Super Resolution Microscopes
A brand new era in super resolution

The N-SIM S Super Resolution Microscope utilizes a unique high-speed structured illumination system to achieve acquisition speeds of up to 15 fps*, enabling fast biological processes to be captured at twice the spatial resolution of conventional light microscopes (~115 nm** in XY).

The N-STORM Super Resolution Microscope achieves a 10-fold improvement in resolution compared to conventional light microscopes (~20 nm in XY), enabling observation at the true molecular level.

The N-SIM S and N-STORM can be easily combined within the same imaging system for greater flexibility in nanoscale imaging experiments. They can be further combined with confocal microscope system such as the AX to create a single versatile platform for multi-scale imaging. Powered at the core by the industry-leading Ti2 inverted microscope with its ultra-flexible design, nano-precision Z-drive and Perfect Focus System, the possibilities for experimental design are limitless.

* TIRF-SIM/2D-SIM mode, 512 x 512 pixels, 2 msec exposure time

** FWHM of 100 nm bead images collected in 3D-SIM mode, using 488 nm excitation laser. In TIRF-SIM mode, 86 nm is achieved using 40 nm beads excited by a 488 nm laser.

N-SIM S
Structured illumination microscopy
15 fps image acquisition
Lateral resolution of ~115 nm
Axial resolution of ~269 nm

N-STORM
Stochastic optical reconstruction microscopy
Lateral resolution of ~20 nm
Axial resolution of ~50 nm
The N-SIM S combines innovative structured illumination microscopy technology with unparalleled optics to double the resolution of conventional light microscopes. With acquisition speeds of up to 15 fps, the N-SIM S enables high-speed super resolution imaging of dynamic events in live cells.

- Captures rapid changes in live cells at 15 fps*
- Twice the resolution of conventional light microscopes (approx. 115 nm**), utilizing structured illumination microscopy
- Automatic switching between different illumination modes
- Two-channel TIRF-SIM acquisition
- Acquires larger fields of view (66 µm x 66 µm***)
- Compatible with easy-to-use dry objectives
- Simultaneous two-channel imaging (optional)

* TIRF-SIM/2D-SIM mode, 512 x 512 pixels, 2 msec exposure time
** FWHM of 100 nm bead images collected in 3D-SIM mode, using 488 nm excitation laser. In TIRF-SIM mode, 86 nm is achieved using 40 nm beads excited by a 488 nm laser.
*** Field of view using a 10X objective
Capture rapid changes in live cells

High-speed super-resolution imaging at 15 fps

Nikon's new high-speed structured illumination system utilizes a novel pattern modulation technology to generate fast and precise switching of illumination patterns. The N-SIM S Super Resolution Microscope achieves incredible acquisition speeds up to 15 fps*, enabling super-resolution time-lapse imaging of live cells and intracellular dynamics. Discover a new level of live-cell imaging with the N-SIM S.

* 15 fps: 512 x 512 pixels, 2 msec exposure time
Easily switch between imaging modes for optimal results

Automatic switching between illumination modes

Newly-developed, high-speed structured illumination technology not only enables fast acquisition rates but also automatic switching between illumination modes and automated optimization of structured illumination patterns for different wavelengths and magnifications. This expanded automation enables fast 2-color TIRF-SIM imaging as well as multiplexing of different SIM modalities. The N-SIM S provides easy-to-use, streamlined workflows, whether it be for single-mode or multi-modal imaging experiments.

Acquire larger fields of view

The N-SIM S can acquire super-resolution images with a large field of view of 66 µm square. This larger imaging area enables very high throughput for applications/samples that benefit from larger fields of view, such as a neurons, reducing the amount of time and effort required to obtain data.

Simultaneous two-channel imaging

Simultaneous two-color imaging is possible by utilizing an optional Two Camera Imaging Adaptor* and two sCMOS cameras.

*Hamamatsu Photonics K.K.

2D-SIM mode/TIRF-SIM mode

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

3D-SIM mode

The 3D-SIM mode generates structured illumination patterns in three dimensions to deliver a two-fold improvement in lateral and axial resolution. Two reconstruction methods (“slice” and “stack”) are available to optimize results for application requirements (e.g. sample thickness, speed, etc.).

Images courtesy of: Dr. Minami Tanaka, Dr. Kaoru Katoh, Biomedical Research Institute Molecular Neurobiology Group, National Institute of Advanced Industrial Science and Technology

Images courtesy of: Dr. Reinhard Windoffer, RWTH Aachen University

Photos courtesy of: Drs. Henrik Strahl and Leendert Hamoen, Centre for Bacterial Cell Biology, Newcastle University

Images courtesy of: Drs. Shizuha Ishiyama and Kaoru Katoh, The National Institute of Advanced Industrial Science and Technology

Images courtesy of: Dr. Alexey Khodjakov, Wadsworth Center, Albany NY

Images courtesy of: Dr. Alwyn Noojin, Blackbox Genes, Albany NY
The principle of 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 light 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 into the optical system aperture (Fig. B).

