2P290 創薬スクリーニングのための心筋細胞ネットワークにおける空間パターンと集団サイズの重要性
Importance of spatial arrangement and community size on cardiomyocyte network for precise and stable in vitro drug screening measurement

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We have investigated the community size and spatial arrangement dependence of cardiomyocyte network response against pharmaceutical compounds. Typical four types of cell networks, small cluster, small closed loop, two-dimensional sheet, and a large closed loop, were formed in those fabricated agarose microchambers on a multielectrode array chip and comparison of their responses against Quinidine. The beating rhythm of the two-dimensional sheet was most durable and stable against administration of Quinidine even 100 μ M, whereas the large closed loop network was most sensitive and showed lethal arrhythmia from 1 μ M. The results indicate that the cell community for drug screening should be tuned to the proper community size and spatial arrangement pattern.

2P291 マニフォールドを用いた投影イメージの分類プロトコル：コヒーレント X 線イメージングによる粒子ダイナミックスの解析へ向けて
Classification protocol of projection images by manifold: Toward analysis of dynamics of particles with coherent x-ray diffraction imaging

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Coherent x-ray diffraction imaging enables us to collect huge amounts of diffraction images of a single particle for a short time. Since images represent projections of snapshots of a particle, they reflect its dynamics. Thus, dynamics can be analyzed using the images. One of the requirements for the analysis is a classification of images. Usual classification methods, however, would generate a great deal of classes because there are images arising from snapshots with a subtle difference as well as those with a large difference. This makes the analysis difficult. Here we propose a classification protocol of projection images using manifold, by which the issue above can be solved. We demonstrate the usefulness of the protocol using the images constructed in a computer.

2P292 振動和周波検出赤外超解像顕微鏡による毛髪 α -ケラチンの分子配向観察
Observation of molecular orientation of human hair α -keratins by VSFG detected IR super-resolution microscopy

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IR super-resolution images of cross-sections of human hairs were measured by using a vibrational sum-frequency generation (VSFG) detected IR microscope with a sub-micrometer spatial resolution. For the amide III band, the cross-section sample gave clear strong VSFG signals at the cortex area. This enabled us to measure the distribution of an α -helix structure of keratin proteins (α -keratins) in the hair. On the other hand, the VSFG signal disappeared completely when the amide I band was monitored by the same polarization of incident light. From the polarization dependence of VSFG, it is concluded that the α -keratins are well aligned along the axial direction in human hair. In the presentation, the results of the oblique-sections will be also reported in detail.

2P293 Determination of dissociation constants of NF κ B p50/p65 heterodimer using fluorescence cross-correlation spectroscopy in the living cell

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The p50/p65 heterodimer plays various roles in gene regulation. The quantitative value of affinity, namely the K_d, for the heterodimer in living cells is not known yet. We used fluorescence cross-correlation spectroscopy to quantify the heterodimerization of p50/p65 in the living cell. We determined the K_d of the transiently expressed mCherry tandem dimer (mCherry2) and green fluorescent protein (EGFP) protein fused with IPT domains of p50 and p65, respectively, in living cells. The K_d values of mCherry2-p50 and EGFP-p65 were determined to be 0.46 μ M in the cytoplasm and 1.06 μ M in the nucleus. These results suggest the different binding affinities of the p50/p65 heterodimer in the cytoplasm and nucleus of the living cell.

2P294 蛍光・発光イメージングによる OPN5 発現細胞の Ca²⁺ 応答測定
Bioluminescent Imaging Revealed a Rapid Ca²⁺ Response in OPN5-expressing Cells

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Fluorescence imaging is a powerful tool for investigating the intracellular signaling, but it may be difficult to apply to photo-receptive or photo-sensitive cells. In this report, we demonstrated that a light-induced Ca²⁺ response was different between fluorescence imaging and bioluminescence imaging in human opsin5 (OPN5)-expressing cells. In addition, the irradiation induced the phosphorylation of MAPK when OPN5 was stimulated under dark condition, but this phosphorylation was altered when OPN5 was irradiated under the bright condition. These findings suggested that comparison of the responses in these imaging provide a new technique to investigate the differences of physiological characteristics between light-adapted and dark-adapted photoreceptors.

2P295 ライブセル超解像イメージングに向けた多重分子用アルゴリズム“Wedge Template Matching”
Localization Algorithm of High-Density Fluorophores, “Wedge Template Matching” for Live Cell Super Resolution Imaging

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Localization microscopy excites a statistical fraction of molecules over time, allowing the sub-diffraction location of each molecule to be mathematically determined. A limitation of this method is the need for many (>5,000) raw frames to produce meaningful reconstructions. To address this issue we developed a localization algorithm of high density fluorophores, “Wedge Template Matching (WTM)”, which can localize simultaneously overlapping molecules. On simulated data, WTM can localize overlapping molecules at greater than 20 molecules/ μ m² density with localization precision (FWHM) of 24 nm. We demonstrate WTM for live cell super resolution imaging.