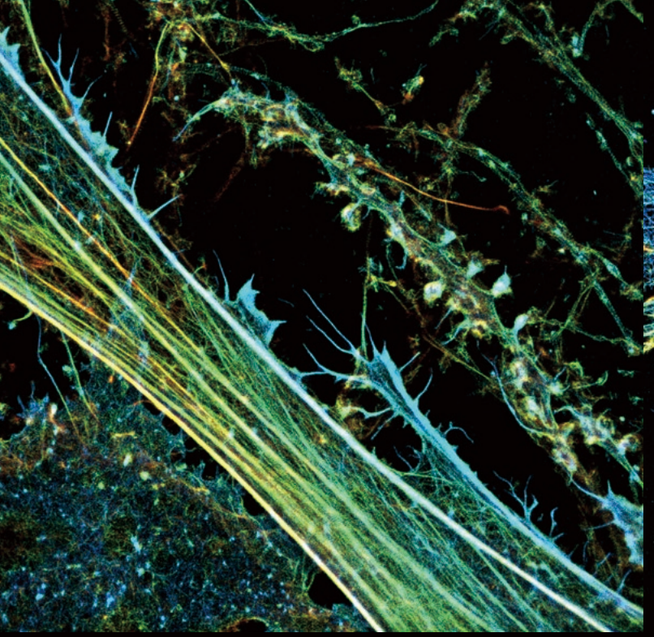
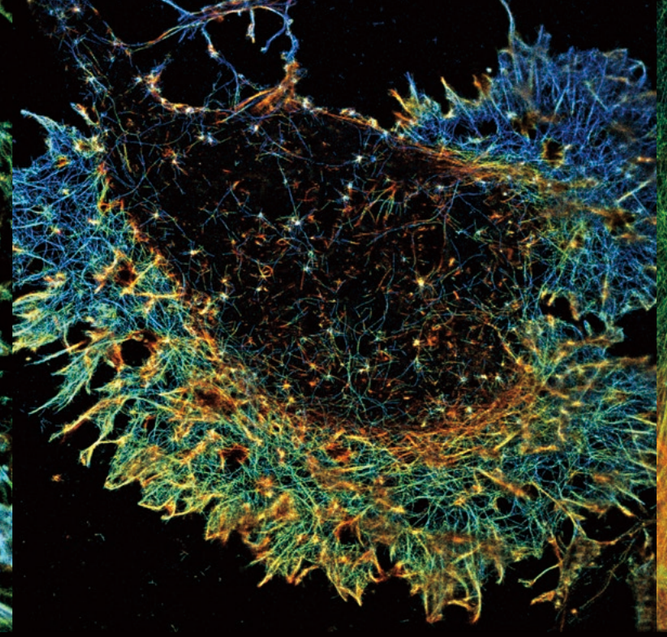
N-STORM

