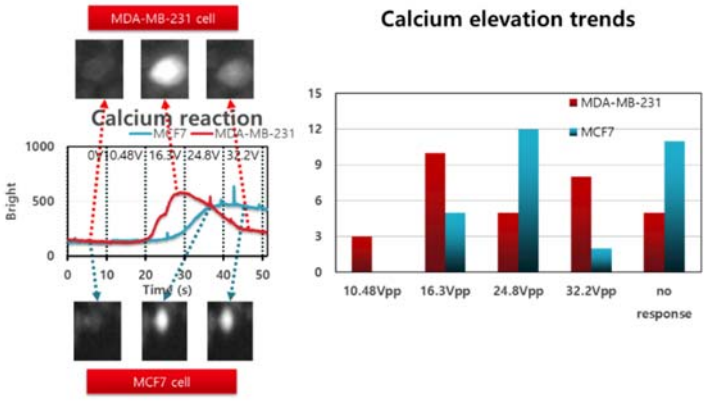


**Figure 17 Calcium elevation of MDA-MB-231 and MCF7 cell**

The cell was pressed by acoustic pressure then the cell exhibited calcium elevation. This mechanotransduction effect was related to the cell invasiveness. The mechanisms of the mechanotransduction in a cell was not clarified yet. However, this phenomenon could be used to determine the cell mechanics under external force stimulation. Here, we supplied acoustic pressure to the cell and monitor the calcium reaction. The input voltages were from 0Vpp to 32.2Vpp. The cell was monitored by fluorescence microscopy with CCD camera in 200ms exposure time. The power of acoustic pressure had kept increasing at each 100 images acquired. The highly invasive breast cancer cell MDA-MB-231 mainly exhibited calcium elevation at 16.3Vpp. The weakly invasive breast cancer cell MCF7 mainly exhibit calcium elevation at 24.8Vpp. Also, the MDA-MB-231 cell was likely to react more frequently than MCF7 cell.

