

# Quick Guide

## **LSM 710, LSM 780 Systems and ConfoCor 3** Laser Scanning Microscopes



**LSM Software ZEN 2012**  
November 2013



We make it visible.



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## Introduction

This Quick Guide describes the basic operation of the LSM 710, LSM 780, LSM 710 NLO, LSM 780 NLO and ConfoCor 3 Laser Scanning microscopes with the ZEN 2012 software.

The purpose of this document is to guide the user to get started with the system as quick as possible in order to obtain some first images from his samples.

This Quick Guide does NOT replace the detailed information available in the full user manual or in the manual of the respective microscopes (Axio Imager, Axio Observer, Axio Examiner).

Also, this Quick Guide is written for a user who is familiar with the basics of Laser Scanning Microscopy.



**For your safety!**  
**Observe the following instructions:**

- The LSM 710, LSM 780, LSM 710 NLO, LSM 780 NLO and ConfoCor 3 Laser Scanning Microscope, including its original accessories and compatible accessories from other manufacturers, may only be used for the purposes and microscopy techniques described in this manual (intended use).
- In the Operating Manual, read the chapter *Safety Instructions* carefully before starting operation.
- Follow the safety instructions described in the operating manual of the microscope and X-Cite 120 lamp / HBO 100 mercury lamp.

## Starting the system

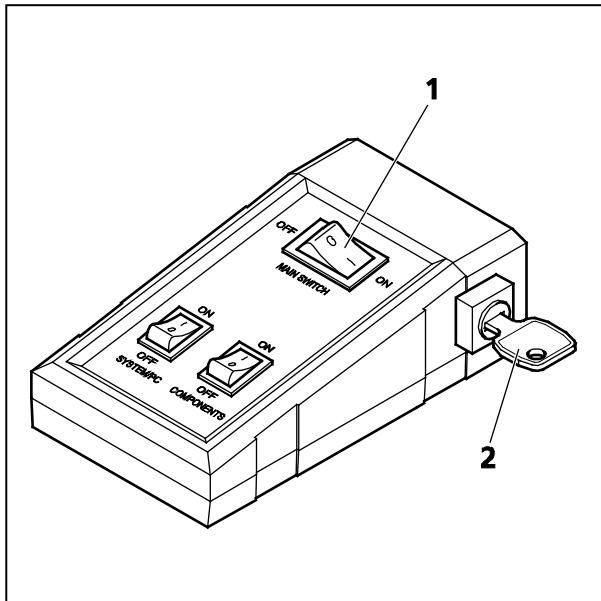


Fig. 1 Power remote switch

### Switching on the LSM system

- Switch on the main switch (Fig. 1/1) and the safety lock (Fig. 1/2).
- When set to **ON** the power remote switch labeled **System/PC** provides power to the computer. This allows use of the computer and ZEN software offline

After complete startup, the computer is ready for the components start.

- To completely switch on the system, now press the **Components** switch to **ON**. This starts the other components and the complete system is ready to be initialized by the ZEN software.

### Switching on the X-Cite 120 or the HBO 100 mercury lamp

- Switch on the main switch of the X-Cite 120 / HBO 100 lamp for reflected light illumination via the power supply as described in the respective operating manual.

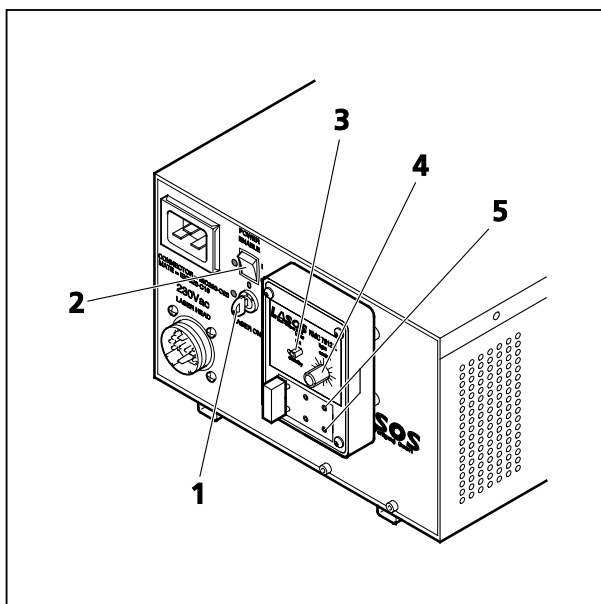


Fig. 2 Power supply of Ar-ML laser

### Switching on the Ar-ML Laser

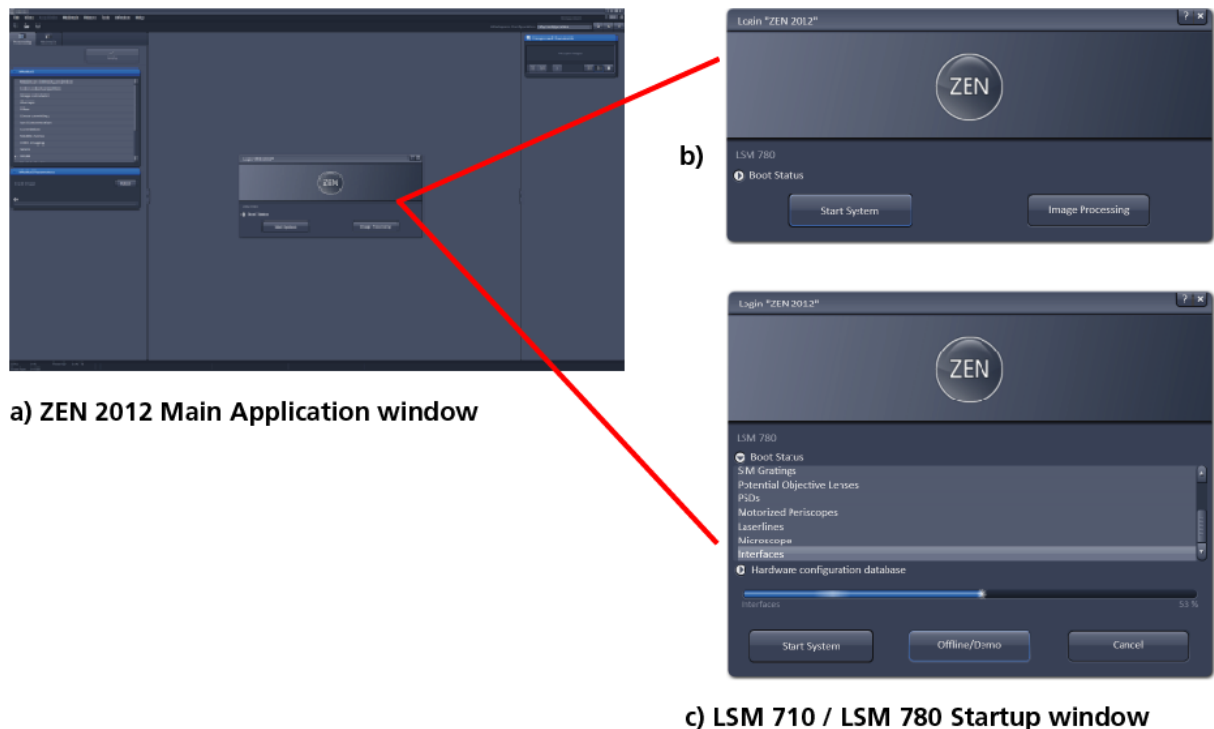
- If the Ar-ML laser is required, switch it on via the toggle switch (Fig. 2/2) on the power supply and turn the key (Fig. 2/1). The laser is automatically kept in standby mode for 5 minutes to warm up.
- Set the idle-run-switch (Fig. 2/3) to run. It takes about 50s until the laser has reached the set output power (green LED) provided the warm up time of 5 minutes is already completed.
- Adjust the required power level with the control knob (Fig. 2/4) (default position should be 11 o'clock).

## Starting the ZEN software




- Double click the **ZEN 2012** icon on the WINDOWS desktop to start the Carl Zeiss LSM software.