Super Resolution Microscope

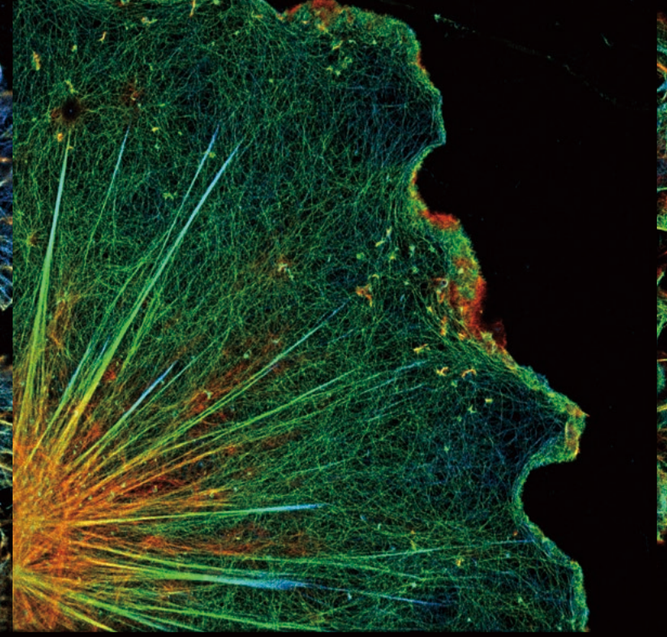
Shedding New Light On **MICROSCOPY**



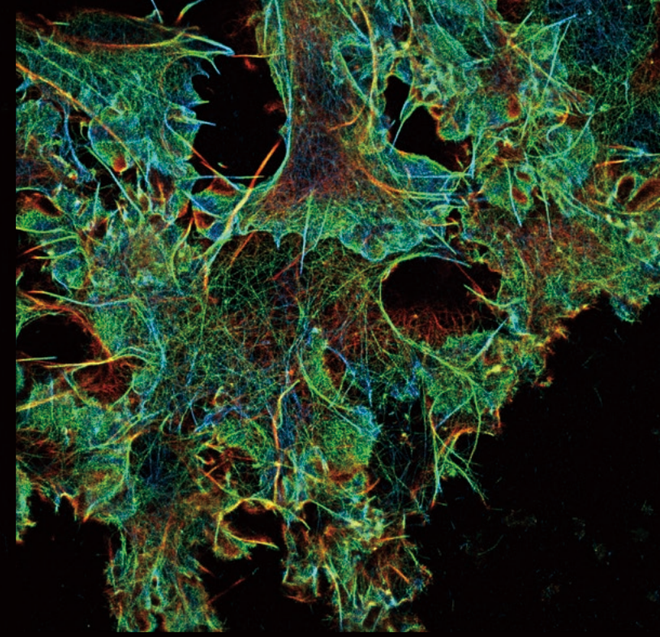
a: Hippocampal neurons and glia in culture
3D-STORM imaging of actin labeled with Alexa Fluor® 647 Phalloidin using depth-code pseudo color. Image "a" shows four types of actin organization, from bottom left to top right: the cell body of a neuron, a glial cell with stress fibers, a neuronal dendrite with spines, and an axon.
Photos courtesy of: Dr. Christophe Leterrier, NeuroCyto team, NICN CNRS-AMU UMR7259, Marseille, France



b: Growth cone of a neuron in culture



c: Glia in a neuronal culture

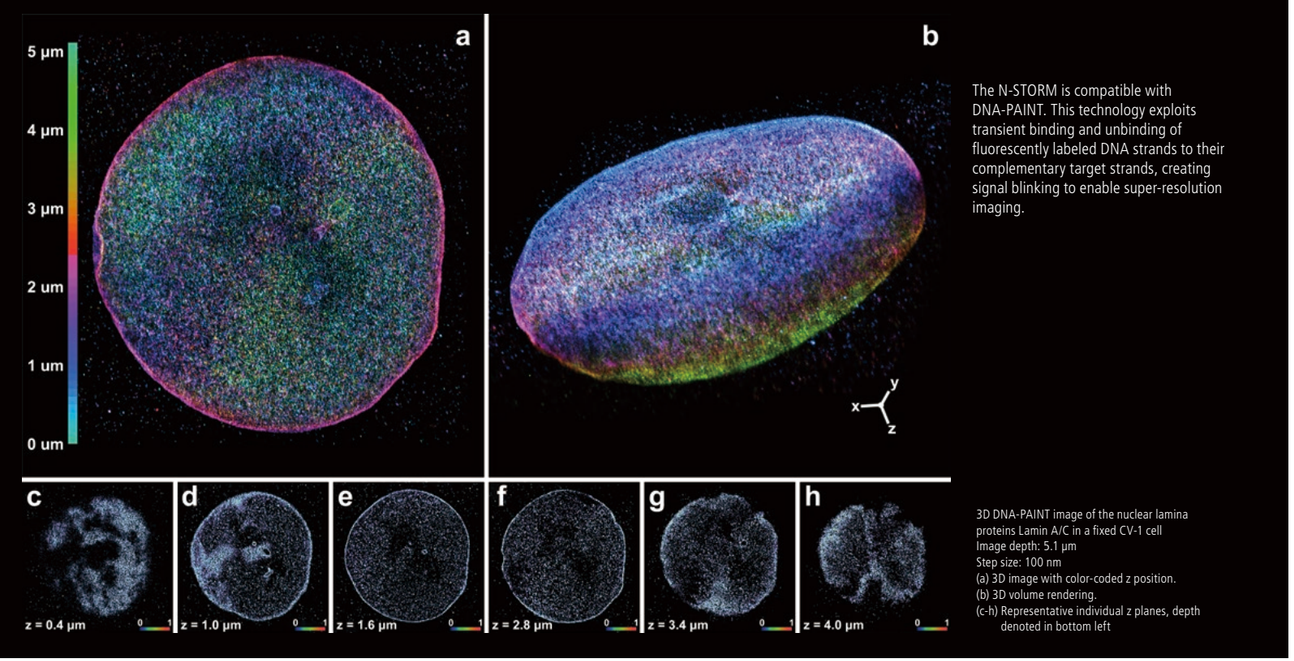


d: COS cells

Experience the nanoscale universe

STochastic Optical Reconstruction Microscopy (STORM) reconstructs a super-resolution image by combining precise localization information for individual fluorophores in complex specimens. 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). This enables super-resolution imaging at tenfold the resolution of conventional light microscopes (up to 20 nm in xy). This powerful technology allows visualization of molecular interactions and organizations at the nanoscopic scale, opening up new worlds of scientific discovery.

- 10 times the resolution of conventional light microscopes in x, y and z directions
- Dynamic super resolution imaging at the molecule level
- Multi-color imaging capability
- High definition, high label density images
- Large image acquisition area



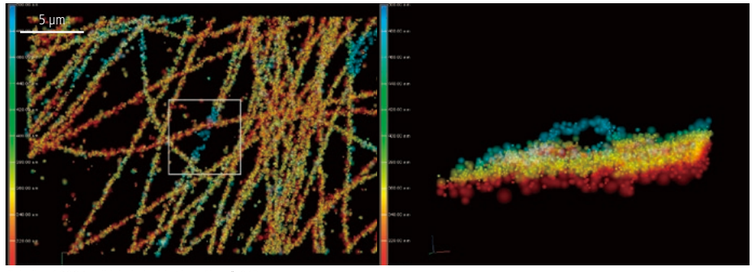
The N-STORM is compatible with DNA-PAINT. This technology exploits transient binding and unbinding of fluorescently labeled DNA strands to their complementary target strands, creating signal blinking to enable super-resolution imaging.

