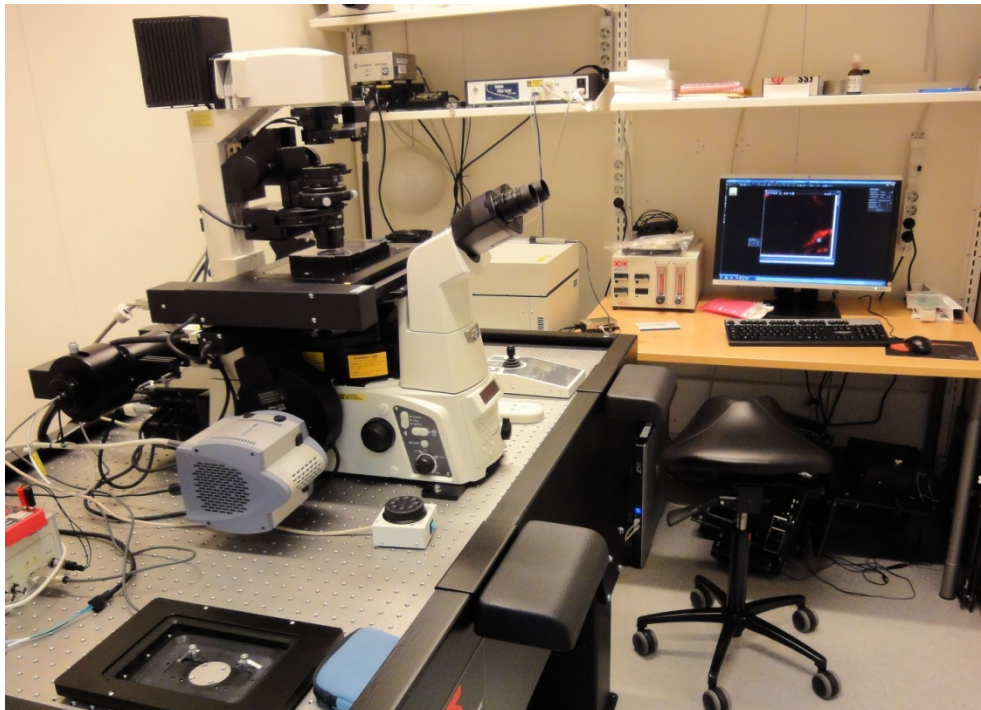


# Nikon eclipse Ti-E Microscope and NIS-AR Software

For STORM, TIRF and Live Cell Imaging

**DRAFT VERSION**

Room B115a2



User Guide

Biomedicum Imaging Unit (BIU)

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# 1 General

## 1.1 Instruments

This user guide covers the use of the Nikon Eclipse Ti-E microscope. <http://research.med.helsinki.fi/biu/instruments/nikontirf.htm>.

## 1.2 Reservations

### Common reservation rules

Instructions for Scheduler online reservation system can be found on the BIU web page: <http://www.biu.helsinki.fi/reservations.htm>. You can make your Nikon reservations two weeks in advance. If you need to cancel your reservation, you have to do it two hours before your reservation starts. Uncancelled reservations will be charged as normal. Make sure you are familiar with the use of Scheduler and the BIU fees and user policy (<http://www.biu.helsinki.fi/fees.htm>).

When you make reservations, always remember to indicate in the Event name field what temperature you will be using the system at (RT or +37°C). The first reservation for a particular day decides at what temperature the system will be kept for the entire day.

If you want to extend your session and there are no reservations after you, you can make a new reservation right after the current one. It is not possible to make a single reservation that extends beyond 24:00. In such a case reservation can be extended by starting a second one at 00:00.

## 1.3 Billing

The billing is based ONLY on the reservations made in Scheduler. There are two prices for the use of the system, one including lasers (TIRF, STORM) and the other without (live cell and other epifluorescent imaging). Please remember to choose the appropriate option when reserving the system (Service: 'with lasers' or 'no lasers'). There is also a separate fee for the training session (2h).

## 1.4 Unauthorized use

BIU periodically checks the logins of the computer. If your logon/off times exceed your reserved time in Scheduler, BIU will consider that as unauthorized use of the instrument and ask for an explanation. For unauthorized use of the instrument, BIU may issue a warning. After three warnings, BIU may revoke the user's license. Also, misuse/neglect of the instrument may lead either to a warning or cancellation of the user license and the repair costs may be charged.