The **ZEN Main Application** window and the **LSM 710 / LSM 780 Startup** window appear on the screen (Fig. 3)

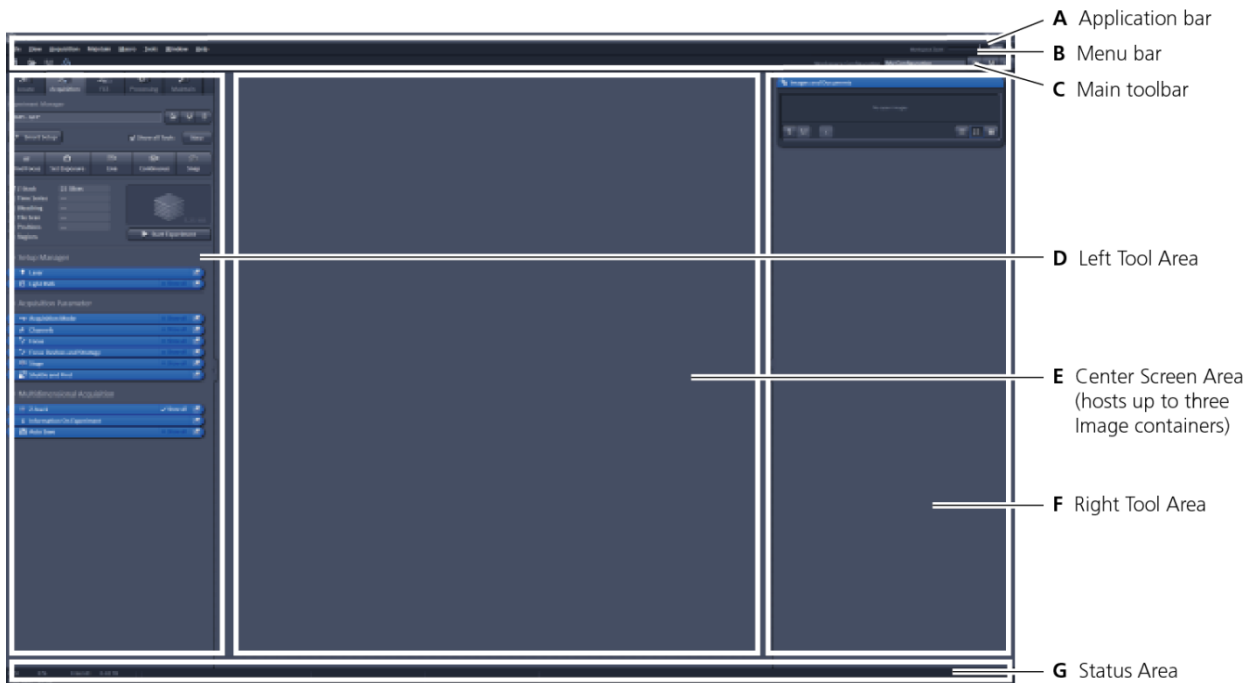


**Fig. 3** **ZEN Main Application window at Startup (a) and the LSM 710 / LSM 780 Startup window (b and c)**

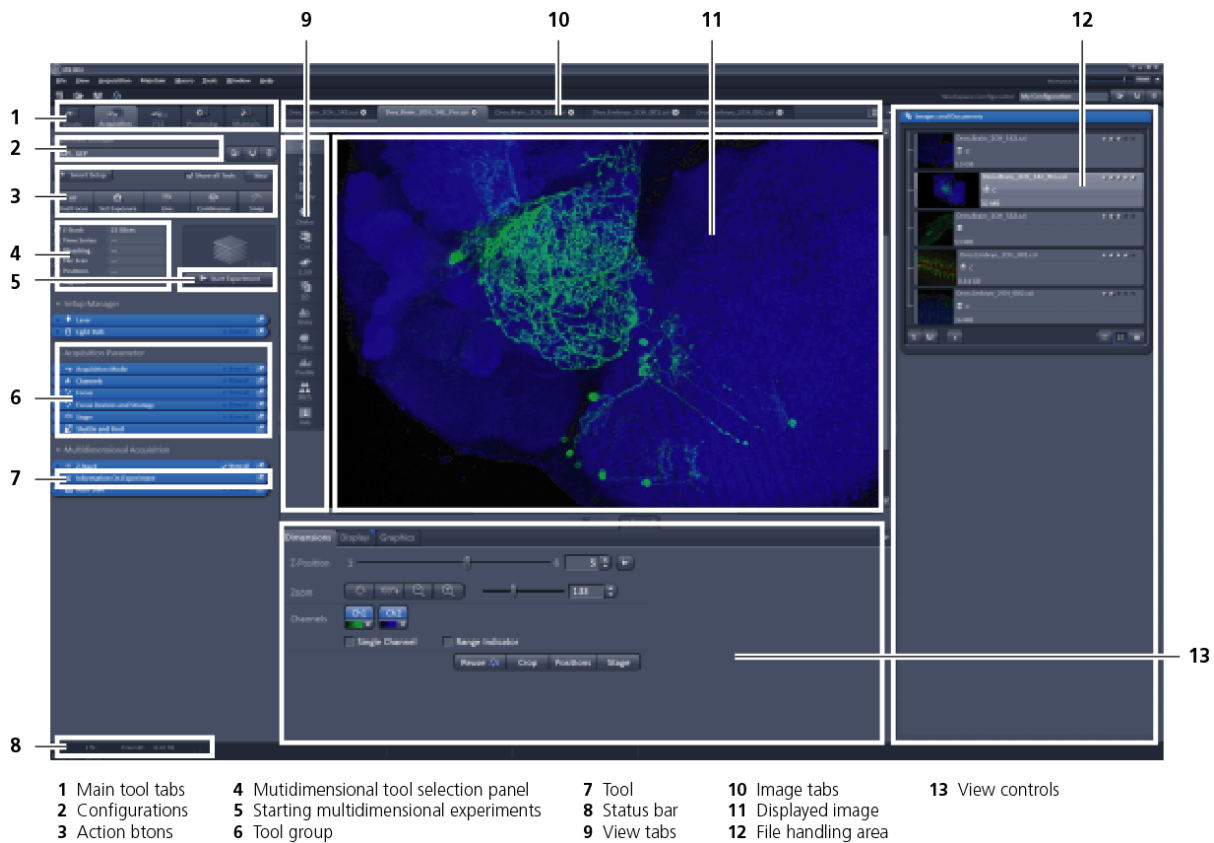
In the small startup window, choose either to start the system (**Start System** hardware for acquiring new images) or in **Image Processing** mode to edit already existing images. Toggle the little  symbol to view the Boot Status display and get the additional **Offline / Demo** button option:

- Choosing **Start System** initializes the whole microscope system and activates the entire software package for new image acquisition and analysis.
- The **Image Processing** mode ignores all hardware and activates only data handling and image processing functionality for already acquired images.
- The **Offline / Demo** mode reads the current hardware database but does not activate the system hardware for use. Instead, it simulates the system hardware for training purposes.
- Upon clicking the **Start System** button, the **Image Processing** button changes to a **Cancel** button. Click **Cancel** to interrupt/stop the **Startup** of the system.

After Startup, the ZEN Main Application Window (Fig. 4 and Fig. 5) opens. To benefit from all of ZEN's features, run the window in its full screen mode.



**Fig. 4 ZEN Main Application Window after Startup with empty image container**



**Fig. 5 ZEN Main Application Window after Startup with several images loaded**

## Introduction to ZEN – efficient navigation

The **ZEN 2012** interface is clearly structured and follows the typical workflow of the experiments performed with confocal microscopy systems:

On the **Left Tool Area** (Fig. 4/D) the user finds the tools for sample observation, image acquisition, image processing and system maintenance, easily accessible via four **Main Tabs** (Fig. 5/1). All functions needed to control the microscope can be found on the **Ocular Tab**, to acquire images use the **Acquisition Tools** (Fig. 5/3 and 4). Arranged from top to bottom they follow the logic of the experimental workflow. The area for viewing and interacting with images is centered in the middle of the **Main Application Window**: the **Center Screen Area**. Each displayed image can be displayed and/or analyzed with many view options available through view tabs which can be found on the left side of the image. According to the chosen view tab, the required view controls appear in View Control Tabs below each image. File management and data handling tools are found in the **Right Tool Area** (see Fig. 4 and Fig. 5).

Color and brightness of the interface have been carefully adjusted to the typical light conditions of the imaging laboratory, guaranteeing optimal display contrast and minimal stray light for high-sensitivity detection experiments. The **ZEN** software is optimized for a 30" TFT monitor but can also be used with dual-20" TFT setups.

