“Ti2 Pad” menu

Nosepiece: Shows the objectives. The currently selected one is shown with a green selection color (40x in image). Click on an objective to select it.

Escape: When pressed, this button moves the objective as far down as possible. When pressed again, the objective moves back up to the previous z-coordinate.

OBS: Be very careful when **exiting** “Escape”. If there is a new sample/slide, it may sit lower in the holder, causing the objective to crash into it.

Light Path: The pathway the light takes. Currently, only “*EYE*” (eyepiece) and “*L100*” (camera, left side of microscope) are available. Note that only one can be selected at a time (you can not view the sample in the eye-piece and on camera at the same time).

PFS: The “**P**erfect **F**ocus **S**ystem”. When PFS is on, and the sample is in focus, the microscope will maintain the focus while moving the stage in x- and y-directions. Note that while PFS is on, you can not change the z-coordinate of the objective (i.e. you can’t raise or lower it).

Z Drive: Here you can move the objective up and down (i.e. change the z-coordinate) in the indicated step sizes, instead of manually turning the knob on the microscope. The last one has a custom size step length you can define.

DIA: The shutter and intensity slider of the white-light illumination

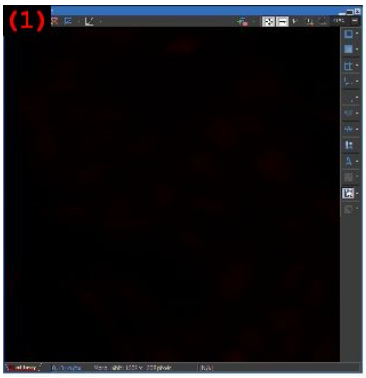
Shutters: The main shutter for the microscope only (the EPI and laser have their own shutters as well, the buttons for those are elsewhere). If this shutter is off, no light will be visible.

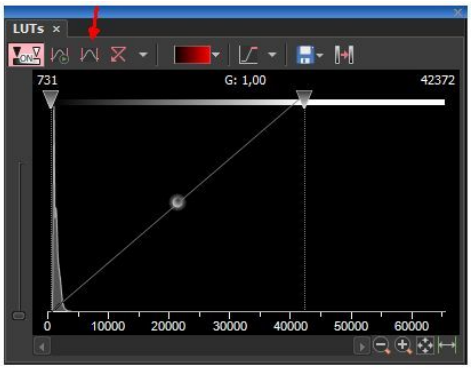
Filters: The filter cubes in the microscope. Currently selected cube is highlighted in blue (rainbow multi-bandpass cube on figure). Multi-bandpass cube works for most of the wavelengths and is the only one that works when doing experiments with multiple wavelengths.

"LUTs" menu

The **LUT**s window (**L**ower **U**pper **T**hreshold**s**) is a window where you can set the thresholds and contrast for the image being measured with the microscope. A histogram of the current image is displayed, and the lower and upper threshold are overlaid on this histogram

In figure (1), the upper threshold is set very high, compared to the histogram peak. This essentially means that all the pixels have too low brightness to be seen in the image window. A way to fix this is to manually move the upper threshold down to the peak or press the "Autoscale" button (shown in figure with a red arrow). This will (usually) set both thresholds to a suitable level.

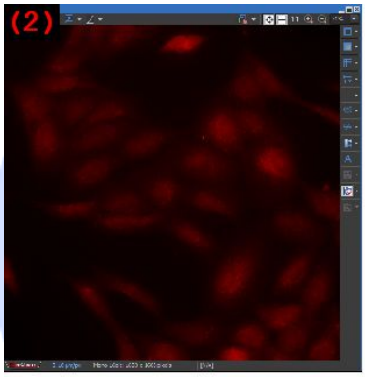


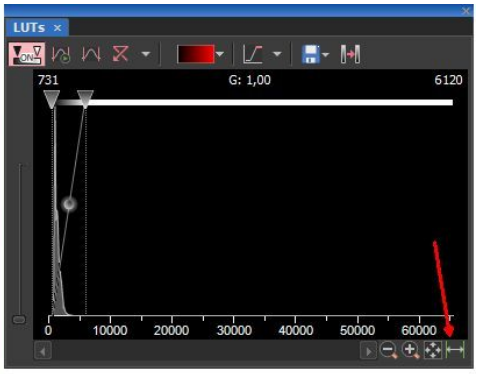


OBS: If there are pixels with extremely high intensity values compared to the rest of the image, the autoscale button will not set the upper threshold low enough to make the image visible. If there are very few high intensity pixels, you also wont see anything on the histogram. In this case, the only way to get a good image is to manually set the LUTs.

