STED imaging of mouse erythroblasts reveals ring-shaped structure of centrioles

Application Note

Staff members to contact: Fadwa Joud, Stefanie Reichelt
Sample by: Julia Haefner, Fanny Gergely Group, CRUK Cambridge Institute

1 STED MICROSCOPY

STimulated Emission Depletion (STED) microscopy is a super-resolution fluorescence optical microscopy technique that allows to circumvent the optical diffraction limit and makes it possible to image subcellular structures and organelles with previously unattainable detail. STED works by reducing the effective fluorescent spot using two superimposed lasers. The first beam excites fluorophores to a higher energy level and is directly followed by a donut-like red-shifted STED beam which will deplete the fluorescent molecules at the periphery of the focal spot inducing ground state relaxation. Emission from this process is resonant with the STED source and can therefore easily get filtered out. This prevents molecules outside the spot from contributing to the build-up of the image. The result is a small super-resolution spot. Gated STED (g-STED) allows to push the achievable resolution even further (more than 50% increase) by detecting the signal only in a certain time gate after the excitation pulse.

2 SAMPLE

Mouse erythroblasts were grown on glass coverslips and fixed with ice-cold methanol. Centrosome markers γ-Tubulin and Cdk5rap2 were labeled with Alexa 488 and Alexa 568, respectively. Both primary and secondary antibodies were used at twice the usual concentration. Prolong Diamond was used as mounting media. STED imaging reveals ring-shaped pattern for both γ-Tubulin and Cdk5rap2, while only γ-Tubulin additionally localises to the centriole lumen (central dot staining).

3 IMAGING

STED imaging was performed using a Leica TCS SP8 STED 3X microscope (inverted) coupled to a White Light Laser (tunable from blue to red with an AOBS), three STED lines (592, 660 & 775 nm) and tunable spectral detectors. STED donut is created by a vortex phase mask and a second optical path with a different phase mask forms a z donut. Resolution becomes then tunable in x, y and z allowing for 3D STED. A STED-specific 100 × 1.4 NA oil objective (Leica) was used to focus the excitation beam and collect the signal on Leica’s hybrid detectors used in gating mode (1 – 6 ns).

STED acquisition was done in the following order: first, Alexa 568 was excited with \( \lambda_{\text{exc}1} = 568 \text{ nm} \) and depleted with a strong radiation of \( \lambda_{\text{depl}1} = 660 \text{ nm} \), then Alexa 488 was excited with \( \lambda_{\text{exc}2} = 488 \text{ nm} \) and depleted with a strong radiation of \( \lambda_{\text{depl}2} = 592 \text{ nm} \). In addition, for resolution comparison purpose, a conventional confocal image was acquired for each channel before taking the STED image. Due to the strong depletion laser powers, excitation powers were increased approximately 2 fold compared to confocal mode in order to compensate for signal loss. A frame accumulation of 3 was also used to further amplify the signal.

4 IMAGE PROCESSING

The gain in resolution with STED imaging in the two channels can be further improved by deconvolution, a process to reverse the optical distortion created during image acquisition, using SVI Huygens Professional software. Deconvolution Wizard was used with an automatic recognition of the acquisition parameters. The software package calculates the STED PSF based on these imaging parameters.

![Figure 1. Comparison between Confocal, STED and deconvolved STED images showing the resolution increase. Images are 2.52 × 2.10 μm](image)

STED Application Note | 17-04-2018 | Fadwa Joud