報告番号	甲 第 13166 号

主 論 文 の 要 旨

論文題目 **Measurement System of Cellular Environment Using Fluorescence Microsensor**
(蛍光マイクロセンサを用いた細胞環境の測定システム)

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論 文 内 容 の 要 旨

Investigation of intracellular properties is very important since deemed to provide significant information that contributes to the biology applications such as drug delivery and cancer diagnosis. The purpose of this study is to realize various manipulation and injection of fluorescence sensor into a selective target cell using optical control of zeta potential and utilizing multiple wavelength lights, and development of a hydrogel fluorescence sensor for prolonged stable temperature measurements for achieving the measurement system of physiological parameters in the cellular environment.

Chapter 1 introduces the background of the research and the previous study of this thesis. After explaining the importance of manipulation, injection, and measurements of micro-nanoparticles in single cell analysis, the fluorescence-based sensors using micro-nanoparticles, and the problems of conventional manipulation and

injection of micro-nanoparticles into cells for intracellular measurements were discussed. Besides, the features of optical control in manipulation and injection of micro-nanoparticles into cells for intracellular measurements also described. Finally, the purpose of the research and the outline of the thesis are described.

Chapter 2 presented a new method of manipulation and injection of fluorescence microsensors into cells using glass nanoprobe and optical control of zeta potential. As a demonstration, a single microsensor of 750 nm diameter was picked-up using a glass nanoprobe with optical control of the zeta potential. Then, the microsensor was transported and immobilized onto a target cell membrane. After that, it was injected into the cytoplasm using NIR laser at 1064 nm wavelength. The success rate of pick-up and cell immobilization of the microsensor were compared, and the cell injection and cell survival rates were evaluated.

In Chapter 3, the author proposed the manipulation and injection of the fluorescence microsensor into cells using multiple wavelength lights. The sensor made of 1 μm diameter polystyrene particle-containing Rhodamine B and an infrared (IR: 808 nm) absorbing dye. The polystyrene particle can be manipulated with optical tweezers by 1064 nm laser to the target cell. After being transported to the cell membrane, the fluorescence microsensor is heated by 808 nm laser and injected into the cell by melting the cell membrane. The manipulation and injection of the microsensor to the MDCK cell by 1064 nm and 808 nm laser were demonstrated. The result showed a high success rate (70%), low invasive and rapid injection within 10 s. From these results, the effectiveness of the proposed cell injection of fluorescence microsensor using multiple wavelength lights were confirmed.

In Chapter 4, the author presented a hydrogel fluorescence microsensor for prolonged stable temperature measurements. In this work, a photobleaching compensation method based on the diffusion of fluorescent dye inside a hydrogel microsensor is proposed. The factors that influence compensation in the hydrogel microsensor system are the interval time between measurements, material, the concentration of photo initiator, and the composition of the fluorescence microsensor. These factors were evaluated by comparing a polystyrene fluorescence microsensor and a hydrogel fluorescence microsensor, both with diameters of 20 μm. The effect of microsensor size on the stability of the fluorescence intensity was also evaluated. The hydrogel fluorescence microsensors, with sizes greater than the measurement area determined by the axial resolution of the confocal microscope, showed a small decrease in fluorescence intensity, within 3%, after 900 measurement repetitions. The temperature of deionized water in a microchamber was measured for 5,400 s using both a thermopile and the hydrogel fluorescence microsensor. The results showed that the maximum error and standard deviation of error between these two sensors were 0.5 °C and 0.3 °C, respectively, confirming the effectiveness of the proposed method.

Chapter 5 gives the conclusions of this study and the future works were discussed.

In conclusion, the proposed methods allow the manipulation and cell injection of a single microsensor to be used as a carrier for intracellular and extracellular measurement, especially in biological and biomedical applications such as drug delivery and cancer diagnosis.