LCAM startup manual, Andor microscope (A2.42)

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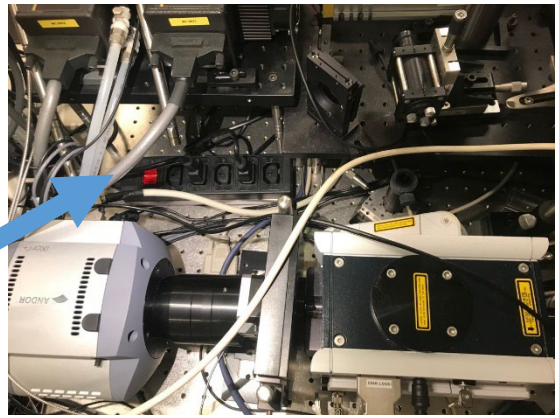
2nd contact person: Mark Hink, C2.264 (+ 6211), M.A.Hink@uva.nl
Information microscope: http://www.lcam-fnwi.nl/facilities/andor

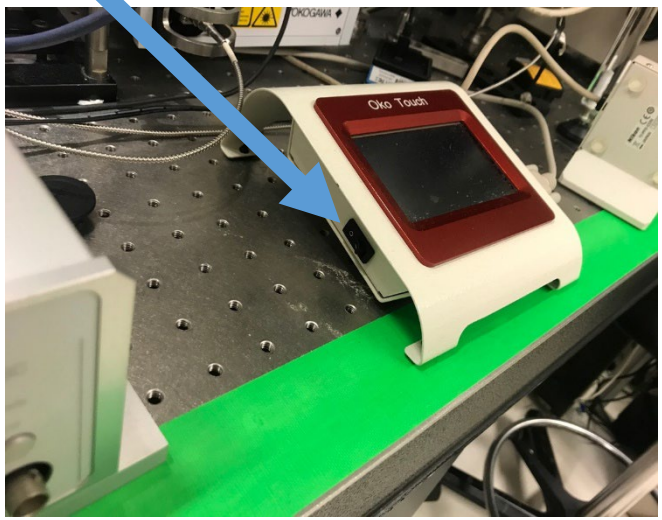


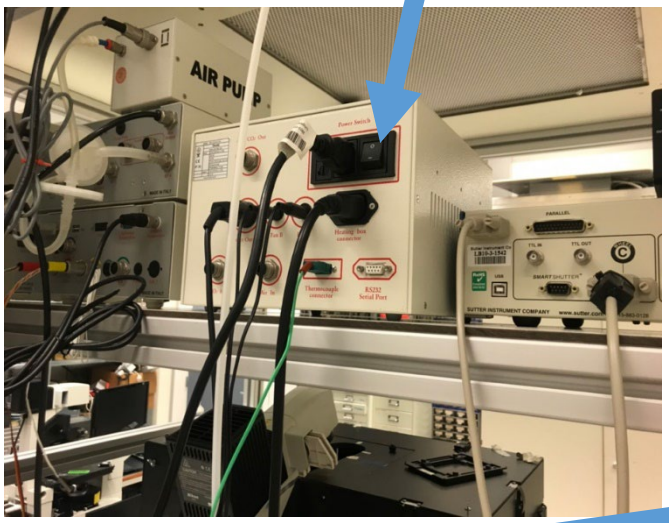
Before using the Andor microscope, the user (and co-workers) should have had the official intake discussion with the LCAM-staff, succeeded the LCAM-confocal training course & exam and had an individual training at the Andor microscope. ***Bookings*** *can be requested via cam.microscopy@gmail.com*

Start-up procedure Andor confocal

1. Switch on the powersocket at the optical table (between lasers and camera) using the red switch.
2. Switch on the computer: user: **imcb** password: **Microscopy@23**
3. If needed switch on the Oko Touch temperature controller and CO_2 regulator.