## 3.4 Cell viability test

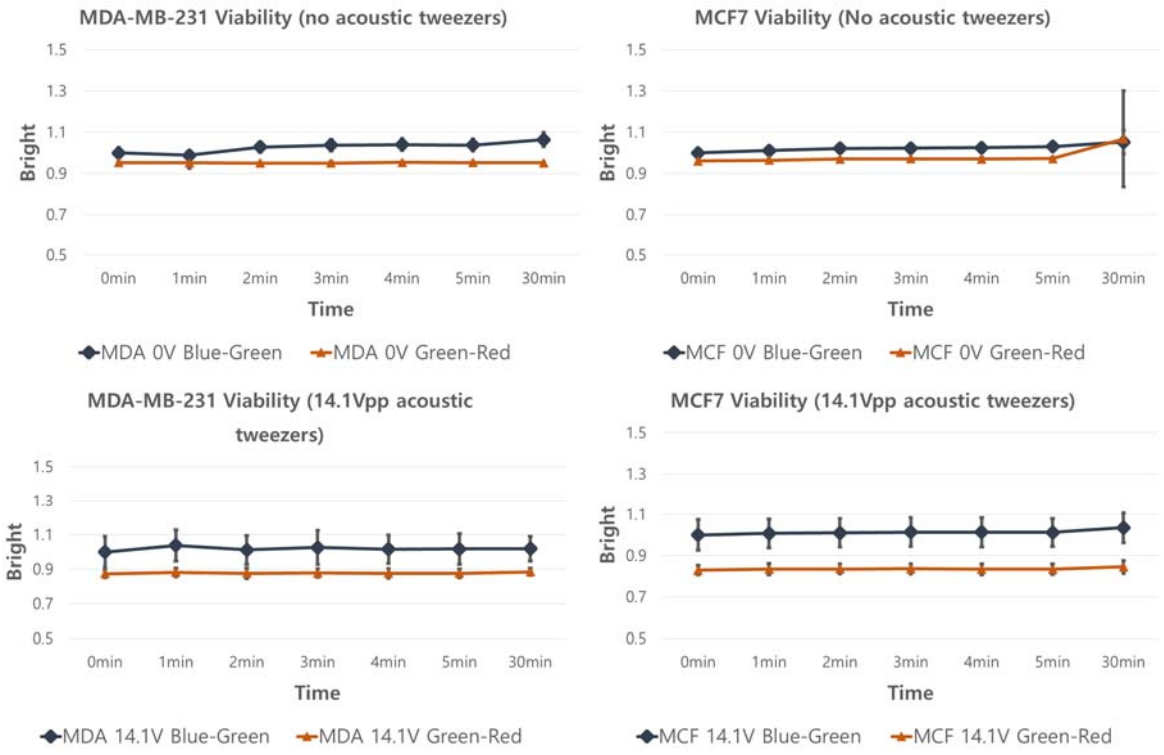


**Figure 18 Cell Viability Test result**

The one of the advantages of the acoustic tweezers system was that the high frequency ultrasound microbeam could manipulate the cell without any contact which had a potential damage to the cell. In order to prove that there was no significant damage, we labeled the cell with calcein 1uL ethidium 4uL in order to monitor the cell with fluorescence microscopy. The cells were monitored by the blue light excitation and green fluorescence emission light and by the green light excitation and red fluorescence emission light. Previous study [35] reported that the green emission light is declined and red emission light was improved when the cell going to be dead. The result in Figure 21 indicated that the acoustic trapping force had no significant effect on the suspended live cell. The results proved that the high frequency ultrasound microbeam system could be ideal tool to manipulate the cell without any potential damage

# IV. DISCUSSION

The high frequency ultrasound microbeam system was capable of eliciting calcium elevation on suspended cell by acoustic pressure. The high frequency ultrasound microbeam was utilized to focus a beam tightly and stimulate the cell with high power. Actually, the high frequency ultrasound microbeam allowed for the cell to be stimulated with high power acoustic pressure. A cancer cell typically had allowable mechanical index MI of 1.9 [36]. We could calculate the allowable maximum acoustic pressure power by the equation $\text{MPa} = \text{MI} \times \sqrt{center\ frequency}$ [17]. The allowable maximum acoustic pressure is ~26.87Mpa with 30MHz ultrasound transducer. It was the reason why the high frequency ultrasound is suitable for cell manipulation.

In order to manipulate the cell, there were existing techniques [37] such as optical tweezers [38, 39], magnetic tweezers [40, 41], micropipette aspiration [42-44] and atomic force microscopy [3]. However, these techniques had common problem that the additional materials should be contacted to the cell membrane to stimulate a cell with the existing techniques. In order to overcome these problems, we employed the high frequency ultrasound microbeam system to trap the cell with label-free. The high frequency ultrasound microbeam system generated the trapping force and pressure force to a cell directly. Therefore, we applied the high frequency ultrasound microbeam system to a suspended cell to stimulate a cell using it effectively. The cell was automatically positioned at the focus point of ultrasound and automatically pressed by acoustic radiation force generated while acoustic trapping. Hence, we thought that the high frequency ultrasound microbeam system is an ideal non-contact cell stimulation method. Also, the high frequency ultrasound microbeam system could be applied to an object in turbid media. We could prove that acoustic trapping was working very well in turbid media. The focus was not significantly changed

since the sound speed in intralipid was very little faster [34]. However, the ultrasound is highly attenuated by intralipid [34]. We could monitor the degradation of trapping performance using the high frequency ultrasound microbeam system in turbid media. The acoustic power should be adjusted to higher power than clear media's one for the same performance.

