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Cover photo (top): Luminal surface of the organ of Corti at postnatal day 1. (Mouse) Photographed with the cooperation of: Drs. Kanoko Kominami, Hideru Togashi, and Yoshimi Takai, Division of Molecular and Cellular Biology, Kobe University Graduate School of Medicine/Faculty of Medicine

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Monitor images are simulated. Company names and product names appearing in this brochure are their registered trademarks or trademarks AB: Export of the products' in this brochure is controlled under the Japanese Foreign Exchange and Foreign Trade Law. Appropriate export procedure shall be required in case of export from Japan. \*Products: Hardware and its technical information (including software)



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Super Resolution Microscope





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# Nikon's super-resolution microscopes bring your research into the world of nanoscopy beyond the diffraction limit.

Nikon's Super Resolution Microscope N-SIM/N-STORM enables elucidation of the structures and functions of nanoscopic machinery within living cells. The resolution of conventional optical microscopes, even with the highest numerical aperture optics, is limited by diffraction to approximately 200 nm.

Using high-frequency structured illumination, the N-SIM can achieve an image resolution of 115 nm\*, which was previously considered impossible with optical microscopes. Furthermore, with a temporal resolution of up to 0.6 sec/frame\*\*, N-SIM enables super-resolution time-lapse imaging of dynamic molecular interactions in living cells. Live samples can be maintained at optimal environmental conditions using a stage top incubator that was designed for use with the N-SIM.

N-STORM trades off temporal resolution for spatial resolution, realizing an incredible image resolution of approximately 20 nm, which is 10 times or more than that of conventional optical microscopes. Utilizing STochastic Optical Reconstruction Microscopy (STORM), it is now possible to gain insight into protein-protein interactions at a molecular level.

Nikon's super-resolution microscopes with unrivaled optical technologies integrate powerful proprietary technologies into streamlined platforms that are designed to be easy to use. N-SIM/N-STORM can dramatically enhance the ability to address questions in the nanoscopic realm, and instill confidence in the conclusions that can be drawn from your data.

\*Excited with 488 nm laser, in 3D-SIM mode \*\* With 2D-SIM/TIRF-SIM mode



# See like you have never seen before





kiwami The Japanese calligraphy on the above reads as "kiwami," which means to master or pursue excellence.

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# N-SIM

In structured illumination microscopy (SIM), the unknown cellular ultra-structure is elucidated by analyzing the moiré pattern produced when illuminating the specimen with a known high-frequency patterned illumination. Nikon's Structured Illumination Microscope (N-SIM) realizes super resolution of up to 115 nm in multiple colors. In addition, it can continuously capture super-resolution images at a temporal resolution of 0.6 sec/frame, enabling the study of dynamic interactions in living cells.

## Live-cell imaging at double the resolution of conventional optical microscopes

N-SIM utilizes Nikon's innovative new approach to "structured illumination microscopy" technology. By pairing this powerful technology with Nikon's renowned CFI Apochromat TIRF 100x oil objective (NA 1.49), N-SIM nearly doubles (to approximately 115 nm\*) the spatial resolution of conventional optical microscopes, and enables detailed visualization of the minute intracellular structures and their interactive functions.

\* Excited with 488 nm laser, in 3D-SIM mode

## Temporal resolution of 0.6 sec/frame—amazingly fast super-resolution microscope

N-SIM provides ultra fast imaging capability for Structured Illumination techniques, with a time resolution of up to 0.6 sec/frame, which is effective for live-cell imaging (with TIRF-SIM/2D-SIM mode; imaging of up to approximately 1 sec/frame is possible with Slice 3D-SIM mode).

## Various observation modes

## TIRF-SIM/2D-SIM mode

This mode captures super-resolution 2D images at high speed with incredible contrast. TIRF-SIM mode takes advantage of Total Internal Reflection Fluorescence observation at double the resolution as compared to conventional TIRF microscopes, facilitating a greater understanding of molecular interactions at the cell surface.