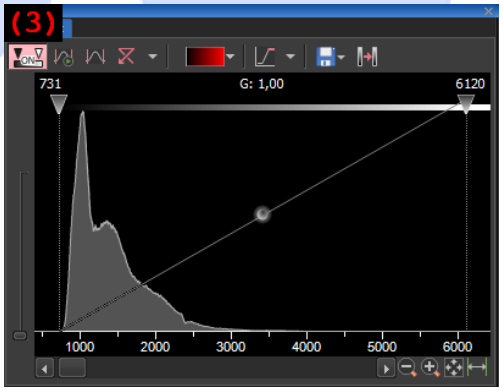
In figure (2), the "Autoscale" button has been pressed. The upper threshold has been lowered significantly, from 42372 to 6120. Correspondingly, the image is now visible.

If desired, you can scale the horizontal axis by pressing the button marked with the red arrow. This scales it to proximity to the threshold values (see figure (3)).

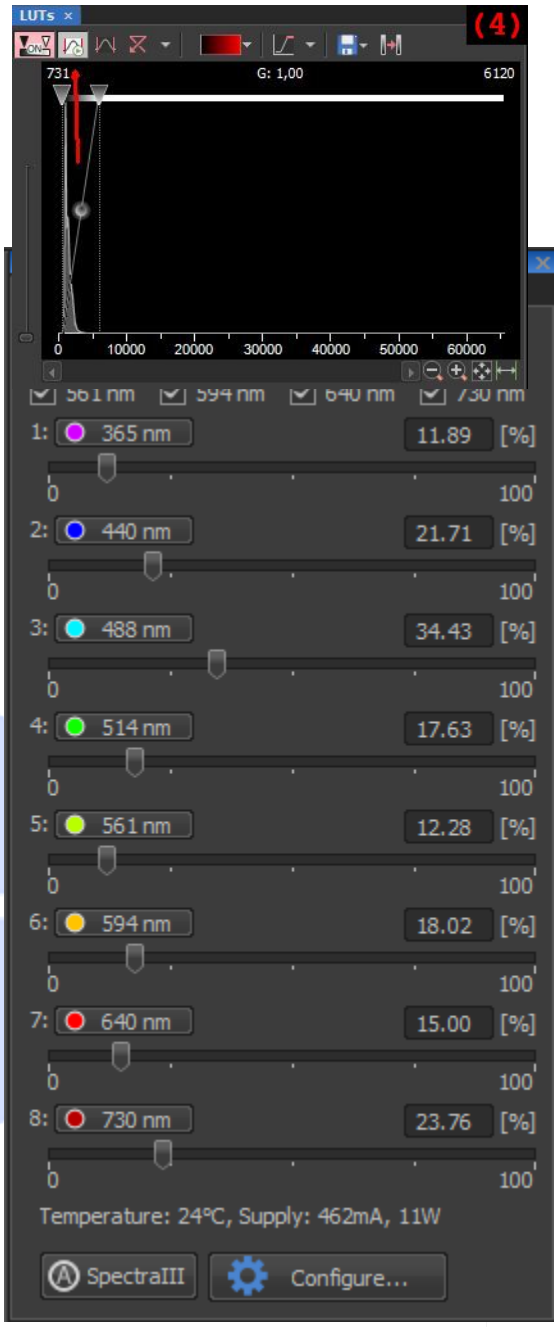




Finally, if you're trying to find focus, or moving the objective, you can activate the "Keep Autoscale On" setting (see figure (4)). This way, the software will autoscale the image automatically every frame. You can also keep this on while recording.



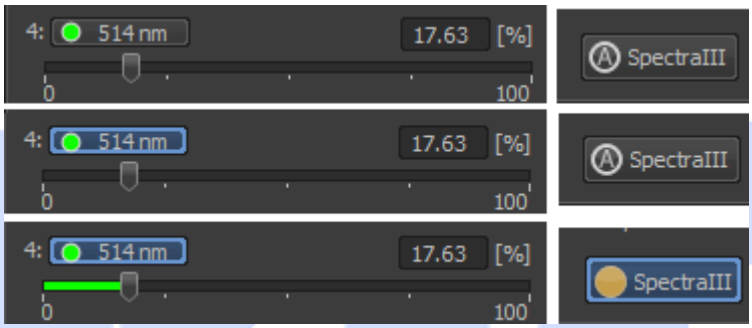
NOTE: The LUTs are purely for visualization of the image being recorded by the camera. Changing the LUT settings has no effect on the image acquisition, just on how the image is presented on the monitor. You can think of the image acquisition as a photon count per pixel. Changing the LUTs doesn't affect the photon count, just how bright a given photon count appears on the monitor.