CO_2 switch (on the left side of remote control) and temperature switch (on the backside)

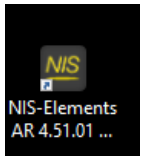


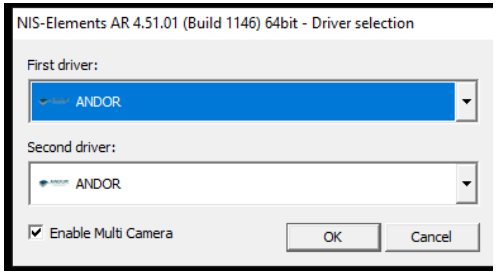


4. If needed switch on the Nikon Intensilight fluorescence lamp.

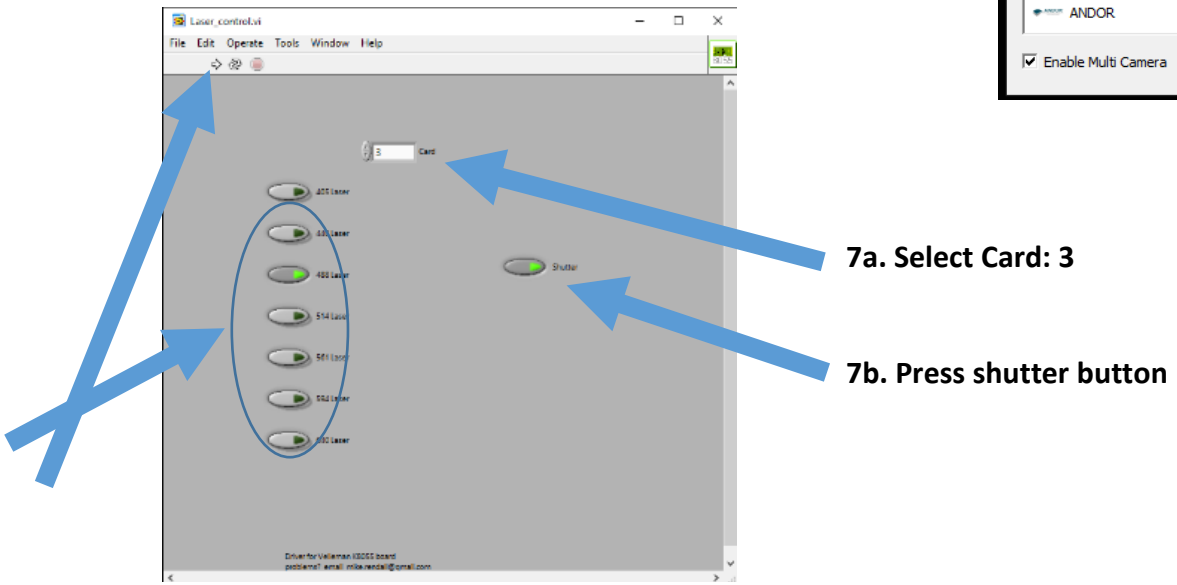
5. Start software, NIS Elements (from desktop) and Select OK for ""Andor""

6. Start Laser control software (from desktop)





7. Switch on all the laser-lines you will need in the laser program from 7a->7d



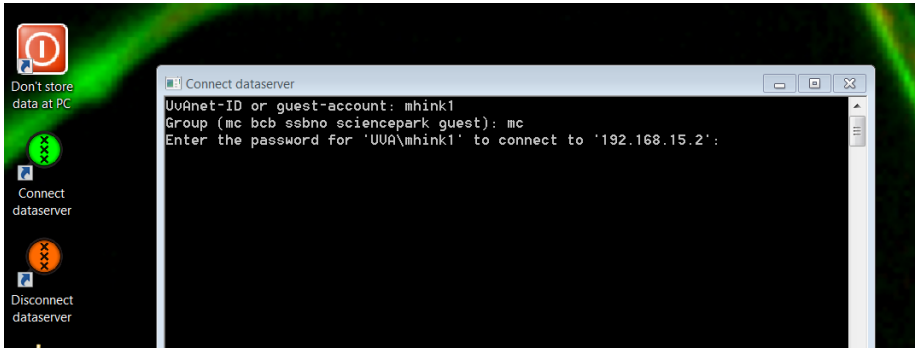
7a. Select Card: 3

7b. Press shutter button

7c. Select laser line(s)
7d. Select Run button

8. **Data storage**

Never store data on the local harddisk, all data should be stored at the dataserver. Data present on the local PC will be deleted without further notice. Be aware that the storage of data on the sever will be your own responsibility as well. Although there is a regular backup of the server we will not take any responsibility for lost or damaged data, so make backups yourself. Contact Mark Hink in order to get access to the data-server from your office computer.



