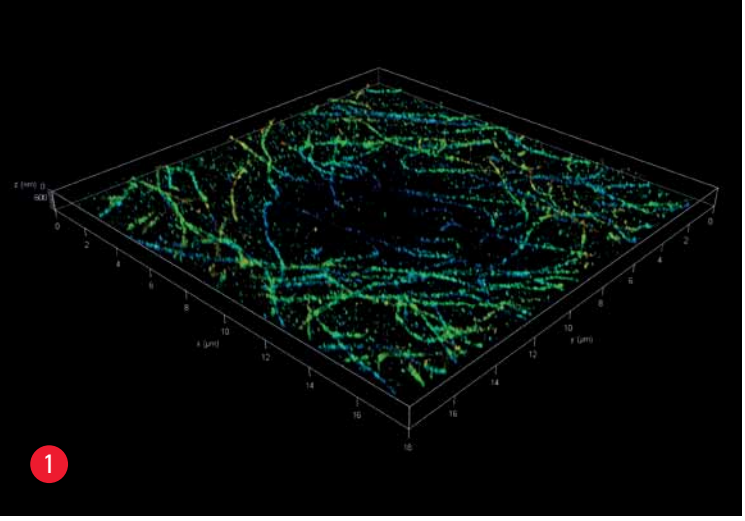
From Eye to Insight

Leica
MICROSYSTEMS

Leica SR GSD 3D

Redefine the Limits of Super-Resolution Microscopy

3D Localization with Ground State Depletion



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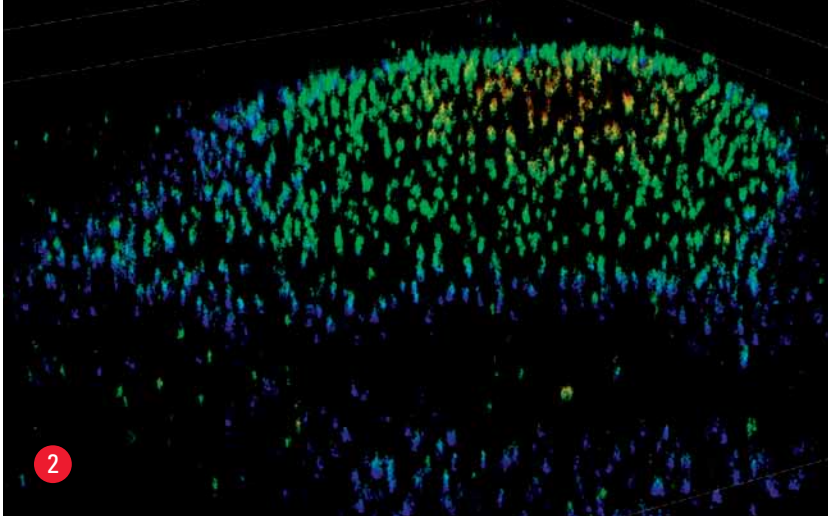

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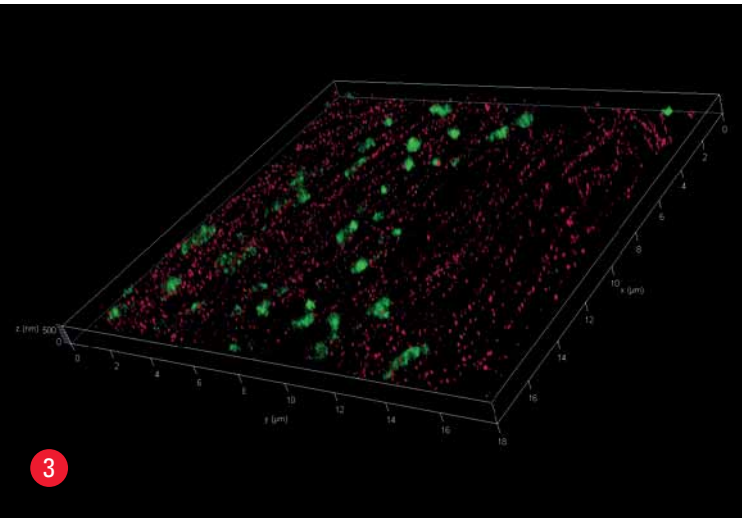

