3P295 生細胞内における長鎖非翻訳 RNA の一分子イメージング法の開発

A method to visualize endogenous long non-coding RNA with single molecule sensitivity

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Increasing knowledge about long non-coding RNAs (lncRNAs) from in vitro experiments highlights a need to study lncRNAs in live cells. We here present a method for imaging repeat regions of lncRNA in living cells. A mutant PUM-HD labels tandem repeats in a RNA sequence. Each split fragment of EGFP is connected to N and C terminal of mutant PUM-HD. When two probe molecules come close to each other on the repeat regions, split fragments of EGFP between the probes reconstitute an EGFP. Single particle tracking of TERRA revealed heterogeneities of the dynamics and its transient localization to telomere. Single molecule imaging of endogenous lncRNA provides quantitative verification of in vitro results and moreover, leads to the hypothesis by investigating unseen phenomenon.

3P296 複数の光遺伝学ツールとの組み合わせが可能な化学発光膜電位センサーの開発

Genetically-encoded chemiluminescent voltage indicator applicable in conjunction with multiple optogenetic tools

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Genetically encoded voltage indicators (GEVIs) are powerful tools to extract information from large number of neurons and combination with optogenetic tools must provide insights into the new function of neural circuit. However, excitation light irradiating GEVIs often restrict optogenetic tools we can use due to spectral overlap. In the conference, we will introduce a chemiluminescent GEVI. Since this novel GEVI emits very bright chemiluminescence without excitation light, it enables 333 frames / sec imaging. Besides, our GEVI can visualize the voltage change triggered by ChR2 and HR. Our results demonstrate the chemiluminescent GEVI are very useful for particularly long-term imaging with multiple optogenetic tools to understand the mechanism of neural circuit.

3P297 電子顕微鏡画像処理システム Eos への Web ブラウザと PIONE の統合による、ユーザビリティの向上

Development of a user-friendly system for image processing of electron microscopy by integrating web browser and PIONE with Eos

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Eos (Extensible Object-oriented System) is one of the applications for image processing of electron microscopy. In usual cases, Eos works with only character user interfaces (CUI) under the operating systems (OS) such as OS-X or Linux, not user-friendly. Thus we extended Eos to a web system independent of OS with graphical user interfaces (GUI). The part of system implemented properly and produced image analysis, but remained a problem of workspace for analysis: We need to provide common workspace for analysis because the client is physically separated from server. To solve workspace problems, we have developed the system employing PIONE, our developing platform that works under cloud environments.

3P298 コヒーレント X 線回折イメージングにおける回復電子密度図の多変量解析を利用した分類と評価

Classification and assessment of reconstructed electron density maps in coherent X-ray diffraction imaging using multivariate statistics

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Coherent X-ray diffraction imaging (CXDI) is expected to bridge a serious gap between optical and electron microscopy in terms of spatial resolution and sample thickness. However, CXDI is still premature for structure analyses even fifteen years after its experimental demonstration. This is mainly attributed to difficulty in reconstruction of correct electron density maps from recorded diffraction patterns. Although many reconstruction algorithms have been proposed, there are still few criteria to evaluate obtained maps adequately. In this presentation, we introduce a way to classify and assess reconstructed electron density maps in multi-dimensional image space for finding global solutions applying multivariate statistics.

3P299 コヒーレント X 線回折イメージングにおけるフリーデル対称性を拘束条件とした暗視野位相回復法の開発とシングルショット回折データ解析への応用

Dark-field phase-retrieval method under the constraint of Friedel's symmetry for structure analyses in coherent X-ray diffraction imaging

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In coherent X-ray diffraction imaging, electron density maps of non-crystalline particles are reconstructed by applying the phase-retrieval method to the Fraunhofer diffraction patterns. In practice, the lack of diffraction patterns in very small-angle by beam-stopper and detector saturation results in the loss of information on the overall shapes and total electrons of particles, and makes the reconstruction difficult. To overcome this problem, we have developed the dark field phase retrieval method under the constraint of Friedel's symmetry in small-angle diffraction. Here we report the theory, a set of simulation to assess the method and the application to structure analyses for the single-shot diffraction patterns collected using X-ray free electron laser.

3P300 Trafficking of membrane protein PAR-1 carried by endocytotic vesicles in cancer cells

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Protease activated receptor 1 (PAR-1), has been known to be one of the most essential membrane protein that mediates intracellular signals promoting cell motility, which is closely related to cancer metastasis. Since the machinery of vesicles carrying this PAR-1 proteins plays a key role in signal transfer, we imaged the trafficking of PAR-1 carrying vesicles, mainly focused on the moment of endocytosis, to analyze the movement of activated PAR-1 after internalization. Our triple-view method consisting of dual-focus fluorescence and phase contrast optics, enabled us to track endocytotic vesicles in 3-dimension. Also, using confocal microscopy, we were able to diagnose the characteristic movements of PAR-1 proteins with respect to their relative position in a cell.