When this “super-resolution" information is then mathematically combined with the standard information captured by the objective lens, it results in resolutions equivalent to those captured with objective lenses with approximately double the NA (Fig. C).

"N-SIM provides the resolution necessary to identify and evaluate the structural organization of the nuclear lamina.** It’s ease of use and stable performance has made N-SIM an integral research tool in my laboratory.


N-SIM S Specifications

- Lateral resolution (FWHM of beads in xy) 115 nm*1 in 3D-SIM mode, 86 nm*2 in TIRF-SIM mode
- Axial resolution (FWHM of beads in z) 269 nm*1 in 3D-SIM mode
- Image acquisition time Up to 15 fps (105-SIM/2D, 2D SIM, 2D SIM) 250 fps (time to line)
- Reconstructed image size 1024 x 1024 pixels, 2048 x 2048 pixels
- Imaging mode TIRF-SIM, 2D-SIM, 3D-SIM (Reconstruction method: slice, stack)
- Multi-color imaging Up to 6 colors
- Simultaneous multi-color imaging Two colors
- Compatible Laser UD-UV Series laser unit Standard: 405 nm, 488 nm, 561 nm, 640 nm Option: 445 nm
- LED-F series laser unit (TIRF-SIM) Standard: 405 nm, 488 nm, 561 nm, 640 nm Option: 445 nm, 515 nm
- Z-Stack time series laser unit (2D-SIM) 405 nm, 466 nm, 470 nm, 518 nm, 540 nm, 567 nm
- Objective CFI Plan Apochromat Lambda 40XC (NA 0.95)* 3 CFI Plan Apochromat Lambda 60XC (NA 0.95)* 3 CFI SR Plan Apochromat IR 60XAC WI (NA 1.27) CFI SR Plan Apochromat IR 60XC WI (NA 1.27) CFI SR HP Apochromat TIRF 100XAC Oil (NA 1.49) CFI SR HP Apochromat TIRF 100XC Oil (NA 1.49)
- Software NIS-N-SIM analysis
- Camera ORCA Fusion BT sCMOS camera (Hamamatsu Photonics K.K.)
- Ziva Light Engine series laser unit (2D/3D-SIM)
- Ti2-E with double layer configuration with Perfect Focus Unit
- Ti2-FT24 Motorized filter cube turret
- NIS-A 6D and N-SIM analysis
- N-SIM S 3D illuminator
- LED-FLi Illuminator
- Motorized filter cube turret
- N-STORM module 2
- Motorized N-STORM kit 3
- Perfect Focus Unit

Operating conditions

- 20 °C to 28 °C (± 1.5 °C)
- 10 °C to 30 °C (± 5 °C)

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

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*** Supplied with microscope base body.
STochastic Optical Reconstruction Microscopy (STORM) reconstructs a super-resolution image by combining precise localization information for individual fluorophores in complex fluorescent microscope specimens. The N-STORM takes advantage of Nikon’s powerful Ti2-E inverted microscope and applies high-precision multi-color localization and reconstruction in three dimensions (xyz) to enable super-resolution imaging at tenfold the resolution of conventional light microscopes (up to 20 nm in xy). This powerful technology enables visualization of molecular interactions and organizations at the nanoscopic scale, opening up new worlds of scientific understanding.
Tenfold increased resolution in \(x\), \(y\) and \(z\)

Up to 50 nm axial resolution

In addition to lateral super-resolution, the Ni-STORM utilizes proprietary methods to achieve a tenfold enhancement in axial resolution over conventional light microscopes and provide nanoscale information in 3D. The 3D-stack function allows multiple Ni-STORM images from different \(z\) positions to be captured and stitched into one image to create thicker STORM images.

Up to 20 nm lateral resolution

The Ni-STORM utilizes high-precision localization information from thousands of individual fluorophores present in a field of view to create breathtaking “super-resolution” images, exhibiting spatial resolution 10 times greater than conventional light microscopes.
**Dynamic super resolution imaging**

Newly developed optics and illumination systems, optimized for CMOS technology, have increased image acquisition speeds by up to 10 times. With acquisition times reduced from minutes to seconds*, dynamic events in live specimens can now be captured with molecular level resolution.

* Using high-speed mode (20 μm x 20 μm imaging area)

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**High definition, high density images**

Newly developed excitation optics and improved image acquisition rates provide increased molecule localization density, resulting in clearer images of macromolecular structures.

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**Large image acquisition area**

New intermediate zoom lenses in the imaging system have been developed and optimized for a wide field of view. The wide-view mode achieves 80 μm x 80 μm, a 4-fold increase in imaging area compared to previous models.

