

Living up to Life



APPLICATION NOTE | 07/2012

# Leica SR GSD – Super-Resolution Microscopy with GSDIM

Widefield

GSD

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Cover:

Ptk2-cells. NPC-staining: anti-NUP153/AlexaFluor 532

Microtubule-staining: anti- $\beta$ -tubulin/AlexaFluor 647

Courtesy: Wernher Fouquet, Leica Microsystems in collaboration with  
Anna Szymborska and Jan Ellenberg, EMBL, Heidelberg, Germany.

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## Introduction

# The New World of Resolution

Great advancements in biology have been possible by using fluorescence microscopy. So far, the resolution of the images was limited due to physical constraints. In the past couple of years, new methods evolved circumventing these limitations and bringing fluorescence microscopy to a new level of resolution, boosting the possibilities in science with fluorescence microscopes.

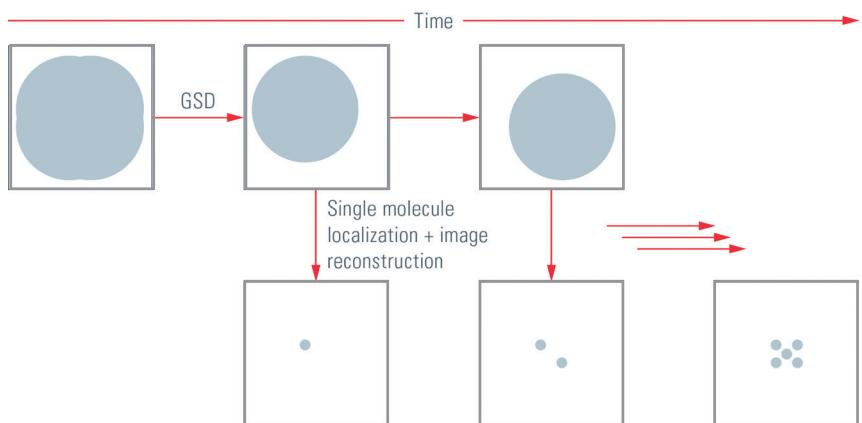
The resolution of a regular fluorescence microscope image is limited by diffraction to approximately half the wavelength of the emitted light. To separate fluorescently labeled structures that are closer together, a solution is needed to overcome Abbe's diffraction limit. The most common super-resolution methods for imaging beyond the diffraction limit are localization microscopy, SIM (structured illumination) and STED (Stimulated Emission Depletion Microscope). Localization microscopy does not look at an ensemble of simultaneously emitting fluorophores, but at clearly separated, individual fluorophores which can be localized with nanometer precision. Over time, the position of each fluorophore labeling a biological structure is determined and the image is re-constructed in silico based on the position information of each fluorophore. The challenge is to effectively singularize an ensemble of fluorophores to allow single molecule detection.

### THE LEICA SR GSD

The Leica SR GSD offers the highest lateral resolution with light microscopy and is a superb tool for determining the structure of fluorescently labeled specimen in neurobiology, cell biology, virology, microbiology and physiology. The workflow is usually based on standard immunostaining techniques and integrates perfectly into existing workflows for fluorescence microscopy.

The Leica SR GSD is using the localization microscopy technique Ground State Depletion followed by individual molecule return (GSDIM) to achieve superior super-resolution images. GSDIM is an innovative and reliable technology for achieving super-resolution images with a resolution of down to 20 nm.

The crucial step in GSDIM is to temporarily switch the majority of fluorophores off to allow the precise localization of single fluorophores. To this end, excitation light of high intensity is used in such a way that almost all fluorophores in the sample are turned dark, leaving only single, well-separated fluorophores emitting fluorescence, which is a prerequisite for nanometer precision localization.



**Fig. 1:** Creating a super-resolution image is a multi-step process: A diffraction limited image consists of overlapping fluorophores (left). Using GSD, these fluorophores are imaged one-by-one over time by switching all molecules in the region temporarily off except one. The image of a single molecule allows a precise localization of the fluorophore with nm accuracy. By repeating this process for (theoretically) all molecules of the structure, detailed position information of all fluorophores forming the structure is obtained. Finally the super-resolution image is calculated based on the position information of each fluorophore, displaying the structure with an improved resolution down to 20 nm.

## Calculation of localization precision – why organic fluorophores are better

The final resolution of a super-resolution-image obtained with the Leica SR GSD system is dependent on a) the precision of the localization of each individual fluorophore (localization precision) and b) the minimal distance between individual fluorophores marking the structure of interest (staining density). The staining density can be influenced e.g. by higher antibody concentrations used during immunostaining.

The localization precision is dependent on the number of photons collected from an individual fluorophore and can be approximated by the following formula:

$$\Delta r_{\text{SMS}} \approx \frac{\Delta r}{\sqrt{N}}$$

With  $\Delta r$ : diffraction limit of the microscope / objective  
 $N$ : Number of photons emitted by an individually localized fluorophore

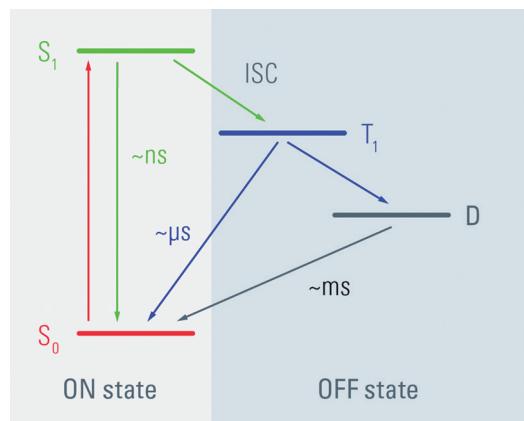
$\Delta r_{\text{SMS}}$ : localization precision of the individual fluorophore

Therefore, to achieve a localization precision of 10 nm, 400 photons have to be collected from the fluorophore with a microscope delivering a diffraction limited resolution of 200 nm.

