LSM700 user manual

If you have any problems or questions with the system, contact Janne Capra (office Sn3209, janne.capra@uef.fi, +358 50 516 5268) or Kirsi Ketola (office Sn3210, kirsi.ketola@uef.fi, +358 50 3299984)

Please note that you need training to be allowed to operate this confocal microscope independently. If you need a user training, please contact Janne Capra.

1. Turning on the system:

All the switches are numbered. First, turn on the main power switch (1, arrow pointing at the white switch) on the laser unit cart located left of the microscope.

Next, turn the key (2) one quarter clockwise. The key is located directly underneath the main power switch, and it powers the laser unit.

Third is the auxiliary unit power switch (3) on the extension cord. The extension cord is located underneath the microscope table. This will also power up the computer, located underneath the computer table. You don't need to press the power button on the computer front panel.

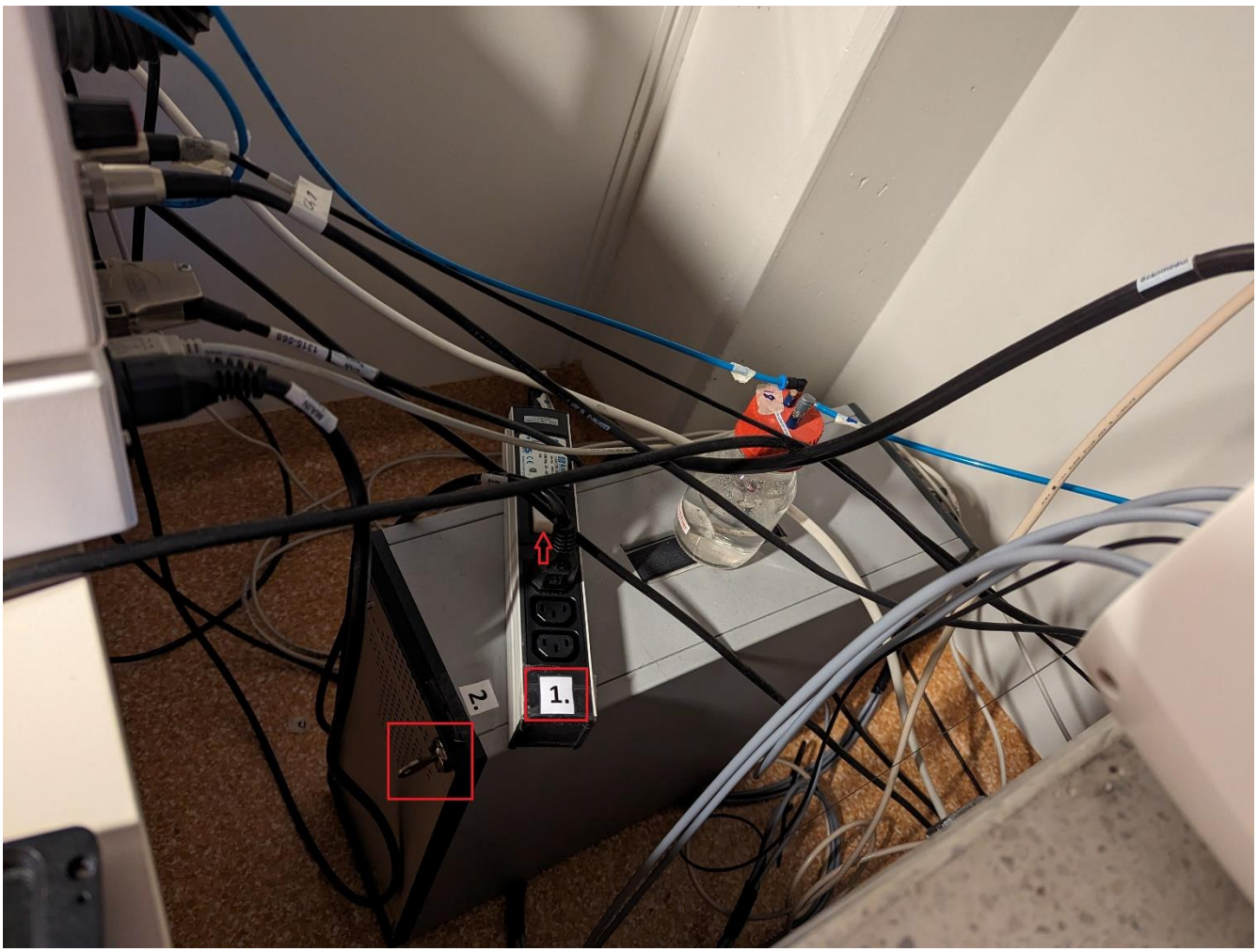
Fourth, turn on the Colibri fluorescent light unit located on the computer table, next to the monitor (4). In addition, each colour LED needs to be turned on and powered up separately. To do this, press each of the discs on the Colibri control box down (each LED line will say ON after this is done), and then power the LED from 25% to 100% by pushing the disc up until the corresponding LED line says 100%.

Please note that you can leave the LED power to less than 100% to protect your sample from photobleaching, especially if it bleaches very easily. Just make sure you use a high enough power to actually be able to see the fluorescent signal.

Fifth and final step is to turn on the microscope hull by pressing the grey button on the left side of the microscope hull (5). This will turn on the small blue light on the bottom front of the microscope, and the touch screen.

Once the computer is on, log on to LSM700 account. There is no password. Start Zen software and when prompted, select Zen System. During the initialization of the software, an error log window will pop up, unless the environmental control units are

not switched on. If environmental control is not required (i.e. if the sample is not alive), this error log can be ignored.

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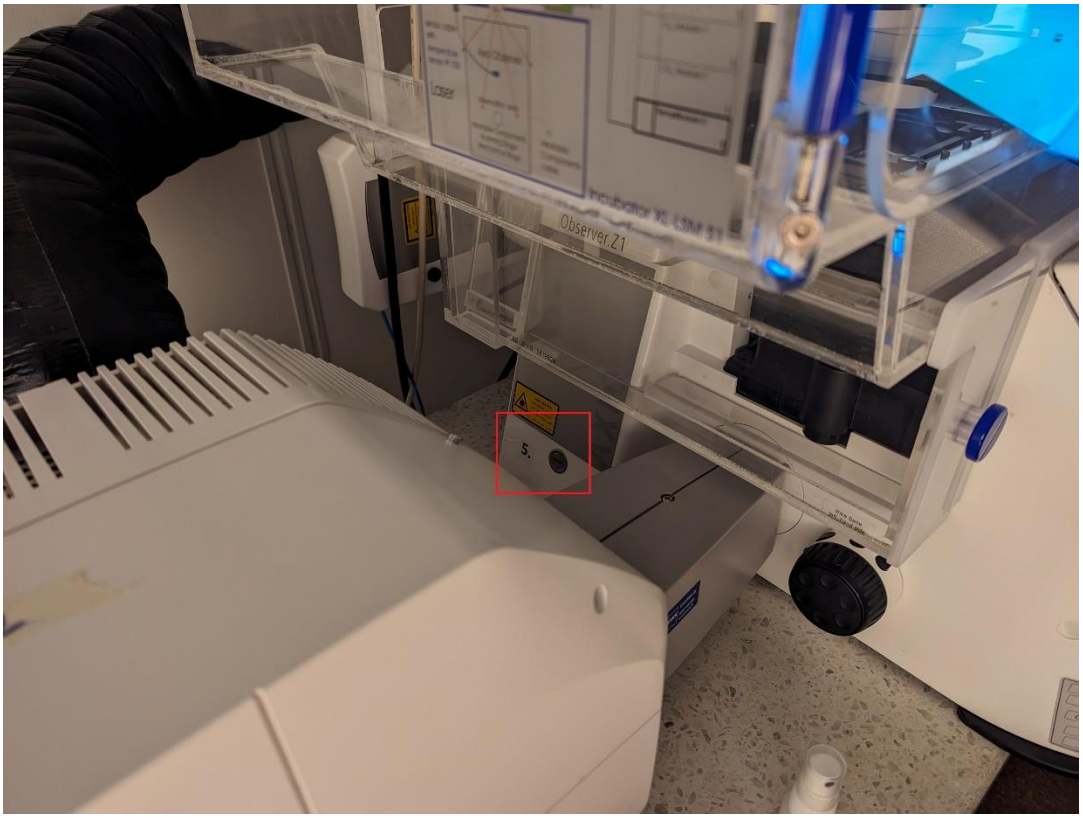
Observer.Z1
5.

