Nikon TE2000 fluorescence microscope

NIS-Elements User manual

AR 4.20.00

MIC

Molecular Imaging Center

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MIC
Molecular Imaging Center

Do you want to take images of your sample?

No, just observing

I will analyse some data

yes

No need to start up the pc and software. Just make sure you are in visual mode on the control box.

Start up the computer and choose «no grabber»

Start up pc and software. Log onto the server. Focus your sample in the microscope. Will you need the fluorescent lamp?

Yes, I have fluorescing samples

No

Choose DS-U2 and turn on the mercury lamp. If you need brightfield as well, adjust for Köhler (page 27).

Choose DS-U1 and check for Köhler (page 27). Do you need DIC or HMC?

Timelapse Go to page 16-17.

Large scan Go to page 11-14.

Multipoint imagaing Go to page 18.

Regular multichannel imaging Go to page 8-9.

yes

Initiate stage (page 11)

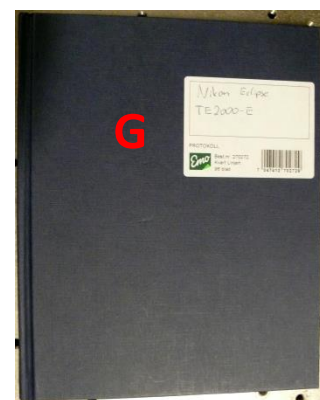Warm up stage (30 min before). Start the CO2 mixer (page 17).

Setting up DIC (page 32) Setting up HMC (page 30)

Saving data from DS-U2. This camera takes 12 bit images. For quantification analysis, keep the 12 bit. For localization observation, save as tiff or jpeg and change bit size to 8. You can permanently change it to 8 bit (page 6).

Saving data from DS-U1. Choose any format as this is a 8 bit camera (page 23).

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Switch on the system

1. Turn on the main switch (A).

2. Start up the computer (B).

3. Log in with your user account at the PC.

   – If you do not have a user account yet, ask MIC personnel to get one.

4. If you need the mercury lamp,

   – check online in the booking system (F) and in the logbook (G) whether it has been used in the last 30 min (is it warm?).

   – turn on the power switch (C) and press ignition (D).

   – note the run time (E) in the logbook (G).

5. Start "NIS-AR" (H), the NIS-Elements software.

Switch off the system

1. Check online in the booking system whether there is a user coming after you (A).

    –   If yes, phone her/him and ask whether he/she needs the mercury lamp.

    –   If nobody uses the mercury lamp for the following 30 minutes, switch it off at the power button (B).

2. Close the software and shut down the PC.

3. Fill out the logbook (C).

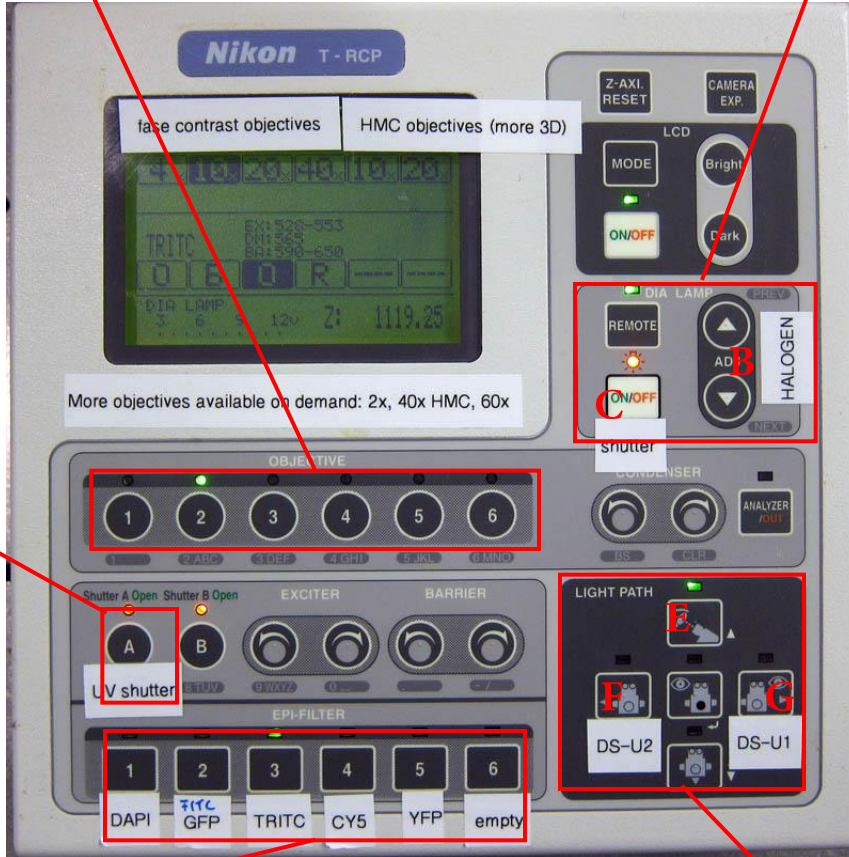4. Turn off the main switch (D).
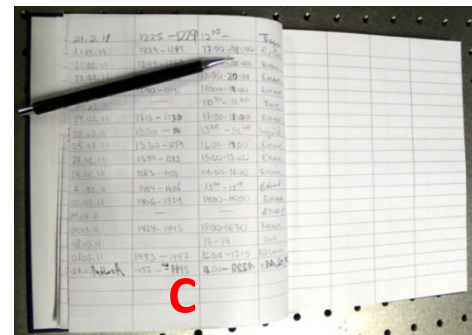
The TE2000 pad

Objective switch

Halogen light control. The main switch on the power supply needs to be on (A). Regulate the intensity here (B). Halogen shutter (C).

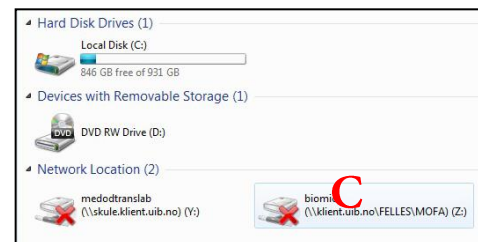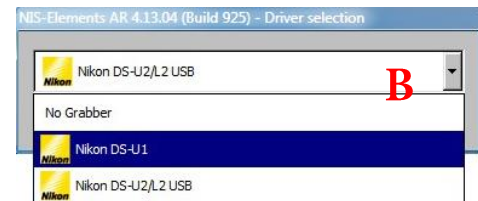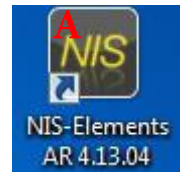Shutter for the UV light. The power supply needs to be on (D) and lamp ignited (E).

Fluorescence filter positions. No. 6 is empty and used for imaging BF, Ph, Pol, HMC, and DIC.

Light path control. Eyepiece (E), B/W-camera for fluorescence and DIC (F), and colour camera (G).

1. Double-click the "NIS-AR"-button on the Desktop (A).

2. Select the camera control unit you want to use (B):
   - For the colour camera, choose "Nikon DS-U1".
   - For the B/W-camera, choose "Nikon DS-U2/L2 USB".

3. Log on to Biomic on the "Skule" server (- to be able to save images directly onto the server).
   - Double-click the "biomic" icon on the klient server which you can find on the desktop (C).
   - Enter your UiB username in the following way: username: `uib\username`, and then the password (D).
   - If you do not have access to „biomic" yet, contact Torstein Ravnskog (☎ 86323, room 6C114fF, teknisk fellesavd.).
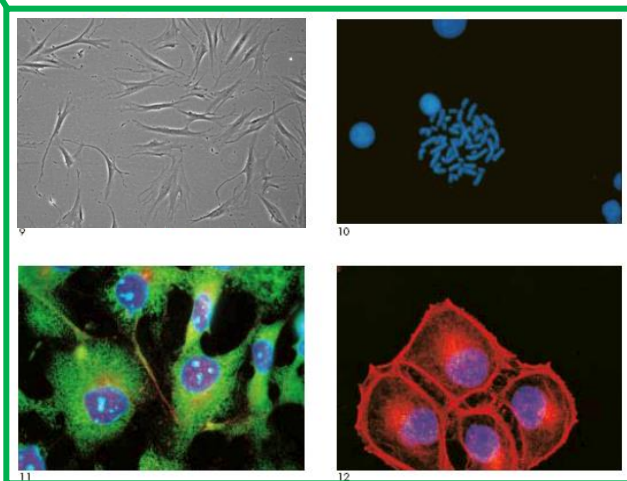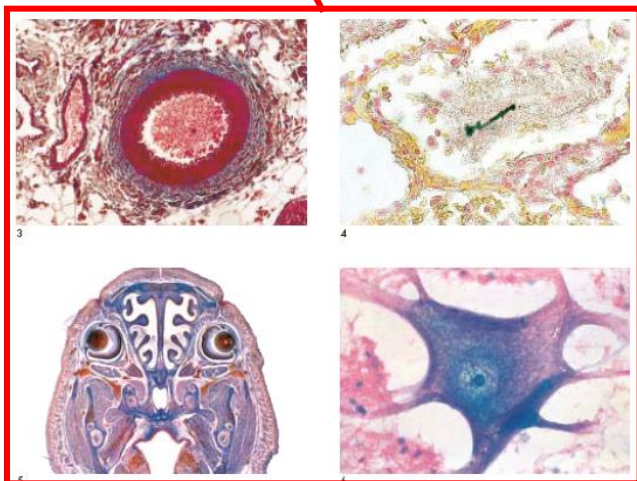
Camera selection

NIS-Elements AR 4.20.00 (Build 967) - Driver selection

Nikon DS-U2/L2 USB

No Grabber

Nikon DS-U1

Nikon DS-U2/L2 USB
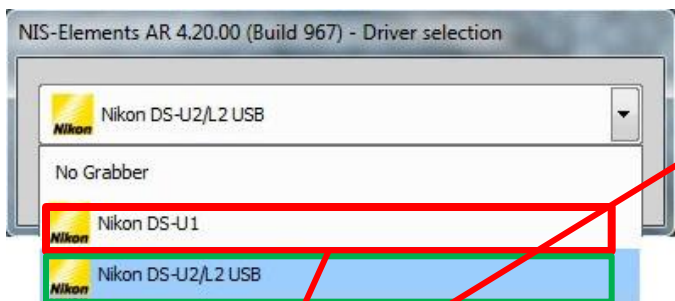
Colour camera DS-5Mc

BW-camera DS-Qi1

Turn on the main switch (A, colour camera; B, BW-camera) before use.

- histological sections
- BF + phase contrast images
- classical stainings (Golgi, Nissl etc.)

- fluorescence acquisition
- DIC + HMC images
- timelapse imaging

Pixels:	2560 x 1920 pixels; binning 2x2, 4x4
Cooling:	Peltier cooling 20° below ambient
A/D conversion:	8-bit
Exposure time:	1/1000 – 600 s
Sensitivity:	Equivalent to ISO 64

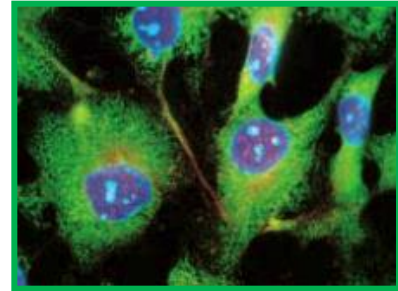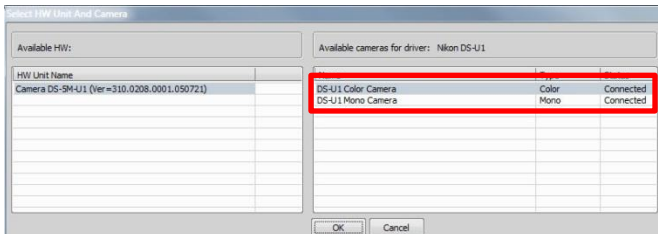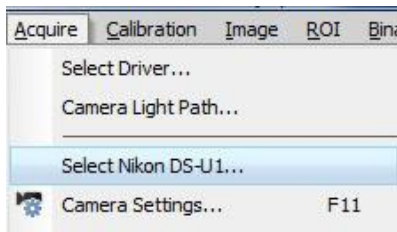| Pixels: | 1280 x 1024 pixels; binning 2x2, 4x4 |
Cooling:	Peltier cooling 10° below ambient
A/D conversion:	12-bit
Exposure time:	1/1000 – 600 s
Sensitivity:	Equivalent to ISO 800

Adjusting camera depth/color/bit

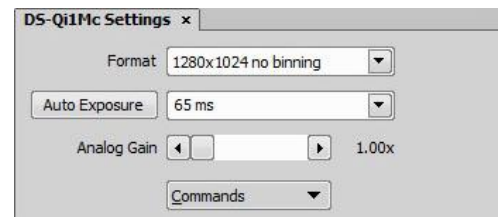If you need to change camera bit: Acquire → Select driver

DS-U1

Color camera can image B/W or color images.
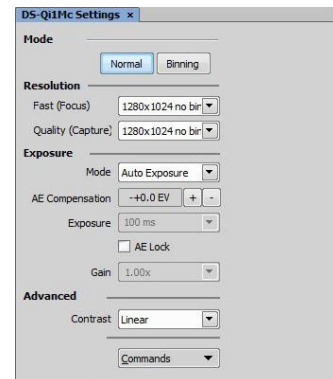

DS-U2/L2 USB

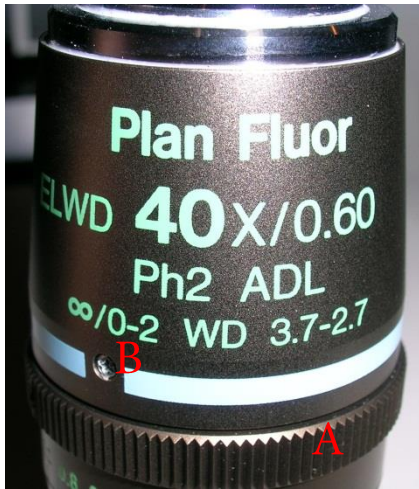For the B/W camera you can choose to image 8 or 12 bit.