The emitted number of fluorophores is in the first instance a property of the fluorophore in combination with the embedding medium. As a rule of thumb, organic fluorophores display higher brightness and photostability than fluorescent proteins. In consequence the number of photons delivered by these dyes is much higher and the localization precision significantly improved. Finally, a better super-resolution image can be obtained with "stronger" dyes like organic fluorophores due to the improved localization precision!



Step by Step Guide  
to the Molecular  
Basics of GSDIM  
Microscopy



**Fig. 2:** The emission of fluorescent light is caused by electrons circulating between the ground and the excited state. Unlike these ON states, fluorophores in the OFF state are not able to emit light. When enough molecules are in the OFF state, it is possible to detect individual molecules in the sample.

## THE MOLECULAR BASICS OF GSDIM

As soon as the sample is excited by (laser) light, electrons are catapulted by photons into the 1st excited state. On returning from the 1st state into the ground state, light of slightly lower energy (red-shifted) is emitted.

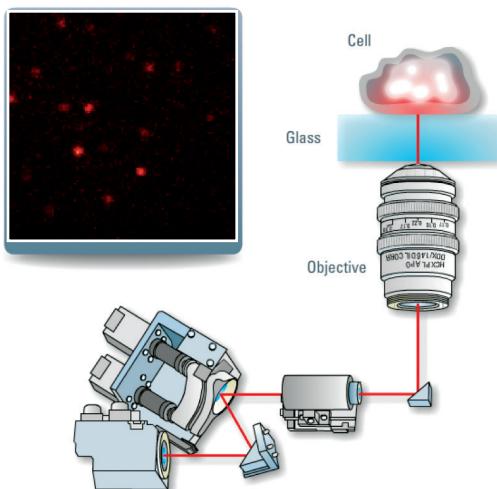
Higher laser power increases the number of photons that can hit an electron in the ground state and transfer the electron into the excited state. As a result, more electrons in the sample cycle between the ground and excited state in a given time. The number of emitted photons increases and a brighter sample is visible.

A further increase of the excitation light (e.g. from a high power laser source) can lead to a dimmer sample due to reduced fluorescence emission without bleaching the fluorophores. At the beginning of this process, the increase in excitation light leads to a higher number of electrons cycling between the ground and the 1st excited state. From the excited state a certain percentage of electrons enter the off state. The only accessible sub-state from the 1st excited state is the triplet state. Entering the triplet state requires a spin-flip. A spin-flip has a very low probability of occurrence and is therefore a very rare event. In consequence the off state is practically unpopulated at low light exposure of the sample. Off states have a long lifetime (in the range of  $\mu$ s to s) and fluorophores with electrons in the off state cannot participate in fluorescence emission of the sample any more. During the excitation with a high power laser, more and more electrons end up in the off state and the sample

appears dimmer although the overall number of fluorophores has not changed. Just the number of "active" or "accessible" fluorophores has been altered. "Pumping" electrons in the off state can be seen as a reversible switch. Fluorophores are turned off for the time the electron resides in the off state. When the electron returns to the ground state, the fluorophore is rendered "active" and can again emit fluorescent light. All transitions between molecular states of electrons are probability-governed processes and therefore the exact occurrence of a transition cannot be predicted for a given electron.

For super-resolution imaging the laser power is kept at a high level until only a few fluorophores in the field of view emit photons. The "active" fluorophores can cycle up to a few thousand times between the ground and 1st excited state, creating a "burst" of photons before the electron returns to the off state. The photon "burst" is recorded with a high-sensitive EMCCD camera. Furthermore, only a few electrons populate the ground state for a short time, leading to the situation of "ground state depletion with individual molecule return" (GSDIM).

In general, fluorophores emitting photon bursts are spatially well separated, which allows the allocation of all photons in a certain region to a single molecule. In this situation the center of the recorded, diffraction-limited signal is the same as the position of the fluorophore. The position of the fluorophore can be calculated up to nanometer precision.



**Fig. 3:** The Leica SR GSD uses laser to obtain the super-resolution image. The laser light is directed to the sample via an optimized light-path, including motorized beam-steering and a dedicated 100x objective.

# The Leica SR GSD System for brilliant Super-Resolution Images

## KEY FEATURES OF THE LEICA SR GSD

- › GSDIM-technology –  
Wider range of standard fluorophores
- › Leica SuMo-Stage –  
Effective hardware drift suppression
- › Super-resolution in both TIRF - and Widefield-Mode
- › High Power Lasers –  
Higher flexibility in fluorophore selection and shorter imaging time
- › Multi-purpose widefield system –  
including TIRF / DIC / Live cell imaging
- › Optimized localization algorithm –  
Superb image quality
- › LAS AF GSD Wizard –  
Intuitive handling minimizes user training and makes SR imaging easier



Fig. 4: Leica SR GSD System

## THE SUMO STAGE – PREVENTING DRIFT

In microscopy, it is always necessary to minimize sample drift. This is of paramount importance in super-resolution imaging, due to the resolution (down to 20 nm) and the acquisition times (in the range of minutes) in question. The SuMo Stage (Suppressed Motion Stage) for the Leica SR GSD introduces a brand new technology in drift reduction. The drift is suppressed to such an astonishing level that no post-processing to correct for drift is necessary to obtain brilliant super-resolution images. Therefore the sample preparation process is tremendously simplified because a correction of drift using gold beads or other fiducial markers is not necessary. The SuMo Stage ensures maximum performance and minimum lateral drift, by closely coupling the objective with the sample holder and by using a specially designed combination of carefully selected materials for sample holder and sample stage. Coupling the objective to the stage and not placing it into the nosepiece minimizes drift between sample and objective. Otherwise these components would drift independently and in the worst case lead to an additive effect of the respective drift movements.