## 1.5 Users' mailing list

All registered users are automatically added to the users' mailing list. Please read those e-mails to know what is going on!

## 1.6 Acknowledging BIU

Whenever you are using instruments and/or services maintained/provided by BIU, you are expected to acknowledge BIU in your publications and inform BIU about the papers.

## 2 Components

### 2.1 Lightsources

Transmitted	12V Halogen lamp with Sutter shutter	
Epifluorescent	Intensilight 130W Hg lamp	
TIRF/STORM lasers	405 nm	100mW
	Argon (457, 488, 514nm)	65mW
	561 nm	150mW
	647nm	300mW

### 2.2 Objectives

Magnification	Objective type	NA	Medium	Coverglass thickness (mm)	Working distance (mm)	Perfect Focus compatibility	Transmitted Light Contrast
4×	PlanFluar	0.13	Air	0.17	16.5	-	PhL
10×	PlanFluar	0.30	Air	0.17	16.0	+	Ph1
20×	PlanApo $\lambda$	0.75	Air	0.17	1.0	+	Ph2
40×	PlanFluar	0.75	Air	0.17	0.66	+	Ph2
60×	PlanFluar	0.85	Air	0.11-0.23	0.40	-	

#### Also available on request:

Magnification	Objective type	NA	Medium	Coverglass thickness (mm)	Working distance (mm)	Perfect Focus compatibility	Transmitted Light Contrast
20×	Plan Fluar	0.50	Air	0.17	2.1	-	Ph1
20×	Plan Fluar	0.50	Air	0.17	2.1	-	DIC
40×	Plan Fluar	0.75	Air	0.17	0.66	+	DIC
63×	Plan Apo VC	1.40	Oil	0.17	0.13	+	
100x	Apo TIRF	1.49	Oil	0.13-0.19	0.12	+	

## 2.3 Filters

### Excitation filters

Pos	Ex. Filter
1	D405/40x
2	ET470/40x
3	HQ545/30
4	ET560/40x
5	S565/28x
6	ET620/60x
7	ET640/30x
8	357/44

### Turret:

Pos	Ex.Filter	Dichroic Mirror	Em. Filter	Laser Quality ?
1	None	440DCXR	D470/40m	No
2	None	488TIRF	525/50	Yes
3	None	585DCXR	ET630/75m	Yes
4	None	T660LPXR	ET700/75	No
5		Variable on request		
6	None	Transmitted light	Empty	

Addition filters on request (primarily placed in position 5)

	Ex.Filter	Dichroic Mirror	Em. Filter	Laser Quality ?
a	None	STORM	STORM	Yes
b	None	GFP/dsRED double	525/50 + 630/75	Yes
c	Quad (405-488-561-647)	Quad	Quad (use clean-up filters 4-7 if needed)	Yes

### Emission filters

These are usually not used (set position 1) as the turret filter block already contains an emission filter in most positions.

Pos	Em. Filter
1	Empty
2	525/50
3	630/75
4	488 Notch
5	ET450/50m
6	ET525/50
7	ET605/52
8	ET700/50m

### Commonly used filter combinations (single fluorophore)

Fluorophore	Excitation filter	Turret	Emission filter
DAPI	1	1	1
GFP	2	2	1
dsRed	4	3	1
Cy5	7	4	1

## 2.4 Camera

There are two Andor EM-CCD cameras connected to the system, the Andor iXon DU-897 and the iXon DU-885.

Camera	Port	Pixels	Pixel size	Chip
DU-885	Left	1004×1002	8 $\mu$ m×8 $\mu$ m	Front-illuminated
DU-897	Right	512×512	16 $\mu$ m×16 $\mu$ m	Back-illuminated

N.B. The pixel size is the physical size of the pixel on the chip, not the size of the pixel in your image. That depends on the optics used.

While the DU-897 is the more sensitive camera with a superior signal to noise ratio the low pixel count means that you will be unable to fulfill Nyquist sampling with most (if not all) optical configurations. It is mostly meant for STORM imaging or other single fluorophore / low light applications. If you are unsure of what camera to use you will most likely want to use the DU-885 camera.

## 2.5 Motorized Stage

Nikon motorized stage with encoders.

