LCAM startup manual, Nikon A1 confocal microscope (A2.34)

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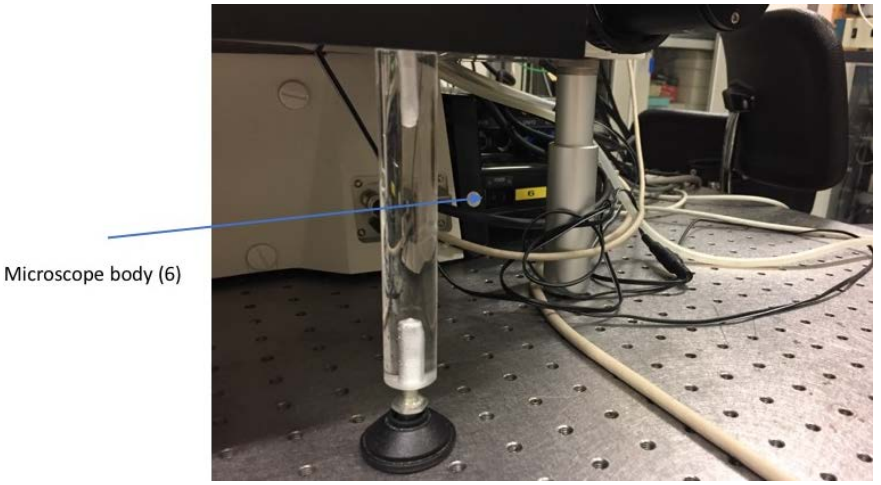
Information microscope: http://www.lcam-fnwi.nl/facilities/nikon-a1

Before using the Nikon A1 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 A1 confocal. ***Bookings*** *can be requested via cam.microscopy@gmail.com*

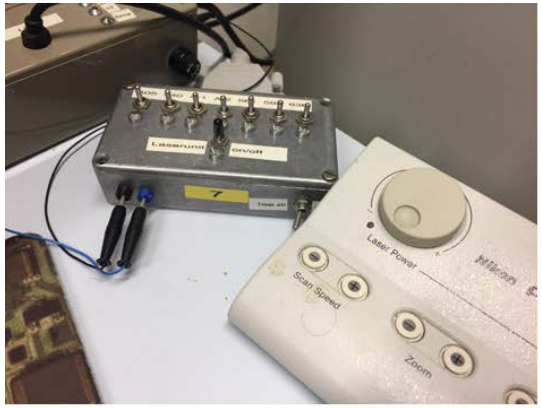
Start-up procedure A1 confocal

1. Switch on Halogenlamp powersupply (1)
2. Switch on XY-driver unit (2)
3. If needed switch on the temperature controller and CO2 regulator (3 & 4)
4. If needed switch on the Fluorescence lamp (5, far right)



5. Switch on the microscopebody at the backside (6)

6. First switch on all the laserlines you will need (indicated by Yellow LEDs) at the switchboard laserunit (7). Then turn ON the laserunit itself by the single separate switch (green LED).



Laser control box (7)

Select the laser (toggle switch, yellow led will turn on)
(Ar1 -> 488, 514)
Switch on the laserunit (toggle switch, green led will turn on)

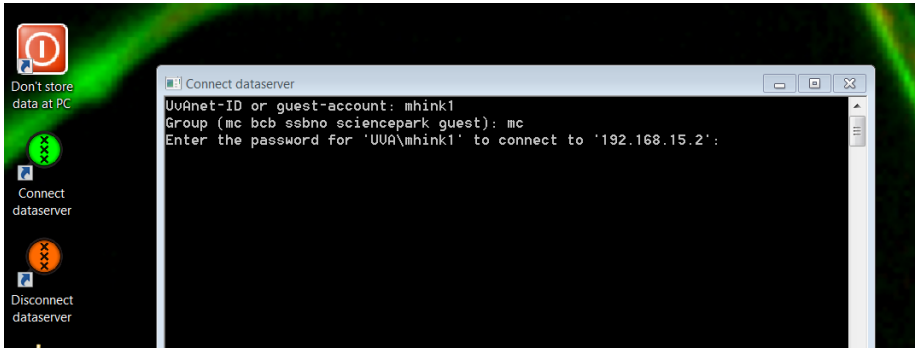

Switch A1 control unit (8)