The highly invasive cell (MDA-MB-231) and weakly invasive cell (MCF7) were prepared to compare the differences of deformability and calcium reaction by acoustic trapping. The power for acoustic trapping were adjusted by controlling an input voltage to the ultrasound transducer with range from 0 $V_{pp}$ to 32.2 $V_{pp}$. The ultrasound transducer was positioned by 3-axis motorized stage with 1 um precise resolution. We monitored the deformability and calcium reaction of the cell while increasing the inserted voltage from $0V_{pp}$ to 32.2 $V_{pp}$. We could finally observe the different deformability and calcium reaction of the different breast cancer cell under acoustic trapping.

In order to measure the area deformation caused by acoustic trapping, we developed the new equation for measuring the area using the integration of sectors area of an object. This equation guarantee minimized the pixel errors. The pixel error was induced when the images are acquired by low resolution CCD camera. This low resolution images exhibited the various area result with same microbead. Therefore, we developed the algorithm to minimize this error and we could successively measure the microbeads and the cell deformability. The deformation of the cell was monitored by fluorescence microscopy proportionally to the acoustic power. This result implied that the high frequency ultrasound microbeam system could be an ideal non-contact stimulation method for the cell. For measuring the area deformability, we should consider that the most ideal method to measure the deformation was that both a high resolution CCD camera and a high-resolution microscope were prepared to acquire the image.

The calcium elevation effects were monitored by fluorescence microscopy. This result had no significant differences with the previous research on the cultured cell manipulation with 200MHz ultrasound microbeam [19]. The highly invasive cell MDA-MB-231 exhibited more frequent calcium reaction than weakly invasive cell MCF7. The MDA-MB-231 cell also exhibited the trend to react the calcium elevation at relatively lower acoustic power than MCF7 cell. However, each breast cancer cell exhibited calcium reaction at various acoustic power condition. The cells basically included the heterogeneous properties [45]. In order to overcome the heterogeneous problem, we experimented 30 times and plotted the statistical result. The statistical result clearly indicate there was different mechanotransduction characteristics. These results could be the biomarker for determination of the metastatic potential of the breast cancer.

Interestingly, we could monitor the calcium responses on the cell which placed on the next side of target. We guessed that there was strong fluid shear stress caused by the acoustic radiation force. Also, we could monitor these fluid shear stress could cause calcium elevation very well. The calcium elevations are exhibited at relatively low acoustic power rather than a trapped object's one at the focus point. These calcium elevation caused by fluid shear stress caused by an ultrasound microbeam should be researched in the future.

# V. CONCLUSION

In this thesis, I demonstrate the development of a high frequency ultrasound microbeam system which is capable of manipulating and quantitatively characterizing a cancer cell in suspension. The system I developed enables to trap a cancer cell in suspension. It was here found that the deformation and calcium elevation of the trapped cancer cell may depend on its phenotype. Previous studies [18, 19] showed that pulling of the acoustically trapped microbeads attached to a cell membrane results in the membrane deformation of a cultured cell and also the high frequency ultrasound microbeam stimulation elicits calcium elevations in the cultured cancer cells, not in suspension. The main drawbacks in the studies were 1) needing additional materials to be attached to cell membrane in order to quantify the mechanical properties of a cultured cell and 2) culturing cancer cells to receive high frequency ultrasound microbeam stimulation. In contrast, the system I developed is capable of trapping a suspended cell and stimulate the cell after isolation from other adjacent cells in suspension. Moreover, by dong that, the changes in morphological and molecular features of cancer cells may be simultaneously monitored, allowing to obtain multiple information in the characterization of a cancer cells. Therefore, the results shown in this thesis demonstrate that the high frequency ultrasound microbeam system can be a promising tool in a non-contact manner to manipulate and characterize a cancel cell in suspension,

