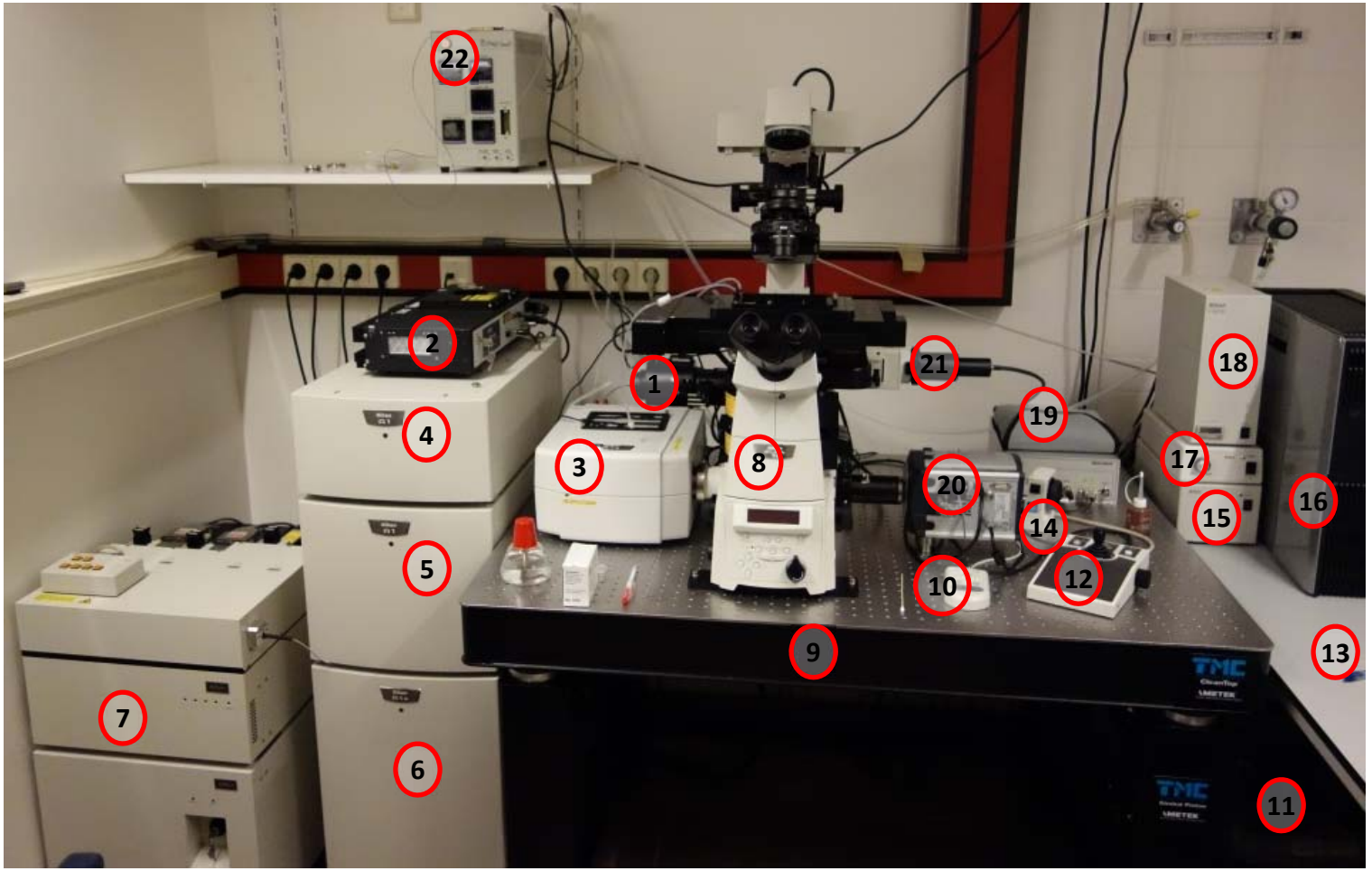
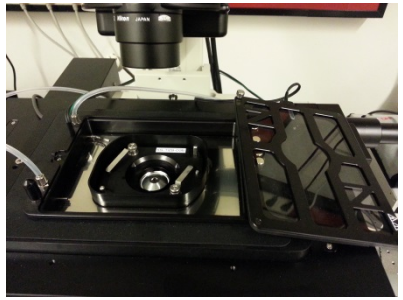
Overview NIKON A1R-STORM microscope:



(1) TIRF illumination arm
(2) Pump laser for 640 nm
(3) A1R scan head
(4) A1R detector unit
(5) A1R spectral unit
(6) NIS control unit
(7) Du4 laser unit
(8) Microscope Ti-E body
(9) Vibration isolation table
(10) Intensilight remote
(11) Compressor for table
(12) Joystick for xy table
(13) A1R remote control
(14) Pfs control unit
(15) Control unit xyz table
(16) PC/monitors
(17) Halogen lamp
(18) Fluorescence lamp-intensilight(dia)
(19) MCL Z-piezo nano-stepper
(20) Andor iXon Ultra EM CCD camera
(21) Diascopic illumination arm
(22) Tokai Hit climate control unit

Accesories:

1. TOKAI hit culture chamber, different inserts to fit -3 cm dishes and object glass sized slides


2. SANYO CO2 incubator (not humidified, use humid enclosed box or dish to keep samples)
3. Vacuum pump

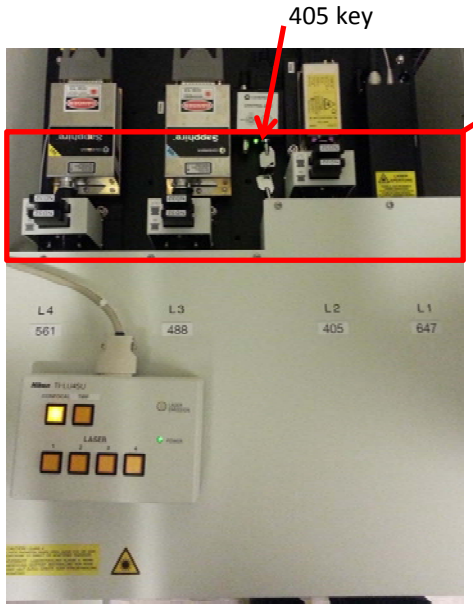
4. Workstation NIS elements (reserve via reserve-a-scope)