View from backside

7. Switch on the A1 control unit at the backside (8)
8. Start software, **NIS Elements**

Data storage

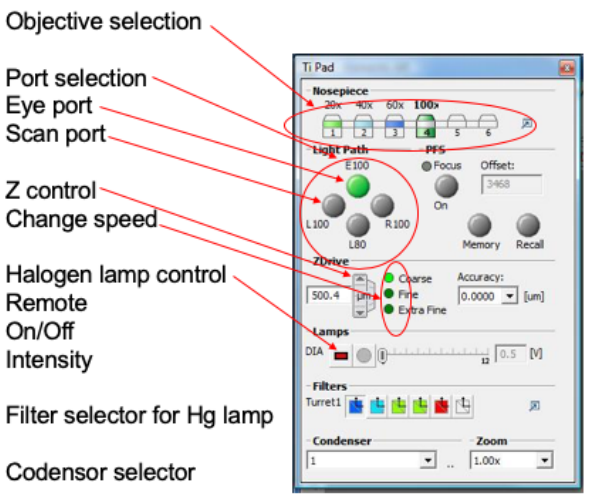
Never store data on the local harddisk, all data should be stored at the dataserver. Data present on the local PC will be deleted without further notice. Be aware that the storage of data on the sever will be your own responsibility as well. Although there is a regular backup of the server we will not 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.

Basic handling of the A1 confocal microscope

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

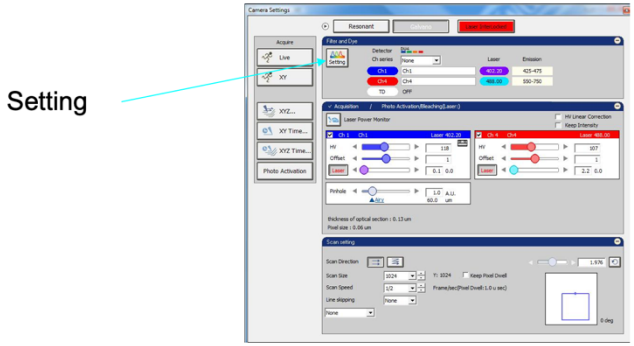


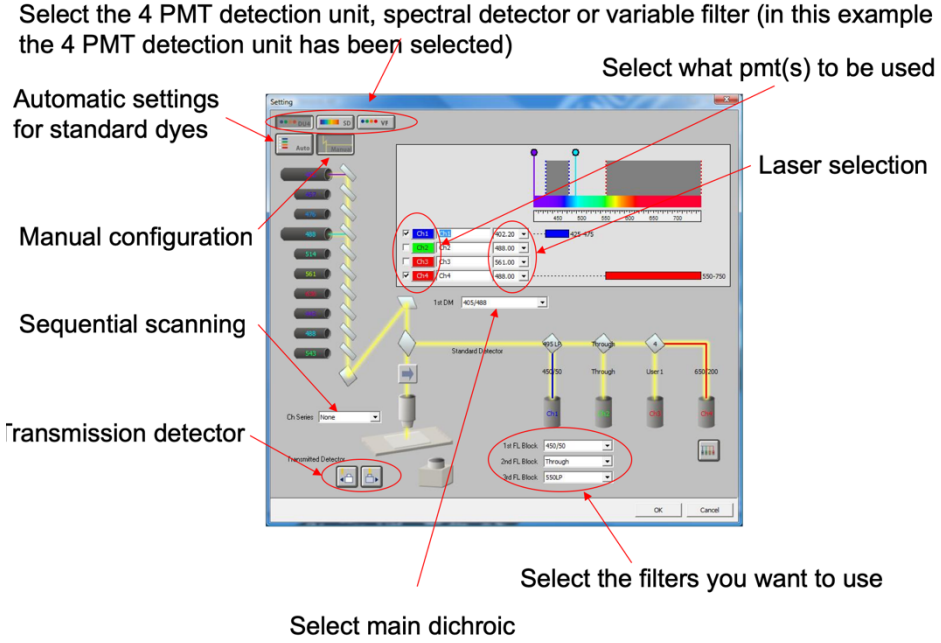
1. Double click the ***NIS Elements*** icon. When the software has started the main screen appears. Note: If a fault appears, close the software & check if the Microscope body and Control unit are switched ON and restart the software

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*.