2. How to get started?

First, find the area of interest on the sample using the oculars. For this, make sure Zen software is turned on and has finished initialization. Click Locate tab (1.1.) on the main view of Zen software (Figure 1.)

To observe fluorescence light, the fluorescence light source shutter can be opened by clicking On button on the Reflected Light menu (1.2.). To switch the filter, click the Filter menu button (1.5.), and select the desired filter.

Transmitted light (for bright field imaging) can be turned on by clicking the light bulb icon (1.3.) and clicking the On button from the pop-up menu. The brightness of the light can be controlled from the slider. The lamp can be off by clicking the Off button.

To change objective, click the Objective menu button (1.4.) and select the desired objective. Please note the different immersion medium requirements (air, water, oil) between the objectives. If switching between objectives with different medium requirements, a notification will pop up alerting about the changing medium requirement. Clicking Ok will proceed with the objective change, clicking Back will return the objective revolver to the previous position.

To control the light path settings from the touch screen, please see the Additional information part of this manual.

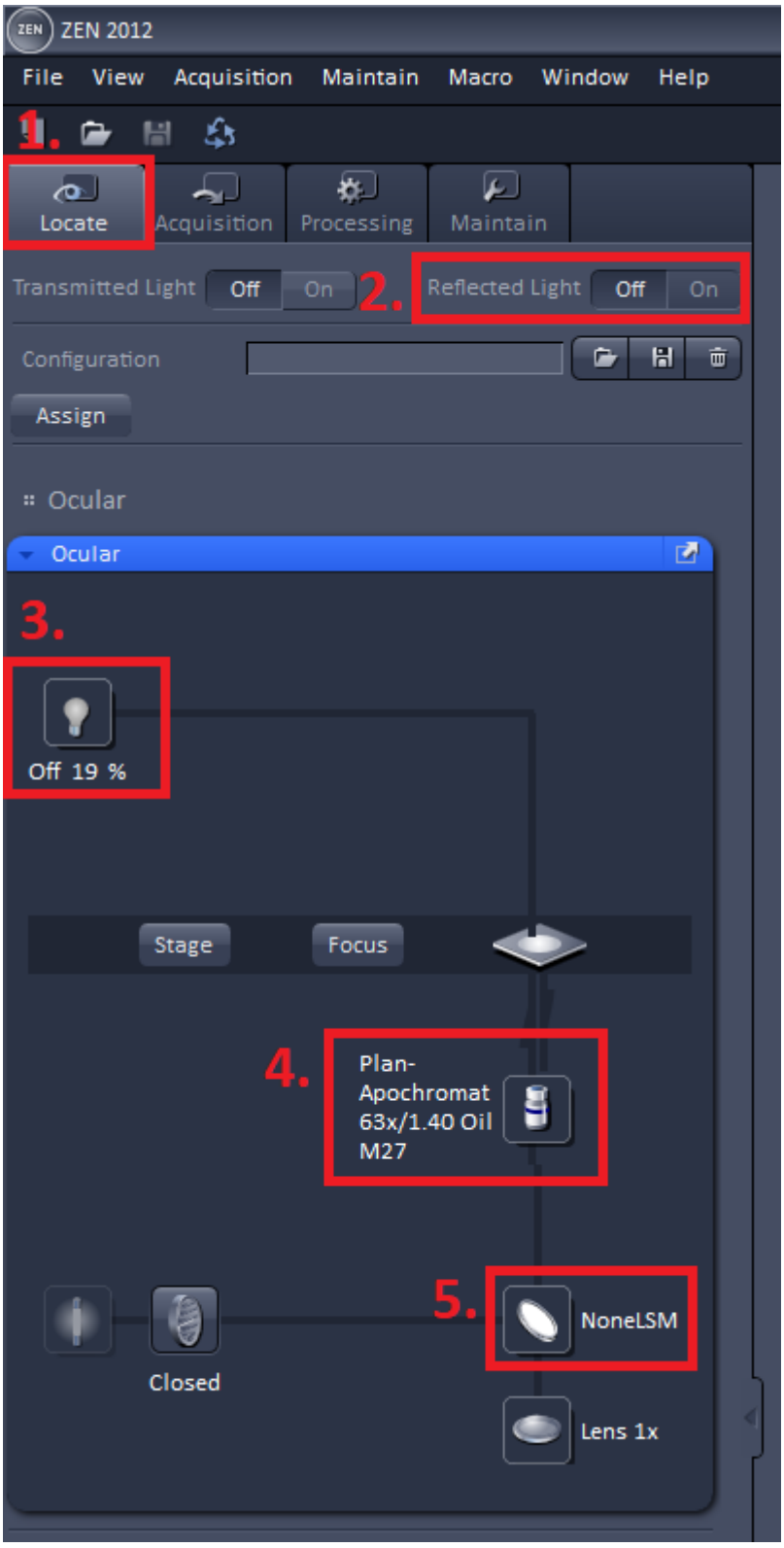

Figure 1. Locate tab.

3. Image settings

Everything needed for taking images can be found on Acquisition tab (1. on Figure 2). To start with a "clean slate", select Basic from the Experiment configuration drop-down menu (2.2.). Once imaging parameters have been setup, those parameters can be then saved by clickin the cog symbol on Experimental configuration, and selecting Save As. **Do not override any parameters in Basic by saving on it!**

Smart Setup (2.3.) should be used to select the channels (fluorophores) needed and imaging mode desired. For instructions on how to use the Smart Setup menu, please refer to part 4. Smart Setup. Channels selected on Smart Setup will appear on Channels menu. It is strongly recommended to **use the Smart Setup menu** instead of adding tracks manually!

Configuration of image parameters can be done in the Acquisition Mode. For instructions on how to use the Channels and Acquisition Mode menus, please refer to part 5. Acquisition Mode and Channel menus.

