Alkyne has a unique Raman band that does not overlap with Raman scattering from any endogenous molecule in live cells. Therefore, alkyne-tag Raman imaging (ATRI) is a promising approach for visualizing small molecules in live cells. An examination of structure-Raman shift/intensity relationships revealed that alkynes conjugated to an aromatic ring and/or to a second alkyne (conjugated diynes) have strong Raman signals in the cellular silent region and can be excellent tags. As a proof of concept, imaging of 5-ethyl-1,2-deoxyuridine (EdU) in living HeLa cells has been demonstrated. Simultaneous imaging of two small molecules, EdU and a CoQ analogue, with distinct Raman tags was also demonstrated.

Cellular fingerprints to distinguish and identify the various cellular states with Raman spectroscopy
Tomonobu Watanabe1,2,4 (RIKEN, Quantitative Biology Center, Immunology Frontier Research Center, Osaka University, Graduate School of Frontier Bioscience, Osaka University, PRESTO, Japan Science and Technology Agency)

Self renewal and differentiation capability of ES cells are maintained as a result of complex physico-chemical interactions between various elements, such as DNA, protein and lipids, within a nano-scale volume. Microscopy is a powerful tool to observe biological events because of the applicability for observation of living specimens, however, diffraction limit of light restrict the spatial resolution of the microscope to few hundred nanometers. To overcome the problem, we have been challenging to detect the nano-scale events with light that includes the information in nano-scale. Raman scattering is one of the scattered lights, and inhere all the vibration mode of the molecule that scattered. In this meeting, we would like to show our challenge to describe potential landscape of ES cell differentiation by Raman’s “cellular fingerprints”.

The cell cycle plays a pivotal role in reproduction of all living organisms. Dysregulation of the cell cycle components may lead to tumor formation. By analyzing the diffusion properties of type-A GABA receptors (GABA_A) on the cell surface using single molecule imaging technique with quantum dots, we found that Ca^{2+} influx evoked by neuronal excitation increased GABA_A diffusion dynamics and contributed to rapid and plastic reduction in GABAergic synaptic transmission. Conversely, another intracellular Ca^{2+} signaling pathway, i.e. Ca^{2+} release from the intracellular Ca^{2+} stores reduced the surface GABA_A mobility and had an effect to stabilize the GABAergic synapses. These results indicate that Ca^{2+} from different sources could have the opposite effect on the regulation of GABAergic synapses.

The function of CaMKII for synaptic plasticity, we developed genetically encoded light-inducible CaMKII inhibitor and photo-activatable CaMKII (CaMKII) is one of the most important signaling molecules for long-term potentiation and associated spine enlargement underlying learning and memory. Here, to understand the function of CaMKII for synaptic plasticity, we developed genetically encoded light-inducible CaMKII inhibitor and photo-activatable CaMKII by using LOV2 derived from phototropin. We applied these newly developed optogenetic tools for the study of structural plasticity of single dendritic spines by using 2-photon fluorescence microscope and 2-photon glutamate uncaging, and found that 1) ~60 s of CaMKII activation is sufficient for inducing transient and sustained spine enlargement, 2) CaMKII activation alone is sufficient for triggering structural plasticity.

In Vivo Raman Spectral Imaging of Cell Cycle Dynamics:
Adding a New Dimension to Cell Cycle Research
Chuan-Keng Huang, Jen-Fang Hsu, Shinsuke Shigeto (Dept. Appl. Chem., National Chiao Tung Univ.)

The cell cycle plays a pivotal role in reproduction of all living organisms. Dysregulation of the cell cycle components may lead to tumor formation. Detailed molecular-level study of the cell cycle dynamics will not only deepen our understanding of life, but it will also open up new possibilities of diagnosis/prognosis of cancer cells. In this work, we use a hybrid of time-lapse Raman imaging and multivariate curve resolution to disentangle complicated spatiotemporal behaviors of the major intracellular components during the cell cycle of a single fission yeast cell. We have detected a protein component associated with tyrosine phosphorylation, which cannot be seen with the univariate approach. Further we extend our work to the cell cycle of colon cancer cells.

Spectral analysis is essential to bio-Raman research to disentangle Raman data since they are complicated and fluctuated multivariable. This talk, we sought to predict cell fates, including cellular differentiation of multicellular system. Two types of cellular systems were used, that is, filamentous cyanobacteria and human breast cancer cell line. For cyanobacteria, Raman technique yielded marker vibrations characteristic to differentiation. The multivariable analysis suggested that the diverse states of undifferentiated cells were converged into a specific state through differentiation.

Inhibitory GABA receptor diffusion dynamics: a single molecule study

By analyzing the diffusion properties of type-A GABA receptors (GABA_A) on the cell surface using single molecule imaging technique with quantum dots, we found that Ca^{2+} influx evoked by neuronal excitation increased GABA_A diffusion dynamics and contributed to rapid and plastic reduction in GABAergic synaptic transmission. Conversely, another intracellular Ca^{2+} signaling pathway, i.e. Ca^{2+} release from the intracellular Ca^{2+} stores reduced the surface GABA_A mobility and had an effect to stabilize the GABAergic synapses. These results indicate that Ca^{2+} from different sources could have the opposite effect on the regulation of GABAergic synapses.

Spectral Analysis for Bio-Raman Research
Shin-ichi Morita (Cellular Informatics Laboratory, RIKEN)

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