"SpectraIII pad"

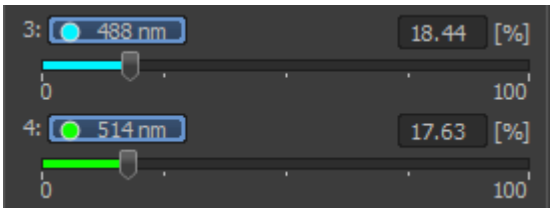
The "SpectraIII pad" is where you control the EPI light source. The EPI has 8 different possible wavelengths, and can fire multiple ones simultaneously, at different intensities.

If you need to set a custom illumination (i.e. not a predefined optical configuration), you do so with this pad. Select the wavelength/s you want to use by pressing the corresponding button.



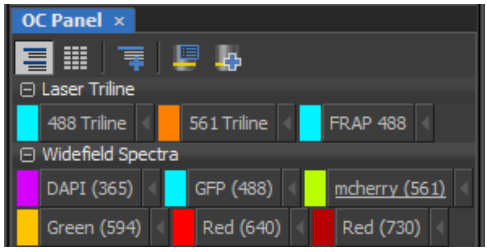
Here we see the three possible selections for the 514 nm light.

- In the first figure, the wavelength isn't selected, so the light is OFF.
- In the second figure, the wavelength has been selected. However, the SpectraIII shutter (bottom left) is off, so the light is OFF.
- In the third figure, the 514 nm wavelength is selected, and the SpectraIII shutter is open. Now the light will be ON, indicated by the green slider.



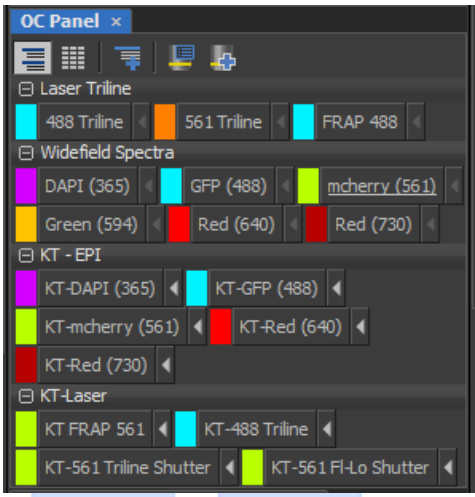
In this figure, both the 488 nm and 514 nm lights are on. The light from the microscope will be a combination of both.

“OC Panel”



When you have found a light-and camera settings that are suitable for your measurements, you can save the setup as an **O**ptical **C**onfiguration (OC). This saves the selected wavelength and intensity, and creates a button for it under the “OC Panel” tab.

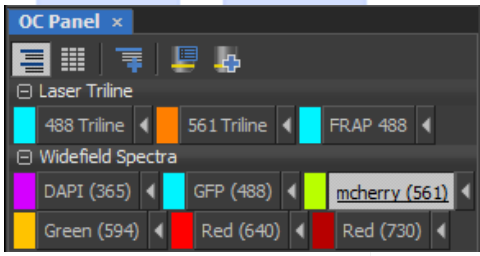
All user accounts have access to a couple of shared OC’s. These OC’s are locked; they cannot be changed by anyone. These are grouped under “Widefield Spectra”. If everything gets messed up, you should be able to select one of these and know that it is always the same settings. More advanced users will also have access to shared laser OCs, grouped under “Laser Triline”. These also cannot be changed.



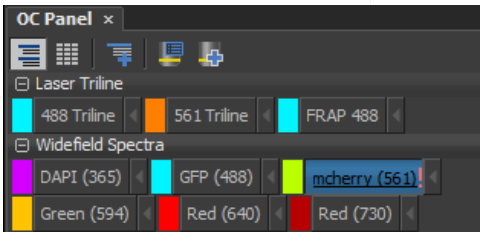
As a user, you can also make your own personal OC’s that you can save for yourself, and no one else can edit. To the left you can see an example of this. Under the two shared groups “Laser Triline” and “Widefield Spectra”, there are two more groups, “KT – EPI” and “KT – Laser”, which are my private configurations. I can set those any way I like and change them whenever I want.