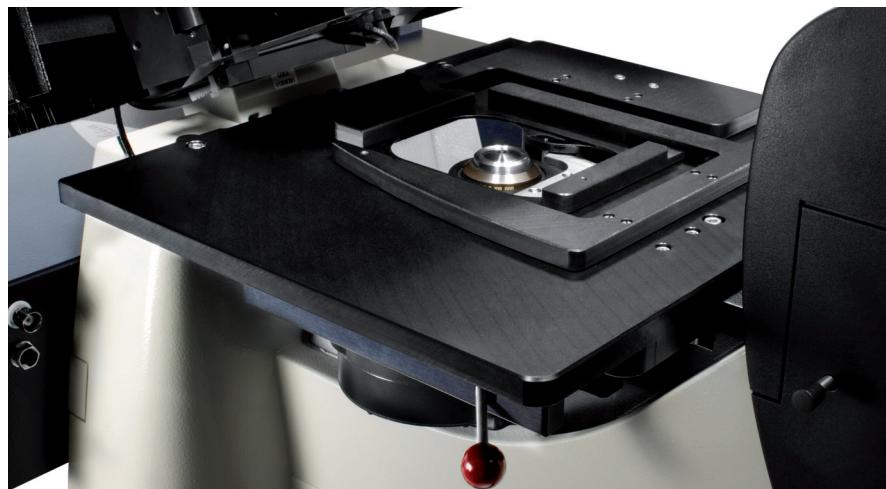


Fig. 5: The SuMo Stage (Suppressed Motion Stage), with suppressed motion technology, minimizes drift for accurate localization of molecules, by closely coupling the objective with the sample holder and other highly sophisticated design features.

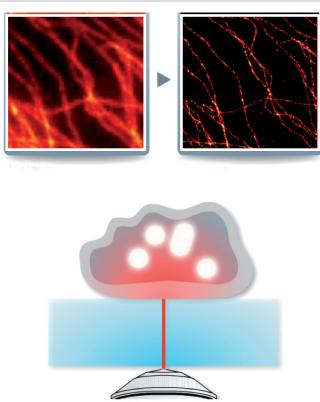
## Formation of a super-resolution image with GSD

The super-resolution image (SR) is acquired and reconstructed in a multi-step process.

1. The sample is excited with a laser to drive fluorophores into the off state. This process can be seen as a decrease in fluorescence intensity in the image. The process is reversible and has nothing in common with bleaching, which leads to the irreversible destruction of the fluorophore.

2. As the overall fluorescence intensity decreases, the emission of well-separated individual fluorophores becomes visible for a short period until the molecule returns to the off state. Other molecules will exit the off state and become visible at the same time, leading to the impression that the image is "blinking".

3. The position of individual molecules is determined in each image with nanometer precision. The coordinates of the fluorophore are used to reconstruct the final super-resolution image. Since only a few fluorescent molecules can be analyzed in each frame it is necessary to acquire thousands of images to detect the entire set of hundreds of thousands of fluorophores labeling a biological structure. This leads to imaging times in the range typically between 1 – 5 min min per super-resolution image.



**Fig. 6:** The fluorescently labeled cell is illuminated with high intensity laser light, resulting in single fluorophore "blinking". Based on analyzing the single fluorophores a super-resolution image is reconstructed (right) with a 10x higher resolution than the diffraction-limited widefield image (left).

# Suitability of Samples

GSD is suitable for a wide range of samples. Cells grown on standard glass coverslips can be used e.g. after chemical fixation. Furthermore tissue slices can be prepared for GSD and placed on a glass coverslip. The samples are normally fluorescently labeled by immunostaining or other suitable techniques.

mechanical stability for high-resolution imaging. The respective products should be tested by the researcher for their suitability. A better flatness and high stability can be generally achieved by stress free mounting of a glass coverslip, e.g. in specially designed coverslip holders.

## FLUORESCENT PROTEINS

GSDIM is suitable to create super-resolution images using fluorescent proteins. E.g. the fluorescent protein YFP / Venus displayed astonishing results. Furthermore, other fluorescent proteins should work with this technology (including GFP). To combine the power of organic fluorophores with the advantages of genetically encoded fusion proteins (e.g. if no suitable antibody is available), the use of tags like SNAP-, Halo- or CLIP-Tag is recommended. These tags can be genetically linked to the protein of interest and later on labelled with organic fluorophores (e.g. after fixation and permeabilization).

## SUPER-RESOLUTION IMAGING OF LIVING CELLS

Recent reports have studied the suitability of GSDIM (also known as dSTORM) to image living cells. In general, it is possible to study living cells with the Leica SR GSD, but phototoxicity - as a result of the high laser power applied - and the dynamics of structure of interest during the observation period should be carefully controlled.