12 bit imaging (quantification; 4096 tones)

8 bit imaging (visualization; 256 tones))

Correction collar adjustment

A

B

C

Some objectives have a correction collar (A) to adjust for cover slip thickness between 0 and 2 mm (B).

In order to adjust:
- look through the eyepiece at all times.
- turn the correction collar slightly (might be somewhat stuck at the beginning).
- refocus (C).
- turn the correction collar again slightly.
- refocus again.
- repeat until you get a sharp, crisp image (D).
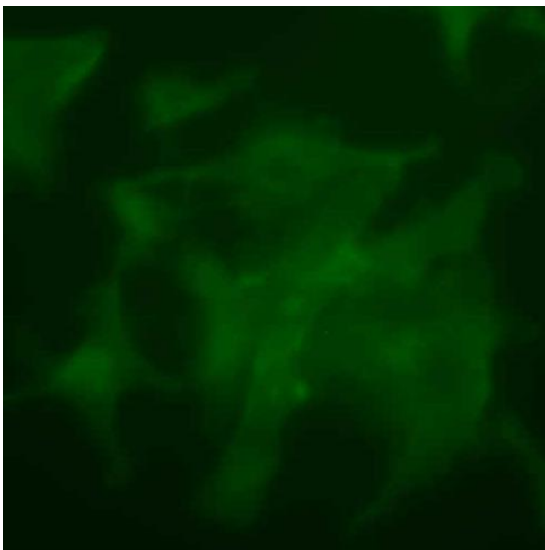
Correction collar not adjusted


D
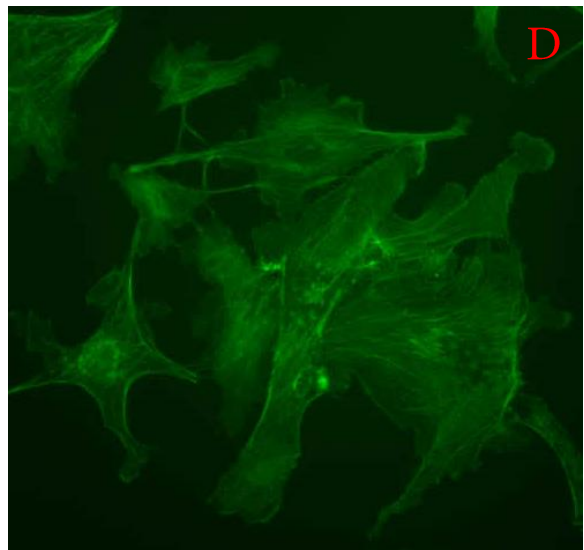
Correction collar adjusted

Manual multichannel acquisition (I)

1. The ND Acquisition window should be on the right screen. If not, make sure you are in the locked MIC folder (A).

2. Open the lambda window. Tick the optical configurations you need (B). If the one you need is not there in the list, just select the channel and choose another configuration by clicking on the arrow (C).

3. For automated saving, tick «Save to File» (D), set the File Path to an appropriate folder on the klient server, and give the data to be recorded a sensible name.

4. Use the «M» in the left toolbar to start capturing. Focus when needed.

5. Click «continue» to record the image.
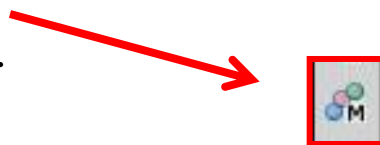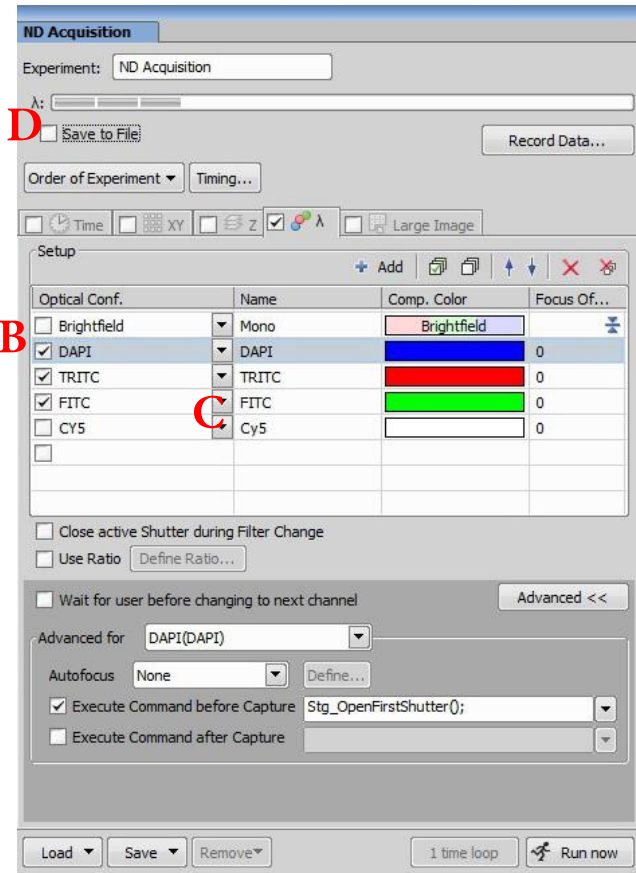
If your samples are bleaching a lot, it is adviced to select the longest wavelength first (CY5 or TRITC), then green and blue last.

# Manual multichannel acquisition (II)
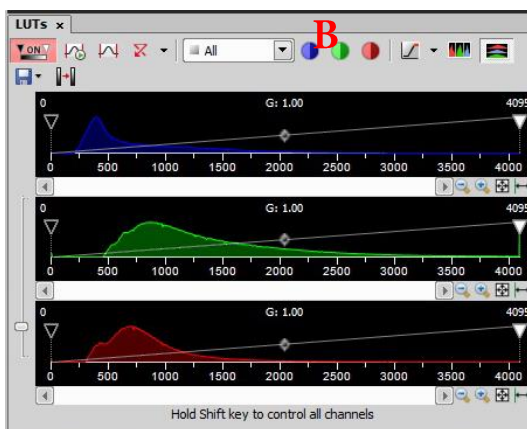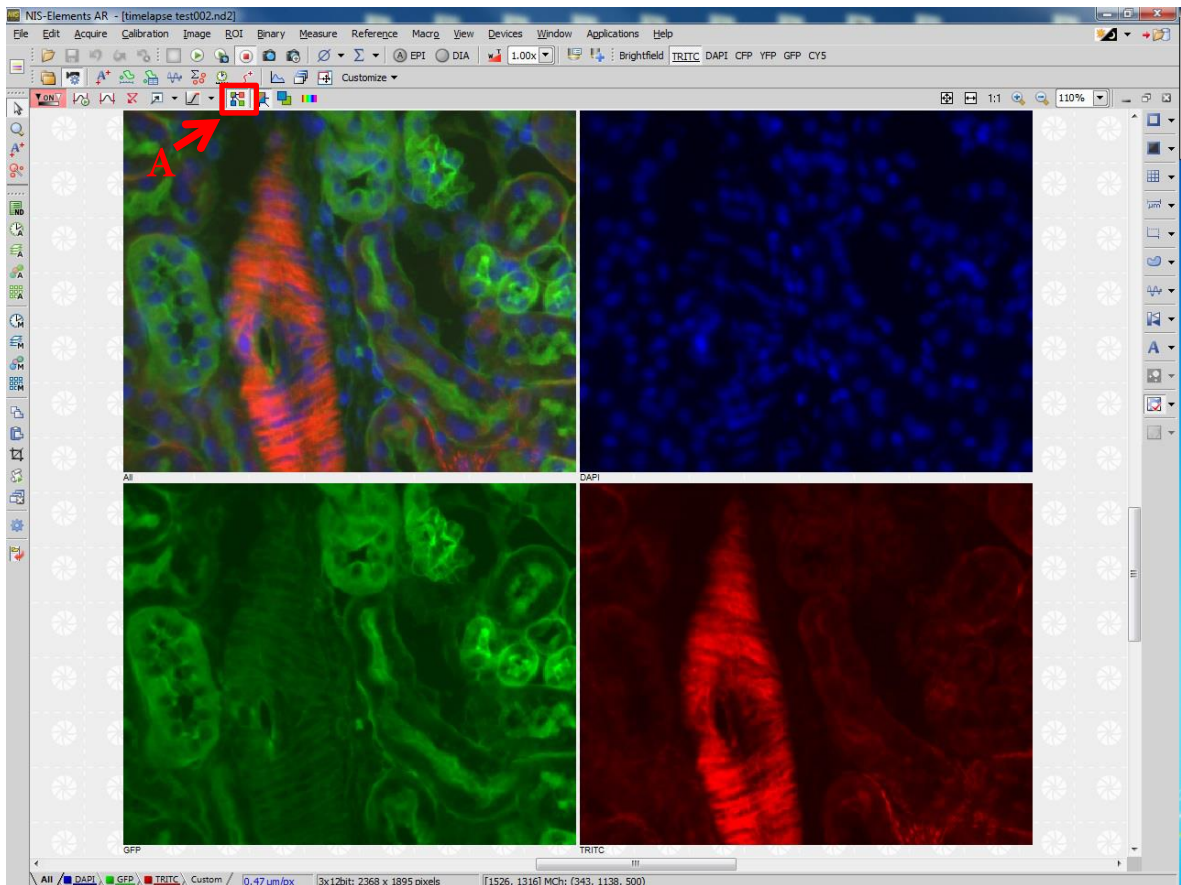
- In the "Split Channel" view (A), you can see how the different channels built up during acquisition . You can zoom in using the middle mouse button.
- After acquisition, use the LUTs to adjust the contrast for each channel individually. You can also try the automatic adjustment (B).

