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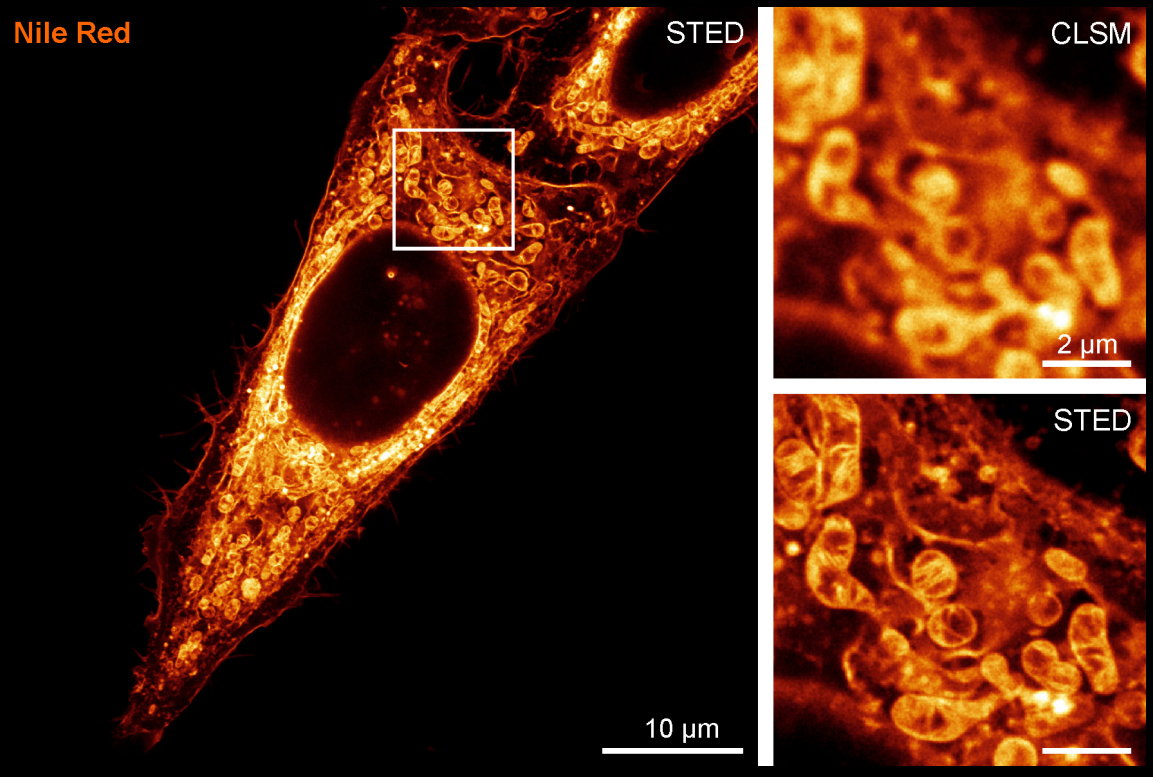
Review Article

EXTENDING NANOSCOPY POSSIBILITIES WITH STED AND EXCHANGEABLE FLUOROPHORES

A smart strategy for whole-cell, 3D, multi-color and live-cell STED imaging



Authors

Dr. Julia Roberti

Introduction

When it comes to STED Nanoscopy, keeping high signal-to-noise is key to achieve the best possible resolution in fixed and living cells. This can be challenging in the case of experiments in 3D and/or with time series, where the sample undergoes many rounds of image acquisition and photobleaching becomes an issue.

If fluorophores were completely immune to photobleaching, it should be possible to perform STED indefinitely using the same molecules over and over. In practice, one performs STED with the best available fluorophores in terms of brightness and photostability (Grimm, Muthusamy et al. 2017) [1], and at high labeling densities. However, there is a clever alternative to come closer to the ideal situation: if "fresh" fluorophores replenish the sample in each round of STED, imaging will take place with intact fluorophores every time. This concept is the basis of STED with exchangeable fluorophores, developed in Mike Heilemann's lab in partnership with Marko Lampe from the EMBL in Heidelberg. This approach enables whole cell, 3D, multi-color STED imaging as well as live-cell STED microscopy for long acquisition times and at 1 Hz imaging rates. This work has recently been published in the journal Nano Letters (Spahn, Grimm et al. 2019) [2].

STED Nanoscopy with an "infinite" pool of fluorescence labels

The idea of using fluorophores that transiently bind to their target was adapted from the single-molecule super-resolution method termed PAINT (Point Accumulation for Imaging in Nanoscale Topography) (Sharonov and Hochstrasser 2006) [3]. A key advantage of PAINT is its independence of photobleaching: fluorophores repeatedly and transiently bind to their target, while otherwise freely diffusing in the imaging buffer.

