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

Mikiko Sodeoka\(^1,2\) (RIKEN, ERATO, JST)
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-deoxyribose (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.

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 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.

In Vivo Raman Spectral Imaging of Cell Cycle Dynamics: Adding a New Dimension to Cell Cycle Research

Chuan-Keng Huang, Jen-Fang Hu, 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 new possibilities 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.

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 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.

Imaging and controlling the activity of signaling molecules in dendritic spines of hippocampal neurons

Hideji Murakoshi\(^1,2\) (National Institute for Physiological Sciences, PRESTO, JST)
Ca\(^2+\)/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 inducing transient and sustained spine enlargement.