

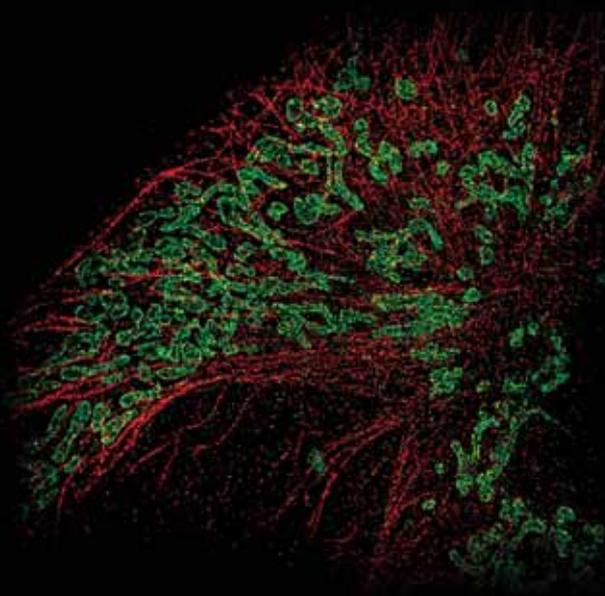


Super Resolution Microscope N-SIM/N-STORM



N-SIM/N-STORM

Super Resolution Microscope





New Vision, New Horizons

See like you have never seen before



Nikon's Super-Resolution Microscopes bring your research into the world of Nanoscopy beyond the diffraction limit.

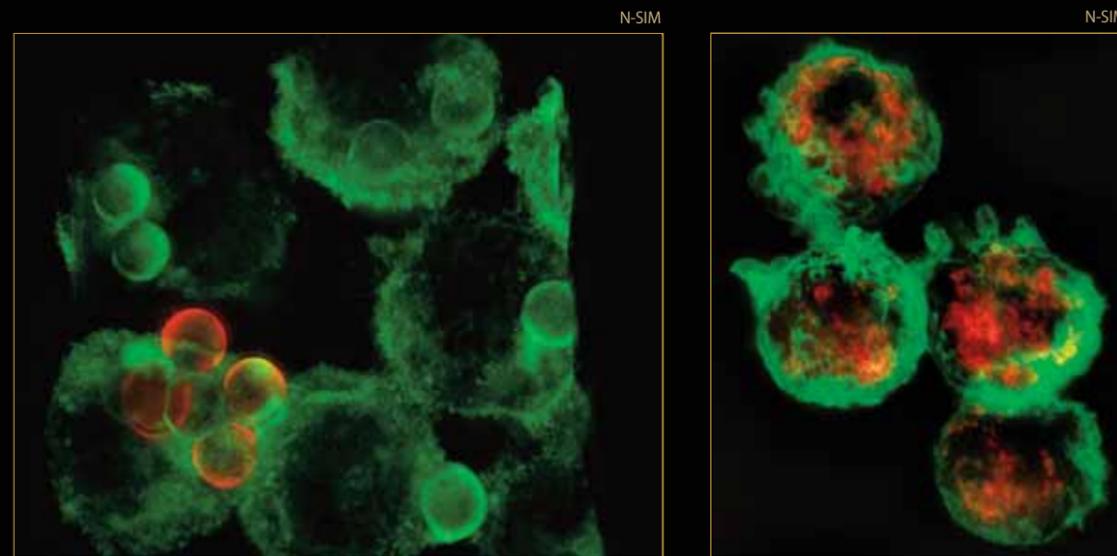
Nikon's new Super-Resolution Microscope N-SIM/N-STORM enables elucidation of the structures and functions of nanoscopic machinery within living cells. The resolution of conventional optical microscopes, even with the highest numerical aperture optics, is limited by diffraction to approximately 200nm.

Using high frequency Structured Illumination, the Nikon N-SIM can achieve an image resolution of 85nm*, 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 on the N-SIM.

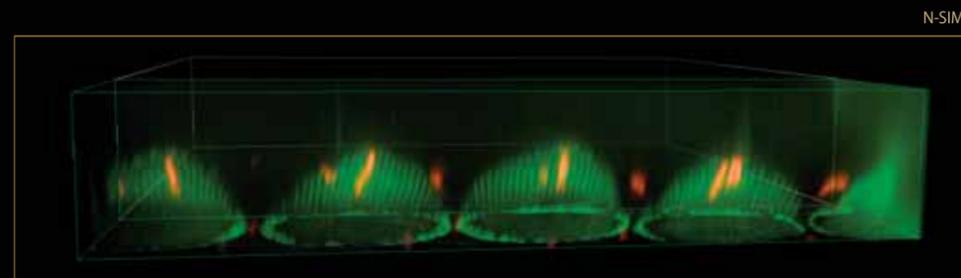
N-STORM trades off temporal resolution for spatial resolution, realizing an incredible image resolution of approx. 20nm, which is 10 times or more than that of conventional optical microscopes. Utilizing STochastic Optical Reconstruction Microscopy (STORM) it is now possible to gain insight into protein-protein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

Nikon's super-resolution microscopes integrate powerful proprietary technologies into streamlined platforms that are designed to be easy to use. N-SIM and 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.

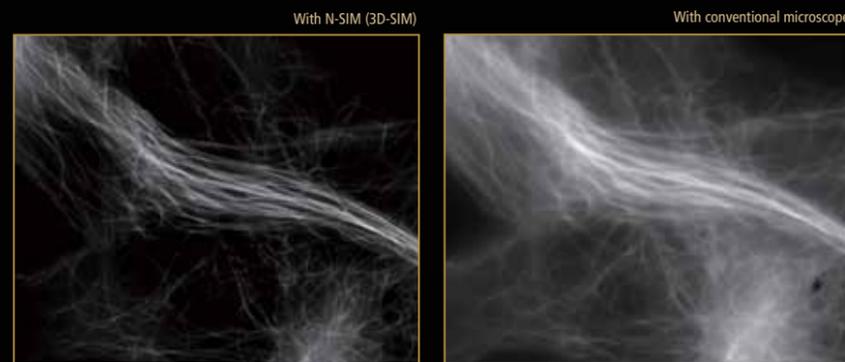
*Excited with 488nm laser, in TIRF-SIM mode
** With 2D-SIM/TIRF-SIM mode



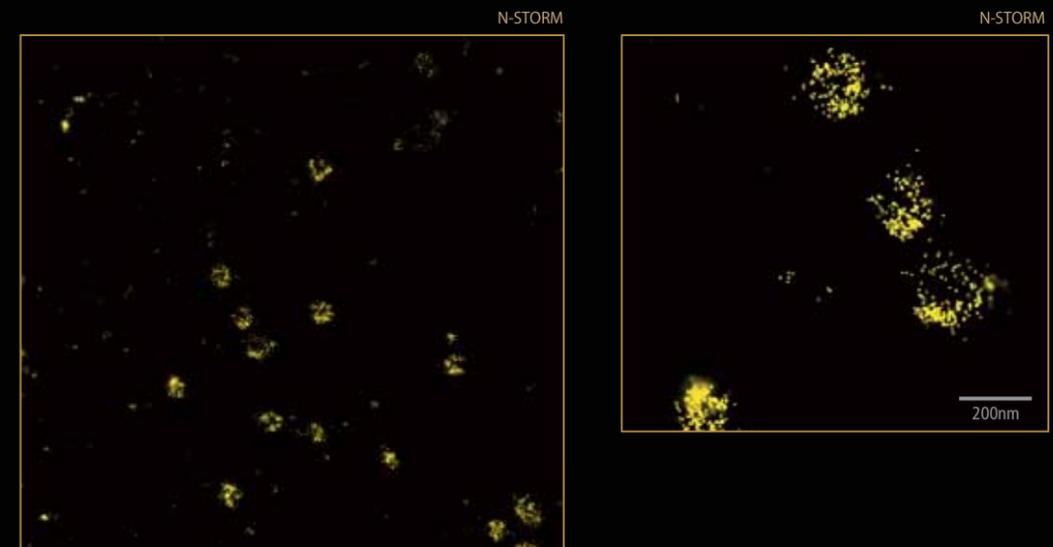
Macrophages (J774 cells expressing mVenus-SNAP23) phagocytosing opsonized beads that were incubated with Alexa555 labeled secondary antibodies after fixation. The beads without red signals are in phagosomes containing mVenus-SNAP23. Photographed with the cooperation of: Drs. Chie Sakurai, Kiyotaka Hatsuzawa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



