

*Super Resolution Microscope*

# N-SIM/N-STORM



# See like you have never seen before

Super Resolution Microscope  
N-SIM/N-STORM

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

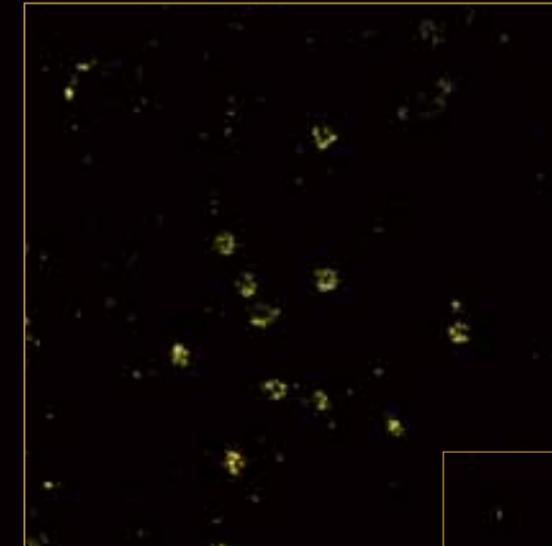
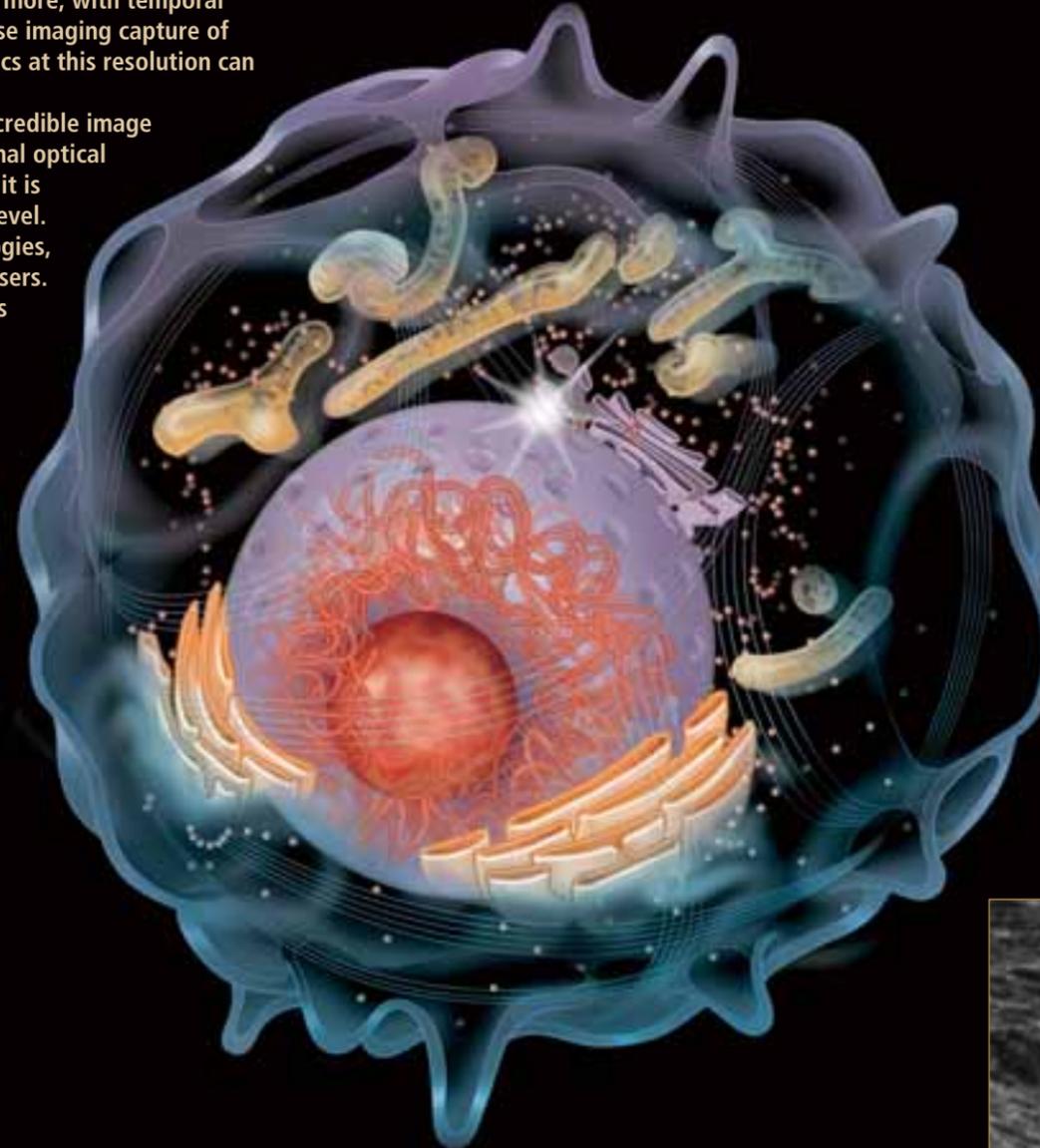
Nikon's new Super Resolution microscope N-SIM/N-STORM enables elucidation of structure and function of the nanoscopic machinery within living cells.