If Z-stack, time-lapse and/or tile imaging is needed, enable the corresponding option in the Multimodal Imaging menu (2.7.).

Once image settings have been setup via Smart Setup, Live mode (2.5.) can be enabled to get a quickly and continuously scanning view of the current location on the sample. Live mode does **not** use parameters from Acquisition Mode menu, but it does use parameters from Channels menu. Live mode is a good way to find an area of interest, focus and to check that the Channels menu settings are satisfactory. Continuous mode (a button next to the Live mode) also continuously scans the imaging area but uses parameters from both Acquisition Mode and Channels menus. This gives a more realistic representation of the final image but takes longer and causes more photobleaching to the sample.

Once the parameters are all set up and area of interest and the right focus have been found, a single image can be taken by pressing the Snap button (2.6.). This takes a single scan from the current location and focus, using parameters from both Acquisition Mode and Channels menus. After the scan is completed, it appears as a new tab in the Viewing Area. The image is only temporarily saved, and it is highly recommended that, if the image is as desired, it is saved as soon as possible. **Please note that if another image is taken with the Snap function or using the Live mode before saving a**

previously taken Snap, the unsaved Snap will be overwritten! Instead of saving, another Snap can be taken without overriding the non-saved Snap by clicking the New button (2.4.) to create a new tab.

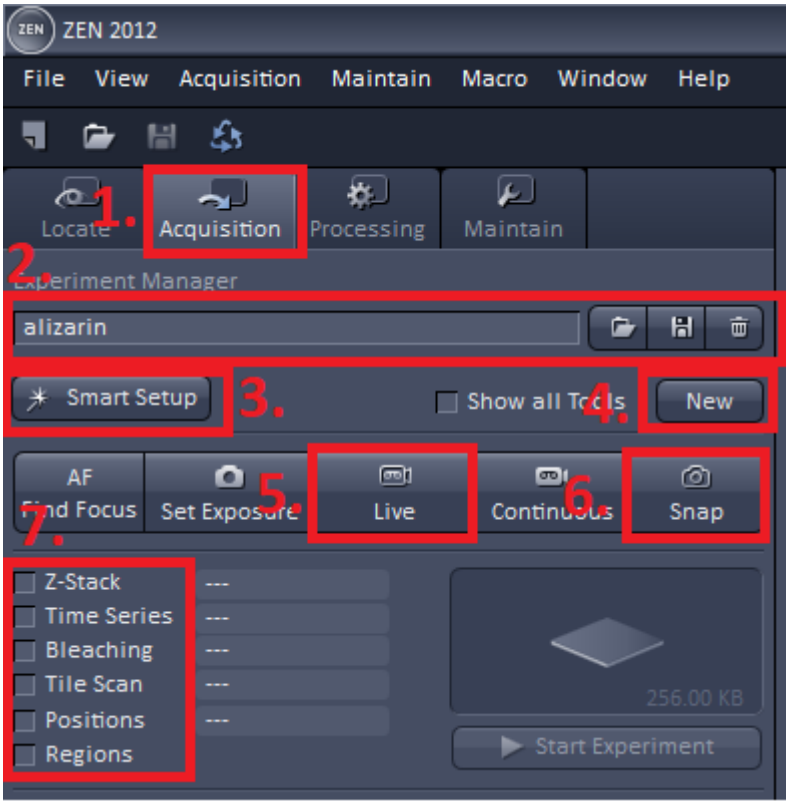

Figure 2. Acquisition tab

4. Smart Setup menu

When the Smart Setup button is pressed on the Acquisition tab (Figure 2.3.), the Smart Setup menu opens (Figure 3).

New dyes can be added by clicking the small black triangle (3.1), which opens Add Dye or Contrast Method menu. Select the dye from either the list of Recently Used (3.3.) or from the Dye Database (3.5.). Search bar can be used to search for the right dye (3.4.). The dye list shows the default colour that the system is using for the dye along with the emission maxima of the dye. Click the dye to add it to Smart Setup. The default colour of the fluorophore for this imaging session can be changed by clicking black triangle under Color tab (3.2.)

From the Preset Options menu (3.3.) keep the Current option. Smart Setup presents three Proposals (3.8.): Fastest, Best Signal and Smartest.

The proposal **Fastest** aims to record all dyes simultaneously as a single track with multiple channels whenever possible. If the number of fluorescent dyes exceeds the number of available detectors, Smart Setup switches to a speed-optimized multi-channel strategy with frame-wise switching between the acquisition tracks. This proposal favors speed over cross talk.

The proposal for **Best Signal** follows a strategy that maximizes the efficiency of signal recording and also minimizes the level of cross talk. Multi-color acquisitions using this strategy are typically performed as sequential acquisitions of each dye species with frame-wise switching between tracks.

Smartest (line) combines the advantages of Fastest and Best Signal. This proposal aims to minimize the number of acquisition tracks as well as cross talk. This is achieved by recording dyes with low spectral overlap as a single acquisition track. In addition, Smartest (Line) improves the acquisition speed also by performing line-wise switching between tracks whenever applicable.

Once the desired proposal has been selected, click Apply (3.9.) to close the Smart Setup menu. Selected channels and dyes will now appear in the Channels menu.