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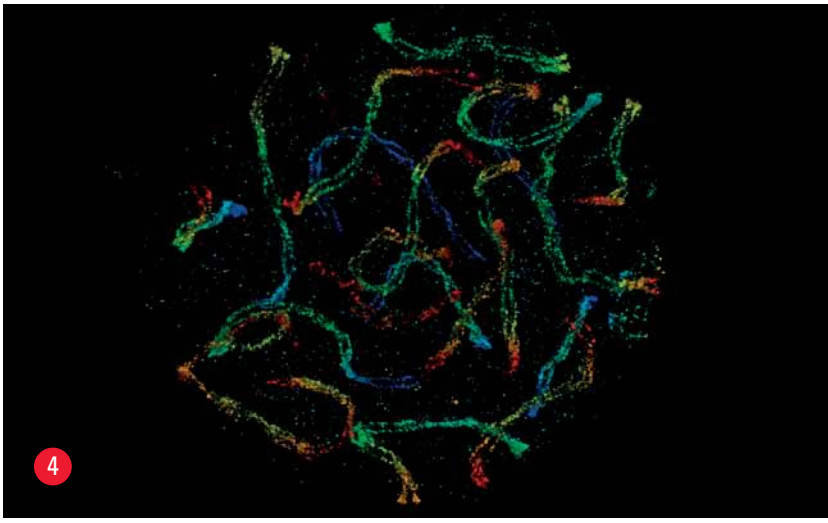

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Leica Design by Christophe Apothéloz

Setting New Standards in 3D Localization Precision

Stochastical optical reconstruction microscopy (GSDIM or dSTORM) can achieve super-resolution images with a lateral resolution of down to 20 nm. However the z-resolution of a widefield microscope limited resolution around several hundred nanometers. As a pioneer in super-resolution microscopy, Leica Microsystems developed the Leica SR GSD 3D to overcome this limit. This system reaches resolution down to 50 nm in axial direction, setting a new standard in 3D localization precision.

FROM 2D TO 3D

Working in close collaboration with top scientists and building on the success of the confocal super-resolution STED systems, Leica Microsystems has added the Leica SR GSD widefield super-resolution microscope to its portfolio. A winner of the R&D100 Award and other prizes, it has now been further advanced. The Leica SR GSD 3D offers not only highly precise and reproducible localization of single molecules in 2D, but also in 3D.

The Leica SR GSD 3D harnesses the technique of GSDIM (Ground State Depletion followed by individual molecule return) using a wide range of fluorophores.

TRUSTED LOCALIZATION

The Leica SR GSD 3D is optimized in several ways to achieve highly precise and reliable localization results. All optical components are apochromatically corrected and conform to the highest Leica Microsystems' standards.

The Leica SR GSD 3D is equipped with a 160x high-performance objective specifically developed for super-resolution microscopy. Its design is optimized for high-power laser emissions. The extremely low autofluorescence ensures a high signal-to-noise ratio, which is ideal for single molecule detection applications. The outstanding apochromatic correction further improves the image results.

YOUR BENEFITS

- Maximum resolution in X,Y down to 20 nm
- Maximum resolution in Z down to 50 nm
- Highest localization precision and system stability by optimal alignment of all optical components
- High power 160x objective for maximum laser stability, lowest auto fluorescence and highest color correction
- The SuMo Stage, with **Su**ppressed **Mo**tion technology, minimizes drift
- Online super-resolution image projection – see results as they are acquired
- Full application flexibility offered by combining super-resolution with TIRF and epifluorescence

Fig. 1: MDK cells: Tubulin stained with Alexa 647. Courtesy of R. Jacob, Marburg, Germany

Fig. 2: Hela cells: NUP153 stained with Alexa 647

Fig. 3: MDCK cells: Tubulin stained with Alexa 532 (red) and Mitochondria stained with Alexa 647 (green). Courtesy of R. Jacob, Marburg, Germany (sample preparation)

Fig. 4: Synaptonemal complex protein 3 (SYCP3) stained with Alexa 647. Courtesy of M. Lessard, Maine, USA (sample preparation)

Maximum Optical Performance for Reproducible, High-Quality Results

The Leica SR GSD 3D provides not only the maximum possible resolution in widefield microscopy so far, but also scores with its unique precision for localizing single molecules, its system stability, optical performance and its easy operation. Leica Microsystems' latest technologies enable researchers to achieve fast, reproducible, and high-quality super-resolution results.