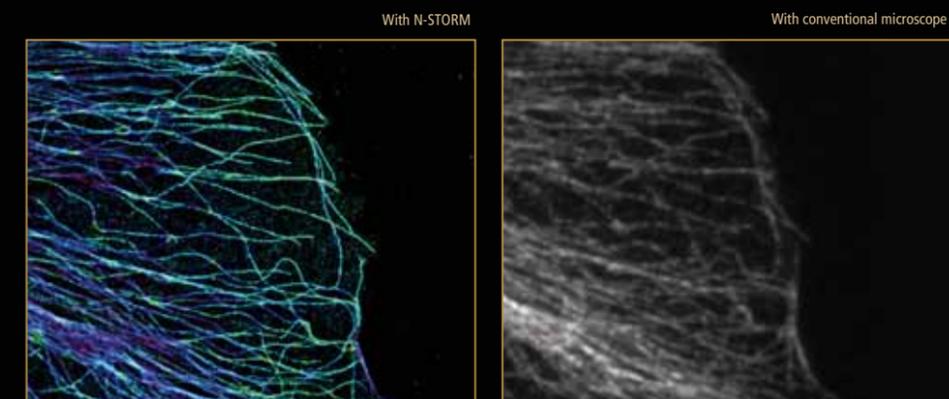
Luminal surface of the organ of Corti at postnatal day 1. (Mouse)
Green: F-actin, red: acetylated-tubulin
Photographed with the cooperation of: Drs. Kanoko Kominami, Hideru Togashi, and Yoshimi Takai, Division of Molecular and Cellular Biology, Kobe University Graduate School of Medicine/Faculty of Medicine



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



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



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

N-SIM Temporal resolution of 0.6 sec/frame enables super-resolution time-lapse imaging of dynamic live cell events

In Structured Illumination Microscopy, 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 Microscopy (N-SIM) realizes super resolution of up to 85nm 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 (to approx. 85nm) the resolution of conventional optical microscope

The N-SIM super resolution microscope utilizes Nikon's innovative new approach to "Structured Illumination Microscopy" technology.

By pairing this powerful technology with Nikon's renowned CFI Apo TIRF 100x oil objective lens (NA 1.49), N-SIM nearly doubles (to approx. 85nm*) the spatial resolution of conventional optical microscopes, and enables detailed visualization of the minute intracellular structures and their interactive functions.

* Excited with 488nm laser, in TIRF-SIM mode

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

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 approx. 1 sec/frame is possible with 3D-SIM mode).

Various observation modes

TIRF-SIM/2D-SIM mode

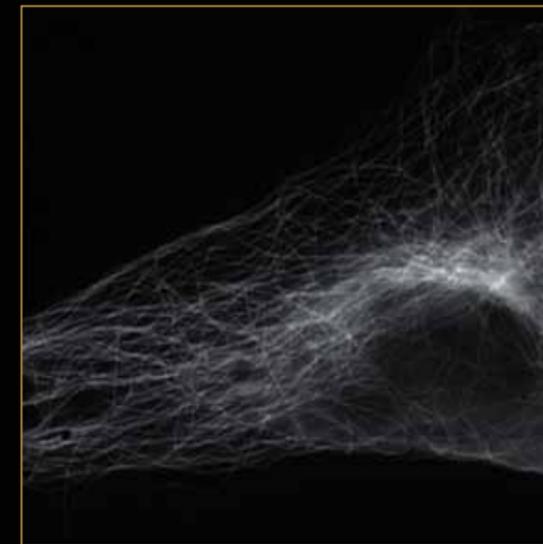
This mode captures super-resolution 2D images at high speed with incredible contrast. TIRF-SIM takes advantage of Total Internal Reflection Fluorescence observation at double the resolution as compared to conventional TIRF microscopes, facilitating a greater understanding of molecular interactions at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

3D-SIM mode

Axial super-resolution observation using the N-SIM system enables optical sectioning of specimens at 300nm resolution in cells and tissues of up to 20µm thickness. Additionally 3D SIM eliminates out of focus background fluorescence resulting in breathtaking contrast.

5 laser multi-color super-resolution capability

The Nikon LU-5 is a modular system with up to 5 lasers enabling true multi-spectral super resolution. Multi-spectral capability is essential to the study of dynamic interactions of multiple proteins of interest at the molecular level.



Microtubules in B16 melanoma cell
Mode: 3D-SIM
Objective: CFI Apo TIRF 100x oil (NA 1.49)
Image capturing speed: approx. 1.8 sec/frame
Photographed with the cooperation of: Dr. Yasushi Okada, Laboratory for Cell Polarity Regulation, Quantitative Biology Center, RIKEN

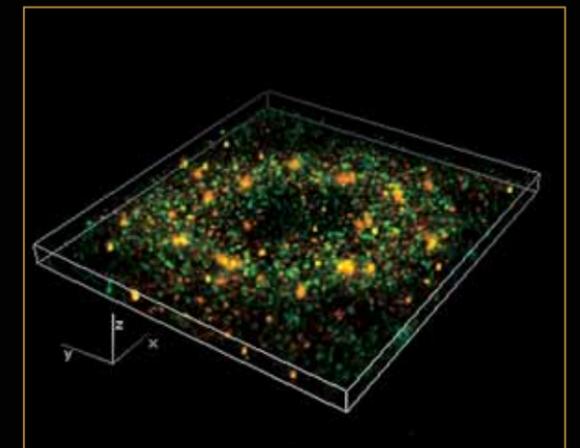
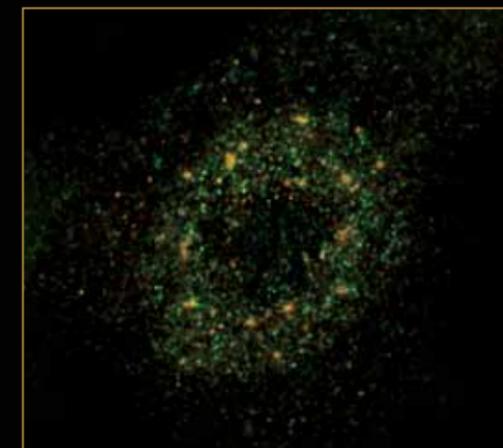


With conventional TIRF



With TIRF-SIM

Plasma membrane of B16 melanoma cell labeled with YFP
Objective: CFI Apo TIRF 100x oil (NA 1.49)
Photographed with the cooperation of: Dr. Yasushi Okada, Laboratory for Cell Polarity Regulation, Quantitative Biology Center, RIKEN

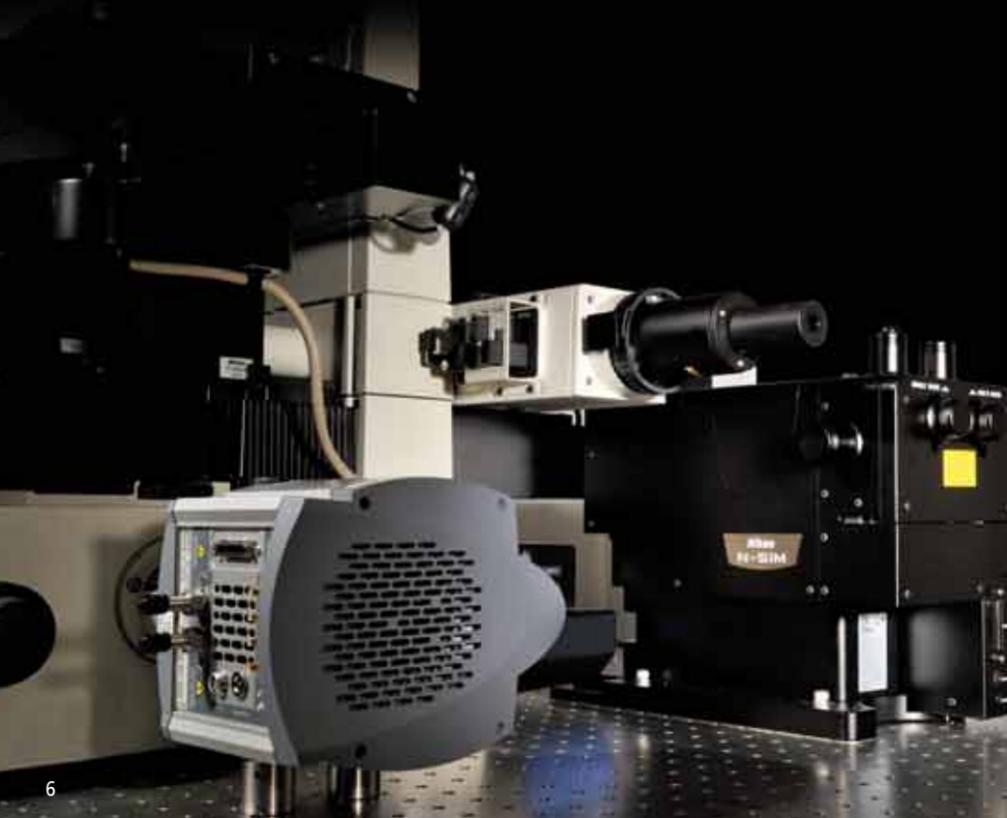


Co-localization images of a target protein of VEGF signaling (Cy3) and its ubiquitin E3 ligase (FITC)
Unprecedented insights are gained into the localization and organization of these structures inside the nucleus
Mode: 3D-SIM, Z-stack
Objective: CFI Apo TIRF 100x oil (NA 1.49)
Photographed with the cooperation of: Drs. Hidetaka Ohnuki and Shigeki Higashiyama, Ehime University Graduate School of Medicine