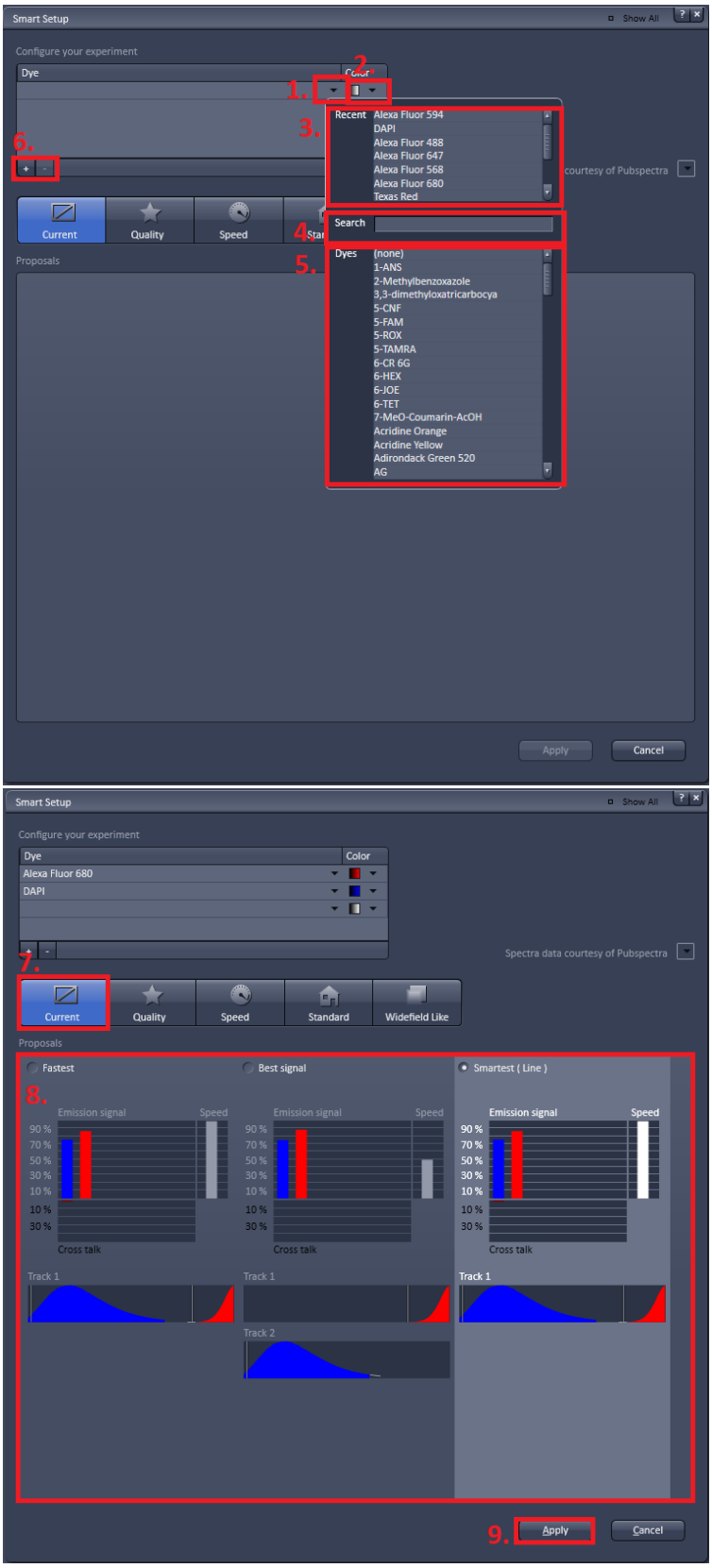

Figure 3. Smart Setup menu

5. Acquisition Mode and Channels menus

Acquisition Mode and Channels menus are blue header menus that can be found in the Acquisition tab. Acquisition Mode contains settings for image resolution and quality, while Channels menu contains settings for image brightness. Zen software hides some of the options, so be sure that the Show All box is checked for both Acquisition Mode and Channels menus (4.5.)!

Acquisition Mode settings are usually selected once at the start of the imaging session and then kept the same throughout it. Frame Size (4.1.) dictates the final resolution of the image in pixels. The X x Y drop-down menu gives commonly used values. The more pixels there are in the picture, the smaller are the details that it can capture, but the longer it takes to take each image. Remember, however, that if the source of light (the fluorophore of interest) is bigger than one pixel, then increasing the number of pixels does not increase picture quality! Thus, 1024x1024 frame size is usually a good choice, unless the picture will be cropped later. For faster imaging smaller frame sizes can be used.

Direction drop-down menu (4.4.) allows the selection of scanning direction. By default, this is set to single direction scan (an arrow pointing to the right). This is slower than bi-directional scan (an arrow with heads pointing both left and right), with no real benefit, thus bi-directional scanning would be better.

Scan speed (4.2.) slider dictates how long the laser is staying in an area the size of a single pixel. This setting is similar to exposure time in wide-field microscopes. Bigger number means shorter time spend on each pixel, which means faster imaging. Scan Time shows the time it takes to take one image with all the current Acquisition Mode and Channels settings.

Averaging (4.3.) is a tool to reduce noise in the image. When set to 1, the images are taken as normal. If set to any other number, each line in the image will be scanned the indicated number of times, and the mean intensity value will be taken for each pixel in the final image. This will reduce the noise but increase scanning time.

Channels menu settings are often adjusted multiple times during the imaging. Track list (4.6.) shows the dyes selected via Smart Setup. If the box next to the track name is unselected, those channels will not be imaged. Only the options affecting the selected

track are shown in the menus below it, and all the parameters need to be set separately for each track and channel. Dyes can also be given false colours that do not correspond to their actual emission wavelength from the drop-down menu on the right. The false colouring will then appear in all subsequent images. For a smoother Live mode scanning (see Figure 2), only one track should be selected at a time. Otherwise the system will try to image all the tracks, and this will slow down the scanning process significantly, making it very difficult to focus and move around in the sample. Remember to check all the tracks back on before you take an image with Snap function!

Lasers menu (4.7.) shows which lasers will be used for each track. These are automatically set by Smart Setup, so they do not need to be changed manually.

Laser strength sliders (4.8.) indicate how much of the full power of the laser is directed into the sample. Each laser has its own slider. The higher laser power produces brighter images, but also photobleaches the fluorophores faster, and can damage living cells. Low laser power is especially recommended when searching for a region of interest or focus. Laser strength can be adjusted either via the slider, by writing the number into the field next to the slider, or using the up and down arrows (4.9.).

Pinhole menu (4.10.) allows you to change the size of the pinhole. Pinhole size dictates the optical thickness of the sample. Increasing the pinhole size will allow more light from the sample to get into the detector, but will also reduce spatial resolution in Z direction, meaning objects that are actually very far from each other in depth appear to be on the same level. Decreasing the pinhole size below 1 Airy unit (AU) will slightly increase the optical resolution, btu will drastically decrease the brightness. Use of 1 AU as the pinhole size is recommended. To set the pinhole size to 1 AU, click the 1 AU button

Master Gain (4.11.) slider sets the amplification level of the detected signal. Each dye in the track has its own Master Gain slider. Higher levels of Gain will increase the image brightness but will also increase the noise. Zen Black will use a starting value of 180 V, which is way too low. A good starting value is around 600-700, but can be adjusted when needed. Since Master Gain increases image brightness without damaging the fluorophores or the cells, it is recommended for sensitive dyes and/or cells.