## 2.6 Environmental Chamber

The environmental chamber encases the microscope and allows you to control the temperature that the sample and optics are at. The Nikon Eclipse TiE is primarily used for visualization of living cells at 37°C so you must clearly mark the intended temperature in your scheduler reservation. As it takes approximately 2h to heat up the optical components and 2h to cool down the first reservation made for a particular day decides at what temperature the system will be at **all** day. The person requesting 37°C is the one responsible for turning the temperature controller on (and off).

## 3 Turning on the system

### 3.1 Turning on the hardware

All peripheral equipment except lasers and environmental chamber are controlled by the Nikon TiE hub. They must all therefore be turned on **before** turning on the microscope, otherwise they will not be controllable.

1. Epifluorescent lightsource
2. Stage and transmitted light controllers
3. Sutter shutters (both epi and transmitted light)
4. Piezo stage (if used)
5. Microscope hub
6. Turn on the computer and log in. The computer is not connected to the network and does not use university accounts

### 3.2 Starting the acquisition software

The software used for controlling the system is the Nikon NIS-Elements Advanced Research (NIS) software package. There are two shortcut icons on the desktop. The one with reading “NIS” is the full package for controlling the microscope. The one marked “A” is for the analysis of previously acquired images only.

On start-up the system will ask you to choose a camera driver. Normally you will want to use the regular Andor driver. Do not use the Andor for NSTORM option unless you are running a STORM experiment.

## 4 Basic use

### 4.1 Optical configuration

The use of the NIS software is centered on the use of “optical configurations”. All the required settings for the acquisition of a single image are stored as a single pre-selection: filters, light source, shutters, camera settings etc.

#### Creating a new configuration

You can either create a new optical configuration from scratch or duplicate an old configuration, rename it and use that as a base.

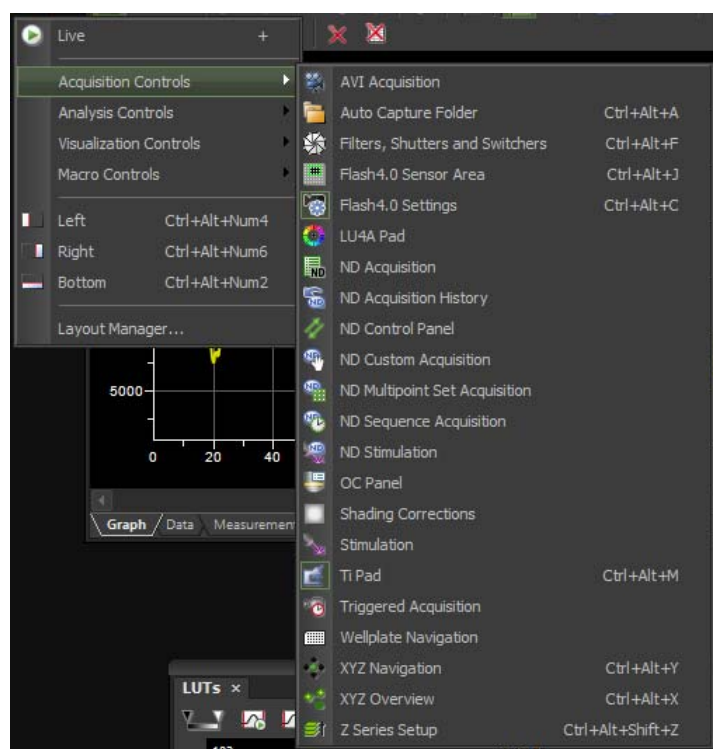
## 4.2 ND acquisition

The NIS Elements software has multiple tools for acquiring image sequences. Usually it will be enough to use the most versatile and general purpose tool: ND Acquisition. Once you have set up optical configurations for your experiment you will be able to use this tool to take time-lapse, multi-point, z-stack, multichannel, and/or large montage images.

## 5 Advanced use

### 5.1 Acquisition controls

The acquisition Controls can be accessed by right clicking on an empty area of the program background and choosing from the drop down menu. You will need atleast the camera settings (Ctrl+Alt+C) and the TiPad (Ctrl+Alt+M), LU4A Pad if you are using the lasers (TIRF, STORM).



### 5.2 Triggered image acquisition

Currently there is only one triggering available on the Nikon system; the LU4 laser box can trigger the Andor iXon 897 camera. To optimally use this option you will have to activate two tools:

*Fast Time-lapse:* Activated from the Acquire menu. This will allow you to maximize camera frame rate. Only when using one image setting or in combination with “Triggered Acquisition”.