3D reconstruction image



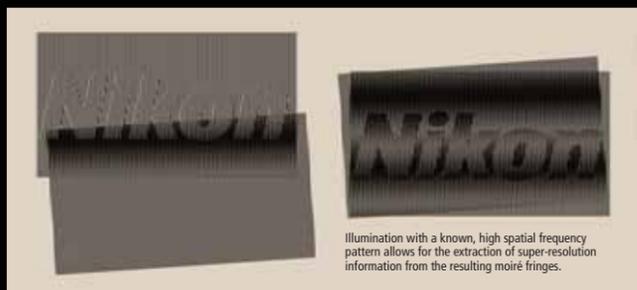
Live-cell N-SIM imaging of mitochondria labeled with Mito-Tracker red.
Live-cell imaging with N-SIM reveals dynamics of mitochondria at twice the spatial resolution. Cristae in mitochondria are also clearly observed.
Mode: 3D-SIM
Objective: CFI Apo TIRF 100x oil (NA 1.49)
Image capturing interval: approx.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



The principle of the Structured Illumination Microscopy

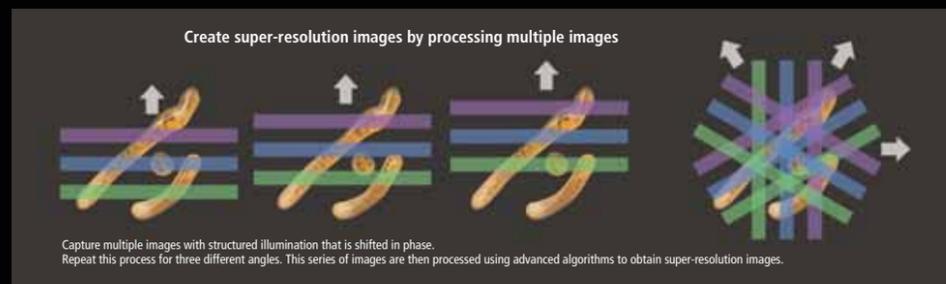
Analytical processing of recorded moiré patterns, produced by overlaying a known high spatial frequency pattern, mathematically restores the sub-resolution structure of a specimen.

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

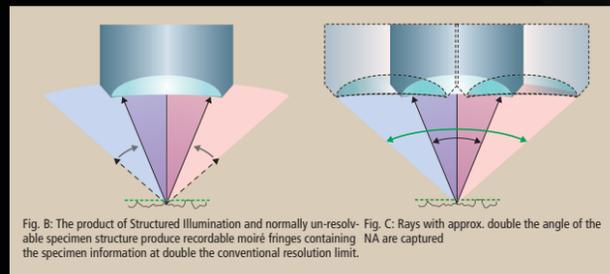
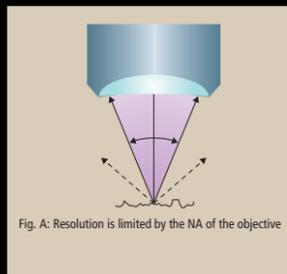
An image of moiré patterns captured in this process includes information of the minute structures within a specimen. Multiple phases and orientations of structured illumination are captured, and the displaced "super-resolution" information is extracted from moiré fringe information. This information 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

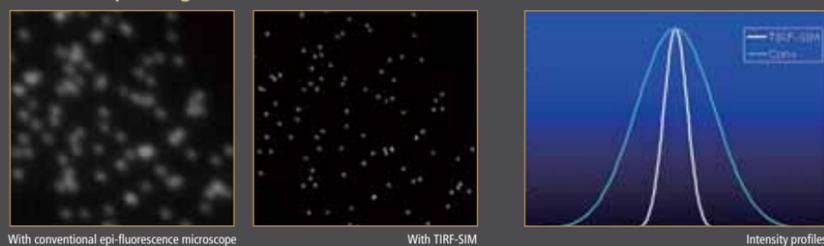
The capture of high resolution, high spatial frequency information is limited by the Numerical Aperture (NA) of the objectives, and spatial frequencies of structure beyond the optical system aperture are excluded (Fig. A).

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).

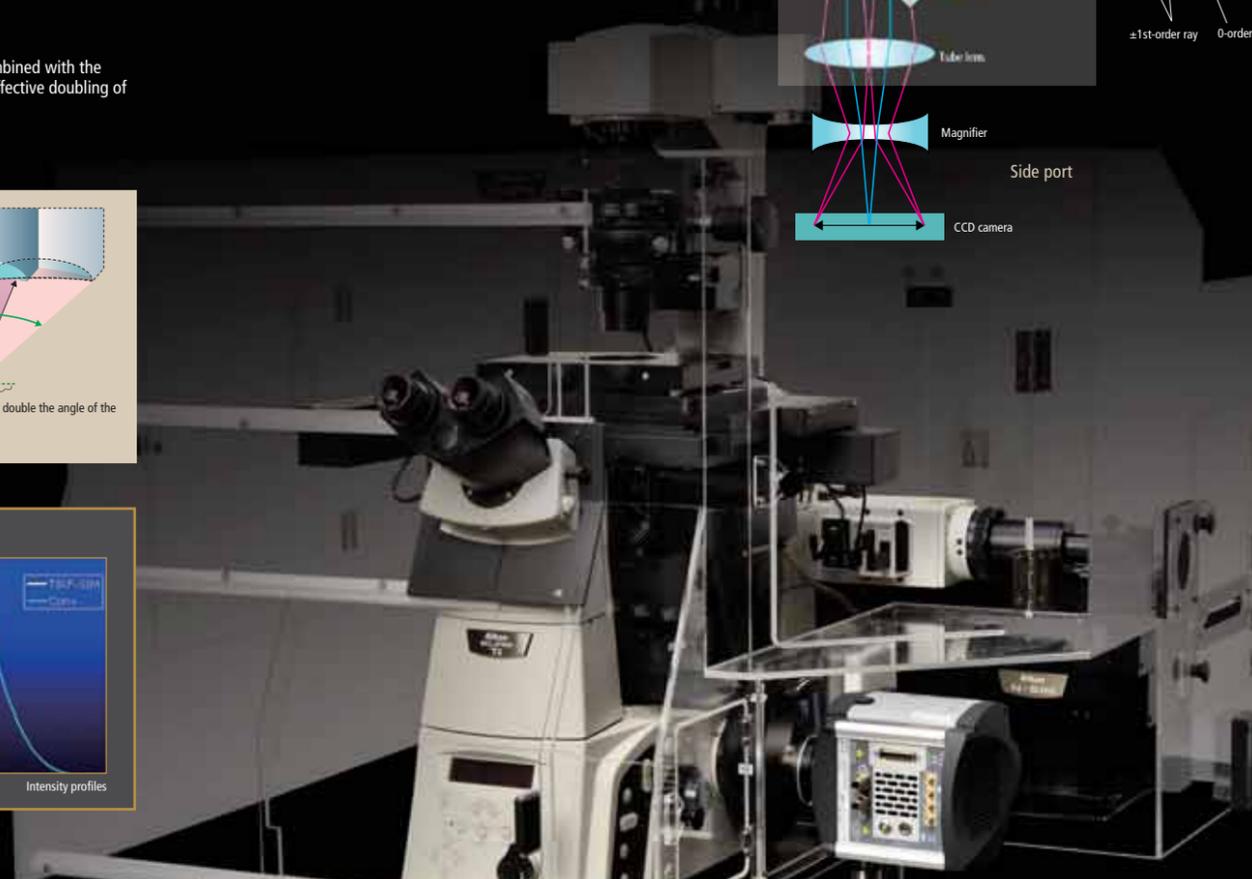
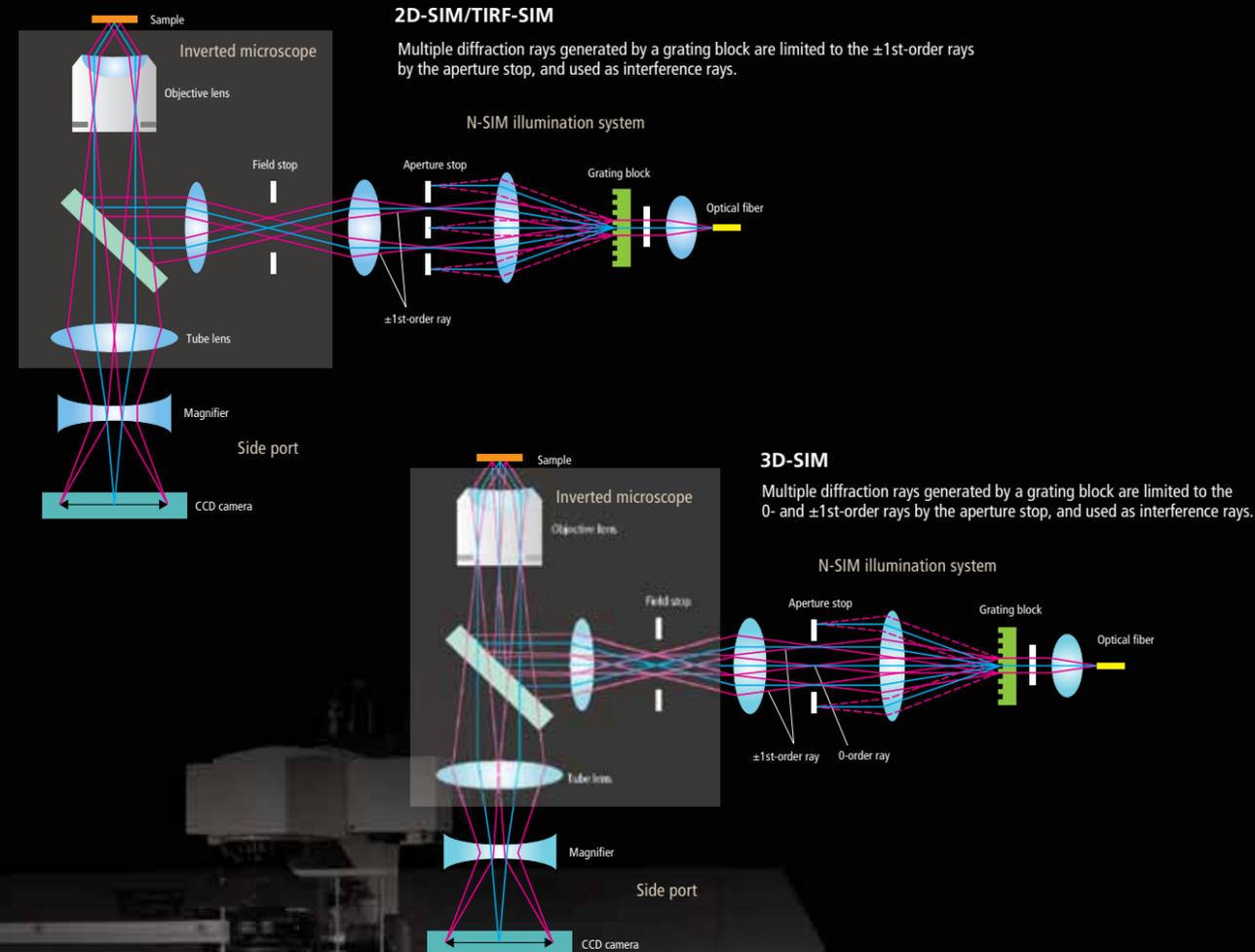


