Abbelight SAFe 360 SMLM Guide

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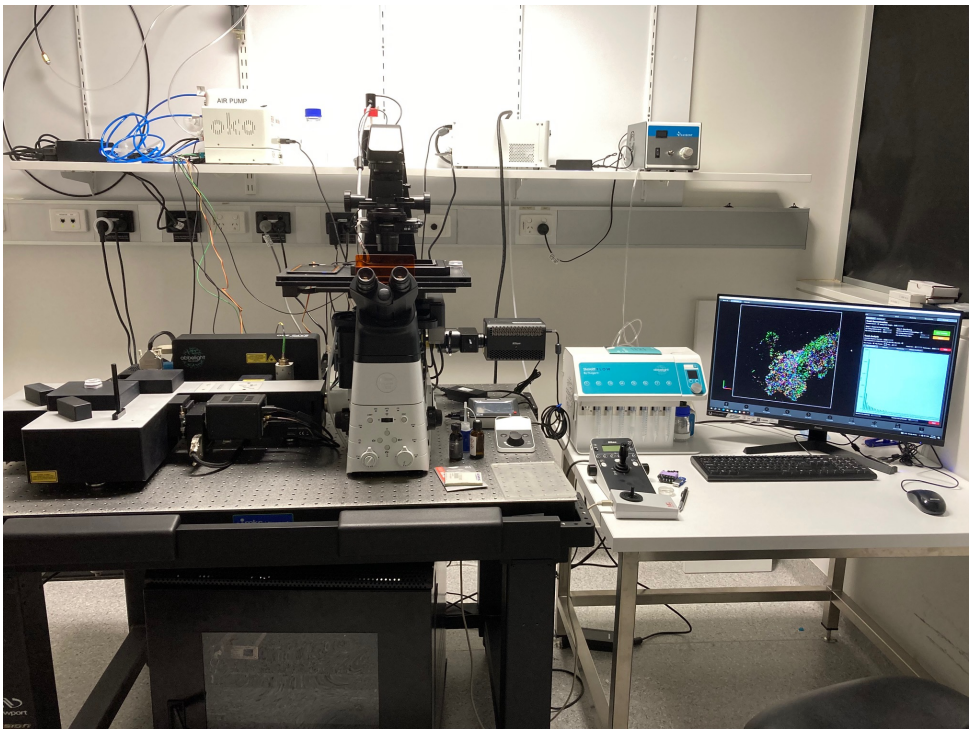
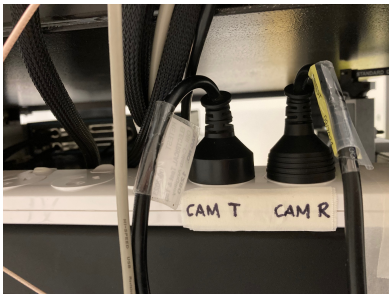
Getting started

Turn the microscope and its peripherals (Fig. 1a) on in the following way:

1. Turn the two Hamamatsu ORCA-Fusion BT sCMOS cameras on at the power board at the back and underneath the optical table (Fig. 1b). Wait 30 seconds.



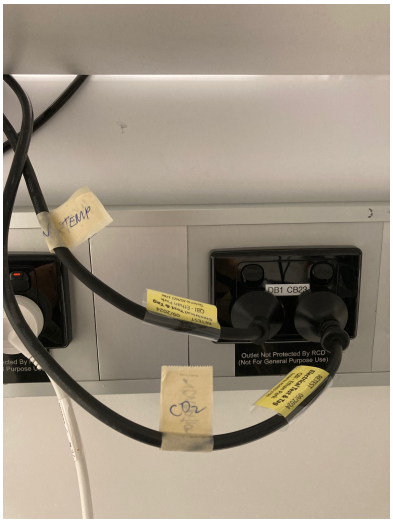
(a) Microscope body and peripherals.



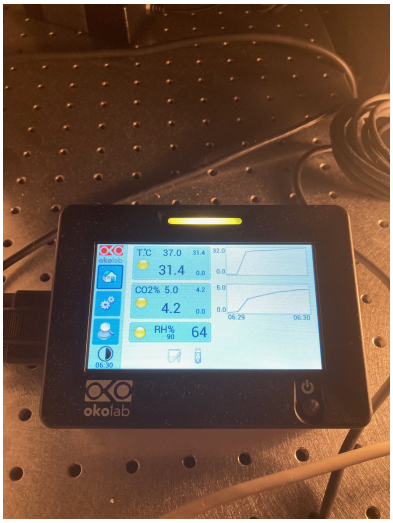
(b) Power board for the Hamamatsu ORCA-Fusion BT sCMOS cameras.

Figure 1: The Abbelight SAFe 360 single-molecule localisation microscope.

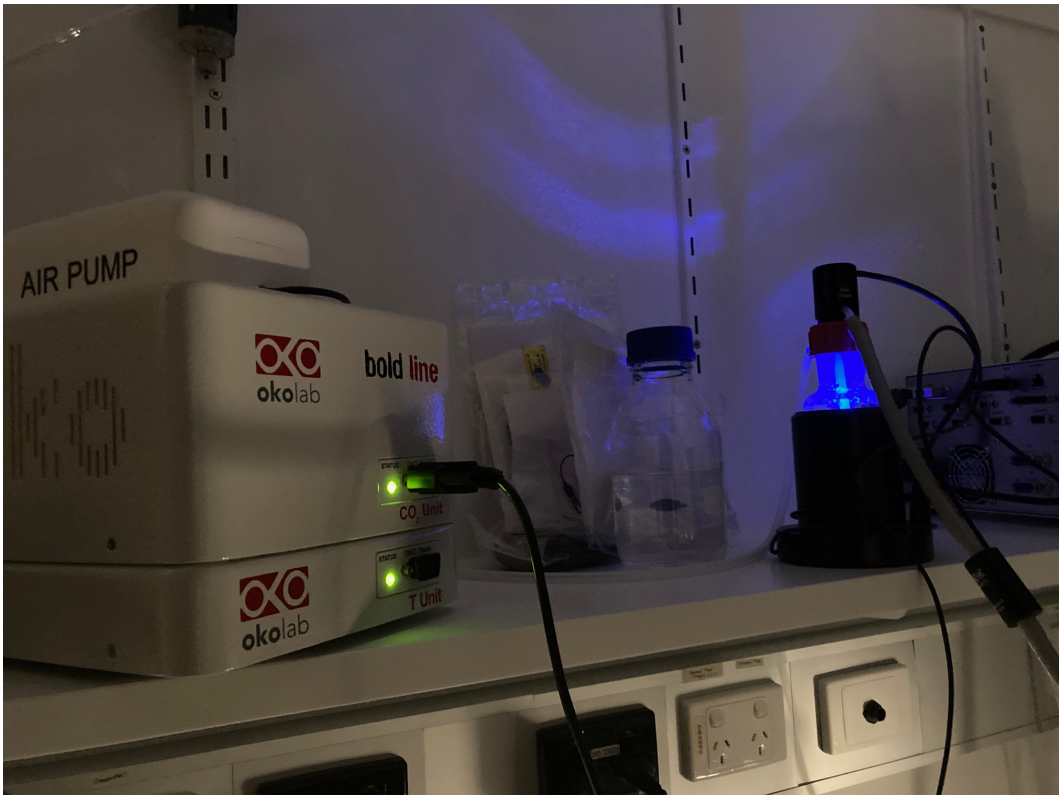
2. If performing a live-cell experiment, turn the Okolab stage-top incubator on at the power outlet on the back wall (Fig. 2a). On the Okolab touch pad, set the temperature and CO_2 level accordingly (Fig. 2b).



(a) Power for the Okolab modules.



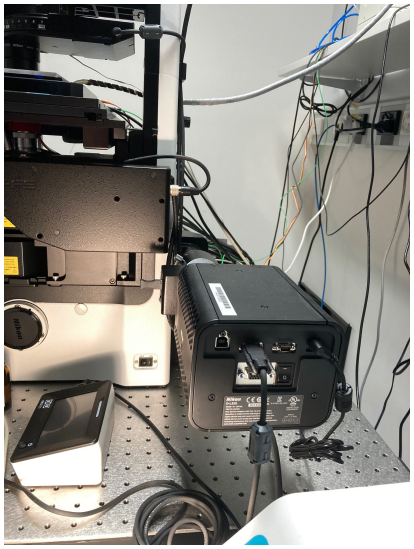
(b) The Okolab touch pad.



(c) Okolab modules and humidity bottle and heater.

Figure 2: The Okolab stage-top incubation system.

3. Turn the Nikon Eclipse Ti2 microscope on. The power switch is on the right side of the microscope body (Fig. 3a). If viewing fluorescence through the oculars, turn the Nikon D-LEDI source on. The power switch is at the back of the LED module (Fig. 3a). This also turns on the LED controller (Fig. 3b).



(a) Power switches for the Nikon body and LED.

(b) The Nikon D-LEDI controller.

Figure 3: Nikon Eclipse Ti2 microscope body and D-LEDI power switches, and the D-LEDI controller.

4. Turn the Abbelight module power switch on (red indicator lit when on, Fig. 4). Turn the laser emission key to the "On" position and open the laser shutter. Turn the SAFe Electronic main switch on. Wait for 15 seconds. Turn the scanner switch on.

   Turn the computer on. To log on to Windows, use ".\Workstation", and leave the password blank.

   Log on to PPMS when prompted.

Figure 4: Abbelight tower showing (top to bottom): Abbelight module, Oxxius laser controller, SAFe Electronic module, and acquisition PC.

Visualising a specimen through the oculars

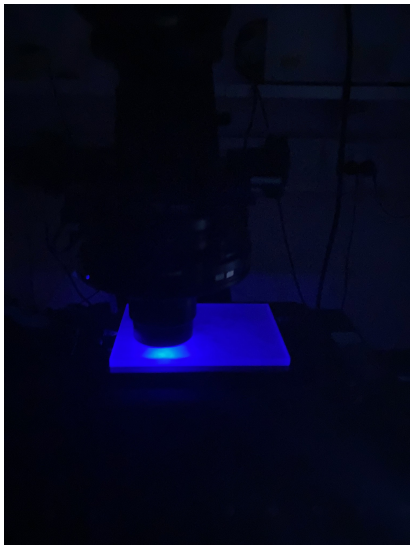
1. To protect the objective from damage, press the “ESC” button on the front of the microscope body. This brings the objective down, away from the specimen plane. Place the specimen on the stage insert and bring the objective up towards the specimen until the immersion oil comes into contact with the coverslip.