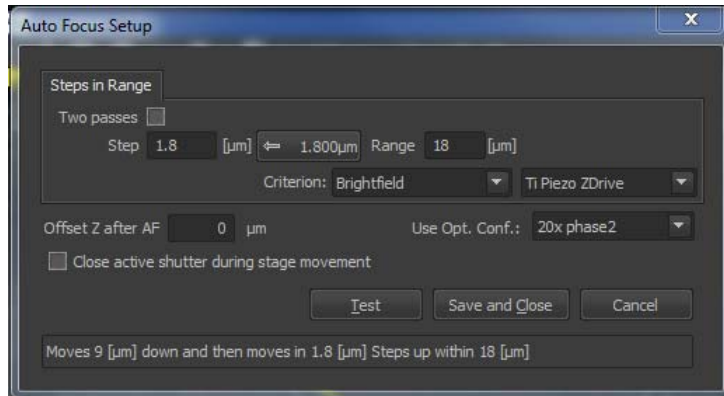
*Triggered Acquisition:* Activated from the “Acquisition Controls” menu. From here you will be able to choose triggered devices and set laser power and camera exposure.



## 5.3 Autofocus

If you are unable to use the PFS hardware autofocus (unsuitable objective, large offset etc.) there is also a traditional software autofocus available.

### Setup



#### *Two passes*

Select this option to perform auto focus in two steps: Coarse and Fine.

#### *Step, Coarse Step, Fine Step, Recommended step button*

Define the step size (distance between two Z positions) in micrometers. There is a button left of the Range field which indicates a step size recommended for the current objective magnification. Click the button to insert the value to the “step” field.

#### *Range*

Define the total distance which the Z drive will use for auto focus.

#### *Criterion*

Defines which focus criterion is used:

- Brightfield - standard contrast based criterion
- Fluorescence - suitable for fluorescence microscopy
- Max sum intensity - criterion based only on the intensity values. Can be useful in confocal microscopy.
- Yeast, Bacteria (Ph) - a criterion optimized for yeasts under phase contrast (Ph) microscope.

#### *Offset Z after AF*

This option may be useful in fluorescence applications for the cases where the auto focus algorithm produces offset (the system states it is focused but you would like to focus on some other objects within the image, which are blurry). After performing auto focus, the Z drive will move by the distance defined within this field.

#### *Close active shutter during stage movement*

The active shutter will close between image acquisitions. Typically, this option is used to reduce the photo-bleaching effect on live cells but extend total time of autofocusing.

#### *Test*

Press this button to start focusing immediately. If everything works fine, click the Save and Close button to apply the setting.

#### **Note**

When working with low-magnification objectives (4x for example) use higher steps (like 10 microns). For higher magnifying microscopic objectives (for example 40x) use lower steps (like 3 microns). Also, the travel range should be adjusted for each objective. For example the travel range for a 20x objective should be about 100 micrometers. Be sure not to enter higher travel range than half of the working distance of the objective.

### **5.4 Mouse Joystick and Focus**

You can use the computer mouse to move the stage and manually focus the image if enabled under Devices.

#### **XY Movement**

Right click on the live image on the spot you want to center and choose '*Move this Point to Center*' from the drop down menu.

#### **Z Focus**

Use the Mouse scroll while hovering over the live image.

## **6 Ending your session**

Always start by checking the reservation system to see if there are still other users of the Nikon TIRF/STORM system. If yes proceed to step 6.1, otherwise step 6.2.

### **6.1 There are users after you on the same day**

Store all image data and click FILE/EXIT to terminate the NIS-Elements software. Close all open windows and exit the user profile you are using but do not turn the computer off.

Clean up the objectives; have the light turned on so that you can see properly. Use lens paper to wipe up the oil. **Do not rub** it against the top lens of the objective.

### **6.2 You are the last user of the day**

#### **To completely turn off the system:**

1. Turn laser (argon) power level to minimum.
2. Clean objectives as above, wipe oil objectives with ethanol. **Do not rub!**
3. Exit NIS-Elements software and turn off computer.
4. Turn off shutters, microscope controllers and microscope.
5. Turn off hubepifluorescent lightsource.

Clean up whatever mess or spills may have occurred during your session.