Comparison of TIRF-SIM versus conventional microscope images

Images of diameter 100nm fluorescent beads captured with a conventional microscope and super-resolution microscope N-SIM. The intensity profiles of single point images indicate that the resolving power of the super-resolution microscope is about double that of the conventional epi-fluorescence microscope.

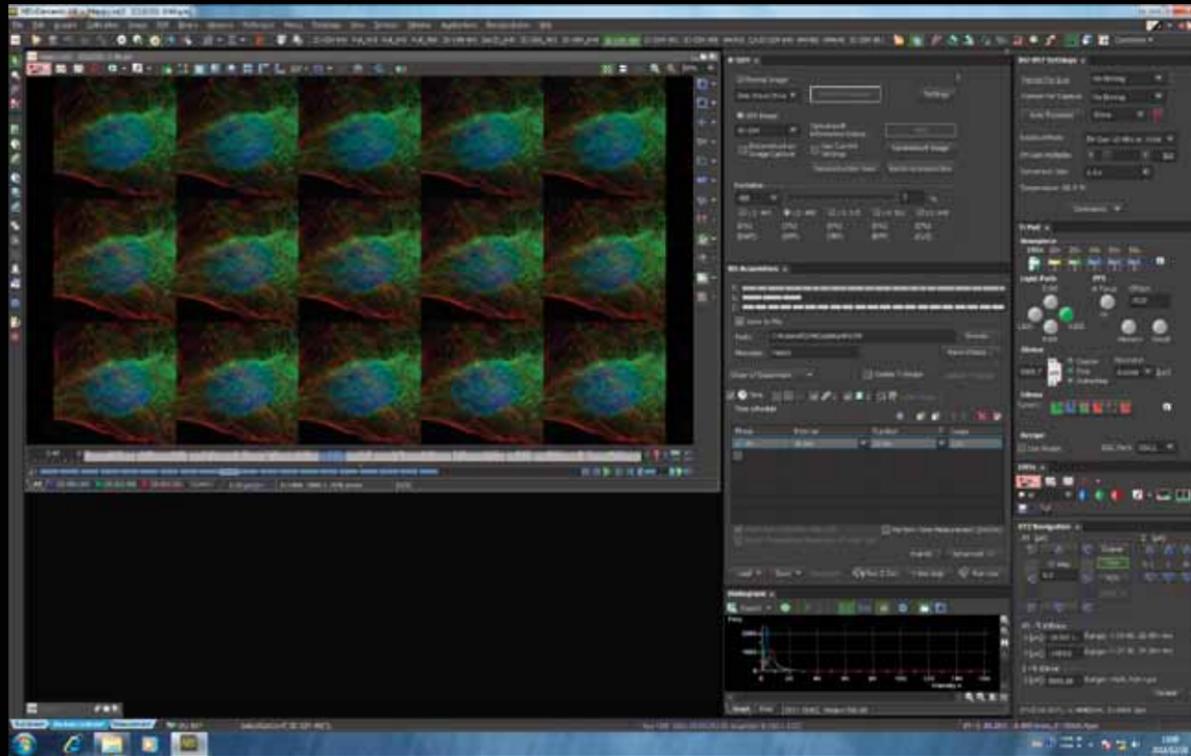


Optical layout of N-SIM



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, cross-platform 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.



N-SIM image acquisition (3D-SIM)

Image acquisition

- N-SIM mode selection
- Laser power control
- Setting imaging options



N-SIM main GUI

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

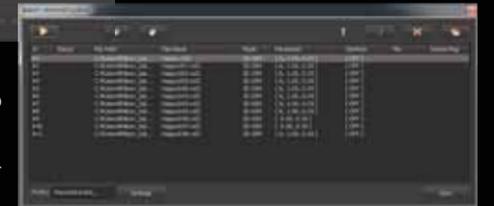
Setting image reconstruction

Auto settings allow the software to automatically 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

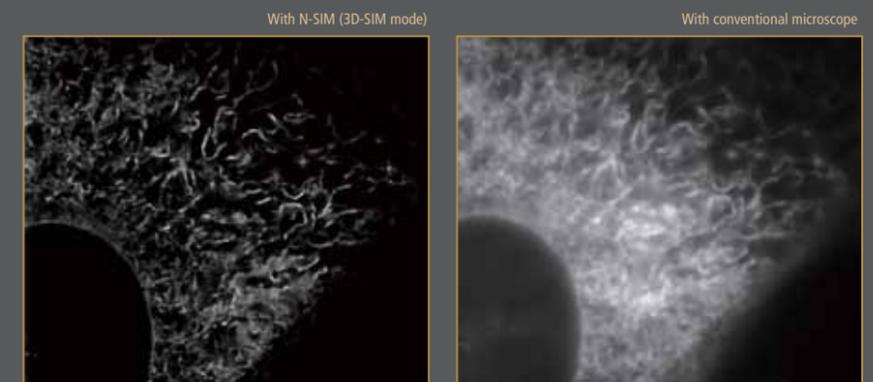
Reconstruction view allows users to preview the results of the selected reconstructed parameters on the current/selected frame, allowing for efficient reconstruction parameter determination.



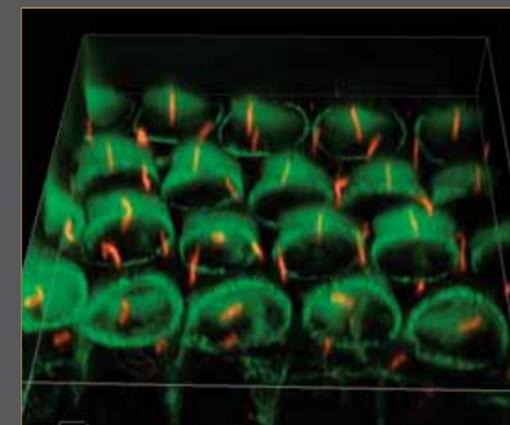
Batch reconstruction

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

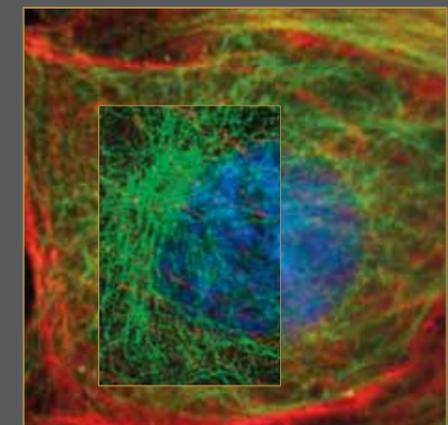
N-SIM sample images



Endoplasmic reticulum (ER) in living HeLa cell labeled with GFP
Objective: CFI Apo TIRF 100x oil (NA 1.49) Image capturing speed: approx. 1.5 sec/frame (movie)
Photographed with the cooperation of: Dr. Ikuro Wada, Institute of Biomedical Sciences, Fukushima Medical University School of Medicine