SWITCH ON:	***where's the switch?***
i. *Laser unit (7)	power on (back right top and middle box) key clockwise (bottom unit front 561, 487), 405 , 647 separate key
ii. Control unit (6)	left side push button
iii. *Intensilight Fluo-lamp (18)	tumble button
iv. *Halogen lamp (17)	tumble button
v. Xyz driver (15)	
vi. MCL piezo Z stage (19)	tumble button
vii. Microscope body (8)	tumble button back right
viii. PC (16)	
ix. *Tokai Hit control (22)	push button top front (flip switches for component controls)
x. *Andor Ultra EM-CCD	push button facing you (off when PC=off)
xi. NIS	different icons are used to start with or without lasers
SWITCH OFF: (reversed order as above)	
i. Close NIS	shut down rest during ***backup*** of your data
ii. Lasers (7) 	Start "NIKON laser control", toggle off lasers in the script, turn key to vertical position ***and*** power off middle and top boxes of DSU4
iii. Control unit (6)	
iv. Intensilight Fluo-lamp (18)	
v. Halogen lamp (17)	
vi. Xyz driver (15)	
vii. MCL piezo Z stage (19)	
viii. Microscope body (8)	- clean lenses , lower lens by coarse focus (NOT using ESCAPE option)
ix. Tokai hit control(22)	- clean humid chamber (96% EtOH 20 min, dry using vacuum system)
x. *Andor Ultra EM-CCD	
xi. Shut down PC(17)	
xii. Cover objectives by Tokai hit lid and cover microscope with cover sheet	

* Only switch on elements you need, consult CCI staff when you 're lost

Laser control (1):

NOTE: When active lasers pass the eye/E port, a shutter will block the lasers. The block can be lifted by pressing the large RED ‘Remove interlock’ button.



ND filters: UP position

!!!NOTE: only place down when in low power mode, hi power mode will destroy them!!!

Power switches at the back right of middle and top box.

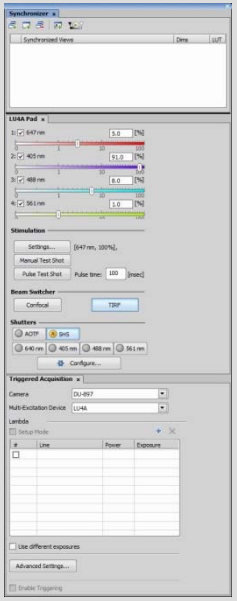
Laser power and status controlled via script:
405: can be switched off using key on remote switch
487 and 561 on by default, off by script
640 requires pump laser (on the A1R controller)

pump laser to feed 640 nm line
Power “on”: tumble switch
Key: “on” > green LED, laser in ’stand by’
Script controls “on” status (LED turns white)

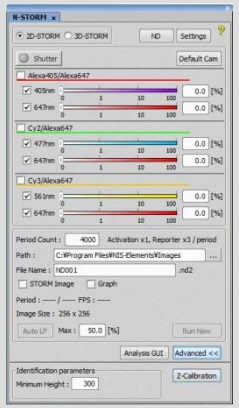
NIS control

In A1R GUI

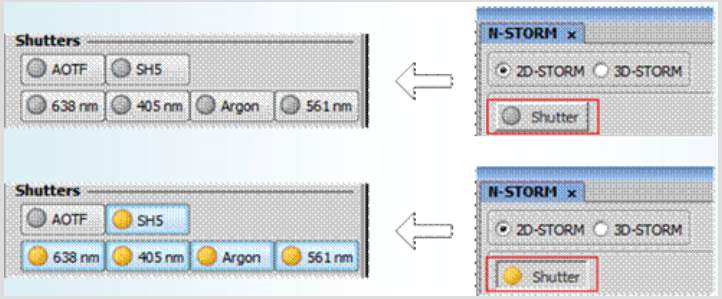
In Lu4A pad



In STORM GUI



Shutters in TIRF GUI couple to STORM GUI



Laser control (2):

NIS laser script: *Set laser power of the lasers you need (set others to "off"):*

1). low (for live A1R or TIRF imaging, set by staff to >10% of max)

2). high for STORM imaging (!!lift ND filters on laser unit)

3). For wide field imaging, start "NIS no lasers"

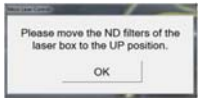
NB: ND filter settings are not monitored, log them

On the desktop: 3 options to start NIS

1). Laser power set only: works when NIS is running

>> 

>> 

>> 

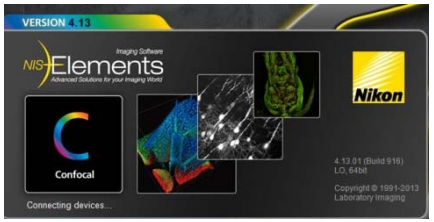
>> Continue using NIS

NOTES:
ND32 transmits 3.125% of input

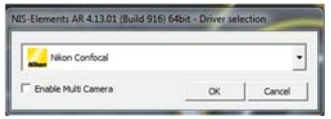
2). Laser power set and start NIS

>> Starts laser power dialogue as above in 1). >> Continues after ND filter dialogue with NIS startup as in 3).

3). Start NIS w/o (re-)adjusting laser

>> 

>> 

>> 

left

Z-sensitivity

Change Objective

focus

on/off

intensity

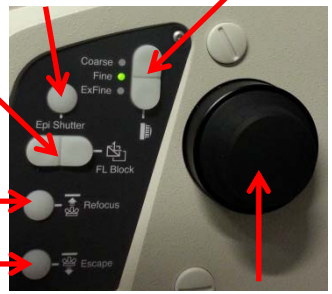
Halo-lamp

right

Change Fluocubes top turret (WF)