## 3D-SIM mode

Two modes are available. Slice 3D-SIM mode allows axial super-resolution imaging with optical sectioning at 300 nm resolution in live-cell specimens; Stack 3D-SIM mode can image thicker specimens with higher contrast than Slice 3D-SIM mode.

## **5** laser multi-color super-resolution capability

LU5 N-SIM 5 Laser Module is a modular system with up to five lasers enabling true multi-color super resolution. Multi-color capability is essential to the study of dynamic interactions of multiple proteins of interest at the molecular level.



Volume view



Macrophages (J774 cells expressing mVenus-SNAP23) phagocytosing opsonized beads that were incubated with Alexa555 labeled secondary antibodies after fixation. The beads without red signals are in phagosomes containing mVenus-SNAP23. Photographed with the cooperation of: Drs. Chie Sakurai, Kiyotaka Hatsuzawa and Ikuo Wada, Fukushima Medical University School of Medicine.



Luminal surface of the organ of Corti at postnatal day 1. (Mouse) Green: F-actin, red: acetylated-tubulin Photographed with the cooperation of: Drs. Kanoko Kominami, Hideru Togashi, and Yoshimi Takai, Division of Molecular and Cellular Biology, Kobe University Graduate School of Medicine/Faculty of Medicine





antibody



Maximum projection





Microtubule architecture of epithelial cell Microtubules are immunostained with anti-tubulin

Photos courtesy of: Dr. Ulrike Engel, Nikon Imaging Center at the University of Heidelberg

## Super-resolution imaging of live cell dynamics



Live-cell N-SIM imaging of mitochondria labeled with Mito-Tracker red. Live-cell imaging with N-SIM reveals dynamics of mitochondria at twice the spatial resolution. Cristae in mitochondria are also clearly observed. Mode: Slice 3D-SIM mode Objective: CFI Apochromat TIRF 100x oil (NA 1.49) Image capturing interval: approximately 1 sec. (movie)

## N-SIM images (TIRF-SIM)









192 min.

240 min.

FoLu cells (fox lung) expressing eGFP-vinculin Mode: TIRF-SIM mode

Photos courtesy of: Dr. Michael W. Davidson, National High Magnetic Field Laboratory, Florida State University

## Slice 3D-SIM mode images

Slice 3D-SIM mode is suitable for capturing time-lapse activities of living cells at specific depths.

N-SIM image (Slice 3D-SIM mode)







Bacillus subtilis bacterium stained with membrane dye Nile Red (red), and expressing the cell division protein DivIVA fused to GFP (green). N-SIM enables accurate localization of the protein during division. Photos courtesy of: Drs Henrik Strahl and Leendert Hamoen, Centre for Bacterial Cell Biology, Newcastle University

N-SIM image (Slice 3D-SIM mode)

## Conventional widefield image





Microtubules in B16 melanoma cell labeled with YFP Objective: CFI Apochromat TIRF 100x oil (NA 1.49) Image capturing speed: approximately 1.8 sec/frame (movie) Photographed with the cooperation of: Dr. Yasushi Okada, Laboratory for Cell Polarity Regulation, Quantitative Biology Center, RIKEN

## Stack 3D-SIM mode images

Stack 3D-SIM mode constructs 3D images based on Gustafsson's theory, and is suitable for acquisition of volume data.

Volume view





Width: 26.19 µm, Height: 27.11 µm, Depth: 3.36 µm

Mouse keratinocyte labeled with an antibody against keratin intermediate filaments and stained with an Alexa 488 conjugated second antibody. Photos courtesy of: Dr. Reinhard Windoffer, RWTH Aachen University



Width:16.00 µm, Height: 13.36 µm, Depth: 6.00 µm

Human U2OS cell during mitosis metaphase The cell is labeled green (kinetochore protein CENP-B), red (alpha-tubulin) and blue (DNA). Photo courtesy of: Dr. Alexey Khodjakov, Wadsworth Center, Albany NY







## The principle of the Structured Illumination Microscopy

Analytical processing of recorded moiré patterns, produced by overlaying a known high spatial frequency pattern, mathematically restores the sub-resolution structure of a specimen.