Following this concept, the authors considered exchangeable labels typically used for PAINT also for other imaging modalities, e.g. confocal microscopy and STED nanoscopy. The prerequisite for these fluorophore labels are fast exchange kinetics and high-density labeling of the target structure. Different to single-molecule PAINT imaging, this requires higher concentrations, as well as suitable binding affinities (~ µM) and off-binding kinetics (~ 10-100 s-1). The authors evaluated the performance of five labels, including two membrane stains (Nile Red, FM4-64), two fluorogenic DNA stains (JF646-Hoechst, SiR-Hoechst), and the actin labeling peptide Lifeact-AF594. They determined the ability of the fluorophores to exchange between the target structure and the free pool, the fluorescence signal over time, and the resulting STED image quality. Importantly, some of the fluorophores are cell permeable (e.g. Nile Red and SiR-Hoechst) and thus suitable for live-cell experiments. All of the dyes investigated are compatible with the same depletion wavelength (775 nm) and allow simplified two color STED imaging: Nile Red, FM4-64 and Lifeact-Alexa 594 can be easily combined with one of the DNA dyes (JF646-Hoechst or SiR-DNA).

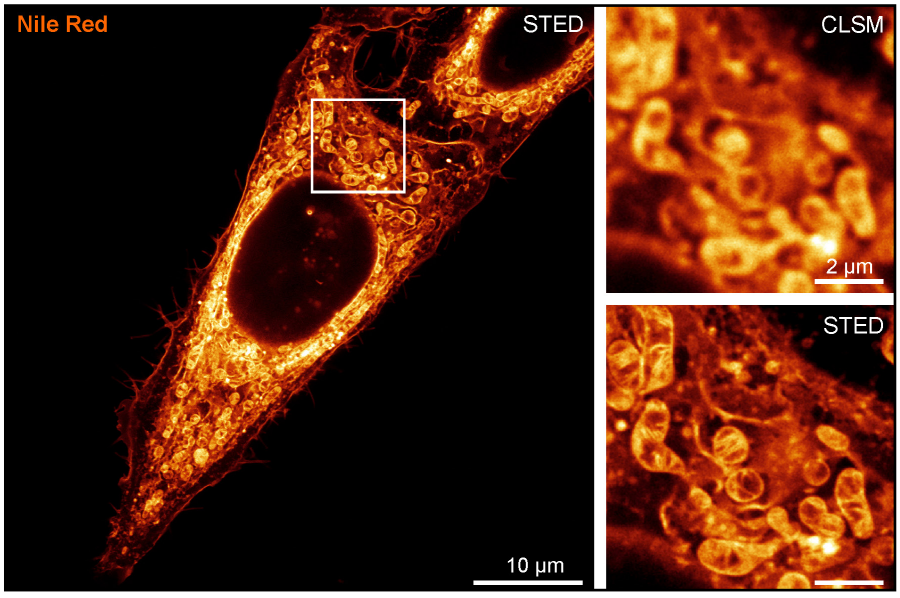


Figure 1: STED imaging of mitochondria in fixed cells stained with the exchangeable fluorophore Nile Red, using one depletion wavelength (775 nm). The zoom-in region (white square) shows the difference between confocal and STED, in which mitochondrial cristae are clearly resolved. Images courtesy: Prof. Dr. Mike Heilemann, Johann Wolfgang Goethe-University, Frankfurt, Germany, and Dr. Marko Lampe, EMBL, Heidelberg, Germany.

The results demonstrated that exchangeable fluorophores enabled labeling densities that allowed for high-quality STED imaging with low background. The constant exchange allowed using optimal imaging conditions for fixed cell imaging in 2D and 3D (Figure 1). A particular benefit is STED imaging of large volumes (such as entire cells): exchangeable fluorophore labels, other than permanent labels, do not show out-of-plane photobleaching.
The combination of Lifeact-AF594 and JF646-Hoechst in eukaryotic cells showed the advantage of STED for multicolor: the Lifeact peptide labeled actin, while JF646-Hoechst targeted DNA features at the nanoscale (Figure 2, page 4). The STED approach was also successful for multicolor, 3D STED imaging both in mammalian and bacterial cells. Hereby, the images on whole-cell, two-color 3D STED along the axial direction showed a constant fluorescence signal over time. In both cases, the dynamic fluorophore exchange circumvents photobleaching issues that can compromise signal-to-noise ratio.