Resolution of conventional optical microscopes even with the highest numerical aperture optics are limited by diffraction to approximately 200nm.

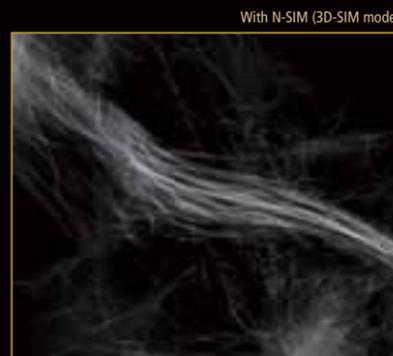
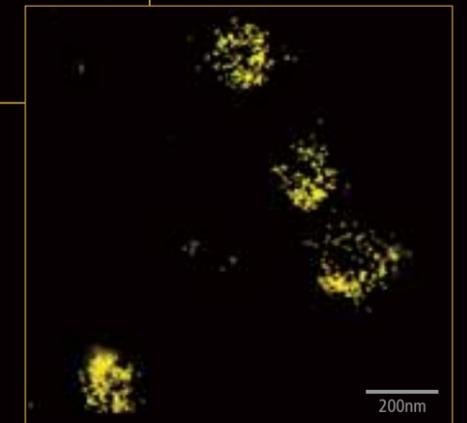
Using high frequency Structured Illumination, the Nikon N-SIM can achieve image resolution of 85nm\*, which was previously considered impossible with optical microscopes. Furthermore, with temporal resolution of up to 0.6 sec./frame\*\*, N-SIM enables super-resolution time-lapse imaging capture of dynamic molecular interactions in living cells. The observation of such dynamics at this resolution can open new worlds of discovery.

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, yet bring them to the laboratory in a form designed to be simple for system users. 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



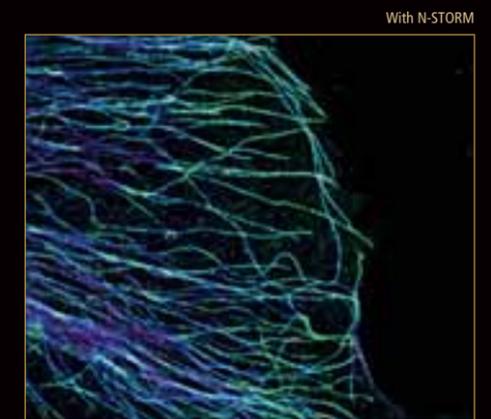
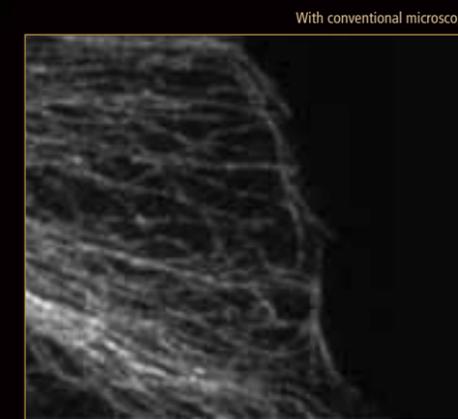
Single color STORM image of a clathrin-coated pit in a mammalian cell labeled with Cy3-Alexa647  
Objective: CFI Apo TIRF 100x oil (NA 1.49)



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: Yasushi Okada, Ph.D., Department of Cell Biology and Anatomy, Graduate School of Medicine, University of Tokyo



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: Ikuo Wada, Ph.D., Institute of Biomedical Sciences, Fukushima Medical University School of Medicine



Fluorescence labeled microtubule

# 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 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: Yasushi Okada, Ph.D., Department of Cell Biology and Anatomy, Graduate School of Medicine, University of Tokyo

