Evaluation of Cells Activity Using Non-invasive Dielectrophoresis Method

Ryosuke komai, Keishu Aritoshi, and Kozo Taguchi
Science & Engineering, Ritsumeikan University, Kusatsu, Japan
Email: ro0021ps@ed.ritsumei.ac.jp, taguchi@se.ritsumei.ac.jp

***Abstract*—The advance of biotechnology has provided various benefits to our lives. There is an increase in the opportunity of cell fusion and cultivation for using optical tweezers. Although optical tweezers is useful technique, cells are damaged by laser irradiation. Therefore, in our study, we estimated the cells damage by Ar laser (514nm) and semiconductor laser at 980nm. As a preliminary stage to our study, we trapped cells using optical tweezers at 980nm laser and Ar laser and showed manipulation data of polymer microspheres and yeast cells. Furthermore we calculated trapping power and trapping efficiency from the results of manipulation data. In the next stage, we focused on activity evaluation method using Dielectrophoresis (DEP) as estimating yeast cells damage. We trapped yeast cells by optical tweezers at 980nm laser and Ar laser and observed the movement of cells while changing the time of irradiation. We set the initial value to voltage of 1V and frequency of 500kHz and applied such parameter settings to the electrode by function generator(KENWOOD, FG-281). This experiment proved that optical tweezers at Ar laser was harmful for yeast cells, while optical tweezers at 980nm laser was harmless for yeast cells.**

***Index Terms*—Dielectrophoresis (DEP), optical tweezers, cell damage, ar laser, 980nm laser, frequency**

I. Introduction

Optical tweezers is useful technique in cell manipulation. Using this technique, it is possible to freely move the cells in non-invasive and non-contact [1]-[3]. In our experiment, we used this technique for taking target that were microsphere and yeast cells (Saccharomyces cereviase). Dielectrophoresis (DEP) is caused by non-uniform electric field and it is useful to judge the viability and activity of cells. It is generally that healthy cells attach to electrode, while damaged cells repel electrode. Another way to judge cells viability is using methylene blue. However, it is not suitable for manipulating healthy cells because it is poisonous [4]. In our experiment, we tried to use DEP for judging the viability of cells so we needed to demonstrate whether our bipolar electrode was suitable for judging the viability or not. After the demonstration, we started the main experiment about estimating the cells damage by Ar laser and semiconductor laser at 980nm. However, it was necessary to select healthy cells for cell fusion and cultivation. Thus we used both optical tweezers and DEP effectively to estimate the cells damage. Using such technique, we studied the cells damage by using optical tweezers and DEP.

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II. Theory

A. Dielectrophoresis (DEP)

DEP is used to manipulate dielectric particle in the solution. It caused by non-uniform electric field so dielectric particle like cells moved between two electrodes with AC field [5]. The cells movement that shows between two electrodes is known to be in proportional to the applied voltage of the DEP [6], [7]. The movement depends on complex permittivity of dielectric particle and solution [8]. It does not matter whether the cells are charged or not. The movement of dielectric particle is changed by frequency dependence. Fig. 1 shows dielectric particle that repels electrode in strong electric field.

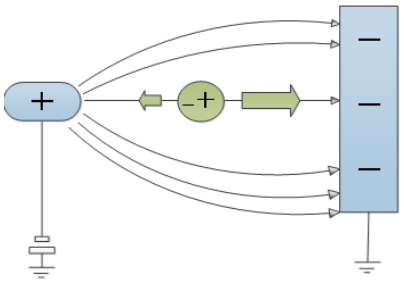

Figure 1. DEP in strong field.

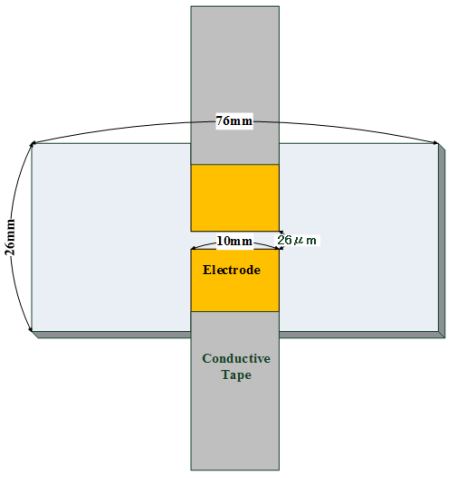

Figure 2. Bipolar electrode with conductive tapes.

