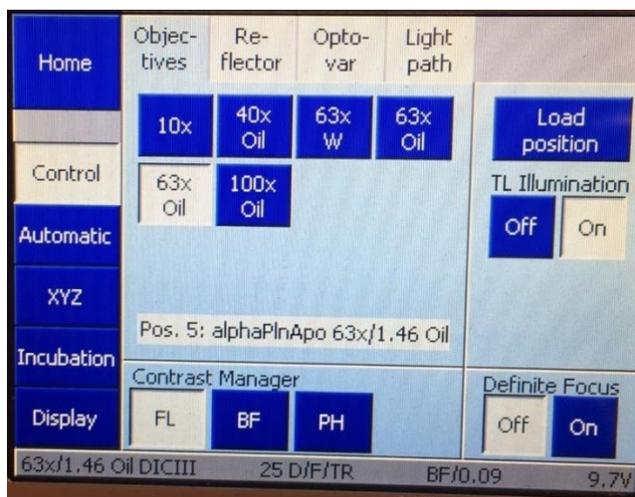


Zeiss Elyra 7 TIRF set up (Oct 2022)

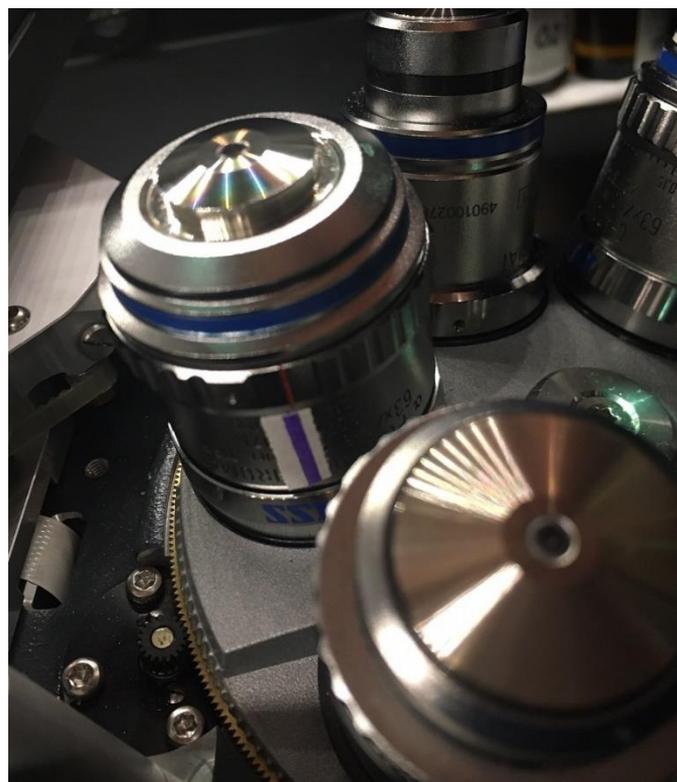
Setting up the Zeiss Elyra for TIRF imaging

Follow the start-up procedure described elsewhere. If you require Temperature and CO₂ control follow the relevant procedure.

TIRF imaging should be done with the *63x Oil NA 1.46 lens*. This has a correction collar which will need to be adjusted when working at 37°C.

