

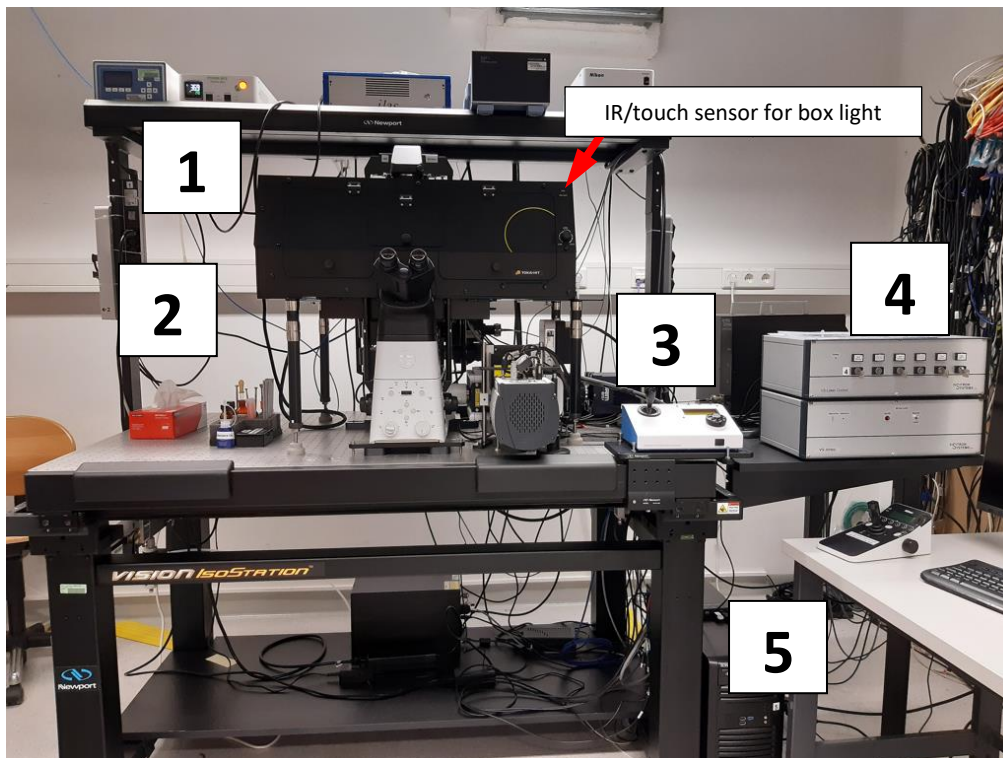
Visitron LIVE Spinning Disk

Startup and Shutdown

January 20, 2023

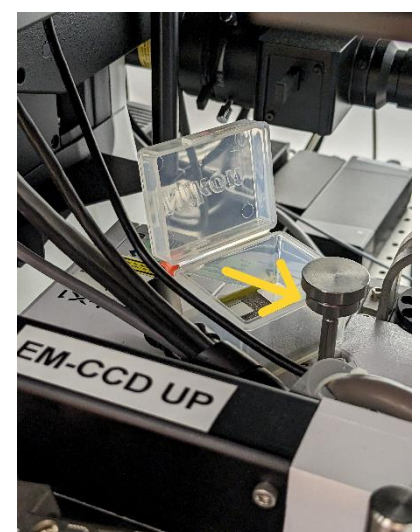
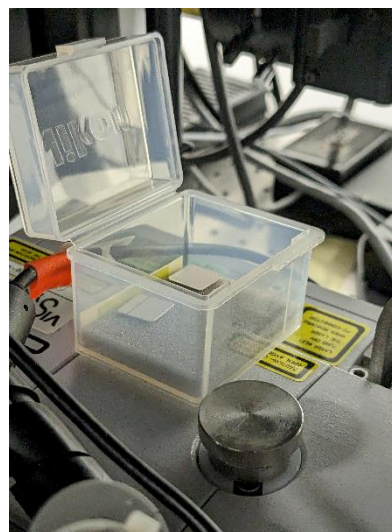
1. Powering on the system

- Switch on power strips **1**, **2** and **3**, in this order. Wait a second between powering on the next.
- Switch on the lasers you need via the keys **(4)**.
- Turn on the computer **(5)**.



