**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 | **NIS-Elements Ar (v4.60)** |
| Epifluorescence | **Nikon Intensilight E** |
| Laser excitation wavelengths | **405 nm, 445nm, 488 nm, 561 nm, 647 nm** (all cw) |
| Filter turret 1 | Filter cubes for:  **DAPI** (excitation 340-380, emission 435-485)  **CFP** (426-446, 460-500)  **FITC** (465-495, 515-555)  **TRITC** (528-553, 590-650)  **Cy5** (625-650, peak 670 nm) – not inserted,  **continuous STORM**  (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:  **SIM488** (470-490, 500-545)  **SIM561** (556-566, 570-640)  **SIM647** (590-650, 663-738) |
| Objectives | Nikon CFI **HP Apo TIRF 100x Oil, NA 1.49**, WD 0.12 mm, Temperature Correction Ring 23-37°C (for STORM)  Nikon CFI **SR Apo TIRF 100x Oil, NA 1.49**, WD 0.12 mm, Temperature Correction Ring 23-37°C (for SIM)  Nikon CFI **Plan Apo 60x WI, NA 1.27**, WD 0.17 mm, Correction Collar 0.15-0.19 (for SIM)  Nikon CFI **Plan Apo Lambda 20x, NA 0.75**, 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 | **EM CCD Andor iXon Ultra DU897** (Andor Technologies)   * right port * available for both SIM and localization super-resolution techniques, live-cell imaging and TIRF imaging * 512x512 pixels * pixel size: 16x16 μm * frame rate 56 fps for 512x512 pixels, standard mode * frame rate up to 1 fps for 512x512 pixels, 3D-SIM acquisition * frame rate up to 1.6 fps for 512x512 pixels, TIRF-SIM acquisition   **sCMOS Hamamatsu ORCA 4.0 V2** (Hamamatsu Photonics)   * left port * available for both localization super-resolution techniques, live-cell imaging and TIRF imaging * 2048x2048 pixels * pixel size: 6.5x6.5 μm * frame rate 100 fps for 2048x2048 pixels, standard scan |
| 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)   * inserted on demand * available beamsplitters: 565 nm (T565lpxr), 505 nm (T505lpxr), Polarization/Anisotropy module |