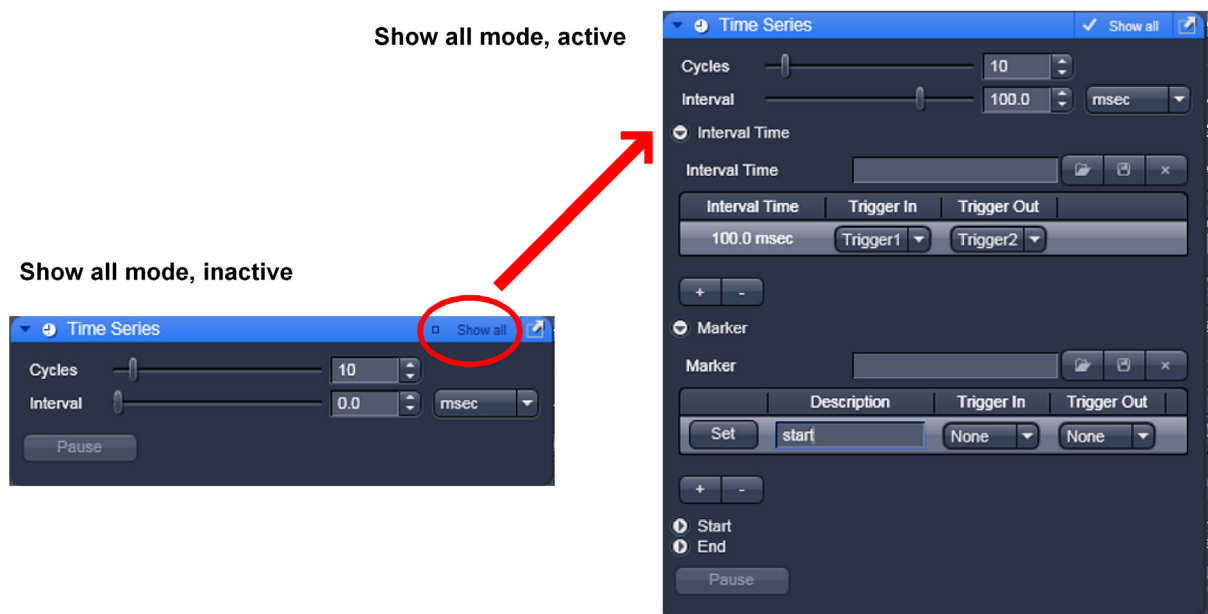
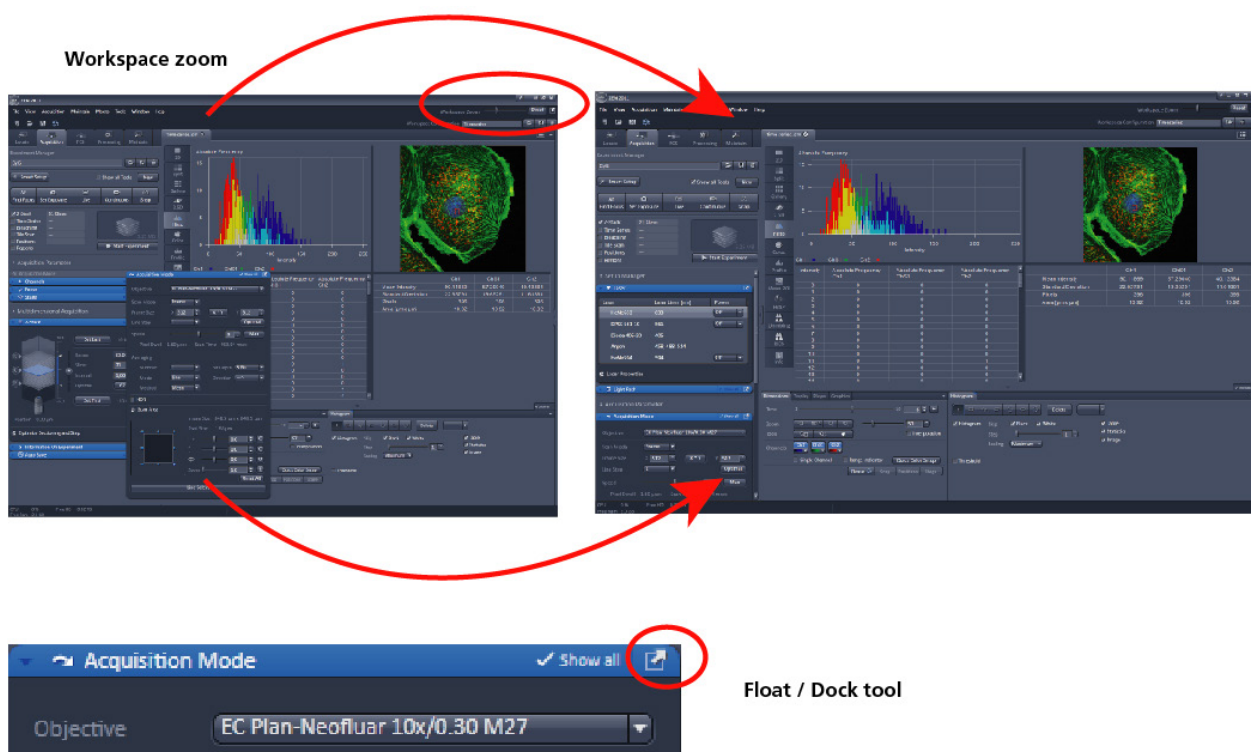


Fig. 6 Show all mode

A focus in the development of **ZEN 2012** was to fulfill the needs of both basic users and microscopy specialists. Both types of users will appreciate the set of intuitive tools designed to make the use of a confocal microscope from Carl Zeiss easy and fast:

The **Show all** concept ensures that tool panels are never more complex than needed. With **Show all** deactivated, the most commonly used tools are displayed. For each tool, the user can activate **Show all** mode to display and use additional functionality (Fig. 6).



**Fig. 7 ZEN Window Layout configuration**





More features of **ZEN 2012** include:

- The user can add more columns for tools to the **Left Tool Area** or detach individual tools to position them anywhere on the monitor. To add a column, drag a tool group by the title bar (e.g., Acquisition Parameter) to the right and a new tool column automatically opens. Alternatively use the context menu "Move Tool group to next column". To detach a tool, click on the little icon on the very right end of the blue tool header bar (Fig. 7).
- Another unique feature in Imaging Software is the scalable ZEN interface. This **Workspace Zoom** allows adjustment of the **ZEN 2012** window size and fonts to the situational needs or your personal preferences (Fig. 7).
- Setting up conventional confocal software for a specific experiment can take a long time and is often tedious to repeat. With **ZEN** these adjustments have to be done only once – and may be restored with just two clicks of the mouse. For each type of experiment one can now set-up and save the suitable **Workspace Layout**. These configurations can also be shared between users.
- For most controls, buttons and sliders, a **tool tip** is available. When the mouse pointer is kept over the button, a small pop-up window will display which function is covered by this tool/button.

These are just some of the most important features of the ZEN interface. For a more detailed description of the functionality for the **ZEN 2012** software, please refer to the User Manual that is provided with your system.




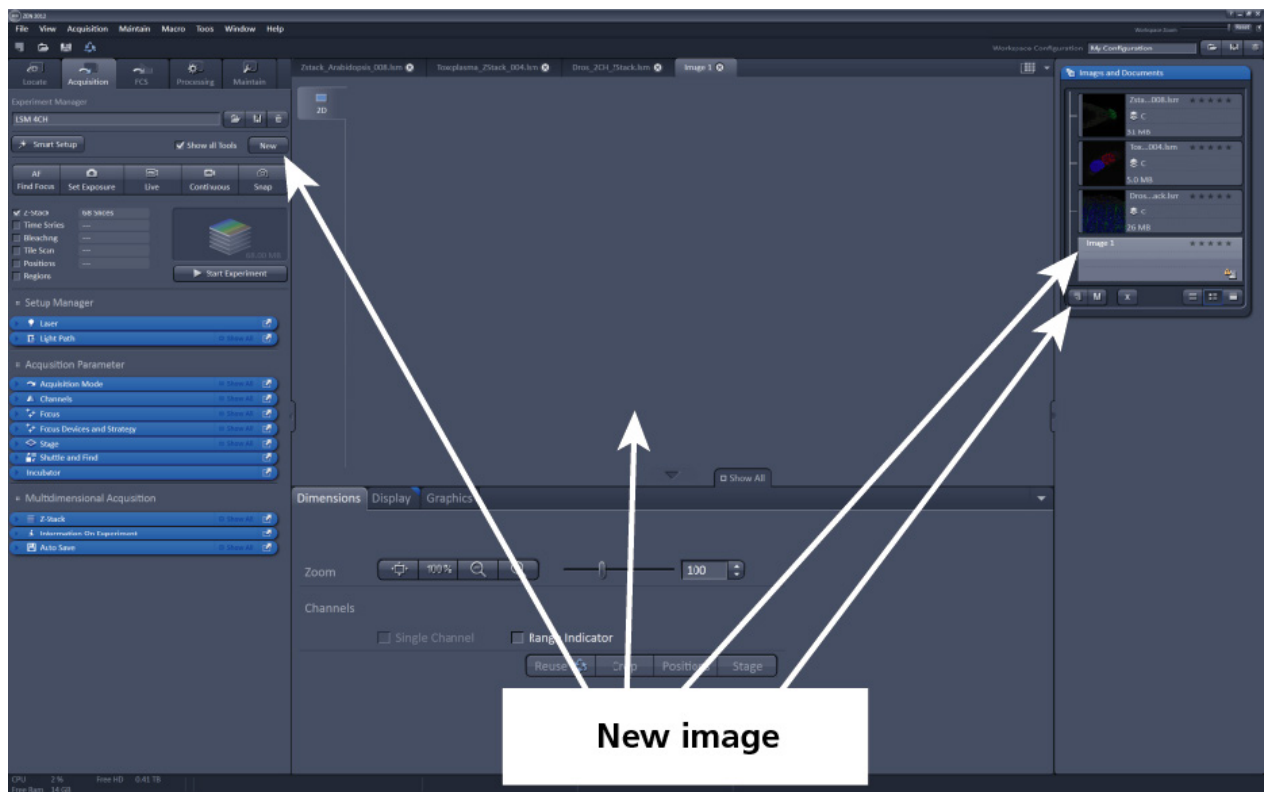
## Setting up a new image document and saving your data

To create a new image document in an empty image container, click the **Snap**  or the **Set Exposure**  button. For an empty image document press the **New**  or  buttons.