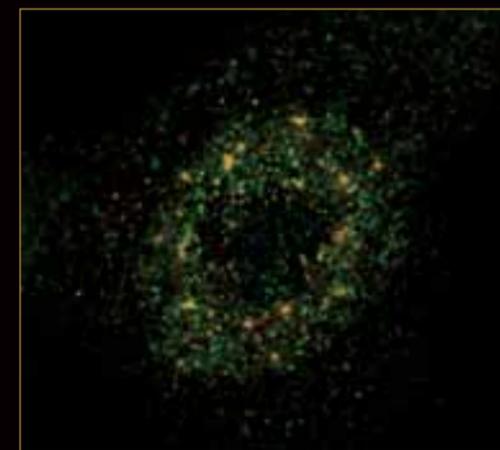


With conventional TIRF

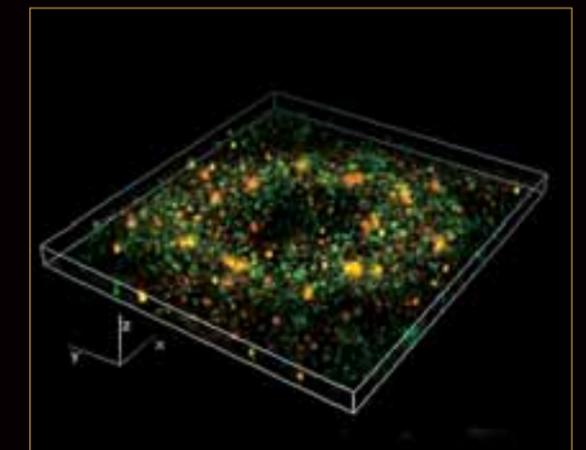


With TIRF-SIM

Plasma membrane of B16 melanoma cell labeled with YFP  
Objective: CFI Apo TIRF 100x oil (NA 1.49)  
Photographed with the cooperation of: Yasushi Okada, Ph.D., Department of Cell Biology and Anatomy, Graduate School of Medicine, University of Tokyo



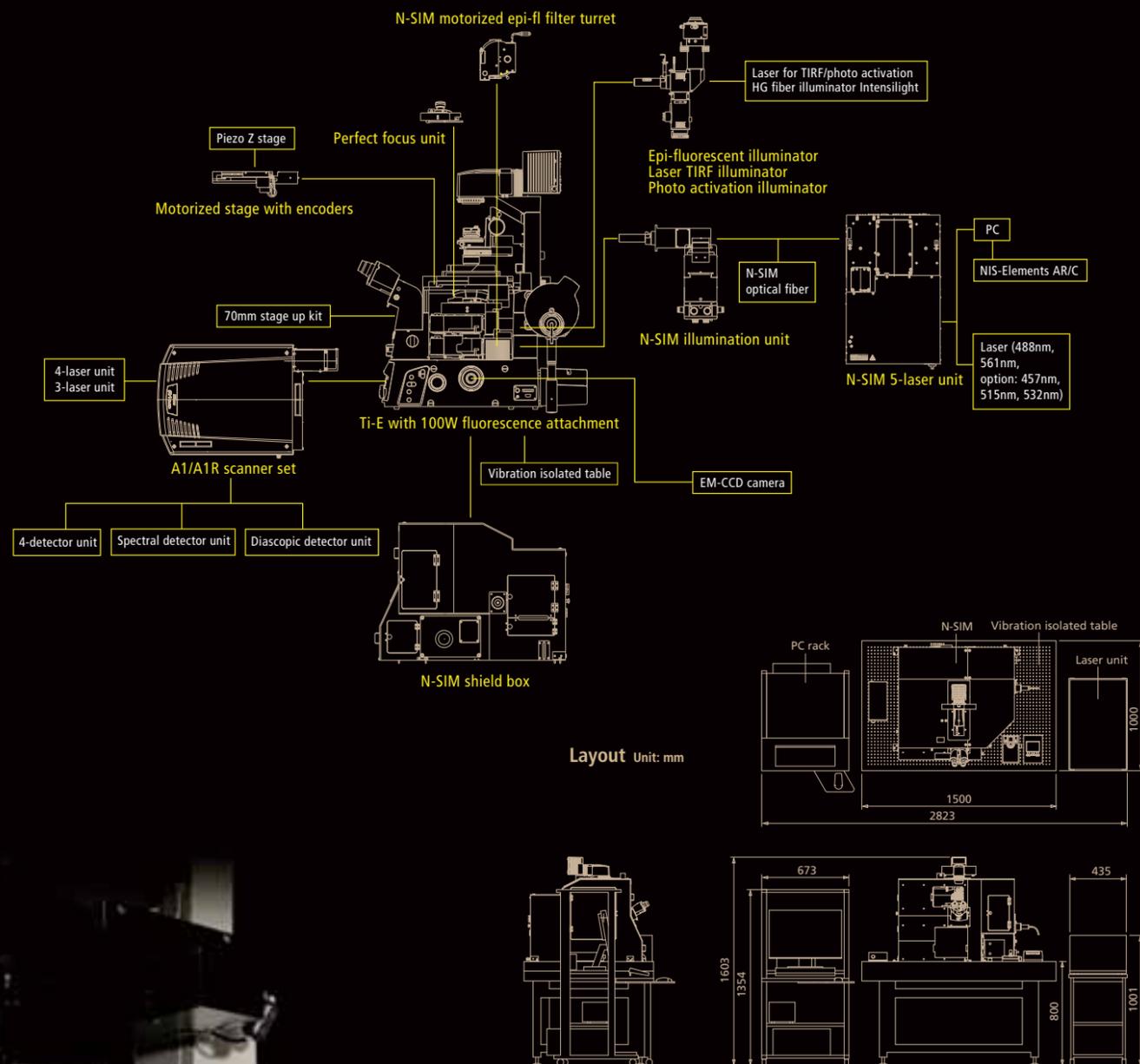
Co-localization images of a target protein of VEGF signaling (Cy3) and its ubiquitin E3 ligase (FITC)  
Unprecedentedly detailed structure of the nuclear body can be observed  
Mode: 3D-SIM, Z-stack  
Objective: CFI Apo TIRF 100x oil (NA 1.49)  
Photographed with the cooperation of: Hidetaka Ohnuki, Ph.D., Shigeki Higashiyama, Ph.D., Ehime University Graduate School of Medicine