## COVERSLEIPS

Super-resolution imaging requires excellent optical performance throughout the whole optical system – not only within the microscope and objective. The coverslip is a major parameter with high impact on imaging results. To obtain optimal super-resolution image quality a flat coverslip is required. Unfortunately, while being uncritical for standard widefield applications, not all chambered coverslip products can guarantee the degree of flatness optimal for GSD. Furthermore, chambered coverslip products may not provide the necessary

## IMMUNOFLUORESCENCE

Starting with samples prepared with standard immunofluorescence protocols optimized for diffraction-limited fluorescence should already provide stunning super-resolution images. To obtain optimal GSD images it might be necessary to increase the concentration of primary and secondary antibodies to achieve a higher labeling density. Increasing the labeling density ensures that the structure of interest is sufficiently stained with fluorophores. A sparse labeling of structures can lead to a point-like appearance of your image. In order to reduce unspecific background signals in the GSD image, it might be necessary to increase the time for blocking your sample as well as the concentration of the blocking reagent. It is advised to wash your samples thoroughly (e.g. by increasing the number and time of washing steps).

## STRUCTURE PRESERVATION

The preservation of a specific cellular structure strongly depends on the fixation method. The exact fixation protocol should be optimized for the structure of interest. For the majority of structures a Paraformaldehyde / Formaldehyde fixation is sufficient. Small amounts of Glutaraldehyde (0.05% to 0.2% (v/v)) in addition to Paraformaldehyde / Formaldehyde as a fixant can strongly improve structure preservation. Glutaraldehyde can induce a hazy fluorescent background therefore quenching with ammonium, NaBH<sub>4</sub> or other suitable reagents is recommended. Methanol fixation is common for microtubule preservation, but might not be sufficient for other structures.

# Embedding Medium 1

## REAGENTS

- β-Mercaptoethylamine (MEA) (e.g. Sigma-Aldrich, # 30070, also known as Cysteamine)
- Phosphate Buffered Saline (PBS)
- HCl / NaOH for pH adjustment

## BUFFER

PBS containing 10 mM β-Mercaptoethylamine (MEA) adjusted to pH 7.4.

Dissolve the β-Mercaptoethylamine in PBS and adjust to pH 7.4. The β-Mercaptoethylamine concentration can be varied over a wide range to optimize the GSD image – the usable range is in general between 10 and 100 mM (recommended concentrations for testing 10 / 30 / 100 mM).

### β-Mercaptoethylamine solutions must be freshly prepared before use.

Alternatively, stock solutions can be prepared and stored at -20°C and freshly thawed before use. The frozen aliquots can be stored for several weeks.

Illumination	EPI ✓	TIRF ✓
Laser [nm]	Dye	Manufacturer
<b>488</b>	AlexaFluor 488	life technologies
	Atto 488	Sigma
	Chromo 488	Active Motif
	Oregon Green 488	life technologies
	Chromo 505	Active Motif
<b>532</b>	Atto 520	Sigma
	AlexaFluor 532	life technologies
	AlexaFluor 568	life technologies
<b>642</b>	Atto 633	Sigma
	AlexaFluor 647	life technologies
	Atto 647N	Sigma
	Atto 655	Sigma
	AlexaFluor 680	life technologies
	AlexaFluor 700	life technologies

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# Embedding Medium 2

## REAGENTS

- Glucose Oxidase (e.g. Sigma-Aldrich G2133)
- Catalase (e.g. Sigma-Aldrich C1345)
- Glucose
- Phosphate Buffered Saline (PBS)
- HCl / NaOH for pH adjustment

## BUFFER

PBS containing 10% (w/v) glucose, 0.5 mg/mL glucose oxidase and 40 µg/mL catalase adjusted to pH 7.4.

**Note:** It is recommended to prepare a glucose stock solution and mix the reagents freshly before mounting the coverslip.

Illumination	EPI ✓	TIRF ✓
Laser [nm]	Dye	Manufacturer
<b>532</b>	Atto 532	Sigma
	AlexaFluor 532	life technologies
	Rhodamine 6G	Active Motif
	Atto565	Sigma
	AlexaFluor 568	life technologies
<b>642</b>	Atto 655	Sigma
	AlexaFluor 680	life technologies

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## Embedding Medium for YFP

The following aqueous embedding was successfully used to image YFP in fixed and permeabilized mammalian cells:

- |                                     |           |
|-------------------------------------|-----------|
| – Catalase SIGMA, C1345             | 40 µg/mL  |
| – Glucose Oxidase SIGMA G2133       | 0.5 mg/mL |
| – Glucose                           | 10 mg/mL  |
| – β-Mercaptoethylamine (Cysteamine) | 1 mM      |

PBS adjusted to pH 7.4.

# Embedding Medium 3

## REAGENTS

- Polyvinyl alcohol (PVA) with a molecular weight of 25,000, 88 mol% hydrolyzed, e.g. Polysciences, #02975-500
- Phosphate Buffered Saline (PBS)
- HCl / NaOH for pH adjustment

## HARDWARE

Spincoater

## BUFFER

Prepare a solution of 1% PVA in PBS. Adjust the pH to 7.4.

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## SPINCOATING

Place the coverslip on the spincoater and dry the coverslip with brief 5-10 sec spin at approx. 3000 rpm. Drop 50 µl of the PVA-solution onto the sample and spin the sample at approx. 3000 rpm for approximately 30 s. Let the PVA-film dry for a few minutes and mount the sample. The sample can be stored at room temperature and used for GSD imaging for up to three months.

No additional mounting medium is required. The PVA-film is protecting the sample.