"The GSDIM system from Leica Microsystems is an easy-to-use, straightforward super-resolution microscope that opens new possibilities for our research on membrane traffic that we could so far not achieve with alternative super-resolution systems."

Dr. Rainer Pepperkok
Team Leader and Head of Advanced Light Microscopy Core Facility, EMBL, Heidelberg, Germany

MINIMUM DRIFT

In microscopy, it is always necessary to minimize drift. This is of paramount importance in super-resolution imaging, due to the resolution and the acquisition times in question. With the SuMo stage (SUppressed MOtion) Leica Microsystems has introduced a new technology in drift compensation which puts the maximum system drift below the resolution during acquisition. This makes it possible to observe the super-resolution image as it is being acquired. The special SuMo technology ensures not only minimum drift but also maximum stability during detection.

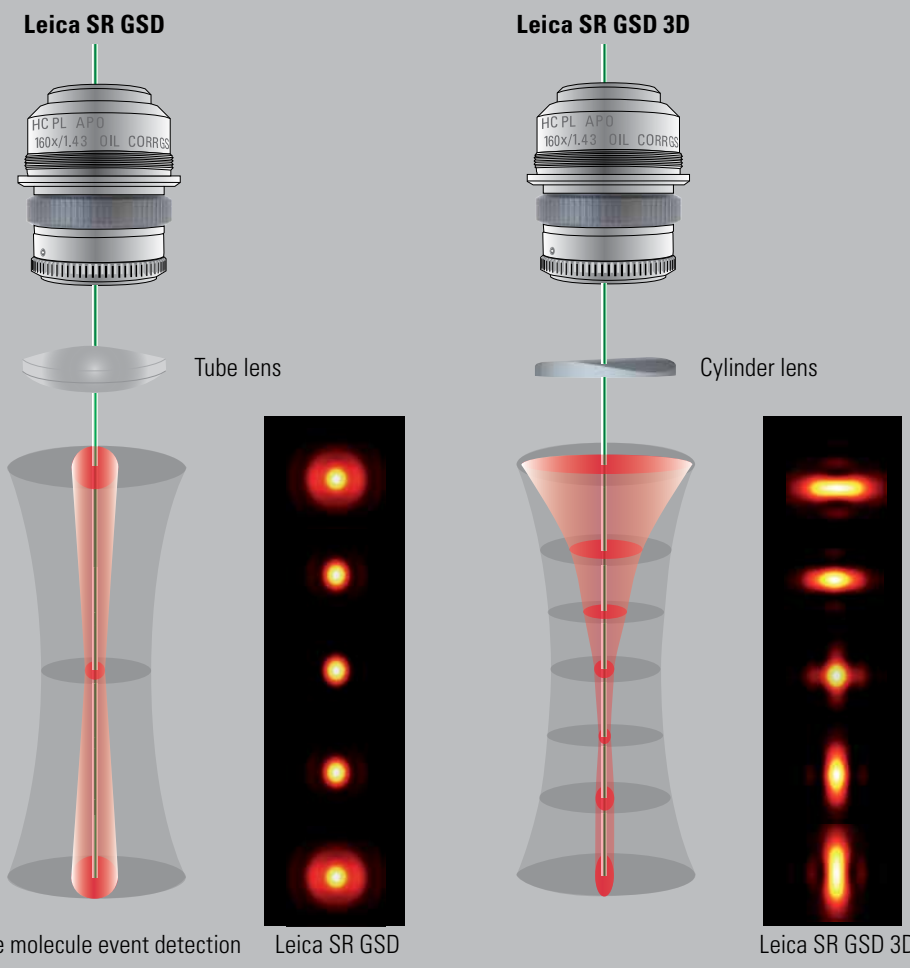
COLOR SPECIFIC CALIBRATION

The GSD software enables precise calibration of the system for the localization of molecules in x, y and z. Besides the standard calibration with gold beads, color-specific calibrations with fluorescence beads or dye molecules of the specimen are available.