3D reconstruction image approx. 5µm thick (part)



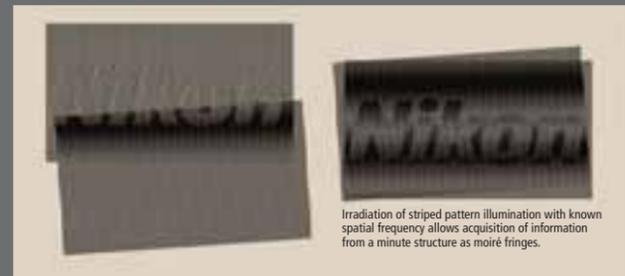
Dynamics of mitochondria stained with Mito-Tracker red  
Cristae in mitochondria are visualized and the dynamics of mitochondria can be observed.  
Mode: 3D-SIM  
Objective: CFI Apo TIRF 100x oil (NA 1.49)  
Image capturing interval: approx. 1 sec. (movie)



## Principle of the Structured Illumination Microscopy

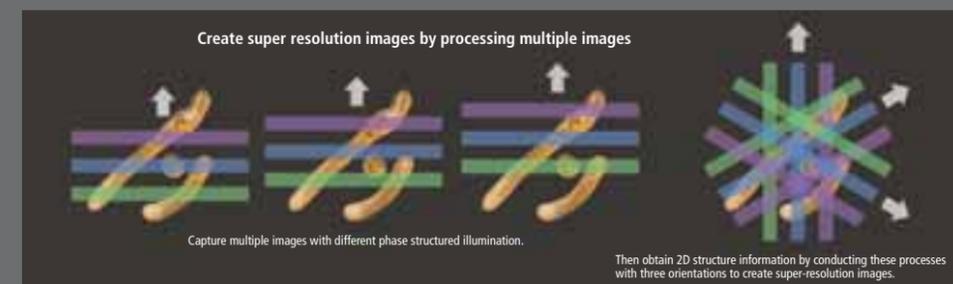
Analytical processing of recorded moiré patterns produced by overlay of a known high spatial frequency pattern mathematically restores sub-resolution structure of a specimen.

Utilization of high spatial frequency laser interference to illuminate sub-resolution structure 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 then transformed back into image space creating an image at double the conventional resolution limit.