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In our experiment, we made bipolar electrode shown in Fig. 2. The bipolar electrode was made by Au and the distance between electrodes was 26μm and their edges were covered with conductive tapes. Conductive tapes were connected to function generator via conductive line. The power was supplied from a function generator and we prepared yeast cells as object.

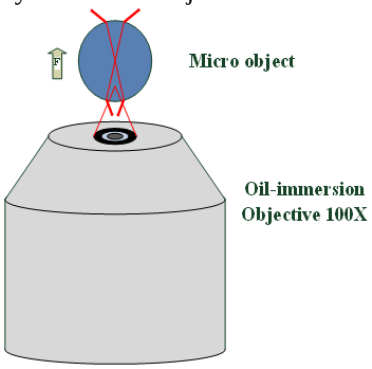

Figure 3. Micro object trapped by optical tweezers.

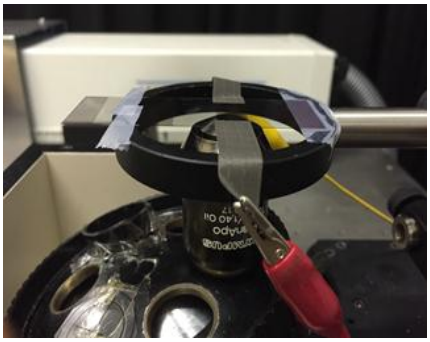

Figure 4. Bipolar electrode and objective lens.

B. Optical Tweezers

Optical tweezers is possible to manipulate micro object like cells with non-invasive and non-contact [9]. Generally, microscope object lens or optical fiber is used for focusing laser in optical tweezers. Reflection and refraction, absorption, emission is caused when micro object exposed to the focused laser by microscope object lens. According to the phenomenon, momentum of focused laser is changed at surface. Thus radiation pressure works upward and the object are trapped like Fig. 3. The trapped point depends on where buoyancy and gravitation are balanced. Fig. 4 shows bipolar electrode that was made by Au and we trapped micro objects for using such objective lens.

C. Trapping Power and Efficiency

Definition of trapping power is F and trapping efficiency is Q and μ is viscosity coefficient. We used manipulation data so we calculated F and Q by using "equation (1), equation (2), and equation (3)".

$$F=6\pi\mu av \tag{1}$$

F: trapping power
π: the circular constant
μ: viscosity coefficient
a: radius of object
v: velocity(manipulation data)

$$F=QPn/c \tag{2}$$

F: trapping power
Q: trapping efficiency
P: average laser power
n: refractive index
c: the velocity of light

$$\mu=\frac{0.1}{2.1842\{t-8.435+\sqrt{8078+(t-8.435)\text{^}2\}-120}} \tag{3}$$

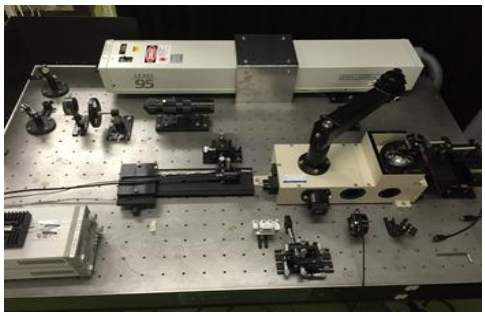

Figure 5. Optical system used for experiments.

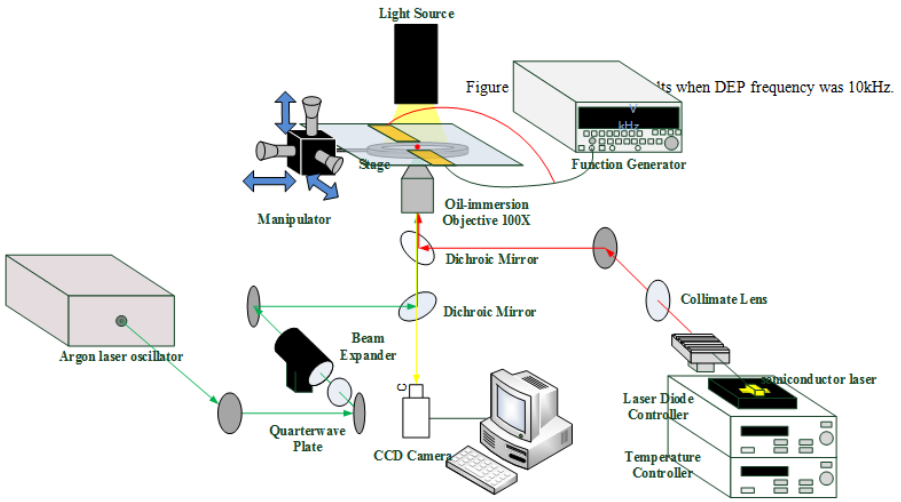

Figure 6. Experimental setup using DEP and optical tweezers.

