Whole-cell, 3D and multi-color STED imaging with exchangeable fluorophores

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Introduction

Photobleaching is a major challenge in fluorescence microscopy, in particular if high excitation light intensities are used. Signal-to-noise and spatial resolution may be compromised, which limits the amount of information that can be extracted from an image. Photobleaching can be bypassed with exchangeable labels, which transiently bind to and off a target and thereby replenish destroyed labels by intact ones from a reservoir. Here, we demonstrate **STED microscopy with the exchangeable dyes** Nile Red and JF_{646}-Hoechst / SiR-Hoechst (Lukinavičius et al., Nat. Commun, 2015), as well as the short peptide lifeact-AF594 (Kiuchi et al., Nat. Methods, 2015). We demonstrate STED imaging of large volumes, in multi-colour and in live cels. We next **generalized the concept of exchangeable labels for STED microscopy** by implementing short, fluorophore-labeled oligonucleotides that transiently bind to complementary oligonucleotides attached to protein-specific antibodies. The constant exchange of fluorophore labels in bypasses photobleaching that occurs with covalent labels. We show that this concept is suitable for targeted, two-color STED imaging of whole cells.

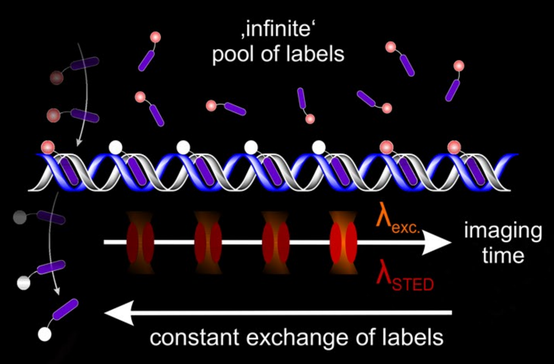
Summary

Exchangeable fluorophores render STED (and confocal) microscopy insensitive to photo-bleaching. This facilitates imaging of large volumes in fixed cells at optimal resolution, multi-color and live-cell STED imaging.

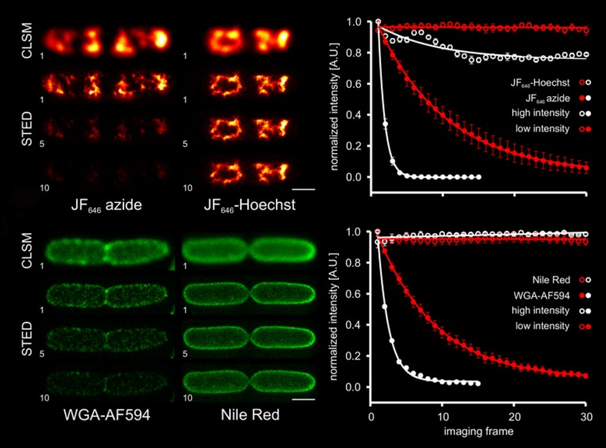
Outlook

The concept of exchangeable dyes enabling bleaching-independent imaging of mammalian cells and bacteria can be applied to almost any fluorescent or fluorogenic probe reversibly binding the target structure at suitable binding and dissociation rates. We expect that a targeted development of novel **fluorophore labels with appropriate binding affinity and kinetics** will be beneficial for super-resolution microscopy.

Concept

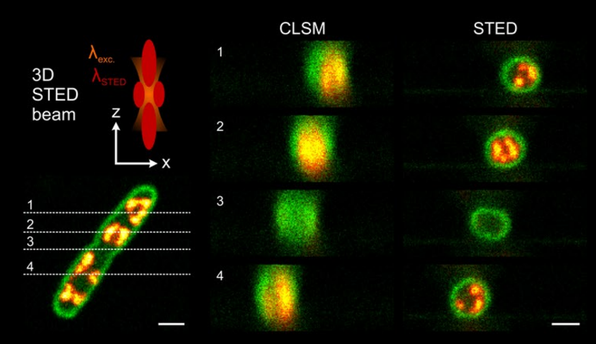


Bleaching-independent



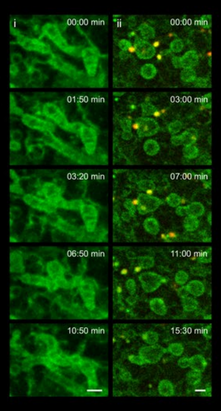
E. coli labelled with covalently bound (JF_{646}-azide, WGA-AF594) and exchangeable dyes; Scale bars: 1 µm