Illumination	EPI ✓	TIRF not possible
Laser [nm]	Dye	Manufacturer
<b>488</b>	AlexaFluor 488	life technologies
	Atto 488	Sigma
	Chromo 488	Active Motif
	Oregon Green 488	life technologies
<b>532</b>	Atto 520	Sigma
	AlexaFluor 532	life technologies
	AlexaFluor 680	life technologies

# Embedding Medium 4

## REAGENTS

- Glycerol (analytical grade)
- Mowiol 4-88 (e.g. Calbiochem #475904)
- Distilled water
- TRIS
- HCl / NaOH for pH adjustment

## EMBEDDING MEDIUM

- Start with 6 g Glycerol (analytical grade)
- Add 2.4 g Mowiol 4-88 (Calbiochem #475904)
- Add 6 ml Aqua dest.
- Add 12 ml 0.2 M TRIS buffer pH 8
- Stir for 4 hours (magnetic stirrer)
- Let suspension rest for 2 hours
- Incubate for 10 min at 50 °C (water bath)
- Centrifuge at 5000x g for 15 min
- Take the supernatant and freeze it in aliquots at -20 °C

Illumination	EPI ✓	TIRF not possible
Laser [nm]	Dye	Manufacturer
<b>488</b>	Atto 488	Sigma
	Chromo 505	Active Motif
<b>532</b>	AlexaFluor 532	life technologies
	Rhodamine 6G	Active Motif
	Atto 647N	Sigma
<b>642</b>	Atto 655	Sigma
	AlexaFluor 700	life technologies

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# Dual-Color Stainings

Leica Microsystems recommends the combination of a) AlexaFluor 532 and AlexaFluor 647, b) AlexaFluor 488 and AlexaFluor 647 and c) Atto 488 and AlexaFluor 647 for sequential dual color imaging. Please image the red channel (AlexaFluor 647) first. Leica Microsystems does NOT recommend imaging the green/orange channel (e.g. AlexaFluor 488) at the

beginning to avoid bleaching of AlexaFluor 647, which can be - although at very low levels – excited by a 488 nm (or 532 nm) laser. If it is intended to use other fluorophores for double-staining of the sample, please check that both fluorophores can be imaged under the same GSD imaging conditions (embedding media) and crosstalk is negligible.

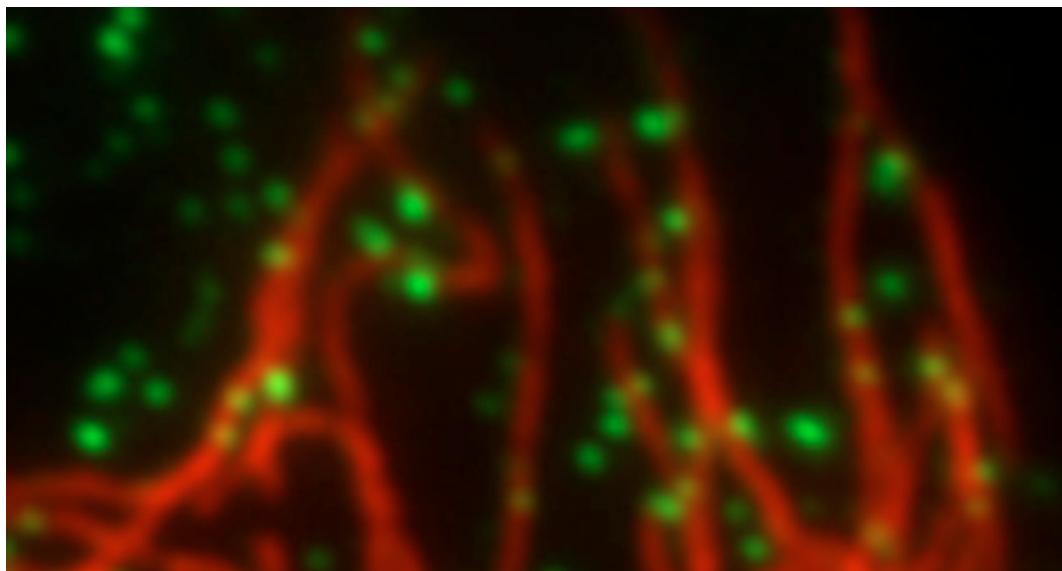
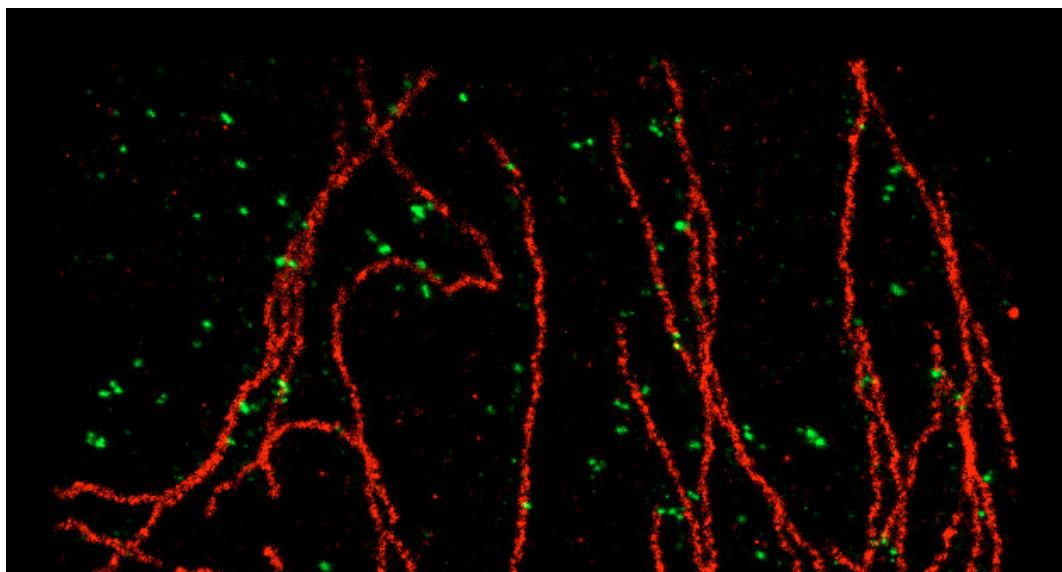
## Use of 405 nm laser