Login using the **green *Connect dataserver*** icon at the desktop**:** Type your userID (UvAnetID), group and password. After succesful login a network drive U:\ will be visible where you should store your data.

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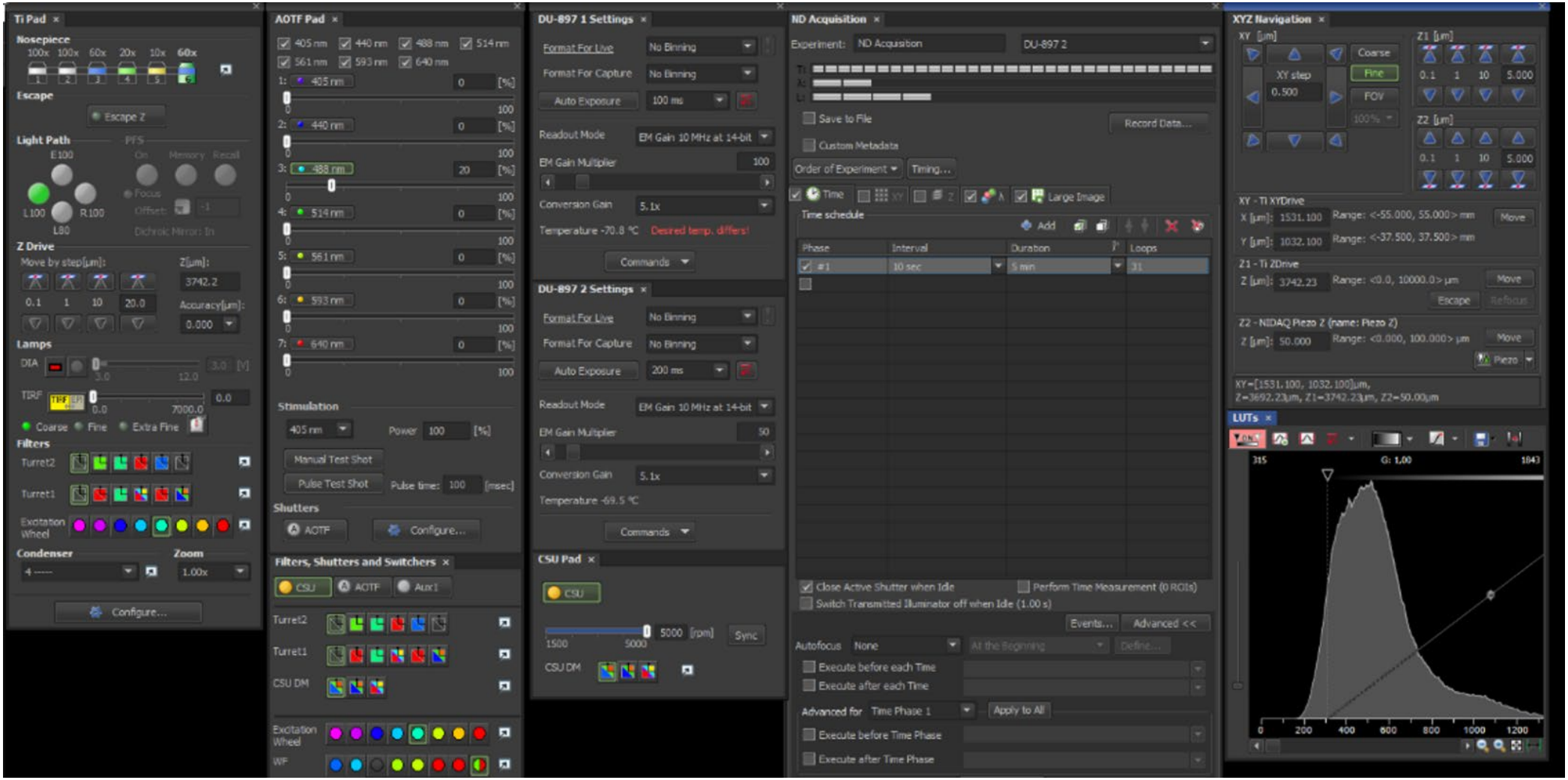
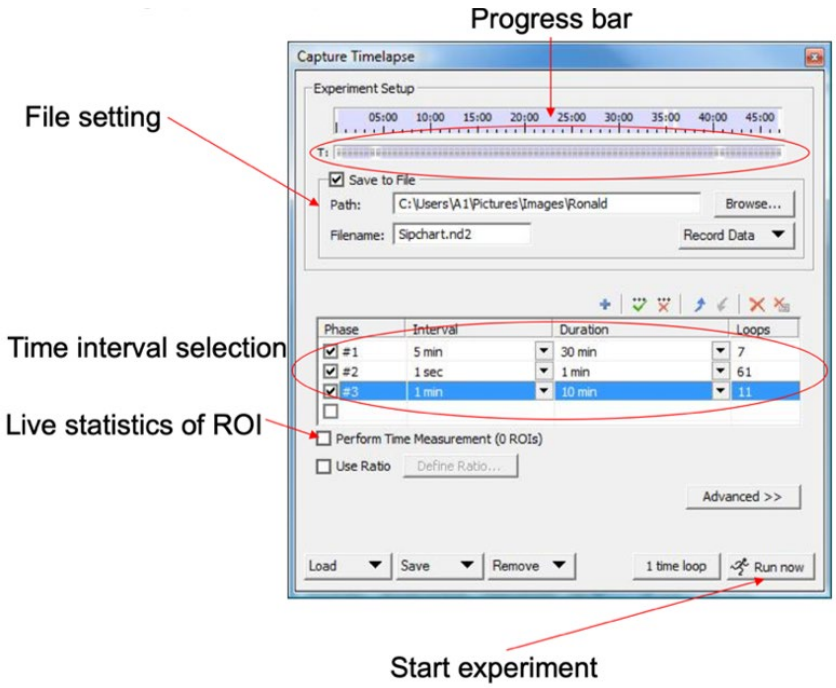
Basic handling of the Andor microscope

