The Biophysical Society of Japan General Incorporated Association

2SH-01 KcsA カリアミオンイオンチャネルの溶液条件変化応答 1 分子間隔ダイナミクスの解析
Analysis of Single Molecular Gating Dynamics of the KcsA Potassium Channels Responding to Rapid Changes of Solution Conditions
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Recently, we succeeded in recording single molecule gating transitions of KcsA potassium channel from closed to open state with the modified diffraction X-ray tracking (DXT) method. In the conventional method the motions were recorded at video rate under equilibrium conditions. To reveal a whole picture of the gating dynamics we introduced a fast measurement system, pH jump measurement system, optimized X-ray for the method, and an efficient spot tracking program. The channel was fixed on a glass plate at the extracellular part and a gold nanocrystal was attached to the intracellular end of the channel molecule as a probe. The sample was irradiated with an optimized white-beam from a synchrotron and the motions of the Laue diffraction spots from the nanocrystal were tracked on a CMOS detector at the speed of 5000 frames/s. Solution pH condition was jumped during the measurements from neutral to acidic pH by laser-photolysis of caged proton, which can trigger the gating motions of pH-gated KcsA channel. The diffusion spots in the sequential image data were extracted and tracked by a developed data analysis program. The trajectories of the diffraction spots were translated into movements of the channel. With this modified DXT method we succeeded in revealing the different dynamic status of the channel depending on the change of pH condition and the gating transitions starting from the closed state with a larger spatial range at the speed of 5000 frames/s which is comparable to the speed of single channel current recordings.

2SH-02 中赤外吸収領域の観察が可能な赤外超解像顕微鏡の開発と生物材料への応用
Development of a mid-IR super-resolution microscope and its application to biological samples
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Infrared (IR) microscope is a powerful tool to measure molecular images of biological samples based on molecular information. However, microscopic objects such as biological cells cannot be measured by conventional IR microscope, because of its low spatial resolution about 10 μm. This is a serious problem for a microscope.

To overcome this problem, we have developed a novel type of IR super-resolution microscope based on vibrational sum-frequency generation (VSFG) detection. VSFG is a non-linear optical process and we irradiate visible and IR beams on the sample. If IR beam is resonant to vibrational mode of target molecule, VSFG signal photon will strongly generate. Therefore, we can detect IR absorption of the molecule by monitoring VSFG. In addition, IR absorption can also be imaged at the diffraction limit of visible light (several 100 nm) because VSFG signal has visible wavelength. By using this method, we succeeded in obtaining IR super-resolution images of biological cells such as onion root cells and human lung cancer cells in the 3 μm IR region. The spatial resolution is approximately 1 μm, that is smaller than the diffraction limit of IR light.

In this study, we improved an observational wavelength range of it up to the 6-9 μm mid-IR region to observe amide bands, and applied this IR microscope to the biological samples such as sugar crystals, amino acids, and human hairs. In the presentation, the result of IR super-resolution micro-spectroscopy of human hairs will be discussed in detail.

2SH-03 白色レーザーを用いた CARs 分光イメージングによる生細胞の動描画
Tracing dynamical behavior of a single cell by CARs microscopy using a white-light laser source
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The mechanism of a solubilization process of living organisms with surfactants has so far been attributed to the surface adsorption and subsequent disruption of cellular membranes. In this study, we applied quantitative CARs microscopy to investigate the molecular dynamics of surfactant molecules and intracellular molecules in living cells after the exposure of surfactant with low concentration (0.1 w%). By using an isotope labeled surfactant with CD bonds, the dynamic behavior of the surfactant in living cells was clearly observed. The simultaneous CARs imaging of the cell itself and surfactant molecules can address the details of the solubilization process with the surfactant. We observed the accumulation of the surfactant molecules inside a living cell and a subsequent sudden leak of cytosolic components such as proteins. This indicates the surfactant uptake prior to the solubilization of the cells. Our observation revealed that the surfactant molecules are accumulated in the lipid rich part of the cells probably due to the similarity of the composition. Quantitative CARs microscopy enables us to estimate the molecular concentration of the surfactant molecules accumulated in a cell quantitatively. We also investigated the drag response to the surfactant uptake dynamics. As the result of the inhibition of tubulin polymerization, the surfactant uptake rate was significantly lowered. This suggests that intracellular membrane trafficking contributes to the surfactant uptake mechanism.

2SH-04 イメージングトススペクトロトロピートよって初めて明らかになった、細胞内表面分布の分子種特異的な性質について
Imaging mass spectrometry revealed the polarized intracellular distribution of specific lipid molecular species
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Fluid mosaic model of cell membrane tells that fluid part phospholipids flow like liquid. This model predicts the evenly distributed phospholipids in cell membrane. However, the distribution of phospholipids was not well investigated experimentally at intracellular level because it was very difficult to visualize the distribution of distinct lipid molecular species regarding its variety of length of fatty acids. We applied high resolution imaging mass spectrometry on cultured neuron to visualize the gradient of phospholipids along the axon. Surprisingly, the distribution is distinct between the molecular species of phospholipids along the cellular polarity. We focus on the distribution of Arachidonic acid (AA)-containing PC (AA-PC), which we found to be enriched within the axon and is distributed across a proximal-to-distal gradient. Inhibitors of actin dynamics (cytochalasin D and phallacidin) disrupted this gradient, while microtubule dynamics inhibitors did not. A possible model of this newly found phenomena will be presented and discussed.

2SH-05 隨意運動中のマウス運動器官 2 光学イメージング
Two-photon imaging of the mouse motor cortex during voluntary skilled movement
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Motor cortical neurons are activated at multiple stages during voluntary skilled movement. However, the fine-scale organization of these activities remains poorly understood. To address this issue, we developed a self-initiated lever-pull task using a forelimb for two-photon calcium imaging of the mouse cortex in vivo. In layer 2/3 of two forelimb motor areas, the rostral forelimb area and caudal forelimb area, we identified several types of cortical cells according to their temporal activity patterns. We found that the trial-to-trial variability of the activity of individual cells is associated with that of other cells. This variability is not independent among neurons; rather, pairs of neurons covary their activities, depending on their spatial distribution and the extent of their firing rates. Our results suggest that sequential reconfiguration of functional ensembles with different correlation structures occurs in the motor cortical microcircuit.

2SH-06 Chemiluminescence imaging of flow-induced ATP release at caveolae in vascular endothelial cells
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Endothelial cells (ECs) alter their functions in response to hemodynamic shear