Utilization of high spatial frequency laser interference to illuminate sub-resolution structures within a specimen produces moiré fringes, which are captured. These moiré fringes include modulated information of the sub-resolution structure of the specimen. Through image processing, the unknown specimen information can be recovered to achieve resolution beyond the limit of conventional optical microscopes.



## Create super-resolution images by processing multiple moiré pattern images

An image of moiré patterns captured in this process includes information of the minute structures within a specimen. Multiple phases and orientations of structured illumination are captured, and the displaced "super-resolution" information is extracted from moiré fringe information. This information is combined mathematically in "Fourier" or aperture space and then transformed back into image space, creating an image at double the conventional resolution limit.



## Utilizing high-frequency striped illumination to double the resolution

The capture of high resolution, high spatial frequency information is limited by the Numerical Aperture (NA) of the objectives, and spatial frequencies of structure beyond the optical system aperture are excluded (Fig. A).

Illuminating the specimen with high frequency structured illumination, which is multiplied by the unknown structure in the specimen beyond the classical resolution limit, brings the displaced "super-resolution" information within the optical system aperture (Fig. B).









## **N-SIM analysis software**

N-SIM image processing, reconstruction and analysis are carried out using the N-SIM module that resides within Nikon's universal, crossplatform imaging software NIS-Elements. The NIS-Elements platform allows for the same level of intuitive operation of N-SIM that exists for other Nikon imaging systems such as confocal microscopes.



## Image acquisition

●N-SIM mode selection ●Laser power co

## Setting image acquisition

Up to five different laser wavelengths are available. User-customized spectral, z-stack, and time-lapse acquisition settings are automatically managed to allow for a simple workflow from acquisition to N-SIM image reconstruction. N-SIM image reconstruction can be further optimized by modifying reconstruction parameters post-acquisition/offline.

## Image processing

- Manual setting of N-SIM image
- reconstruction parameters Optimization of N-SIM image
- reconstruction parameters Reconstruction view
- Batch reconstruction

select the most appropriate reconstruction parameters for the acquired images to reconstruct N-SIM images. Users can further optimize reconstruction by manually adjusting these parameters.

Reconstruction view

Batch reconstruction images, and post-image acquisition.





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Setting image reconstruction Auto settings allow the software to automatically

Reconstruction view allows users to preview the results of the selected reconstructed parameters on the current/selected frame, allowing for efficient reconstruction parameter determination.

This function allows for the reconstruction of multiple N-SIM image files, including time-lapse and z-stack

## N-SIM system diagram



N-SIM specifications			
Lateral resolution (FWHM of beads in xy)	115 nm* in 3D-SIM mode		
Axial resolution (FWHM of beads in z)	269 nm* in 3D-SIM mode		
Image acquisition time	Up to 0.6 sec/frame (TIRF-SIM/2D-SIM) Up to 1 sec/frame (Slice 3D-SIM) (needs more 1-2 sec. for calculation)		
Imaging mode	TIRF-SIM (TIRF XY super resolution) 2D-SIM (XY super resolution, up to 3µm deep) Slice 3D-SIM (XYZ super resolution, up to 20µm deep) Stack 3D-SIM (XYZ super resolution, up to 50µm deep)		
Multi-color imaging	Up to 5 colors		
Compatible Laser	Standard: 488nm, 561nm Option: 405nm, 458nm, 514nm, 532nm, 640nm Laser combination: 405 nm/488 nm/514 nm/532 nm/561 nm, 405 nm/488 nm/514 nm/561 nm/640 nm, 458 nm/488 nm/514 nm/532 nm/561 nm, 458 nm/488 nm/514 nm/561 nm/640 nm		
Compatible microscope	Motorized inverted microscope ECLIPSE Ti-E Perfect Focus System Motorized XY stage with encoders Piezo Z stage		
Compatible objective	CFI SR Apochromat TIRF 100×oil (NA1.49) CFI Apochromat TIRF 100×oil (NA1.49) CFI SR Plan Apochromat IR 60×WI (NA1.27) CFI Plan Apochromat IR 60×WI (NA1.27)		
Camera	Andor Technology iXon3 897 EMCCD camera		
Software	NIS-Elements Ar/NIS-Elements C (for Confocal Microscope A1+/A1R+) Both require optional module software NIS-A N-SIM Analysis		
Operating conditions	20 °C to 28 °C ( ± 0.5 °C)		