On/off intensilight

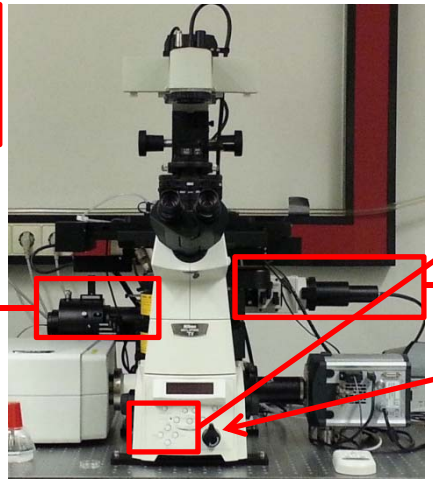
Z-sensitivity



Objective-up again

Objective-down

focus



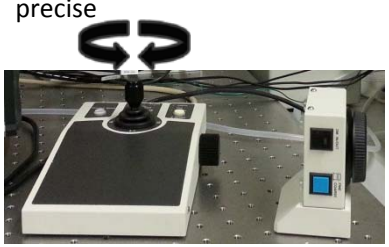
Eye pieces

TIRF

diascopic

Zoom lens 1/1.5

precise



shutter

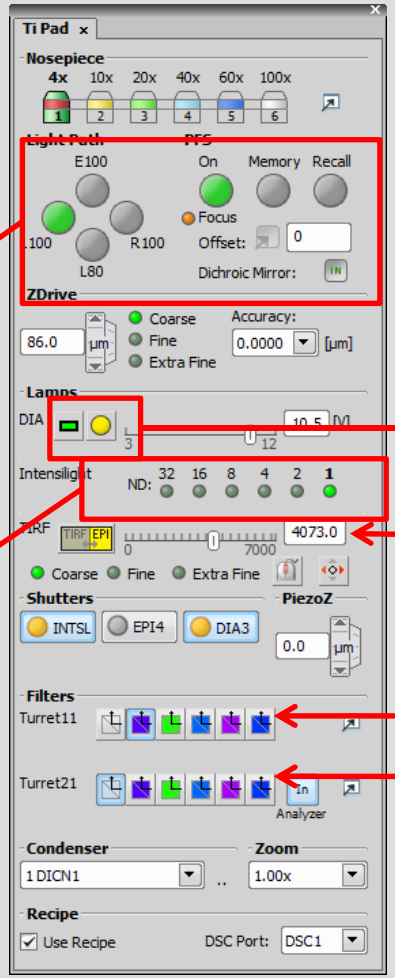
ND control

Table remote (XY,Z) Pfs(Z) Intensilight remote

When pfs=on, MCL focus controls Z and mic-Z is disabled

NIS control

Ti pad acquisition control



Red: mic control of halo

Green: NIS control of halo

3300 = straight up

Diascopic TOP turret

TIRF BOTTOM turret

Outline of steps in different imaging modes

detailed settings are outlined later in this handout or are clearly explained in NIKON docs (how-to or handouts)

NOTE: use Layout tabs to get preset ‘pads’ for controlling hardware, right click in preview area to show additional ‘pads’

A1R

- Choose objective
- Choose optical configuration
- Check INTERLOCK
- Set layout tab to A1R
- Set gain/offset (AG-sets this quickly and requires little tweaking)
- Set timelapse, Z-setting, optional channel series setting etc.
- START

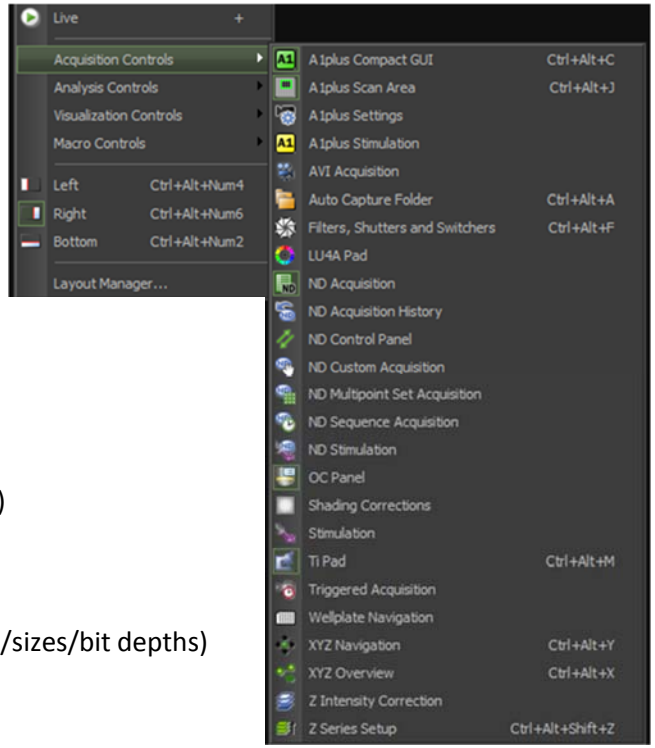
TIRF imaging

- halogene/intensilight if you diascopally inspect the sample
- Use 100x or high (>1.4) NA objective
- Koehler, Check INTERLOCK
- Set layout tab to TIRF layout
- Align TIRF spot (on TIRF arm, STORM lens “up”) (Y-manually, X via NIS)
- Focus (pfs “on”)
- TIRF mode on Ti-panel
- AOTF/SH5 and laser line on in Du4 laser control panel
- Change angle (coarse>fine) to optimal intensity (toggle autointensity to see effect
- Set Andor sensitivity and speeds
- Save setting on each optical config
- Set timelapse, lambda etc.
- START

WF

- Choose objective
- Choose optical configuration
- Set Intensilight strength (ND setting on remote or in panel)
- Set Andor sensitivity and speeds
- Save setting on each optical configuration
- Set timelapse, lambda etc.
- START

Layout tabs



Tips:

- Dialogue views are present in layout tabs but can be changed and remain user specific

  (access controls using: Acquisition>Analysis>Visualization controls or right click in viewer window)
- Windows controls (Shift/Ctrl/Alt/right-click context controls are available)
- Drag-n-drop works for channels or series to extract and combine image groups (for compatible type/sizes/bit depths)
- Interlock is activated when lasers are accessing the eye (top) port. Manual deactivation is needed
- Make sure status of zoom lens is noted (its setting is not automatically read by NIS)
- Make sure status of ND filters on the laserbox are noted (again these are not automatically read by NIS)
- Setting of laser setting by script is not automatically read by NIS (note into labjournal to allow quantitative imaging)
- Store your data on your own HD, large sets are easily generated and can clog up the machine in less than a week, make sure datatransfer is fast (USB3).
- An APP (iOS, Win, Android) was made to track settings and mail notes of your experiment, the CCI website also provides forms to do this (BOOKMARK)

Useful docs on the desktops of PCs (workstation and mic):