3D-STED of *E. coli*



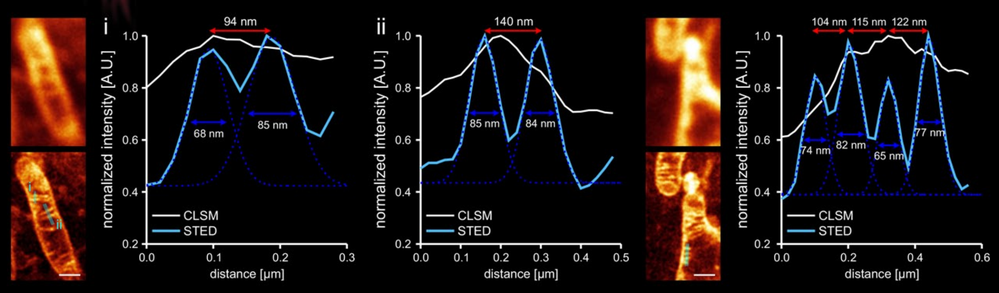
E. coli cell labelled with the exchangeable dyes Nile Red (membrane, green) and JF_{646}-Hoechst (DNA, red); Scale bars: 1 µm

Live-Cell 2D-STED

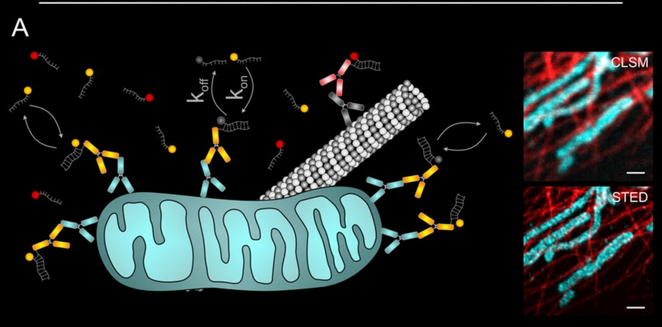


Live cell imaging with the exchangeable dye Nile Red (left) and in combination with SiR-Lysosome (in order to highlight the organelle with this non-exchangeable dye). Scale Bar: 2 µm

