Alkyne-Tag Raman Imaging for Visualization of Small Molecules in Live Cells

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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-ethynyl-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.

Adding a New Dimension to Cell Cycle Research

Chuan-Keng Huang, Jen-Fang Hsu, Shinshuke 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 for 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 for Bio-Raman Research

Shin-ichi Morita (Cellular Informatics Laboratory, RIKEN)

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 fate, 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.

Spectral Analysis for Bio-Raman Research

Katsuhiko Mikoshiba

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

Spectral Analysis for Bio-Raman Research

Hideji Murakoshi

Ca\textsuperscript{2+}/Calmodulin-dependent kinase II (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.