(様式 5)

指導教員 承認印	

2017 年　12 月　14 日
Year　Month　Day

学位（博士）論文要旨

(Doctoral thesis abstract)

論文提出者 (Ph. D. candidate)	工学府博士後期課程　機械システム工学　専攻 (major) 平成 24 年度入学(Admission year) 学籍番号　12833004　氏名　金指　康明 (student ID No.)　(Name)　(Seal)
主指導教員氏名 (Name of supervisor)	梅田　倫弘
論 文 題 目 (Title)	ミトコンドリア活性評価のための 2 光子吸収蛍光励起近接場 pH 測定法の開発 Development of Near-field pH Measurement excited by Two-photon Absorption Fluorescence for Evaluation of Mitochondrial Activity

論文要旨（2000 字程度）
(Abstract(400 words))
※欧文・和文どちらでもよい。但し、和文の場合は英訳を付すこと。
(in English or in Japanese)

A proton distribution in the vicinity of mitochondria attracts interest due to its influence to a necrotic cell death. Mitochondria produce adenosine triphosphate, ATP using a proton concentration gradient generated across the inner membrane. Therefore, the pH distribution near the mitochondria changes from time to time. However, when the mitochondria is exposed to physical or chemical stress, the proton concentration gradient disappears and ATP can not be produced, resulting in necrotic cell death. This process is considered as a part of the reason for necrotic cell death. Therefore, it is expected to clarify the mechanism of necrotic cell death by measuring the pH distribution in the vicinity of mitochondria. However, since there is a limit to the spatial resolution of the conventional microscope, it is very difficult to accurately measure the pH value in the vicinity of the mitochondria.

In this study, we propose pH measurement method based on two-photon fluorescence excitation of a dual wavelength pH sensitive dye and scanning near-field optical microscopy (SNOM) to improve spatial resolution and to avoid reabsorption. In this method, two-photon absorption is generated using a femtosecond pulsed laser, and fluorescence at 580 nm and 650 nm is emitted by locally exciting the dye. The fluorescent signals are collected by a near-field probe of SNOM. Fluorescent signals of each wavelength are separately detected by a cooled photomultiplier, and the fluorescence intensity ratio (FIR=I 580 nm / I 650 nm) is calculated as an index of pH

value. As a result of obtaining the pH calibration curve using this proposed method, the good correlation coefficients of 0.984 and 0.993 were obtained for two photon and data sheet, and two photons and one photon, respectively. Then, a time resolution of 0.1 sec in pH change was measured by the time response to the addition of acid. In addition, mitochondrial activity was observed by pH change at three different mitochondrial concentrations.

Next, a multi-probe system with two near-field probes was proposed to evaluate the difference in the activity between a single mitochondrion and mitochondrial aggregation. First, we measured the FIR at the different pH value from 5.0-8.5 and prepared a calibration curve of pH-FIR. The FIR dynamic responses were then measured by dropping hydrochloric acid (HCl) into the buffer solution. As a result, we can simultaneously measure the pH changes at two different points in the SNARF-4F solution by the measurement system. Therefore, it is possible to measure the pH change in the near-field region of mitochondria and to measure the activity difference between single mitochondrion and mitochondrial aggregation. In addition, mitochondrial samples were prepared using optical tweezers and mitochondrial activity was evaluated by the two near-field probes detection system. As a result, it was concluded that the pH change of mitochondria depends on the number of mitochondrial individuals by clarifying the change in pH of mitochondria after addition of nutrient substrate.

（英訳）※和文要旨の場合(400 words)