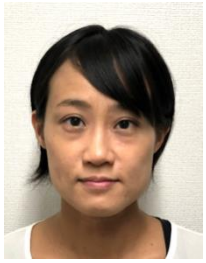
【Grant-in-Aid for Transformative Research Areas（B）】

Section II



Title of Project： Innovative multiplex imaging with functional Raman probes

KAMIYA Mako
(The University of Tokyo, Graduate School of Medicine, Associate Professor)

Number of Research Area：20B201 Researcher Number：90596462

【Purpose of the Research Project】

Fluorescence imaging has been used as an indispensable life science research tool that allows observing dynamics and functions of various biomolecules in live cells and organisms. However, fluorescence imaging suffers from a 'color barrier' (i.e. the number of simultaneously resolvable targets is limited to 4-5) owing to the intrinsically broad fluorescence spectra of fluorescent molecules. On the other hand, in recent years, Raman imaging with specific Raman tags such as alkyne, nitrile, and polyyne have been attracting attention as a method for highly multiplexed detection, because the Raman spectral linewidth can be 50-200 times smaller than the fluorescence peak width and can be tunable by structural modification such as isotope editing. However, multiplex imaging with these reported Raman tags generally requires more than 10 minutes for acquiring one image, which makes it difficult to track the biological phenomena in real time. Further, most of the pre-existing Raman tags show constant Raman signal intensity ("always-on" Raman probes), and thus their application has been limited to the labeling of molecules of interest in cells. Although there are a few reports of Raman probes that show a shift in Raman frequency or show Raman signal activation by chemically producing alkynes by externally added reagents, it has generally been believed that the Raman signal cannot be switched on and off, since it arises from molecular vibration.

In this research project, we aim at establishing a first-in-class general design strategy of "activatable-type" Raman probes that can react with endogenous biological targets and show an increased Raman signal under physiological conditions. We will also optimize and sophisticate our stimulated Raman scattering (SRS) microscopy to achieve real-time observation of biological phenomena, then validate the utility of our method for simultaneous detection of plural biomolecules in live cells and tissues. For this purpose, three groups with different expertise are working closely together to develop new functional Raman imaging probes (Mako Kamiya Group: Chemical Biology), to optimize and upgrade the high-speed, multi-color Raman spectroscopic microscopy (Yasuyuki Ozeki Group: Optics), and to apply the developed technology to biological samples (Fumiaki Obata Group: Molecular Biology).

【Content of the Research Project】

In this research project, we aim to establish a molecular design strategy to precisely control Raman signal (to switch Raman signal on and off), and to establish a method for multiplexed detection of biomolecules in live cells and tissues with superior functionality compared to conventional methods. Specifically, we will focus the following three projects.

(Project 1) Multiplex imaging of enzyme activities

We will develop activatable Raman probes whose Raman signal can be activated upon reaction with target enzymes, and establish a method for multiplex detection of multiple enzyme activities in living cells and tissues.

(Project 2) Multiplex detection of target structures

We will develop Raman probes whose Raman signal can be activated upon labeling to target proteins and structures, and develop a method for multiplex detection of target structures in live cells and tissues without washing procedure.

(Project 3) Multiplexed super-resolution imaging

We will develop photoswitchable Raman probes and Raman microscopy that induces photoswitching of the developed Raman probes to confine Raman signals to a small area to establish multiplexed super-resolution Raman imaging method.

【Expected Research Achievements and Scientific Significance】

The multiplex imaging methods using functional Raman probes to be established in this research project will dramatically expand the applicability of Raman imaging as a bioanalysis tool. Therefore, we can expect that our research project will lead to the creation of new interdisciplinary research fields, and the technologies to be developed will affect a wide range of research fields.

【Key Words】

Raman tags/probes: A general term for molecules with unique molecular vibrations that can be detected by Raman scattering. Raman tags/probes with different molecular vibration frequencies allows us to perform multiplex detection of multiple molecules. Since the molecular vibration spectrum is narrower than the fluorescence spectrum, Raman probes are advantageous for multiplexed detection.

【Term of Project】 FY2020-2022

【Budget Allocation】 121,500 Thousand Yen

【Homepage Address and Other Contact Information】

https://sites.google.com/view/i-raman/