Automated ND acquisitions

Using the ND acquisition , you can set up experiments that include

- timelapse
- Multipoint (XY)
- z-series
- Multichannel (lambda)
- large image

If you need multipoint/ large image, you have to initialise the stage first. Then, tick all the modules you need (A) and define as described on the following pages. Do not forget to define the order of the experiment (B). A progress window (C) will inform you about the progress. Use "advanced" (D) to define autofocus and usefull commands for shutter control.
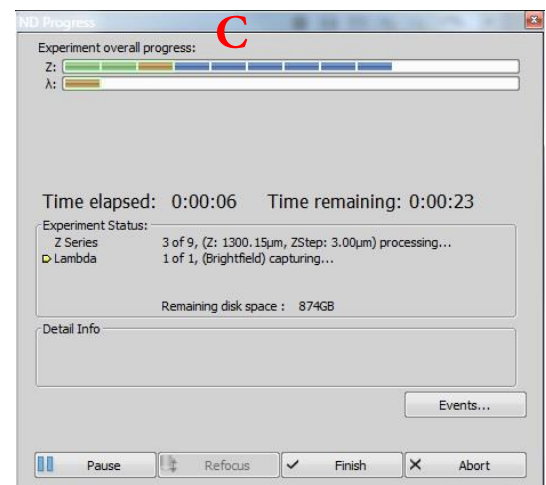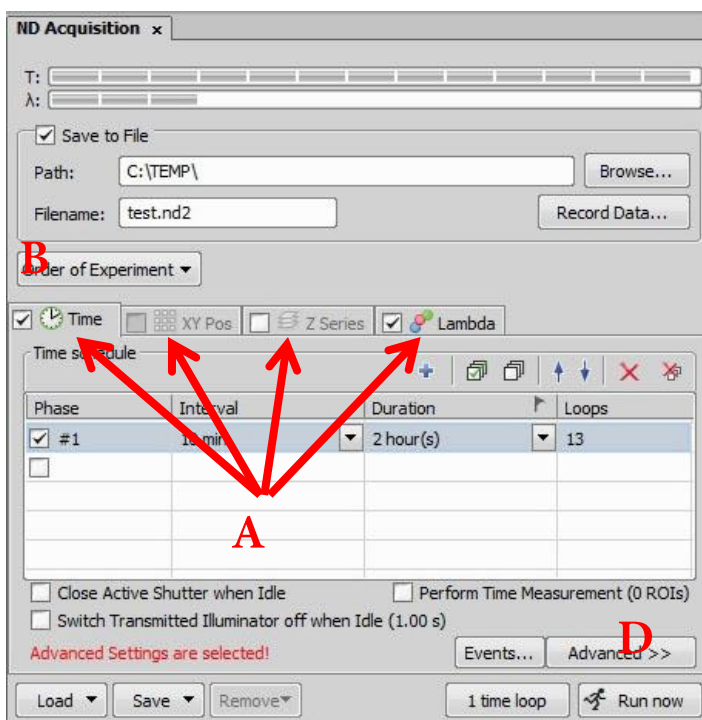
1. Initiate stage (Devices →
   Initiate stage).
2. Make sure the objective is
   not in the top position
   before you hit «escape &
   init» (A).
3. In the ND acquisition
   window you check for λ
   and define the channels
   you want to image (B).
4. You can choose to save
   the image automatically
   if you define where the
   image should be saved
   and give it a filename
   (C).
5. You need to define an
   additional command
   (open the UV shutter)
   before the first channel
   (D) or else your first
   image will be dark. Find
   the command (E):
   Stg_OpenFirstShutter
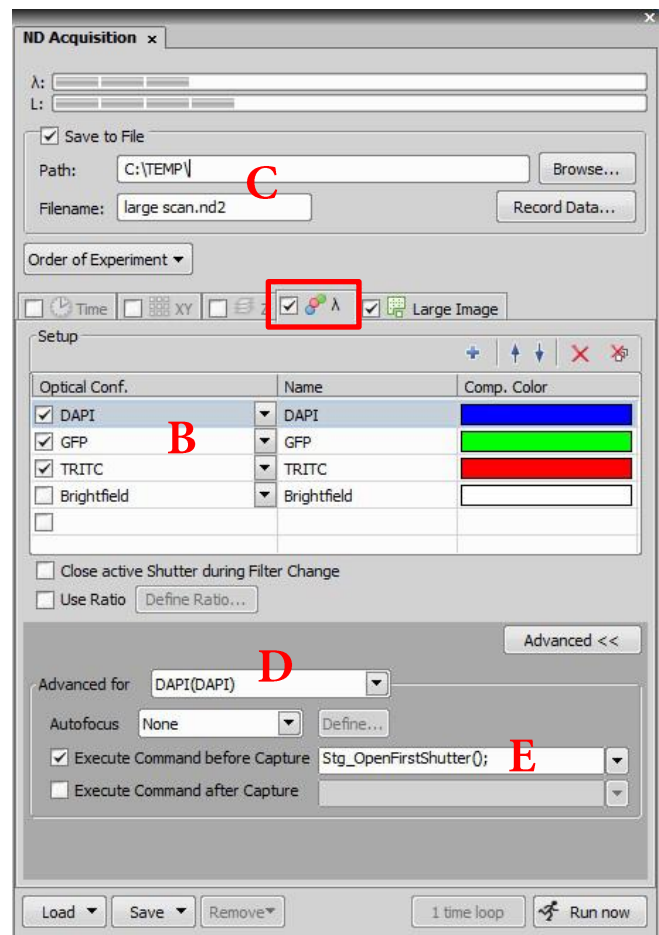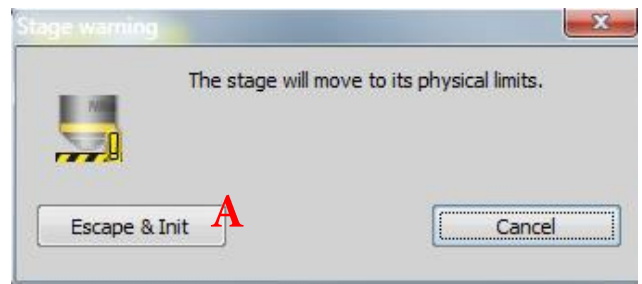
Large image acquisition (II)

1. Before you start an automatic large image acquisition, make sure the exposure time is correct for all the channels you want to image. Go to the different channels by clicking on the setting maked on the topp and open the camera (click + on the keyboard).
2. Find a correct exposure and gain. Switch over to manual exposure mode (A).
3. If a «!» shows up next to the setting (red arrow), then click on it and «assign current camera setting». If you don't do this the old camera settings will be used.
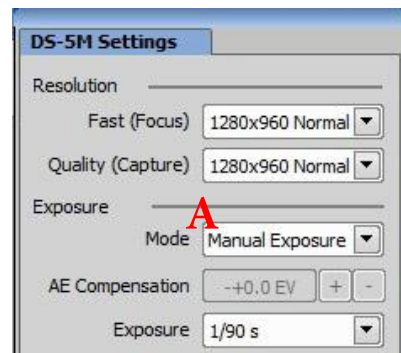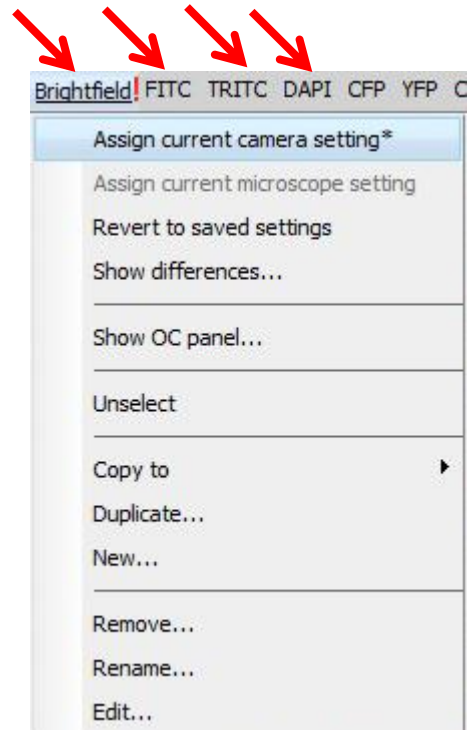
If you are using the brightfield channel I recommend you to do a shadow correction as the halogen illumination is not perfect in the centre (see page 15)

5. Tick the «large image» window and define the amount of images to be scanned (A), the first number indicates the x direction.
6. Move to the centre of your object and adjust for focus.
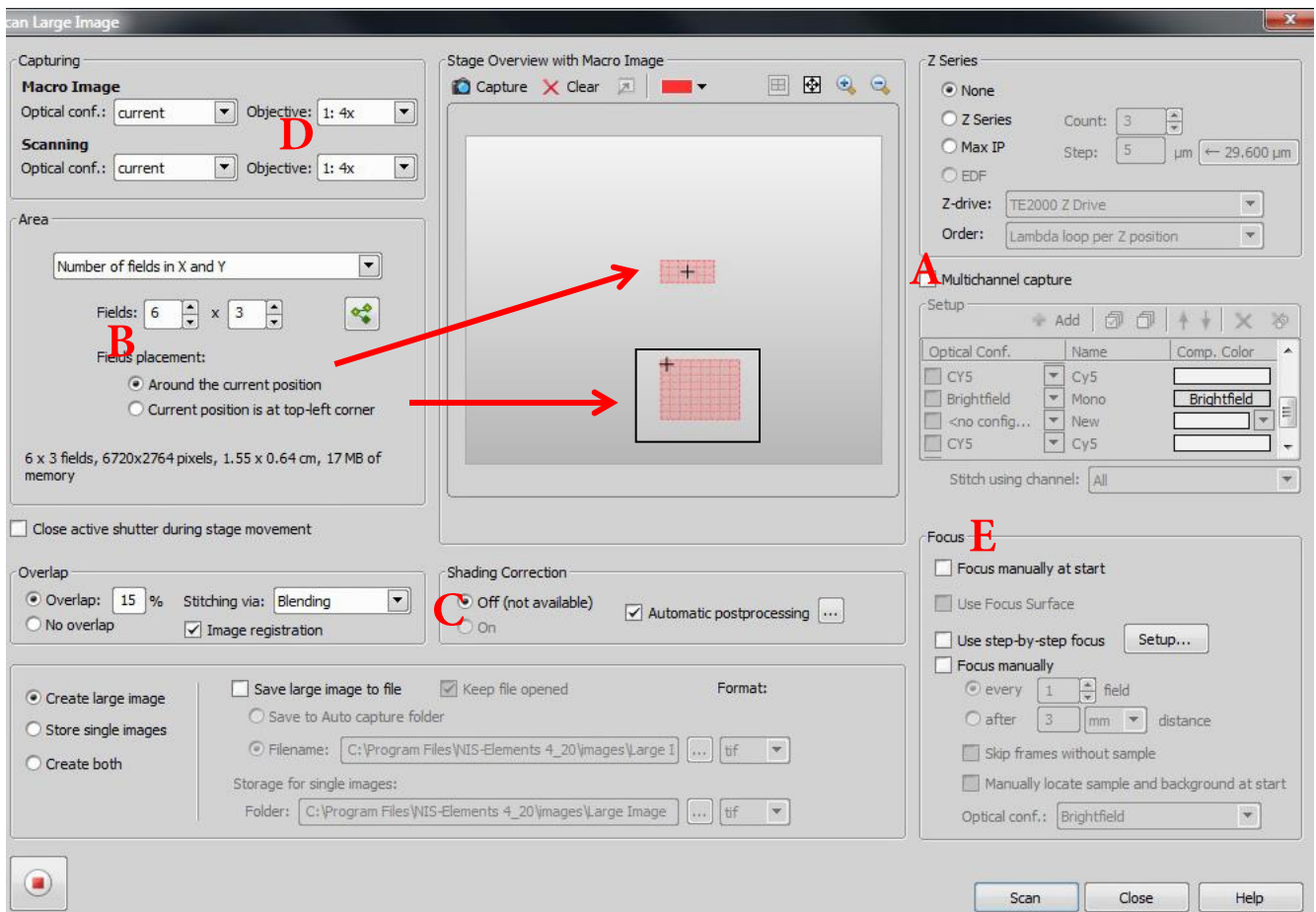7. If you want autofocus, you need to choose «define by steps» (C) either in lambda or timelapse mode.
8. Define the stepsize (D) and total z range (E).
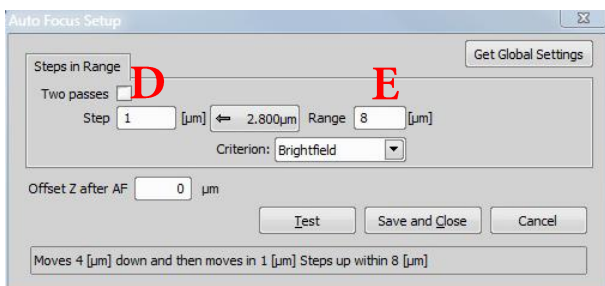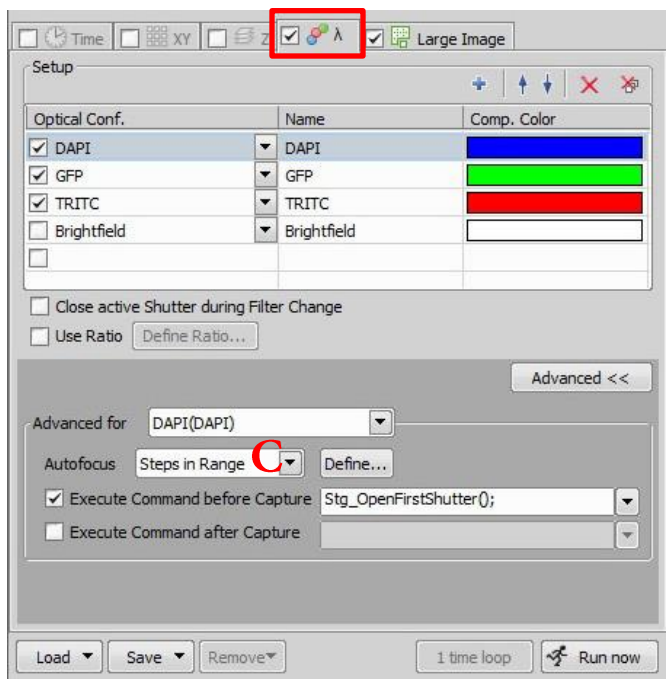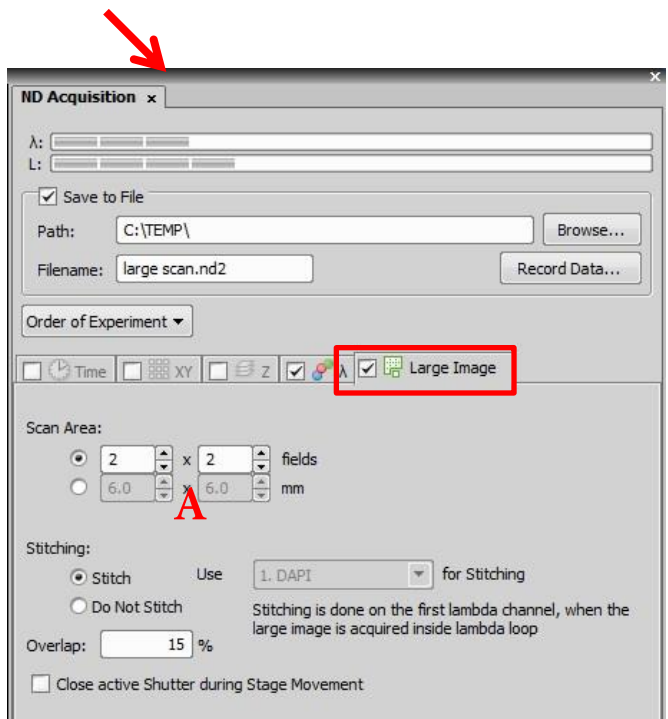
9. Find «scan large image» under the «acquire» meny.
10. If you need multichannel capture, define your channels (A).
11. Define the size of the field (B) and the placement of start postion (B)
12. Asign shading correction (C) if you are using brightfield (you need to capture correction image beforehand).
13. Choose which objective you want to use (D).
14. Define how you want to focus (E).