BRIDGING THE RESOLUTION GAP

Visualizing the precise localization of cellular processes is crucial to understanding the interplay between molecules, structures and functions. The additional insight given by GSD super-resolution microscopy is extremely useful for a range of applications, since a number of structures currently in the research focus are smaller than the diffraction limit. This includes endo- and exosomes, viruses and nuclear pore complexes, to name but a few. Bridging the resolution gap between light and electron microscopy has the advantage of being able to use straightforward sample preparation protocols for fluorescence microscopy.

The Evolution of Re/olution



Reproducible 3D results: To enable the localization of molecules in z direction, the Leica SR GSD 3D uses the astigmatism approach. By adding a cylindrical lens to the emission beam path of the system, a different PSF is obtained (right). This astigmatism serves to localize the fluorescence molecule in axial direction. The amount of distortion allows determination of the exact position of the detected molecule in the z dimension and thus the 3D reconstruction. To ensure reproducibility of results, the cylindrical lens is precisely positioned into the beam path as soon as the system is switched from 2D to 3D recording – not by hand, but automatically by a single mouse click through software control.

SuMo Stage for minimum lateral drift

160x high power super-resolution objective

Greatest Flexibility for Your Live Cell Applications

"Leica Microsystems turned our idea of switching standard fluorophores by ground state depletion into a nanoscope that delivers stunning images."

Prof. Dr. Stefan Hell
Director at the Max-Planck-Institute for Biophysical Chemistry, Department of NanoBiophotonics, Göttingen, Germany

MULTI-PURPOSE SYSTEM

The Leica SR GSD 3D is based on the highly regarded Leica AM TIRF MC fully automated TIRF system (Total Internal Reflection Fluorescence), allowing super-resolution to be combined with TIRF microscopy. The system can also be used for a wide variety of applications in all fields of live cell or advanced fluorescence microscopy. As a multi-functional system, the Leica SR GSD 3D gives you the freedom to tailor the system exactly to your needs.

INTEGRATED WORKFLOW

The GSD workflow is based on standard immunostaining techniques and integrates perfectly into existing workflows for fluorescence microscopy. This enables the viewing of samples from the incredible perspective of super-resolution microscopy using a wide range of compatible fluorochromes. Use existing primary and secondary antibodies, adapt the staining protocol and start benefitting from the ultimate resolution in light microscopy!

FULLY AUTOMATED WIZARD

The fully automated experiment wizard guides you to the perfect super-resolution image step by step. Many automated functions are available to help with settings or alignments, but can be switched off to keep operation as simple as possible. The system software, Leica Application Suite Advanced Fluorescence (LAS AF), offers the familiarity and ease of use already appreciated on our confocal, compound and stereo microscopes.

FAR BELOW THE DIFFRACTION LIMIT

The resolution of a regular fluorescence microscope image is limited to approximately half the wavelength of the emitted light by diffraction. To separate fluorophores that are closer together, the solution is to ensure that not all illuminated fluorophores are able to emit simultaneously. To this end, the excitation light is used in such a way that almost all fluorophores in the samples instantly turn dark. The continuously shining excitation light removes fluorophores from their ground state, leaving only a few, sparsely distributed molecules capable of emitting fluorescence.

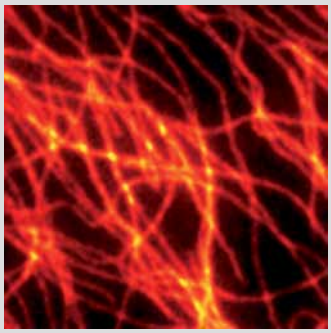
These molecules are imaged on a camera so that their position can be localized with high precision – before being turned off. As other molecules return back to their ground state, the microscope registers the position of many sequentially emitting molecules. The position of each fluorophore is accumulated into a single image, revealing details with unmatched resolution down to 20 nm. This is the basic principle of the GSDIM technique.