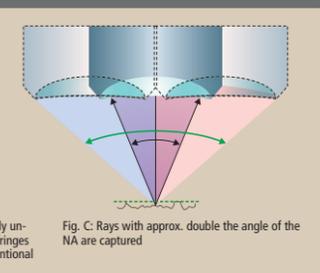
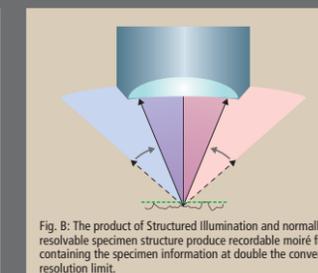
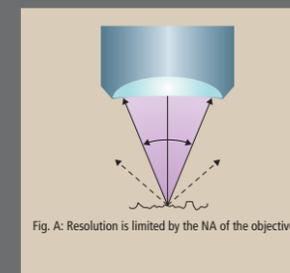


## Utilizing High Frequency striped illumination to double the resolution

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).

When this "super resolution" information is then mathematically combined with the standard information captured by the objective lens, it results in an effective doubling of the NA, and therefore resolution of the optical system (Fig. C).



## Comparison images: with TIRF-SIM and with conventional microscope

Images of  $\phi 100\text{nm}$  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.



With conventional epi-fluorescence microscope



With TIRF-SIM



Intensity profiles

# 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 precisely localized information of each fluorophore detected within a complex microscope specimen. N-STORM applies high-accuracy multi-channel molecular localization and reconstruction in 3 dimensions taking full advantage of Nikon's powerful Ti-E inverted microscope, realizing super resolution of 10 times (approx. 20nm laterally) greater than conventional microscopes. This powerful technology can bring to view nanoscopic molecular interactions opening new worlds of understanding.

**Super resolution at 10 times (approx. 20nm laterally) greater than conventional optical microscopes**

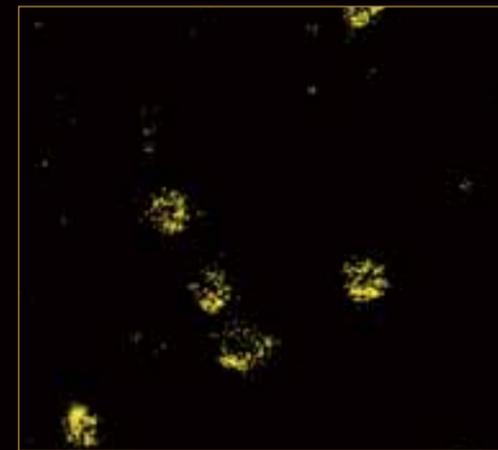
N-STORM utilizes highly accurate localization information (2D or 3D) of 1000's of discrete fluorophor molecules within a microscope specimen to create breathtaking "super-resolution" images, exhibiting spatial resolution 10 times greater than conventional optical microscopes.

**N-STORM can uniquely generate greater than 10 times standard optical resolution axially as well (approx. 50nm)**

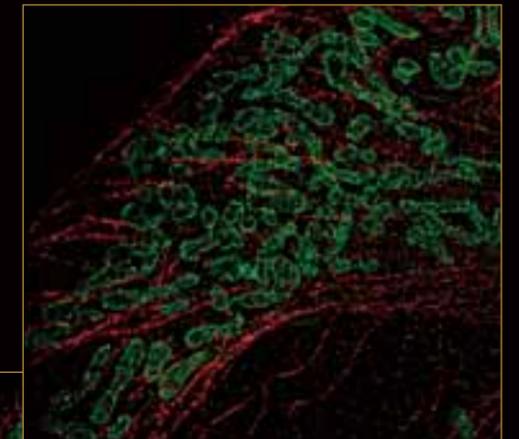
In addition to lateral super-resolution, N-STORM utilizes proprietary methods to achieve a 10 fold enhancement in axial resolution, effectively providing 3D information at a nanoscopic scale.

**Multi-color imaging using various fluorescent probes**

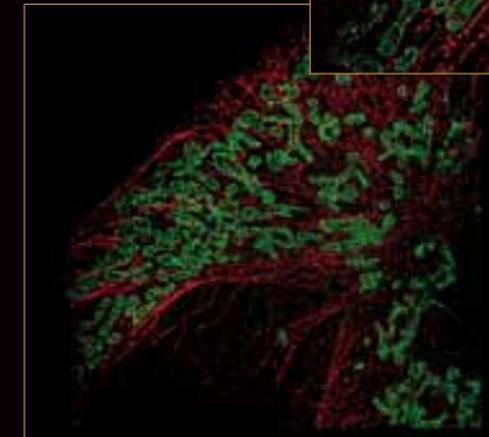
Multi-color super resolution imaging is possible by cleverly combining various "activator" and "reporter" probes. This makes it possible to gain critical insight into the co-localization and interaction of multiple proteins at the molecular level.