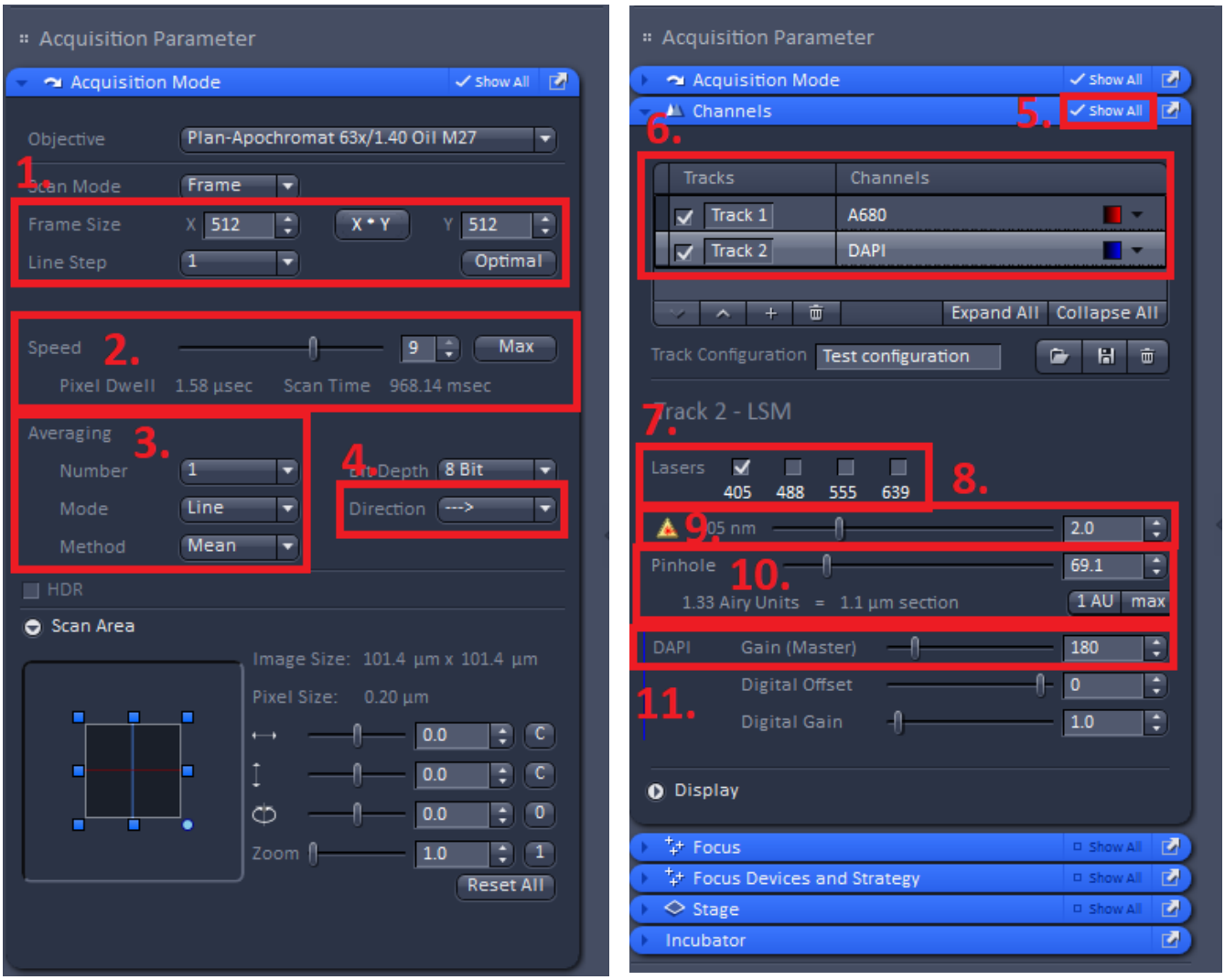

Figure 4. Acquisition Mode and Channels menus

6. Live-cell imaging

For live-cell imaging the heating unit needs to be turned on (Figure 6). Heating unit is located on the left side of the microscope, connected to the microscope by a thick, black tube. The power switch is located behind the lowest unit. In addition to this, the CO_2 gas bottle main valve, located next to the LSM800 microscope on the other side of the curtain, needs to be opened. Other valves on the gas bottle do not need to be adjusted.

The incubation settings can be changed from the Incubator menu below the Channels menu. Both the heating insert and the XL Unit should be set to the same temperature. The incubator needs at least 30 minutes to warm up, but about an hour for the temperature to stabilize. Make sure that all three side hatches and the sliding panels on top of the incubation chamber are closed to maintain the desired conditions.

Figure 6. Environmental control unit

7. Additional Information – Touch screen

The light path settings can also be controlled via the touch screen (Figure 7). **These settings only affect how the samples look when viewed through the oculars!** The actual confocal microscope image settings are set from the Zen software (see parts 4 and 5).

To start, press the Control button on the left side of the screen (7.1). From the Control menu, select Objectives (7.2) to select the desired objective from the available selection (7.3). To visualize the sample with brigh-field illumination, press the On button under TL Illumination (transmitted light, 7.4). To visualize the sample with fluorescent light, press the On button under RL Illumination (reflected light, 7.5). For fluorescent light, the desired filter needs to be selected as well from the Reflector menu (7.6), and pressing the name of the filter.

Please note that if you wish to use brigh-field illumination, you should select one of the “empty” filters from the Reflector menu.

The light-path that the visualization light takes can be viewed from the Light-path menu (7.7).