2. On the right side of the microscope body, move the the lower filter turret knob (labelled “1”) to the right or left until the desired filter cube is in place (Fig. 5a). Move the the upper filter turret knob (labelled “2”) to an empty position. The turret positions are indicated in the microscope controller (Fig. 5b). In this example, we have used the FITC cube.

3. Open the epi-fluorescence shutter by pressing “FnR.” This illuminates the specimen (Fig. 5c). To visualise the specimen through the oculars, press the ”Eye” icon on the front of the microscope body. Press “PFS” on the microscope controller to activate Nikon’s Perfect Focus System. The indicators on the Nikon body should show that the PFS is active, the light path selected is towards the oculars, and the epi- shutter is open (Fig. 5d).

(a) Turret knobs and epi- shutter, “FnR.”

(b) Microscope controller.



(c) Epi-fluorescence illumination.

(d) PFS, ocular path, and epi- shutter indicators.

Figure 5: Controllers and indicators for visualising a specimen through the oculars.

Simultaneous dual-channel acquisition

1. After the relevant specimen region and focus are found using the oculars, switch to the camera light path by pressing “Camera L” on the front of the microscope body (Fig. 5d). This configures the light path so that the fluorescence emission is directed to the left port of the microscope, to be detected by the cameras.
   Use the turret knobs so that both turrets are on empty positions. This is indicated by the text, “no data,” on the Nikon controller display (Fig. 5b).

2. Start NEO Live Imaging by double-clicking on its shortcut icon on the desktop (Fig. 6).

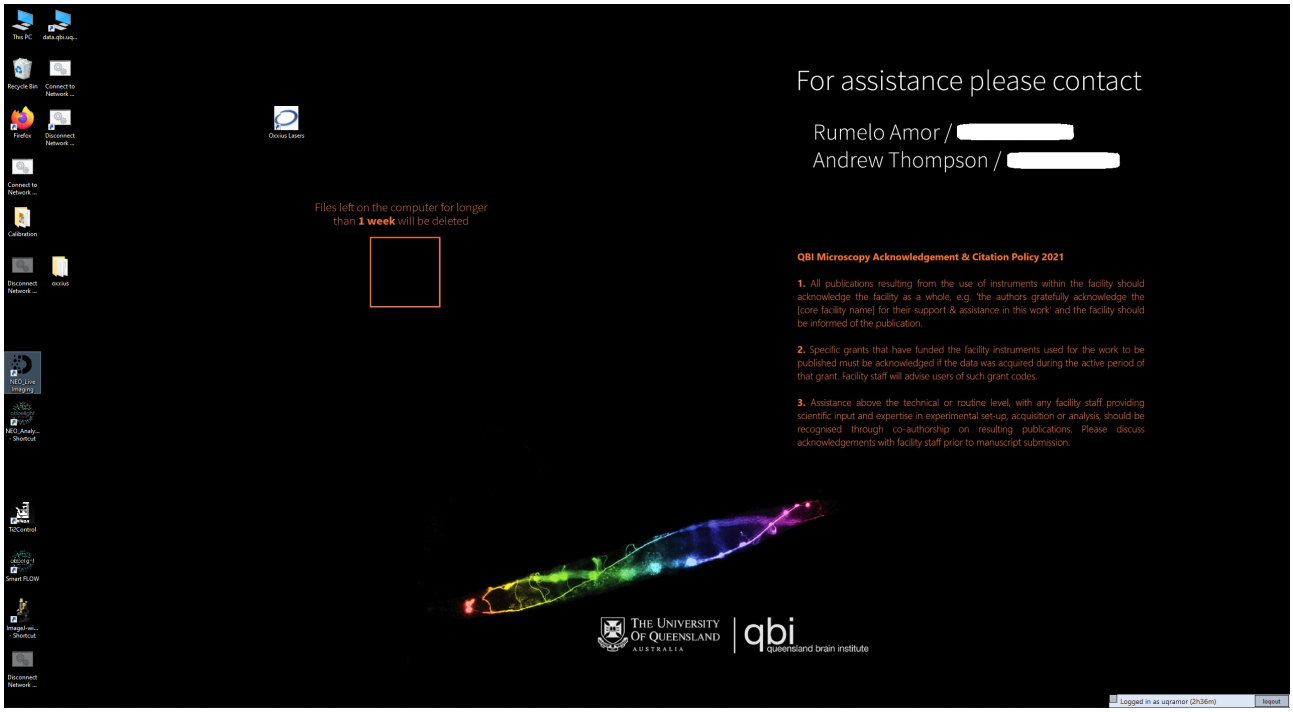


Figure 6: Desktop with NEO Live Imaging shortcut highlighted.

3. On the NEO Live Imaging GUI, click on “System,” then “Hardware Configuration” (Fig. 7).

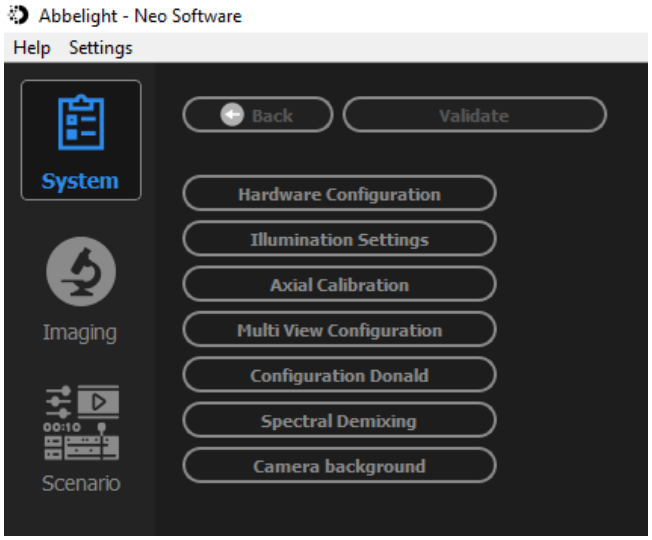


Figure 7: Accessing the hardware configuration on the NEO Live Imaging GUI.

4. In the resulting hardware configuration window, under “Saved configurations,” select “SAFe 360 - Simultaneous 2 colors - Nanoscopy - 2D” (Fig. 8).
   Make sure that “Camera transmitted:” is on “Hamamatsu 0,” and “Camera reflected:” is on “Hamamatsu 1.”
   Click “Validate.”

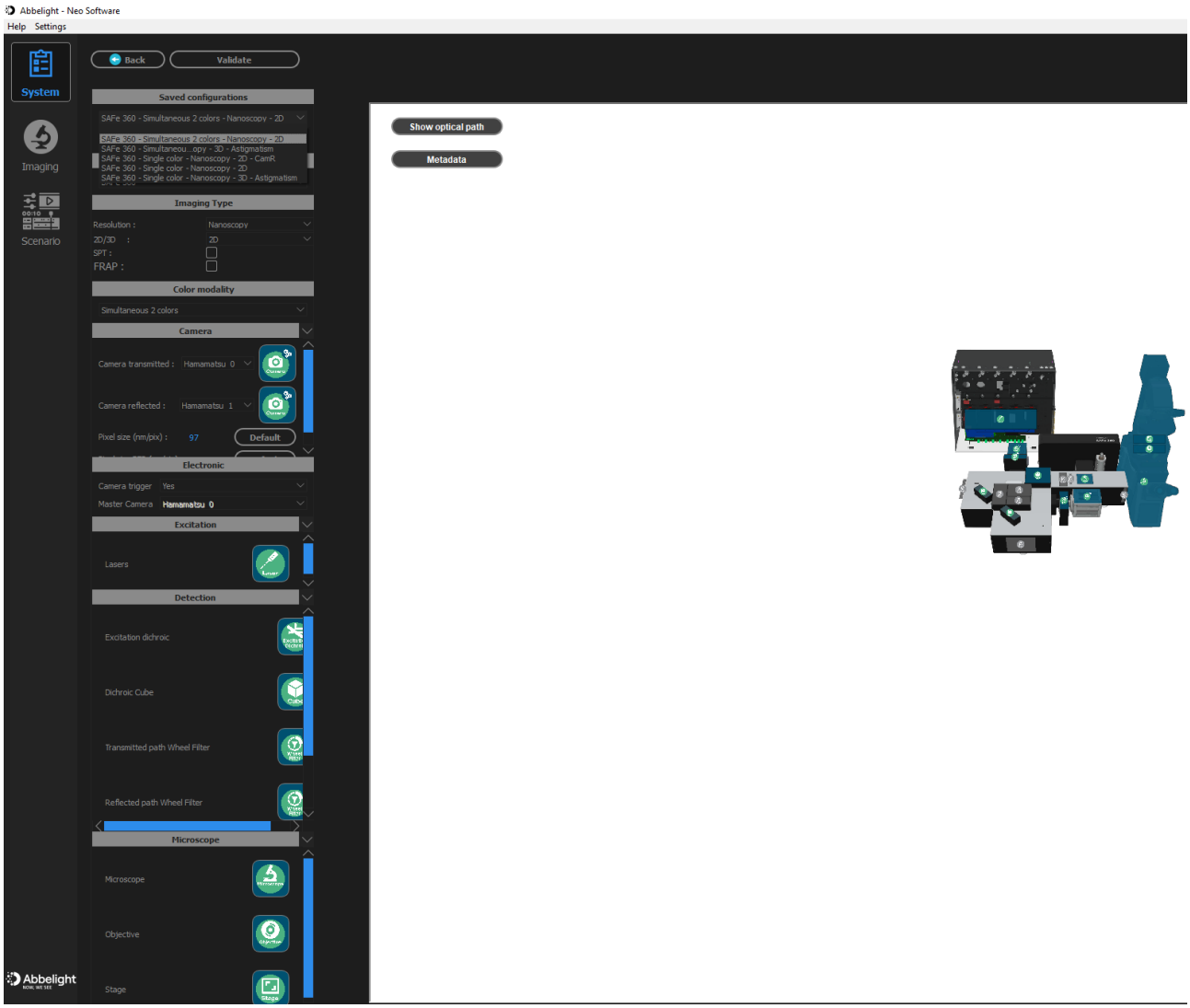


Figure 8: The hardware configuration window.