3D DNA-PAINT image of the nuclear lamina proteins Lamin A/C in a fixed CV-1 cell
Image depth: 5.1 µm
Step size: 100 nm
(a) 3D image with color-coded z position.
(b) 3D volume rendering.
(c-h) Representative individual z planes, depth denoted in bottom left

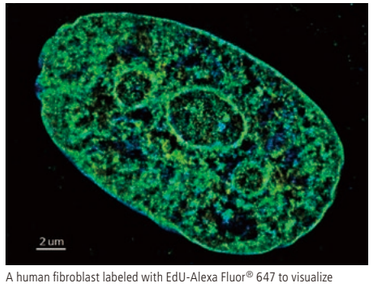
Tenfold increased resolution in x, y and z

Up to 50 nm axial resolution

In addition to lateral super-resolution, the N-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 3D-STORM images from different Z positions to be captured and stitched into one image to create thicker STORM images.



Tubulin of BSC-1 cell labeled with Alexa Fluor® 647



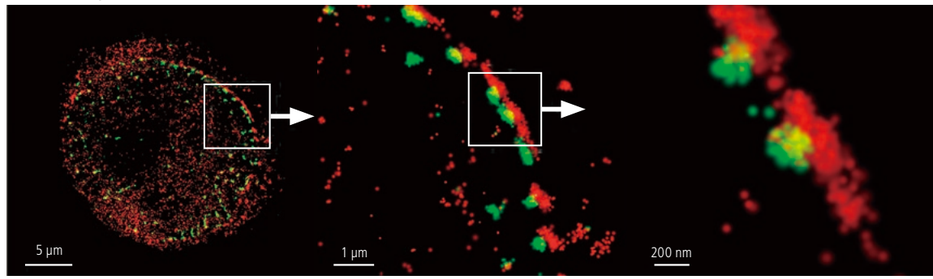
A human fibroblast labeled with EdU-Alexa Fluor® 647 to visualize DNA with 3D-STORM.
Photo courtesy of: Jason Otterstrom, Ph.D., Melike Lakadamyali, Ph.D., The Institute of Photonic Sciences (ICFO), Castelldefels, Spain

Primary cell culture of Drosophila brain
3D-STORM image of EdU-labeled DNA in Drosophila melanogaster neuroblast
Photo courtesy of: Anna Oddone, Ph.D., Melike Lakadamyali, Ph.D. group, The Institute of Photonic Sciences (ICFO), Castelldefels, Spain

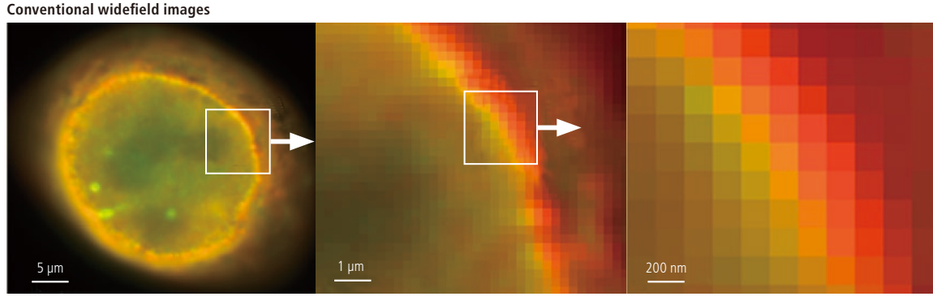
Up to 20 nm lateral resolution

The N-STORM utilizes high-precision localization information from thousands of individual fluorophores present in a field of view to create breathtaking "super-resolution" images. This allows spatial resolution 10 times greater than that of conventional light microscopes.