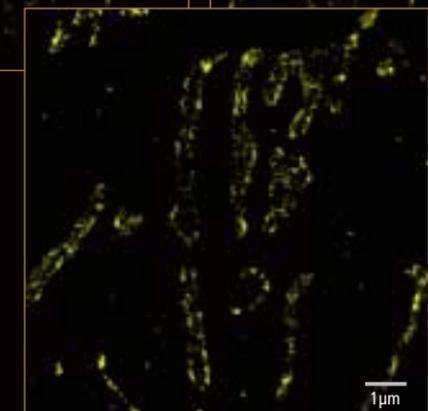
Single color STORM image of a clathrin-coated pit in a mammalian cell labeled with Cy3-Alexa647  
Objective: CFI Apo TIRF 100x oil (NA 1.49)



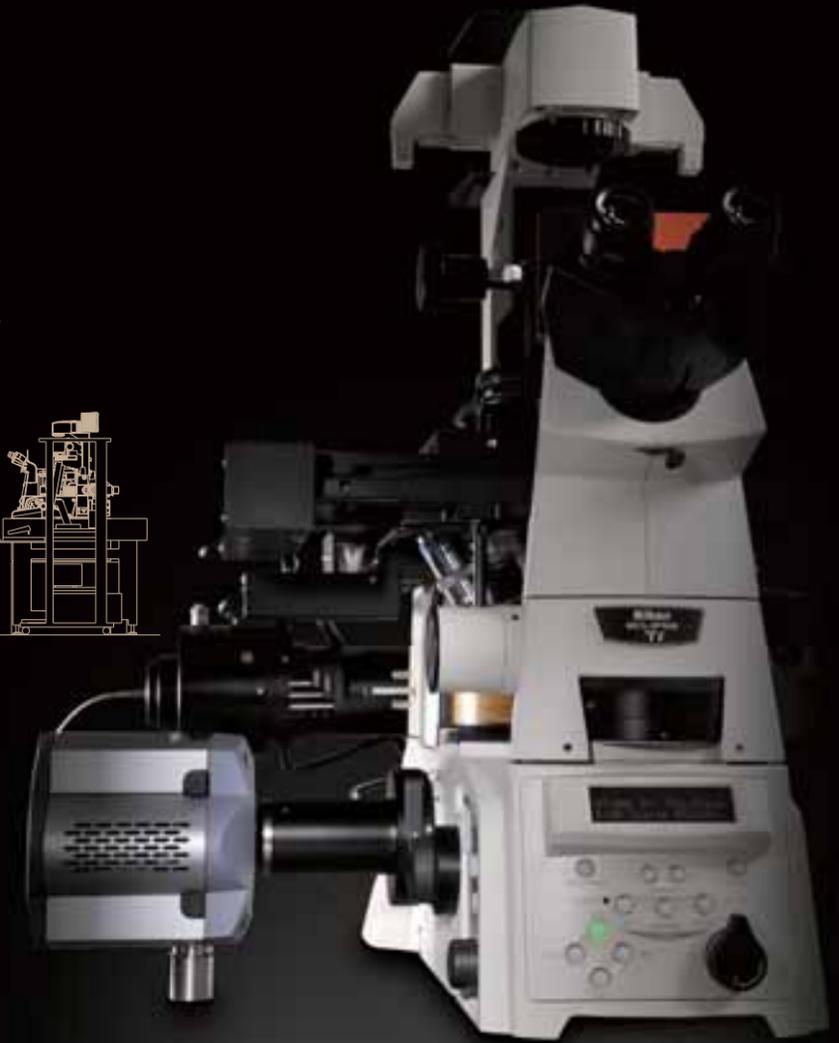
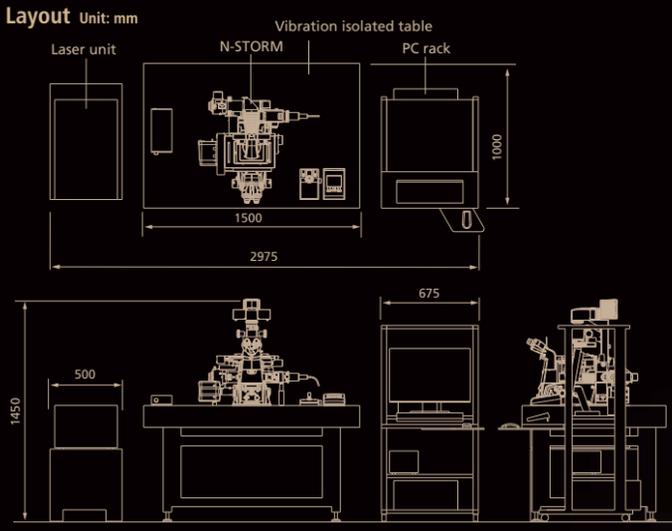
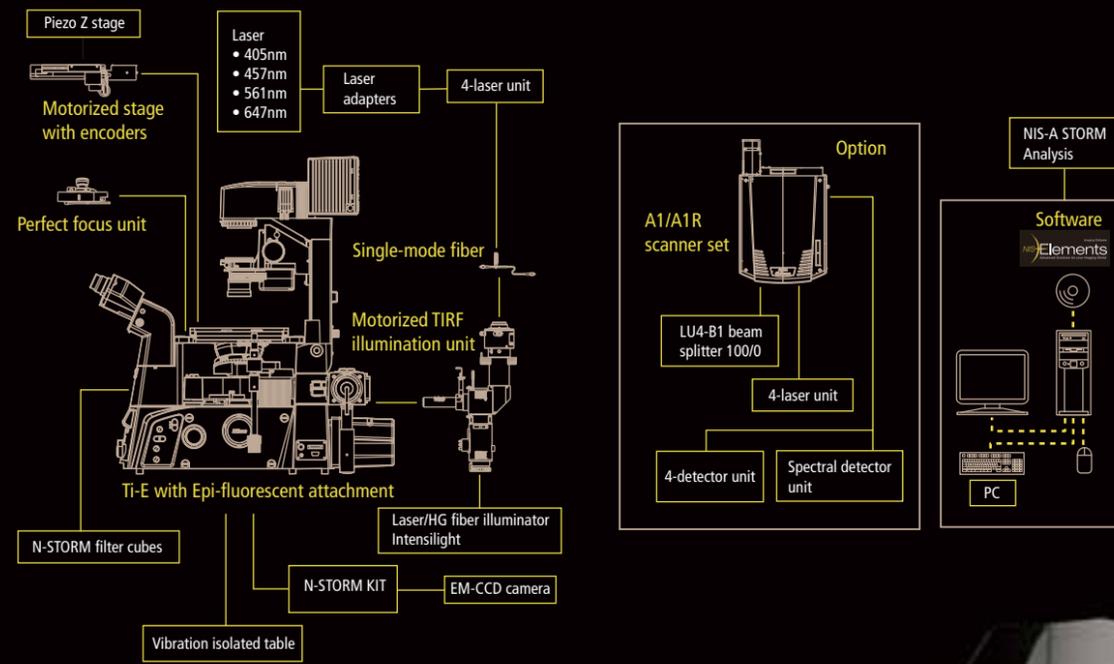
Dual color STORM image of microtubule (Alexa405-Alexa647) and mitochondria (Cy3-Alexa647) in a mammalian cell.  
Objective: CFI Plan Apo VC 100x oil (1.40)