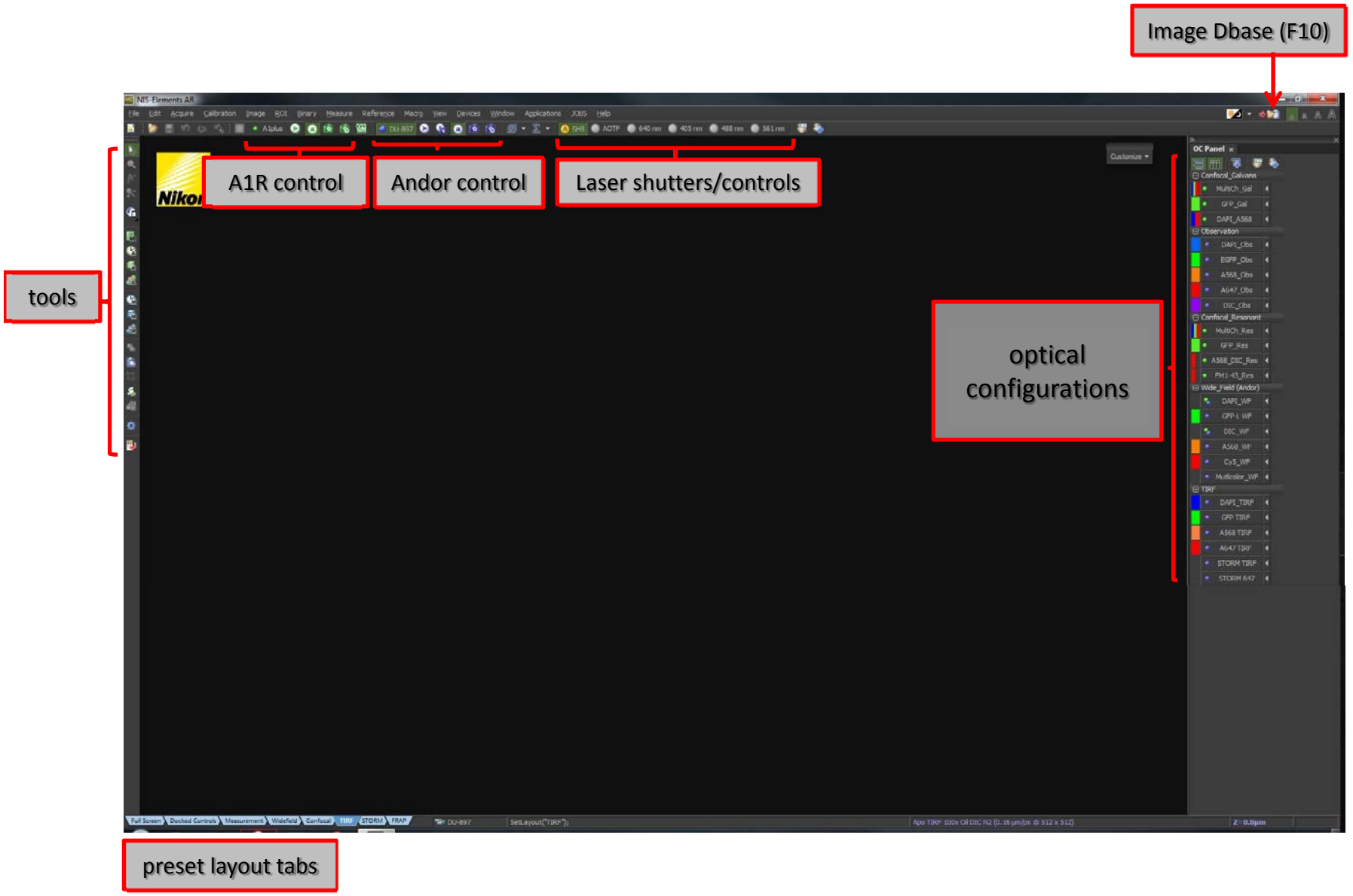
- This Quick guide to get you started and help choose between the Confocal, TIRF, wide field or STORM modi
- A1R handout for controlling confocal via the NIS software
- Koehler alignment procedure
- Ti handout to guide you through microscope stand specifics
- 'HOWTO' docs quickly guide you through various procedures
- STORM handout for controlling STORM modules via the NIS software
- Registration/ checking forms to keep track of settings and files for your labjournal

NOTE: Not everything is saved in the image metadata:
The ND filters , 1/1.5 magnifying lens on Ti stand, objective correction collars, STORM lens, 3D STORM lens, 1/4λ-plate.

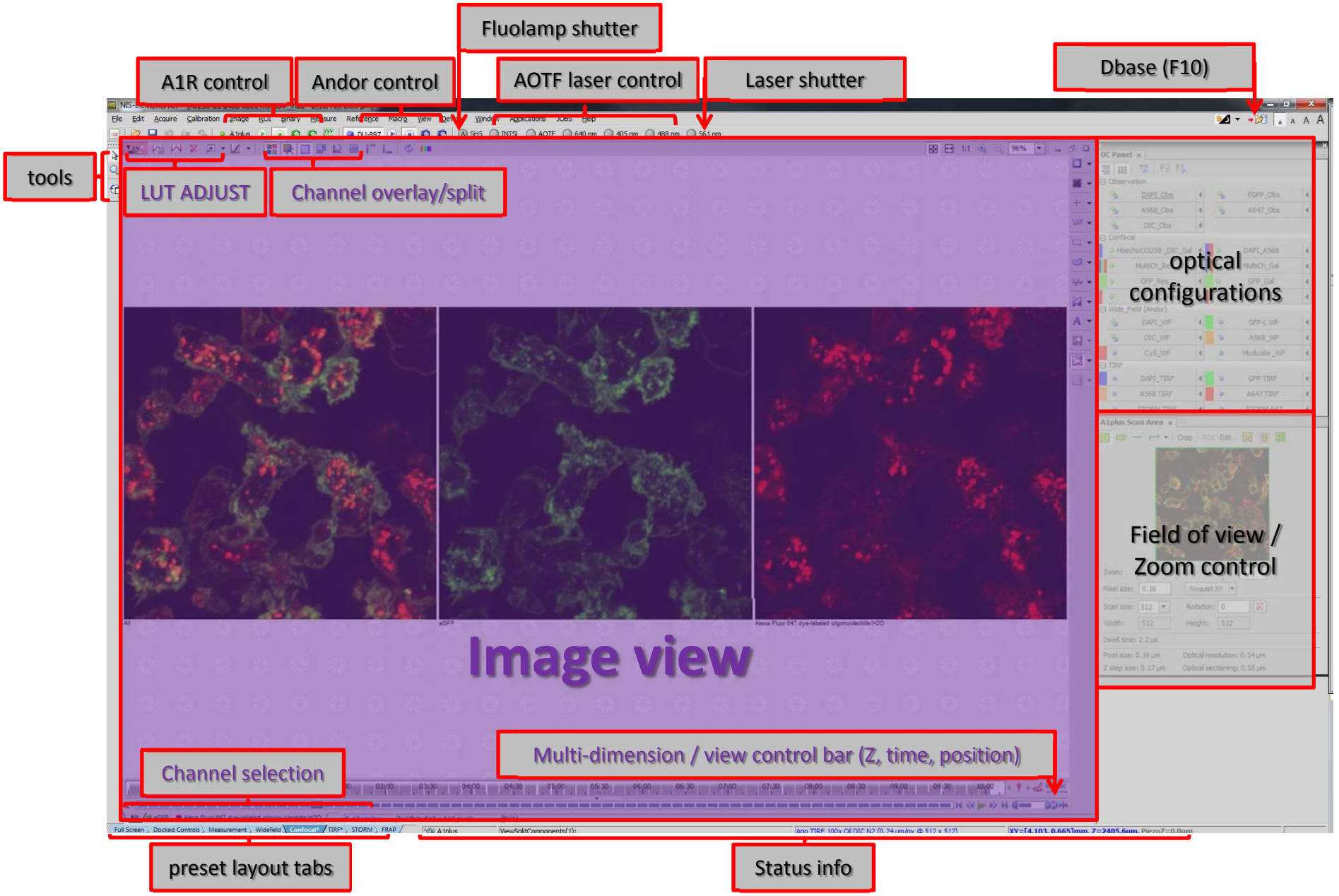
*A **CCI app** was made to:*
1. track these settings, 2. make experiment notes and 3. mail these to (on of) your mail adress(es)
(it is a cross platform HTML app: not as snappy as native apps but working on all devices)