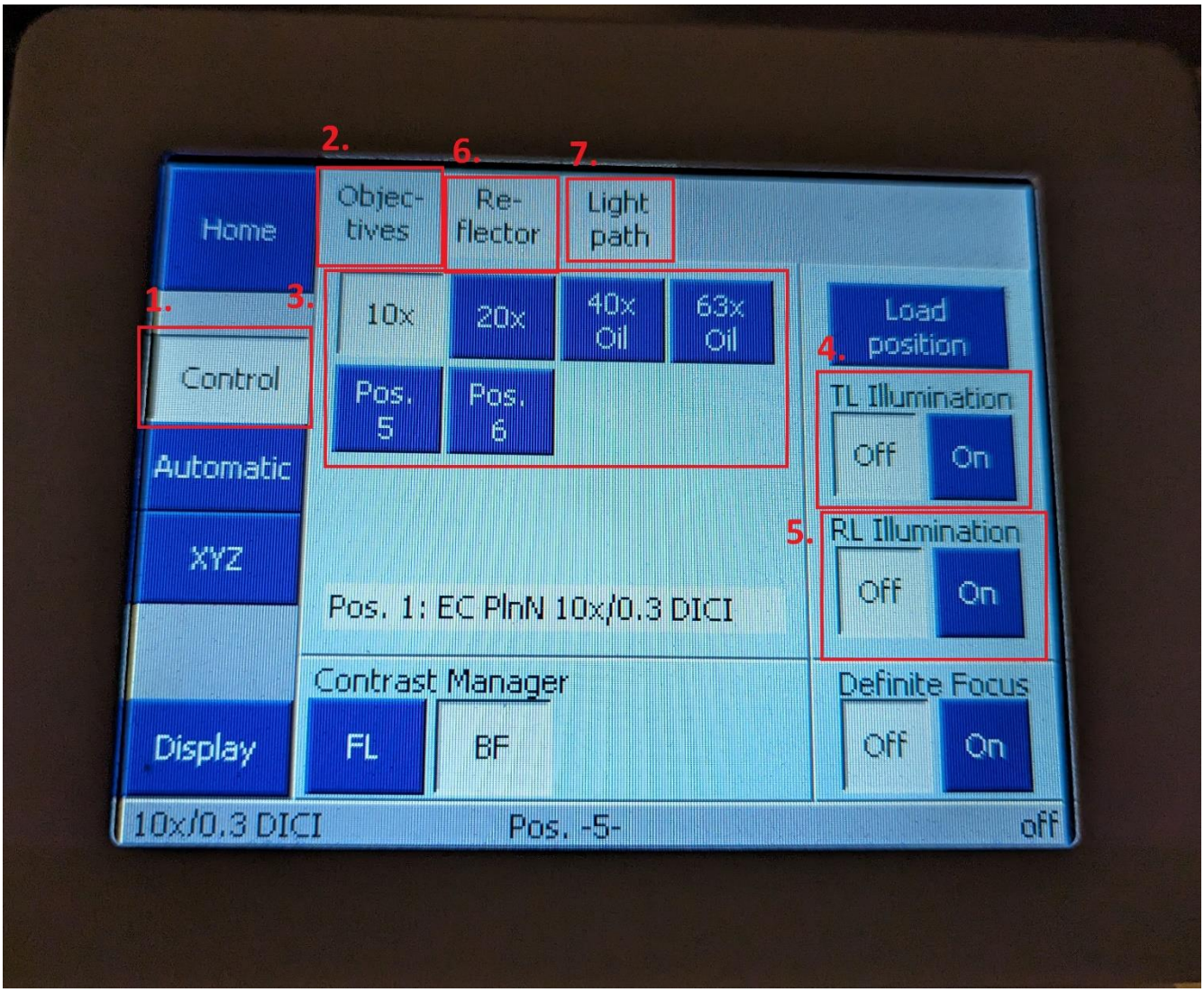

Figure 7. The touch-screen

8. Additional Information – Graphics and Display settings

Graphics tab (8.1) can be used to add annotations and measurements to the image, and also to change image brightness and contrast. Different annotation tools (8.2) include text tool, measuring tool, arrows, and different shapes, including squares, circles and free from. The shapes also give the area of the shape and average intensity of the fluorescent signal inside it for each channel. The annotation settings can be changed by clicking the More button (8.3). The added annotations are shown on Annotations/Measurements list (8.4), where they can be turned invisible/visible, or the name of the annotations and the measurements can be hidden. The annotations can

also be saved and loaded to a new image, and they will keep their relative positions. The intensity measurements will be updated according to the new position.

On the Display tab, the image brightness and contrast can be manipulated. With Histogram selector (8.5) the channel to be manipulated can be selected. If All is selected, all the channels will be manipulated in the same manner. To change image brightness, the slider on the right end of the histogram (8.6) can be dragged towards the left. Similarly, the slider on the left slide can be dragged to right to change the image contrast. **These settings only change how the image looks on the screen, they don't change the intensity values!** Changes made with the histogram sliders can be reseted by clicking the Reset button (8.8). It is recommended that you save your images without any changes made to the histogram, as they will be kept the next time the image is opened, and this might cause confusion and lead to errors in image interpretation. If any of the tools shown in the pictures in this manual are not visible, make sure that the Show All box has been checked (8.9).

Dimensions tab (8.10 and Figure 9) can be used to view different dimensions of the image. Available tools depend on the selected image settings. Channels tools (9.1) allow applying a Look-Up Table (LUT) to change the colour of the channel. Pressing the name of the channel allows hiding that channel from the viewing area. Single Channel box can be checked to force only channel to be visible at any given time. Range Indicator box (9.2) can be checked to apply a special LUT that colours black pixels (pixels with intensity value of 0) with blue, and maximum brightness pixels (depends on the bit depth of the image, for 8-bit image it is 255) with red. Red indicates that pixels are overexposed and the imaging settings should be set to minimize this. Other values are shown in shades of gray. Range Indicator can also be used when setting the imaging parameters in Live mode (see part 3). Reuse button (9.3) can be used when settings from a previously saved image are wanted to use. It will copy Channels and Acquisition Mode settings and additional imaging mode (Z-stack, Time-series etc.) settings.

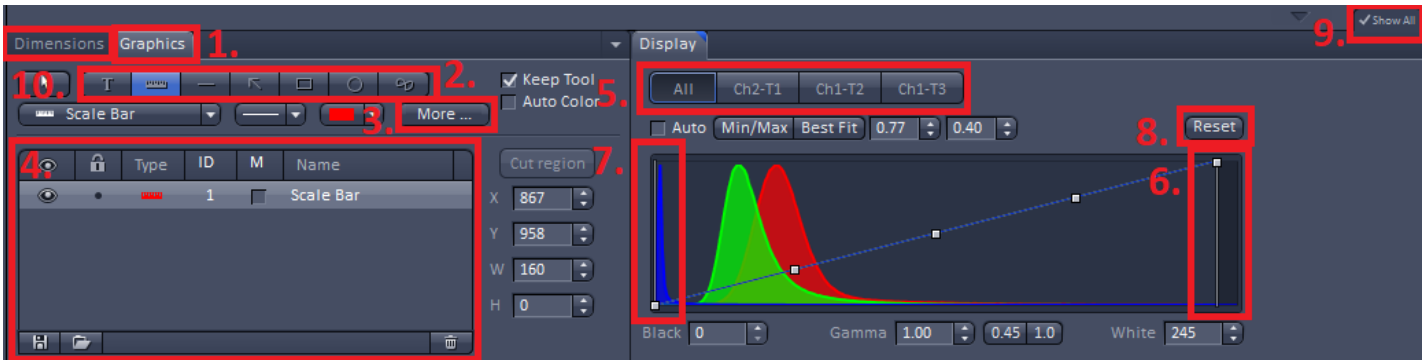

Figure 8. Graphics and Display settings

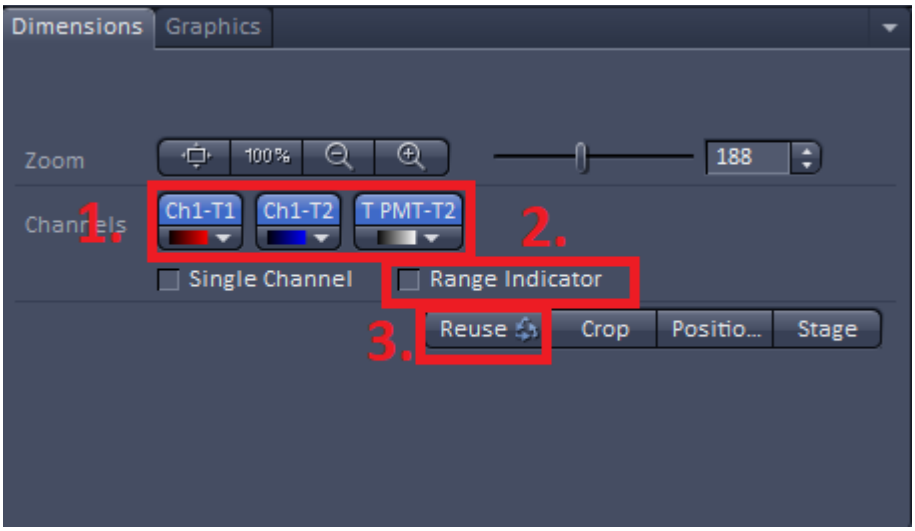

Figure 9. Dimensions

9. Additional information - Sample adapters

LSM700 has two adapters for holding different sample types. The most commonly used one is the variable size adapter (Figure 10. on the right) for microscope glass slides, ibidi 8-well plates and such, and for 35 mm dishes (when the sample holders are brought close together). To load a microscope glass slide, move the sample holders apart from each other, place the sample against the groove on the sample holder (10.2), and move the opposing sample holder against the sample so that it rests firmly between the sample holders.

The other adapter is for well-plates. It has a tightening hand on the top left side (10.4) that will hold the well-plate steady. Pull the hand back and place the well-plate onto the adapter. Release the hand. When removing the well-plate, pull the tightening hand

back before trying to remove the well-plate, otherwise the whole adapter might pop out. **Once you are finished with the well-plate adapter, please switch back to variable size adapter!** There is only one well-plate adapter, so if it is not on the LSM700 table, check the LSM800 table.