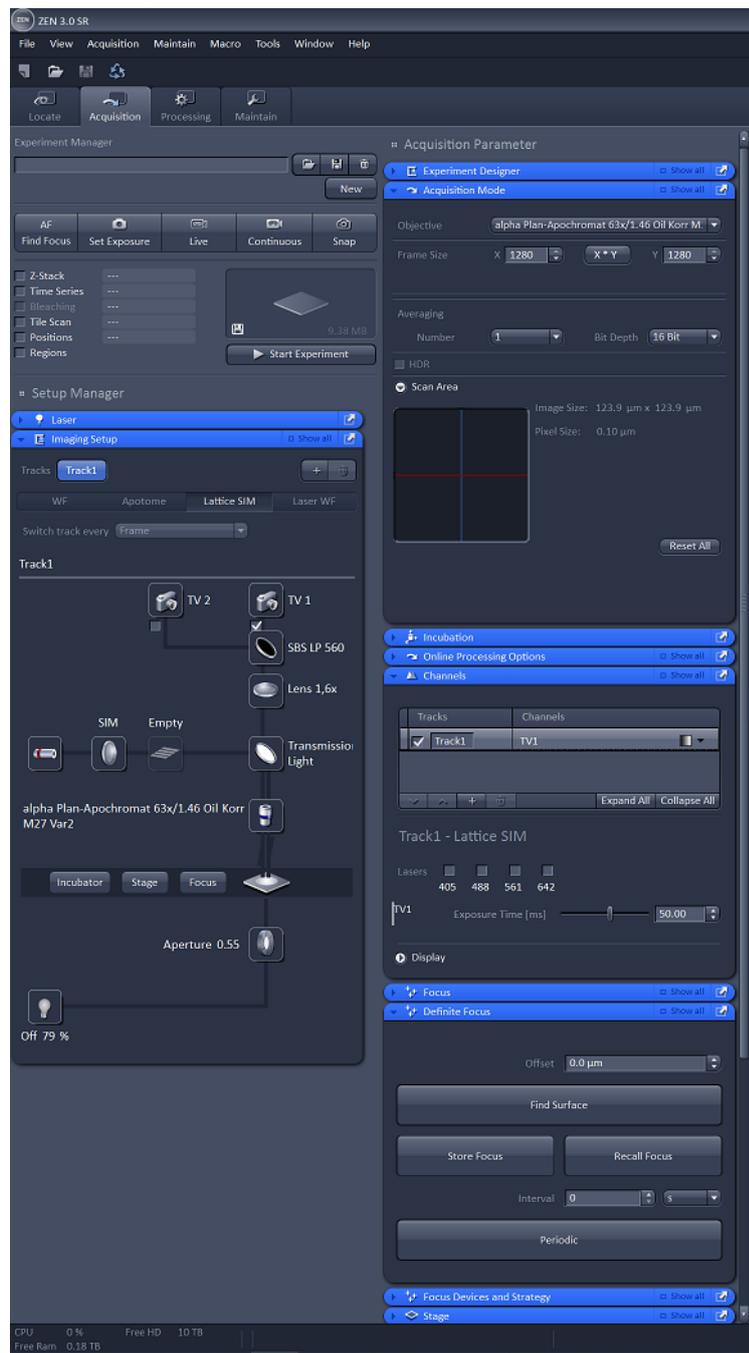


For the adjustment use the additional mark on the objective barrel. The small black mark (left image below) on the correction rings refers to the RT setting and the red mark is for 37°C. The additional mark (thicker Edding mark, no photo) on the correction ring refers to the setting for 27°C, the standard temperature we run the microscope at. **You should set the correction collar back to this thicker mark at the end of the session, when setting back the temperature.**



Zeiss Elyra 7 TIRF set up (Oct 2022)

Adjusting Zen Black controls



On the *Acquisition tab* arrange and open the following windows as shown to the left if they do not appear after the software has started.