5. Click on “Imaging,” then “Project,” then “New Project” (Fig. 9).

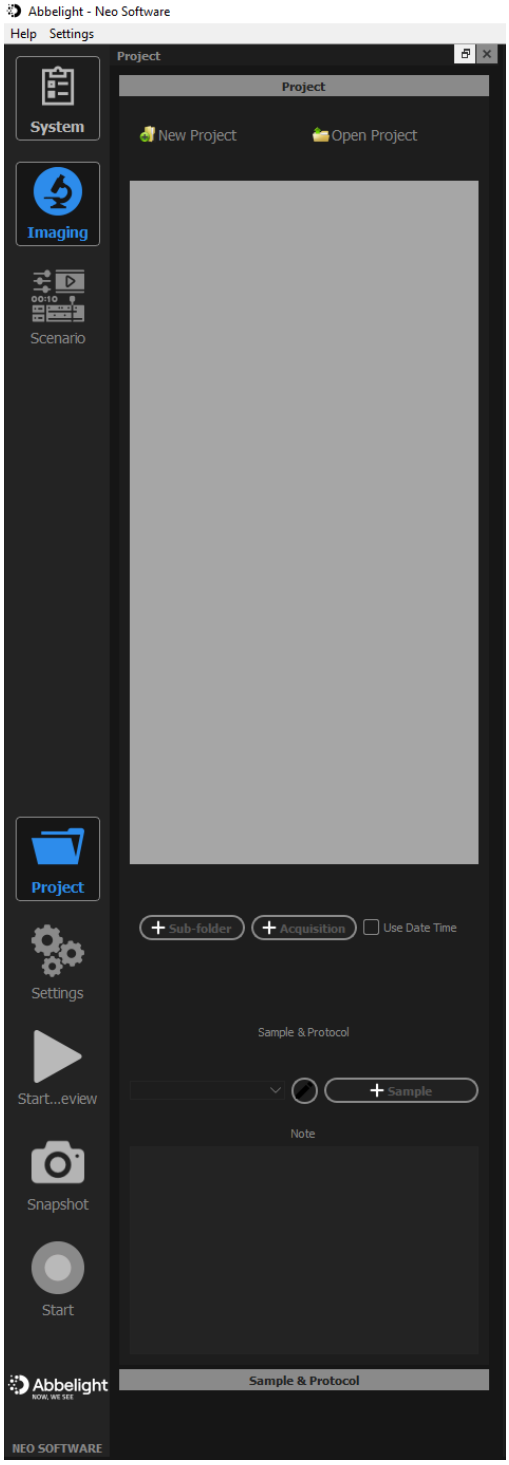


Figure 9: Project window.

6. Create a new project directory in C:/Users/Workstation/Documents (Fig. 10) as the C: drive is the fast drive. Click “Finish.” Click on “Project” again so it’s not highlighted and the project tab is hidden.

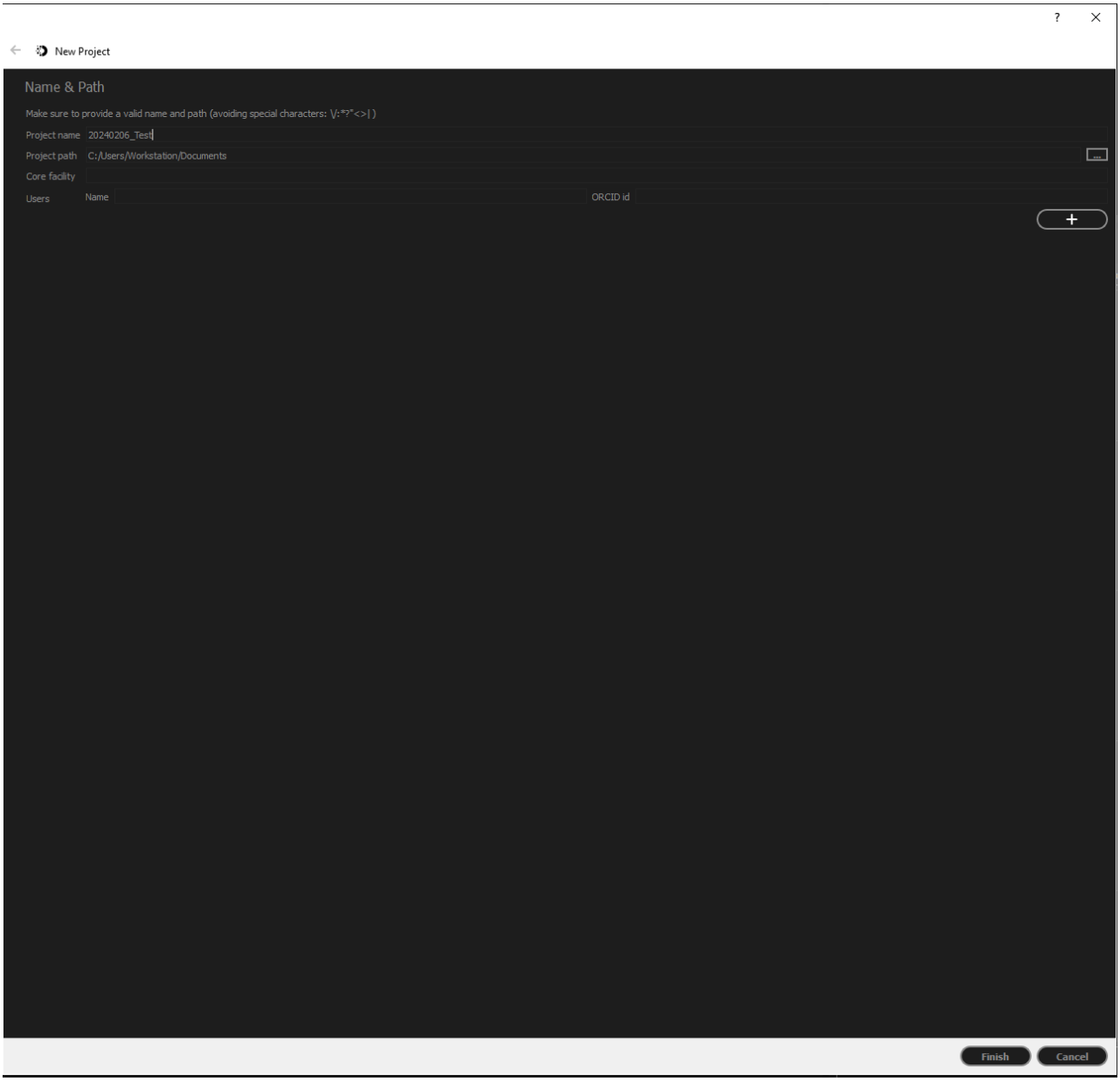


Figure 10: Project directory.

7. Click on “Settings” to show the settings tab (Fig. 11).

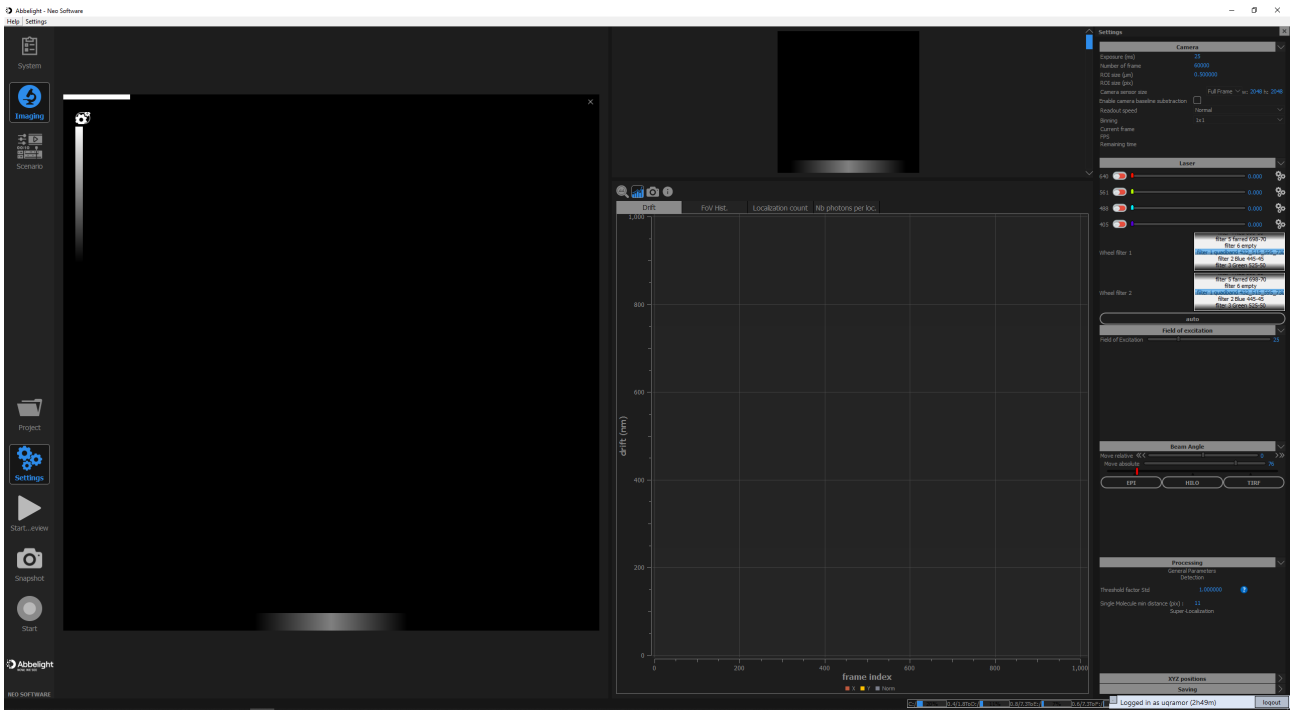


Figure 11: Settings tab.