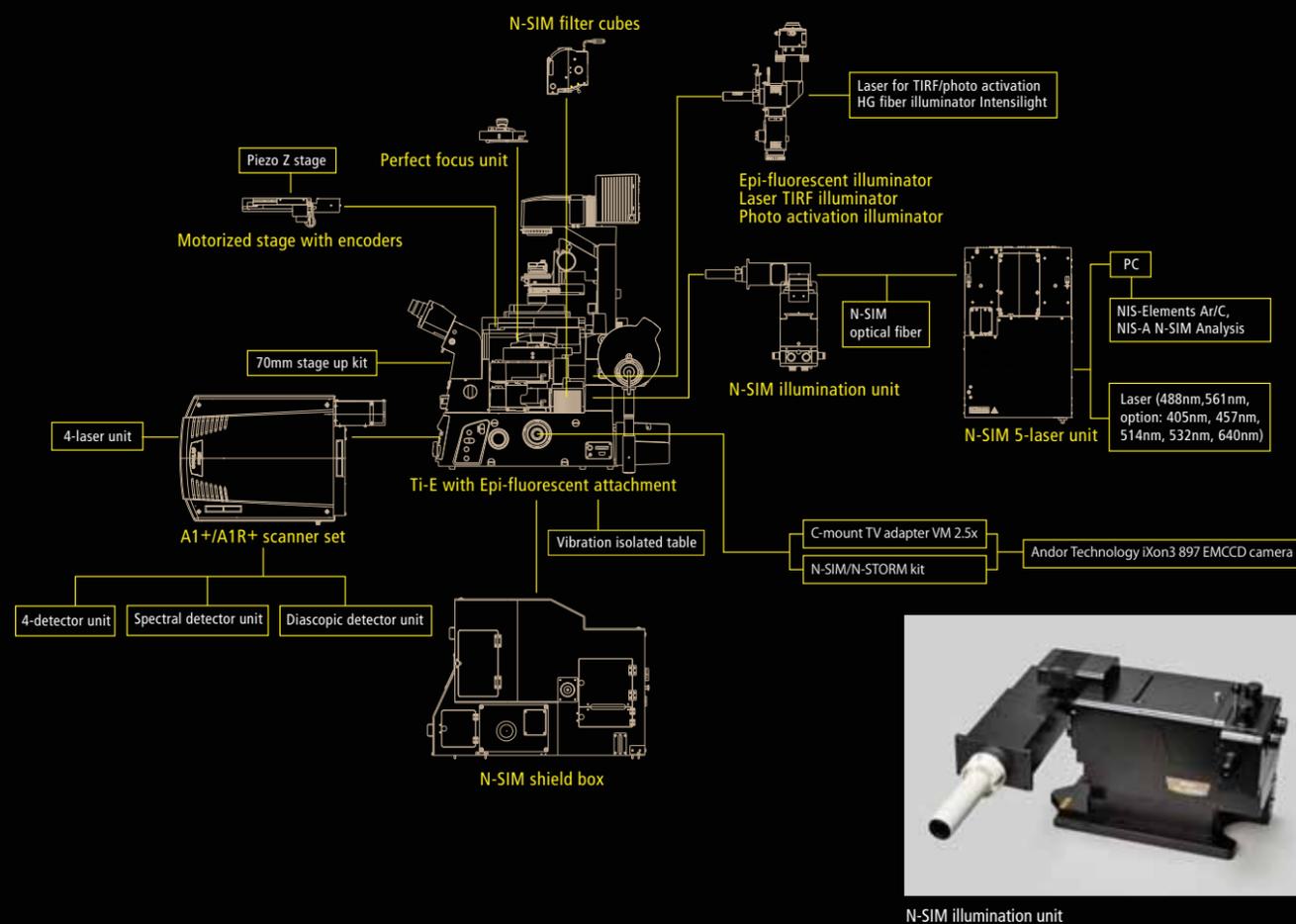


Luminal surface of the organ of Corti at postnatal day 1. (Mouse)
Green: F-actin, red: acetylated-tubulin
Photographed with the cooperation of: Drs. Kanoko Kominami, Hideru Togashi, and Yoshimi Takai, Division of Molecular and Cellular Biology, Kobe University Graduate School of Medicine/Faculty of Medicine



Comparison image of conventional fluorescent image (outside) and 3D-SIM reconstruction image (inside).
Laser: 405nm, 488nm, 561nm

N-SIM system diagram



N-SIM specifications

XY resolution	Approx. 100nm (up to 85nm: theoretical, in TIRF-SIM mode 488nm excitation)
Z-axis resolution	Approx. 300nm
Image acquisition time	Up to 0.6 sec/frame (TIRF-SIM/2D-SIM) Up to 1 sec. (3D-SIM) (needs more 1-2 sec. for calculation)
Imaging mode	TIRF-SIM (TIRF XY super resolution) 2D-SIM (XY super resolution, up to 3 μm deep) 3D-SIM (XYZ super resolution, up to 20 μm deep)
Multi-color imaging	Up to 5 colors
Compatible Laser	Standard: 488nm, 561nm Option: 405nm, 457nm, 514nm, 532nm, 640nm Laser combination: 458nm/488nm/514nm/532nm/561nm, 405nm/488nm/514nm/532nm/561nm, 405nm/488nm/514nm/561nm/640nm, 458nm/488nm/514nm/561nm/640nm
Compatible microscopes	Motorized inverted microscope ECLIPSE Ti-E Perfect Focus System Motorized XY stage with encoders Piezo Z stage
Objectives	CFI Apo TIRF 100×H (NA1.49) CFI Plan Apo IR 60×WI (NA1.27)
Camera	Andor Technology iXon3 897 EMCCD camera
Software	NIS-Elements Ar/NIS-Elements C (with confocal microscope A1+/A1R+) Both require NIS-A N-SIM Analysis
Operating conditions	20 °C to 25 °C (± 0.5 °C)

Stage top incubator for N-SIM TiZ-SH (optional)

Feedbacks sample temperature directly to temperature control unit to provide accurate and stable sample temperature control. PC connection allows monitoring and logging of temperature and CO₂ concentration. (Tokai Hit Co., Ltd.)



Features

- Sample temperature range: 7°C to 40°C (at 20°C to 25°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

Layout Unit: mm



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 10 times the resolution of conventional microscopes (~20nm 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 20nm lateral resolution, a tenfold improvement over conventional optical microscopes.**

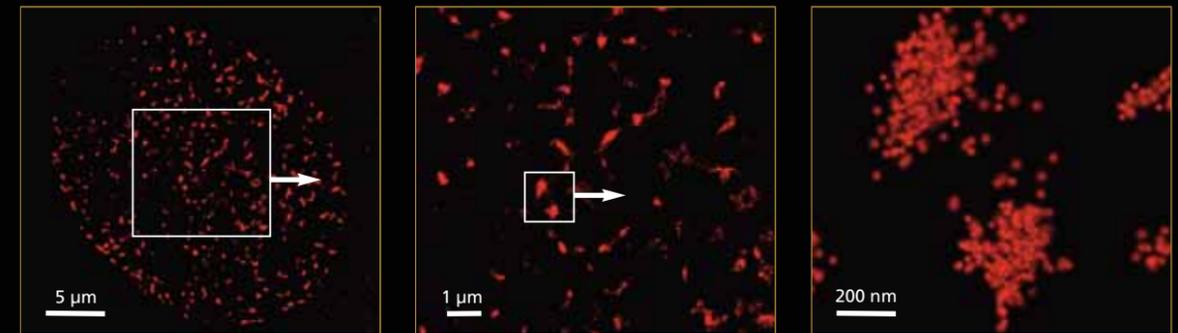
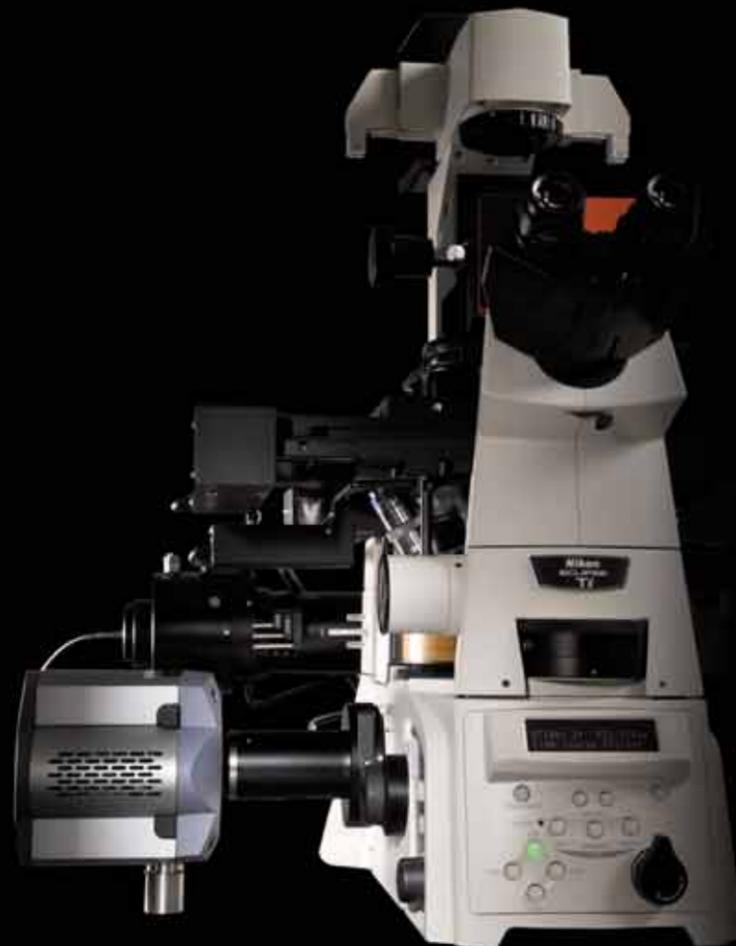
N-STORM utilizes high accuracy localization information for thousands of individual fluorophores present in a field of view to create breathtaking "super-resolution" images, exhibiting spatial resolution that is 10 times greater than conventional optical microscopes.