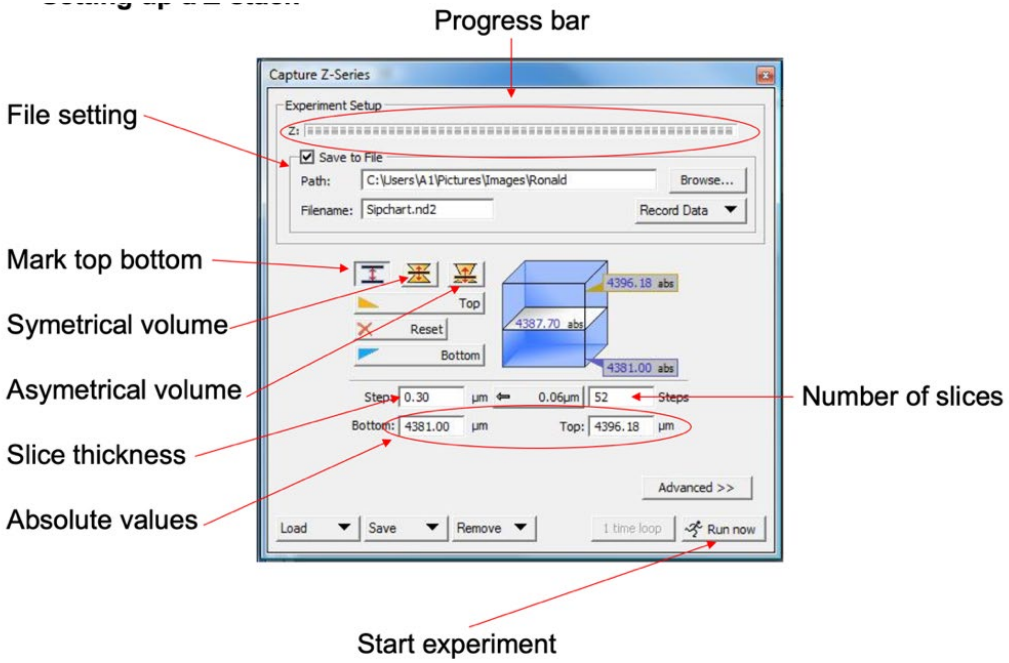
Put a droplet of the correct immersion liquid on top of the objective (air, water or oil). A small bottle of **Nikon** oil can be found nearby. In case the oil bottle is empty: **NEVER** use the immersion oil from other brands (Leica/Zeiss/Olympus) but contact Ronald or Mark for a refill.