The Leica SR GSD is equipped with a 405 nm laser. The lifetime of fluorophores in the off state can be shortened by illuminating the sample additionally with UV-light. The shorter lifetime leads to an increase of fluorophores in the on state and therefore can be used to increase the number of detected events per frame. By keeping the number of events per frame in an optimal range, it is possible to

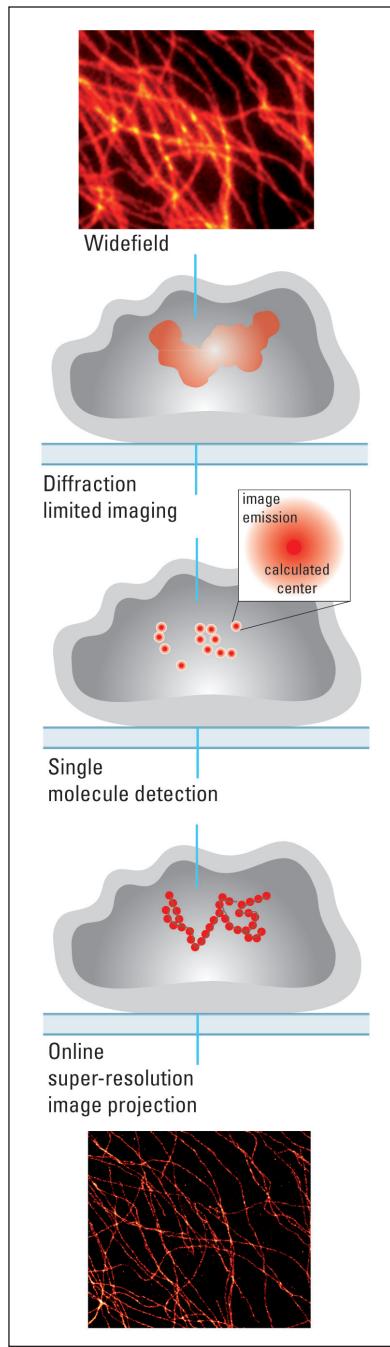
a) efficiently detect well-separated single fluorophores and

b) keep the time to acquire a super-resolution image short, because it is possible to accumulate the required number of events within fewer images.

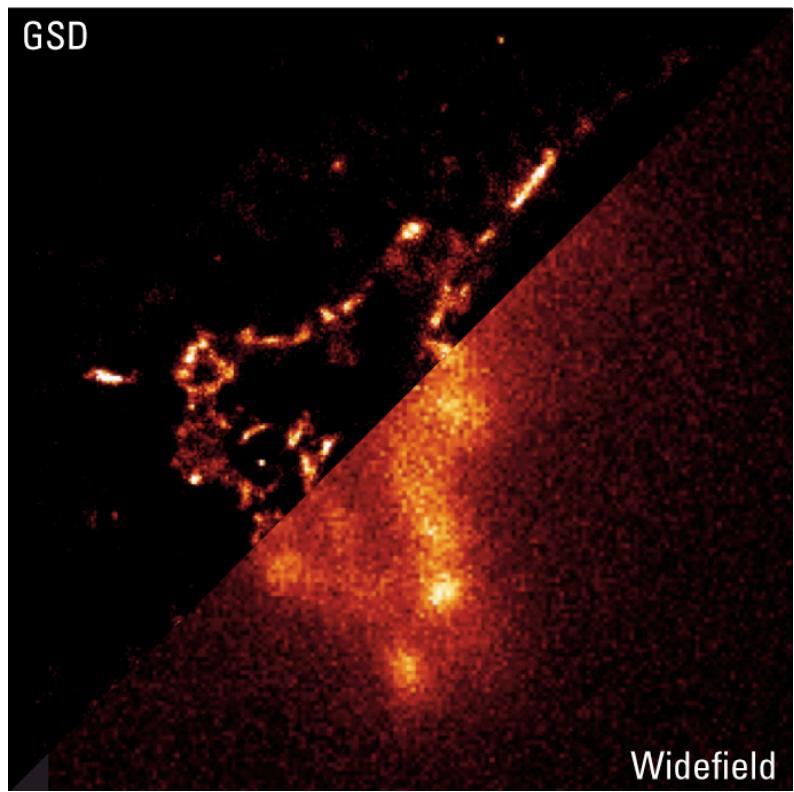
When acquiring the longer wavelength channel of dual-color images, it is important to use the 405 nm laser carefully! The 405 nm laser can excite green dyes and therefore bleach the shorter wavelength fluorophores while acquiring the longer wavelength super-resolution image. The optimal settings should be determined for each sample type separately.