1. Look at your sample and adjust the Köhler illumination (!).
2. Open live camera view. Find a clean, dustfree, sample free area on your slide/well. Focus!
3. Go to «Acquire – Shadow correction».
4. Select capture correction image (A).
5. Choose 2-3 areas to to record correction images. Click «next» in between each recording and click «finish» when you are done.
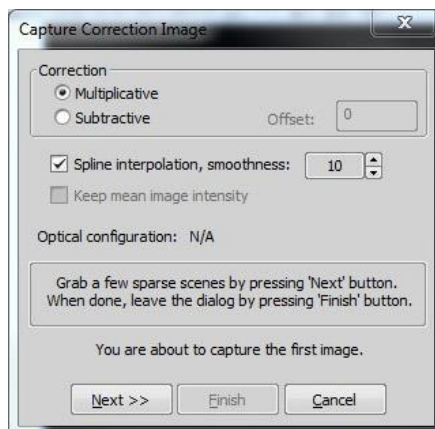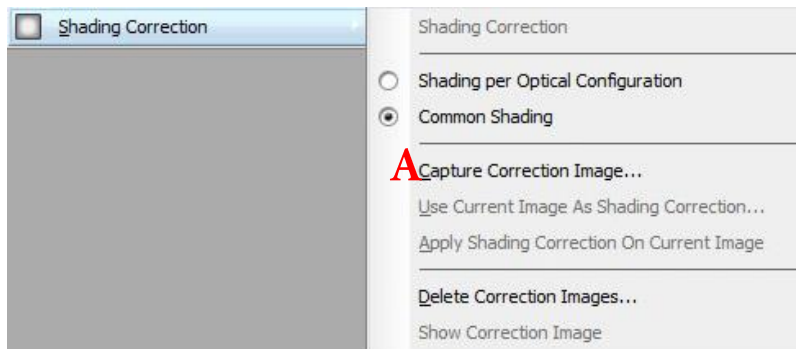
6. The correction image (B) will be saved and used in the automatic large image acquisition.

Timelapse acquisition (I)

1. Check the "time" flag in the ND acquisition window. Define the interval (B) at which the images should be taken. Define the "duration" of the experiment (C).
2. You can define different loops of timelapse experiments. If you want to acquire faster imaging for an hour before doing a slower imaging, then define a second "phase" (D).
3. For long term experiments, use autofocus under "advanced" (E).
4. If you are doing imaging with multiple channels, check the lambda flag (A) and define the channels as before described.
5. You might have to define some advanced settings (F) in order for the first image to be exposed correctly.
6. For opening UV shutter or opening and closing halogen light.
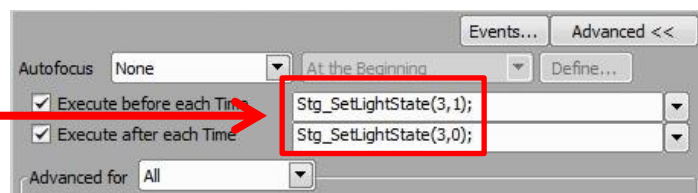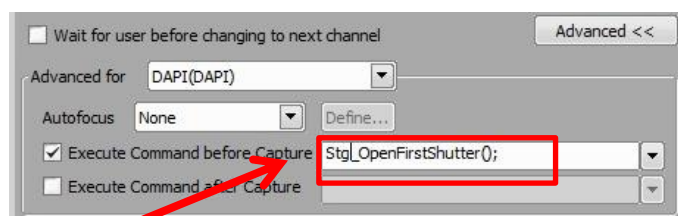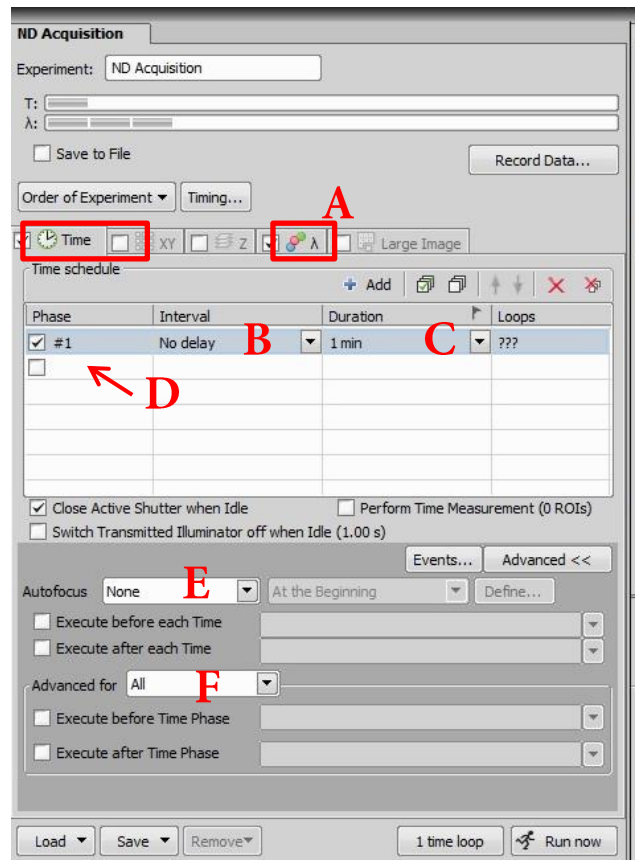
Timelapse acquisition (II)

7. Turn on the temperature/ CO_2 controller (switch behind box).
8. Put the insert on the microscope stage (A) in order for the metal to warm up. Leave this in place for at least ½ hour (careful with the lid!). Make sure there is water in the bottle (D).
9. There are two different insert to choose from: a slide size or a 35 mm petri dish (B).
10. Place cells in posistion and use clams to fix the well/s (C).
11. Turn on the pump (E) and open the CO_2 valve (F) by lifting up the top and turn the wheel till you see the needle rise. Do not touch the wheels on the front! The CO_2 level has been fixed to 5%.
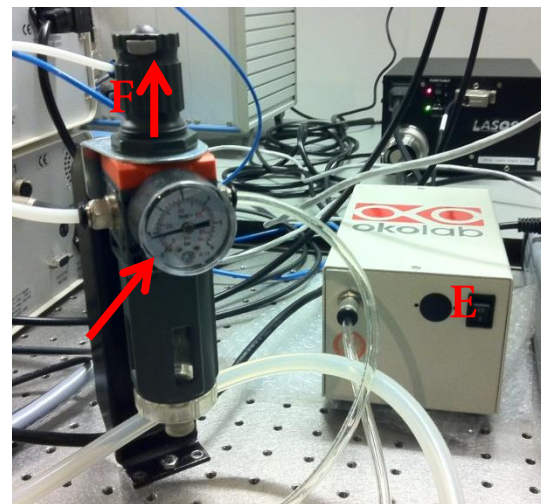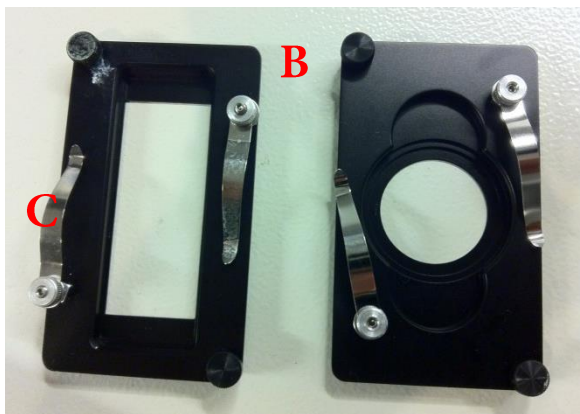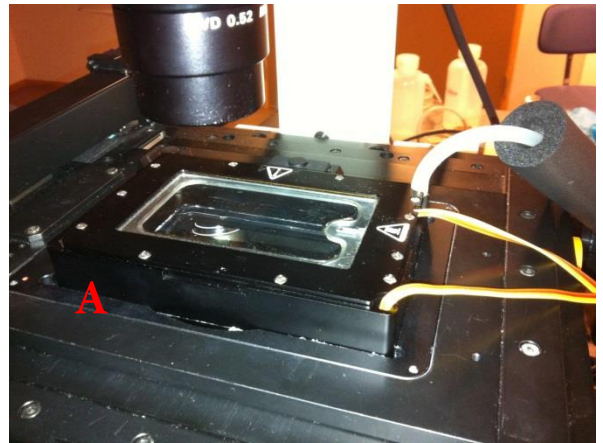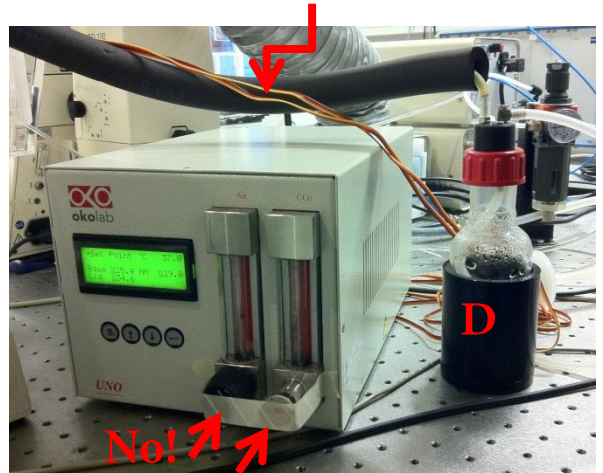
Multipoint acquisition

1. Initialize the stage (Devices – initiate stage).
2. Check the multipoint flag in the ND acquisition window (A).
3. Define your multipoint positions in the XY window with a tick into an empty square (B). Make sure "include Z" is checked off.
4. Define which channels (C) you want to image and the interval of your timelapse (D).
5. You can define where the data is going to be saved (E), preferably on the biomic server.
6. If you are doing a long timelapse, use autofocus (under advances (F)).
7. You might have to advanced setting also for the shutter control (se previous page).
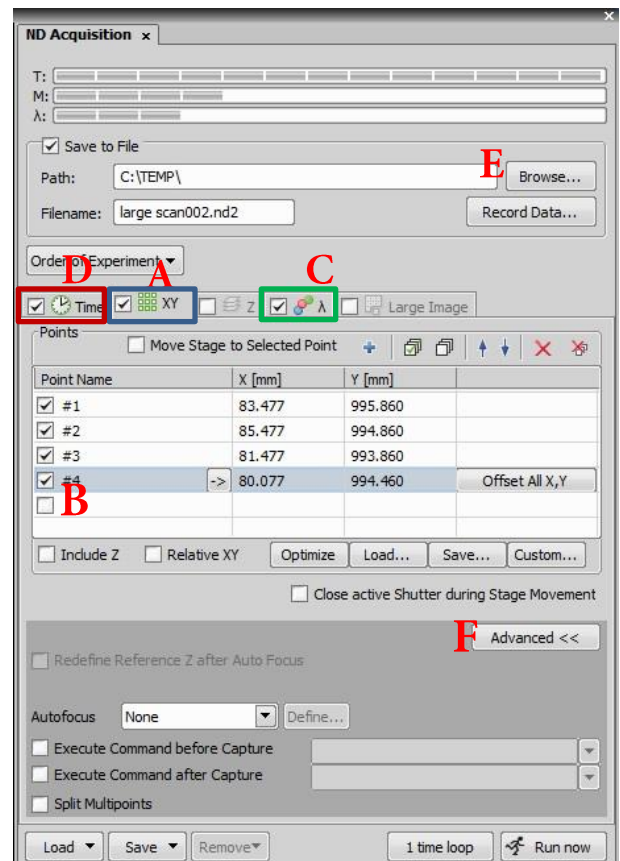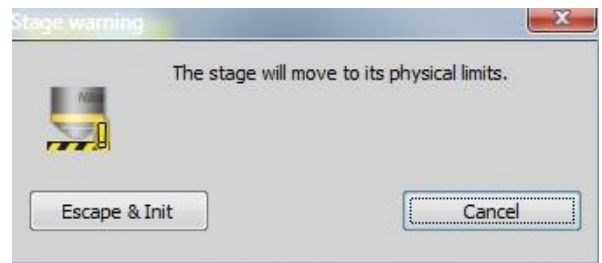
In order to avoid bleaching, the shutter has to be closed when no image is being acquired. To do so automatically in multidimensional experiments,

- *untick* "Close Active Shutter when Idle" (A).