\* These values are measured using 100nm diameter beads excited at 488nm. Actual resolution is dependent on laser wavelength and optical configuration.

## **N-SIM illumination unit**

This unit illuminates a specimen with a high spacial frequency pattern generated by a grating block and illuminates the specimen multiple times while shifting the phases and orientations of the structured illumination.



## Optional accessories for N-SIM

## Stage Top Incubator TIZSH

Seedbacks sample temperature directly to temperature control unit to provide accurate and stable sample temperature control. PC connection allows monitoring and logging of temperature and CO<sub>2</sub> concentration. (Tokai Hit Co., Ltd.)



Combining super-resolution microscope with other imaging modalities

## A1+ with N-SIM

By using the Confocal Microscope A1<sup>+</sup> and Super Resolution Microscope N-SIM in tandem, multilateral observation of the dynamics of a single live cell is possible by switching between A1+ and N-SIM. A1+ enables high-speed image acquisition, low-magnification observation and photostimulation, while N-SIM enables approximately 100 nm-resolution live-cell observation.



With N-SIM



## **Objectives for super-resolution microscopes**

The SR (Super Resolution) objectives have been designed for new applications that break the diffraction barrier.

The most recent optical designs and the best selection of optical glasses have been applied to yield optical performances with the lowest possible remaining spherical and cylindrical aberrations.



CFI SR Plan Apochromat IR 60x WI CFI SR Apochromat TIRF 100x oil

### Features

- Sample temperature range: 30°C to 40°C (at 20°C, ±2°C room temperature)
  Heater setting temperature:
- Top heater: room temperature to 50°C . Bath heater: room temperature to 50°C Stage heater: room temperature to 55°C . Feedback sensor: room temperature to 40°C Lens heater: room temperature to 45°C Accuracy: ±0.3°C (on the plate)
- Chamber humidity: RH 99% or more

## Included accessories

- UNIV-D35 dish attachment for 35mm dish
  D35-200F sensor lid for 35mm dish
- Neco temperature and gas management software

- Optional accessories TID-NA stage adapter for Ti motorized XY stage UNIV-SC dish attachment for slide glass and chamber slide UNIV-CGC dish attachment for chambered coverglass CS-200F sensor lid for chamber slide

- CGC-200F sensor lid for chambered coverglass



# N - STORM Achieving a resolution 10 times greater than a conventional optical microscope enables molecular level understanding

STochastic Optical Reconstruction Microscopy (STORM) reconstructs a super-resolution fluorescent image by combining precise localization information for individual fluorophores in complex fluorescent microscope specimens. N-STORM takes advantage of Nikon's powerful Ti-E inverted microscope and applies high-accuracy, multi-color localization and reconstruction in three dimensions (xyz) to enable super-resolution imaging at tenfold the resolution of conventional optical microscopes (up to 20 nm in xy). This powerful technology enables the visualization of molecular interactions at the nanoscopic level, opening up new worlds of scientific understanding.

## N-STORM offers 20 nm lateral resolution, a tenfold improvement over conventional optical microscopes.

N-STORM utilizes high accuracy localization information for thousands of individual fluorophores present in a field of view to create breathtaking "super-resolution" images, exhibiting spatial resolution that is 10 times greater than conventional optical microscopes.