To change sample adapters, lift the adapter up until it pops out of the frame. Turn the new adapter so that the red dot is closest to you, or that the text has the right side up (10.1 and 10.3). Place the adapter on to the frame so that the left side of the adapter is resting on the small ledge on the bottom left side of the frame. Hold the adapter steady against the ledge and push it towards the left side of the frame. On the right side of the stage there is a black lever (Figure 11). Pull it back to allow the adapter to settle in and release it to tighten the adapter in place. Finally, check that the adapter is flush against the frame.

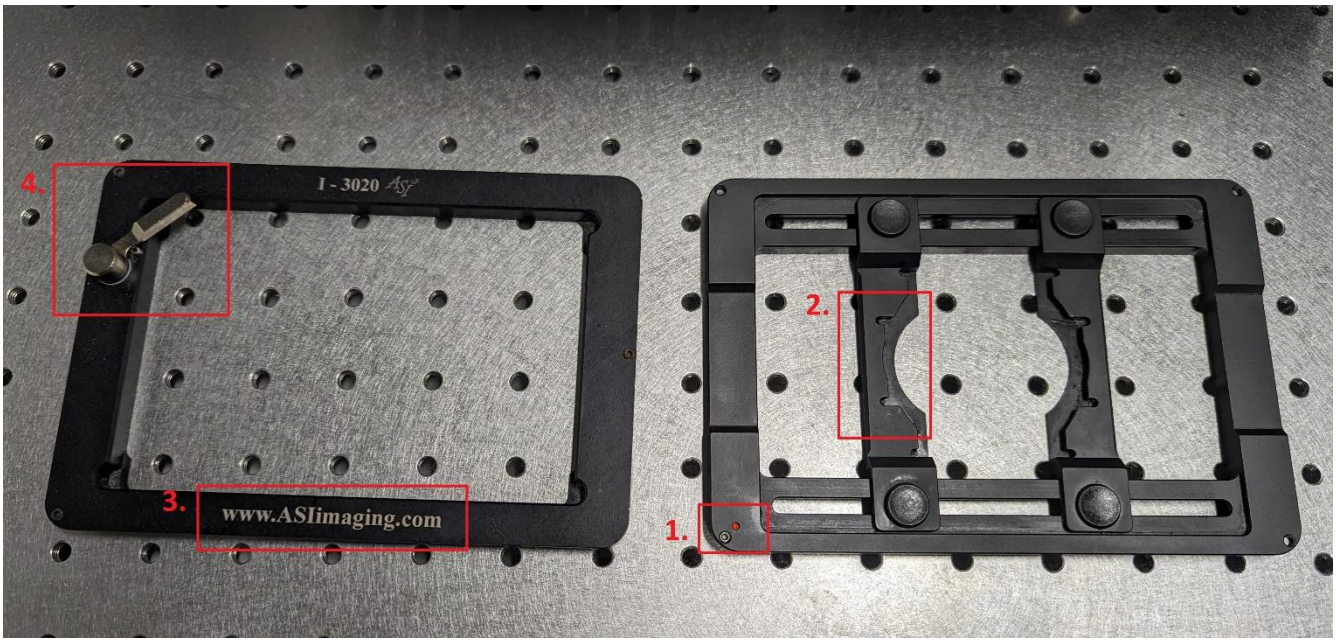

Figure 10. Sample adapters

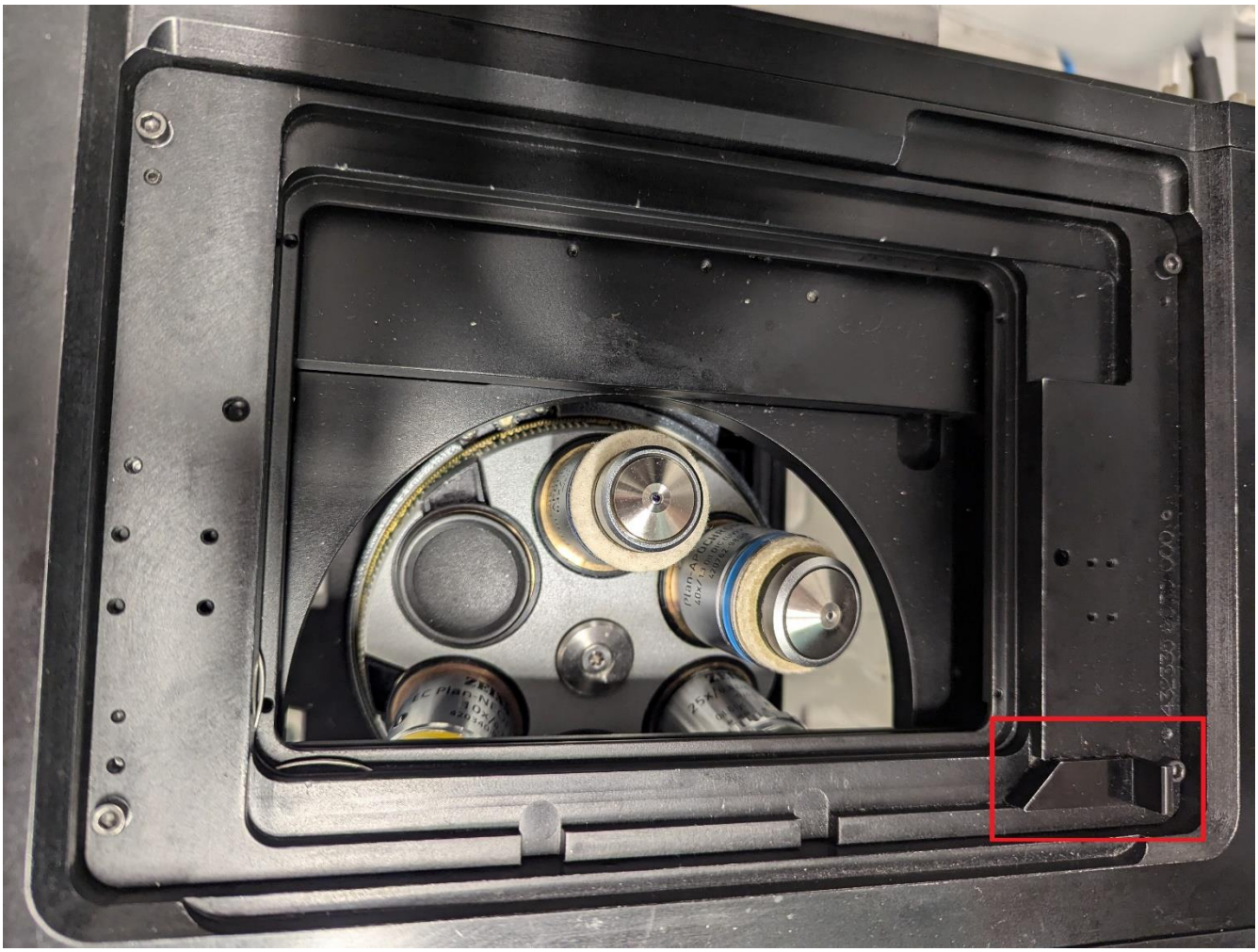

Figure 11. Changing adapters

10. Taking brightfield images

To include a brightfield image with your fluorescent channels, a special brightfield T-PMT detector needs to be enabled. First, check the Show all Tools box (12.1) on Acquisition tab. This will show two new blue frame menus. Open the Light Path menu (12.2). Select which Track the brightfield image should be added by clicking the Track number (12.3). Brightfield imaging uses the same laser as the selected Track. Thus, it's beneficial to select a Track that can be used with the lowest laser strength to minimize photobleaching.