TECHNICAL DATA OF THE LEICA SR GSD 3D

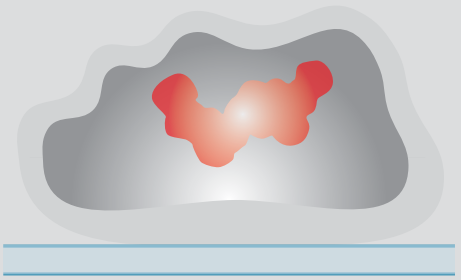
Lateral resolution*	› Maximum 20 nm › Typical 40 nm
Axial resolution*	› Maximum 50 nm › Typical 70–100 nm
Laser	› 488 nm/300 mW › 532 nm/500 mW › 561 nm/500 mW › 642 nm/500 mW › 405 nm/30 mW
Imaging modes	› 3D super-resolution › TIRF (also available with GSD) › EPI fluorescence (also available with GSD) › Brightfield › DIC/PH
Laser safety	› System class 1
Field of view	› 18 x 18 µm (GSD high power mode) › 40 x 40 µm (GSD large field of view mode) › 50 x 50 µm (TIRF)
Supported dyes	› Alex Fluor® 488 › Rhodamine-6G › Atto 532 and 488 › Alexa Fluor® 532 › Alex Fluor® 546 › Atto 565 and 568 › Alexa Fluor® 568 › Alexa Fluor® 647 › YFP › …
Imaging	Real-time image processing and display of results as they are acquired
Software	› Full set of software tools for configuration, acquisition, processing and image analysis

* Performance of the system is dependent on the appropriate room and sample conditions

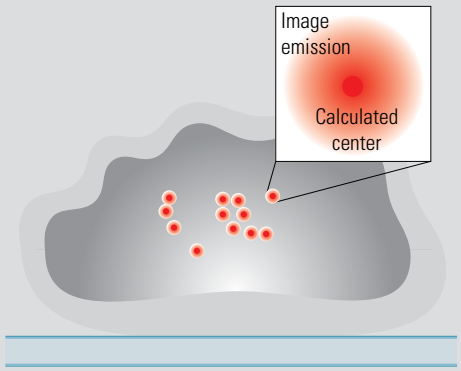
The principle of GSD/dSTORM



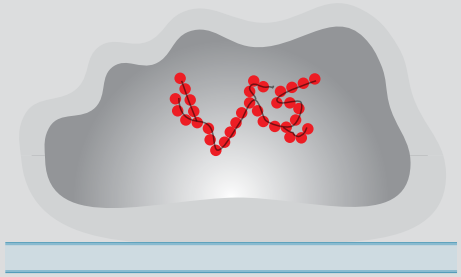
Widefield



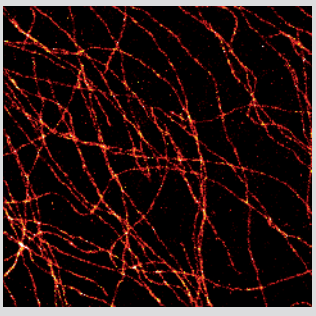
Diffraction limited imaging



Single molecule detection



SR image projection during acquisition



GSD

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Leica MICROSYSTEMS

Leica Microsystems operates globally in three divisions, where we rank with the market leaders.

LIFE SCIENCE DIVISION

The Leica Microsystems Life Science Division supports the imaging needs of the scientific community with advanced innovation and technical expertise for the visualization, measurement, and analysis of microstructures. Our strong focus on understanding scientific applications puts Leica Microsystems' customers at the leading edge of science.

INDUSTRY DIVISION

The Leica Microsystems Industry Division's focus is to support customers' pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

MEDICAL DIVISION

The Leica Microsystems Medical Division's focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

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Learn more about the basic principle of GSDIM, how GSDIM images are created, 3D Localization with GSD and many more super-resolution issues on Leica Science Lab: http://www.leica-microsystems.com/science-lab/topics/super-resolution/