3P-332 過渡蛍光検出赤外分光法に基づく赤外超解像顕微鏡の開発と細胞への応用

IR super-resolution microscope based on transient fluorescence detected IR spectroscopy and its application to cells

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IR microscope has been used widely for following reasons: 1) IR absorption spectrum of small samples and domains can be measured, 2) without causing any damage, and 3) spatial mapping of specific materials by IR is possible. However, the spatial resolution of IR microscopes is very poor relative to other optical microscopes. This is because the spatial resolution of a conventional IR microscope is restricted by the diffraction limit, which is almost the same as the wavelength of IR light. The diffraction limit prevents a conventional IR microscope from achieving a better spatial resolution, because the IR wavelength is very long, ranging from 3 to 25 μm in the mid-IR region. If a specific IR absorption band can be mapped with sub-micron spatial resolution, visualization of the molecular structure and reaction dynamics in a non-uniform environment such as a cell becomes a possibility. In this study, we developed a novel IR super-resolution microscope combining a laser fluorescence microscope and transient fluorescence detected IR spectroscopy and succeeded to perform IR imaging on the cells labeled by rhodamine-6G. We also demonstrated picosecond time-resolved IR imaging of vibration relaxation of rhodamine-6G in the cells, and found an abnormally long-lived component on vibrational relaxation. In order to understand the characteristic dynamics occurring within cells, vibrational relaxation of probe molecules in specific parts of cells have been measured by using other probe fluorescent dyes. The results will be discussed in details.

3P-333 非晶質凍結状態細胞内超微小構造の位相差電子顕微鏡観察

Phase contrast observation of ultrastructures inside vitrified cells.

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The ultimate goal of electron microscopy is the observations of ultrastructures inside living cells, however, conventional electron microscopy has drawbacks requesting chemical fixation and heavy metal staining of the specimens. A newly developed phase contrast electron microscope enabled the observation of ice-embedded vitrified living cells without fixation and staining.

Escherichia coli were suspended for the storage in a freezer. Spores of *Bacillus subtilis*, which can be alive at low temperature, were suspended in ethanol. Spermatozoa were suspended in a medium for artificial insemination. They were rapidly frozen in liquid ethane cooled with liquid nitrogen. The observation was carried out on a JEOL JEM 3100FFC electron microscope operated at 300kV acceleration voltage with a Hilbert differential interference contrast phase plate.

E. coli: Ultrastructures in the whole cell were well visualized. The cytoplasm consisting of granular substances on the cell wall were visualized. *Bacillus subtilis*: The ultrastructural cell wall was well visualized but that of cytoplasm was hardly visible because of the TEM invisibility for the thick core part of spores. Spermatozoa: their ultrastructures in the middle piece with circumferentially oriented mitochondria and in the principal piece with axoneme were clearly visualized. Phase contrast electron microscopy of vitrified cells for *E. coli*, *Bacillus subtilis* and spermatozoa, which were frozen but able to revive again after melting, enabled the observation of actually living creatures.

3P-334 2 + 3 次元画像を用いた、可視化・可聴化による特徴像の解析補助ツールの開発 2

A new molecular viewer with visualization of 2+3 dimensional images and audibilization II

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It is important to compare electron microscopic images with atomic models, analyze 2D micrographs and/or reconstructed 3D maps from them and elucidate the relationship between their structures and functions; it is also necessary to decide the projection angle of molecules by comparing each molecule in EM images with atomic models. Particles with each projection angle, thus, are collected to produce the molecule's three-dimensional reconstruction image. However, a general viewer for 2D images and 3D atomic models wastes much time for comparing. In addition, this requires much skill and is inefficient for 2D-3D comparison. Therefore, a new software to compare 2D-EM images with 3D-EM reconstructions and/or atomic coordinates is necessary. In addition, current molecular viewers can not supply some information by audio expression. However, aural is an important sense for human next to the sight. Therefore, we would find something not gained by visual expression by passing information through ear to brain. Therefore, I have developed a new molecular viewer to display atomic models of molecules and EM images at the same time, with aiding molecular structure analysis by sight and aural with coming and going to 2+3D display and labeling/search of the feature region in color and sound. We here show that our software can successfully facilitate comparison between each molecule in EM images and atomic models to understand molecular structure more intimate than ever before.