## N-STORM also offers more than tenfold improvement in axial resolution (up to 50 nm)

In addition to lateral super-resolution, N-STORM utilizes proprietary methods to achieve a tenfold enhancement in axial resolution, effectively providing 3D information at a nanoscopic scale.

## Multi-color imaging using various fluorescent probes

Multi-color super-resolution imaging can be carried out using either tandem dye pairs that combine "activator" and "reporter" probes or standard secondary antibodies that are commercially available for continuous activation imaging. This flexibility allows users to easily gain critical insights into the localization and interaction properties of multiple proteins at the molecular level.



## Tenfold improvement in axial resolution

## N-STORM image



Fluorescence labeled microtubule 3D-STORM image of antibody-labeled microtubules. Colors encode z-depth information.



Single color 2D-STORM (continuous activation mode) image of Golgi in a BSC-1 cell labeled with Alexa647 Photos courtesy of: Dr. Michael W. Davidson, National High Magnetic Field Laboratory, Florida State University

## Conventional widefield image



Single color 3D-STORM image of mitochondria in a BSC-1 cell labeled with Alexa405-Alexa647 Color encodes z-position information

## **10 times the resolution of conventional optical microscopes**





Single color STORM image of a clathrin-coated pit in a mammalian cell labeled with Cy3-Alexa647 Objective: CFI Apochromat TIRF 100x oil (NA 1.49)

## N-STORM images



Sites of DNA synthesis in a pig kidney epithelial cell (LLC-PK1) visualized at super resolution with continuous activation imaging using Alexa647-labeled EdU. Photos courtesy of: Dr. Michael W. Davidson, National High Magnetic Field Laboratory, Florida State University

## N-STORM images









African green monkey kidney cells (BSC-1) labeled with Alexa Fluor 647 (Tubulin) and ATTO 488 (Calreticulin) Photos courtesy of: Dr. Michael W. Davidson, National High Magnetic Field Laboratory, Florida State University

## N-STORM images





Conventional widefield images





Human cervical cancer cells (HeLa S3) labeled with Alexa Fluor 647 (NUP153) and ATTO 488 (TPR) Photos courtesy of: Dr. Michael W. Davidson, National High Magnetic Field Laboratory, Florida State University











## The principle of STochastic Optical Reconstruction Microscopy

# STochastic Optical Reconstruction Microscopy (STORM) reconstructs a super-resolution image by combining high-accuracy localization information of individual fluorophores in three dimensions and multiple colors

N-STORM uses stochastic activation of relatively small numbers of fluorophores using very low-intensity light. This random stochastic "activation" of fluorophores allows temporal separation of individual molecules, enabling high precision Gaussian fitting of each fluorophore image in XY. By utilizing special 3D-STORM optics, N-STORM can also localize individual molecules along the Z-axis with high precision. Computationally combining molecular coordinates in three dimensions results in super-resolution 3D images of the nanoscopic world.

# Reconstruction of N-STORM images using localization information of individual fluorophores



## High-precision Z-axis position detection

Using a cylindrical lens that asymmetrically condenses light beams in either X or Y direction, Z-axis molecule locations can be determined with an accuracy of about 50 nm. Location in Z is determined by detecting the orientation of the astigmatism-induced stretch in the X or Y direction and the size of the out-of-focus point images. 3D fluorescent images can be reconstructed by combining the determined Z-axis location information with XY-axis location information.



# Dedicated tandem-dye pairs for highest localization accuracy

N-STORM uses dedicated fluorescent dye pairs containing an "activator" (relatively short wavelength excitation) and a "reporter" (relatively long wavelength excitation), which enables various color combinations, facilitating multi-channel super resolution. N-STORM can also be carried out using conventional single-dye conjugated antibodies for continuous activation imaging.



