2-6-12. Fluorescent Dyes for Super-Resolution Imaging

Overview

Generally, the spatial resolution of the conventional optical microscopes is limited to almost a half of wavelength of light due to the diffraction limit of light. Super-resolution fluorescence microscopies have made a breakthrough to overcome this limitation and have enabled highly resolved imaging of micro-/nano-structures in biological systems, which cannot be visualized by the conventional optical methods (Nobel Prize in Chemistry, 2014). This field has been advanced by the development of both cutting-edge methods for super-resolved imaging and outstanding fluorescent dyes.

Current Status and Frontiers

Typical examples of super-resolution fluorescence microscopy are STED (stimulated emission depression) microscopy, RESOLFT (reversible saturable (switchable) optical linear fluorescence transition) microscopy, PALM (photoactivated localization microscopy), and STORM (stochastic optical reconstruction microscopy).

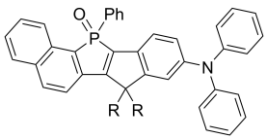

Figure 1. A highly photostable fluorescent dye for STED microscopy.

STED microscopy uses a high-intensity doughnut-shaped beam (STED beam) to eliminate fluorescence around the periphery through stimulated emission, thereby limiting the fluorescent area to the center and increasing the spatial resolution. One problem in STED microscopy is that fluorescent probe molecules are easily decomposed due to the illumination of high-intensity STED beam. Highly photostable fluorescent dyes that contain phosphole *P*-oxide and phospha-rhodamine skeletons have been designed and synthesized [Yamaguchi *et al.*].

In RESOLFT microscopy and PALM/STORM, photoswitchable fluorescent molecules, which interconvert between non-fluorescent (off) and fluorescent (on) states, are used as fluorescent probes. RESOLFT microscopy increases the spatial resolution by illuminating doughnut-shaped beam, similar to STED microscopy. PALM and STORM are based on single-molecule fluorescence detection and construct highly resolved images by the localization analysis of fluorescent spots that show on/off switching and the subsequent integration of the analyzed images. Photoswitchable fluorescent proteins, such as Kaede and Dronpa, have been developed [Miyawaki *et al.*] and have successfully

been applied to super-resolution imaging.

Photoswitchable fluorescent molecules based on photochromic diarylethenes have also been synthesized [Irie, Morimoto]. Oxidized derivatives of bis(benzothienyl)ethene produce highly fluorescent closed-ring isomers by photoisomerization, thus undergoing turn-on mode photoswitching of fluorescence. The photoswitchable fluorescent diarylethenes have been applied to super-resolution bioimaging. One of the characteristics of the fluorescent diarylethenes is that photoswitching properties (photoisomerization quantum yield) can be controlled by appropriate chemical modifications. This enables the rational design of molecular functions that meet the requirement in microscopy principles. Diarylethene molecules that show solvatochromism of fluorescence or a metal-ion sensing function can also be synthesized by appropriate molecular design.

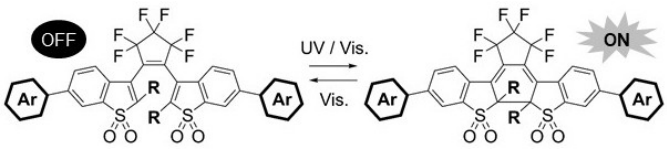

Figure 2. Photoswitchable fluorescent diarylethene.

It has been found that stochastic single-molecule fluorescence switching of a diarylethene derivative is observed under continuous illumination of single-wavelength visible laser, whereby diarylethene molecules are photoexcited at its hot band (Urbach tail) [Miyasaka, Ito]. This phenomenon was applied to super-resolution imaging and single-molecule tracking, successfully visualizing nano-patterns and nano-scopic inhomogeneity of polymer materials.

References

1. C. Wang *et al.*, *Proc. Natl. Acad. Sci. USA*, **116**, 15817–15822 (2019).
2. R. Ando, H. Mizuno, A. Miyawaki, *Science*, **306**, 1370–1373 (2004).
3. M. Irie, M. Morimoto, *Bull. Chem. Soc. Jpn.*, **91**, 237−250 (2018).
4. S. Takaku, R. Nishimura, M. Morimoto, *Dyes Pigm.*, **216**, 111354 (2023).
5. S. Ito *et al.*, *Polym. Chem.*, **13**, 736−740 (2022).

Future Perspectives and Directions

Issues that need to be resolved or achieved within the next 10 years: On-demand design of fluorescent dyes according to microscope principles and observation targets. Generalization as tools for biological and medical research, drug discovery, and pathological diagnosis.

(Author: Masakazu Morimoto)