The new document is immediately presented in the **Documents** panel of the Right Tool Area. Remember, an unsaved 2D image in the active image tab will be over-written by a new scan. Multi-dimensional scans or saved images will never be over-written and a new scan will then automatically create a new image document.

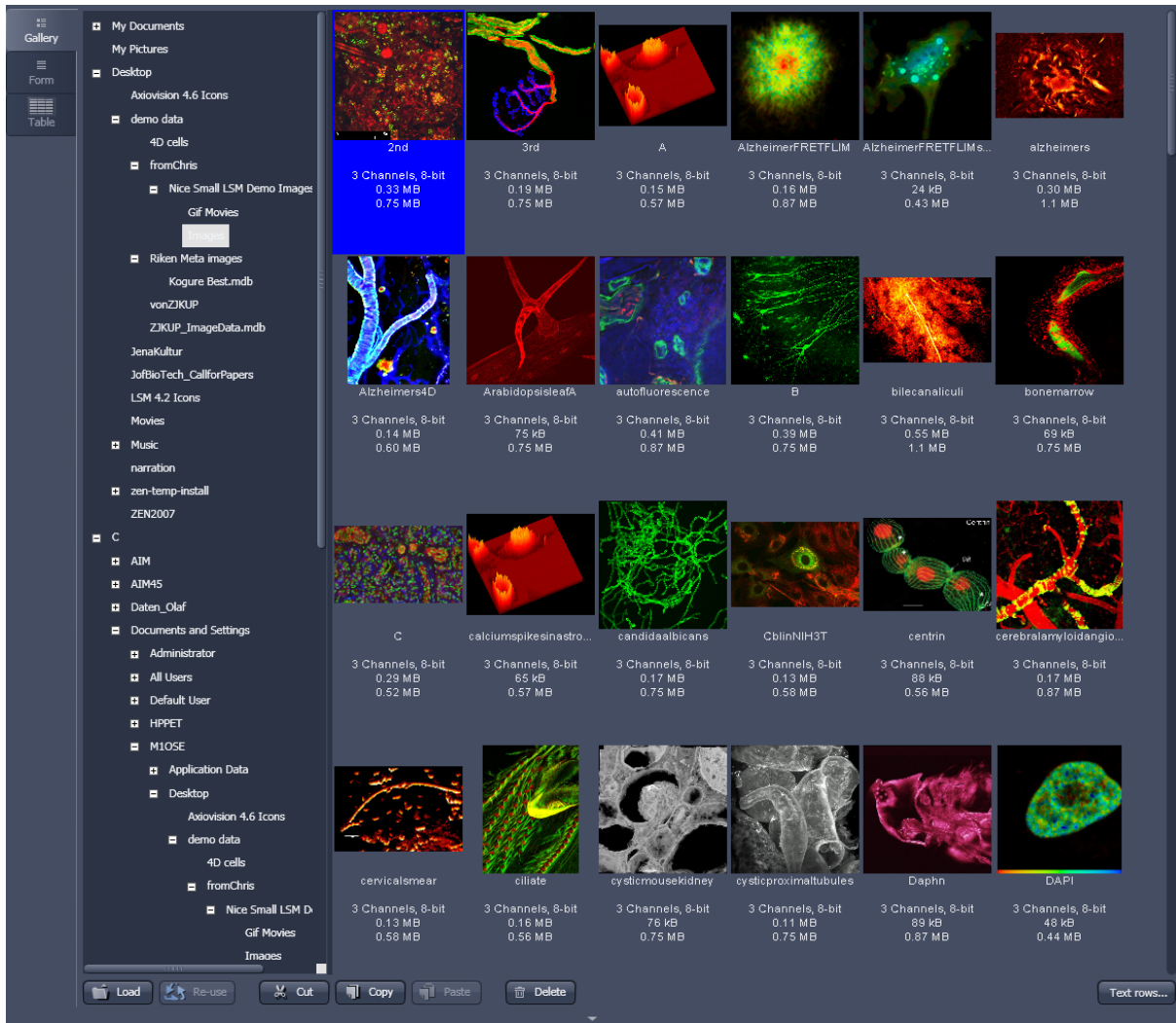
Acquired data is **not** automatically saved to disc. Make sure you save your data appropriately and back it up regularly. The ZEN software will ask you if you want to save your unsaved images when you try to close the application with unsaved images still open.

 There is no image database any more like in the earlier Zeiss LSM software versions.



**Fig. 8** New image document in the Open Images Areas

Advanced data browsing is available through the **ZEN File Browser** (Ctrl + F or from the **File** menu). The ZEN File Browser can be used like the WINDOWS program file browser. Images can be opened by double-click and image acquisition parameters are displayed with the thumbnails (Fig. 9). For more information on data browsing please refer to the detailed operating manual.



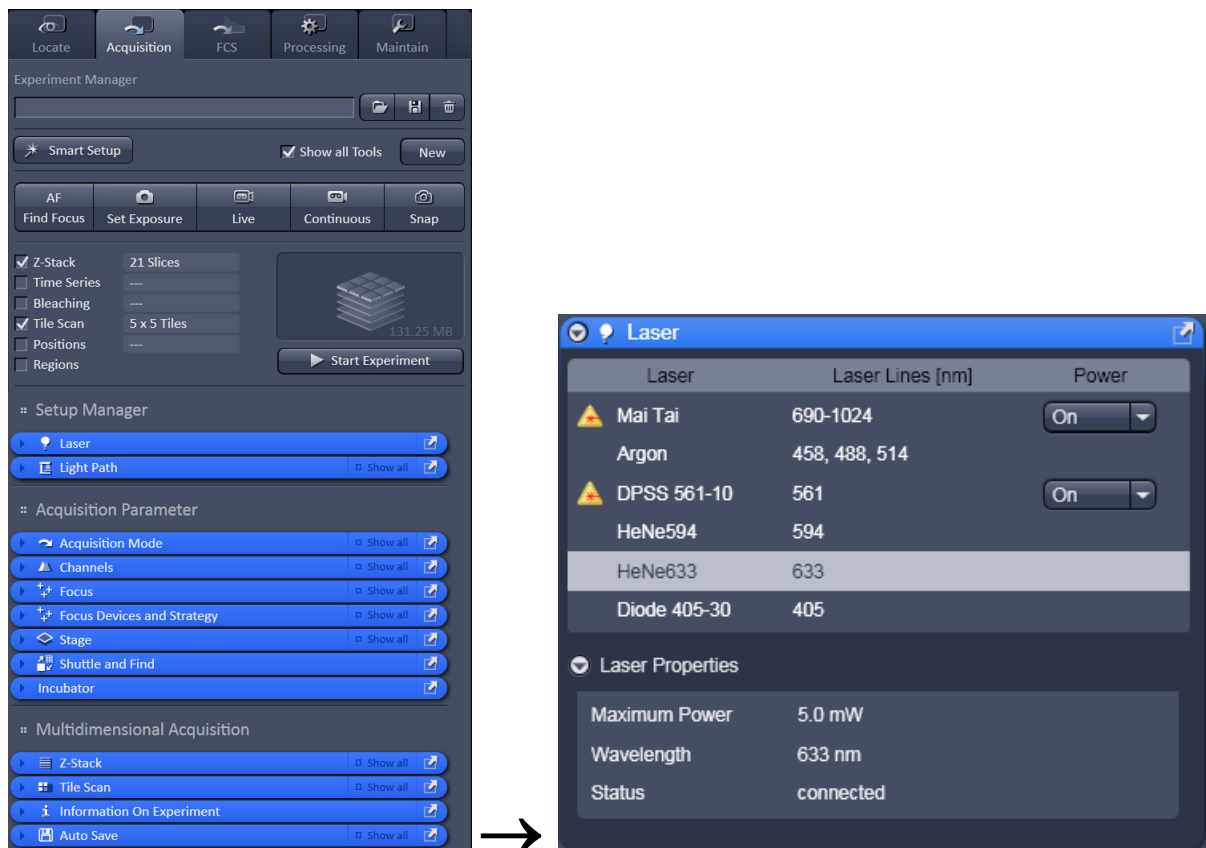
**Fig. 9** File Browser

## Turning on the lasers

**ZEN 2012** operates all lasers automatically. Whenever they are used (manually or by the **Smart Setup** function) the lasers are turned on automatically. The **Laser Life Extender** function of the software shuts all lasers off if ZEN is not used for more than 15 minutes.