3P-335 画像相関分光法によるミトコンドリア形態の定量解析

Quantitative measurement of dynamics of mitochondrial morphology using image

correlation spectroscopy

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Since swollen mitochondrial filaments were found in fibroblasts from patients with several syndromes that had mitochondrial deficiency caused by mutation, mitochondrial morphology appear to be tightly correlated with its function. Several microscopic observations suggest that mitochondrial fraction of a cell sometimes exists as a single interconnected tubular network or reticulum, and that fusion and fission of mitochondria is a dynamic equilibrium. The recent finding that different respiratory states of mitochondria coexist in a single cell would support the heterogeneous structure of mitochondria even if a single reticulum forms. Quantitative measurement of dynamics of mitochondrial morphology is necessary to contain changes in mitochondrial morphology and movement within the whole region of a target cell. In several measured points of a single cell, Koopman et al analyzed green fluorescent protein selectively expressed within mitochondria using fluorescence correlation spectroscopy (FCS) and obtained the slow diffusion coefficient corresponding to mitochondrial mobility. In the present study, we used an image correlation method which was an imaging analog of FCS and measured the mitochondrial mobility in MitoTracker dye-staining cells. Slow moving mitochondria near nucleus ($0.16 \pm 0.07 \mu\text{m}/\text{min}$) masked those moving rapidly in a marginal area ($0.34 \pm 0.12 \mu\text{m}/\text{min}$), therefore, the average mobility of a single cell was $0.17 \pm 0.1 \mu\text{m}/\text{min}$. We plan to use mitochondrial inhibitors and will discuss.

3P-336 蛍光相関分光法と蛍光寿命測定を用いた核内受容体の生細胞内二量体検出

Analysis of intranuclear dimerization using fluorescence correlation spectroscopy and fluorescence lifetime measurement.

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The glucocorticoid receptor (GR) is one of transcriptional regulators that control broad physiological gene networks. Upon ligand binding, GR translocates into the nucleus from the cytoplasm, and then regulates transactivation by association as dimer with specific genomic glucocorticoid response elements (GRE).

Using fluorescence correlation spectroscopy (FCS), we analyzed diffusion properties of green fluorescence protein fused wild type and several mutants of GR before and after addition of ligands in the nucleus of the living cell.

We detected about 6.6-fold slow-moving component of wild type GR in the nucleus in the presence of dexamethasone (Dex) which is a transactivation agonist. On the other hand, one mutant which was impossible to associate with GRE became slow only 1.6-fold, and the other mutant which has no ability of dimerization became slow 2.8-fold by addition of Dex. These results indicate that the slow-moving component is originated by binding to GRE, moreover, the dimerization of GR is not required for association with GRE. Consequently, we propose that dimerization is the final step of transactivational process [1, 2].

However, the direct observation of dimerization of wild type GR by FCS is not still achieved. Therefore, we aim establishment of the analyzing method which combined FCS and fluorescence lifetime measurement for quantitative detection of dimerization.

[1] S. Mikuni et al., FEBS Letter, 581, 389-93, (2007).

[2] S. Mikuni et al., Exp. Mol. Pathol., 82, 163-8, (2006).

3P-337 高輝度LEDを用いた超高速顕微鏡観察用パルス光源の開発

Development of a pulsed light source for fast capturing microscopy using high-intensity LED

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We have already reported about an image recognition based cell sorting system which works based on cell images captured by a high speed camera. We speeded up a throughput of cell sorting process by improving frame rate of the high speed camera and processing time of image analysis. However, an existence xenon flash lamp was not enough to shoot image of particles moving fast at 200 mm/s without photogenes, because its pulse repetition frequency was no more than 400 Hz. To clear up these problems, we have investigated capability of a pulsed LED light source as a high-intensity and 1 μs pulse width light source for microscopy. In general, xenon flash tube is used in a machine-vision application. But we selected a high-intensity LED which has possibility of fast operation and has the same wavelength as the peak of sensitivity of the image sensor. Additionally, we developed a drive circuit to flash the LED in synchronization with the interval of high speed camera. We could take images of polystyrene beads flowing in the micro channel at speed of 200 mm/s clearly. We report detail of the pulsed LED light source and evaluation of captured particle images.