Each OC button consists of three parts.

- First is the color, identifying the wavelength color of the OCs current configuration.
- The name of the OC.
- The save button (the small triangle on the right side of the name)
  This button is greyed out on the shared configurations (you can’t change them).



When an OC is selected it will be highlighted (see “mcherry (561)”). The saved settings have nopw been set.



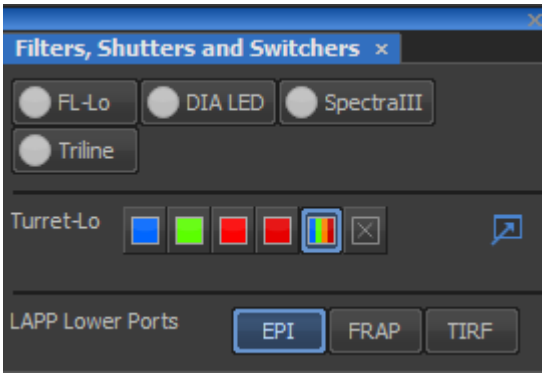
If you on the other hand select an OC, and then change something (say increase the intensity), then button will darken and an orange exclamation mark (“!”) will appear next to the name, notifying you that the settings are not the ones that were saved to this OC.

Currently, most OCs only save the

- Wavelength
- Wavelength intensity
- Camera settings

More things can be saved (such as shutter, multiple active wavelengths, light path, filter cube etc.) but I recommend getting assistance before trying to change those things.

“Filters, Shutters and Switches”



This pad is a compilation of all the shutters, filter cubes, and light-paths, conveniently placed into one window. This one generally doesn’t need to be used but is extremely useful for troubleshooting.

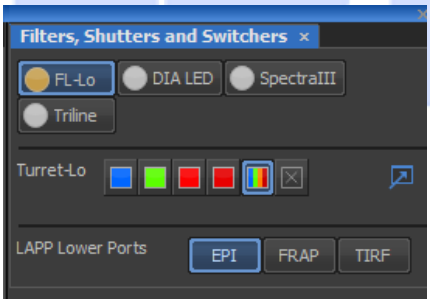
In 99 % of cases where there is no light on the microscope when you want it, the problem is with one of the things on this tab.

Shutters: First are all the shutters of the microscope. If no light is seen when it should, it’s commonly because some shutter is closed.

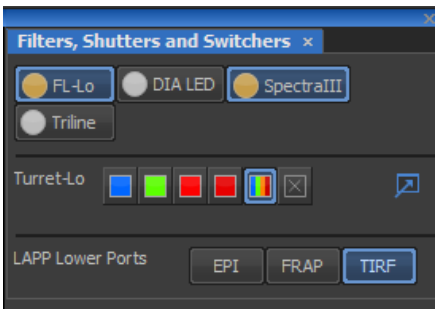
- *FL-Lo*: This is the primary shutter for the microscope itself (not the light sources). When this shutter is off, no light whatsoever reaches the sample.
- *DIA LED*: This is the whitelight to illuminate the sample.
- *SpectraIII*: This is the shutter for the EPI light source. When measuring with EPI, this shutter will be open (along with FL-Lo).
- *Triline*: This is the shutter for the laser light source. When measuring with laser, this shutter will be open (along with FL-Lo).

Turret-Lo: These are the filter cubes. Normally the filter cubes are set when selecting the appropriate predefined optical configuration (almost always the rainbow multi-bandpass cube for the predefined optical configurations). However, if you’re not working with the predefined optical configurations, and hit a problem with no light being visible, check here to see that the correct filter cube is selected.

LAPP Lower Ports: This is the same as the “Ti2 LAPP Pad” window. Here the light source is selected. Like the filter cubes, the light path is set when selecting a predefined optical configuration. However, if you’re not working with the predefined optical configurations, and hit a problem with no light being visible, check here to see that the correct light source is selected.