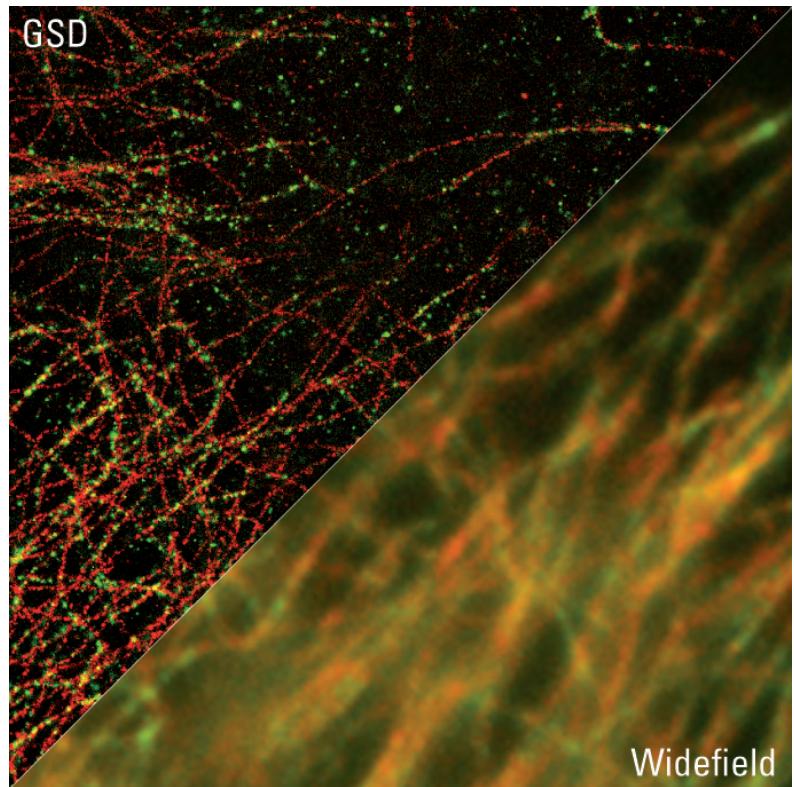
**Fig. 7:** PtK2 cells with AlexaFluor 647 stained microtubules in red and AlexaFluor 488 stained clathrin in green.  
Courtesy: Prof. Dr. Stefan Hell, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany.



**Fig. 8:** The multi-step process to achieve a super-resolution image in a nutshell: The diffraction limited image (top) shows all fluorophores signals overlapping. By localizing individual molecules after switching the majority of fluorophores temporarily off, and continuing this procedure for all fluorophores staining the structure, a super-resolution image (bottom) can be calculated with a 10x better resolution.



**Fig. 9:** Golgi membrane protein Giantin and Golgi matrix protein GM130, immunofluorescence staining with Alexa Fluor 647 and Alexa Fluor 488, respectively. Dr. Yasushi Okada, Department of Cell Biology and Anatomy, Graduate School of Medicine, University of Tokyo, Japan.



**Fig. 10** MDCK cells: Microtubules, AlexaFluor 642 (red) and TyrMicrotubules, AlexaFluor 488 (green). Courtesy of Prof. Dr. Ralf Jacob, Philipps University Marburg, Germany.

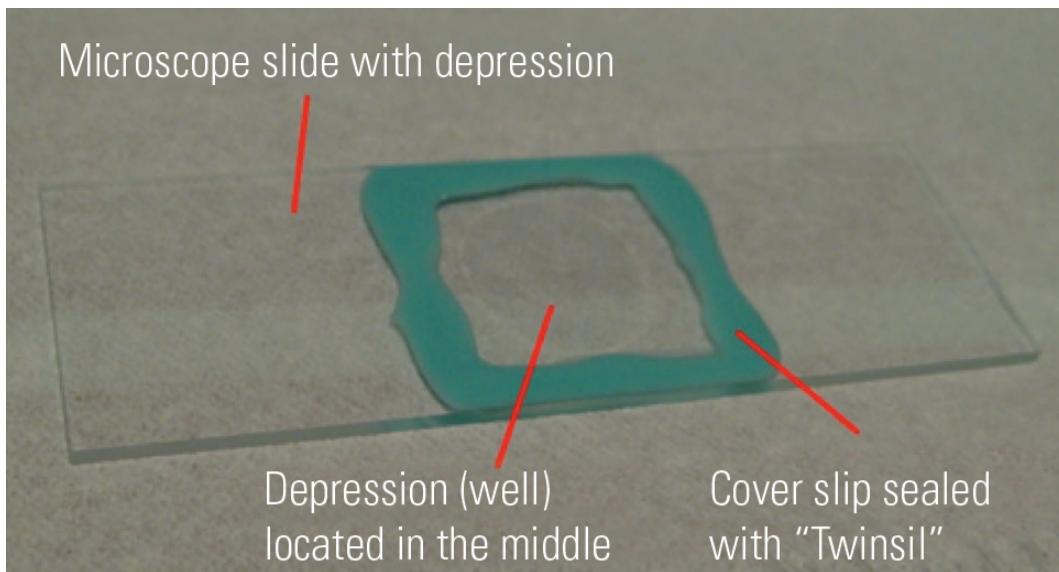
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# Recommended Mounting Procedure

1. Only for imaging with aqueous embedding media 1 & 2 (otherwise proceed with step two): Add 75 – 100 µl of embedding medium into the cavity of a clean depression slide. For embedding media 3 (PVA): leave the cavity of the depression slide empty.
2. Place the glass coverslip with your sample onto the cavity of the depression slide. The sample should face the cavity. The coverslip should cover the depression completely. If aqueous embedding medium is filled in the cavity, ensure that no air bubbles are present. Otherwise carefully remove the coverslip and repeat the procedure.
3. For Embedding Media 1 & 2: Carefully remove excess of liquid using filter paper. Ensure that no buffer is soaked out from the reservoir.
4. Mix the yellow and blue component of Twinsil® in a ratio of 1:1 thoroughly and apply it to the edges of the glass coverslip without directly touching the glass coverslip. For Embedding Media 1 & 2: Seal the coverslip completely with Twinsil®. For Embedding Media 3: Seal the coverslip to approx. 75% and leave the remaining quarter open to allow ventilation and avoid condensation on the sample.
5. After 5 – 10 minutes the two-component glue is hardened and the sample is ready for GSD imaging.
6. The glue can be easily removed without leaving traces on the glass after hardening (e.g. to exchange the imaging buffer).