III. Material and Methods

We made optical systems shown in Fig. 5. The system of Fig. 5 combined Ar laser system with semiconductor laser at 980nm system. The wavelength of Ar laser is 514nm. Quarter wave plate and beam expander and some mirrors were used to introduce Ar laser beam irradiated from the Ar laser oscillator to the dichroic mirror in Fig.6. In semiconductor laser at 980nm (SNO534289, Lumics, Germany), collimate Lens and aome mirrors were used to introduce 980nm laser beam irradiated from semiconductor laser device to the dichroic mirror. Both laser beams were coaxially aligned and then introduced into an objective lens as shown in Fig. 6. Thus two types of laser were focused by oil-immersion objective lens (100X/1.25, Edmund, USA). The motorized translation stage installed on manipulator so we could measure the manipulation data of optical tweezers with microsphere and yeast cells. Fig. 6 shows the whole picture of the device that were used for DEP and optical tweezers. Thus we measured trapping power and efficiency and estimated the cells damage.

IV. Results

A. Judge the Viability

Firstly, we needed to confirm whether bipolar electrode was suitable for judging the viability or not. We steeped cells in hot water of 80 degrees Celsius for 10 minutes to make dead cells and mixed it with methylene blue solution. After that, we applied voltage of 4V, frequency of 10 kHz and 15 MHz to the electrode, and observed the movement of the cells [10].

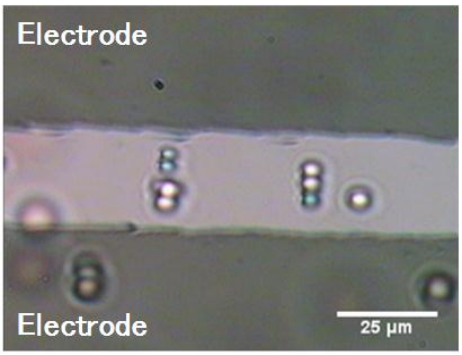

Figure 7. Experimental results when DEP frequency was 10kHz.

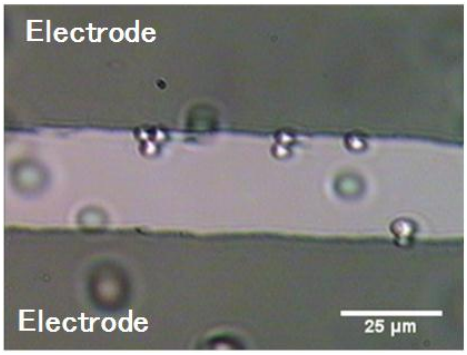

Figure 8. Experimental results when DEP frequency was 15MHz.

As a result, both viable cells and dead cells approached electrode when the frequency was 10 kHz. However, when the frequency was 15MHz, only viable cells were trapped. Fig. 7 and Fig. 8 show the cells movement of each frequency on the electrode. In 10 kHz, viable cells and dead cells that were dyed blue were collected around electrode, however only dead cells that were dyed blue moved away from electrode in 15MHz.

B. Trapping Power and Efficiency of Microsphere and Yeast Cells

We measured data of trapping power and trapping efficiency with microsphere and yeast cells. The size of microsphere was 10μm, yeast cells was about 5μm.

These targets were trapped by focused laser beam and we measured manipulation data by motorized translation stage. As a result, Ar laser was better than semiconductor laser at 980nm in trapping power and trapping efficiency.

In comparison with the data of microsphere and yeast cells, microsphere was better than yeast cells at trapping efficiency. Thus, Ar laser was suitable for manipulating micro object than semiconductor laser at 980nm.