■ **N-STORM also offers more than tenfold improvement in axial resolution (~50nm)**

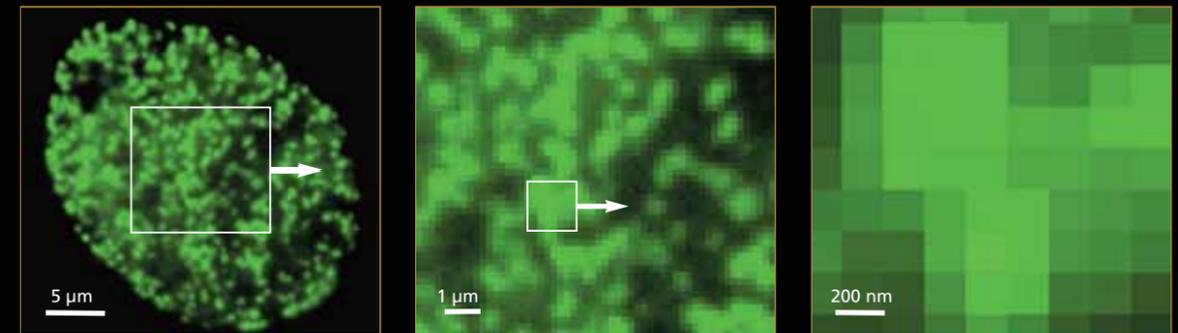
In addition to lateral super-resolution, N-STORM utilizes proprietary methods to achieve a 10 fold enhancement in axial resolution, effectively providing 3D information at a nanoscopic scale.

■ **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.

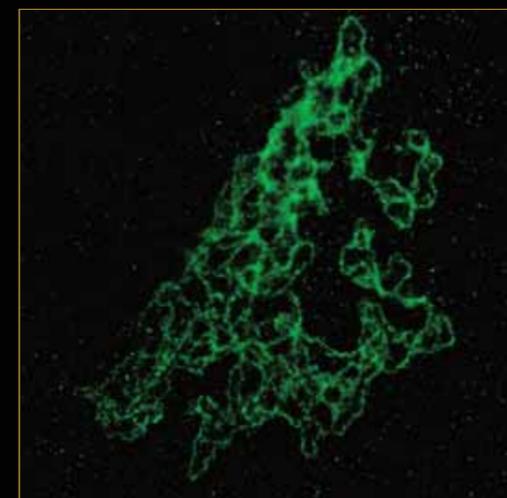


N-STORM images

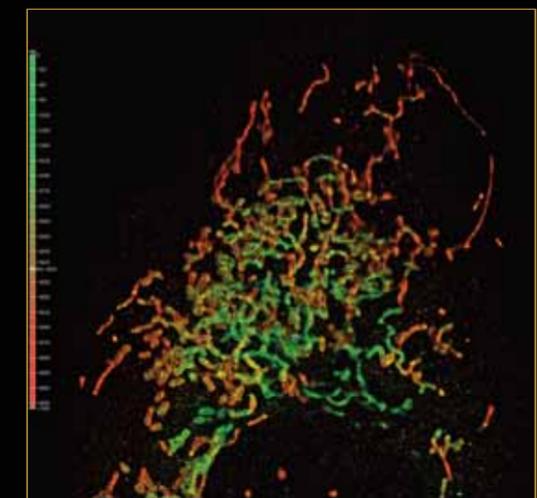


Conventional widefield images

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



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



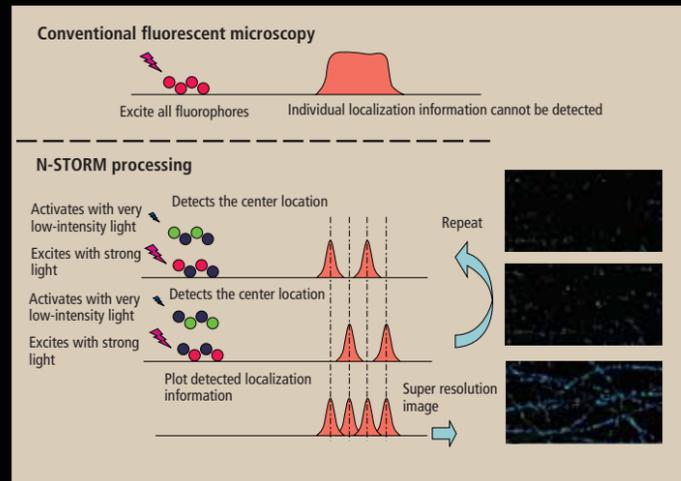
Single color 3D-STORM image of mitochondria in a BSC-1 cell labeled with Alexa405-Alexa647. Color encodes z-position information

The Principle of N-STORM (STochastic Optical Reconstruction Microscopy)

STochastic Optical Reconstruction Microscopy (STORM) reconstructs a super-resolution image by combining high-accuracy localization information of individual fluorophores in 3 spatial dimensions and multiple colors

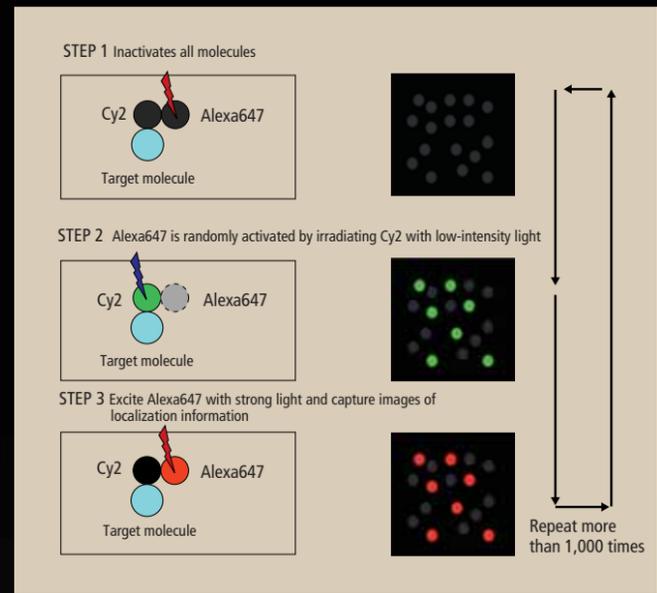
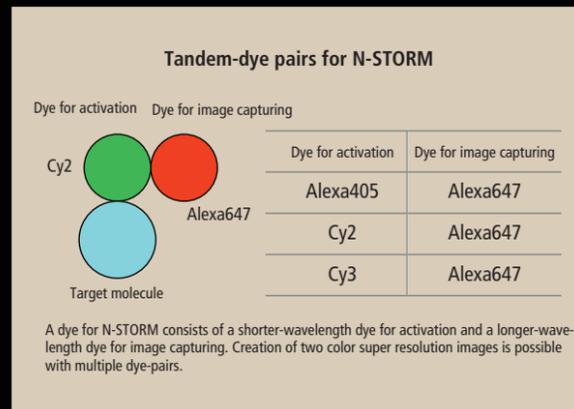
N-STORM uses stochastic activation of relatively small numbers of fluorophores using very low-intensity light. This random stochastic "activation" of fluorophores allows temporal separation of individual molecules, enabling high precision Gaussian fitting of each fluorophore image in XY. By utilizing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

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



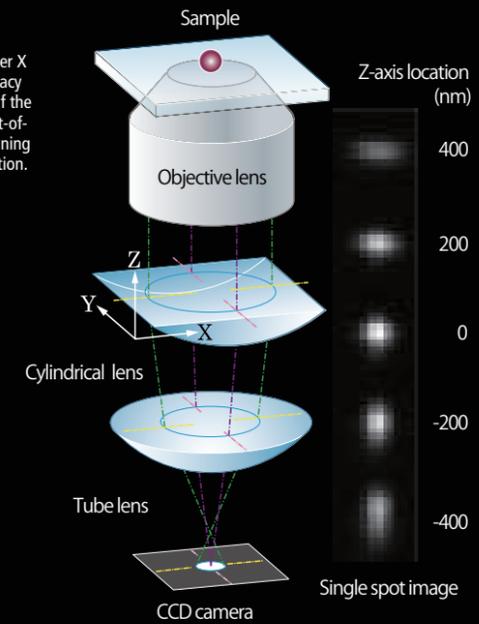
Dedicated tandem-dye pairs for highest localization accuracy

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.

