

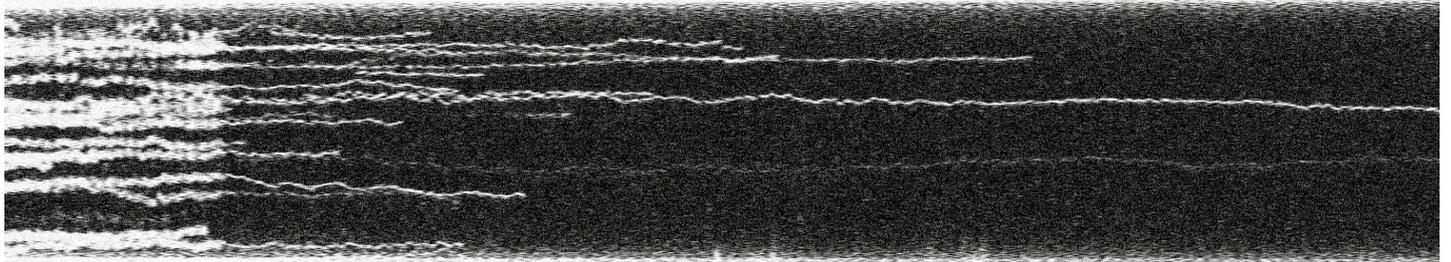
A 3D molecular model of a protein-DNA complex. The DNA is shown as a purple double helix structure. The protein is a large, multi-domain structure with a complex, porous appearance, colored in shades of pink, orange, and yellow. The background is a solid blue color.

Optical tweezers combined with STED reveals protein dynamics on densely covered DNA

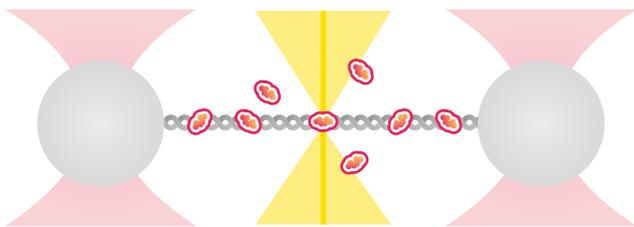
STED Super Resolution
Application Note - C-Trap[®] Dymo

2017

STED SUPER-RESOLUTION



1 Kymograph of the dynamics of human TFAM-Atto 647N at ~5 nM on λ -DNA at a constant force of 4 pN using confocal microscopy (left) and STED microscopy (right).



2 Schematic view of optical tweezers (red cones) combined with STED fluorescence microscopy (yellow beam). Note the single excited protein.

STED Microscopy

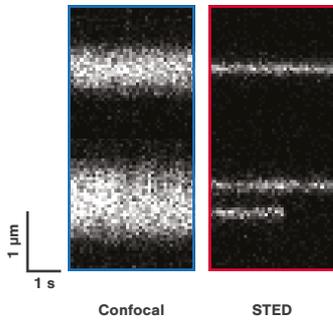
Today's quantitative analysis of DNA-protein interactions at the single-molecule level requires the capability of measuring under biologically relevant conditions with high temporal resolution. This problem can especially be observed when *in vitro* experiments are linked with the *in vivo* situation in which proteins interact highly dynamic with DNA at high protein concentrations. These experiments can be conducted when fast DNA scanning is combined with stimulated emission depletion (STED) microscopy. In this application note we will discuss how LUMICKS' C-Trap[®] Dymo enables the visualization of individual DNA-binding proteins on densely covered DNA.

The real-time observation of the fast dynamics of fluorescently labeled mitochondrial transcription factor A (TFAM) on λ -DNA at a high concentration (~5 nM) can be seen in **Figure 1**. To study these dynamics the C-Trap Dymo SR uses optical tweezers to keep the DNA in place and stretched while 1D STED line-scanning tracks the proteins at high resolution (≥ 50 nm) and high frequency (≤ 200 Hz).

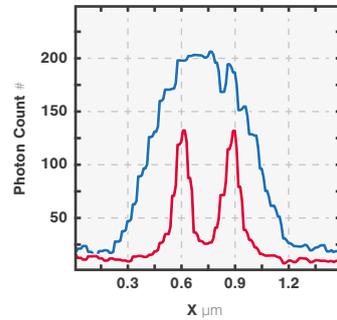
The use of STED enables the tracking of individual protein trajectories, including (un)binding and oligomerization events (**Figure 1**, right), which cannot always be observed with the diffraction limited resolution of confocal microscopy (**Figure 1**, left).

Using confocal fluorescence microscopy only proteins in the diffraction limited confocal spot are excited, making it possible to use fluorescence at high protein concentration, which is not possible when using e.g. wide-field fluorescence microscopy. However, proteins located closely together are all being illuminated while using confocal microscopy. This is resolved by using STED, where the confocal spot is combined with a de-excitation spot, causing only proteins in a sub-diffraction limited band to be illuminated. In **Figure 2** a schematic view of the measurement assay can be seen, showing two optically trapped microspheres tethered by densely covered DNA and illumination by STED fluorescence

microscopy. In **Figure 3** the confocal fluorescence microscopy schematic is shown.



4 A comparison of confocal (left) and STED (right) images of Atto 647N-labeled restriction enzymes bound to optically stretched DNA.



5 Intensity profiles of two proteins located close to each other by using confocal (blue) and STED microscopy (red).

C-Trap SR

The difference between confocal and STED becomes more apparent in **Figure 4**. Here can be seen how two individual proteins, closely located to each other, can clearly be distinguished using STED, whereas they could not be resolved using confocal microscopy. The intensity profiles of two adjacently located proteins clearly show the resolution enhancement and increase in localization accuracy with the use of STED.

LUMICKS' C-Trap Dymo SR allows the user not only to visualize proteins with super-resolution in real-time, but also to simultaneously manipulate and measure molecular interactions with sub-pN force resolution and sub-nm trap-position resolution (the latter two resolution specs are achieved by both the C-Trap Dymo SR and the confocal C-Trap[®] Dymo). An advanced microfluidics system and intuitive software are integrated, which allows experiments to be conducted within minutes.

info@lumicks.com
www.lumicks.com

Or find us on:



LUMICKS HQ

Paalbergweg 3
1105 AG Amsterdam, The Netherlands
+31 (0)20 220 0817



LUMICKS Americas

800 South Street, Suite 100
Waltham, MA 02453, USA
+1 781 366 0380



LUMICKS Asia

Room 577, Block A, Langentbldg Center
No.20 East Middle 3rd Ring Road
Chaoyang District, Beijing, 100022 China
+86 (0) 10 5878 3028

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