Chromeo™ STED Immunofluorescence System

properly prepared samples ensure your STED microscopy yields clear, conclusive high-resolution images

Fluorescent Microscopy with fluorescently labeled antibodies is widely used to determine the sub-cellular localization of specific proteins and to answer questions with regards to protein modification, interactions and life cycle. However, the resolution attainable in immunofluorescence experiments has until recently been limited by a very specific physical property known as the Abbe Law of Diffraction Limiting Resolution. The Abbe limit restricts the ability of the observer to visually resolve objects separated by less than ~200 nm. However, recent advances in super-resolution techniques such as STED (**ST**imulated **E**mission **D**epletion) in combination with confocal scanning enable the observer to exceed the Abbe limit and resolve details as small as 20 nanometers. This facilitates the imaging of sub-cellular structures that previously could not be visualized.

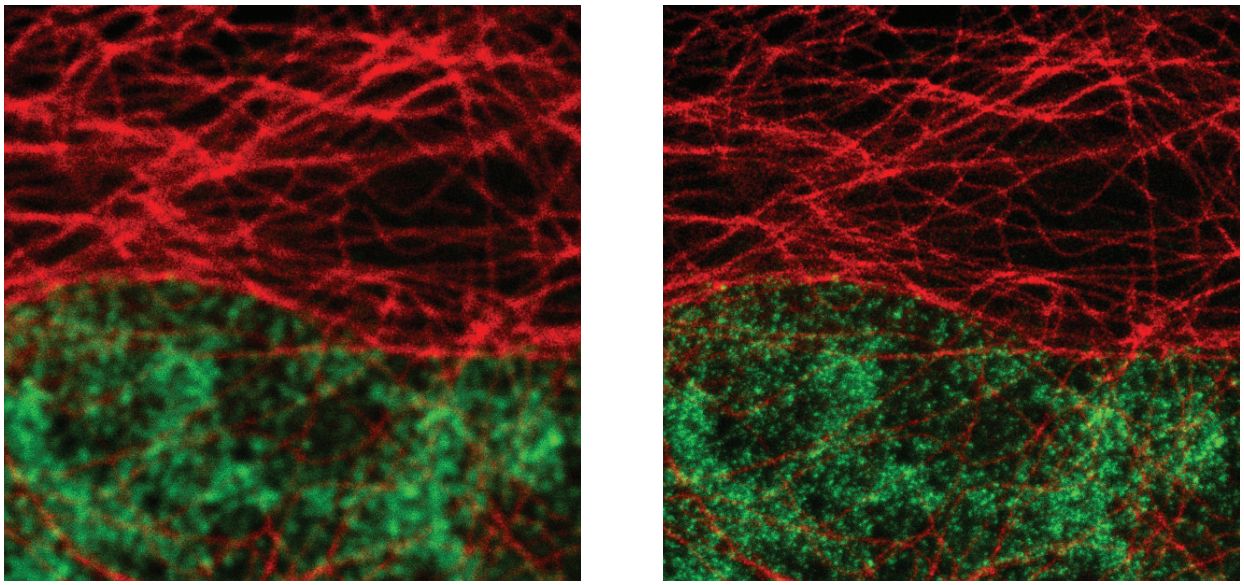

Figure 1: STED microscopy overcomes the Abbe limit and enables a much higher level of resolution than confocal microscopy.
HeLa cells were stained with alpha Tubulin mouse monoclonal antibody (Clone 5-B-1-2) and Chromeo 494 Goat anti-mouse IgG (Catalog No. 15032). Histone H3 was stained with Histone H3 K4me3 rabbit polyclonal antibody (Catalog No. 39159) and the ATTO 647N STED Goat anti-rabbit IgG (Catalog No. 15048) secondary antibody. The left image was prepared using a confocal microscope, while that on the right was produced using a STED microscope. Images courtesy of Leica Microsystems, Germany.

Proper sample preparation ensures high-quality images

For super-resolution microscopy to yield clear, conclusive high-resolution images, it is extremely important to optimize the techniques and reagents used for sample preparation. Proper sample preparation is among the most significant factors for obtaining high-quality images. To help ensure that you consistently achieve the best results possible, Active Motif collaborated with Leica Microsystems to develop the Chromeo™ STED Immunofluorescence System. This kit helps take the guesswork and challenge out of preparing samples for STED microscopy by providing a complete set of proven, QC-tested reagents and an optimized protocol. In addition to certifying this kit for use with its STED microscopes, Leica has tested and recommends many of Active Motif's fluorescent dyes and fluorescent secondary antibody conjugates for use with its instruments because they meet the specifications required for STED microscopy (see page 2).

Advantages of using the Chromeo™ STED System

- Optimized reagents and protocol help ensure proper sample preparation, resulting in the highest quality images
- Certified and recommended by Leica for use in STED microscopy
- Includes optically matched coverslips for maximum resolution

For more complete details, please give us a call or visit us at **www.activemotif.com/sted**.