8. Set the camera parameters. To enable 20-ms exposure times, use a camera sensor size of 1024 x 1024 pixels, or smaller (Fig. 12).
   Activate the required laser lines and set the laser powers. In the optical path, the default cube in place is the Chroma ZT647 dichroic, which sends blue, green, and red signal to the camera in the reflected light path ("CAM R"), and the far red signal to the camera in the transmitted light path ("CAM T"). "Wheel filter 1" is the CAM T filter wheel and "Wheel filter 2" is the CAM R filter wheel.
   For example, if imaging red and far red fluorophores simultaneously, select "filter 6 empty" on Filter wheel 1. This slot is not actually empty, but has the Chroma ET690/50m emission filter for far red (this is a bug in the software that Abbelight will fix). Select "filter 4 Red 600-50" on Filter wheel 2. This sends red signal to CAM R, and far red signal to CAM T.

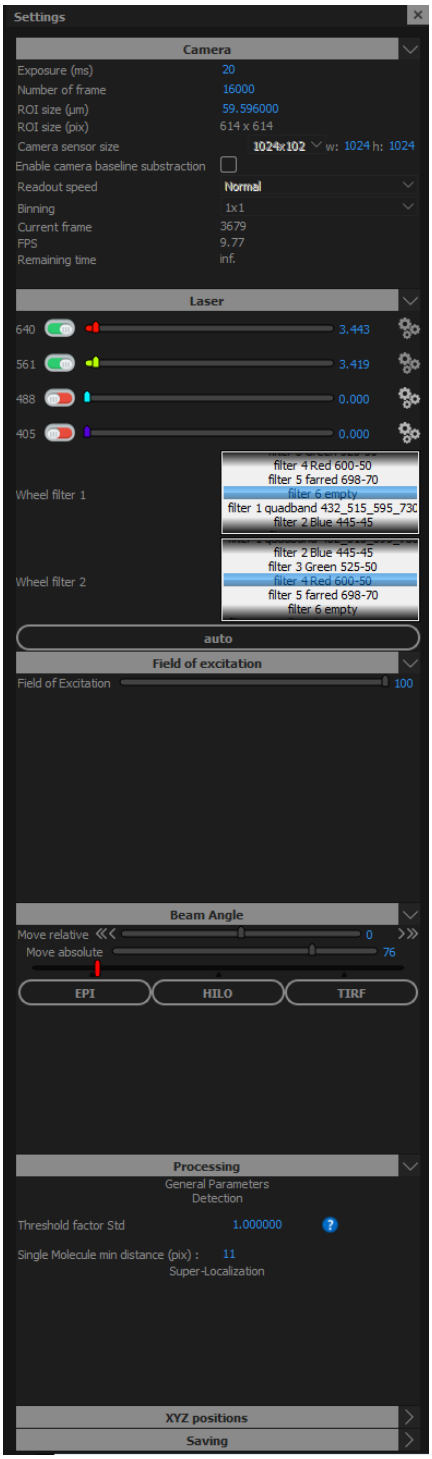


Figure 12: Setting the acquisition parameters.

9. Start Live view by clicking on “Start Preview,” indicated by the play icon on the left side of the GUI. Create the desired region of interest (ROI) size by clicking on the left mouse button and dragging across the field of view (Fig. 13).

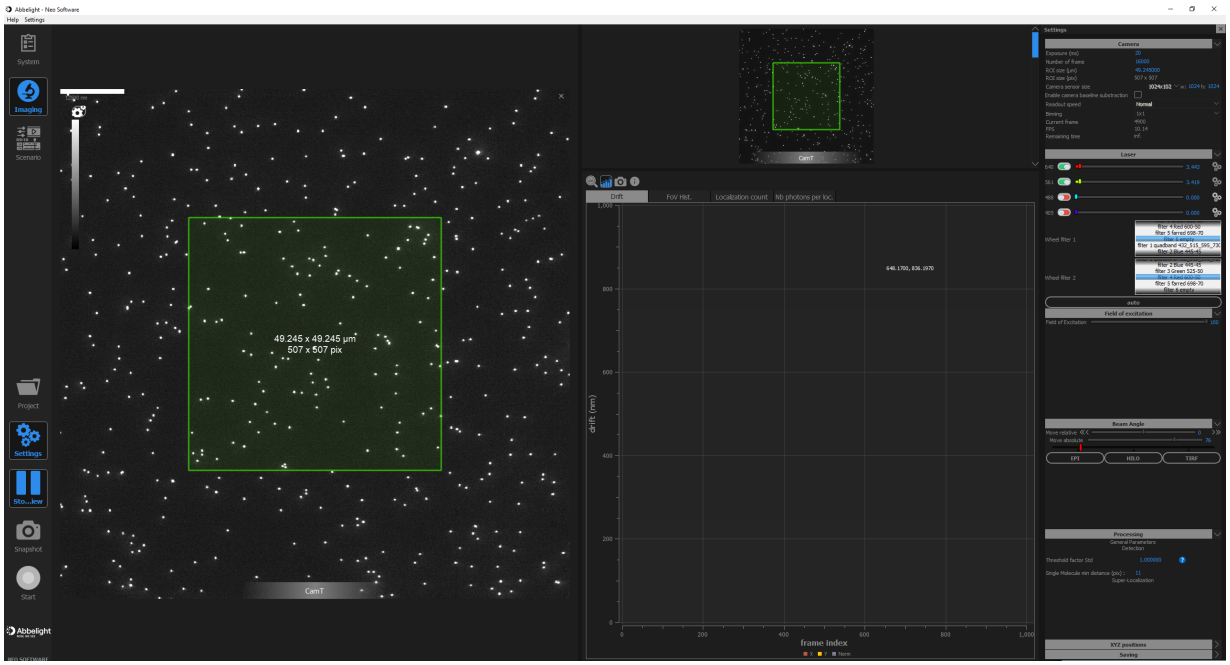


Figure 13: The preview window, and creating a region of interest.

10. Using the “Field of excitation” slider in the Settings tab, set a suatable value for the field of excitation so that the illumination only fills the ROI, but without distortion at the edges (Fig. 14).

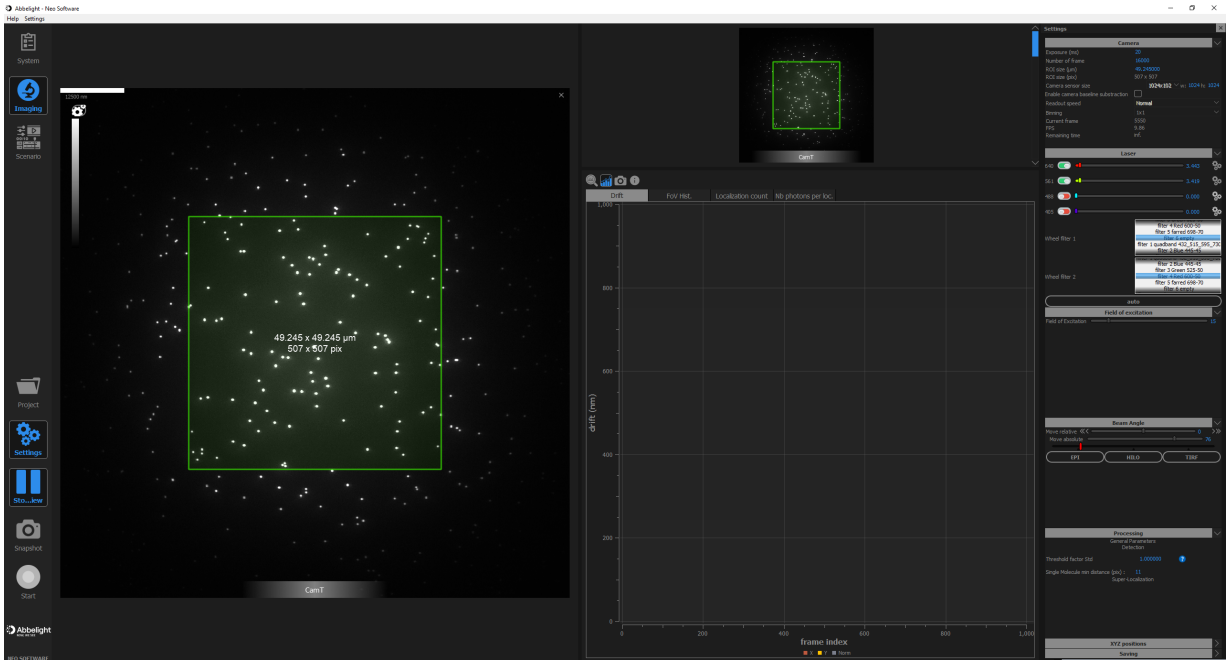


Figure 14: Setting up the field of excitation.

11. Set the desired illumination mode by clicking on “EPI,” “HILO,” or “TIRF” under “Beam Angle” (Fig. 15). To tweak the incidence angle, use the arrows in “Move relative,” or drag the slider.