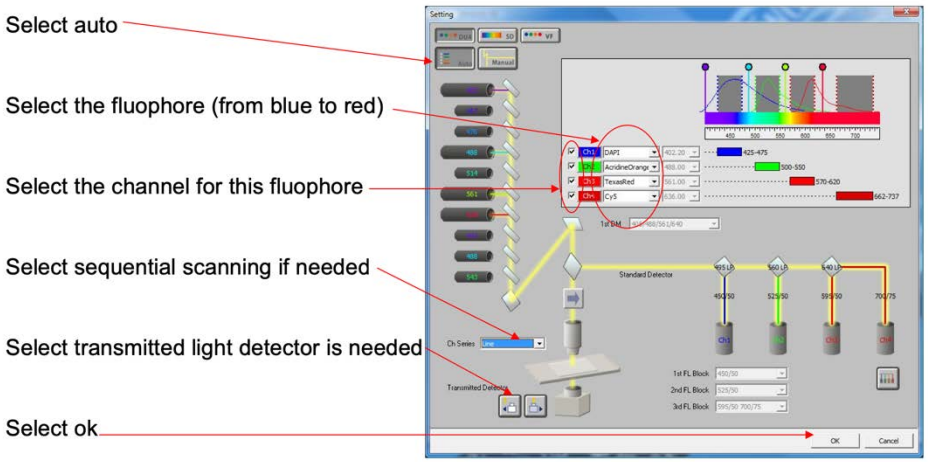
3. The ***Setup*** menu defines a configuration for lasers, detectors and filters:

In the camera setting menu click on settings

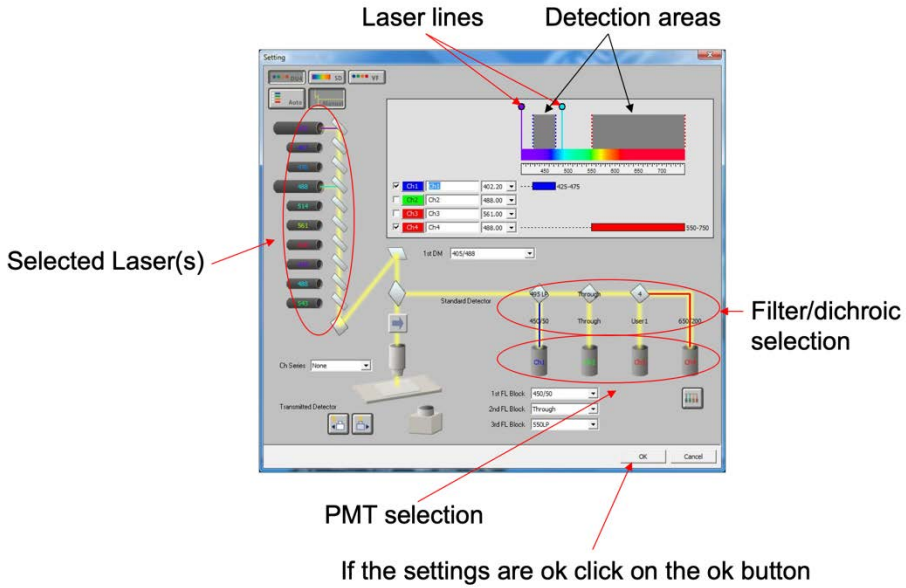




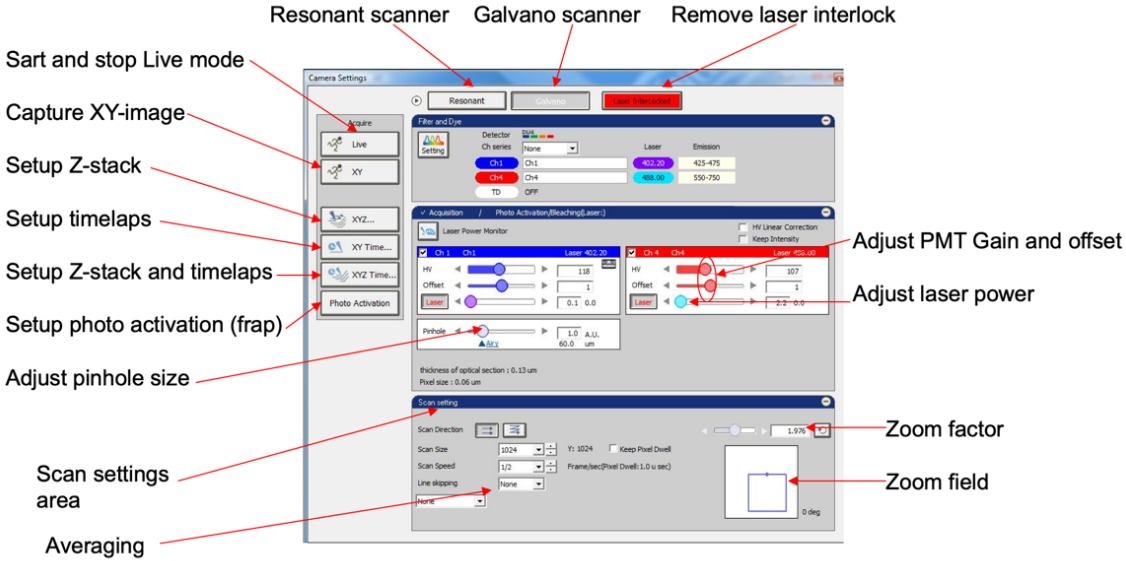
Using the autosetup



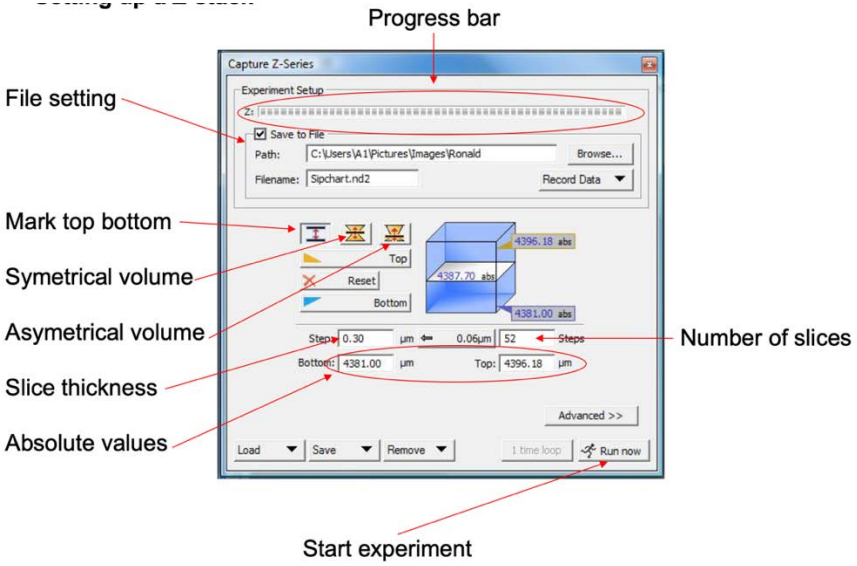
Visualisation of your configuration

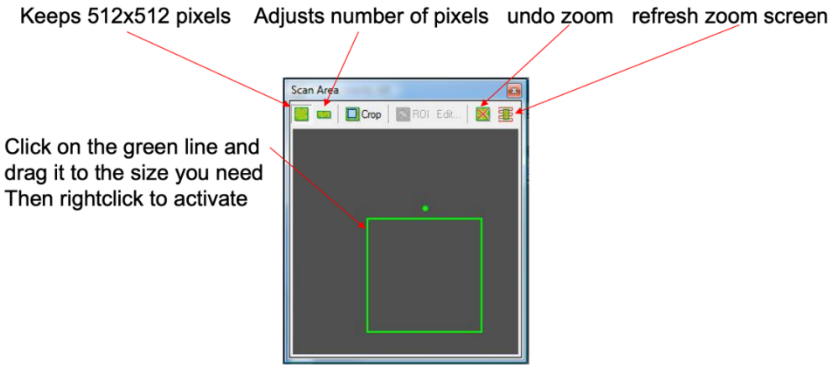


4. The ***Camera settings*** menu defines type of measurement (XY, XYT, XYZ etc.), pinhole size, scan speed, laser power, detector sensitivity and averaging.

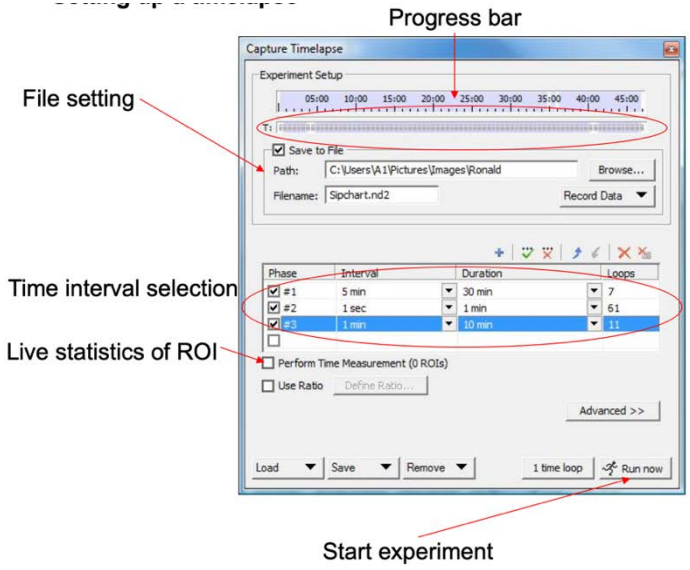


5. Adjusting the zoom: ***Scan Area*** window
6. Setting up a Z-stack in the ***Capture Z-series*** window





7. Setting up a timelapse sequence in the ***Capture Timelapse*** menu.



Components on Nikon A1 confocal microscope

1. Objectives: 20x multi-immersion NA 0.75, 40x oil NA 1.3, 60x oil NA 1.4
2. Filter cubes for visual inspection of fluorescence: BFP, CFP, GFP Band pass, GFP Long pass, RFP
3. Lasers: 405nm, 445nm, 488nm, 514nm, 561nm, 593nm and 635nm

Extra features Nikon A1 confocal microscope

1. The Nikon A1 allows to use a **spectral detector** that consists of a 32 channel PMT module. Using spectral analysis one can for example follow the donor-acceptor signals in FRET experiments or unmix multiple dyes with largely overlapping emission spectra. https://www.microscopyu.com/techniques/confocal/spectral-imaging-and-linear-unmixing
2. The Nikon A1 allows to acquire images via the **Spatially Controlled Intensity Microscopy** (SCIM, previously CLEM) method that significantly reduces the amount of laser power to the sample by lowering the power in background or very strong forground regions. This will significantly reduce photobleaching and improves cell survival while imaging over longer periods. https://www.nature.com/articles/nbt1278
3. The microscope has a CO_2 tube connection and incubator. Ask for details and instructions.
4. The microscope has a Perfect Focus option to keep the sample in focus at higher temperatures and longer measurements. Ask for details and instructions.

Switching off procedure of the A1 confocal microscope

1. Shut down NIS software
2. Logoff using the **red** ***Disconnect dataserver*** icon at the desktop
3. Switch off laser unit (see point 6)
4. Switch off Hg lamp
5. Switch off Nikon control unit
6. Switch off microscope body at the back
7. Switch off halogen powersupply (1)
8. Switch off XYZ driver unit (2)