Technology Offer

High Spatial Resolution Imaging of a Structure of Interest in a Specimen

Ref.-No.: 0707-3657/ 3955/ 3966-BC

Abstract

This technical offer presents two advanced fluorescence microscopy technologies designed to surpass traditional optical resolution limits: Ground State Depletion Microscopy (GSDIM) and high spatial resolution imaging using non-switchable fluorescent dyes. GSDIM leverages ordinary fluorophores, switching them to metastable dark states to achieve high-resolution imaging without the need for photoactivation. The second technology enhances spatial resolution by utilizing the natural transitions of non-switchable dyes between electronic states, thereby enabling precise molecular localization. These innovations significantly broaden the applicability and improve the imaging capabilities of fluorescence microscopy in biological research.

Background

Traditional optical microscopy is constrained by the diffraction limit, which restricts resolution to about half the wavelength of light used. Advancements have introduced techniques like STORM and PALM, which utilize switchable fluorescent proteins and dyes to achieve super-resolution imaging. However, these methods are limited by the availability and performance of photoactivatable compounds. GSDIM and the novel high-resolution imaging technique using non-switchable dyes address these limitations by employing alternative mechanisms for fluorescence modulation, thereby enhancing resolution without the need for specialized dyes.

Technology

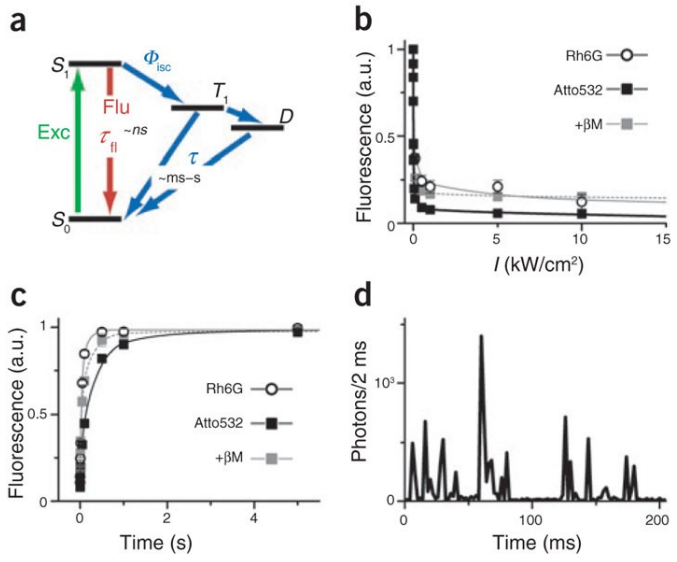

Figure 1: (**a**) Repetitive excitation (Exc) of the singlet ground state S_0 to the first excited singlet state S_1 elicits fluorescence (Flu) emission and switches a molecule to the triplet state T_1 or other dark states D with long lifetime (τ). (**b**) GSD of Rh6G, Atto532 and Atto532 plus a triplet quencher (β-mercaptoethanol; βM) for increasing intensity, I. (**c**) Recovery of the active fluorophores after maximal dark state shelving (**d**) Fluorescence time trace of a single Atto532 molecule in PVA given as the number of detected photons per 2-ms time bin (I = 115 kW/cm^2).

Max-Planck-Innovation GmbH
Arnulfstr. 58
80335 München
www.max-planck-innovation.de

GSDIM:
This patented technology involves switching fluorophores to a metastable dark state and recording those that return to the ground state. Continuous widefield illumination and a high-speed camera capture high-resolution images. The technique works with various fluorophores in different environments, providing flexibility and enhancing imaging capabilities. The method significantly improves resolution, achieving details less than 30 nm.

High Spatial Resolution Imaging with Non-Switchable Dyes:
This method enhances spatial resolution by exploiting the natural transitions of non-switchable dyes between their electronic ground and dark states. By carefully managing the illumination intensity, a significant fraction of the fluorophores is switched to the dark state, allowing the remaining fluorophores to be imaged with high precision. This technique avoids the need for specialized switchable dyes, making it broadly applicable and easier to implement.

Advantages

GSDIM:

- Utilizes ordinary fluorophores, eliminating the need for photoactivatable compounds.
- Achieves super-resolution with simple continuous illumination and camera setup.
- Compatible with various dyes, enhancing versatility.
- Enables dual-color imaging with standard fluorophores.
- Expands the conceptual range and applicability of far-field optical nanoscopy.

This innovative imaging technology unmatched flexibility by working effectively with general fluorophores, unlike many super-resolution techniques that require specialized fluorescent dyes or proteins. This flexibility greatly facilitates broader application across various research fields where specific fluorophores may not be readily available or are prohibitively expensive. This approach not only reduces operational costs but also simplifies experimental setups, making advanced imaging more accessible and versatile.

High Spatial Resolution Imaging with Non-Switchable Dyes:

- Avoids the limitations associated with switchable fluorescent dyes.
- Enhances spatial resolution beyond the diffraction limit.
- Utilizes common, non-specialized dyes.
- Reduces complexity and cost of implementation.

This method optimizes the control of fluorophore excitation states through meticulously managed illumination. This precision allows for a reduction in the number of light sources and system complexity required, offering significant advantages in applications such as medical diagnostics and field applications where simplicity and reliability are crucial. By enhancing the efficiency of the imaging process, our technology streamlines workflows and increases throughput, providing a practical solution that delivers high-resolution imaging without the need for complex infrastructure.

Potential applications

GSDIM:

- Live-cell imaging for studying dynamic biological processes.
- High-resolution mapping of cellular structures.
- Dual-color imaging for differentiating multiple cellular components.
- Research in cell biology, neuroscience, and developmental biology.
- Super-resolution imaging in clinical diagnostics.

Max-Planck-Innovation GmbH
Arnulfstr. 58
80335 München
www.max-planck-innovation.de

High Spatial Resolution Imaging with Non-Switchable Dyes:

- High-precision imaging of biological specimens.
- Enhanced imaging of fixed cells and tissue samples.
- Advanced research in molecular biology and biochemistry.
- Applications in materials science for imaging nanostructures.
- Development of new diagnostic imaging techniques.

Patent Information

GSDIM: priority date: 27.04.2007: EP2605003B1, JP523869B2, CN10148479B, US8084754
High Spatial Resolution Imaging with Non-Switchable Dyes: priority date: 20.05.2029: US8174692, CN102037347B, EP2291641B2, JP587257B2

Publications

Fölling, J., Bossi, M., Bock, H. *et al.* Fluorescence nanoscopy by ground-state depletion and single-molecule return. *Nat Methods* **5**, 943–945 (2008). https://doi.org/10.1038/nmeth.1257

Contact

Dr. Bernd Ctortecka Senior Patent- & License Manager, Physicist
Phone: +49 (0)89 / 29 09 19 – 20
eMail: ctortecka@max-planck-innovation.de

Max-Planck-Innovation GmbH
Arnulfstr. 58
80335 München
www.max-planck-innovation.de