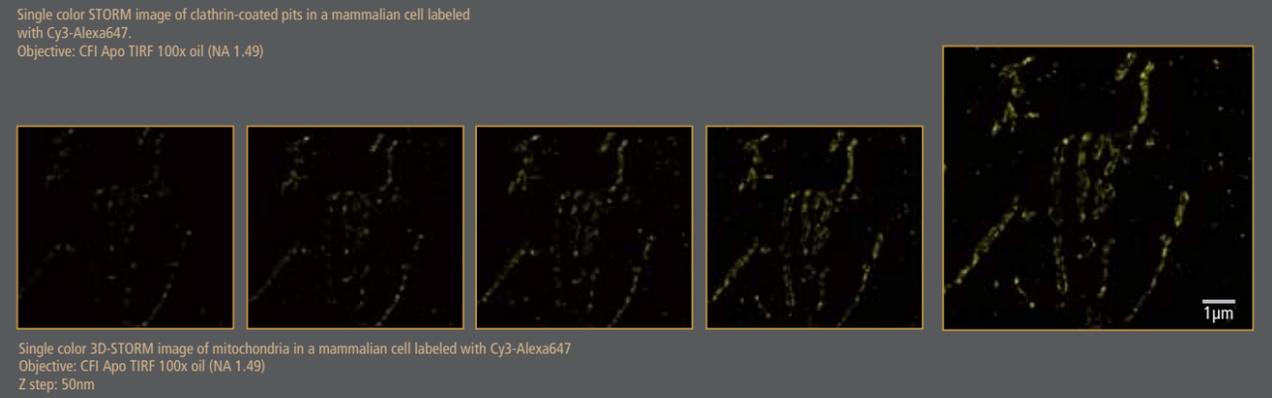
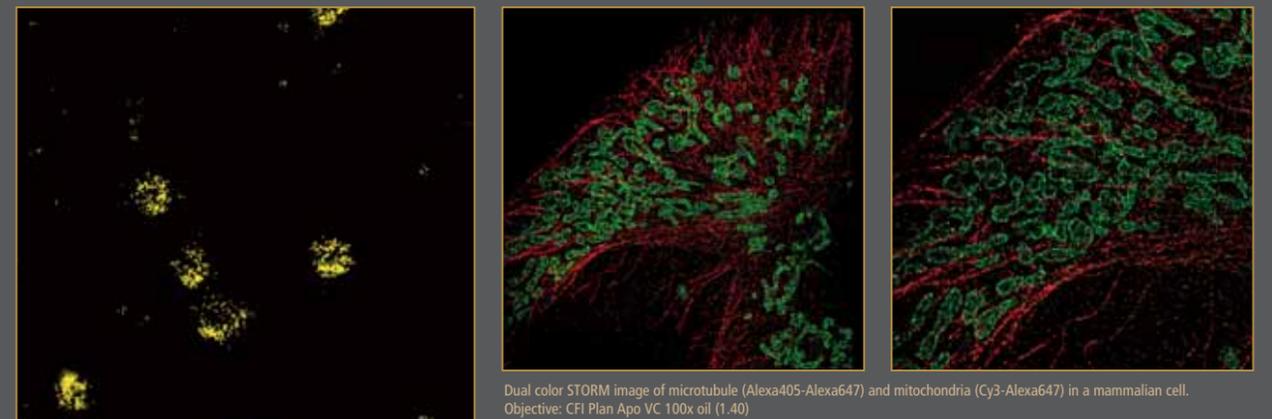


High-precision Z-axis position detection

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 50nm. 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.



N-STORM sample images



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 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.



2D-STORM

3D-STORM

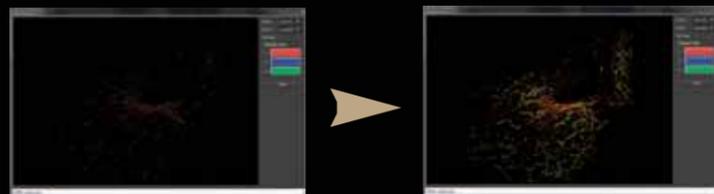
Setting image acquisition conditions

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.



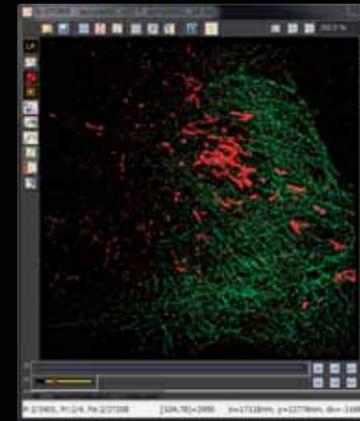
Two minutes after starting image acquisition

Five minutes after starting image acquisition

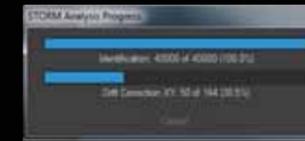


Fluorescent spot number display (graph)

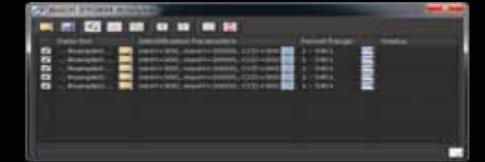
Image analysis



Analysis display



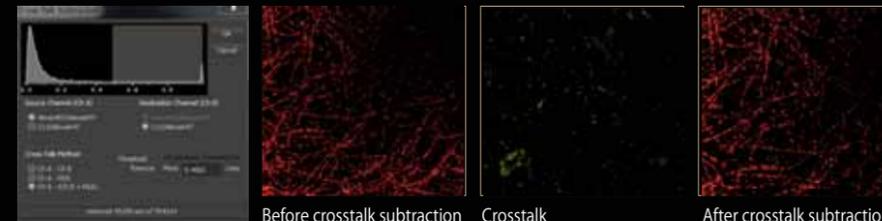
Detects number of fluorescent spots and corrects XY drift, and then constructs N-STORM image.



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.



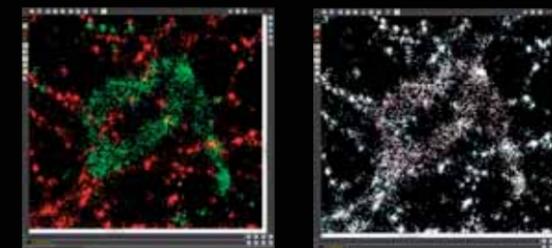
Before crosstalk subtraction

Crosstalk

After crosstalk subtraction

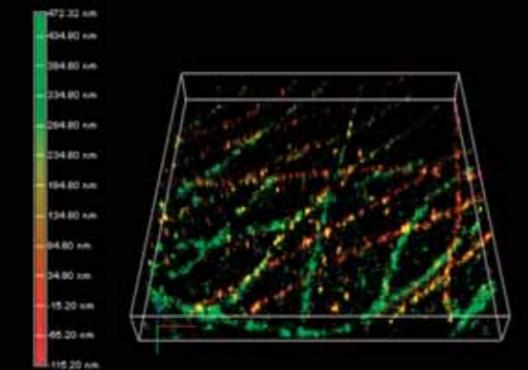
N-STORM image display type

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



Gaussian display mode

Gaussian and cross display mode

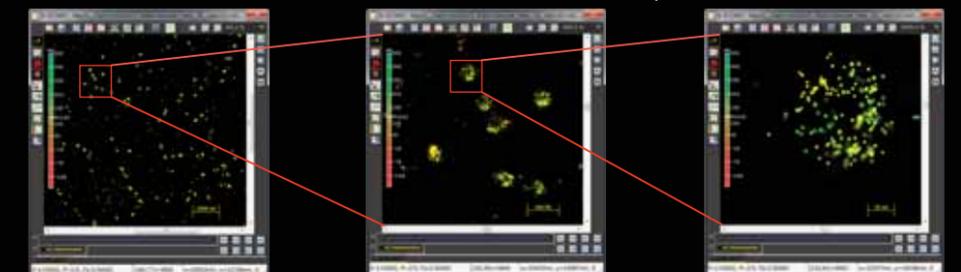


3D display

A major feature of N-STORM is 3D super-resolution image acquisition and analysis. Acquired images can be displayed at any angle after analysis. (Colors of scale bar indicate Z-position)