- open "Advanced >>" (B).

- Select "Execute before Capture" (C) and "Execute after Capture" (D).

- Click on the arrow to the right (E) and select "Command List" (F).

- From the command list (G), select "Stg_OpenFirstShutter()" (H) for "Execute before Capture", and "Stg_CloseFirstShutter()" for "Execute after Capture".
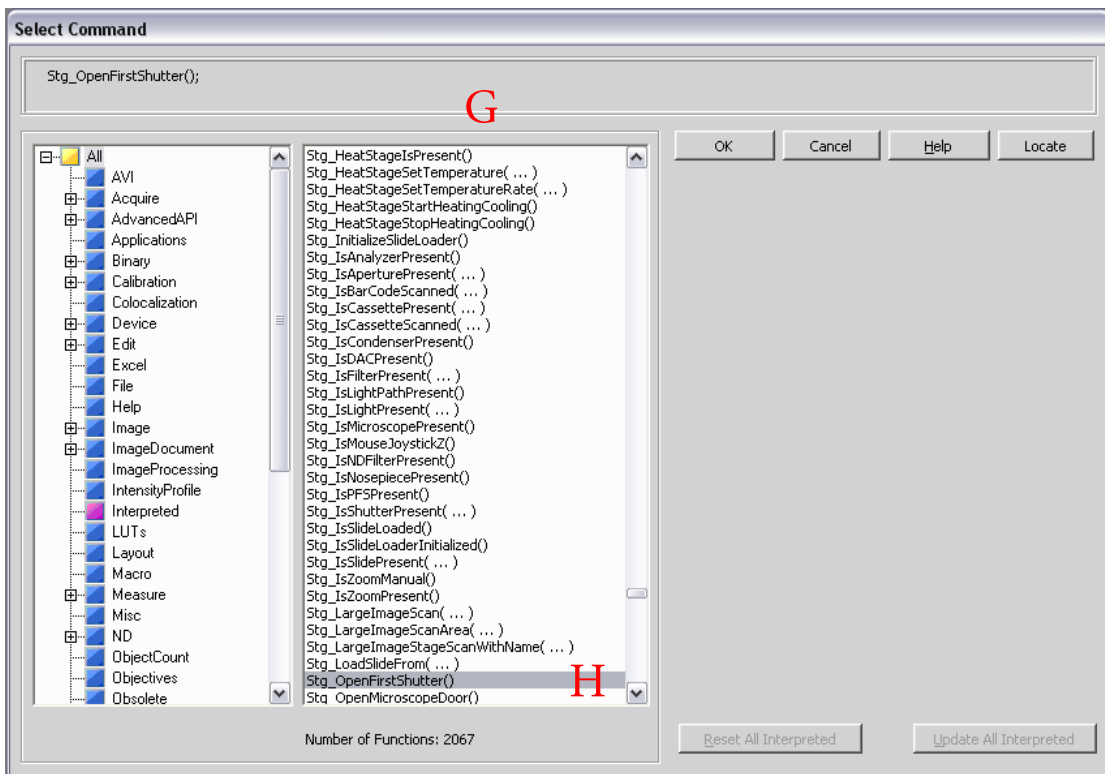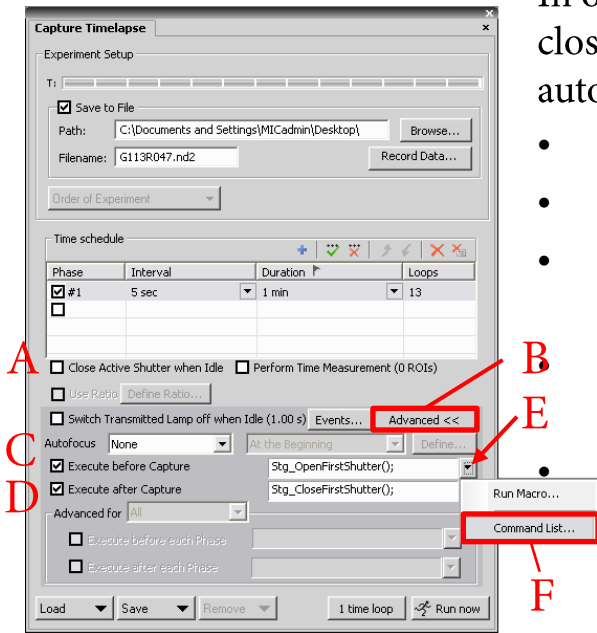
Autofocus

Especially in timelapse experiments, the probe might move out of focus over time. To avoid this, NIS elements provides an autofocus function.

- Click "Advanced" (A) in order to access the autofocus settings.
- Choose either "Steps in Range" or "Adaptive" (B).
- Choose when the autofocus should be applied (C).
- Click "Define" (D) and adjust the settings:
  - in "Steps in Range", you define the z-stack size (E), in which you expect the focal region, and the precision of the scanning (F).
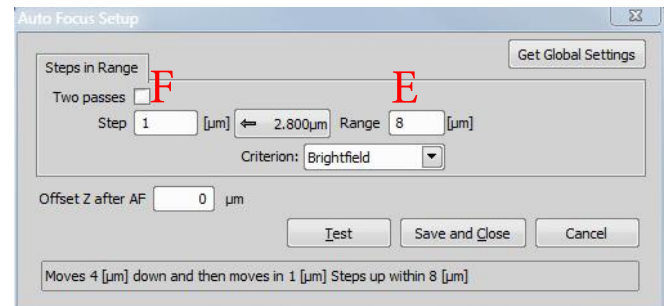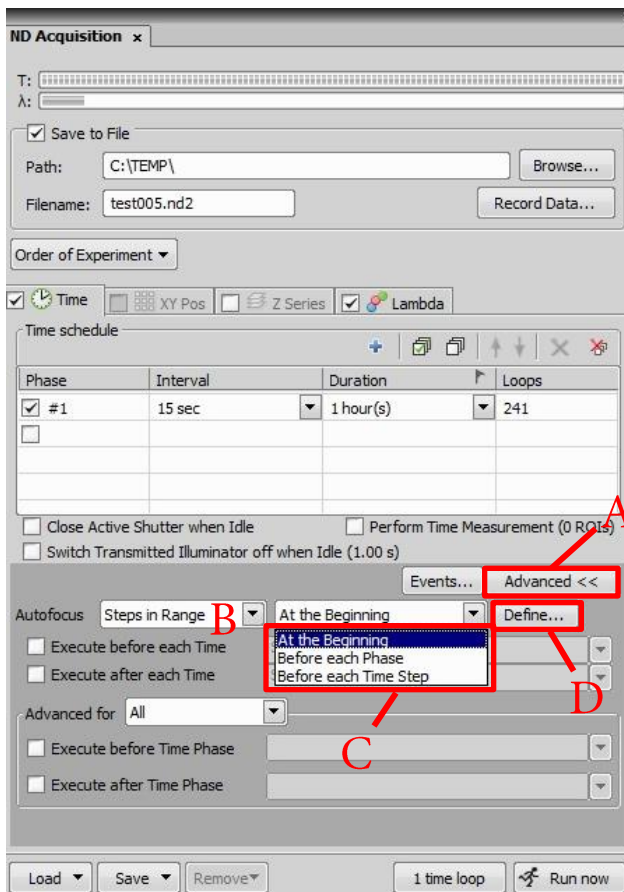
In order to acquire a z-stack, you need to define its thickness and the distance of each slide.

- Open the "z-series" window.
- Focus your sample and define a "top" (A) and "bottom" (B).
- Define the step size (C). The number of steps (D) will change accordingly. It also works vice versa. Furthermore, you can take over the suggestion of the software for

Nyquist sampling (E).
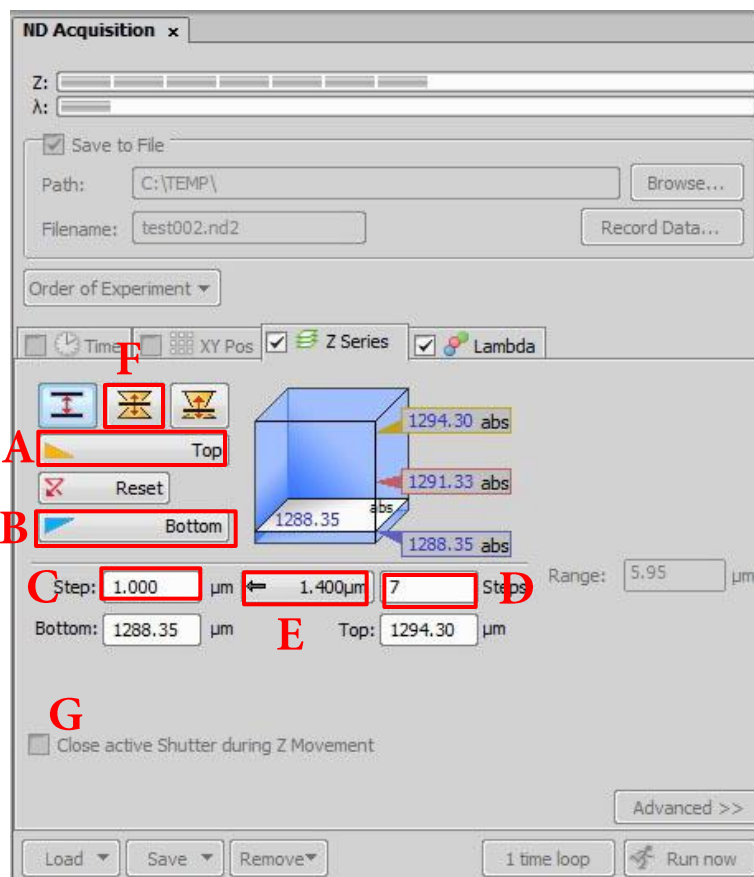- In addition, you can define the size of the image stack by moving to the centre of the stack and setting the size of the half-stacks (F).

Note that you cannot autofocus if you capture a z-stack.

The axial shifting of the objective is very fast; the inbuilt shutter is much slower, so that there is no need for closing during axial movement (G).

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Scale bar

With NIS Elements, you can add a scale bar to your images. Ask MIC personnel to calibrate the objective if required (is most cases, it is calibrated as you find it).
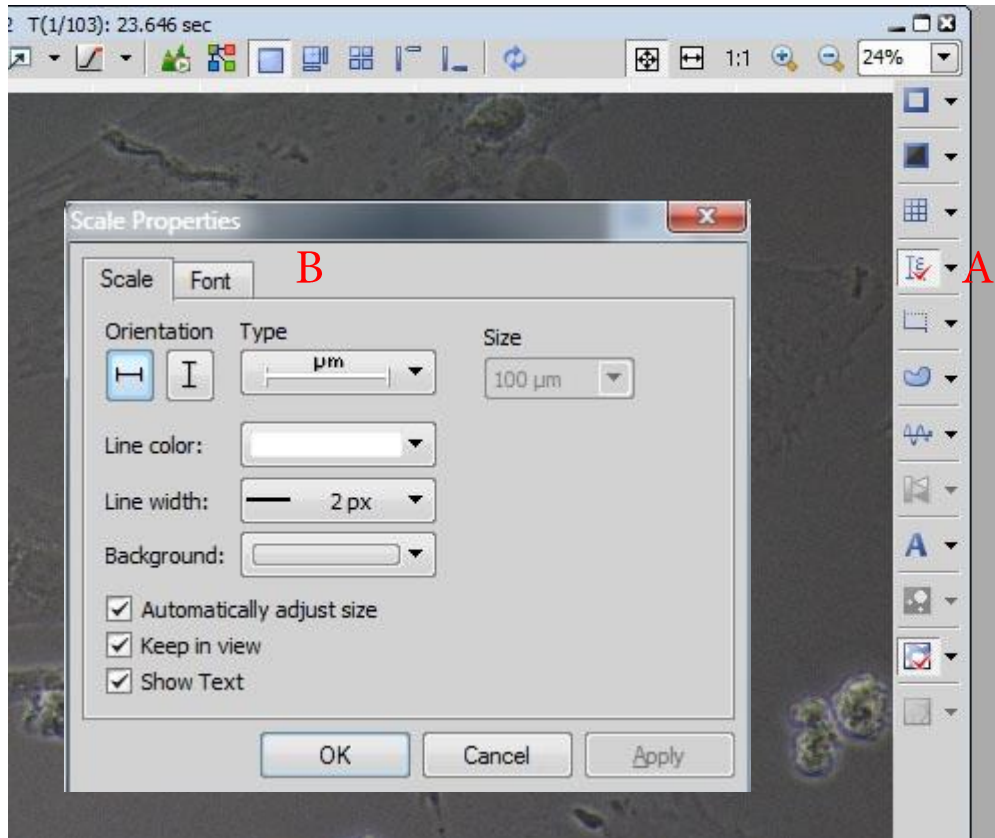
- Tick on the scale bar sign (A) on the right side of your recorded image; the scale bar will appear.
- To change the default, go to scale properties (B).
- „Burn scale" onto image (right click on scalebar).