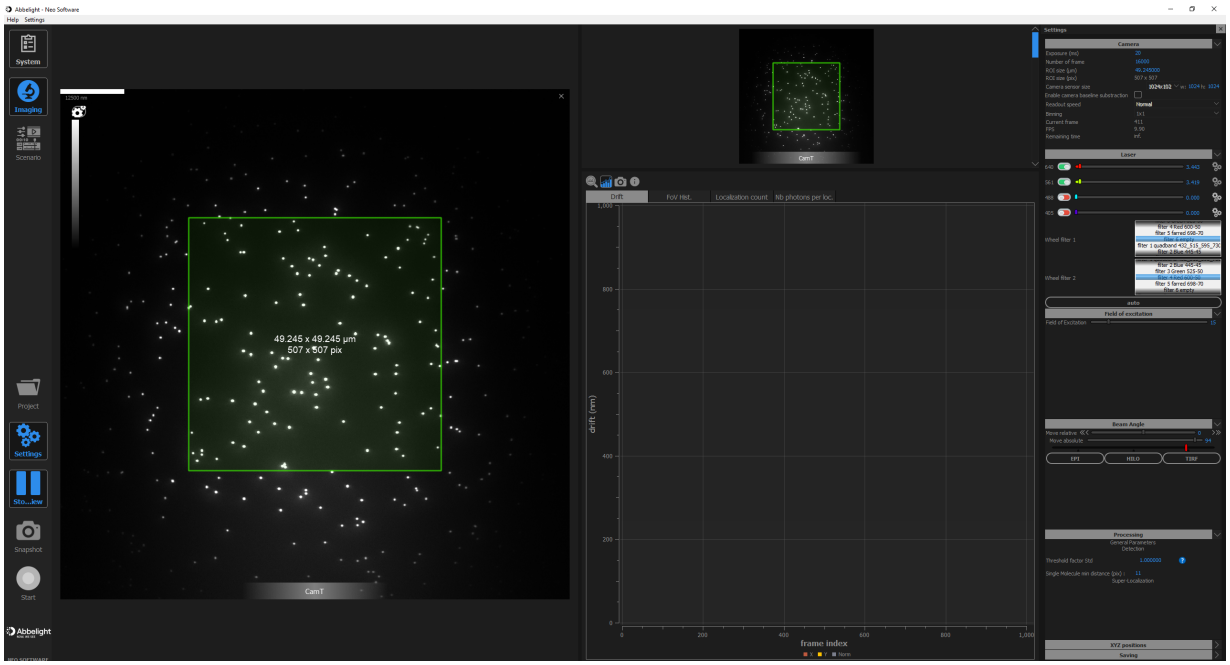


Figure 15: Setting up the beam angle for epi-, HILO, or TIRF.

12. Click on “Snapshot” to acquire and save a snapshot in the project directory (Fig. 16).

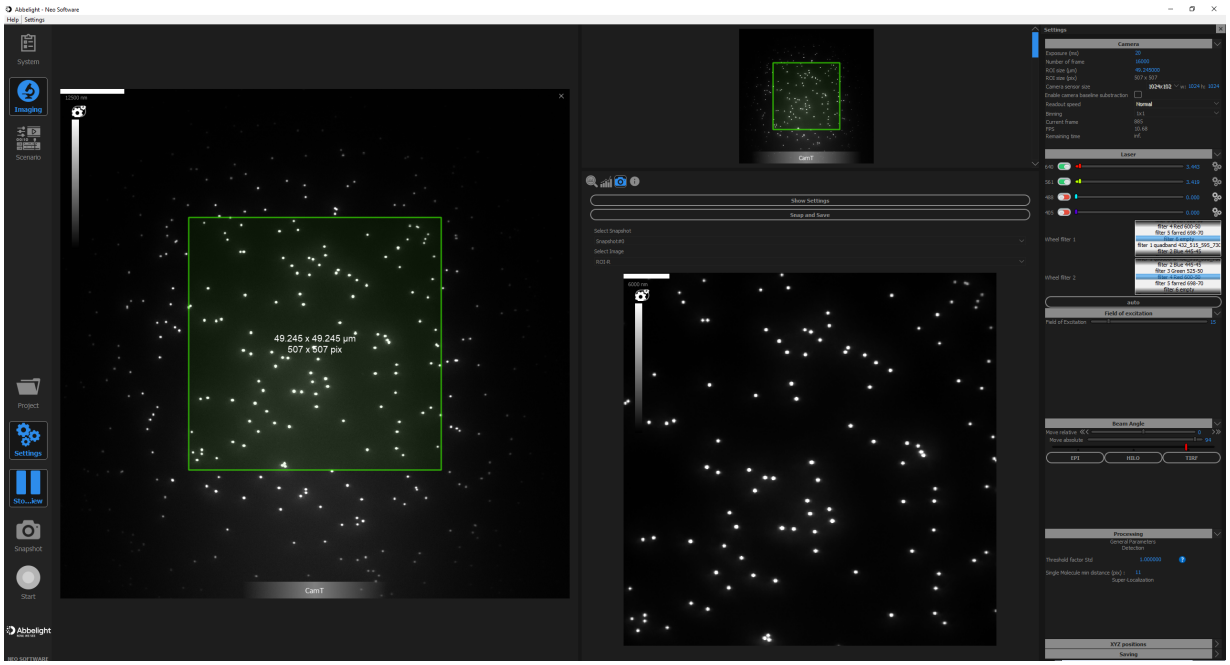
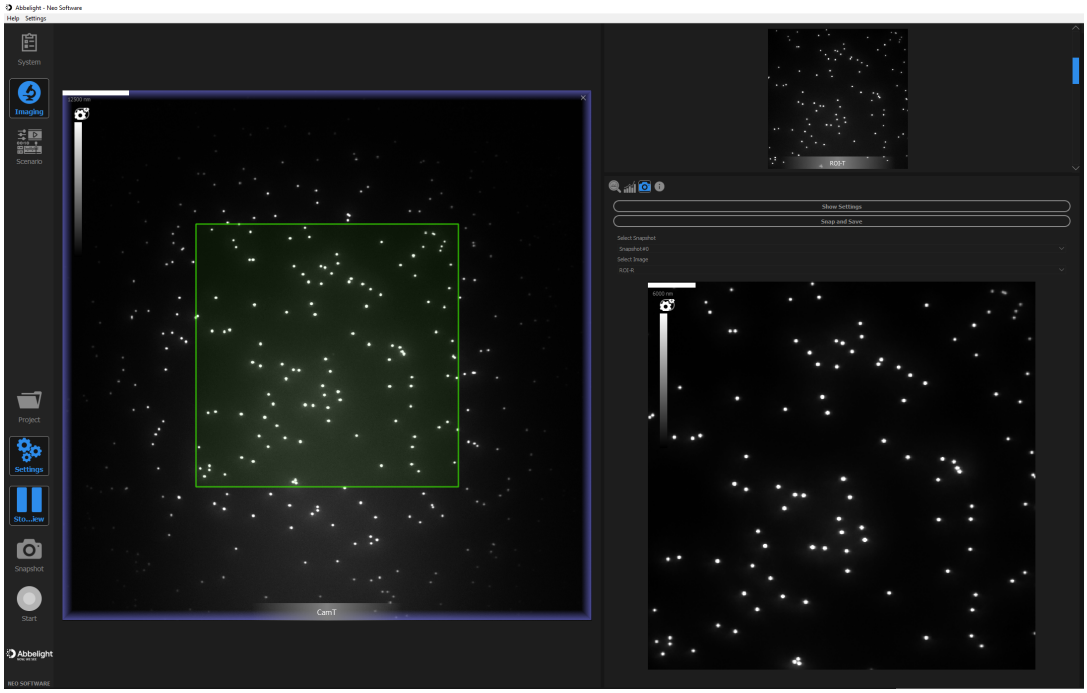
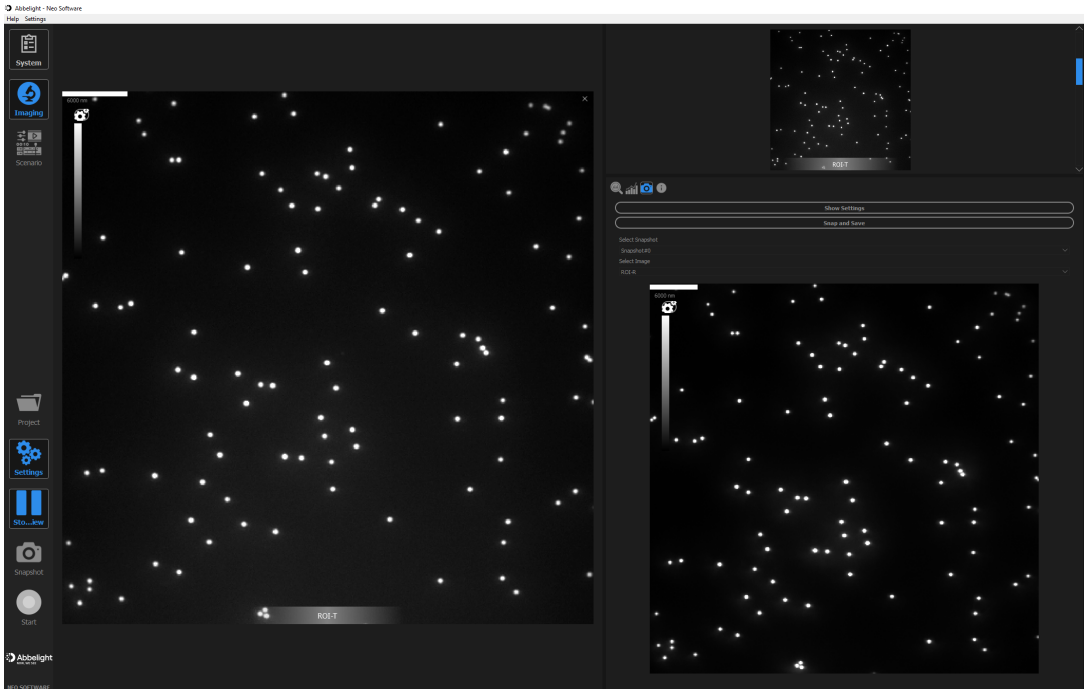


Figure 16: Acquiring a snapshot.

13. To replace the camera view, drag the new camera view over the current view such that all the sides are displayed in blue (Fig. 17a), then release the left mouse button. In this example, we have replaced “CAM T” with “ROI-T” (Fig. 17b).



