measurement system that records the extracellular field potentials and the contractile motions in lined-up cardiomyocyte network simultaneously. Cardiomyocytes were isolated from embryonic mouse heart by collagenase treatment and seeded in rectangular microchambers fabricated on the multi electrode array (MEA) chip for field potential recordings. To detect contractility of cardiomyocytes, the cells were labeled with the fluorescent polystyrene beads and the motions of beads were treated with image-based analysis. Hence, electrophysiological properties (interspike intervals and field potential durations) and mechanophysiological properties (displacements and velocities of beads) were obtained simultaneously. In the presence of verapamil, typical L-type calcium channel blocker, beating rate decreased accompanying with shortening of field potential duration (FPD) and its fluctuation increase, and contractility was weakened significantly with its fluctuation increase. The results indicate that the fluctuation of force generation should also become a biomarker of cardiotoxicity risk more correctly in drug development, also might be used for the clarification of mechanisms.

3PS024 白色 X 線源を用いた X 線 1 分子計測法の開発

Development of Diffracted X-ray Tracking measurement system using various wide-band energy X-ray sources

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Diffracted X-ray tracking (DXT) enable to measure milliradian resolved rotating and tilting motion of single protein molecules using high-brilliant and wide-band energy X-ray source. The tilting (θ) and rotating (χ) angle resolution and temporal resolution in this measurement are 0.9 mrad, 6 mrad and from 5 μ s/frame to 36 ms/frame corresponding to the photon flux. Tracking the diffraction spot from gold nanocrystals attached functional group with protein was developed for revealing the detailed fluctuation and functionalized motions in protein reaction processes at the beamline BL28B2, and BL40XU at the SPring-8 and NW14A at the PF-AR, KEK. (1) We installed the X-ray toroidal mirror at the beamline BL28B2 because the wide-angle diffraction spot tracking of gold nanocrystal markers in the measurement range $\Delta\theta = 7.4^\circ$ can be observed in this beamline. (2) The undulator beamline BL40XU (the measurement range, $\Delta\theta = 4.2^\circ$) allows high speed DXT in microsecond time region using the CMOS CCD camera. (3) New DXT measurement system which was synchronized with pulse laser system (Nd: YAG laser, pulse-width 10 ns, 10 Hz, UV-Vis) has succeeded to observe the open-close motion of membrane protein in reaction process at the beamline NW14A (measurement range, $\Delta\theta = 2.1^\circ$). Thus, the biological science applications of DXT can high profit from observation of the in-vivo functionalized motions of single protein molecules. Here, we will present the development of DXT measurement system and the future plan.

3PS025 CT 撮影用投影型 X 線顕微鏡の開発

Development of Projection X-ray Microscope for Micro-Tomography

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The projection X-ray microscope makes use of a very small X-ray light source excited by the focused electron beam on a thin metal target. If an object is placed just below the metal target, the expanding X-rays enlarge the shadow of the object with infinite focal depth. We have developed the microscope by modifying a scanning electron microscope (SEM) and achieved a resolution about 0.1 μ m. It is advantageous to investigate an opaque sample with better resolution than an optical microscope. And also has additional benefit to investigate three-dimensional structure of the thick specimen which can not studied by an electron microscopy. Taking these advantages, we have applied projection X-ray microscope for three-dimensional (3-D) structure analysis by means of cone-beam computed tomography (CT). To get higher resolution and better contrast, the choice of the metal target is important. The size of X-ray source determines the resolution, thus the electron beam should be focused as small as possible by condenser lens of SEM. And it should have a good balance to get enough strength of X-ray. The electron beam irradiated at the metal target is scattered in the metal and the scattered area is smaller in heavy metal. To get better contrast the wave length of X-ray should be longer utmost it penetrate the sample. In this case light metal target works better because of longer wavelength of K α characteristic X-rays. By accounting these conditions we have optimized the metal target for biological specimens.

3PS026 XFEL を用いた 3 次元分子イメージングのための雑音下における 2 次元回折像の類似度自動同定

Automatic similarity identification of 2D diffraction patterns with noisy background for 3D coherent x-ray diffractive imaging

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We proposed a method for classifying and assembling two-dimensional diffraction patterns to reconstruct the three-dimensional diffraction intensity functions (A. Tokuhisa, et. al., *Acta Crystallographica*, A68, 366 (2012)). In the classification process, similarity of each pair of patterns is judged based on the correlation of corresponding pixels along the concentric circles. When two diffraction patterns are similar to each other, a straight line with high correlations appears from the origin to a certain high scattering-angle region. Theoretically, the integrated value of the correlation value along this line is proportional to the extent of the similarity and can be used as threshold for the classification. This correlation line disappears with increasing scattering-angle due to the quantum noise and the structural irregularity. In our estimation, diffraction images of 10^6 orders will be required to construct the 3D structure with 3 Å resolution for a molecule with a radius of 200 Å. In this case, the necessary number of pattern comparisons is $\sim 10^{10}$ order. To achieve such a huge number of comparisons, we have to develop an automatic system. In this meeting, we will report how to deal with such a huge amount of data and our current status of the system.

3PS027 植物細胞の飛行時間型二次イオン質量分析法と多変量スペクトル分離法による評価

Evaluation of plant cells using time-of-flight secondary ion mass spectrometry and multivariate curve resolution

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Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is one of the most sensitive surface analysis methods providing submicron-scale chemical mapping. It is, however, often difficult to interpret TOF-SIMS spectra of complex samples such as tissues and cells. In this study, multivariate curve resolution (MCR) was employed in order to extract less complex TOF-SIMS spectra from original TOF-SIMS data of plant tissue. As a result, components related to intracellular tissues and cell wall tissues, respectively, were extracted from both samples. By comparing the component of the main sample to that of the reference sample, peaks specific to the main sample were indicated.

3PS028 Shannon エントロピーの可視化による質量顕微鏡データの情報解析 Information analysis by visualizing Shannon entropy using Imaging Mass Spectrometric data

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Microscopic Imaging Mass Spectrometry (MIMS), which we have developed cooperatively with Shimadzu Corporation and others, enables us to analyze spatial distribution of enormous molecules in the resolution of single cell level. MIMS has demonstrated its successful performance by revealing key molecules and their distribution in various research fields, e.g., medical development, agriculture, and material analysis. On the other hand, ageing and some inflammation are difficult to understand using a few key molecules. Principal component analysis and machine learning are also inadequate to elucidate such phenomena overlaying high-dimensional space. In this study, we try to figure out biological state in the framework of overall behavior of molecules, citing the famous argument by Erwin Schrödinger who first utilized entropy for biology to understand living organisms as system that autonomously maintaining their order against disruption.

In our previous presentation in this biophysical society, we visualized Shannon entropy in mouse brain to quantify *in situ* information held in molecular composition. Entropy image reveals characteristic distribution irrespective to anatomical representation. To confirm and discuss such spatial variation in information entropy, we propose a new data processing that takes advantage of