Fluorescent Secondary Antibodies Validated for STED Microscopy

Chromeo™ 488 and Chromeo™ 505 for CW STED microscopy

Leica Microsystems has certified Active Motif's Chromeo™ 488 and Chromeo™ 505 fluorescent dyes and secondary antibody conjugates for use with its TCS STED CW microscope, which uses a continuous argon gas laser (488 nm and 515 nm) for excitation and a continuous 592 nm fiber laser for depletion. The fluorescent properties of Chromeo 488 and Chromeo 505 meet the specifications required to perform STED microscopy with the continuous lasers, and enable live cell imaging below 80 nm. In addition to the validated, high-quality secondary antibody conjugates, both dyes are available as reactive NHS-Esters and as Carboxylic Acids.

ATTO (STED) & Chromeo™ 494 for TCS STED microscopy

The Leica TCS STED system utilizes a pulsed 640 nm excitation laser combined with a 750 nm depletion laser, enabling it to reach a spatial resolution of 50-70 nm. For use in this wavelength range, Leica Microsystems recommends Active Motif's fluorescent ATTO 647N (STED) and ATTO 655 (STED) secondary antibody conjugates. These ATTO dye conjugates have been maximally cross-adsorbed against IgG's of a variety of species to eliminate background caused by non-specific binding.

The TCS STED microscope can also be upgraded for dual color STED simply by integrating a second, 531 nm excitation laser. This enables use of a second dye in high-resolution STED microscopy. With dual color STED microscopy, co-localization of proteins can be studied in a novel and reliable way. Chromeo™ 494 fluorescent dye and secondary antibody conjugates have been certified by Leica Microsystems for use with either ATTO conjugate in dual color TCS STED. Because Active Motif's Fluorescent Antibody Conjugates have been prepared using an optimized protocol that ensures the highest fluorescence intensity and stability, they can be used in such demanding applications.

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The Chromeo™ STED Immunofluorescence System contains sufficient reagents to for the preparation of 24 immunofluorescence slides and includes MAXblock™ Blocking Medium, MAXbind™ Staining Medium, MAXwash™ Washing Medium, MAXfluor™ Mounting Medium S, 24 MAX Stain™ Slides and 50 MAX Stain™ Coverslips. Reagent storage conditions vary from room temperature to 4°C. All reagents are guaranteed stable for 6 months from date of receipt when stored properly.

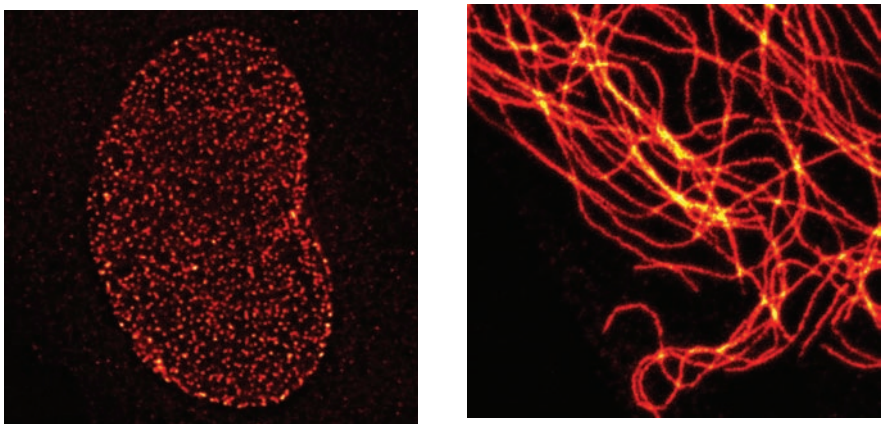

Figure 2: Chromeo 488 antibody conjugates in CW STED microscopy.
Nuclear pore protein-1 (NUP-1) was stained with a primary monoclonal mouse antibody and with Chromeo 488 Goat anti-mouse IgG (Catalog No. 15031) secondary antibody (left). Vimentin was stained with a primary polyclonal rabbit antibody and with Chromeo 488 Goat anti-rabbit IgG (Catalog No. 15041) secondary antibody (right). These STED images are courtesy of Leica Microsystems, Germany.

Dye	Absorption	Emission	STED System
Chromeo™ 488 IgG	498 nm	524 nm	TCS STED CW
Chromeo™ 505 IgG	514 nm	530 nm	TCS STED CW
Chromeo™ 494 IgG	489 nm	624 nm	TCS STED (dual color)
ATTO 647N IgG	644 nm	669 nm	TCS STED
ATTO 655 IgG	663 nm	684 nm	TCS STED

Table 1: Properties of Active Motif fluorescent secondaries certified for use in STED by Leica Microsystems.

Product	Format	Catalog No.
Chromeo™ STED Immunofluorescence System	1 kit	15260
Chromeo™ 488 Goat anti-Mouse IgG	1 mg	15031
Chromeo™ 488 Goat anti-Rabbit IgG	1 mg	15041
Chromeo™ 505 Goat anti-Mouse IgG	1 mg	15030
Chromeo™ 505 Goat anti-Rabbit IgG	1 mg	15040
Chromeo™ 494 Goat anti-Mouse IgG	1 mg	15032
Chromeo™ 494 Goat anti-Rabbit IgG	1 mg	15042
ATTO 647N (STED) Goat anti-Mouse IgG	250 µl	15038
ATTO 647N (STED) Goat anti-Rabbit IgG	250 µl	15048
ATTO 655 (STED) Goat anti-Mouse IgG	250 µl	15039
ATTO 655 (STED) Goat anti-Rabbit IgG	250 µl	15049