Under Setup Manager

- *Imaging Setup*

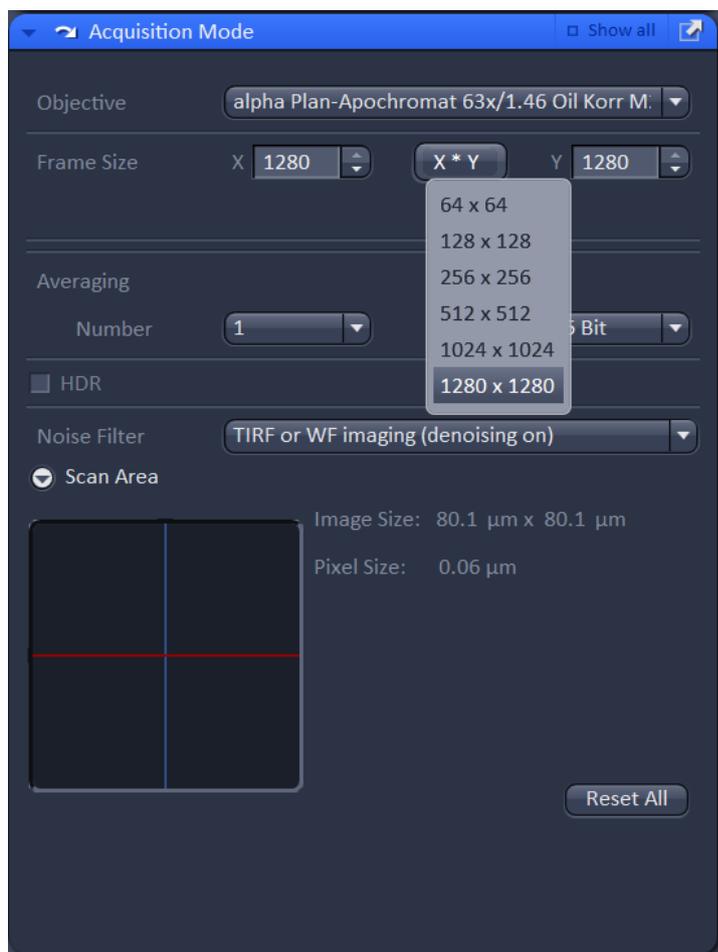
Under Acquisition Mode

- *Acquisition Mode*
- *Channels*
- *Definite Focus*

This will help you when setting up the controls and while imaging.

In the next steps you will make modifications in most of those windows to enable TIRF imaging.

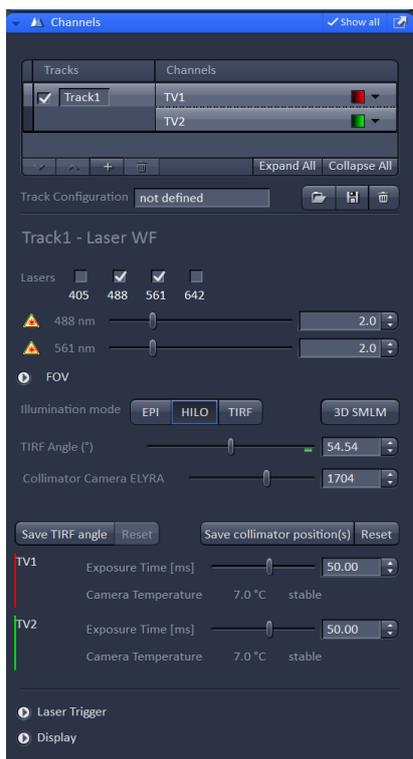
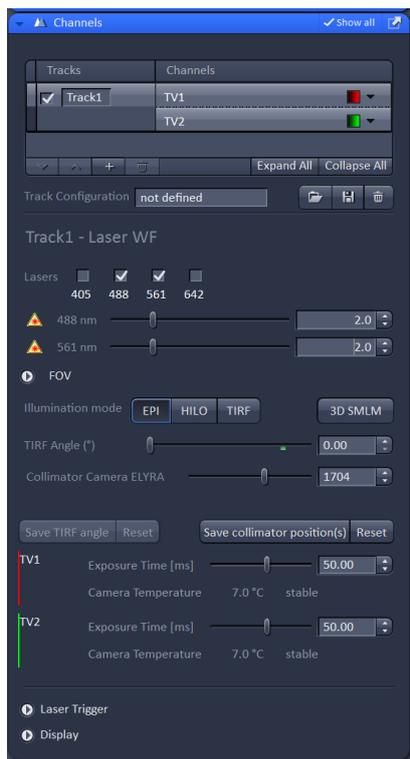
Zeiss Elyra 7 TIRF set up (Oct 2022)



In the *Acquisition Mode* you need to make sure that the *Noise Filter* is set to *TIRF or WF imaging (denoising on)*.

Here you can also control which field of view you use in your acquisition by choosing a suitable value from the drop-down menu. You cannot choose or draw another *Frame Size/ ROI* beside what is suggested.