Scan QR code to get to the app:

View window (dark mode)

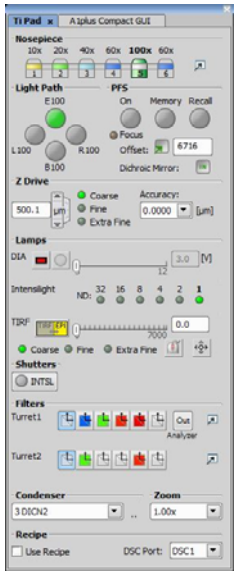


View window with image (light mode)

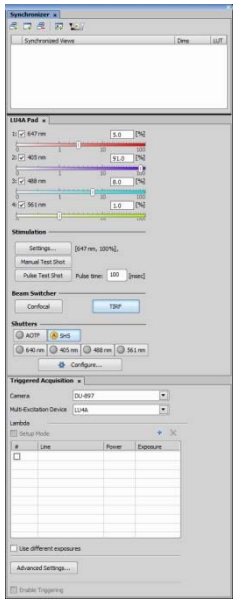


PADs that control units of the mic (preset for optimal use in layout tabs)

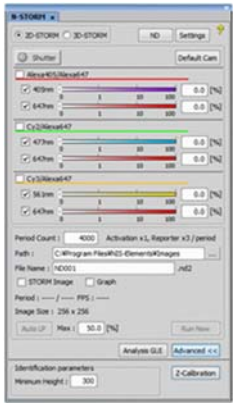
Mic control



LU4A control

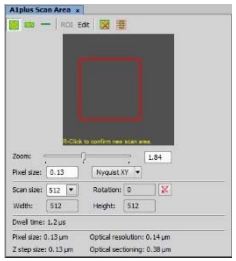


STORM control



A1R control

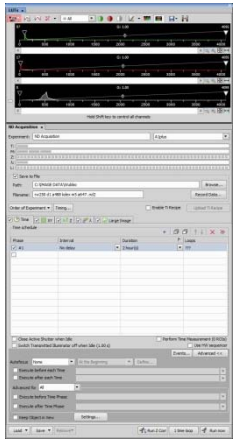
FOV pad



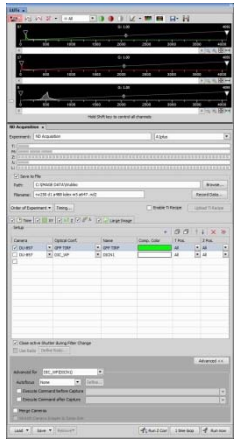
Browser (F10)