N-STORM images

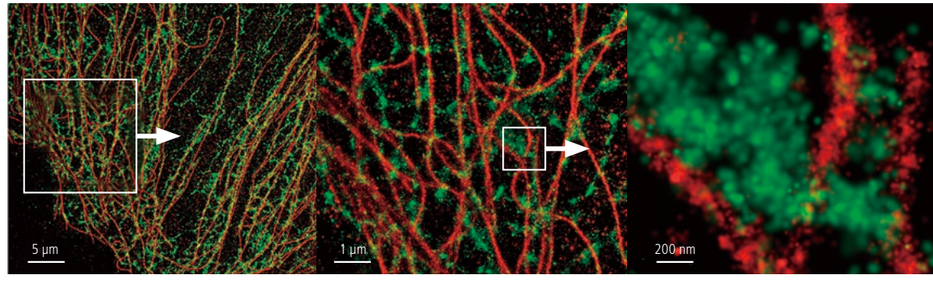


Conventional widefield images

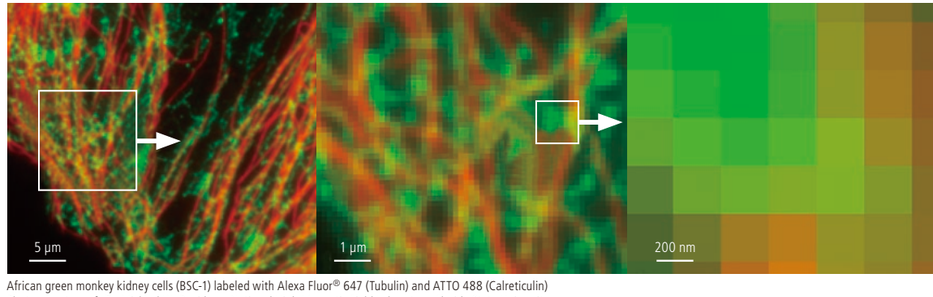


Human cervical cancer cells (HeLa S3) labeled with Alexa Fluor® 647 (NUP153) and ATTO 488 (TPR)
Photos courtesy of: Dr. Michael W. Davidson, National High Magnetic Field Laboratory, Florida State University

N-STORM images



Conventional widefield images

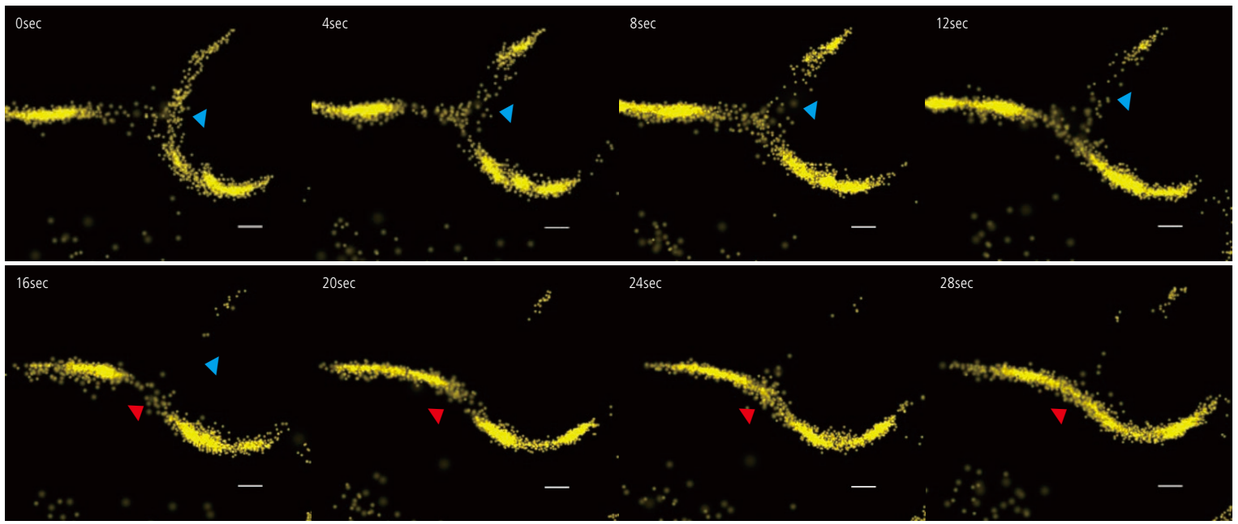


African green monkey kidney cells (BSC-1) labeled with Alexa Fluor® 647 (Tubulin) and ATTO 488 (Calreticulin)
Photos courtesy of: Dr. Michael W. Davidson, National High Magnetic Field Laboratory, Florida State University

Dynamic super resolution imaging

Newly developed optics and illumination systems, optimized for sCMOS 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.

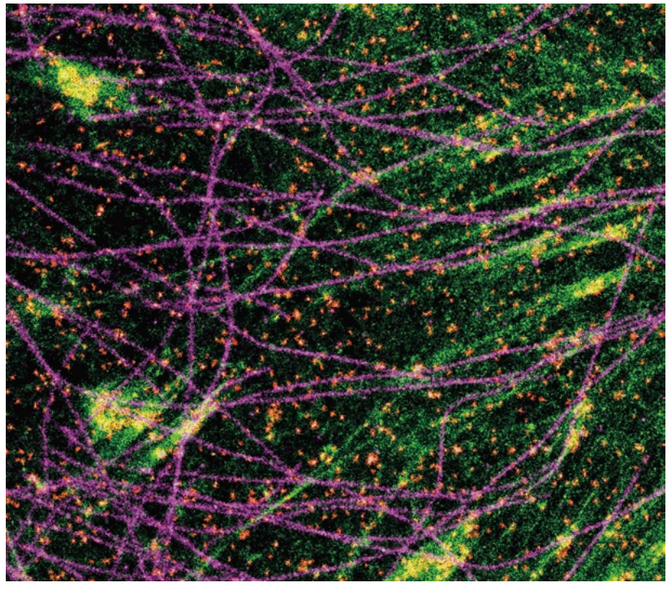
* Using high-speed mode (20 µm x 20 µm imaging area)