Zeiss Elyra 7 TIRF set up (Oct 2022)

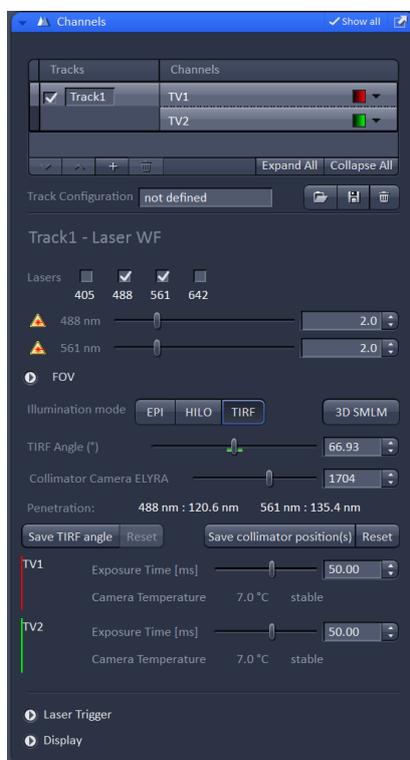


In the *Channels* window you can select which lasers you need to use for excitation. This should obviously match your sample labels and camera selection. You can change the LUT table color here or in the Imaging Set up.

You can control the amount of illumination power for each laser individually.

Between the three illumination modes *EPI*, *HILO* and *TIRF* you can toggle and this will change the values in the *TIRF Angle* slider. The green mark on the *TIRF Angle* slider marks the theoretical optimal

value for the angle. Close to this setting the software will show the calculated penetration depth for the given angle and used excitation wavelength. This will appear in the *TIRF* mode and also in *HILO* if you have moved the *TIRF* angle slider closer to the green mark.



How to use and control the *Collimator slider* will be discussed in the training. You should **never** use the *Save* buttons, neither for *TIRF* nor *Collimator* position.

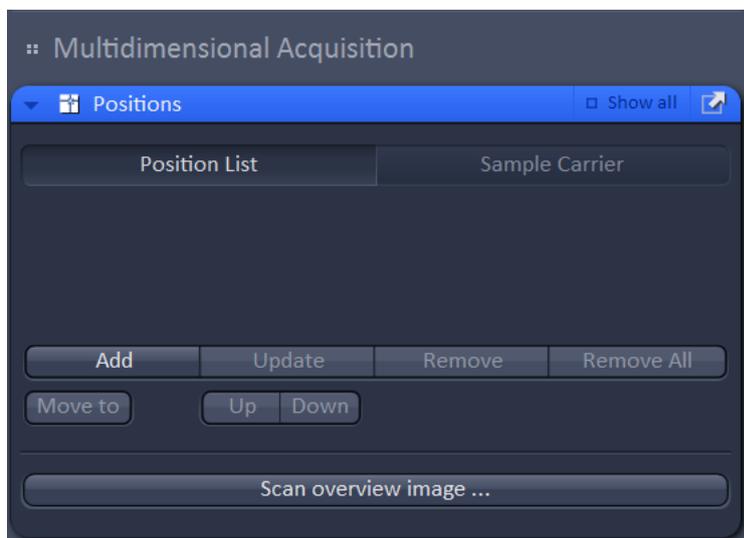
Further below the camera exposure can be controlled. If you are using both cameras they will use the same *Exposure time*, it cannot be set independently.

Zeiss Elyra 7 TIRF set up (Oct 2022)