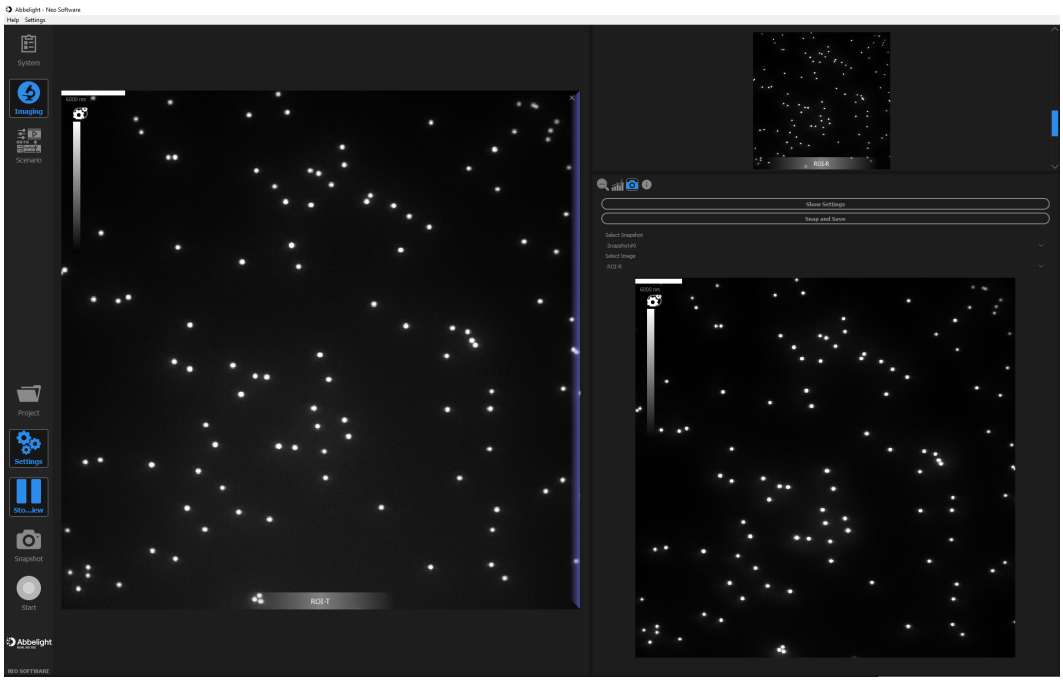
(a) Changing the camera view.



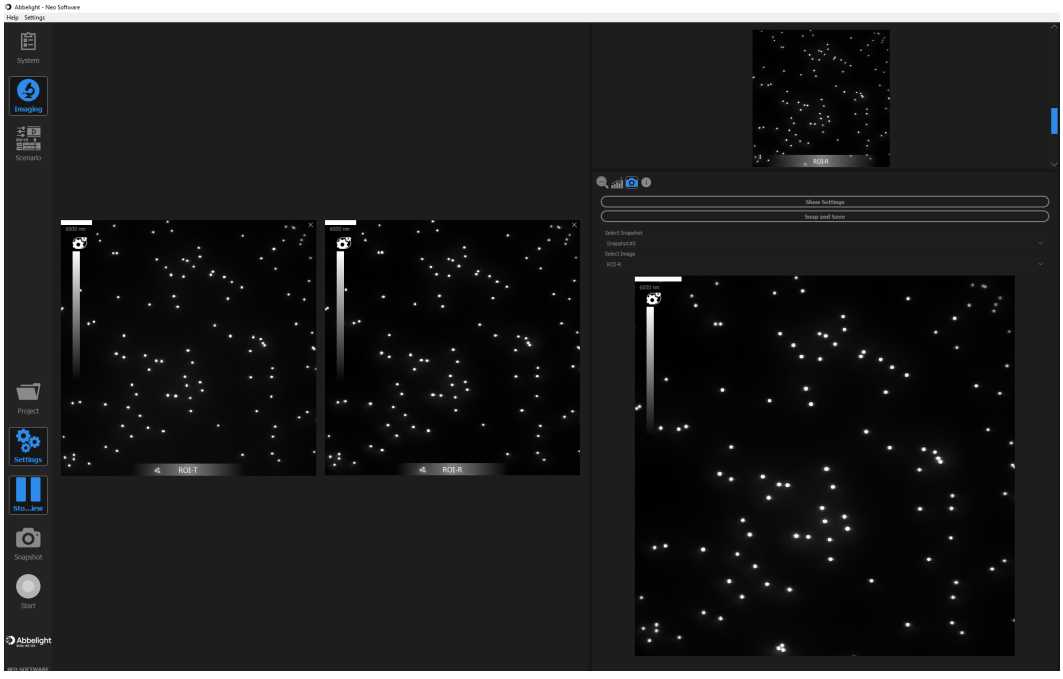
(b) “ROI-T” view.

Figure 17: Replacing the CAM T view with ROI-T.

14. To have two camera views side-by-side, drag the new camera view to the side of the current view such that that side is displayed in blue (Fig. 18a), then release the left mouse button. In this example, we have "ROI-T" and"ROI-R" (Fig. 18b).



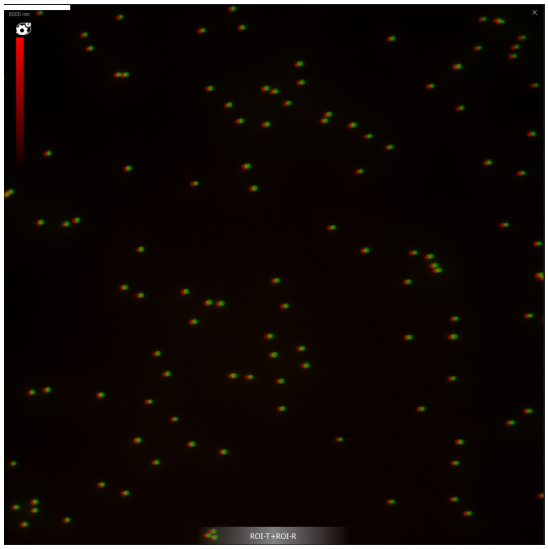
(a) Adding a second camera view.



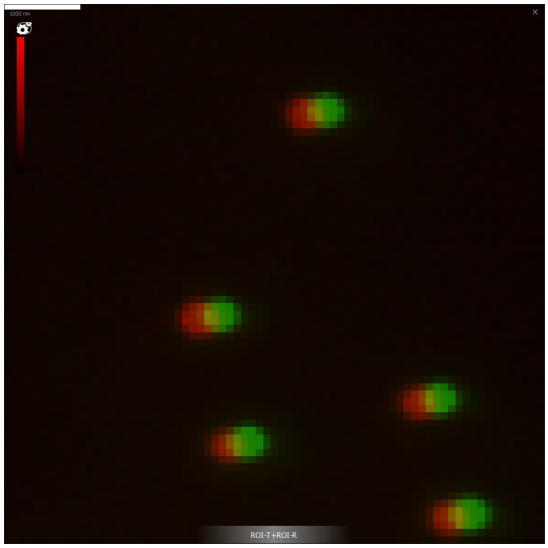
(b) "ROI-T" and "ROI-R".

Figure 18: Setting up two camera views.

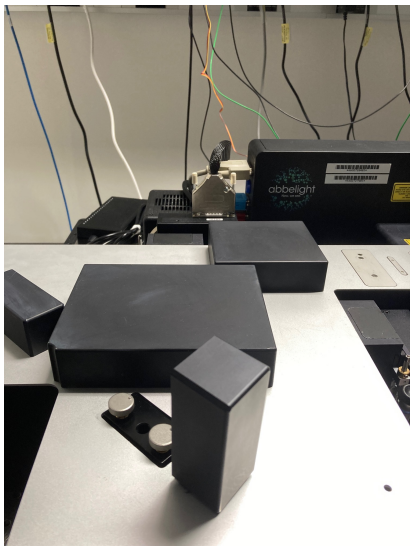
15. To optimise the dual-camera alignment, use a specimen of multi-spectral beads and switch to the camera view "ROI-T+ROI-R" in the preview (Fig. 19a). If there is a slight misalignment, align the cameras in the following way. Use the mouse wheel to zoom in on a few beads (Fig. 19b). Lift the cover on the CAM R steering mirror. This mirror is immediately in front of you when facing the far wall, where the incubation modules are (Fig. 19c). ***Do not modify the CAM T steering mirror position.*** Using the knobs for x- and y-translation, align the CAM R image with the CAM T image until the beads signals are co-located (Fig. 19d).



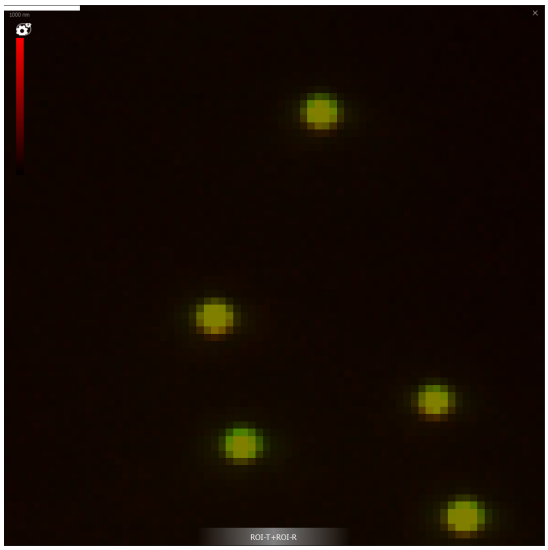
(a) ROI-T+ROI-R camera view.



(b) Zoomed-in view of multi-spectral beads.



(c) CAM R steering mirror.



(d) Beads aligned.

Figure 19: Optimising the dual-camera alignment.

Zoom out and use the full ROI view to confirm that the beads in the field of view are aligned (Fig. 20). If there is rotational misalignment, please contact facility staff to have it corrected.