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

Multi-color imaging capability

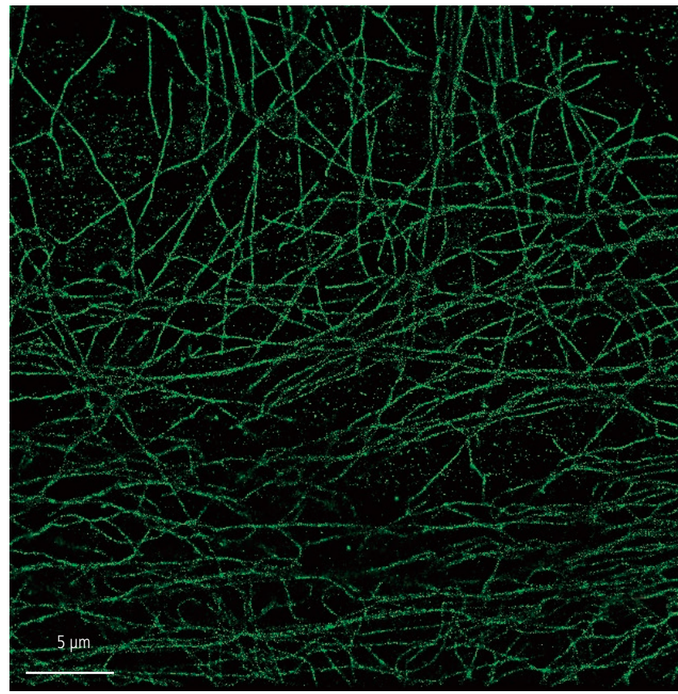
Multi-color super-resolution imaging can be carried out using both activator-reporter pairs for sequential activation imaging and activator-free labels for continuous activation imaging. This flexibility allows users to easily gain critical insights into the localization and interaction properties of multiple proteins at the molecular level.



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

High definition, high density images

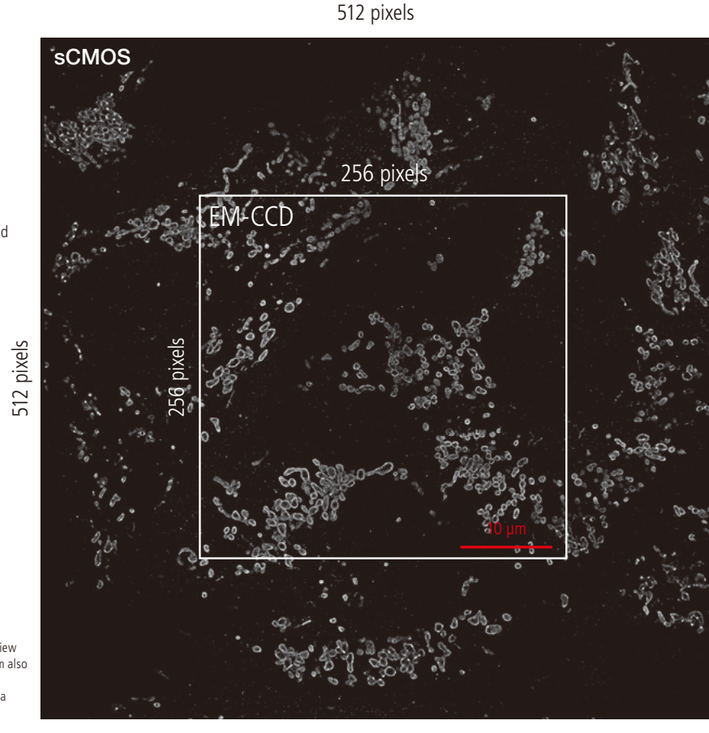
Newly developed excitation optics and improved image acquisition rates provide increased molecule localization density, resulting in clearer images of macromolecular structures.



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

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.



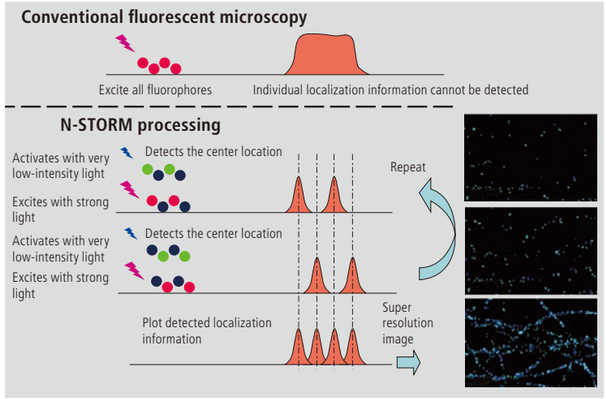
4 times wider imaging area, 80 µm x 80 µm (wide-view mode). Conventional imaging area of 40 µm x 40 µm also shown for comparison.
Sample: Mitochondria TOM20 conjugated with Alexa Fluor® 647

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.