2. Camera selection

- For the EM-CCD (low resolution, high sensitivity), pull the handle on the spinning disk unit up and put the small plastic box underneath to fix it (see image right).
- For the sCMOS (fast, high resolution), push it down. Hold it with your hand while pushing (see image left).



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3. Computer

- Windows login: pick the right user account (*Imaging, FRAP* or *Nanodissection*). *Nanodissection* is password protected, the others are not.
- Launch VisiView. If you have not turned on all lasers, an info message will list inactive lasers being emulated (*Obis, Obis-2, and/or Toptica* for the 405, 488, and/or 640 nm, respectively). Acknowledge by clicking *OK*. Likewise, acknowledge *Hard drive not found* warnings (the startup script will reset the path for saving files to the standard hard drive D:).

- Startup dialog:

Camera & Spinning Disk: enter the selected camera & SD (W1 or X1, *sCMOS* or *EM-CCD*)

Objective: choose the one you wish to use from the drop-down list

Objective to load position:

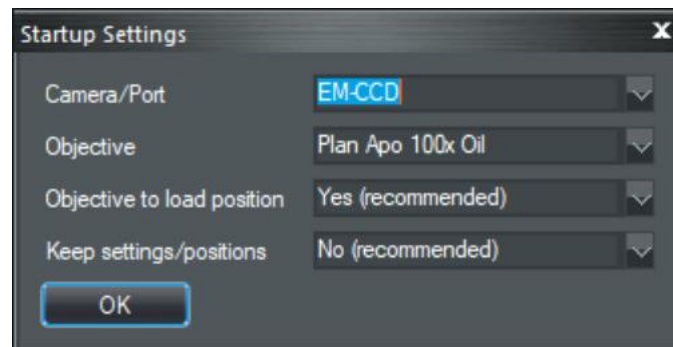
Yes (recommended) moves the objective to the lowest (safe) position

No (are you sure?) should only be chosen if you had to restart VisiView during a session

Keep settings/positions:

No (recommended) clears any previous stage positions and illumination settings

Yes should only be chosen if you had to restart VisiView during a session



4. Focus and escape position

- After running the startup dialog, the objective should be in the lowest (safe) position.
- If the *ESC (Escape)* button on the microscope lights green, the focus is locked. This happens if the previous user has lowered the objective turret at the end of the session using the *ESC* button. Press it and **keep it pressed** until the green light turns off.



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5. Correction collars (Plan Apo 40x Silicone, VC 60x Water)



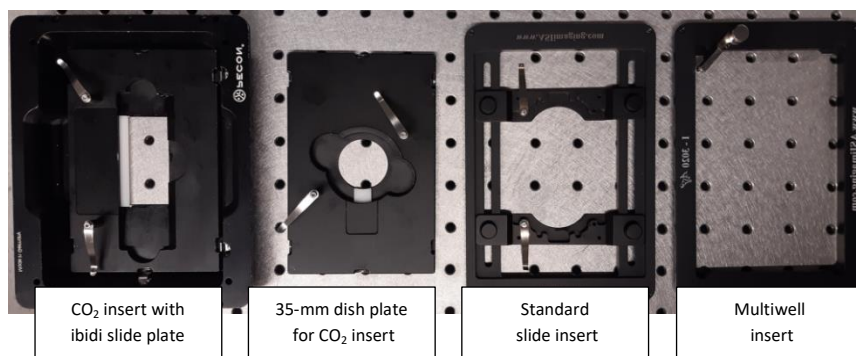
- Launch the webcam in the box with the camera icon on the taskbar.
- Turn on the light in the chamber.
- At 37°C, rotate the collar so that the mark on the objective body coincides with the 0.15-mm scale mark on the collar. On the 40x silicone objective, adjust to the mark halfway between 0.13 and 0.17, on the 60x water to the mark labeled "15".