LUT and multi-dimension setting pads

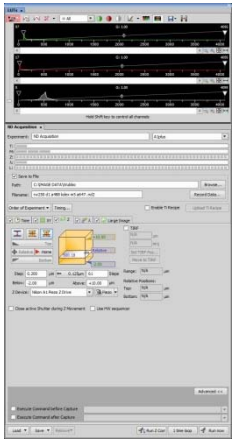
Time series



Channel series

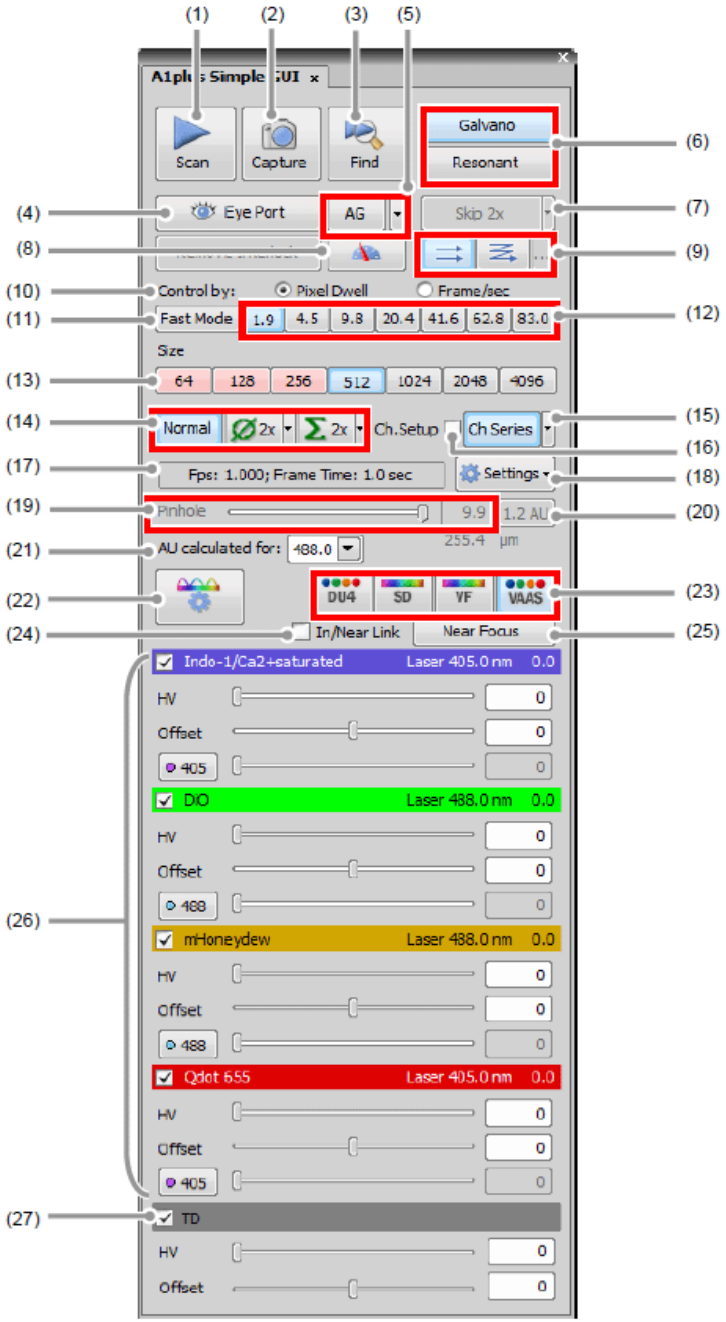


Z series



Tile series

A1R GUI



	Name	Function
(1)	Scan button	Starts/stops live image acquisition.
(2)	Capture button	Captures the image.
(3)	Find... button	Starts/stops live image acquisition in Find mode. Find mode is the mode where the live image acquisition is executed by temporarily switching to the high-frame-rate setting in order to ease the detection of the observation object such as a cell.
(4)	Eye Port button	Changes optical path to eye port.
(5)	Auto Gain (AG)	DU4: Automatically adjusts the HV value (HV gain) of the currently selected channel to the optimum values. (SD/VF: best setting fo all channels)
(6)	Scan mode selection	Enables to alternate between the Resonant scan mode and the Galvano scan mode. Scan modes cannot be alternated while the Live window is displayed. [SD] and [VF] only work in Galvano mode.
(7)	Line skipping	Sets the line skipping mode to be applied during scanning. Line skipping increases scan speed. (Pixel dwell does not change.) Only in Galvano scan mode.
(8)	Laser power monitor button	Displays the laser power value (integer obtained after A/D conversion divided by 10) of the current channel by clicking this button.
(9)	Scan Direction	Toggles between Unidirectional and Bidirectional scan. Bidirectional scan is only selectable if the Square scan area or Band scan area is set.
(10)	Control by:	Switches the Scan Speed selection form.
(11)	Fast Mode	Switches to the Fast Galvano mode making the Galvano scanner processing speed higher than the normal high-speed mode. Only in Galvano scan mode-use.
(12)	Scan Speed	Sets scan speed. (Setting unit: Frame/sec or lines/sec in line scan mode)
(13)	Scan Size	Displays the resolution automatically selected by the scan area. (Display unit: Pixel)
(14)	Average/Integral	Provides options for scanning a given line or area a number of times to display an image of the averaged or integrated values.
(15)	Ch Series button	Selects whether to perform scanning by simultaneously firing all lasers for the channels in use or by sequentially firing one laser after another. Lasers can be selected in the order of Ch1 to Ch4 or Ch4 to Ch1. Only in DU4-use (galvano and unidirectional resonance mode).
(16)	Ch.Setup check box	Displayed when the [Ch Series] button is ON. When checked, the setting by the channel is facilitated. Automatically enters the state where only one channel is selectable.
(17)	Fps:	Indicates the current scan settings.
(18)	Settings button	Displays the menu to open dialog boxes for various settings such as HV Linear Correction.
(19)	Pinhole	Adjusts the pinhole size in Airy units (units of airy disk size).
(20)	AU button	Changes the pinhole to the predetermined home position.
(21)	Reference excitation wavelength for the pinhole size calculation	Selects the excitation wavelength as the reference of the automatic calculation of the pinhole size from the laser wavelengths, or enter it manually in the [A.U. Calculation Settings] dialog box.
(22)	Optical path Setting button	Opens the Optical path window. To use, select the detector and the dichroic mirror, the channels as well as the fluorescence dye, laser, and for each channel.
(23)	Detection mode selection button	Selects the Detection mode for use.
(26)	Brightness adjustment for each channel	For each of the channels (Ch1 to Ch4), use the HV, Offset, and Laser controls to adjust the brightness of the live image.
(27)	Brightness adjustment for transmitted detector	For the transmitted detector, use the HV and Offset controls to adjust the brightness of the live image.

Basic A1R acquisition

1. Preview your slide through the eyepieces and focus on an area of interest
2. Select a suitable optical configuration
3. Click 'Remove Interlock' (changes from red > grey)
4. Choose 'Galvano' for standard imaging or 'Resonant' for fast imaging
5. Select what laser lines you want to acquire (check boxes for DAPI/FITC/TXRED/CY5/TD (transmission)
   NOTE: suggested initial settings: HV-Gain (100); Offset (0); Laser Power (2% (at low laser power startup))
6. Start with a scan size of 512x512 and highest scan speed
   Options: Findmode scans faster, change parameters for this in 'settings'
7. Select 'Normal'
   Options: bidirectional (adjust phase); skip line (speed upscanning); avg; sum; Ch series (set order)
8. Select 1.2 AU for Pinhole
   Options: Optical path setting adjustments (staff can help you)
9. Start "SCAN"
10. Adjust if needed (AG=auto gain: returns good tweaking start)
11. Click "CAPTURE
12. save image to correct folder in c:\image data*YOURNAME*

Multipoint acquisition in Z (and time):

- Setup A1R (NIS start with laser control> layout tab A1R>OC, tweak detectors)
- Make sure ND acquisition (**2**)is active (right-clk on viewer field > acquisition controls(**1**))

XY tab

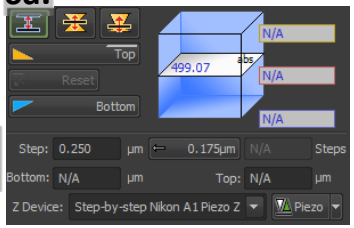
- Mark positions (xyz navigation and xyz overview panes(**3**), from viewer top row or acquisition controls to keep track of things, + (**4**) to add, x (**5**)to remove points; finding positions works best at lower zoom modes)
- Optionally press ' Optimize ' to minimize xy-travel time (not for multi-well imaging)
- Avoid pressing timing… (it sometimes glitches, and gets into an indefinite loop)

Z-tab:

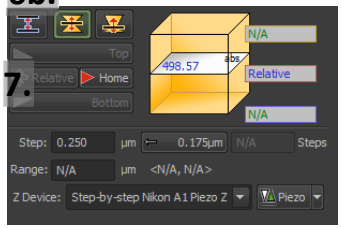
3 methods are available (top/bottom(**6a**); relative (symmetric (**6b**) and asymmetric(**6c**))

- The relative settings requires activation of home (**7**) (default will be symmetric)
- Set top/bottom - range - below/above for the different settings

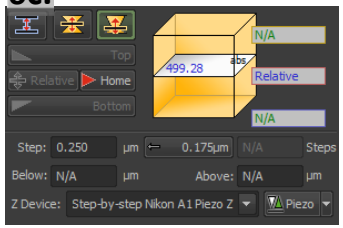
6a.