Single color 3D-STORM image of mitochondria in a mammalian cell labeled with Cy3-Alexa647  
Objective: CFI Apo TIRF 100x oil (NA 1.49)  
Z step: 50nm



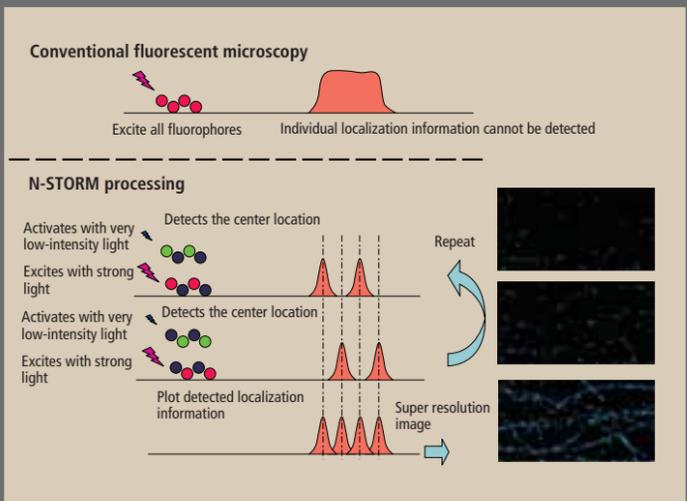
# N-STORM



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

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

N-STORM uses stochastic activation of relatively small numbers of fluorophore molecules using very low-intensity light. This low-level stochastic "activation" of discrete molecules enables high precision Gaussian fitting of each laterally. Additionally, taking advantage of an induced astigmatism via the special 3D-STORM optics, N-STORM localizes each molecule axially. Computationally combining molecular coordinates in 3 dimensions results in high contrast 3D images of the nanoscopic world with molecular specificity.