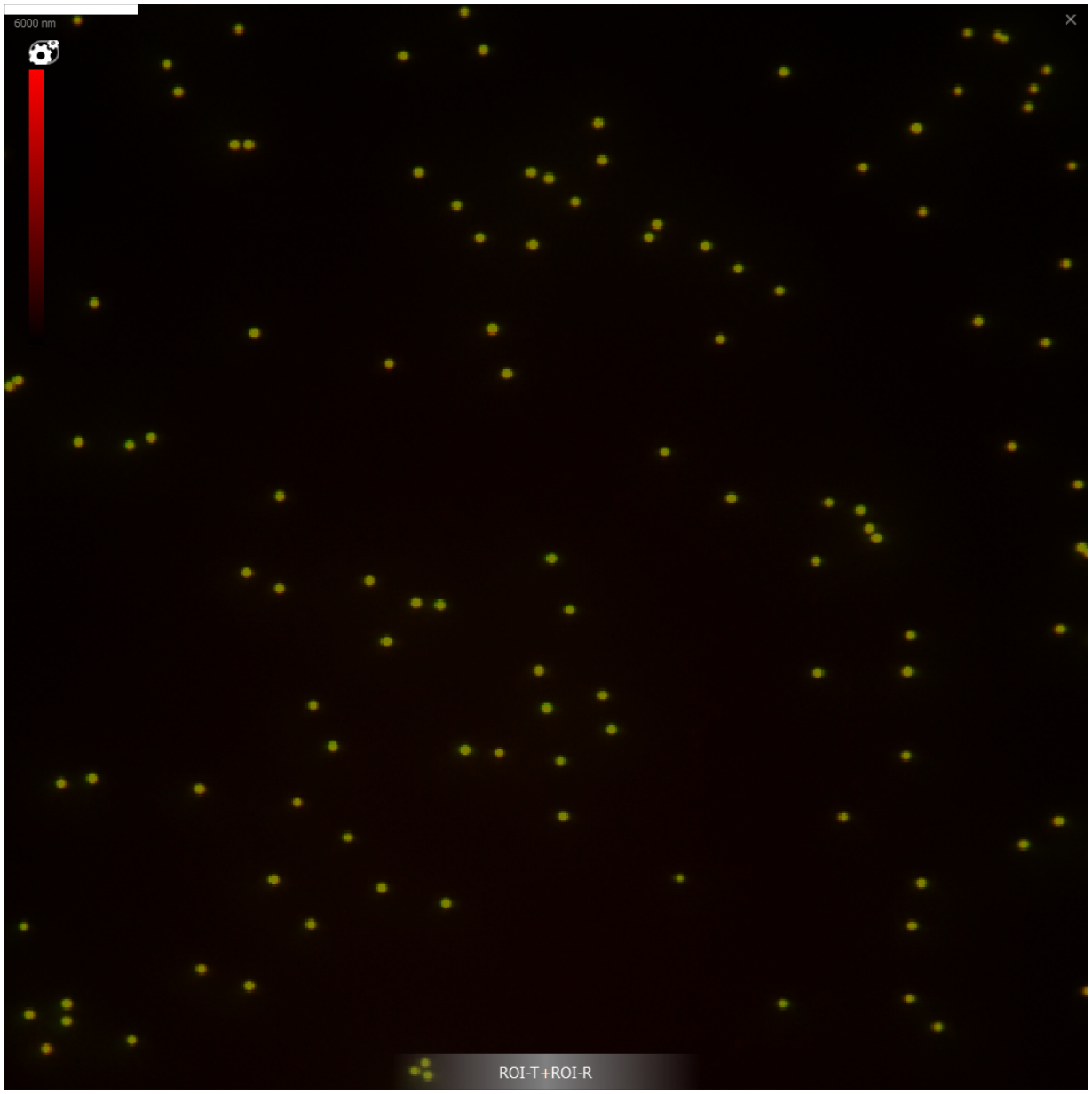
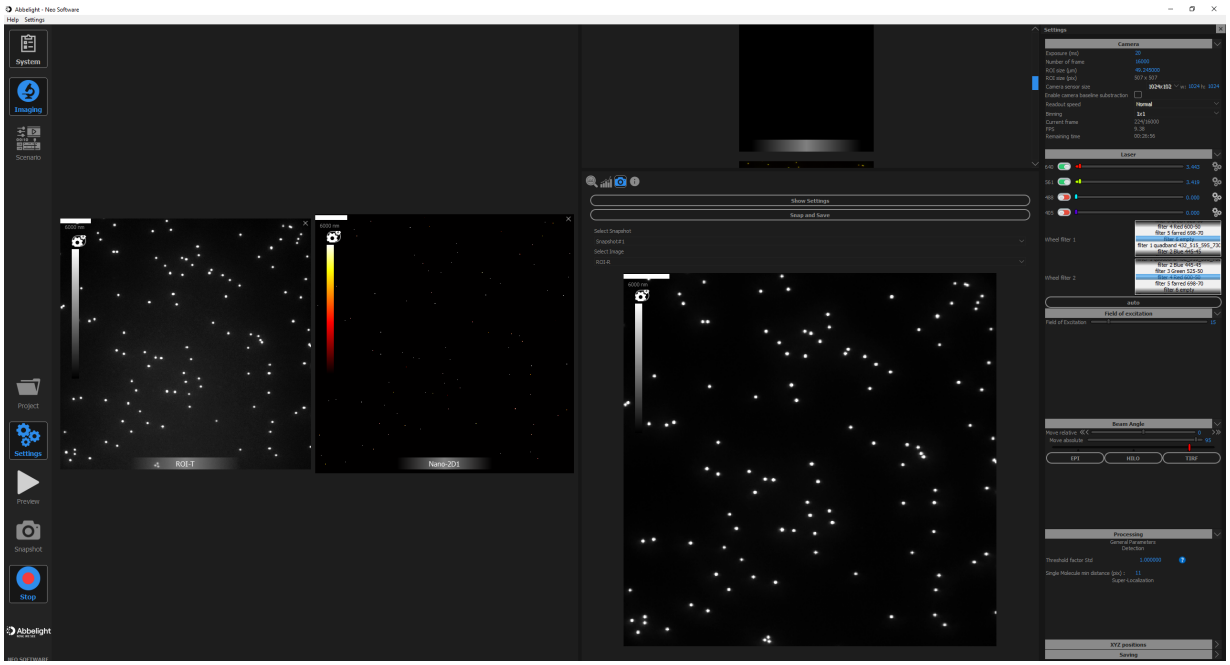
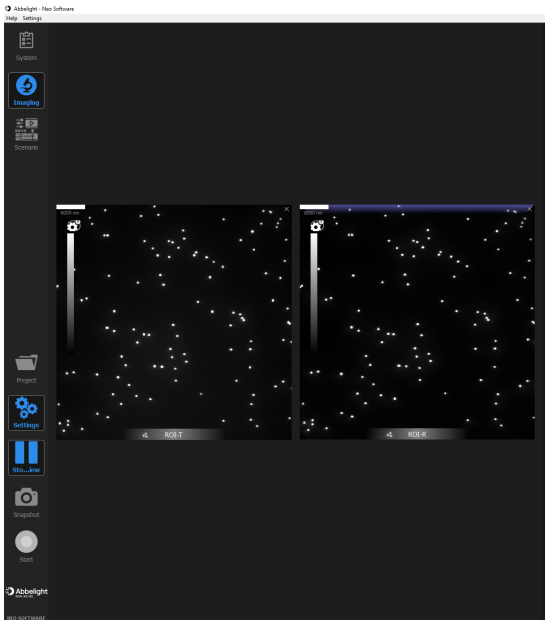


Figure 20: Full ROI field of view of aligned beads.

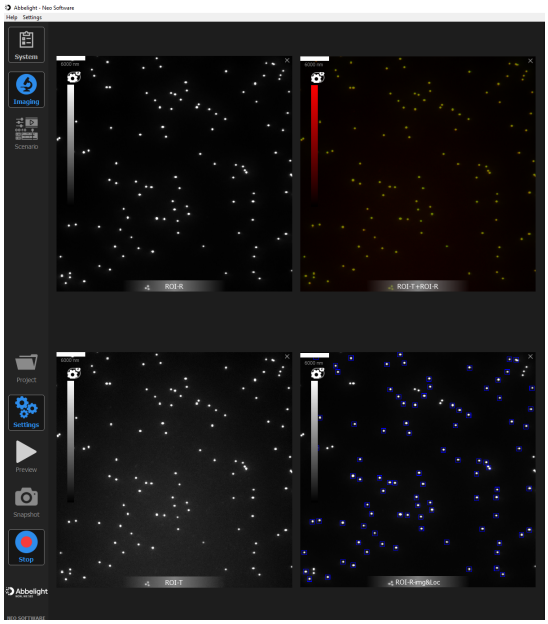
16. To start a stream acquisition, stop the preview by clicking on the pause icon and clicking “Start” (Fig. 21a). This acquires a stream of images with the set number of frames, and saves them in the project directory. To add more camera views during stream acquisition, drag the third (and fourth) camera view to one border of the current views until that border is displayed in blue (Fig. 21b), then release the left mouse button. Figure 21c shows four views, with one showing the image and the localisations in blue squares. Note that the general detection parameters are set in the Settings tab, under “Processing.”



(a) Starting a stream acquisition.



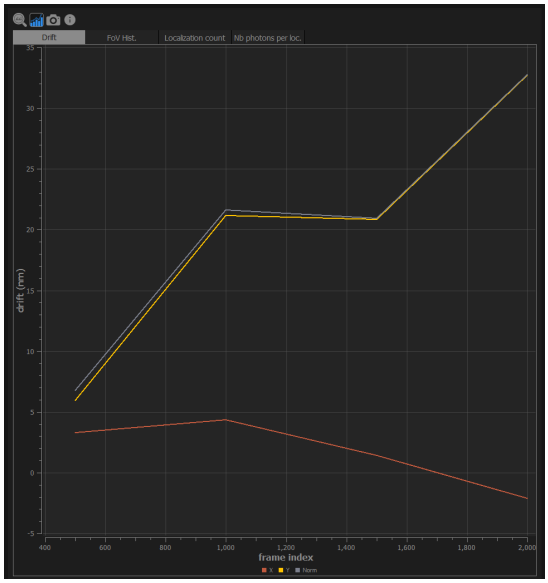
(b) Adding more camera views.



