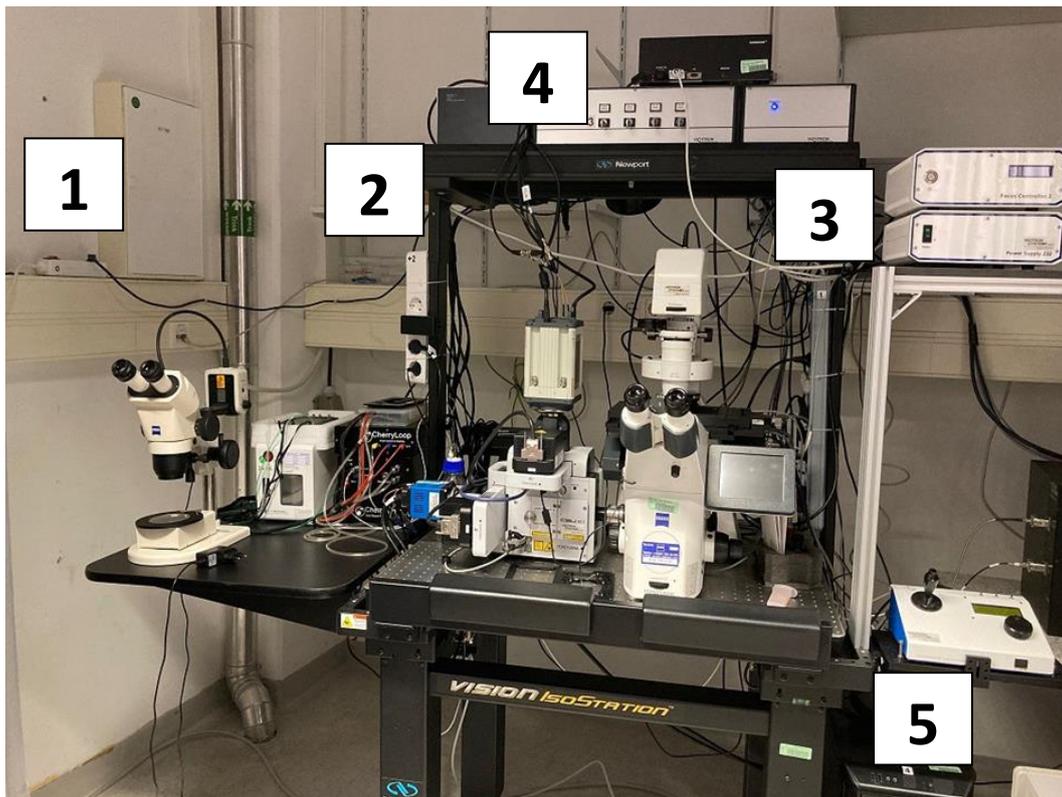


Visitron Spinning Disk

Startup

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

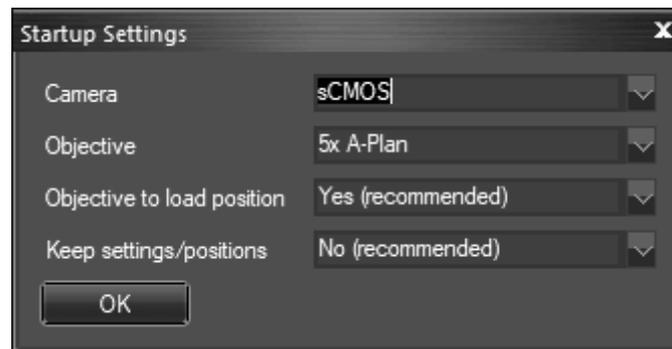


- Select the camera: for the sCMOS camera (fast, high resolution), push in the handle on the spinning disk unit. For the EM-CCD (low resolution, high sensitivity), pull it out.

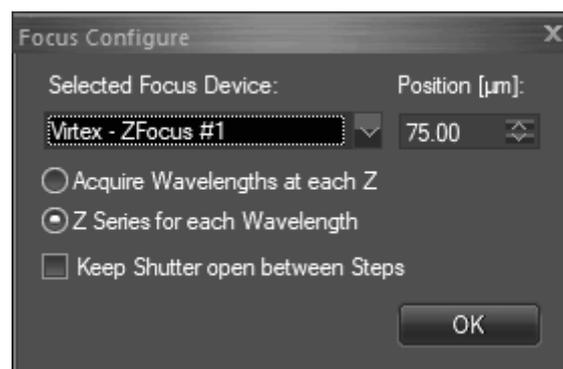


Visitron Spinning Disk

- Windows login: pick the right user account (*FRAP* or *Imaging*).
- Launch VisiView. If you have not turned on all lasers, an info message will pop up, listing inactive lasers. Acknowledge this by clicking *OK*. Likewise, acknowledge *Hard drive not found* warnings with *OK*.
- Startup dialog:
 - Camera: enter the selected camera (*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. Choose *No (are you sure?)* only if you had to restart VisiView and want to keep the current focus.
 - Keep settings/positions: *No (recommended)* clears any stage positions and illumination settings from the previous user. Choose *Yes* only if you had to restart VisiView.



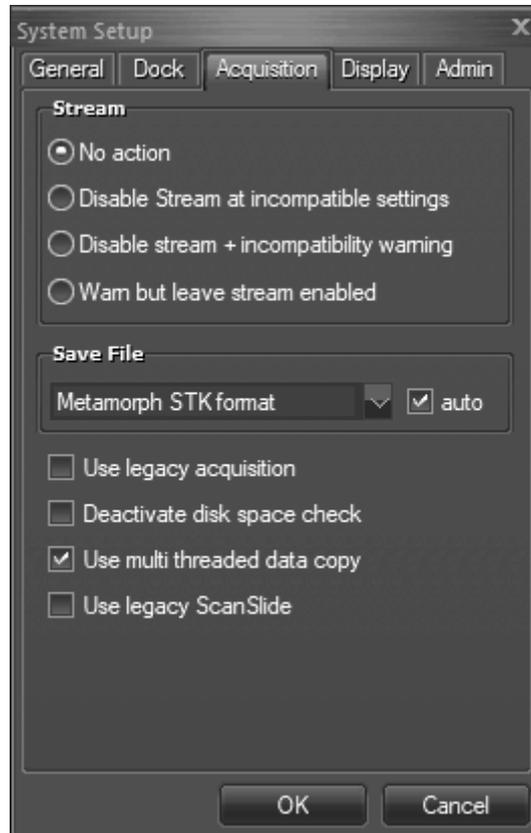
- Channel/wavelength settings: in the top menu, select *Configure* → *Focus*.



- *Acquire wavelengths at each Z* is preferred for z-stacks of rapidly moving objects (shortest possible intervals between different channels).
- *Z Series for each wavelength* is overall faster (requires less movements of the filter wheel).

Visitron Spinning Disk

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



- *Metamorph STK format* is recommended for all kinds of multidimensional acquisitions.
- *OME Tiff format* is recommended only for simple, single-channel FRAP experiments. This format stores FRAP regions within the metadata. Make sure to check the *auto* checkbox. VisiView will then automatically select the proper OME Tiff subtype (32 bit with the file extension *.ome.tif, or the 64 bit version as *.ome.btf).

Shutdown

- On the touch panel of the microscope, press the *Load Position* button.
- 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**). Put the dust cover over the microscope (spare the EM-CCD).