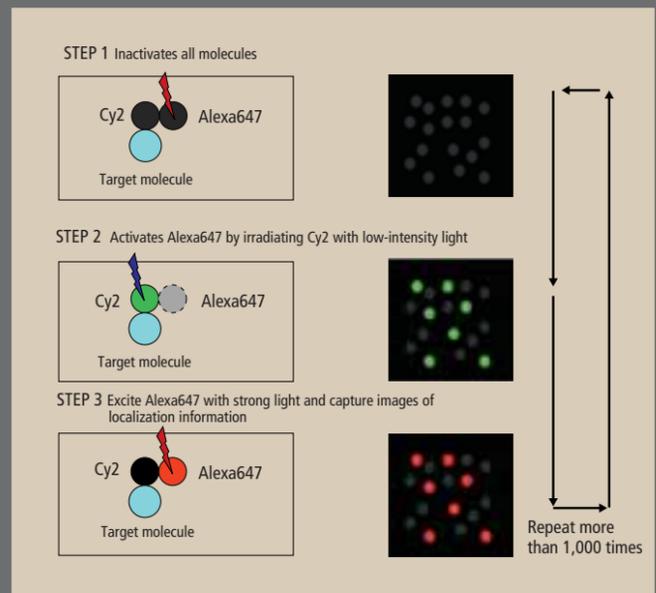
### Dedicated fluorescent dyes

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 true multi-channel super resolution.

**Dyes for N-STORM**

Dye for activation	Dye for image capturing	Dye for activation	Dye for image capturing
Cy2	Alexa647	Alexa405	Alexa647
Alexa647	Target molecule	Cy2	Alexa647
Target molecule		Cy3	Alexa647

A dye for N-STORM consists of a shorter-wavelength dye for activation and a longer-wavelength dye for image capturing. Creation of two color super resolution images is possible with pairs of dye.



## Specifications

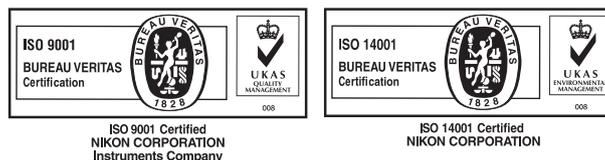
	N-SIM	N-STORM
XY resolution	Approx. 100nm (up to 85nm: theoretical, in TIRF-SIM mode, 488nm excitation)	Approx. 20nm
Z-axis resolution	Approx. 300nm	Approx. 50nm
Image acquisition time	Up to 0.6 sec./frame (TIRF-SIM/2D-SIM) Up to 1 sec. (3D-SIM) (needs more 1-2 sec. for calculation)	10 minutes or more
Imaging mode	TIRF-SIM (TIRF XY super resolution) 2D-SIM (XY super resolution, up to 3µm deep) 3D-SIM (XYZ super resolution, up to 20µm deep)	TIRF-STORM 3D STORM
Multi-color imaging	Up to 5 colors	2 colors simultaneously
Compatible Laser	Standard: 488nm, 561nm Option: 457nm, 515nm, 532nm	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× oil (NA1.49) CFI Plan Apo IR 60× WI (NA1.27)	CFI Apo TIRF 100x oil (NA 1.49) CFI Plan Apo VC 100x oil (NA 1.40)
Camera	Andor Technology iXon DU897 EMCCD camera	
Software	NIS-Elements Ar/ NIS-Elements C (with confocal microscope A1)	NIS-Elements Ar/ NIS-Elements C (with confocal microscope A1) Both need the NIS-A STORM Analysis
Operation conditions	25 °C ± 0.5 °C	20 °C to 25 °C ( ± 0.5 °C )

Cover image (bottom) photographed with the cooperation of: Hidetaka Ohnuki, Ph.D., Shigeki Higashiyama, Ph.D., Ehime University Graduate School of Medicine

Specifications and equipment are subject to change without any notice or obligation on the part of the manufacturer. September 2010 ©2010 NIKON CORPORATION



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