6b.



6c.



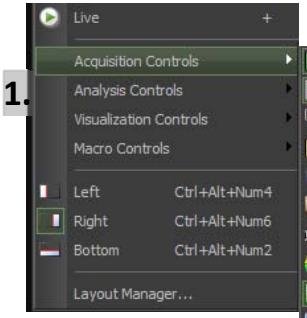
7a. **7.**

- Set step size, suggested step size = Nyquist; press to apply or choose appropriate step, fastest z device= Nikon A1 piezo (**7a**).
- Tick 'Include Z' (**8**) and in Advanced<< tick ' Leave PFS offset ON between points' (**9**)
- Run trough the different multi-positions at higher zoom mode (Nyquist preferably) and tweak xy (**10**) and PFS-Z offset (**11**) arrowheads at each position

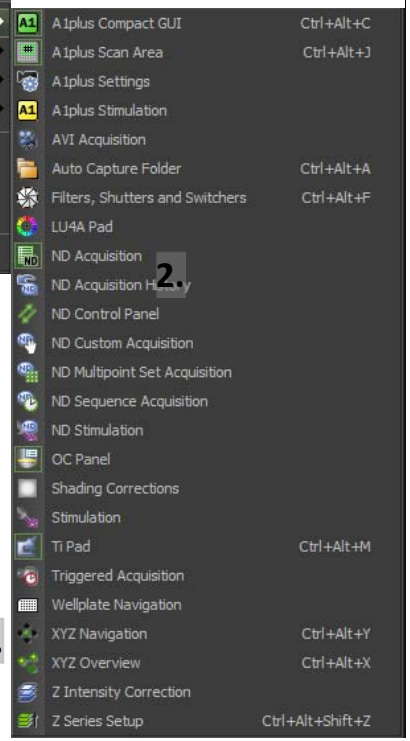
Time tab:

- Set time interval and duration (possible to change at different phases in the time course)
- Run and check the first cycle for correct focusing, xy position return, leave and relax
- Analyse

1.



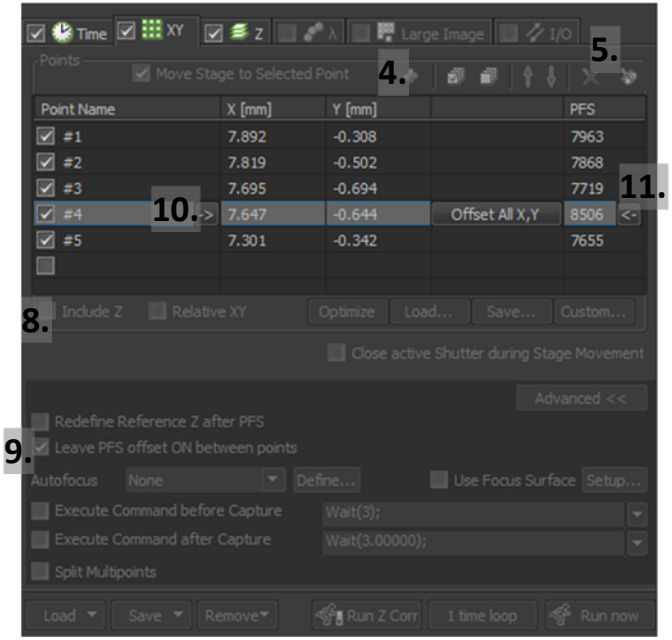
2.



3.

4. **5.**

8. **9.** **10.** **11.**



TIRF alignment:

!!!Work clean, dust causes fringes destroy alignment!!!

!!!aligning requires laser beams to be visible, take care and warn people around you

1. Lasers on at low power (one the lines you need)
2. STORM lens lever up for normal TIRF
3. No sample on the mic
4. Use high NA (>1.4) objective (check temp correction ring 23<>37)
5. TIRF Optical config , it presets:
    a) TIRF on Ti-pad
    b) turret1 pos1(empty), turret2 pos2(dStorm, quadruple HQ block)
    c) activate lasers in control, LU4A pad
    d) open shutter SHS, activate AOTF
    e) set power
    f) opens bottom port to not overexpose one of the imaging detectors
6. Look for spot at the ceiling, adjusting angle (target is exactly above objectives)
    a) top knob on TIRF arm adjusts angle y-axis

    NOTE: place plastic fluorslides to find laser when spot is lost

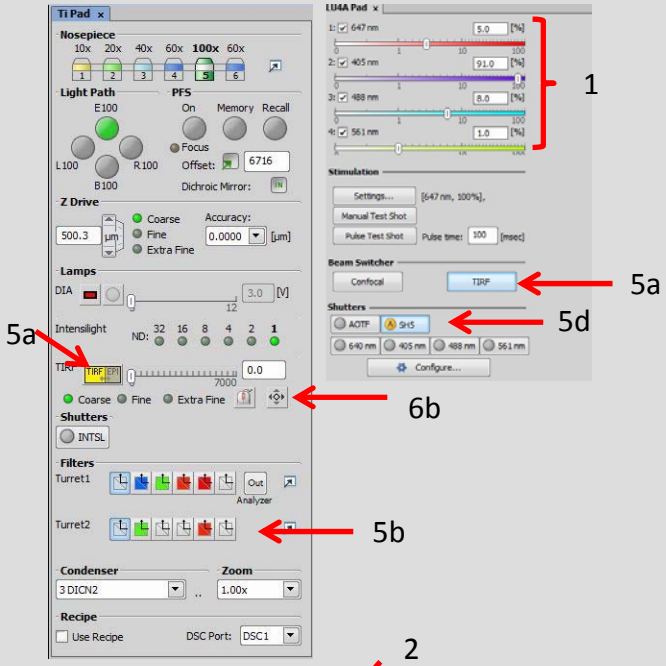
    a) Ti-pad controls angle in x-axis (coarse>>extra fine), 3214 =straight up