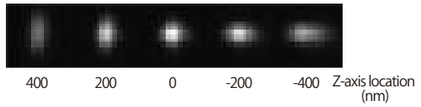
Reconstruction of N-STORM images using localization information of individual fluorophores



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, resulting in a powerful synergy with highly precise Z-positioning provided by the piezo Z stage.

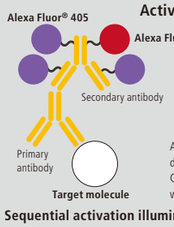
Single spot image



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 localization accuracy between channels by leveraging the same reporter dye for different channels. Each dye pair consists of an activator dye and reporter dye, with the activator dye regulating the activation state of the reporter dye. Activator-free labels consist of imaging dye only, enabling simple labeling and sample preparation techniques such as conventional indirect immunofluorescence using conventional dye-conjugated antibodies.

Activator-reporter pair

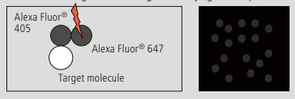


Dye for activator	Dye for reporter
Alexa Fluor® 405	Alexa Fluor® 647
Cy2	Alexa Fluor® 647
Cy3	Alexa Fluor® 647

An activator-reporter pair consists of a shorter-wavelength dye for activation and a longer-wavelength dye for detection. Creation of three-color, super-resolution images is possible with multiple dye-pairs.

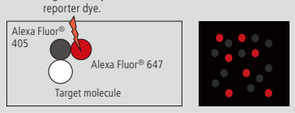
Sequential activation illumination with activator-reporter pair

STEP 1 Most activator-reporter dye pairs are converted to a non-emissive state by combining them with high intensity light and specialized imaging buffer additives.

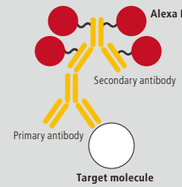


STEP 2 Absorption of light by an activator results in transfer of energy to a nearby reporter dye, accelerating the transition of the reporter dye from a non-emissive to a ground state. The use of spectrally distinct activator dyes allows for the use of the same reporter dye for multiple imaging channels.

STEP 3 High intensity illumination results in fluorescence emission from the activated reporter dye.



Activator-free label

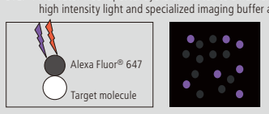


Dye for imaging
Alexa Fluor® 647, CF™ 647, Cy5, ATTO 647N
CF™ 568, Cy3B, Alexa Fluor® 568, Alexa Fluor® 555
CF™ 488, ATTO 488, Alexa Fluor® 488

An activator-free label requires only a reporter dye for image acquisition. Creation of three-color, super-resolution images is possible using dyes with well-separated emission spectra.

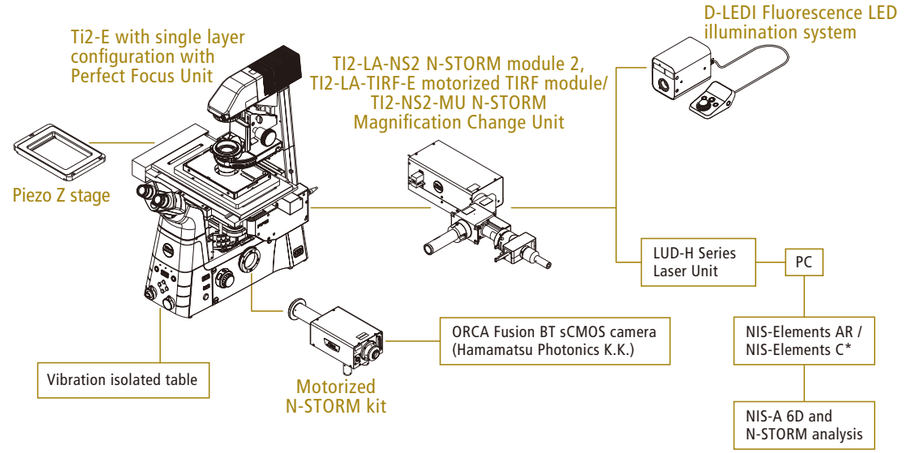
Continuous activation illumination with activator-free label

STEP 1 Activator-free reporter dye is converted to a non-emissive state by combining it with high intensity light and specialized imaging buffer additives.



STEP 2 The reporter dye will stochastically relax to the ground state and then be excited to the fluorescent state by ongoing high intensity illumination.