- Note: when working at room temperature, use the 0.17-mm mark ("17" on the 60x Water).



6. Stage insert

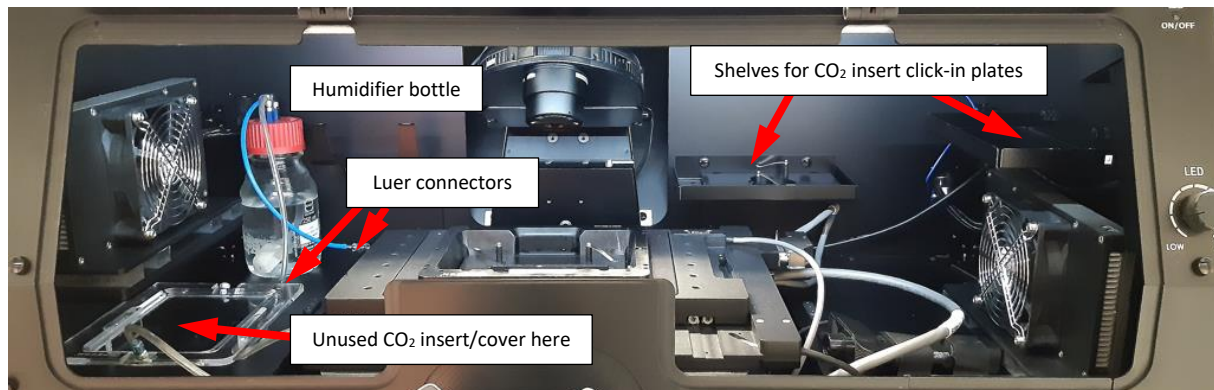


- Install the desired stage insert.
- If the CO₂ insert is not used, it should be stored inside the box (to keep it on temperature) at the front left corner (caution: avoid blocking the travel range of the XY stage). The click-in plates for the CO₂ insert should be stored on one of the shelves inside the box.

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- Other inserts (standard slide, multiwell) should be kept in the small drawer next to the door. In the drawer, there is also a click-in plate for two 35-mm dishes.

Important: when using the click-in plate for two 35-mm dishes, always move the objective down using the *ESC* button before moving from one dish to the other.



7. Immersion and sample

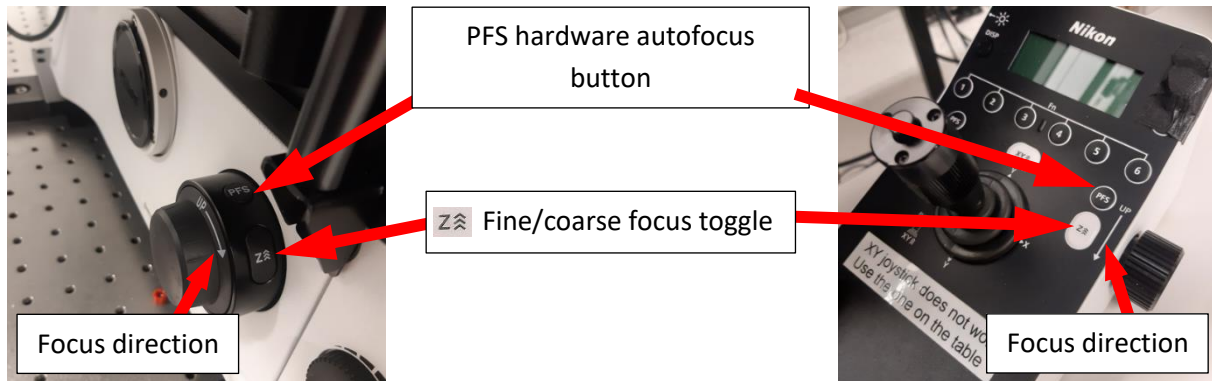
- Place a drop of the appropriate immersion fluid on the objective.