1. Open the ***NIS Elements*** window.
2. For the control of the microscope the ***Ti Pad*** window can be used. If it is not open already you can open it by a right mouse click having the mouse cursor positioned at the grey background of the NIS Elements main screen. Then select ***Acquisition controls*** and then ***Ti Pad***.
3. Select the optical configuration you would like to use.
4. Switch from port 1 to port 0 and back to port 1 (software bug).
5. Optimize Laserpower, exposure time for optimal image quality.


Ti Pad
Nosepiece
Escape
Escape Z
Light Path
PFS
Z Drive
Lamps
Filters
Turret2
Turret1
Excitation Wheel
Condenser
Zoom
Configure...
AOTF Pad
405 nm
440 nm
488 nm
514 nm
561 nm
593 nm
640 nm
Stimulation
Power
Manual Test Shot
Pulse Test Shot
Pulse time:
Shutters
AOTF
Configure...
Filters, Shutters and Switchers
CSU
AOTF
Aux1
Turret2
Turret1
CSU DM
Excitation Wheel
WF
DU-897 1 Settings
Format For Live
Format For Capture
No Binning
Auto Exposure
100 ms
Readout Mode
EM Gain 10 MHz at 14-bit
EM Gain Multiplier
Conversion Gain
5.1x
Temperature -70.8 °C
Desired temp. differs!
Commands
DU-897 2 Settings
Auto Exposure
200 ms
Temperature -69.5 °C
CSU Pad
5000 [rpm]
Sync
CSU DM
ND Acquisition
Experiment:
ND Acquisition
DU-897 2
Save to File
Record Data...
Custom Metadata
Order of Experiment
Timing...
Time
Large Image
Time schedule
Add
Phase
Interval
Duration
Loops
#1
10 sec
5 min
Close Active Shutter when Idle
Perform Time Measurement (0 ROIs)
Switch Transmitted Illuminator off when Idle (1.00 s)
Events...
Advanced <<
Autofocus
None
Execute before each Time
Execute after each Time
Advanced for
Time Phase 1
Apply to All
Execute before Time Phase
Execute after Time Phase
XYZ Navigation
XY [µm]
XY step
0.500
Coarse
Fine
FOV
Z1 [µm]
Z2 [µm]
XY - Ti XYDrive
X [µm]: 1531.100
Range: <-55.000, 55.000> mm
Move
Y [µm]: 1032.100
Range: <-37.500, 37.500> mm
Z1 - Ti ZDrive
Z [µm]: 3742.23
Range: <0.0, 10000.0> µm
Escape
Refocus
Z2 - NIDAQ Piezo Z (name: Piezo Z)
Z [µm]: 50.000
Range: <0.000, 100.000> µm
Piezo
XY=[1531.100, 1032.100]µm,
Z=3692.23µm, Z1=3742.23µm, Z2=50.00µm
LUTs
315
G: 1.00
1843
0
200
400
600
800
1000
1200

6. Setting up a Z-stack in the ***Capture Z-series*** window (see right figure) .





7. Setting up a timelapse sequence in the ***Capture Timelapse*** menu (See left figure)

Components of Andor microscope

1. **Objectives:**
   20x air ELWD NA 0.45
   40x oil NA 1.3
   60x TIRF oil NA 1.49
   100x TIRF oil NA 1.49
2. Filters for visual inspection of fluorescence -> see optical schematic
3. **Lasers:** **405 nm**, **445 nm**, **488 nm**, **514 nm**, **561 nm**, **593 nm** and **640 nm**

Extra features Andor microscope

1. The Andor allows to use a **spinning disk**. This can be used for confocal images. The advantage over standard confocal microscopy is the imaging speed can be much faster.

2. The Andor can also be used for TIRF imaging such that you only "illuminate" the border part of a cell, till ca 100 nm above the glass.

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Switching off procedure of the Andor microscope

1. Shut down NIS software
2. Logoff using the **red** ***Disconnect dataserver*** icon at the desktop
3. Switch off powersocket
4. Switch off Hg lamp
5. Switch off Okolab environmental control (if used)