2D-STED imaging of mitochondrial cristae

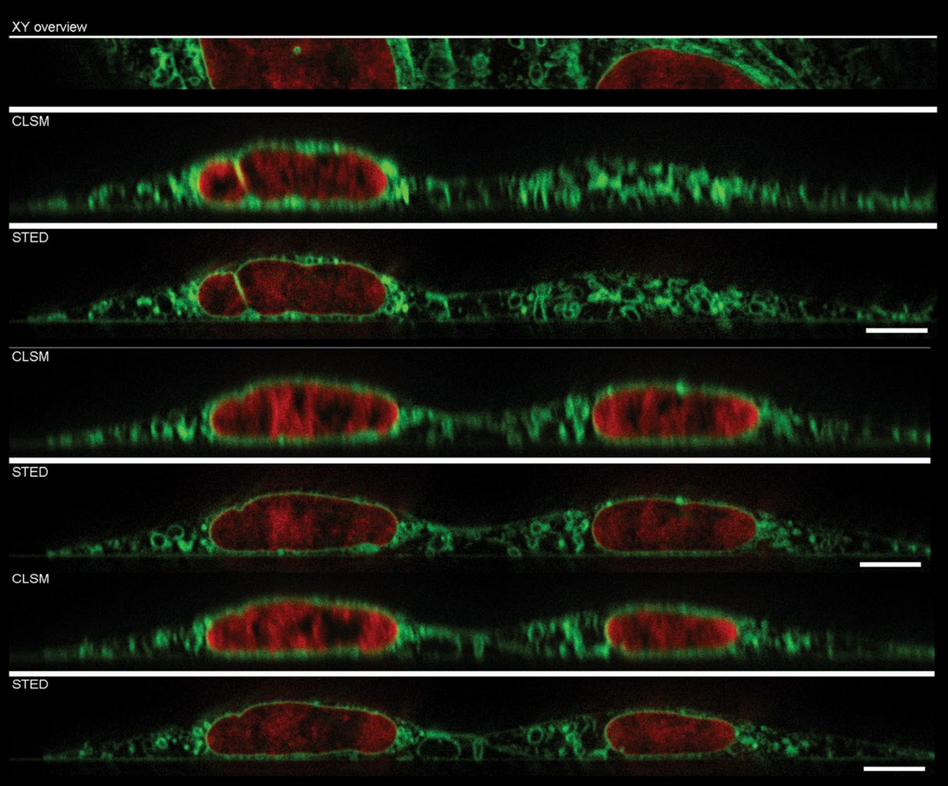


3D STED with DNA-labeled antibodies



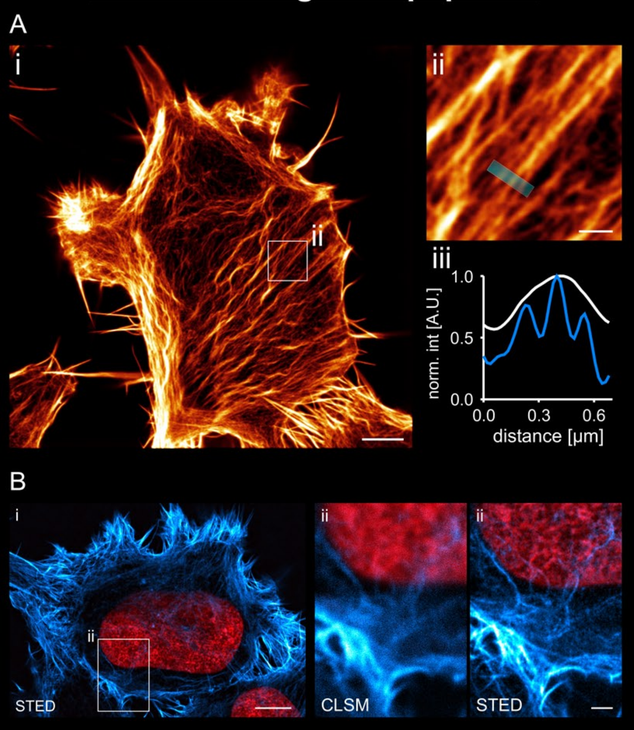
Bleaching-independent STED microscopy with DNA-labeled antibodies targeted by short, fluorophore-labeled oligonucleotides. Scale bars: 2 µm (B), 1 µm (A, Ci), 0.5 µm (Ciii, iv, D).

Volumetric imaging of cells with 3D-STED



Representative xz-scans of a large 3D-STED volume of fixed HeLa-cells labelled with the dyes Nile Red (membranes, green) and JF_{646}-Hoechst (DNA, red); Scale bars: 5 µm

STED imaging using small exchangeable peptides



2D-STED the actin cytoskeleton using lifeact-AF594. Single-color [A] and dual-color STED imaging with JF_{646}-Hoechst (DNA, red). Line profiles in A, iii represent CLSM (white) and STED (cyan) profiles. Scale bars: 5 µm (Bi), 3 µm (Ai) and 1 µm (Aii, Bii)).

Publications

Spahn et al., Nano Letters 2019
Spahn et al., *in revision*