- Plan Apo 20x Air:	None
- Plan Apo 40x Silicone:	Silicone (S on the cap)
- Plan Apo 60x Oil:	Standard Oil (blue bottle)
- Plan Apo VC 60x Water UV:	Immersol W (W on bottle and cap)
- Plan Apo 100x Oil:	Standard Oil (blue bottle)
- Plan Fluor 100x Oil:	Standard Oil (blue bottle)
- Mount your sample in the stage insert.

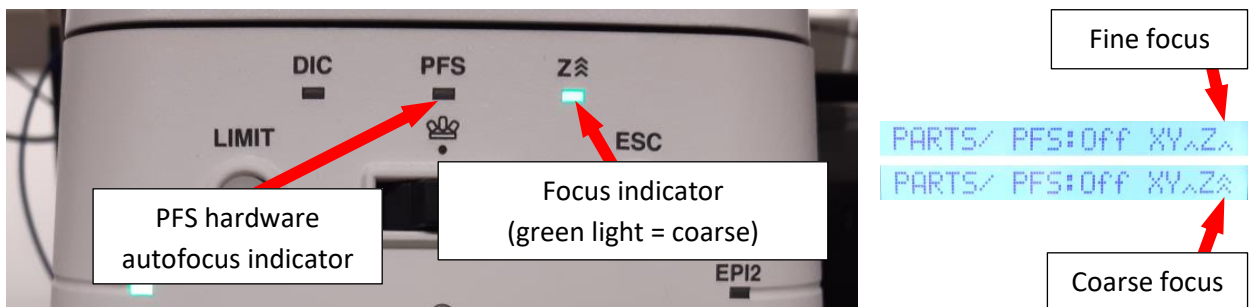


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8. Focusing



- The Nikon focus turns in the opposite direction as compared with Zeiss microscopes. An arrow (UP) on the focus drives and on the Nikon joystick pad indicates the direction.
- The **Z** button toggles between coarse (fast) and fine (slow) mode. In coarse mode, the focus indicator on the frame lights green. The current mode is also indicated on the LED of the joystick.

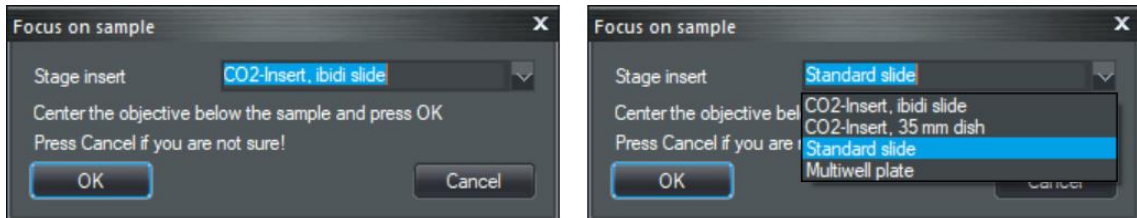


- The *PFS* buttons on the focus drives and joystick control the Perfect Focus hardware autofocus.
 - Pressing the button briefly turns it on or off. The *PFS* indicator on the frame lighting green indicates an active PFS. It blinks if it is off, but detects a surface within its travel range. It remains dark if there is no surface in reach.
 - Pressing it and holding it for >1 sec turns the PFS off and removes the dichroic from the light path. The indicator on the frame turns dark. The LED on the joystick pad displays "PFs:OUT".
- The Nikon XY joystick is inactive. To move the stage in XY, use the ASI controller next to the microscope. Pressing the button on the top of its joystick toggles between slow and fast mode.



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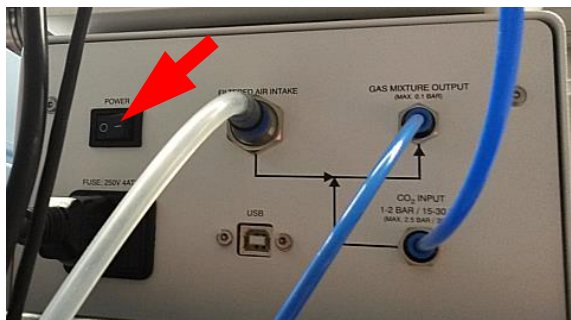
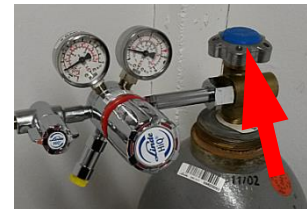
- To ease finding the focus at startup, click on the *Focus Up* button in the VisiView toolbar. In the dialog popping up, select the name of the installed stage insert.