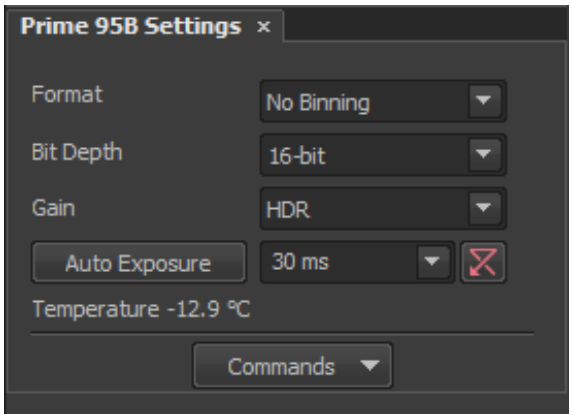


Example 1: No light visible. Here the problem is that the “SpectraIII” shutter (the shutter for the EPI light source) is closed.



Example 2: No light visible. Even though the correct shutters are open, the problem here is that the TIRF light source is selected, instead of the EPI.

“Prime 95B Settings”



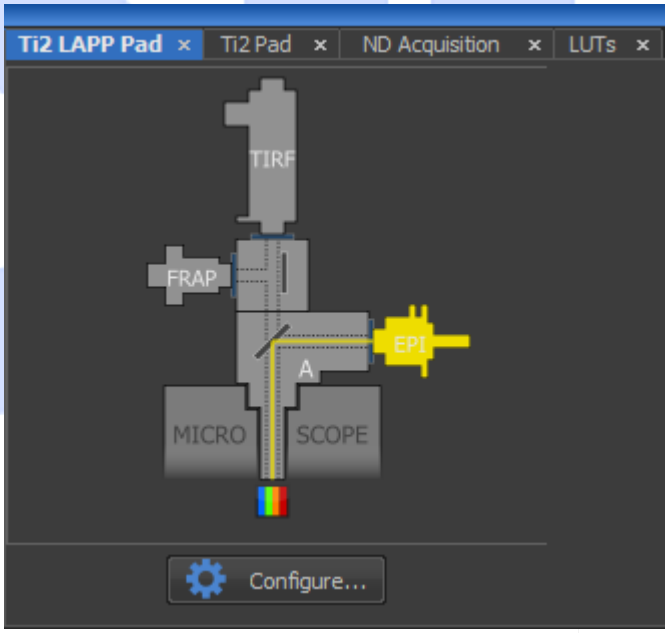
These are the camera settings. Here you can select the binning, bit depth, gain and most importantly, the exposure time.

Binning is when the intensity of several pixels are summed up into one pixel. This allows us for instance to better visualize low-intensity regions of a sample, by binning the brightness of several pixels into one. This comes at a reduction of resolution.

Bin depth is the amount of pixels binned together (??)

Gain.

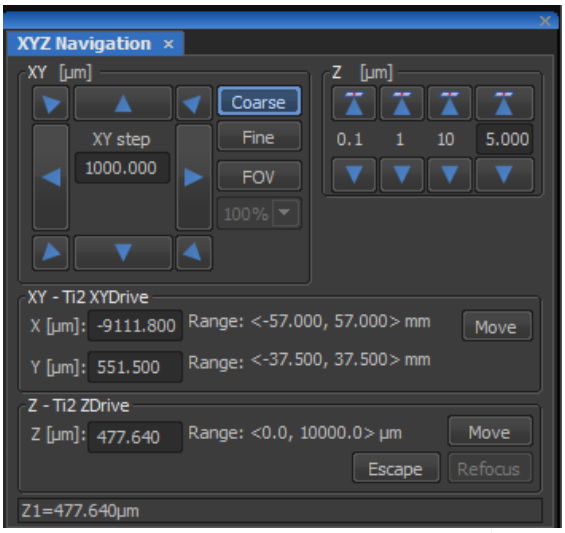
“Ti2 LAPP Pad” menu



The pad shows a schematic of the microscope with the different illumination sources (EPI, TIRF and FRAP) and their corresponding optical pathways. The currently used illumination source will be highlighted in yellow and shows a light path in yellow towards the microscope (EPI is active in the figure). Also shown is the filter cube currently active (rainbow multi-bandpass cube in figure).

By default, when you select an appropriate Optical Configuration, the microscope will automatically select the correct light source. Therefore, this pad is relatively little used unless you’re an advanced user using different light sources. However, if you’re having problems illuminating your sample, you can check this window to see you have the correct source selected.

“XYZ navigation”



This pad allows you to move the stage horizontally (XY coordinates) and the objective vertically (Z coordinate). This can alternatively be done with the joystick and scroller on the microscope. However, here you can move the stage/objective very precisely in stepwise manner (upper half of the window), or to a specific coordinate (lower half of the window).