Image magnification

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

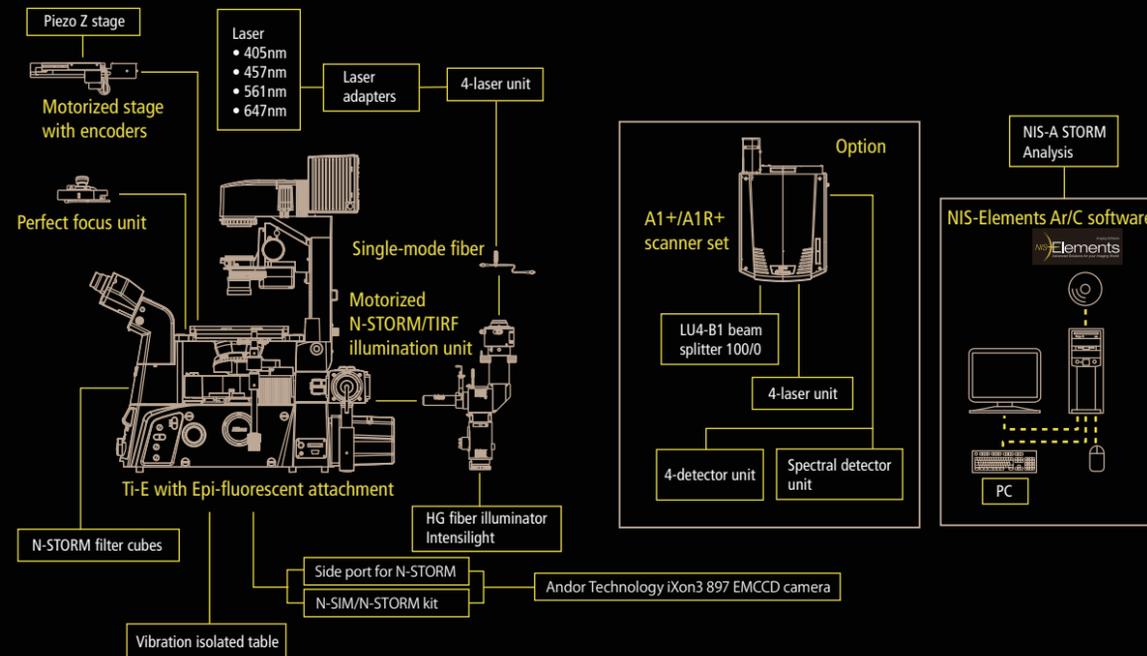


500%

3,000%

20,000%

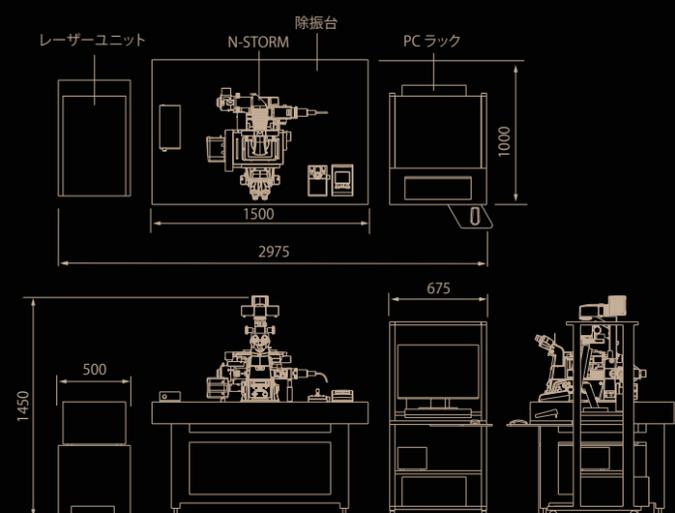
N-STORM system diagram



N-STORM Specifications

XY resolution	Approx. 20nm
Z-axis resolution	Approx. 50nm
Imaging mode	2D-STORM 3D-STORM
Multi-color imaging	2 colors simultaneously
Compatible Laser	405nm, 457nm, 561nm, 647nm
Compatible microscopes	Motorized inverted microscope ECLIPSE Ti-E Perfect Focus System Motorized XY stage with encoders Piezo Z stage
Objectives	CFI Apo TIRF 100×H (NA1.49) CFI Plan Apo VC 100xH (NA1.40)
Camera	Andor Technology iXon3 897 EMCCD camera
Software	NIS-Elements Ar/ NIS-Elements C (with confocal microscope A1+/A1R+) Both need the NIS-A STORM Analysis
Operating conditions	20 °C to 25 °C (± 0.5 °C)

Layout Unit: mm



Motorized N-STORM/TIRF illumination unit



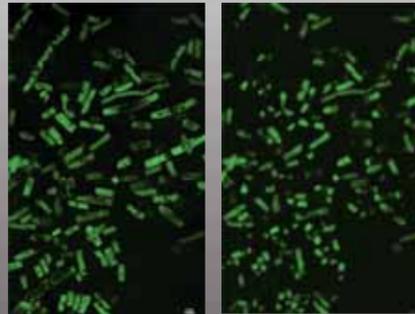
Side port for N-STORM

Combining super resolution with other imaging modalities

Various illumination systems can be combined on the Nikon Ti-E inverted microscope. This flexible platform allows for high-speed live-cell imaging, 6D imaging, and super-resolution imaging in 4D (3D+time) to be all carried out on one integrated system, under the control of the universal NIS-Elements software.

A1+with N-SIM

By using the confocal microscope A1+ 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 photo stimulation, while N-SIM enables approximately 100nm-resolution live cell observation.



With N-SIM

With confocal microscope

E. coli (XL1-Blue) expressing SGFP2
Photos courtesy of: Drs. Takahisa Suzuki and Ikuo Wada, Fukushima Medical University School of Medicine



A1+with N-STORM

With a confocal microscope such as the A1+ or C2+, high-speed image acquisition, low-magnification observation, photo stimulation, etc., of live cells are possible. The super-resolution microscope N-STORM enables acquisition of minute 3D information with 20nm-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
Three positions can be selected for N-SIM, TIRF/2D-STORM and 3D-STORM

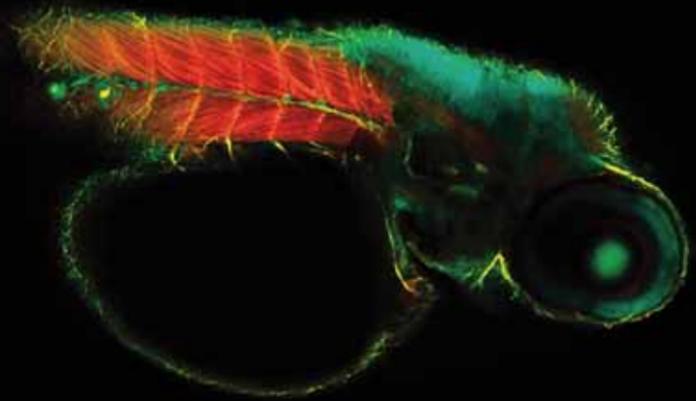
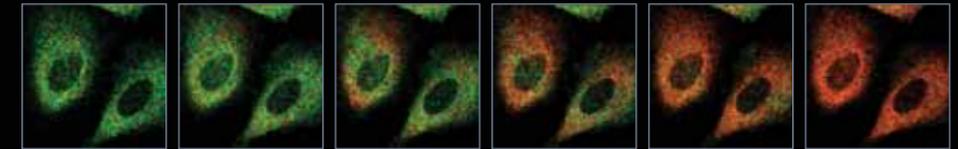


Nikon Confocal Microscope

A1+

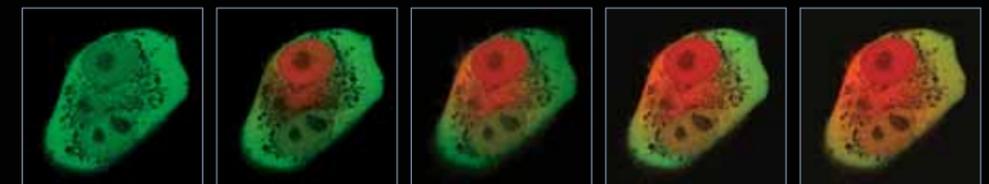
A1+series

Experience the speed and quality



Confocal microscope A1+/A1R+

- A1+ galvano scanner offers high-resolution confocal imaging of up to 16,000,000 pixels
- A1R+ is a hybrid scanning head equipped with both galvano and high-speed resonant scanner. It allows simultaneous photo activation and high-speed imaging of live cells at 420 fps.
- A1si+/A1Rsi+ is equipped with a spectral detector that allows acquisition of a wavelength of up to 320nm in one shot. It enables accurate separation of overlapping fluorescence spectra.
- A1 MP+/A1R MP+ is equipped with non-descanned detectors for multiphoton imaging and allows high-sensitivity acquisition of weak signals in deep areas of living organisms.



Photos courtesy of: Drs. Tomoki Matsuda, Kenta Saito, Kazuki Horikawa and Takeharu Nagai, Hokkaido University