Saving data

All data should be saved on "biomic", and in the following two formats:

- "*.nd2" is the manufacturer's format that contains all the META-data. *This is your raw data that you have to keep to be able to show what you have done.*

- "TIFF" is the scientific format which can be used by almost all software, but is does not contain the full META-data.

You can adjust parts of the META-data in the "Image Fields" dialogue.

The hugely popular "JPEG/JPG" is lossy, produces artefacts, and is *not recommended* in science. Use it *only* for *simple* presentation purposes.
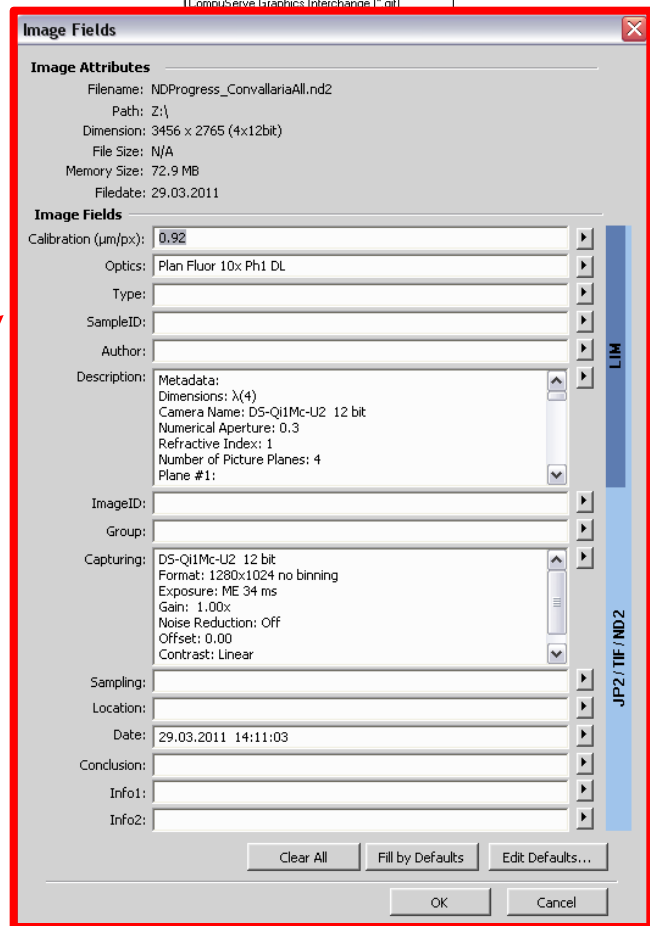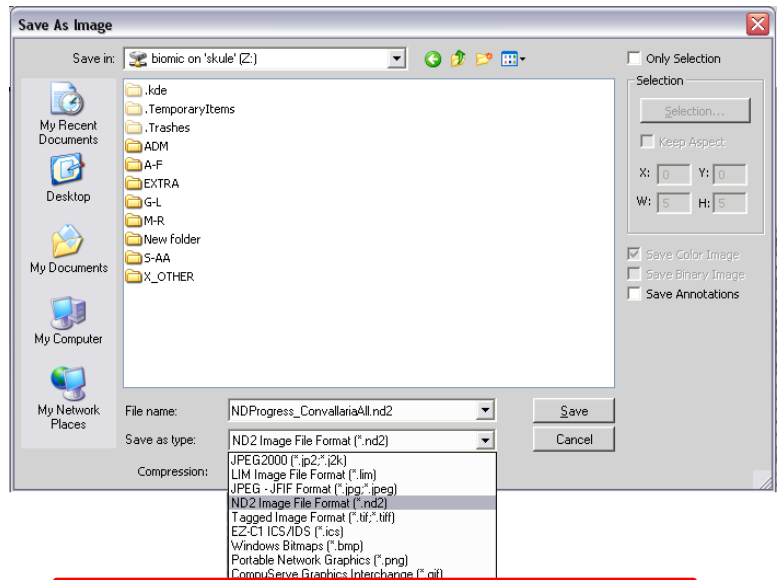
Available objectives

| Position	M	Correction	NA	WD (mm)	Contrast method
1	4	Plan Fluor	0,13	16,4	PhL
2	10	Plan Fluor	0,30	15,2	Ph1
3	20	ELWD Plan Fluor	0,45	7,4	Ph1
4	40	ELWD Plan Fluor	0,60	3,7-2,7	Ph2
5	10	HMC	0,25	6,2	MC1
6	20	HMC LWD	0,40	3,1	MC2
u. r.	2	Plan Apo	0,1	8,5	not recommended
u. r.	40	HMC LWD	0,55	2,7-1,7	MC3
u. r.	60	Plan Apo VC	1.4	0,13	DIC (N2)

Abbreviations: M, magnification; NA, numerical aperture; WD, working distance; Ph, phase contrast; ELWD, enhanced long working distance; HMC, Hoffmann modulation contrast; u. r., upon request; VC, violet-corrected (optimised for multi-colour-staining that includes DAPI and the visible spectrum), DIC, differential interference contrast. All objectives work in brightfield and with polarisation microscopy. All objectives except the ones dedicated for HMC can be used for fluorescence microscopy.

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Available fluorescence filters

Name	λ_{ex}	λ_{em}	Dye examples
DAPI	340-380	435-485	Alexa Fluor 350, BFP, Coumarin DAPI, Indo-1, Hoechst, Marina Blue
CFP	426-446	460-600	Acridine Orange, Alexa Fluor 430, eCFP, Lucifer Yellow, PO-PRO
GFP	450-490	LP500	Alexa Fluor 430/488, BCECF, Calcein, CFDA, Di-8, eGFP, FDA, FITC, Fluo-4, Fluorescein, FM 1-43, FM 4-64, Fura Red, LysoSensor Green, MitoTracker Green, Rhodamine 110/Green, YO-PRO
FITC	465-495	515-555	Alexa Fluor 488, BCECF, Cy2, DiO, eGFP, FDA, FITC, Fluo-4, Fluorescein, FM 1-43, MitoTracker Green, Nissl, Oregon Green 488, Rhodamine 110/Green, YO-PRO, YOYO
YFP	490-510	LP520	Alexa Fluor 488, BCECF, BODIPY, Calcein, CFDA, Calcium Green, eGFP, eYPF, FlAsH, FITC, FM 1-43, Magnesium Green, MitoTracker Green, Oregon Green 488/514, Rhodamine 110/123/Green, TO-PRO, TOTO, YO-PRO, YOYO
TRITC	528-552	577-632	Alexa Fluor 555/610, BODIPY TMR-X, Calcium Orange, dTomato, Ethidium Bromide, evoglow-Bs1/Bs2/Pp1, Magnesium Orange, Nile Red, PO-PRO-3, POPO-3, Propidium Iodide, Rhodamine, TAMRA, Tetramethylrhodamine, TRITC
Cy5	590-650	662-738	Alexa Fluor 647/660, Atto 647, mPlum, NileBlue/Red, Cy5, TO-PRO-3, TOTO-3

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Brightfield filters
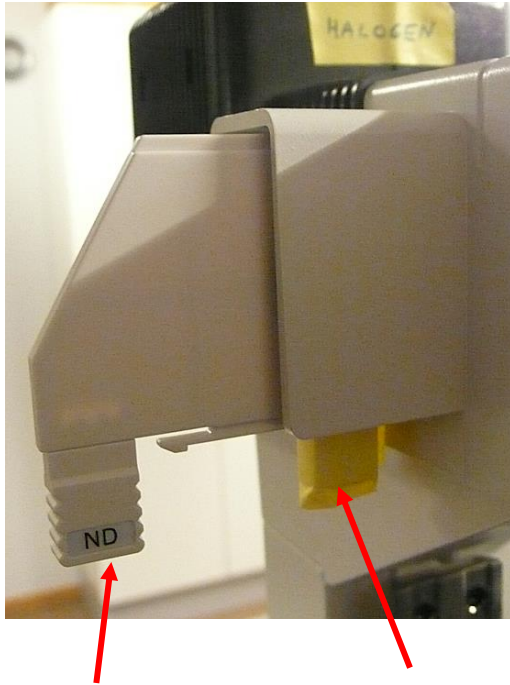
Neutral density filter. Use this when neither the voltage control of the halogen lamp nor the exposure time of the camera suffice.

Diffuser disc. Keep this always in the light path. It decreases the amount of uneven illumination caused by an extended light source (=tungsten wire).

NCB = neutral colour balance. Use this filter if the white balance is not satisfactory at low halogen voltage.

GIF = green interference filter. Use this filter for phase contrast microscopy. This filter removes unwanted halo effects, since phase contrast is calculated for green light.

Köhler illumination

Köhler illumination is *imperative* for all transmission microscopy techniques (brightfield, phase contrast, HMC, DIC, polarisation …).

Adjust also when changing the objective or replacing the sample.
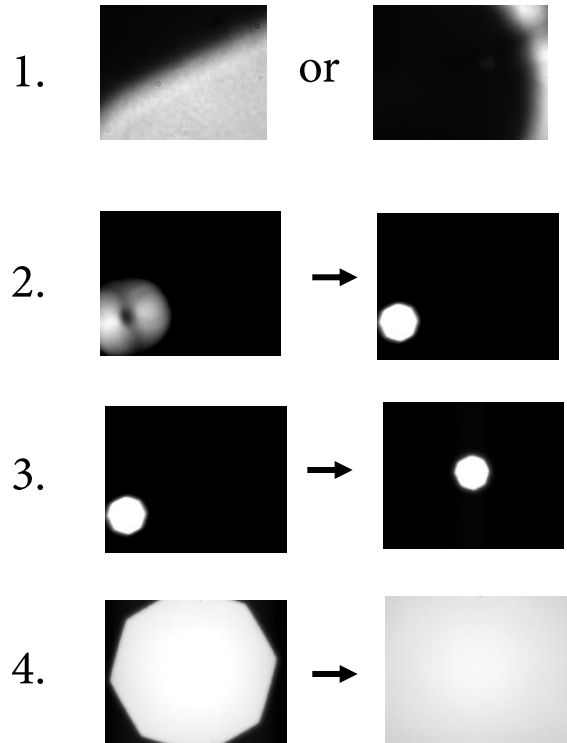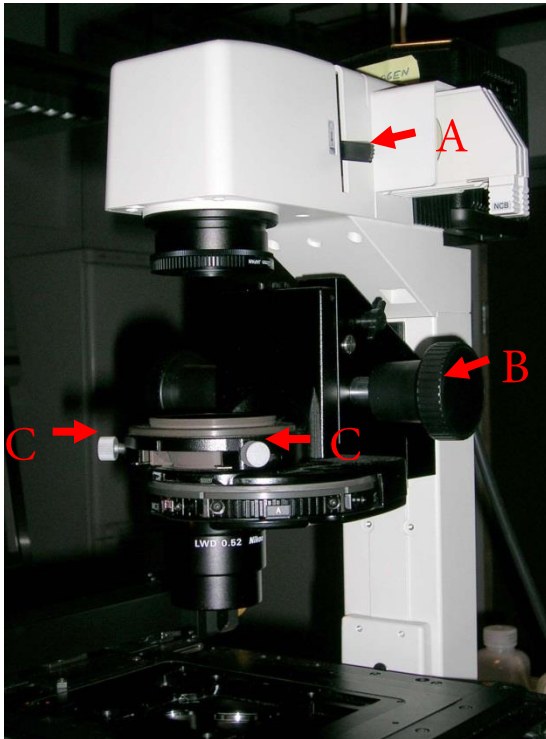
1. Close the field stop diaphragm (A) so that you can see the edges.
2. Move the condensor up and down (B) until you see a *sharp* image of the field stop diaphragm (with sharp edges).
3. Centre the image of the field stop diaphragm by using the knurled condensor centring screws (C).
4. Open the field stop diaphragm (A) until the edges lie just beyond the field of view.

Increasing contrast

- After setting up the Köhler illumination, close the aperture stop (arrow).

- The contrast/depth of field will greatly increase.