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**Multi-color imaging capability**

Multi-color super-resolution imaging can be carried out using both activator-reporter pairs for sequential activation imaging and activator-free labels 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.

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**High sensitivity, high resolution images**

Newly developed intermediate zoom lenses offer increased sensitivity and improved resolution, allowing for clearer imaging of macromolecular structures.

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**3-color STORM image of a CV-1 cell stained with antibodies against alpha tubulin (Alexa Fluor® 488, red), caveolin (Alexa Fluor® 555, red), and with Alexa Fluor® 488-phalloidin (green) for f-actin.**

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**Tubulin of BSC-1 cell labeled with Alexa Fluor® 647, acquisition time: 20 seconds**

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**5 µm**

**512 pixels**

**sCMOS**

**EM-CCD**

**512 pixels**

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**5× wider imaging area, 80 μm x 80 μm (wide-view mode). Conventional imaging area of 40 μm x 40 μm also shown for comparison. Sample: Yeast bud (YOS2) conjugated with Alexa Fluor® 647.**

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**Time-lapse STORM images of African green monkey kidney cell (BSC-1) labeled with Mito-Tracker Red (Mitochondria).**

**Imaging speed: 500 fps**

**28 sec time-lapse imaging with 2 sec interval**

**Scale bar: 0.2 μm**

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**◀ 4sec ▶ ◀ ◀ 12sec */**

**16sec ◀ 11sec ○ 28sec ◀ 24sec ◀ 20sec ◀ 15sec ◀ 30sec**

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**512 pixels**

**256 pixels**

**256 pixels**

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**8 sec**

**0 sec**

**◀**

**4 sec**

**◀ ◀**

**28 sec**

**◀**

**24 sec**

**◀**

**20 sec**

**◀**

**15 sec**

**◀ ◀**

**30 sec**

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**3-color STORM image of a CV-1 cell stained with antibodies against α-tubulin (Alexa Fluor® 647, magenta), caveolin (Alexa Fluor® 555, red), and with Alexa Fluor® 488-phalloidin (green) for f-actin.**
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.

Reconstruction of 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 determined Z-axis location information with XY-axis location information.

Variety of photoswitchable probes and labeling approaches for high localization accuracy

Various types of activator-reporter pairs and activator-free labels are available. The activator-reporter dye pair approach provides consistent the determined Z-axis location information with XY-axis location information.

3D fluorescent images can be reconstructed by combining high-accuracy localization information of individual fluorophores.
Ultra-stable platforms for super-resolution imaging

Slight changes in temperature and minor vibrations in the imaging environment can greatly impact focus stability, which in turn can be detrimental to super-resolution imaging. The ECLIPSE Ti2-E motorized inverted research microscope has been designed with dramatically improved focus stability and an automatic real-time focus correction system to eliminate focus drift, enabling faithful visualization of nanoscopic cell details.

High-stability Z-focusing mechanism

The durable body of the Ti2-E provides a highly stable platform for super-resolution microscopes. The Ti2-E minimizes vibrations by downsizing the Z-focusing mechanism and positioning it adjacent to the nosepiece, providing the superior Z-focusing precision and stability required for super-resolution imaging.

Auto correction collar

Super-resolution imaging is highly sensitive to spherical aberrations. An automatic correction collar enables easy and precise correction collar adjustment to compensate for spherical aberrations, ensuring consistently high-quality super-resolution images.

Real-time focus correction with PFS

The Perfect Focus System (PFS) maintains focus by automatically tracking and maintaining the desired Z position. PFS corrects focus drift, caused by minute temperature changes and vibrations, in real-time. The detector portion of the PFS is separated from the nosepiece to minimize mechanical load and heat transfer, further reducing the potential for Z-drifts.
Water immersion objectives

Silicone immersion objectives

Silicone immersion objectives use high viscosity silicone oil with a refractive index close to that of live cells as an immersion liquid. Because of this imposed refractive index compatibility, these objectives can provide improved photon collection capability and resolution when performing super-resolution imaging deeper into the specimen. They exhibit superior chromatic aberration correction and high transmittance over a broad range of wavelengths.

Silicon immersion objective

Oil immersion objective

Dry objectives

The N-SIM S is compatible with dry objectives, making both super-resolution imaging and confocal imaging available without switching lenses. Low-magnification, wide field-of-view dry lenses enable high resolution observation even at the periphery of sample tissues.