To manually switch lasers on or off:

- Click the **Show all tools** tickbox and open the **Laser** tool. All available lasers can be operated within this tool (Fig. 10).



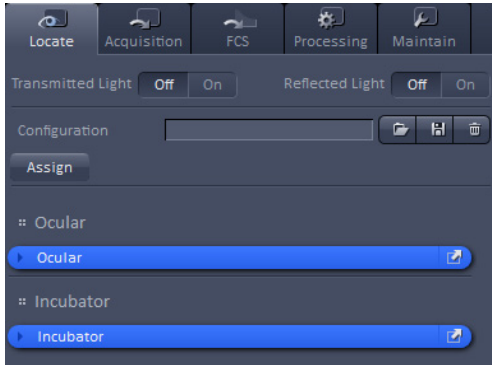
**Fig. 10** Laser Control tool

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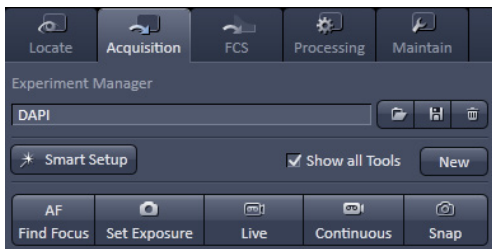
## Setting up the microscope

### Changing between direct observation and laser scanning mode

The **Locate** and **Acquisition** buttons switch between the use of the LSM and the microscope:



- Click on the **Locate** button to open the controls for the microscope beam path and for direct observation via the eyepieces of the binocular tube, lasers are blocked.



- Click on the **Acquisition** button to move back to the LSM system.

## Setting up the microscope and storing settings

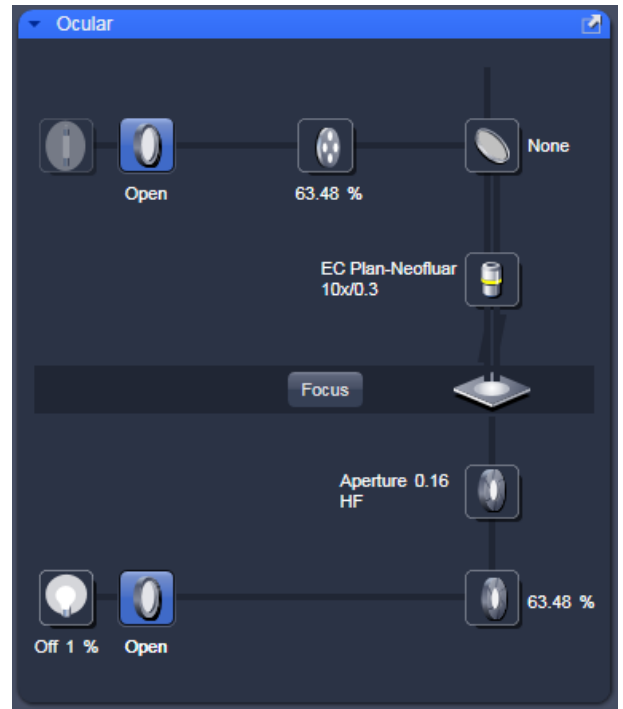
Click on the **Locate** tab for direct observation; press the **Oculars Online** button for your actions to take effect immediately. Then open the **Ocular** tool to configure the components of your microscope like filters, shutters or objectives (Fig. 11).

### Selecting an objective

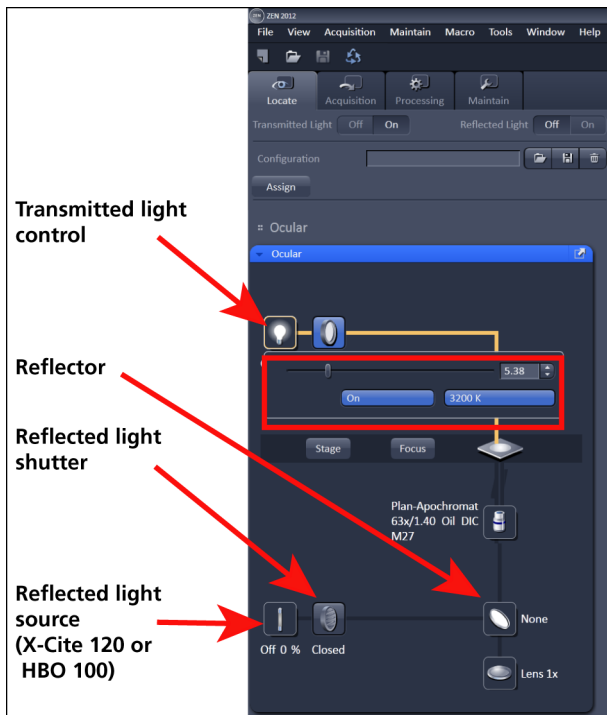
- Open the graphical pop-up menu by clicking on the **Objective** symbol and select the objective lens for your experiment (Fig. 11).
- The chosen objective lens will automatically move into the beam path.

### Focusing the microscope for transmitted light

- Open the graphical pop-up menu by clicking on the **Transmitted Light** icon (Fig. 12).
- Click on the **On** button. Set the intensity of the Halogen lamp using the slider.
- Clicking outside the pop-up control closes it.
- Place specimen on microscope stage. The cover slip must be facing the objective lens. Remember the immersion medium if the objective chosen requires it!
- Use the focusing drive of the microscope to focus the object plane.
- Select specimen detail by moving the stage in X and Y using the XY stage fine motion control.



**Fig. 11** Microscope Control window, e.g.: Axio Imager.Z2






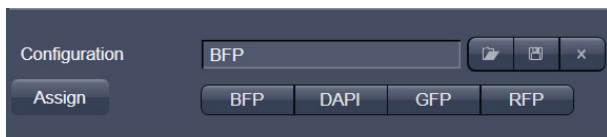
**Fig. 12** Microscope Control window with Transmitted Light pop-up menu

### Setting the microscope for reflected light

- Click on the **Reflected Light** icon to open the X-Cite 120 Controls and turn it on.
- Click on the **Reflected Light** shutter to open the shutter of the X-Cite 120 lamp / HBO100.
- Click on the **Reflector** button and select the desired filter set by clicking on it.

### Storing the microscope settings

Microscope settings can be stored as configurations (Fig. 13) by typing a config name in the pull-down selector and pressing the save  button. Fast restoration of a saved config is achieved by selecting the config from the pull-down list and pressing the  load button. The current config can be deleted by pressing the delete  button.

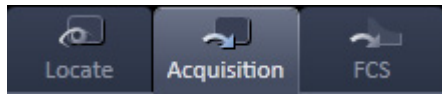


**Fig. 13** Configuration panel

These configurations can be assigned to buttons that are easier to press.

- ☞ Depending on the microscope configuration, settings must be done manually if necessary.