Check the T-PMT box (12.3.) on the bottom of the menu, and the T-PMT channel will appear on the selected Track (12.4). T-PMT channel will also have it's own Gain and

Offset settings (12.5). Use the Gain and Digital Gain to set the brightfield image brightness.

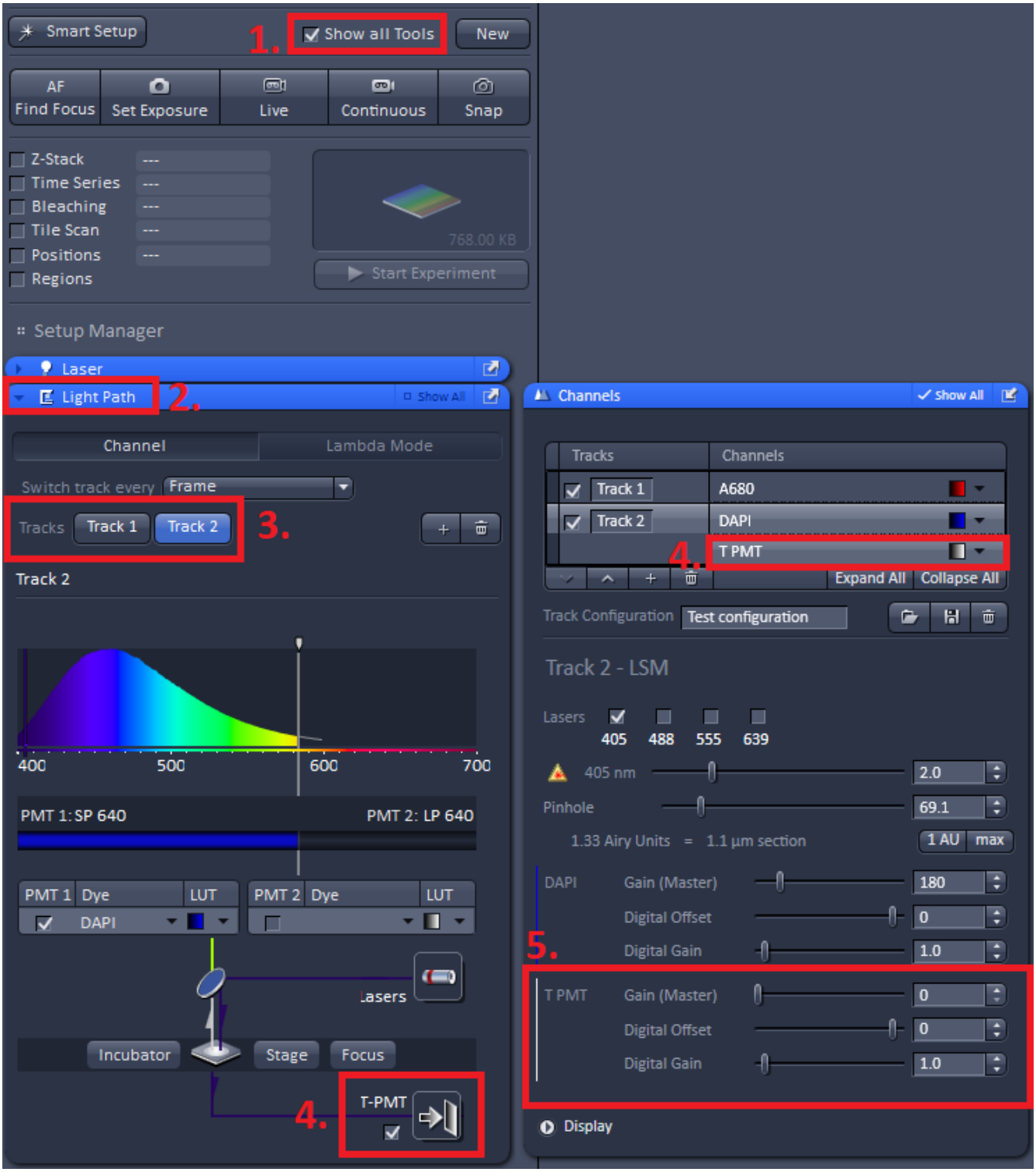

Figure 12. Brightfield imaging using T-PMT detector

11. Troubleshooting

Often a simple turning the system off and then on again solves the problem. This can be done in three different levels: shut down the software and restart to solve software related problems; shut down software and the microscope (auxiliary unit power switch (step 3 on system start up) on the white extension cord) to solve microscope and connection related problems; and shutting down the whole system, waiting couple minutes and then restarting to solve system-wide problems.

If shutting down and restarting does not help, and the problem is not described in the instruction below, please contact Janne Capra (janne.capra@uef.fi).

Problem: The microscope stage is not moving or moves very slowly.

Solution: Press the F1 button on the joystick.

P: When scanning with Live or taking Snaps, the microscope successfully takes pictures, but the usually whining noise is gone.

S: The whining noise comes from the mirrors moving the laser beam continuously across the sample. If the Scanning Speed in the Acquisition Mode is set to slow (Speed 6 and below), the mirrors are simply moving too slow to make that noise. This is normal.

P: Live button was pressed, and the red circle appeared on top of it as normal, but the system is not scanning or doing anything, and the Live scan cannot be stopped.

S: This is a bug that comes when the user moves from the Locate tab to the Acquisition tab and immediately presses the Live button. The microscope needs a few seconds to perform the switch, and if the Live button is pressed before the system is ready, this bug will occur. After about 30 seconds an error message should appear, and the Live scan is interrupted automatically. After this the system can be used normally.

P: Software is acting up in multiple different ways when scanning or taking Snaps, for example images come up with wrong colours, same channels are scanned several times, using Live or Snap gives an error message and nothing happens etc. None of the restarts have helped.

S: These kinds of errors are usually due to a corrupted Experiment, either when using previously saved settings from the Experiment configuration (see part 3. Image settings), or when Reusing the settings from an older image/experiment. Switch the Experiment configuration to Basic and set the channels from start via Smart Setup. Delete the corrupted configuration and save a new one.

P: How can I get the exact same settings that I used previously?

S: Open the image from which the settings are to be copied. Go to in the Dimension submenu located under the image that was opened and click Recycle. This will take the imaging settings (channels, laser settings, pinhole and Gain values, Acquisition Mode settings, Z-stack, Region and Time-lapse settings etc.). It is important to note that it does not change the objective from the current one. This might change some values, for example, the amount of Z-stacks. These will match the values in the original image once the same objective is selected.

P: I'm trying to take an image with Z-stack/tiles/positions, but when I click Snap, all I get is a single picture!

S: When using different imaging modalities that involve taking multiple images, like the ones mentioned above, instead of pressing Snap, you need to press Start Experiment, a bit below the Snap button. Snap always takes a single image from the current location and Z-plane.