Dry objectives support 2D-SIM and 3D-SIM (slice reconstruction) only

<table>
<thead>
<tr>
<th>Model</th>
<th>Immersion</th>
<th>NA</th>
<th>WD (mm)</th>
<th>Correction collar</th>
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Scan the QR code to view a video

N-SIM S/N-STORM

Model Immersion NA WD (mm) Correction collar

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Unified acquisition and analysis software platform

NIS-Elements, Nikon’s unified software platform, provides an intuitive workflow for super-resolution imaging. Combined with graphical programming tools such as JOBS and Illumination Sequence, as well as powerful analysis and visualization tools, NIS-Elements creates a comprehensive operating environment that can be fully customized for a variety of application requirements.

Image Acquisition

**N-SIM S**

**Image acquisition setting**

The N-SIM S can easily switch between 2D-SIM, 3D-SIM and TIRF-SIM modes. The JOBS flexible imaging sequence option enables seamless image acquisition between the N-SIM S, N-STORM and confocal microscopes.

**Image reconstruction**

Auto settings allow the software to automatically select the most appropriate reconstruction parameters for acquired images in order to reconstruct N-SIM S images. Users can further optimize reconstruction by manually adjusting these parameters. The reconstruction view allows users to preview the results of the selected reconstructed parameters on the current/selected frame, enabling efficient reconstruction parameter determination.

**N-STORM**

**Image acquisition setting**

The N-STORM can easily switch between 2D-STORM and 3D-STORM image acquisition modes.

**Batch processing analysis**

Multiple N-STORM images can be simultaneously analyzed.

**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.

**3D display**

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

Display & Processing

**N-SIM S**

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Auto settings allow the software to automatically select the most appropriate reconstruction parameters for acquired images in order to reconstruct N-SIM S images. Users can further optimize reconstruction by manually adjusting these parameters. The reconstruction view allows users to preview the results of the selected reconstructed parameters on the current/selected frame, enabling efficient reconstruction parameter determination.

**N-STORM**

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Multiple N-STORM images can be simultaneously analyzed.

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A major feature of the N-STORM is 3D super-resolution image acquisition and analysis. Acquired images can be displayed at any angle after analysis.
Seamless switching between imaging modalities for multi-scale experiments

Combining the N-SIM S and N-STORM with a single Ti2-E inverted microscope expands the functionality of each individual technology. The N-SIM S enables acquisition of thicker volume images, thereby providing a more comprehensive molecular landscape for interpreting the single-molecule level data acquired by the N-STORM. The N-SIM S and/or N-STORM can be further combined with a confocal microscope such as the AX. A desired location in a sample can be specified in a low-magnification/large FOV confocal image and acquired in super-resolution by simply switching the imaging method. Combining a confocal microscope with a super-resolution system can provide a method for gaining larger contextual views of the super-resolution information.

"Simultaneous equipment of the A1+ Confocal Microscope and the N-SIM Super Resolution Microscope with a single imaging system enables multi-mode analysis. This multi-mode combination is advantageous in the study that requires wide and detailed views in a consistent manner. To analyze giant cells such as cultured mature osteoclasts with diameters of a few hundred microns, the user can acquire an image with a wide view using the A1+, select the area to be observed from that wide image, then switch to the N-SIM for ultrafine observation. Electron microscopy had been commonly used to observe podosomes that assemble to form ring-like structures called actin rings that are functionally associated with cellular adhesion and locomotion of osteoclasts. The N-SIM optically resolves individual podosomes, and enables the quantitative evaluation of podosome functions by analysis of the temporal and spatial dynamics of podosomes."

Dr. Tadahiro Iimura
Division of Bio-Imaging, Proteo-Science Center (PROS), Ehime University

"We found that CCR5, the target molecule for treatment of HIV infections, exists in osteoclasts, and that the functional loss of CCR5 impairs the bone-resorption activity of osteoclasts, resulting in a bone disorder of osteoporosis.* This finding suggests that CCR5 antagonism may prevent bone-disease states such as osteoporosis as well as HIV transmission."

Dr. Takahisa Nozaki
Division of Analytical Bio-Medicine, Advanced Research Support Center (ARASC), Ehime University

"STORM imaging is the most important tool for our research as it is the only technique that allows us to measure the precise position and distribution of important signaling molecules within the chemical synapse on the nanoscale. But this data alone is like looking at stars in a dark sky. We need to use confocal microscopy to show in which context these molecular changes are taking place. After the physiological experiments we use confocal imaging for the morphological characterization of the cell type and the synapse. Then, we switch to STORM mode to gather information on the signaling molecules responsible for the physiological signaling. In this way we can correlate the molecular data, the anatomical data and the physiological data from the very same synapse. And this is very exciting!"

Dr. Istvan Katona
Laboratory of Molecular Neurobiology, Institute of Experimental Medicine of the Hungarian Academy of Sciences

PHOTO CREDIT:
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- N-SIM S
- Vibration isolated table
- Laser unit

**N-STORM layout**

- PC rack
- N-STORM
- Vibration isolated table
- Laser unit

Unit: mm

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