STEP 1 Inactivates all molecules Cy2 Alexa647 Target molecule STEP 2 Alexa647 is randomly activated by irradiating Cv2 with sity light Cy2 Alexa647 Target molecule STEP 3 Excite Alexa647 with strong light and capture images of localization information Cv2 Alexa647 Repeat more Target molecule han 1.000 time

## **N-STORM analysis software**

Nikon's imaging software NIS-Elements and N-STORM Analysis offer various operations, from N-STORM image acquisition to image reconstruction. During image acquisition, live wide-field and reconstructed N-STORM images, as well as the number of localized molecules, can be viewed in real time.



N-STORM image acquisition dialog box

## Image acquisition

Image acquisition setting Simple changeover between 2D-STORM and 3D-STORM image acquisition mode is possible.

## Setting image acquisition conditions

Simultaneous acquisition of multicolor images is possible. In continuous mode, high-speed acquisition of N-STORM images using a single dye is also possible.

## Real time display of localizations per frame

During N-STORM image acquisition, the number of localized fluorescent molecules is displayed in real time using images and graphs. Clicking the Auto LP (Auto Laser Power) button automatically adjusts laser power, depending on the number of localized fluorescent spots.

# N-STORM



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## Image analysis

## Batch processing analysis

Simultaneous analysis of multiple N-STORM images is possible.

## **Crosstalk subtraction**

Subtracts fluorescent spots resulting from excitation crosstalk. After adjusting crosstalk subtraction settings, the resulting image appears immediately.

## N-STORM image display type

Three types of display are available: Gaussian, cross or Gaussian and cross.

## 3D display

A major feature of N-STORM is 3D super-resolution image acquisition and analysis. Acquired images can be displayed at any angle after analysis.

### Image magnification

Selected areas of images can be magnified by up to 20,000%.

## **N-STORM** system diagram



N-STORM Specifications				
XY resolution	Approximately 20 nm			
Z-axis resolution	Approximately 50 nm			
Imaging mode	2D-STORM 3D-STORM			
Multi-color imaging	3 colors simultaneously			
Compatible Laser	405nm, 457nm, 488nm, 561nm, 647nm			
Compatible microscope	Motorized inverted microscope ECLIPSE Ti-E Perfect Focus System Motorized XY stage with encoders Piezo Z stage			
Compatible objective	CFI SR Apochromat TIRF 100×oil (NA1.49) CFI Apochromat TIRF 100×oil (NA1.49) CFI Plan Apochromat VC 100xoil (NA1.40)			
Camera	Andor Technology iXon3 897 EMCCD camera			
Software	NIS-Elements Ar/ NIS-Elements C (for Confocal Microscope A1+/A1R+) Both require optional module software NIS-A N-STORM Analysis			
Operating conditions	20 °C to 25 °C ( ± 0.5 °C)			

## Motorized N-STORM/TIRF illumination unit

### This unit allows laser incident angle adjustment, shutter control and switchover to widefield fluorescence excitation using a Ti microscope control pad or NIS-Elements software.





Combining super-resolution microscope with other imaging modalities

## A1+ with N-STORM

With a confocal microscope such as the A1<sup>+</sup> or C2<sup>+</sup>, high-speed image acquisition, low-magnification observation, photostimulation, etc., of live cells are possible. The Super Resolution Microscope N-STORM enables acquisition of minute 3D information with 20 nm-resolution observation. This system also enables TIRF imaging.

## **N-SIM with N-STORM**

N-SIM and N-STORM can be combined on a single inverted microscope to create the ultimate super-resolution imaging system. Using the N-SIM/N-STORM kit, switching between the two super-resolution modes is possible without having to change the camera adapter.

N-SIM/N-STORM kit ositions can be selected for N-SIM, ional laser TIRF/2D-STORM and





## Side port for N-STORM

Switching between 2D/3D-STORM imaging and conventional widefield imaging is possible with the cylindrical lens IN/OUT across the optical path.

## **Objective for super-resolution** microscopes

The SR (Super Resolution) objective has been designed for new applications that break the diffraction barrier. The most recent optical designs and the best selection of optical glasses have been applied to yield optical performances with the lowest possible remaining spherical and cylindrical aberrations.



CFI SR Apochromat TIRF 100x oil