- Center the objective below the opening of the stage insert using the ASI XY joystick and click *OK*. The objective will move up, close to the sample. It will not be exactly in focus, though, and you will have to focus a bit manually. Clicking the *Cancel* button in the dialog will exit without action.
- To replace the sample once focused, press the *ESC* button on the microscope frame. The objective turret will move down. All focus controls will be locked and the *ESC* indicator will light up green. Briefly pressing the *ESC* button again will move the objective back to last focus position and enable the focus controls (wait until the green light is off). When keeping the *ESC* button pressed until the green light goes off, the focus lock will be released, but the objective turret will remain in the lowest (safe) position.

9. CO₂ control (optional)

- Open the main valve of the CO₂ tank by turning it counter clockwise for 1 or 2 rounds (no need to rotate it up to the stop). Close it clockwise once you are done. ***Don't touch the other valves!***
- Power on the CO₂ controller (power switch on its back side).
- Place the CO₂ cover on the CO₂ insert and make sure that the humidifier bottle is filled with enough distilled water (>50 mL; the bottle can be disconnected with two luer connectors).
- Activate the CO₂ flow on the controller.

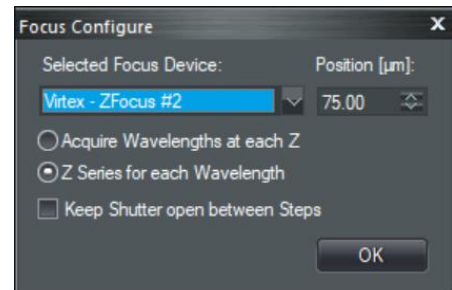


10. VisiView settings

- Focus policy: in the top menu, select *Configure* → *Focus*.

Acquire wavelengths at each Z is preferred for z-stacks of rapidly moving objects (shortest possible interval between different channels).

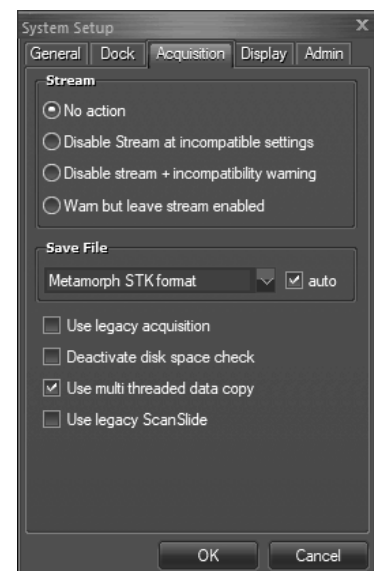
Z Series for each wavelength is overall faster (requires less movements of the slow filter wheel).



- File format: in the top menu, select *Configure* → *System*. In the dialog popping up, select the *Acquisition* tab. Under *Save File*, select file format:

Metamorph STK is recommended for all kinds of multidimensional acquisitions.

OME Tiff is recommended single-channel FRAP experiments. It stores FRAP regions in the metadata. Check the *auto* checkbox. VisiView will automatically select the proper OME Tiff subtype (32 bit as *.ome.tif, 64 bit version as *.ome.btf).



11. Shutdown

- On the microscope, press the *ESC* button to bring the objective in a safe position.
- If using CO₂ control:
 - Turn off the CO₂ flow on the CO₂ controller.
 - Power off the controller.
 - Close the main valve of the CO₂ tank.
- Remove your sample.
- Clean all used immersion objectives with lens cleaning paper.
- Exit VisiView.
- When leaving the system for the next user: sign out from Windows.
- Full shutdown:
 - Shut down the computer.
 - Switch off all laser keys (**4**).
 - Power off the power strips in reverse order (**3, 2, 1**).