

## **Multifunctional imaging by genetically-encoded homo-FRET-based indicator**

**Takeharu Nagai, Sang-Yeob Kim, Tomomi Tani**  
**Research Institute for Electronic Science**  
**Hokkaido University**  
**Kita-20, Nishi-10, Kita-ku, Sapporo 001-0020, Japan**  
**E-mail: [tnagai@es.hokudai.ac.jp](mailto:tnagai@es.hokudai.ac.jp)**

**KEY WORDS:** Homo FRET, fluorescent protein, living cells, microscopy

After identifying the primary sequence of every protein, it is important to elucidate biologically relevant protein-protein interactions. Bioimaging has been used to directly visualize such biological functions for elucidating signal transduction systems. Fluorescence resonance energy transfer (FRET) has been widely used to make functional indicators for intracellular signaling. In spite of the usefulness, conventional FRET imaging, which utilizes two fluorophores with different emission colors, is not suitable for multi functional imaging because broad range of wavelength for the FRET detection perturbs the use of other wavelength probe. This property makes it difficult to simultaneously visualize different biological phenomena in a same cell. To overcome this problem, we developed a homo-FRET-based indicator composed of two spectrally-identical yellow fluorescent proteins as the donor and acceptor. In the indicator, when FRET occurs, the fluorescence anisotropy is altered. To detect the changing in anisotropy, we also invented a fluorescence polarization microscopy which equips an optical device that can compensate fluorescence depolarization by the objective lens with high numerical aperture. By using this system, we successfully visualized multiple physiological phenomena; ie,  $\text{Ca}^{2+}$  changing, and translocation of PKC- $\gamma$  and MARKS upon ligand stimulation in single live cell.