

## Nikon Ti-E microscope with N-SIM and N-STORM modules

Multifunctional fluorescence inverted widefield microscope enabling live-cell imaging, TIRF or HILO illumination and two super-resolution techniques: structured illumination microscopy and single molecule localization microscopy. For more detailed microscope characteristics please see the section "Microscope".

Basic introduction to super-resolution microscopy can be found here:

<http://zeiss-campus.magnet.fsu.edu/articles/superresolution/introduction.html>

### Application

- Multicolor (up to 3 colors) super-resolution images obtained by 3D-SIM technique reaching up to 2x better lateral and axial resolution compared to standard widefield imaging
- Super-resolution 2D images at high speed captured by „TIRF-SIM“ mode for excitation wavelengths 488 and 561 nm for better understanding of molecular interactions at the cell surface
- Two-color super-resolution images obtained by single molecule localization methods (STORM, dSTORM, PALM; TIRF or HILO illumination available) with resolution improvement up to 10x compared to conventional optical microscopes
- Fast and sensitive multicolor widefield imaging with TIRF or HILO excitation option
- Option of simultaneous dual wavelength imaging by single camera
- Long term live-cell imaging available
- Brightfield microscopy

### Microscope

Inverted widefield microscope Nikon Eclipse Ti-E equipped with a piezo Z-stage, motorized XY stage, Perfect Focus System, automatized H-TIRF module, insertable quarter-wave plate, insertable gradation neutral density filter, transmitted light lamp (100 W) and following units:

Software	<b>NIS-Elements Ar (v4.60)</b>
Epifluorescence	<b>Nikon Intensilight E</b>
Laser excitation wavelengths	<b>405 nm, 445nm, 488 nm, 561 nm, 647 nm</b> (all cw)
Filter turret 1	Filter cubes for: <b>DAPI</b> (excitation 340-380, emission 435-485) <b>CFP</b> (426-446, 460-500) <b>FITC</b> (465-495, 515-555) <b>TRITC</b> (528-553, 590-650) <b>Cy5</b> (625-650, peak 670 nm) – not inserted, <b>continuous STORM</b> (excitation, mirror, emission: 387-417, 420-481, 422-478 483-494, 497-553, 502-549 557-570, 575-628, 581-625 636-661, 667-792, 674-786)

Filter turret 2	Filter cubes for: <b>SIM488</b> (470-490, 500-545) <b>SIM561</b> (556-566, 570-640) <b>SIM647</b> (590-650, 663-738)
Objectives	Nikon CFI <b>HP Apo TIRF 100x Oil, NA 1.49</b> , WD 0.12 mm, Temperature Correction Ring 23-37°C (for STORM) Nikon CFI <b>SR Apo TIRF 100x Oil, NA 1.49</b> , WD 0.12 mm, Temperature Correction Ring 23-37°C (for SIM) Nikon CFI <b>Plan Apo 60x WI, NA 1.27</b> , WD 0.17 mm, Correction Collar 0.15-0.19 (for SIM) Nikon CFI <b>Plan Apo Lambda 20x, NA 0.75</b> , WD 1mm
Tube lenses	1x 1.5x
Relay lenses in front of ORCA Camera	1x (widefield) 0.4x (STORM)
Relay lenses in front of EM CCD Camera	1x (astigmatism lens for STORM) 1x (widefield) 2.5x (SIM)
Astigmatism lens (for 3D STORM)	Yes, insertion possible for both cameras
Cameras	<b>EM CCD Andor iXon Ultra DU897</b> (Andor Technologies) <ul style="list-style-type: none"> <li>• right port</li> <li>• available for both SIM and localization super-resolution techniques, live-cell imaging and TIRF imaging</li> <li>• 512x512 pixels</li> <li>• pixel size: 16x16 <math>\mu\text{m}</math></li> <li>• frame rate 56 fps for 512x512 pixels, standard mode</li> <li>• frame rate up to 1 fps for 512x512 pixels, 3D-SIM acquisition</li> <li>• frame rate up to 1.6 fps for 512x512 pixels, TIRF-SIM acquisition</li> </ul> <b>sCMOS Hamamatsu ORCA 4.0 V2</b> (Hamamatsu Photonics) <ul style="list-style-type: none"> <li>• left port</li> <li>• available for both localization super-resolution techniques, live-cell imaging and TIRF imaging</li> <li>• 2048x2048 pixels</li> <li>• pixel size: 6.5x6.5 <math>\mu\text{m}</math></li> <li>• frame rate 100 fps for 2048x2048 pixels, standard scan</li> </ul>
SIM diffraction grating blocks	3D EX V-R (3D-SIM 100x oil objective) 3D EX V-R (3D-SIM 60x water objective) TIRF 488 (TIRF 100x oil objective) TIRF 561 (TIRF 100x oil objective)
Image splitter	DV2 (Photometrics) <ul style="list-style-type: none"> <li>• inserted on demand</li> </ul>

	<ul style="list-style-type: none"><li>• available beamsplitters: 565 nm (T565lpxr), 505 nm (T505lpxr), Polarization/Anisotropy module</li></ul>
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