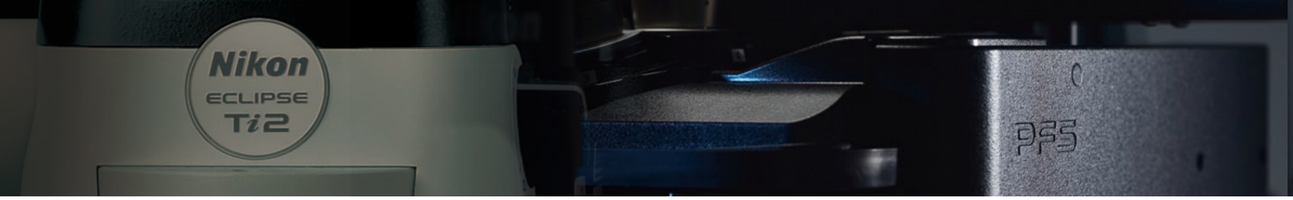
N-STORM system diagram



* Required when used with confocal system

N-STORM Specifications

XY resolution	Approximately 20 nm
Z-axis resolution	Approximately 50 nm
Imaging mode	2D-STORM (normal mode, continuous mode) 3D-STORM (normal mode, continuous mode), 3D-Stack function
Max. field of view	80 µm x 80 µm
Acquisition speed	Up to 500 Hz
Multi-color imaging	Up to 3 colors
Compatible laser	LUD-H series laser unit 405 nm, 488 nm, 561 nm, 640 nm
Compatible microscope	Motorized inverted microscope ECLIPSE Ti2-E Perfect Focus System Motorized XY stage with encoders Piezo Z stage
Objective	CFI SR HP Plan Apochromat Lambda S 100XC Sil (NA1.35) CFI SR HP Apochromat TIRF 100XC Oil (NA 1.49) CFI SR HP Apochromat TIRF 100XAC Oil (NA 1.49)
Camera	sCMOS camera: ORCA-Fusion BT, ORCA-Flash4.0 V3, ORCA-Flash4.0 V2 (Hamamatsu Photonics K.K.) EMCCD camera: iXon3 897, iXon Ultra 897 (Andor)
Software	NIS-Elements AR/NIS-Elements C (for Confocal Microscope AX/AX R) Both require additional software modules NIS-A 6D and N-STORM Analysis
Operating conditions	20 °C to 25 °C (± 0.5 °C)

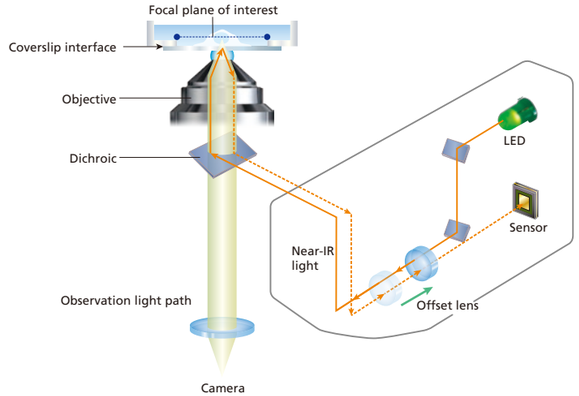


Ultra-stable microscope with super-resolution performance

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.

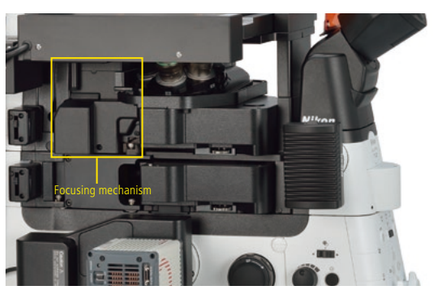
Real-time focus correction with PFS

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.



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.

High-performance optics for super-resolution imaging

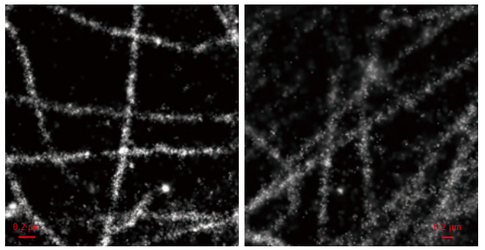
The SR series objectives ensure the lowest possible asymmetric aberration and superb optical performance required for super-resolution imaging. The HP series supports ultrahigh-power lasers required for inducing rapid photo-switching of fluorophores. It provides improved axial chromatic aberration correction to achieve the highest level of precision in localization and image alignment for 3D multicolor STORM imaging.

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 improved 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.

CFI SR HP Plan Apochromat Lambda S 100XC Sil

Silicon immersion objective **Oil immersion objective**



N-STORM images (approx. 6.5 µm depth)
Left: CFI SR HP Plan Apochromat Lambda S 100XC Sil, Right: CFI SR HP Apochromat TIRF 100XC Oil

Oil immersion objectives

These objectives provide the high numerical apertures required for N-STORM imaging and are perfect for flat samples that are in close proximity to the coverslip.

CFI SR HP Apochromat TIRF 100XC Oil

CFI SR HP Apochromat TIRF 100XAC Oil

Model	Immersion	NA	W.D. (mm)	Correction collar
CFI SR HP Plan Apochromat Lambda S 100XC Sil	Silicone oil	1.35	0.31-0.29 (0.30*): 23˚C, 0.30-0.28 (0.29*): 37˚C	Manual
CFI SR HP Apochromat TIRF 100XC Oil	Oil	1.49	0.16-0.10 (0.12*): 23˚C, 0.15-0.09 (0.12*): 37˚C	Manual
CFI SR HP Apochromat TIRF 100XAC Oil	Oil	1.49	0.16-0.10 (0.12*): 23˚C, 0.15-0.09 (0.12*): 37˚C	Auto