- As you close the aperture stop, the resolution will *decrease*!
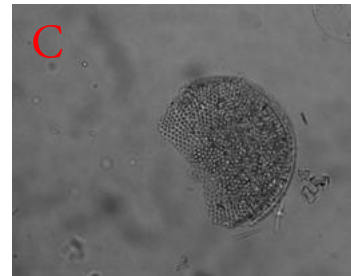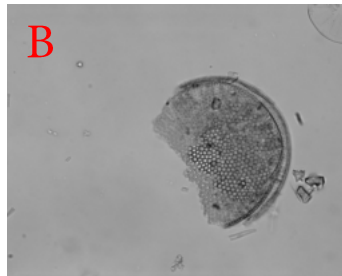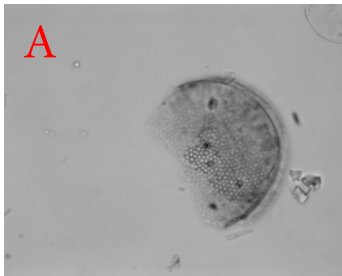
Diatomee in brightfield with different contrast settings: A, aperture open; B, aperture halfway closed; C, aperture fully closed. Note that in A, only a small part of the Diatomee is in focus, whereas in C, you see all in focus, but also dirt in the image pathway.

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Adjusting phase contrast
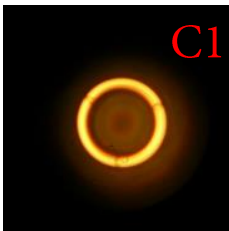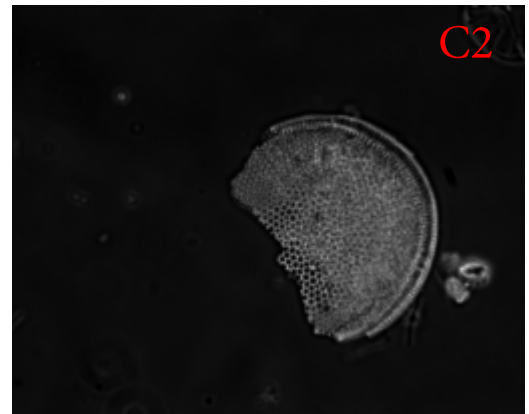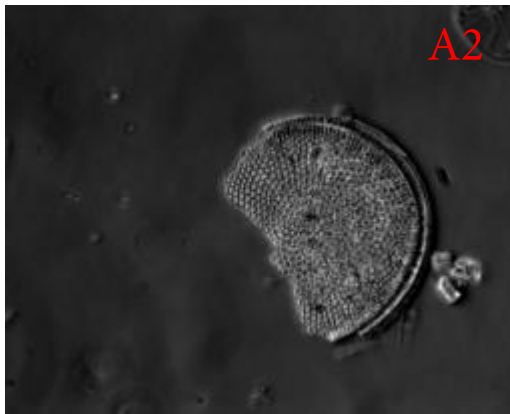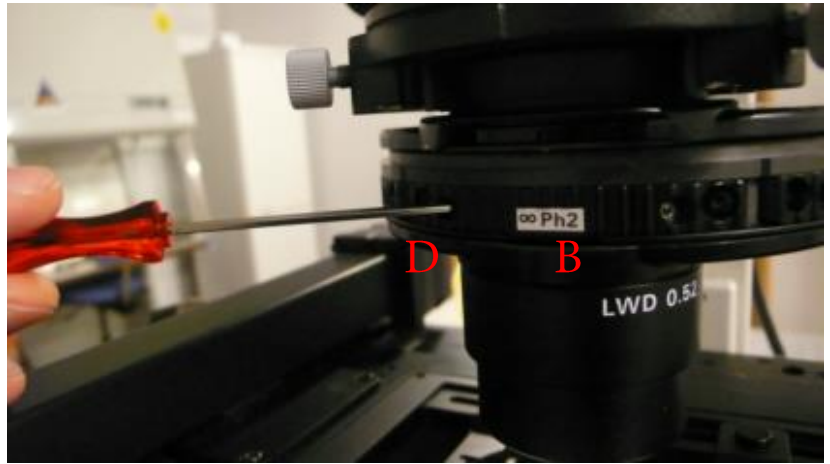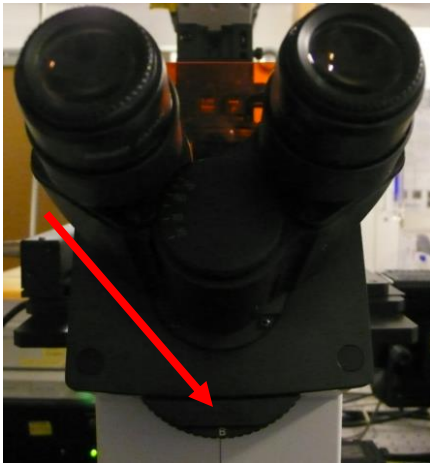
For the adjustment of phase contrast, (i) bring your object in focus and (ii) Köhler. (iii) Switch the condensor (B) to the position matching the objective's inscription. In case of correct alignment (C1), you will see phase contrast with homogeneous halo (C2). (iv) Check the alignment by switching the eyepiece optics to "B" (Bertrand-lens, arrow). The two rings you see (A1) have to overlap. If not, you will have strong artifacts (A2). In order to adjust, (v) move the condensor ring with an allen key(D).

Hoffmann modulation contrast
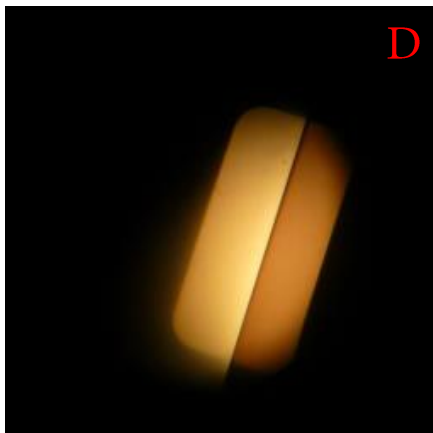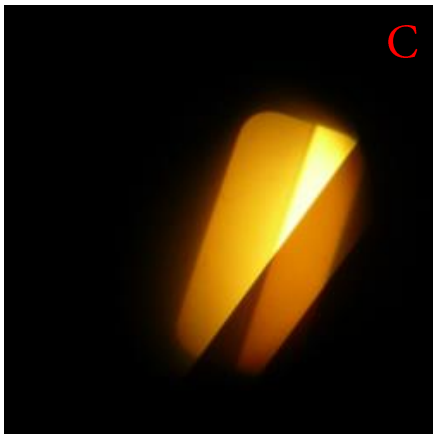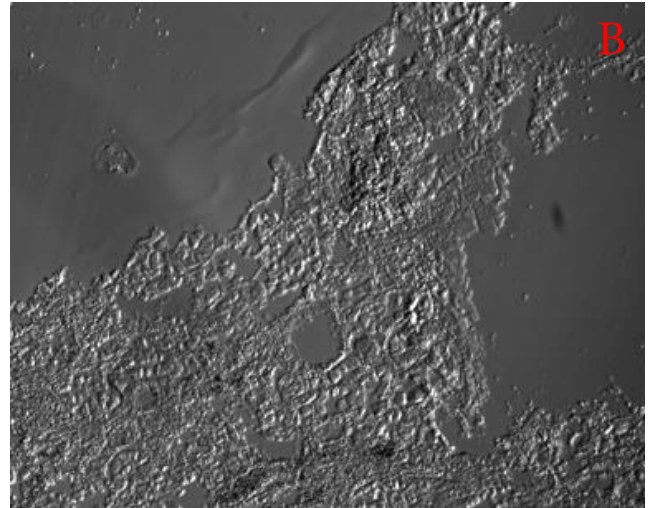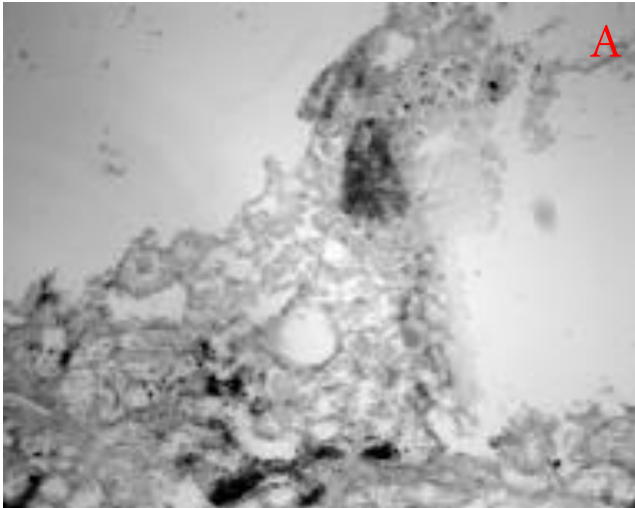
Hoffman modulation contrast creates DIC-like images with less effort, giving the objects a 3D appearance (B). In order to adjust for Hoffmann modulation contrast:

- focus and Köhler your object and lightpath (A).
- at the condensor, switch to the filter corresponding to the objective (MC1-3).
- at the eyepiece, switch from "O" to "B".
- use either the knurled screw at the objective or at the condensor filter to make the two grey-shaded objects parallel (C and D).
- Use an allen key to adjust the distance between the two grey-shaded objects at the condensor filter. They should overlap slightly.

Polarisation microscopy
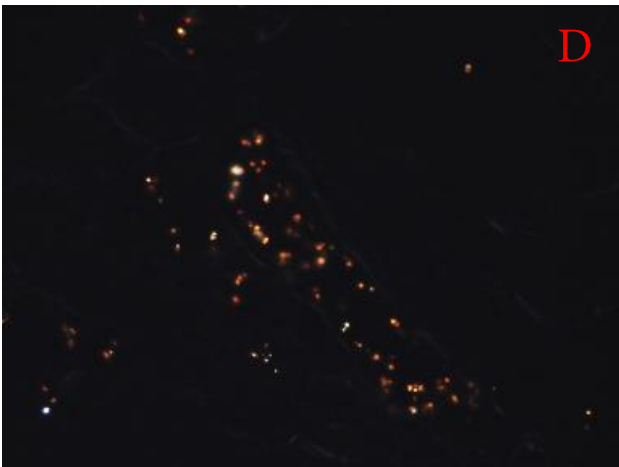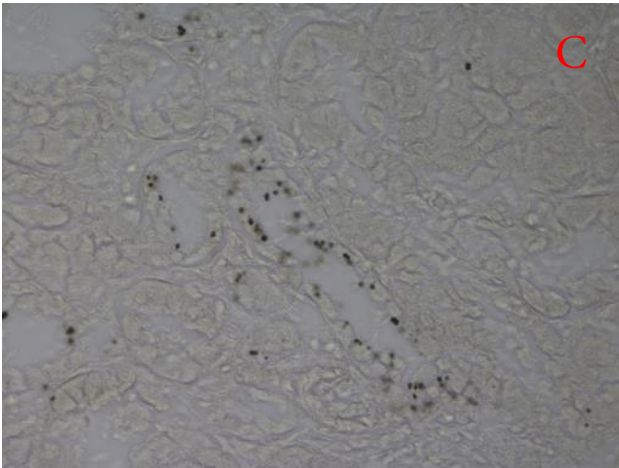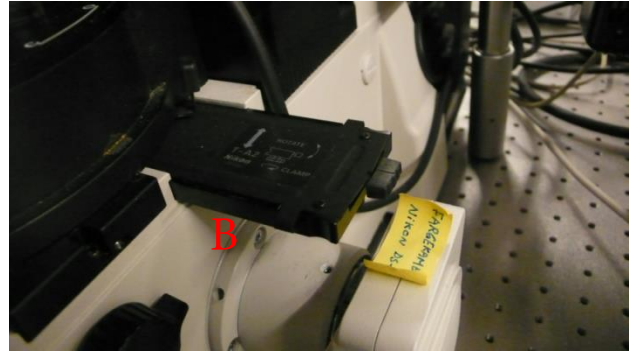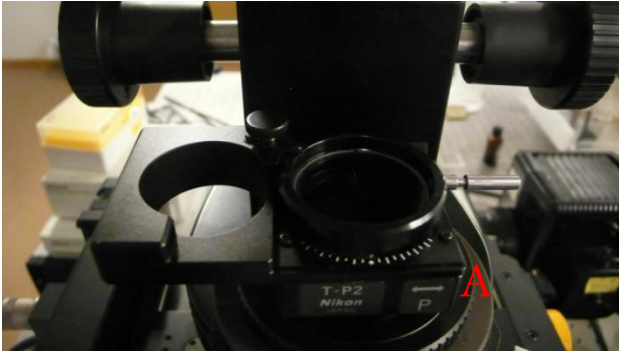
Polarisation microscopy is particularly useful for imaging linear structures like crystals, and can also resemble dark field microscopy. To adjust,

- focus and Köhler your object and lightpath (C).
- set in the polariser (A), which is situated above the consensor.
- set in the analyser (B), which is situated below the filter revolver on the right hand side. Make sure the filters are crossed (background is black).
- image your sample (D). move *both* filters out again after imaging, since each will take 50 % of the light!