In summary, the combination of STED and exchangeable fluorophores delivers high labeling density for whole-cell, multi-color and live-cell STED imaging. The exchangeable labels not only benefit 3D and live-cell measurements, but also allow optimizing STED imaging without losing signal as with permanent labels. The concept can be extended to other labels and opens new opportunities for STED in correlative imaging approaches.

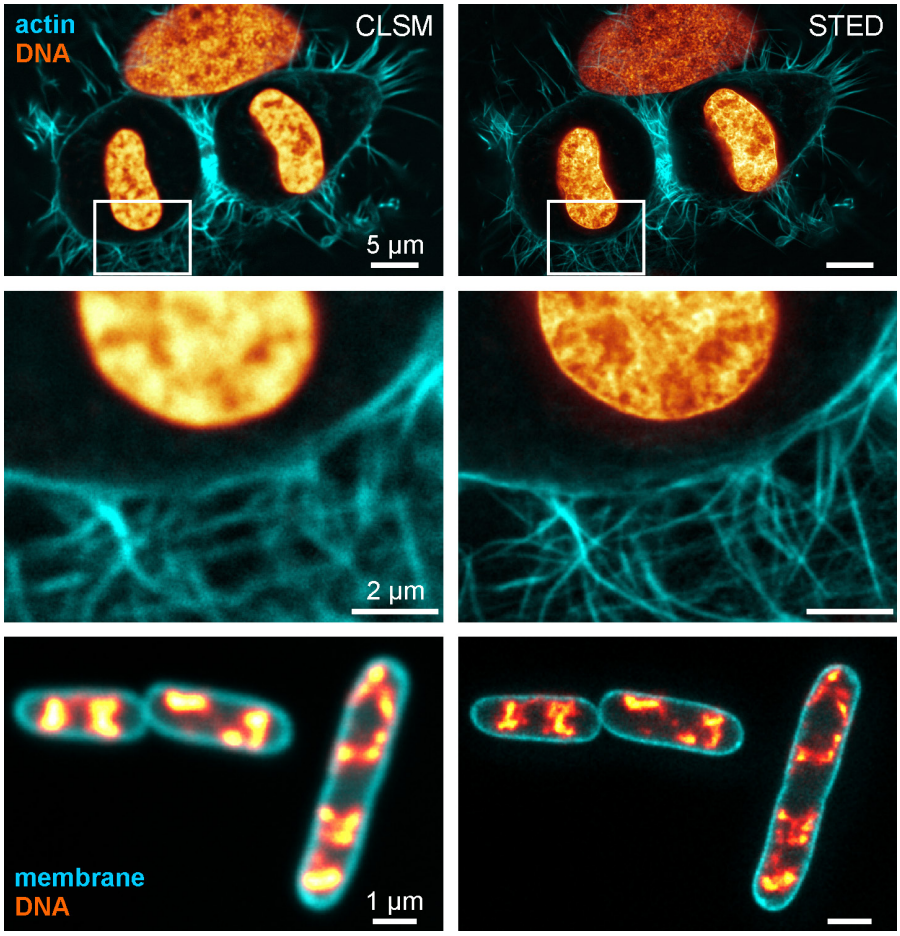


Figure 2: Two-color STED imaging of cells labeled with two exchangeable fluorophores and using one depletion wavelength (775 nm) (top and middle: HeLa cell labeled with Lifeact-AF594 and JF646-Hoechst; bottom: E.coli labeled with Nile Red and JF646-Hoechst). Images courtesy: Prof. Dr. Mike Heilemann, Johann Wolfgang Goethe-University, Frankfurt, Germany, and Dr. Marko Lampe, EMBL, Heidelberg, Germany.

References

[1] Grimm, J. B., A. K. Muthusamy, Y. Liang, T. A. Brown, W. C. Lemon, R. Patel, R. Lu, J. J. Macklin, P. J. Keller, N. Ji and L. D. Lavis (2017). "A general method to fine-tune fluorophores for live-cell and in vivo imaging." Nat Methods 14(10): 987-994.

[2] Spahn, C., J. B. Grimm, L. D. Lavis, M. Lampe and M. Heilemann (2019). "Whole-cell, 3D and multi-color STED imaging with exchangeable fluorophores." Nano Lett. 19 (1), pp 500–505.

[3] Sharonov, A. and R. M. Hochstrasser (2006). "Wide-field subdiffraction imaging by accumulated binding of diffusing probes." Proc Natl Acad Sci U S A 103(50): 18911-18916.

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Leica Microsystems CMS GmbH | Am Friedensplatz 3 | 68165 Mannheim, Germany
Tel. +49 621 70280 | F +49 621 70281028

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