# IV. FUTURE WORKS

In this thesis, I developed a high frequency ultrasound microbeam system which has the potential as a new biophysical tool for manipulation and characterization of a cancer cell. Here we could observe that the morphology and intracellular calcium levels of suspended cells acoustically trapped were changed by the trapping force with the system. More interestingly, highly invasive breast cancer cells exhibited greater morphological deformation and higher calcium elevation than weakly invasive breast cancer cells, thus showing the potential to determine the invasion potential of a breast cancer cell. Note that by using the system, we have performed the experiment on quantification of morphological deformation and calcium elevations in the cells, respectively, but not simultaneously. Therefore, if we could simultaneously quantify the morphological deformation and calcium elevations in the cells, we may achieve better determination of their invasion potentials by using it. In future works, I will therefore redesign our system optimized for the simultaneous monitoring of morphological changes and calcium elevations in a cells and also develop a new program for quantitative analysis of them. In particular, I will develop a novel method capable of reducing the scattering effects, which blearing the contour of a target cell in an acquired image and therefore reducing the accuracy in the quantitative analysis, of fluorescence emitted a target cell on quantitative analysis of morphological and calcium changes of the cell. Furthermore, we will incorporate microfluidic techniques with the system for high-throughput cell analysis.

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# 요 약 문

## 유방암 세포를 조작하고 정량화하기 위한
## 고주파 초음파 시스템 개발

최근 고주파 초음파 마이크로빔을 이용하여 다양한 의료응용을 목적으로 초음파집게, 초음파 현미경, 단일 세포 자극 시스템 등이 다수 개발되고 있다. 본 논문에서 30MHz 단일 초음파 변환자를 이용하여 암세포를 조작하고 그 분자 특성을 분석 하기위한 고주파 초음파 마이크로빔 시스템을 개발함. 이 시스템에서 사용된 단일 소자 LiNbO3 초음파 변환자의 센터 주파수는 30MHz, f-number 값은 0.7 로 세포를 조작하기 위해 초음파를 강력하게 집속할 수 있도록 설계되었다. 초음파를 발생시키기 위해 함수 발생기로부터 펄스화 된 사인파를 발생시키고 이를 10MHz 에서 60MHz 사이로 동작하는 RF 증폭기를 통해 증폭 후 초음파 변환자에 인가함. 초음파 변환자는 3 축 모터제어 스테이지와 함께 IX73 도립 현광 현미경에 부착함. 모터제어 스테이지는 정밀하게 초음파 빔 초점을 조절하기 위한 프로그램으로 제어됨. 또한, 형광 현미경 기술을 사용하여 세포를 관찰하고 정량화 할 수 있는 시스템을 구성함. 구성된 초음파 집게의 성능을 평가하기 위해 마이크로비드를 각기 투명한 용액과 혼탁한 용액 안에서 포획하고 그 성능을 평가하고 비교함. 실험 결과 10um 마이크로비드가 그 용액 안에서 성공적으로 초음파집게에 잡히는 것을 확인하였고, 혼탁한 미디어 내에서 그 초음파 집게 성능이 약화 되는 현상을 관찰함. 더불어, 이 초음파 집게 시스템을 의료용으로 유용하게 응용될 수 있도록 암세포를 조작하고 정량화하는데 적용하였음. 높은 침습성을 가진세포와 낮은 짐습성을 가진 유방암 세포들을 초음파 집게를 이용해 포획 시 그 세포들의 형태학적, 분자적 즉 칼슘 반응을 정량화해서 분별 될 수 있는지 조사함. 여기

서 높은 침습성을 가진 세포 (MDA-MB-231)는 낮은 침습성을 가진 세포 (MCF7)과 달리 낮은 전압에서 강한 칼슘 반응을 나타내는 경향을 보임. 더욱이, 높은 침습성을 가진 유방암 세포는 낮은 침습성을 가진 유방암 세포에 비해 초음파 집게의 음압 하에서 더 크게 형태가 변화하는 모습을 보임. 그러므로, 이러한 결과들은 다른 표현형을 가진 유방암 세포들이 그들의 초음파빔의 포획에 의한 형태학적, 분자적 반응을 정량화함으로써 그 특성들을 파악할 수 있다는 것을 나타냄. 이러한 결과들은 이 시스템의 암세포 조작 및 정량적 분석이 가능한 생물리학 장비로써의 그 잠재성을 나타냄.