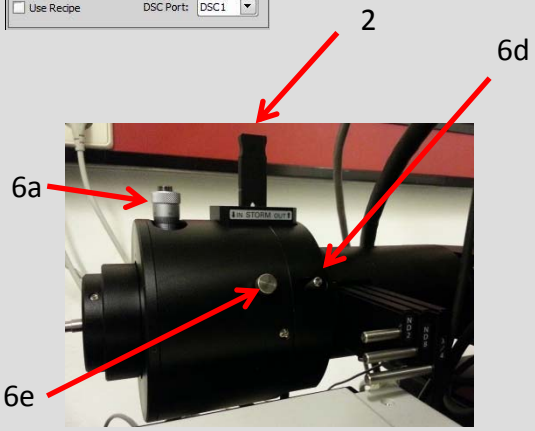
    NOTE: with STORM lens in: straight up ~3387

    a) set straight above mic (scrol wheel moves position)
    b) focus using side slider (should give nice round spot, polish glass surfaces)
    c) optionally lock
    d) adjust angle to left (smaller numbers) to get into TIRF
7. Use correct oil(23<>37), clean cs bottom with EtOH-wetted cotton swabs
8. Further adjust angle on Ti-pad to have optimal S/N.
    a) autoexopure in image window helps at start of tweaking
    b) Later switch it off to tweak signal to optimal S/N

    NOTE: Ultra EMCCD settings: Max EMgain<300, freq 17 mHz, gain3

NIS control





Koehler Alignment

Field aperture diaphragm (3) for Köhler alignment

ND sliders

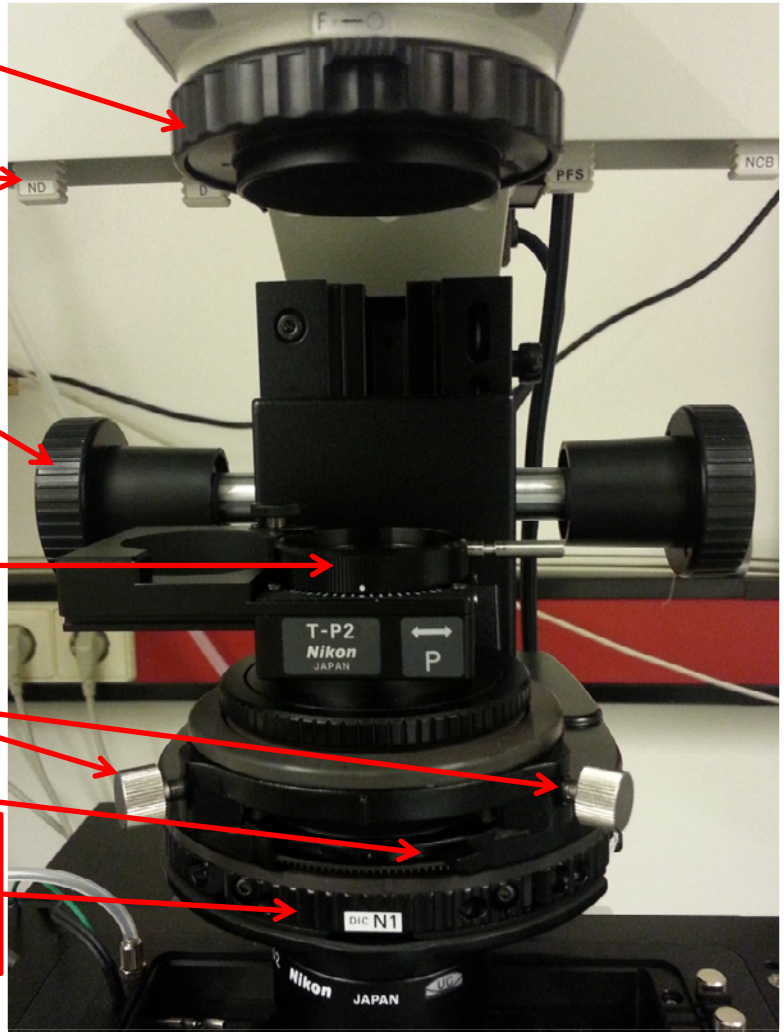
Focus screw condensor (4)

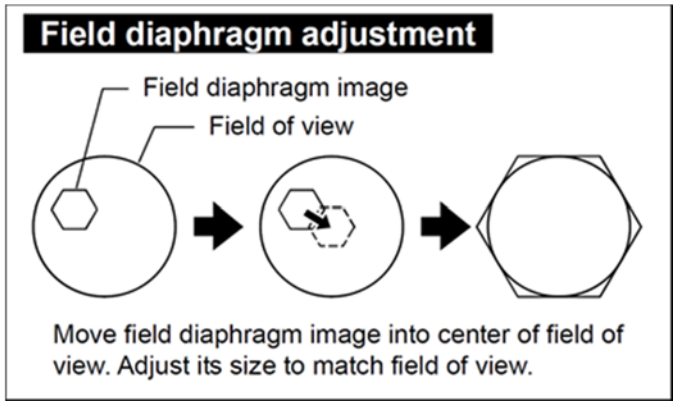
Polarizer for DIC
Right=in / left =in
Rotate using lever

Centering screws (5) for Köhler alignment

Field diaphragm

Condenser ring (1) with prims:
DIC N1 for 10/20x
DIC N2 for 40/60/100x
A open





Köhler alignment

1. Start using 10x objective , set condensor ring to “A”
2. Focus on sample
3. Close field aperture diaphragm until it is visible
4. Focus condensor (use focus screw condensor)
5. Center using centering screws of condensor
6. Open field aperture close to edges and do fine adjustment
7. Open field aperture diaphragm to match field of view
8. Repeat with the higher magnifications, fine tuning easier

Da rules:

1. We are in a ML2 designated area so wear a lab coat and do not eat or drink here
2. Clean up mess before and after use
3. Take your data from the system drive, next users can have a freezing PC when the 1 TB drive is full
4. Be careful with objectives, clean using proper cloths
5. Prevent accumulation of dust by placing TOKAI hit cover and plastic cover over mic after use
6. Cells can be kept in our incubator, yet we choose NOT to humidify it, less contamination result from this. Humidifying your cells is done by placing dishes inside 15 cm dishes together with a pre-wetted tissue or an open milliQ-filled dish.
7. Hourly rates during office hours 15 Euro, fixed overnight pricing 80 Euro.

GMO handling:

1. After live cell experiments, empty TOKAI-hit water basin using vacuum pump, place 70% EtOH in the basin and leave for ~10 minutes. Empty using vacuum pump again and dry the basin with tissues.
2. The right collection flask vacuum pump should contain Chloride solution (10x solution=1 SUMA tablet/liter), and should be emptied the day after imaging.
3. Collect solid waste in plastic bags that are placed in the frame next to the sink, after finishing close it and dispose in yellow bin in the corridor (next to the gas cylinder cabinet)
4. Spill accidents need to be cleaned using EtOH and/or supplied soap solution. Always notify the CCI staff if this occurs