Additional Zen Black controls

Positions

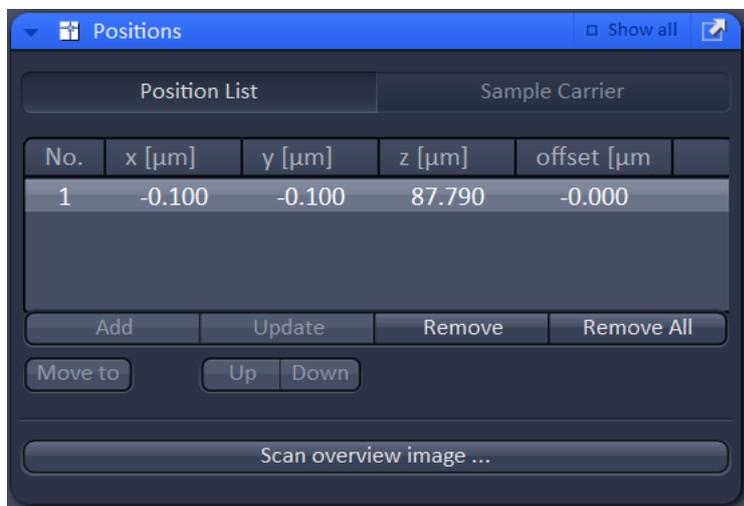
Activating the tick box at *Positions* (left from Start Experiment) will give you an additional window in the *Multidimensional Acquisition* list.



You can make this window freely movable by using the top right white arrow. This allows you to take it over to the Locate tab, where you could use it to find a number of potential imaging positions.

Just click *Add* and the positions will appear in the list.

As you can see xy and z positions are saved. You can visit the positions with the *Move to* function.



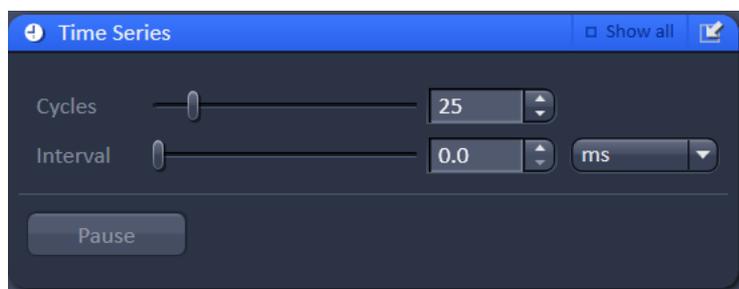
If you use **this make sure to untick positions in the top menu before you say *Start experiment*** – the TIRF angle for each position will vary slightly hence in TIRF you should only ever acquire one position per time-lapse/ experiment.

Zeiss Elyra 7 TIRF set up (Oct 2022)

Time-Series

For a time-lapse you will need to activate the tick box for *Time Series*.

In the times series control window, you can define how many cycles you want to run the time-lapse for, and how long the interval should be.



Interval refers in this case to the time from the start of an image to the start of the next one, you can choose ms, s and min from the drop-down.

Example:

- exposure time is 50ms,
- read/ transfer time is ~2ms (in this example!) and
- the interval time is 100ms.

So, the wait time (no illumination) between images will be ~48ms.

You need to make sure that you enter a reasonable interval time; there will be no warning if your experimental set up requires more time as your planned interval and the system will just image as fast as possible without any wait time.

Cycles refers to how often your experiment will be repeated. If you have a defined timespan you want to run the experiment for, you need to calculate how many cycles you need:

Using the above example:

100ms interval => 10 images/s (1000ms = 1s and 60s = 1min) => 600 images/min

This means if you want to image for 10 minutes you need 6000 cycles.

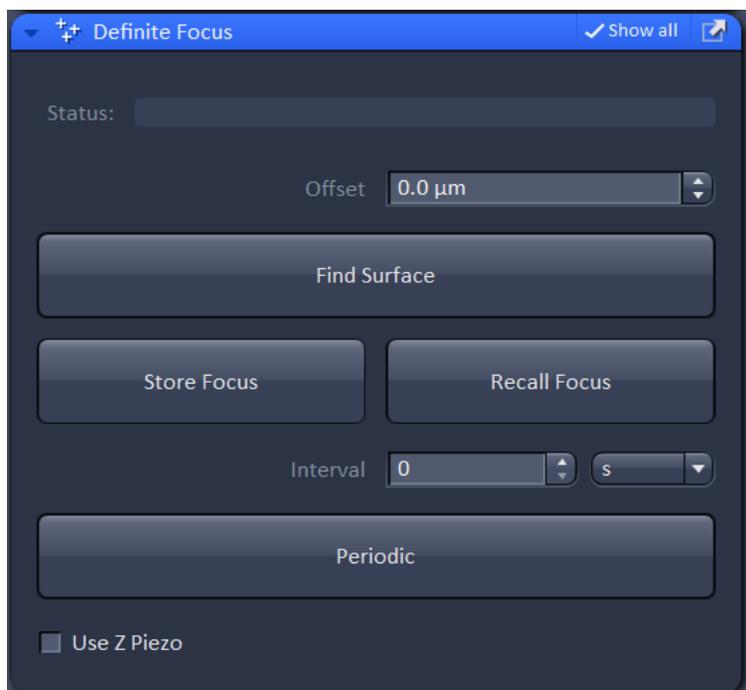
Another examples:

No interval/ 52ms/image (including exposure time and read out) => 19 images/s and 1153/ min

For 15 minutes you will need 17367 cycles.

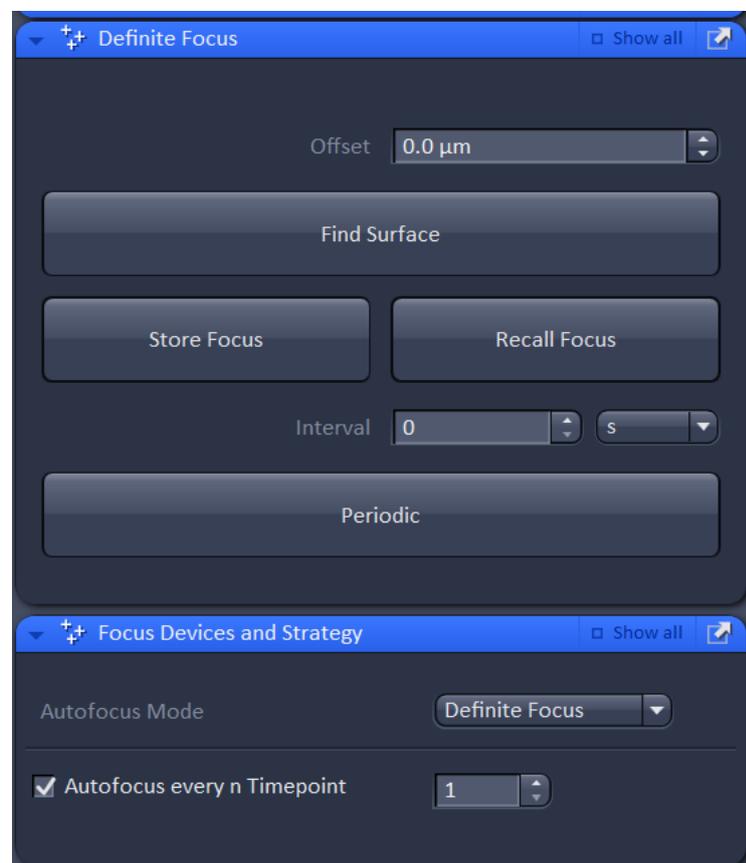
Definite Focus

The control window for the *Definite Focus* system can be found under *Acquisition Parameters*. The *Definite Focus* works stable when using the TIRF lens and a TIRF sample (e.g. a sample in aqueous solution).



Make sure to have *Show all* activated to be able to see the *Status* bar. If you have moved the objective close to the focus/into the oil you can click *Find Surface* and in the *Status* bar you will see a green blinking message while the *Definite Focus* works. Once it is finished *Status* should read *Reflex found*.

Only after the system has found the reflex you can use the *Definite Focus* system to stabilize the focus during a time-lapse experiment.



One needs to define in *Focus Devices and Strategy* that the *Definite Focus* is to be used via the drop-down menu. You will need to define how often the *Autofocus Mode* will be activated during a time-lapse experiment. Any time the *Autofocus* runs it will create a small delay before the next image can be acquired.

If you want to avoid this delay in TIRF imaging you can use the *Periodic* function to have the focus stabilization continuously (with an interval of 0) running during a time-lapse. In this case for *Focus Devices and Strategy* select *None!*

Just active *Periodic* by clicking on it, to stop it click again.