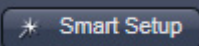
## Configuring the beam path and lasers

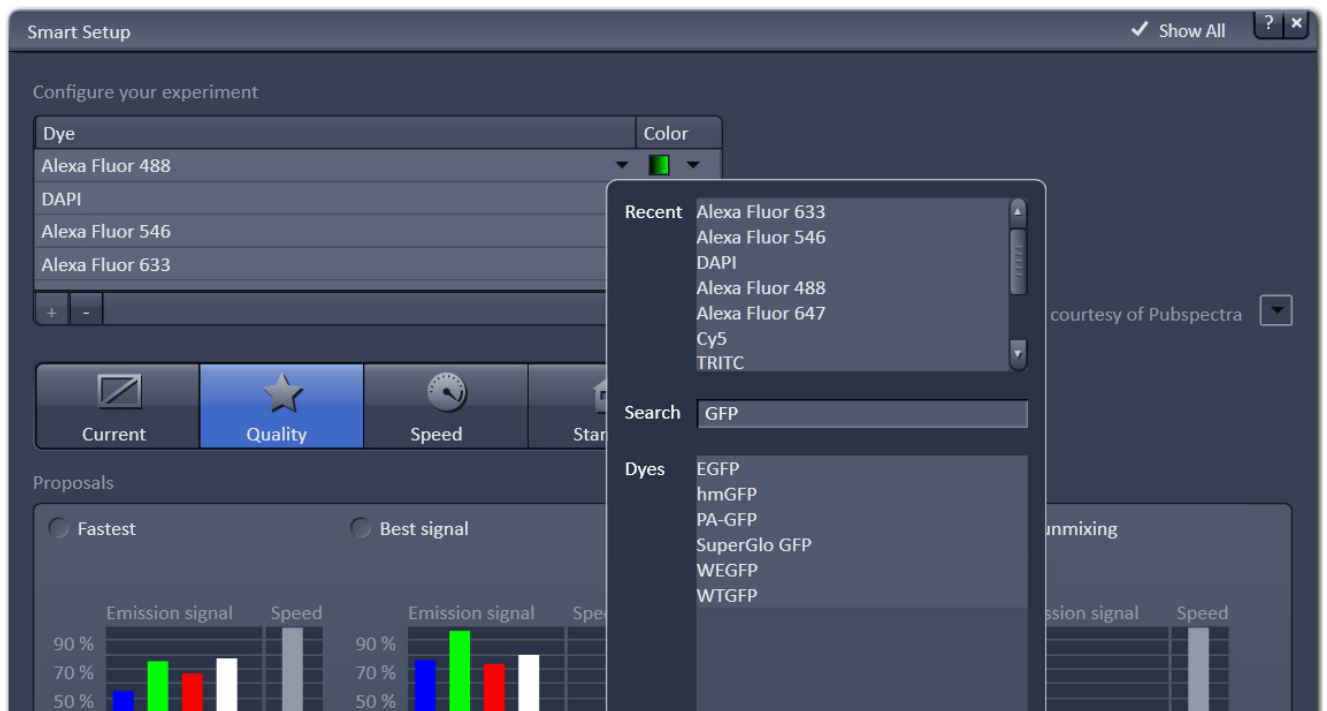


- Click the **Acquisition** button.

## Smart Setup

The tool **Smart Setup** is an intuitive, user-friendly interface which can be used for almost all standard applications. It configures all the system hardware for a chosen set of dyes.

- Click on the **Smart Setup** button  to open the smart setup window. This window can be accessed any time from the software to change dye combinations.
- Click on the arrow in the dye list and simply choose the dye(s) you want to use in your experiment from the list dialogue. In this dialogue, the dyes can be also searched by typing the name in the search field.



**Fig. 14** Smart setup tool

Once finished with the input, **Smart Setup** suggests four alternative considerations (see below): One for **Fastest** imaging, one for the **Best signal, Smartest (Line)** between both speed and best signal and the optimal setup for later **Linear unmixing** of the dyes.

The graphs display relative values for the expected emission signals and cross-talk. The resulting imaging scheme (single or multitrack) is shown below the graphs.

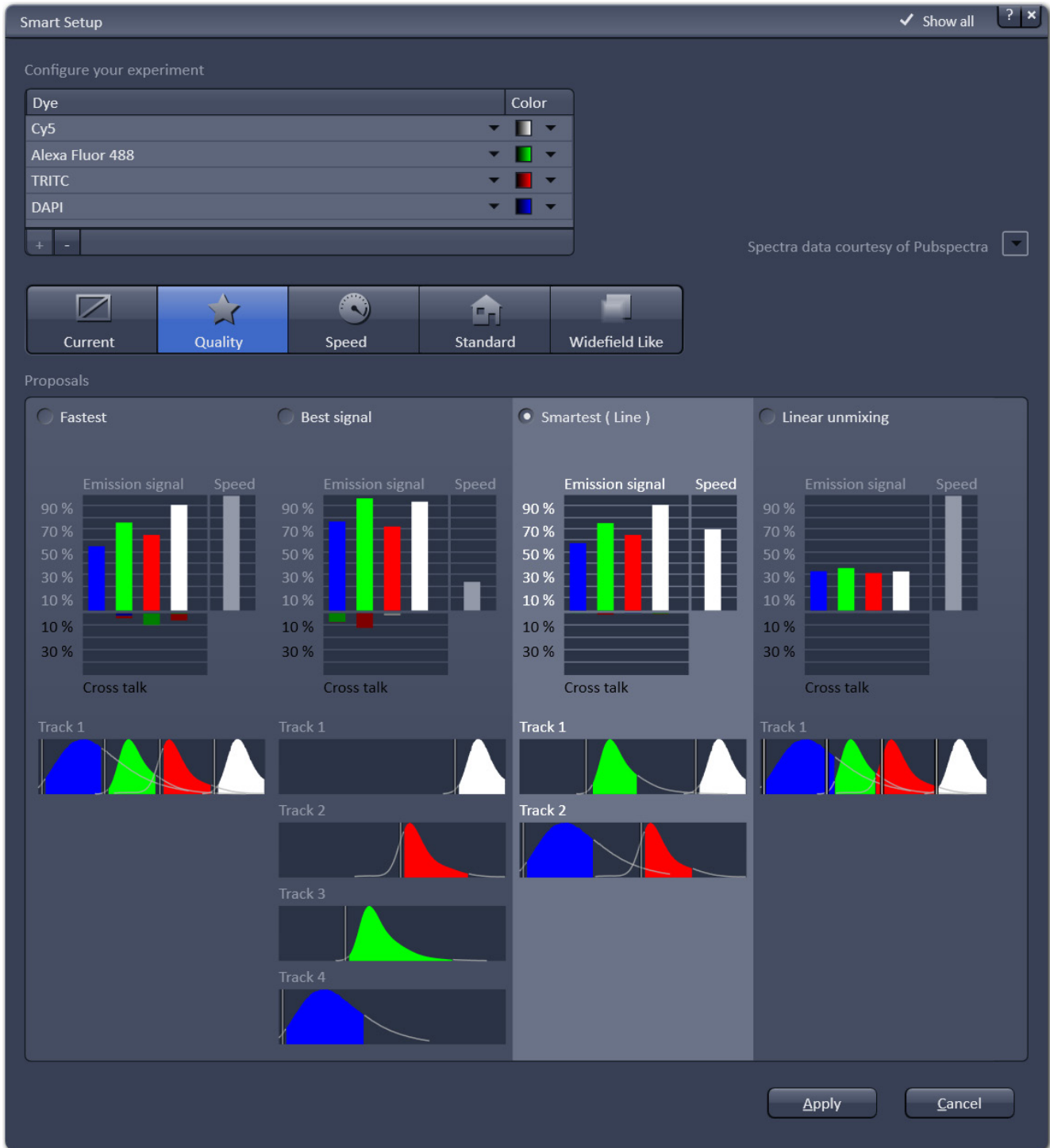


Fig. 15 Proposals panel of the Smart Setup tool



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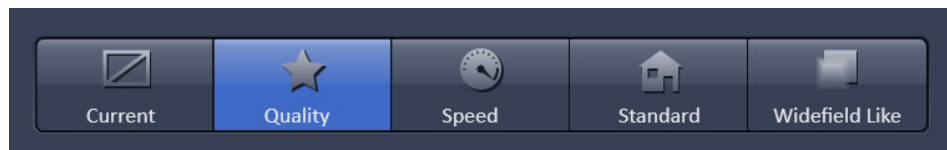
## Motifs

The feature **Motifs** is part of the **Smart Setup** panel. Like in photography, common imaging conditions with typical requirements also exist in laser scanning microscopy.

The different **Motifs** are presets of scanning parameters (pixels size, pinhole diameter, scan speeds etc.) addressing such conditions (Fig. 16).

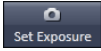
Upon selecting one of these options by mouse click the selected icon will be highlighted. If the motif **Current** is clicked, the current set of scanning parameters will be left untouched.

- Pressing the **Apply** button applies the selected proposal in Smart Setup as well as the motif.



**Fig. 16** The Motifs are presets for typical imaging tasks. The selected motif is highlighted.

If the option **Linear unmixing** is selected, the system is set in the lambda mode automatically.

Pressing the **Set Exposure**  button will then optimize the settings of the Gain (Master) and offset for the given laser power and pinhole size. Further image optimization from this point can be done easily.