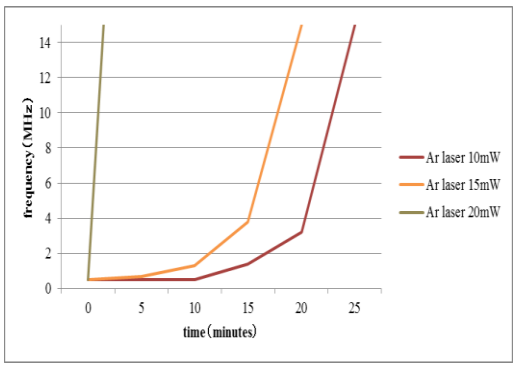

Figure 9. DEP frequency at Ar laser

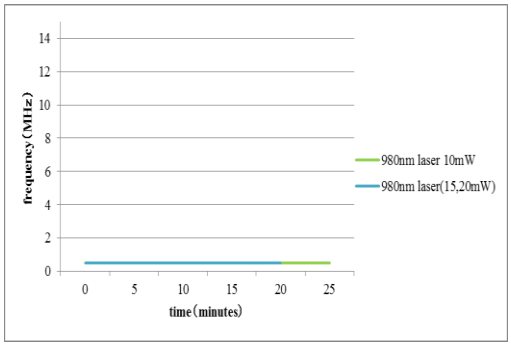

Figure 10. DEP frequency at 980nm laser

C. The Cells Damage at Ar and 980 nm Laser

We set the power of laser at 30mW, 50mW, 70mW and observed the cells movement while changing the frequency. As a result of experiment, the trapping frequency was raised by changing the trapping time in Ar laser. On the other hand, no particular change was seen in semiconductor laser at 980nm. We show these result in Fig. 9 and Fig. 10. Our experiment proved that trapping power, trapping time, and the type of laser were factors of the cells damage by using optical tweezers. In Ar laser, the trapping efficient was better than that of

semiconductor laser at 980 nm, however, it damaged cells as shown in Fig. 9. In semiconductor laser at 980nm, the trapping efficient was less than that of Ar laser, however there was no damage to the cells as shown in Fig. 10. So semiconductor laser at 980nm was suitable for cell fusion and cultivation.

V. Conclusion

Our experiment was to estimate the cells damage by Ar laser and semiconductor laser at 980nm. From these results, it was found that DEP could be used to measure the cells activity. In trapping power and efficiency of optical tweezers, Ar laser was better than semiconductor laser at 980nm in optical tweezers however, Ar laser damaged to the cells. While trapping power and efficiency of semiconductor laser at 980nm were less than that of Ar laser but, semiconductor laser at 980nm was suitable for cell fusion and cultivation.

Acknowledgement

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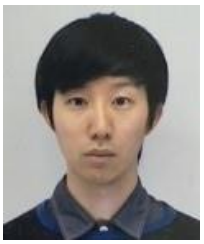
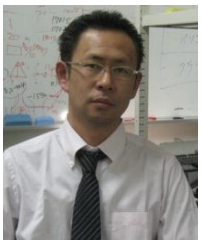


Ryosuke Komai was born in Gifu, Japan, March 2nd in 1993. He received Bachelor's degree in department of science and engineering from Ritsumeikan University, Shiga, Japan on March 2015 and was admitted to a postgraduate course at same University on April 2015. He is belonging to an electronics system course of department of science and engineering. He is using DEP and estimating cells damage by various laser irradiation in the graduate course.

Keishu Aritoshi received the B.E. and M.E. degrees in electrical engineering from Ritsumeikan University, Shiga, Japan in 2013, and 2015 respectively. He is currently working in an industrial company.



Kozo Taguchi was born in Kyoto, Japan, on December 18, 1968. He received the B.E., M.E., and Dr. Eng. degrees in electrical engineering from Ritsumeikan University, Kyoto, Japan, in 1991, 1993, and 1996, respectively. In 1996, he joined Fukuyama University, Hiroshima, Japan, where he had been engaged in research and development on the optical fiber trapping system, semiconductor ring lasers and their application for optoelectronics devices, and polymeric optical waveguides for optical interconnection. In 1996-2003, he worked as an assistant and a lecturer in Fukuyama University. In 2003, he moved to Ritsumeikan University, Shiga, Japan, and currently he is a professor of Department of Electric and Electronic Engineering. From 2006 to 2007, he was a visiting professor at University of St Andrews (Scotland, United Kingdom). From 2014 to 2015, he was a visiting professor at Nanyang Technological University (Singapore). His current research interests include cells trap, microfluidic cell based devices, dye sensitized solar cell, biofuel cells. Dr. Taguchi is a member of the SPIE.