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Differential interference contrast


A

C

D

B

E

In order to adjust for DIC,

- focus and Köhler your object and lightpath to get a nice brightfield image (A).
- insert the polariser above the condensor and the analyser below the filter revolver.
- insert the DIC prisms matching the objective's inscription in the condensor revolver and below the objective (B) to get the typical DIC-image (C). Control the "shadow" by turning the polariser.
- For colour-DIC (D), insert the λ-plate below the polariser (E).
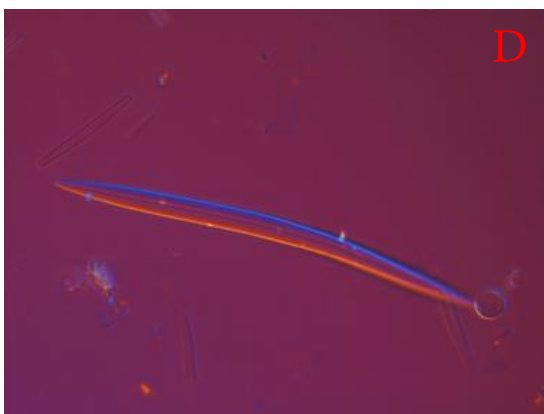
Troubleshooting

- Could not connect to camera!
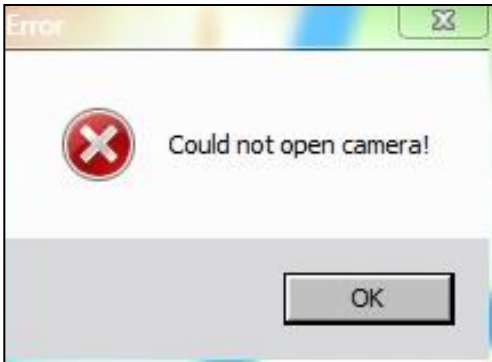
1. Most probably, the switch on the camera controler box is OFF. Check if there is a green light.

2. If control box is OFF, the turn the switch ON and patiently wait for a few seconds before clicking OK on the error message box.

3. If the message continues, try restarting the software .

- I connected to the wrong camera. How do I switch over to the other one?

1. Go to acquire-select driver.
2. Choose from the list.

3. Or, restart software and pick the the correct camera from the list.
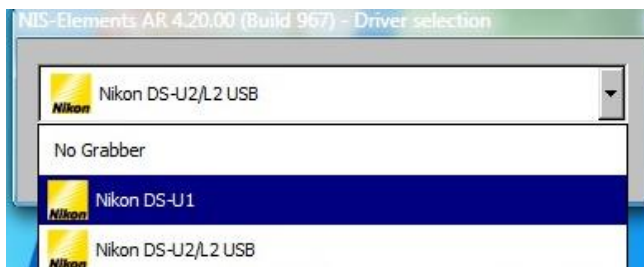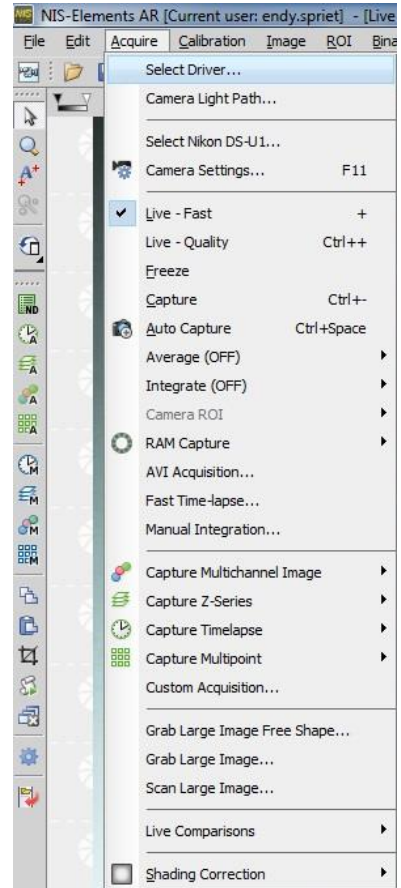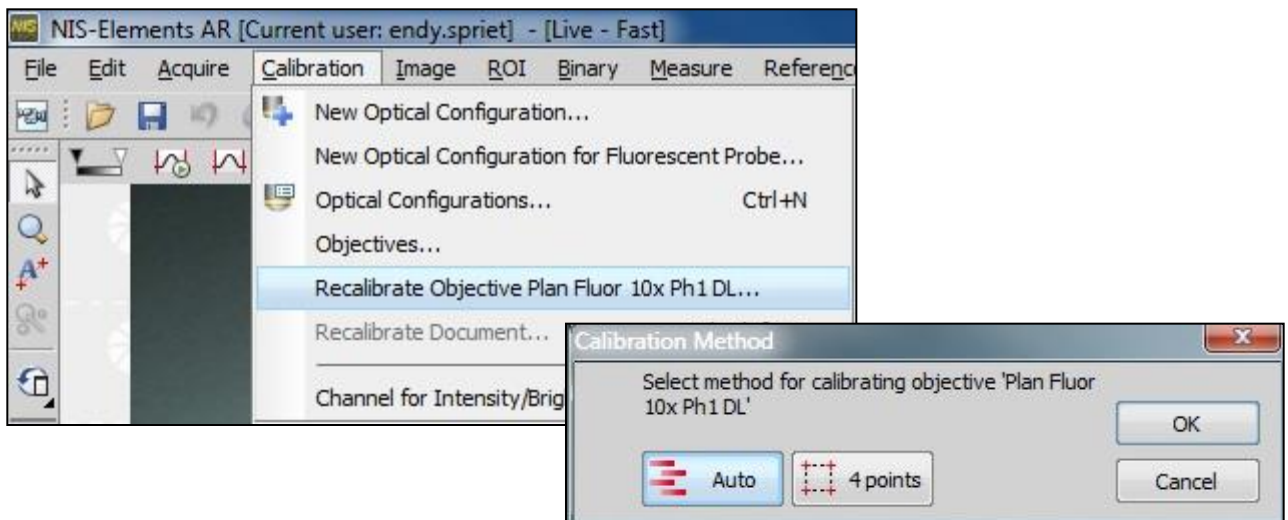
Troubleshooting

- I cannot connect to the skule server.

  You will have to map the server. Do the following:

1. Right mouse click onto «computer» and choose «map nettwork drive».

2. Write: \\klient.uib.no\felles\mofa\biomic

3. Write: uib\username (employees) or student\username

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• After running a large scan, the image does not look perfectly stiched!

1. You need to recalibrate the objective! Do the following:

2. Go to live view (press + on the keyboard). Make sure you see an image on the screen.

3. Go to calibration-recalibrate objective.

4. Choose auto and click OK

5. The stage will now move around automatically. Try a new large scan.

Troubleshooting

- The ND acquistion window looks different. I can't find all the settings there.

1. This does sometimes happen and I don't know why.

2. Simply go to applications-define/run ND acquisition.

3. You window is now back to normal with all settings available!
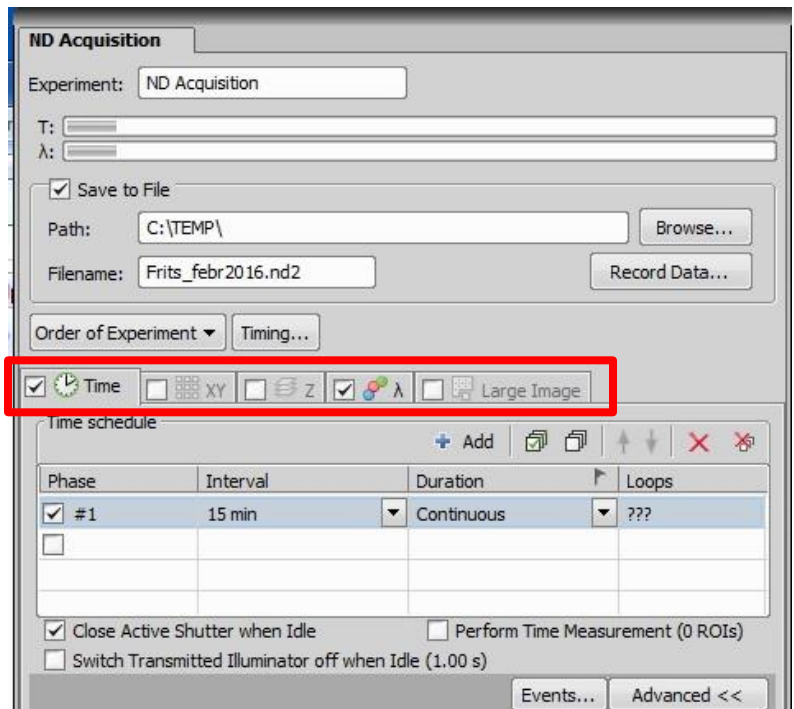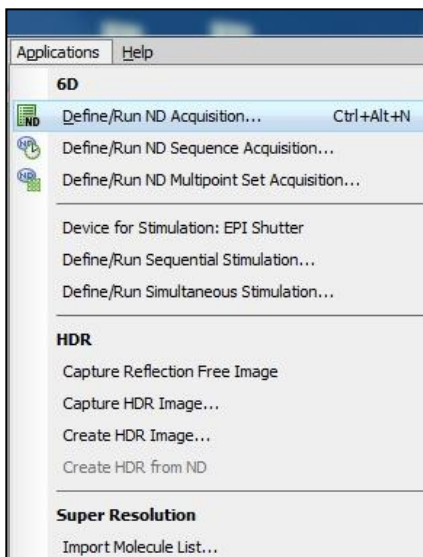
Troubleshooting

- The brightfield image is dimmer or brighter than I expect. It looked fine on the preview image.

1. Most probably you have touched the intensity buttons for the halogen light situated on the control box. This is not saved in the optical setting under camera, but under microscope.

2. Notice the red exclamation mark? This indicated the setting has been change. Right-click on the sign and choose «asign currect microscope setting».
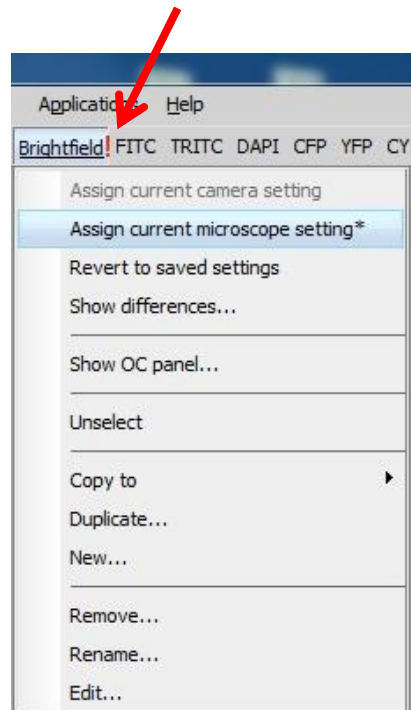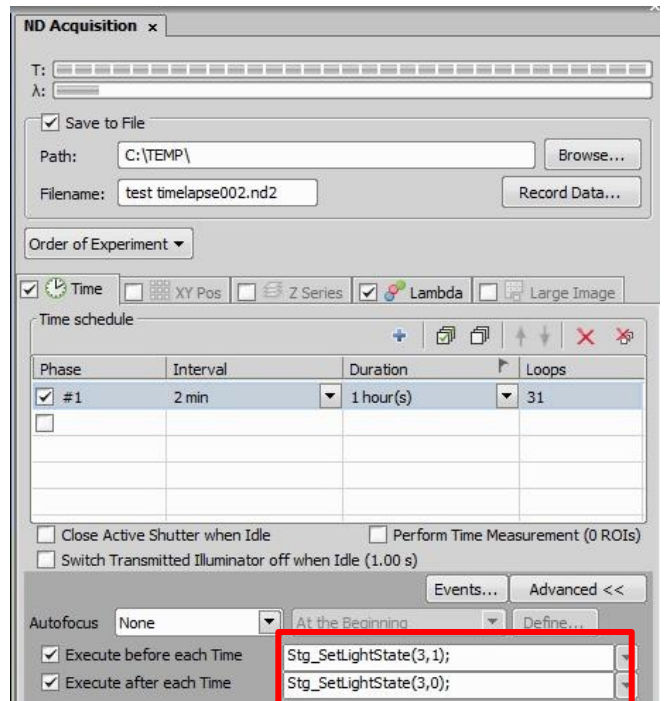
Troubleshooting

- During my timelapse the halogen light does not come ON or OFF as it should!

1. When you run a timelapse you want the halogen light to be turned off after acquisition in order not to create excess heat.

2. In the advanced settings you need to find the floowing commands for before and after every time acquisition:

   STG_SetLightState(3,1) and **STG_SetLightState(3,0)**
   The first command
   turns the halogen light on,
   the last one turns it off.

I'm running a tilescan but the first image is darker than the rest!

Our software runs faster than some of the hardware components. This time the image has been acquired before the shutter had time to fully open.