## Setting up a configuration manually

**Simultaneous** scanning of single, double and triple labeling:

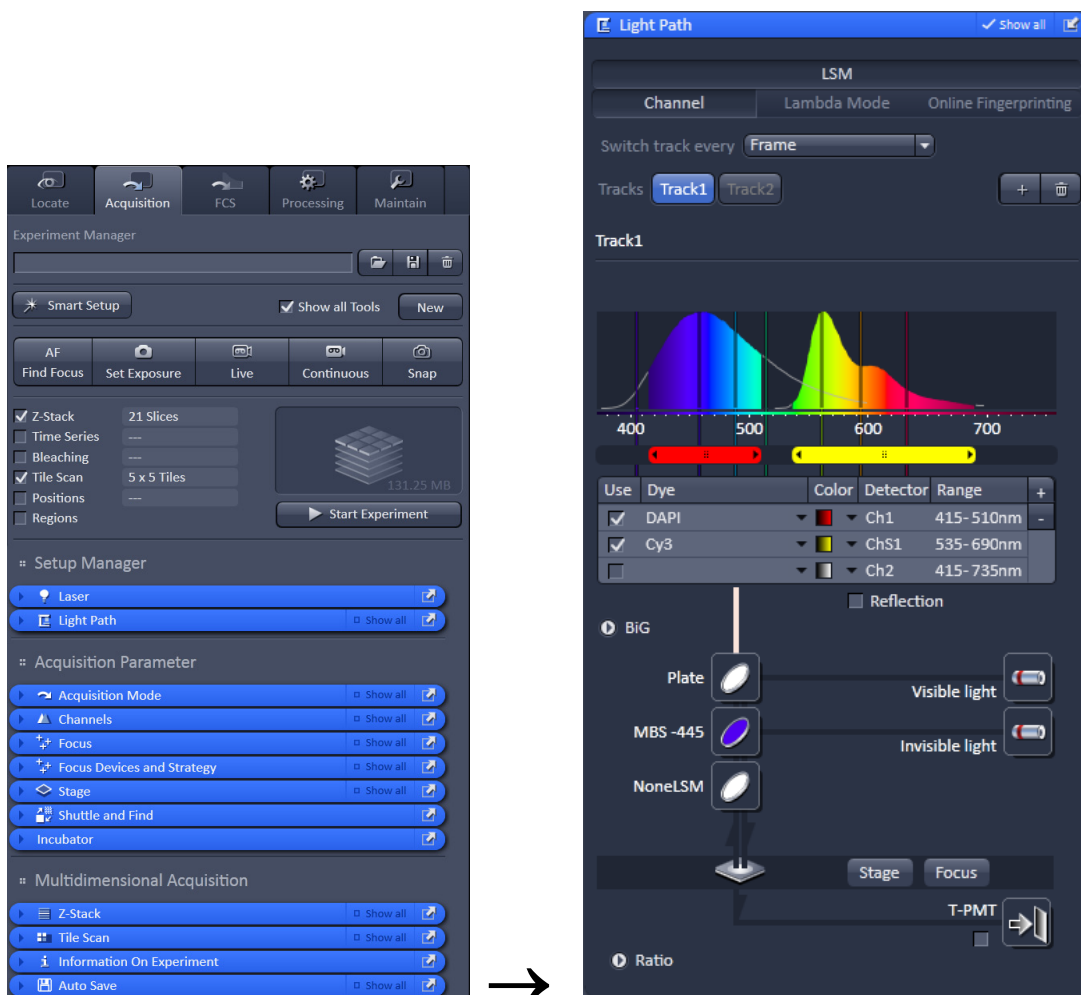
- Advantage: faster image acquisition
- Disadvantage: potential cross-talk between channels

**Sequential** scanning of double and triple labeling; line-by-line or frame-by-frame:

- Advantage: Only one detector and one laser are switched on at any one time. This reduces cross-talk.
- Disadvantage: slower image acquisition

- Open the **Light Path** tool in the **Setup Manager** tool group and the **Channels** tool in the **Acquisition Parameter** tool group to access the hardware control window to set-up the beam path.

The open **Light Path** is shown in Fig. 17.



**Fig. 17** Light Path tool for a single track (LSM)

## Settings for track configuration in Channel mode

- Select **Channel** mode if necessary (Fig. 17).

The **Light Path** tool displays the selected track configuration which is used for the scan procedure.

- You can change the settings of this panel using the following function elements:



Activation / deactivation of the excitation wavelengths for visible and invisible light (check box) and setting of excitation intensities (slider). If necessary open the **Laser Control** tool (see above).



Selection of the main dichroic beam splitter (MBS) for visible and invisible light from the relevant list box.



Selection of an emission filter through selection from the relevant list box.



Activation / deactivation (via check box) of the selected channel (Ch 1-4, monitor diode ChM, QUASAR detectors ChS1-8, transmission ChD) for the scanning procedure and assigning a color to the channel.

- Select the appropriate filters and activate the channels (Fig. 17).
- Click the **Laser** icon to select the laser lines and set the attenuation values (transmission in %) in the displayed window (Fig. 17).
- For the configuration of the beam path, please refer to the application-specific configurations depending on the used dyes and markers and the existing instrument configuration (Fig. 18).
- The Detection Bands & Laser Lines are displayed in a spectral panel (Fig. 18) to visualize the activated laser lines for excitation (vertical lines) and activated detection channels (colored horizontal bars).

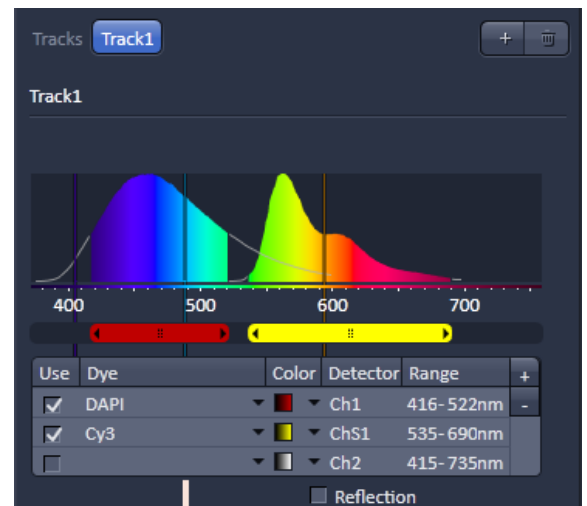





Fig. 18 Detection bands and laser lines display

- For storing a new track configuration open the **Channels** tool in the **Acquisition Parameter** tool group, click  and enter a desired name in the first line of the list box (Fig. 19), then click **Ok** to store the configuration.
- For loading an existing configuration click  then select it from the list box.
- For deleting an existing configuration click  then select it from the list box and confirm the deletion with **Ok**.

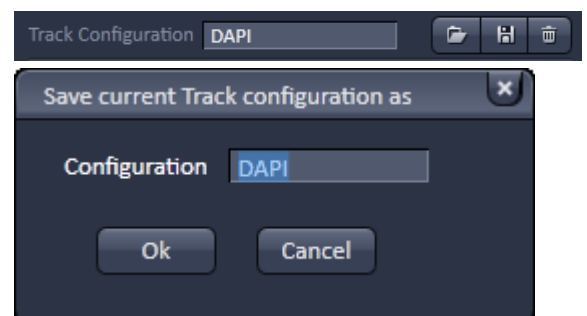


Fig. 19 Track configurations functions

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## Settings for multiple track configurations in Channel Mode

**Multiple track** set-ups for sequential scanning can be defined as one configuration (**Channel Mode Configuration**), to be stored under any name, reloaded or deleted.

The maximum of four tracks with up to eight channels can be defined simultaneously and then scanned one after the other. Each track is a separate unit and can be configured independently from the other tracks with regard to channels, Acousto-Optical Tunable Filters (AOTF), emission filters and dichroic beam splitters.

The following functions are available in the **Light Path** tool of the **Setup Manager** tool group (Fig. 17).

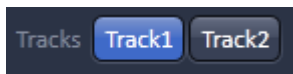
### Switch track every selection box

**Line** Tracks are switched during scanning line-by-line. The following settings can be changed between tracks: Laser line, laser intensity and channels.

**Frame** Tracks are switched during scanning frame-by-frame. The following settings can be changed between tracks: Laser line and intensity, all filters and beam splitters, the channels incl. settings for gain and offset and the pinhole position and diameter.

**Frame Fast** The scanning procedure can be made faster. Only the laser line intensity and the **Amplifier Offset** are switched, but no other hardware components. The tracks are all matched to the current track with regard to emission filter, dichroic beam splitter, setting of Detector Gain, pinhole position and diameter. When the **Line** button is selected, the same rules apply as for **Frame Fast**.

### Tracks line



**Track** buttons: To change between the tracks settings click on the appropriate track button. The selected track is highlighted. The setting table for configuration will be shown behind.



**Add Track** button: An additional track is added to the configuration list in the **Imaging Setup Tool**. The maximum of four tracks can be used. One track each with basic configuration is added, i.e.: Ch 1 channel is activated, all laser lines are switched off, emission filters and dichroic beam splitters are set in accordance with the last configuration used.



