Max-Planck-Innovation

Technology Offer:

MINSTED fluorescence localization and nanoscopy

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The MINSTED method is an innovative technique for determining the position of a singularized fluorophore molecule within an object. Traditional methods in molecular imaging faced challenges in achieving high resolution due to limitations in fluorescence microscopy. Existing solutions like STED (Stimulated Emission Depletion) microscopy improved the spatial resolution but rely on high illumination intensity and/or sample exposure, which can lead to thermal issues and photochemical reactions. MINSTED addresses these limitations by using a novel approach involving fluorescence excitation light and fluorescence influencing light, potentially transforming the resolution capabilities in fluorescence microscopy.

Advantages

1. Enhanced resolution down to about one nanometer, significantly surpassing traditional fluorescence microscopy methods and many optical nanoscopy methods.
2. Reduced photobleaching and photochemical stress on samples due to lowered light exposure, enhancing sample integrity.
3. Ability to singularize and determine the position of fluorophore molecules one by one, facilitating detailed molecular imaging.
4. Compatibility with various fluorescence influencing lights (like STED light), offering versatile application in different microscopy setups.
5. The capability of real-time updates in the estimated position of the fluorophore molecule, allowing dynamic and accurate imaging.
6. Lower thermal impact compared to traditional STED methods, mitigating potential thermal damage to the sample.

Applications

1. Advanced biological and medical research, particularly in studying cellular structures at the molecular level.
2. Pharmaceutical research, especially in drug development and molecular interaction studies.
3. Educational use in microbiology and molecular biology courses, providing students with access to high-resolution imaging technologies.
4. Potential applications in nanotechnology and material sciences, where precise molecular imaging is crucial.
5. Development of new diagnostic tools and techniques in healthcare, enhancing the accuracy of disease detection.

Technology

MINSTED uses a combination of fluorescence excitation light and fluorescence influencing light (such as STED light) to determine the exact position of singularized fluorophore molecules. MINSTED finds and tracks the position of a singularized fluorophore by continuously probing its fluorescence emission near its estimated position and by updating this estimate with each detected photon. This method involves shaping and focusing a light beam to create a light intensity distribution with a central intensity minimum. The technique continuously shifts this distribution relative to the object, allowing for precise localization of the molecule. Photons emitted by the fluorophore are registered individually, and the estimated position is continuously updated based on each registration. This innovative approach results in a significantly improved precision of the estimated fluorophore position while minimizing the required number of detected photons. Due to less photons required, challenges associated with fluorescence and STED microscopy are alleviated, such as photobleaching and thermal effects.

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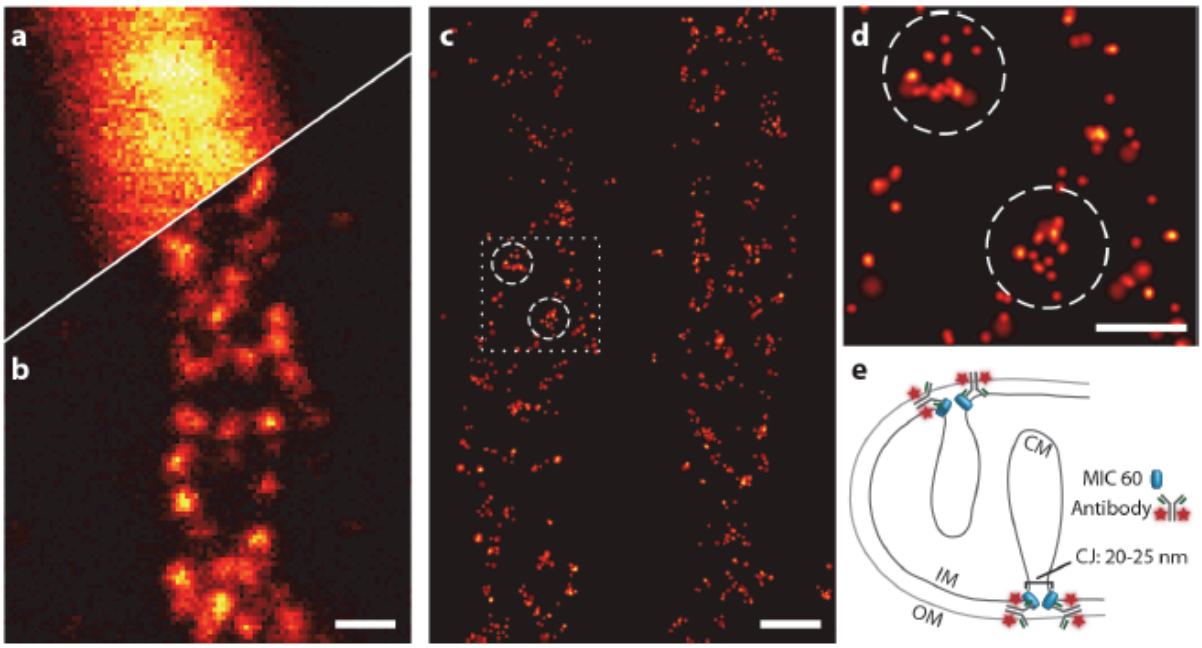

Fig. 1 MINSTED nanoscopy of mitochondrial protein Mic60 [1]. **a**, Confocal and **b**, STED image with about 60 nm resolution of the same mitochondrion taken after simultaneous activation of all fluorophores. **c**, MINSTED nanoscopy image of similar mitochondria resolving the Mic60 clusters. d, Excerpts of data as indicated in c. **e**, Cartoon of the presumed localization of Mic60 in the mitochondrial inner membrane. IM: inner membrane; OM: outer membrane; CM: crista membrane; CJ: crista junction. Scale bars: a–c, 200 nm; d, 100 nm.

Publications

1. M. Weber, M. Leutenegger, S. Stoldt, S. Jakobs, T.S. Mihaila, A.N. Butkevich, S.W. Hell. MINSTED fluorescence localization and nanoscopy. *Nat. Phot.* **15**, 361–366 (2021). DOI: 10.1038/s41566-021-00774-2
2. M. Weber, H. von der Emde, M. Leutenegger, P. Gunkel, S. Sambandan, T.A. Khan, J. Keller-Findeisen, V.C. Cordes, S.W. Hell. MINSTED nanoscopy enters the Ångström localization range. *Nat. Biotechnol.* **41**, 569–576 (2022). DOI: 10.1038/s41587-022-01519-4

Patent Information

- PCT application published WO 2023006176 (A1).
- EP patent application pending.
- US patent application pending.
- JP patent application pending.
- CN patent application pending.

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