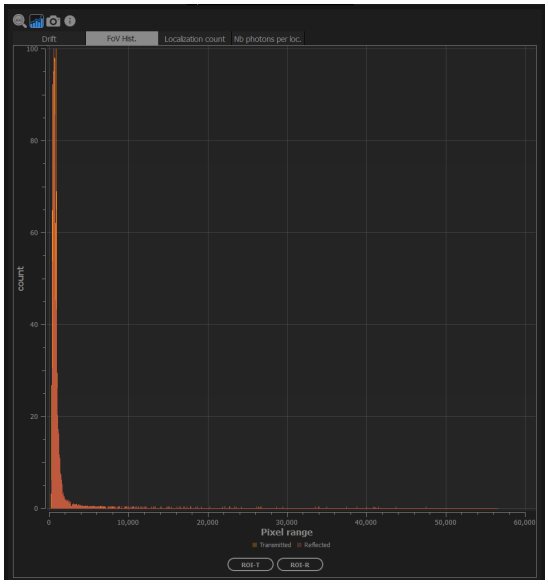
(c) Stream acquisition with four camera views.

Figure 21: Stream acquisition.

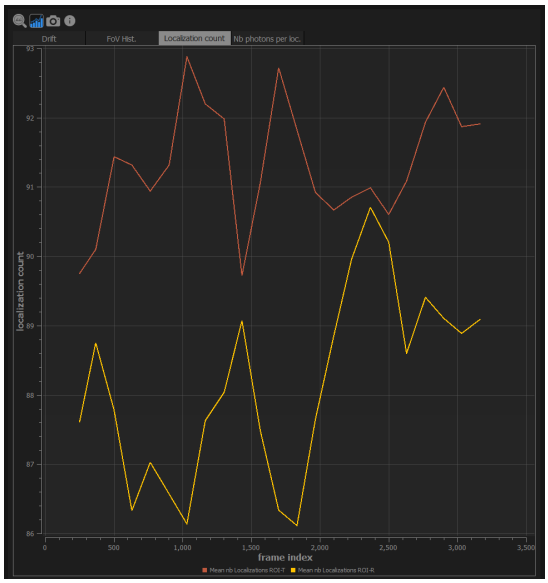
To view aspects of the stream acquisition, click on the graph icon and choose between the drift (Fig. 22a), histogram (Fig. 22b), localisation count (Fig. 22c), and number of photons per localisation (Fig. 22d) curves.



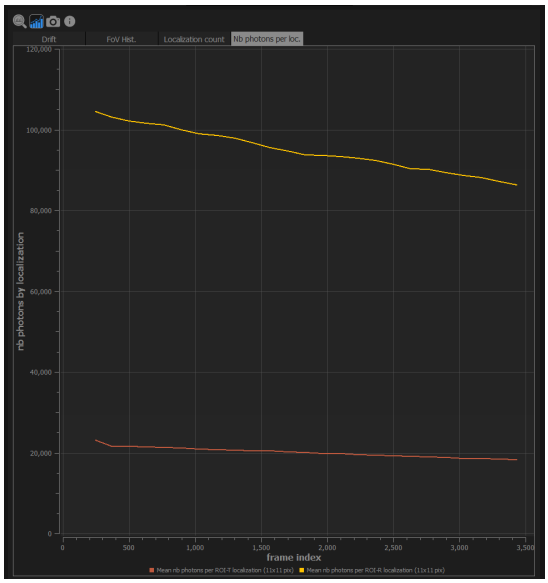
(a) Drift curves.



(b) Histogram curves.



(c) Localisation count curves.



(d) Number of photons per localisation curves.

Figure 22: Stream acquisition curves.

To finish and save the stream acquisition before the prescribed number of frames are acquired, press “Stop.”

Single-channel acquisition: Far red

1. On the NEO Live Imaging GUI, click on "System," then "Hardware Configuration" (Fig. 7). In the resulting hardware configuration window, under "Saved configurations," select "SAFe 360 - Single color - Nanoscopy - 2D" (Fig. 23).
   Make sure that "Camera transmitted:" and "Master Camera" are both on "Hamamatsu 0."
   Click "Validate."

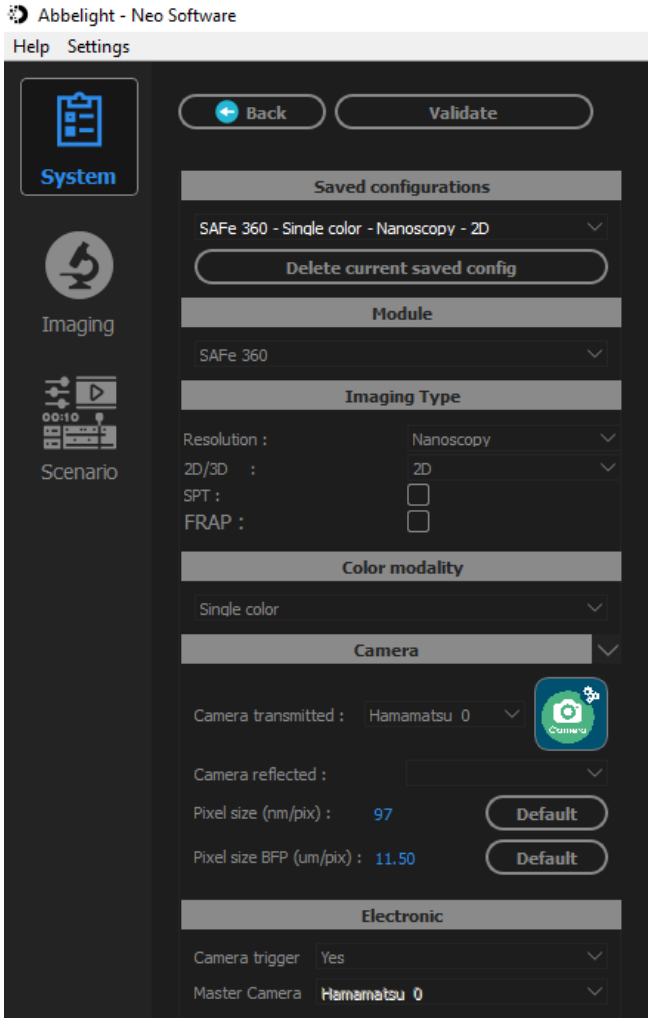


Figure 23: The hardware configuration window for single-channel acquisition of far red signal. The master camera must be set to "Hamamatsu 0."

2. Create a project and set the acquisition parameters in the Settings tab (camera, laser, filter wheel, field of excitation, and beam angle) as described in "Simultaneous dual-channel acquisition."
   Note that the camera uses "Wheel filter 1." Use either "filter 6 empty," which is the Chroma ET690/50m position, or "filter 5 far red 698-70."
   Start a stream acquisition as normal.

Single-channel acquisition: Red, green, and blue

1. On the NEO Live Imaging GUI, click on "System," then "Hardware Configuration" (Fig. 7). In the resulting hardware configuration window, under "Saved configurations," select "SAFe 360 - Single color - Nanoscopy - 2D - CamR" (Fig. 24).
   Make sure that "Camera transmitted:" and "Master Camera" are both on "Hamamatsu 1."
   Click "Validate."

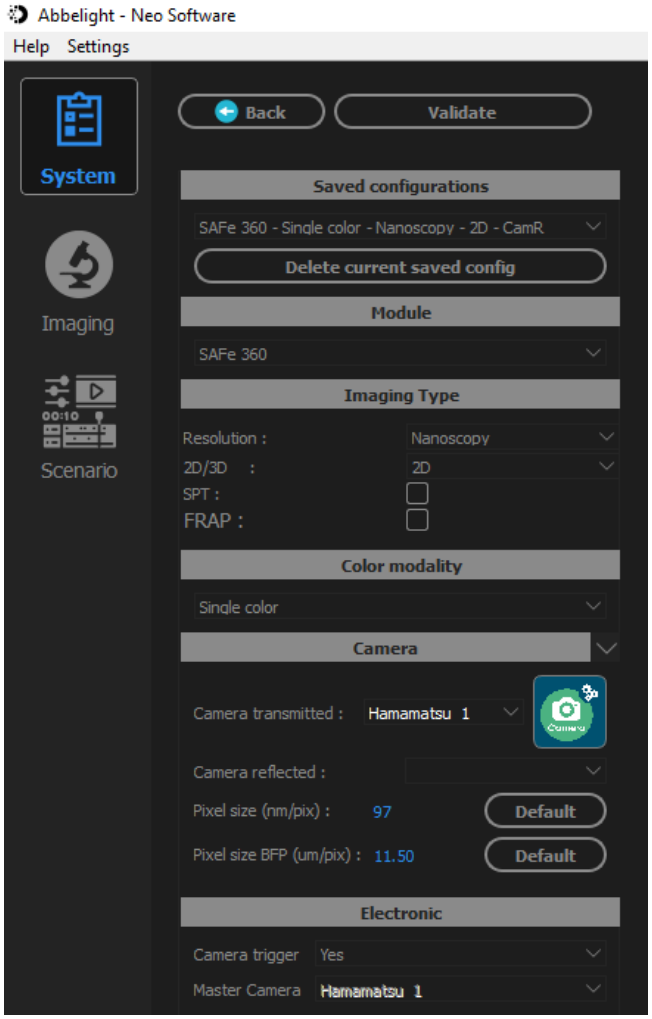


Figure 24: The hardware configuration window for single-channel acquisition of red, green, or blue signal. The master camera must be set to "Hamamatsu 1."

2. Create a project and set the acquisition parameters in the Settings tab (camera, laser, filter wheel, field of excitation, and beam angle) as described in "Simultaneous dual-channel acquisition."
   Note that the camera uses "Wheel filter 2." Use either "filter 4 Red 600-50," "filter 3 Green 525-50," or "filter 2 Blue 445-45."
   Start a stream acquisition as normal.

Ending a session

1. Lower the objective by pressing "ESC" on the microscope body. Remove the specimen.
2. Gently wipe the oil off the objective using lens tissue. *Never use Kimtech Wipes to clean objective lenses.*
3. Close the NEO Live Imaging software, and, if it's a Friday evening, shut down the system by following the steps on page 4 in reverse, and live a happy life.