*With cover glass thickness of 0.17 mm

NIS-Elements — A unified acquisition and analysis software platform

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 setting

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

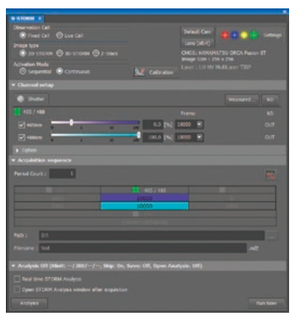
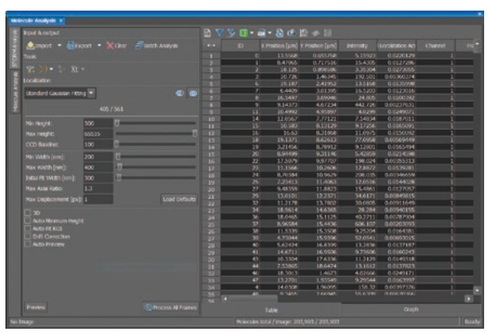
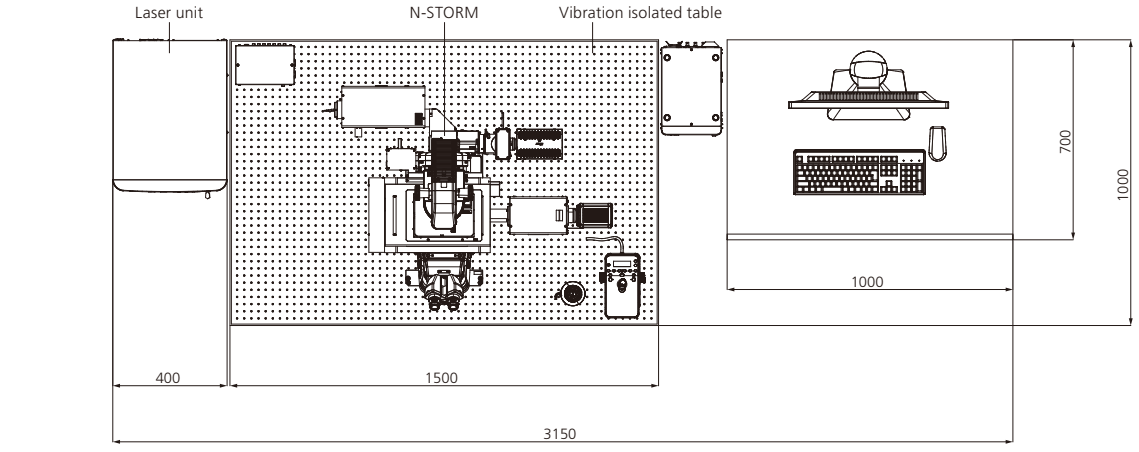


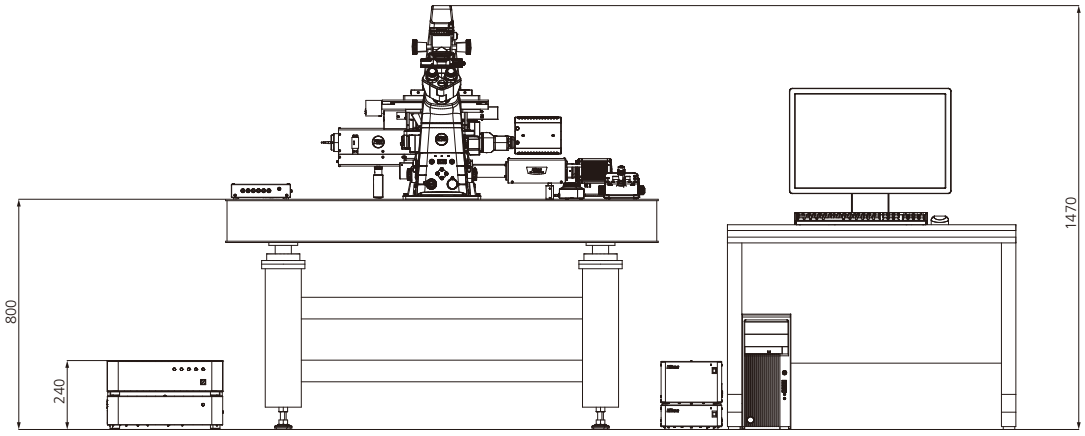
Image analysis setting

Multiple N-STORM images can be simultaneously analyzed.



Layout





Unit: mm

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⚠ WARNING	TO ENSURE CORRECT USAGE, READ THE CORRESPONDING MANUALS CAREFULLY BEFORE USING YOUR EQUIPMENT.

Monitor images are simulated.
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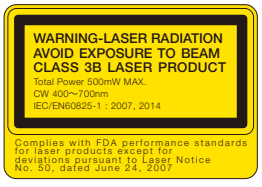
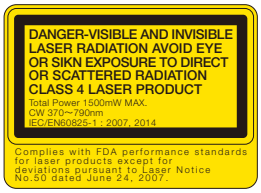
*Products: Hardware and its technical information (including software)





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