Step by Step Guide  
to Optimized Sample  
Preparation for GSD  
Microscopy



**Fig. 11:** Rectangular coverslip mounted on a depression slide and sealed with Twinsil®.

### Summary table

This table provides a short summary of the various fluorophores mentioned in this application letter for GSDIM and the recommendations for suitable embedding media.

Laser [nm]	Fluorophore	MEA in PBS	Glucose Oxidase	PVA	Resin LR-White	Mowiol
488	AlexaFluor 488	+		+		
	Atto 488	+		+		+
	Chromo 488	+		+		
	Oregon Green 488	+		+		
	Chromo 505	+				+
532	Atto 520	+		+		
	AlexaFluor 532	+		+		+
	Atto 532		+	+	+	
	AlexaFluor 546			+		
	Chromo 546			+		
642	Rhodamine 6G	+	+	+	+	+
	Atto 565		+	+		
	AlexaFluor 568	+	+			
	Atto 633	+				
	AlexaFluor 647	+				
	Atto 647N	+				+
	Atto 655	+	+			+
	AlexaFluor 680	+	+			
	AlexaFluor 700	+				+

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# Disclaimer

This Application Letter is designed to guide researchers through the current specimen preparation methods for the Leica SR GSD. All protocols discussed in

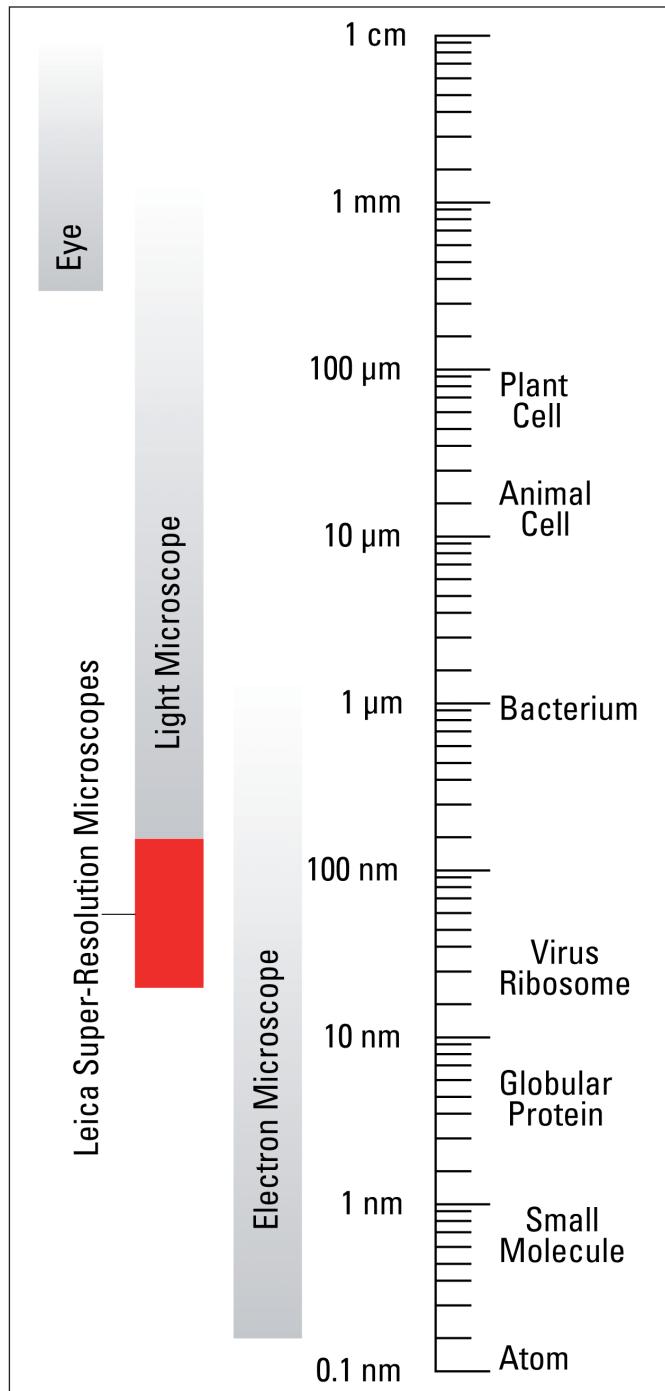
this guide serve as a starting point to establish and perfect your own protocols.

## Important

Read the instruction manual for your Leica SR GSD system and for all other products used in your work before following a protocol.

Your lab may have specific protocols that differ from those in the guide. Leica Microsystems' protocols do not supersede your protocols.

This guide does not replace the instruction manual. Leica Microsystems does not guarantee safety and performance of another company's products that it recommends, nor does it guarantee the safety or successful outcome of experiments using the suggested protocols for your specific work.



**Fig. 12:** Resolving power of different microscopy techniques: The Leica SR GSD is advancing light microscopy to a new level of resolution.

## Leica Science Lab

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The statement by Ernst Leitz in 1907, "**With the User, For the User,**" describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: **Living up to Life.**

Leica Microsystems operates globally in four 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.

### BIOSYSTEMS DIVISION

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

### 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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Belgium · Diegem	+32 2 790 98 50	2 790 98 68
Canada · Concord/Ontario	+1 800 248 0123	847 405 0164
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