**Remove** button: The track marked in the **List of Tracks** panel is deleted.

## Scanning an image

### Setting the parameters for scanning

- Select the **Acquisition Mode** tool from the **Left Tool Area** (Fig. 20).
- Select the **Frame Size** as predefined number of pixels or enter your own values (e.g. 300 x 600) in the **Acquisition Mode** tool. Click on the **Optimal** button for calculation of appropriate number of pixels depending on objective N.A. and  $\lambda$ .

The number of pixels influences the image resolution!

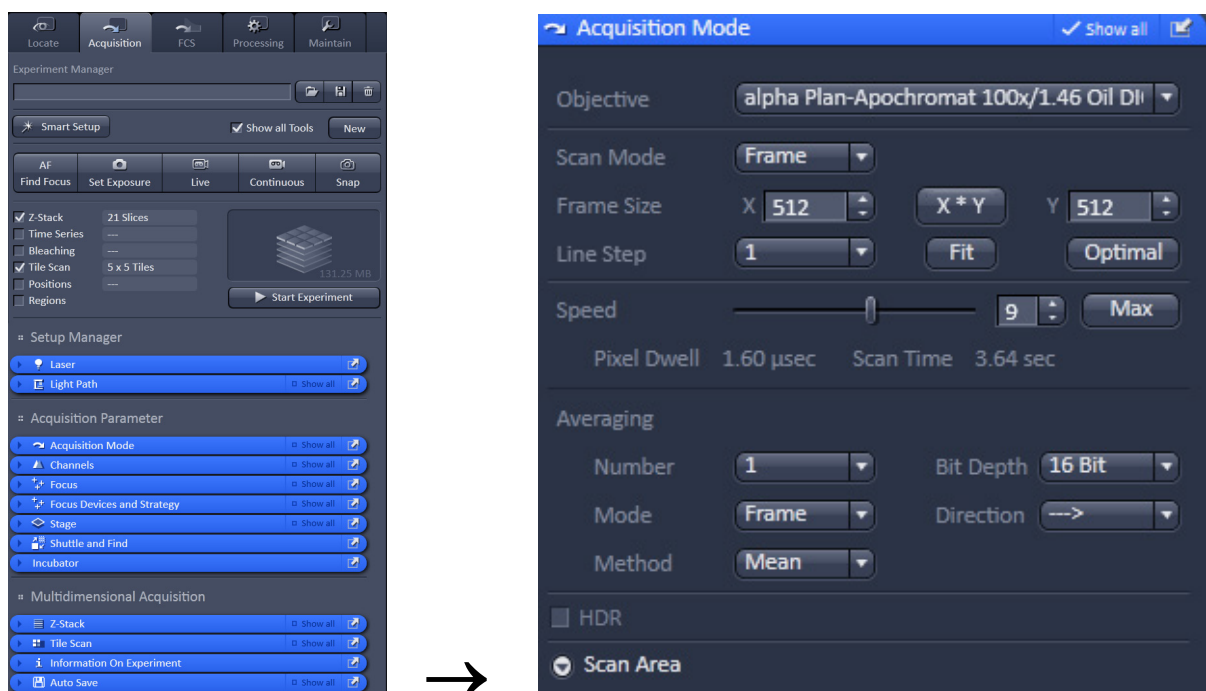


Fig. 20 Acquisition Mode tool

### Adjusting scan speed

- Use the **Scan Speed** slider in the **Acquisition Mode** tool (Fig. 20) to adjust the scan speed.

A higher speed with averaging results in the best signal-to-noise ratio. Scan speed 8 usually produces good results. Use speed 6 or 7 for superior images.

### Choosing the dynamic range

- Select the dynamic range 8 or 12 Bit (per pixel) in the **Bit Depth** pull-down in the **Acquisition Mode** tool (Fig. 20).

8 Bit will give 256 gray levels; 12 Bit will give 4096 gray levels. Publication quality images should be acquired using 12 Bit data depth. 12 Bit is also recommended when doing quantitative measurements or when imaging low fluorescence intensities.

## Setting scan averaging

Averaging improves the image by increasing the signal-to-noise ratio. Averaging scans can be carried out line-by-line or frame-by-frame. Frame averaging helps to reduce photo-bleaching, but does not give quite as smooth of an image.

- For averaging, select the **Line** or **Frame** mode in the **Acquisition Mode** tool.
- Select the number of lines or frames to average.

## Adjusting pinhole size

- Select the **Channels** tool in the **Left Tool Area**.
- Set the **Pinhole** size to **1 AU** (Airy unit) for best compromise between depth discrimination and detection efficiency.

Pinhole adjustment changes the **Optical Slice thickness**. When collecting multi-channel images, adjust the pinholes so that each channel has the same **Optical Slice thickness**. This is important for colocalization studies.

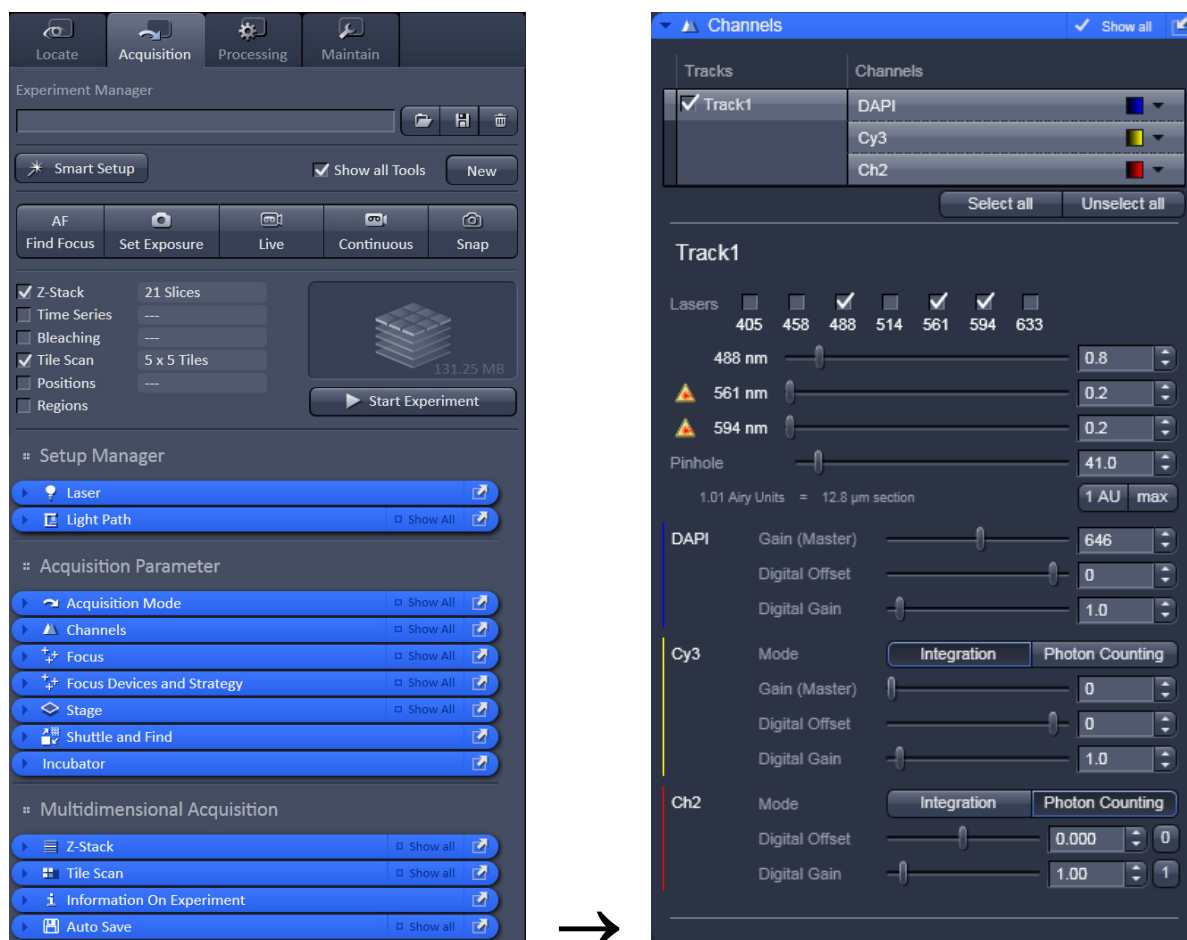
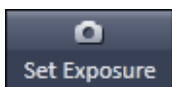


Fig. 21 Channels tool

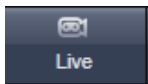
## Image acquisition

Once you have set up your parameter as defined in the above section, you can acquire a frame image of your specimen.

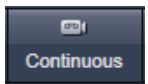
- Use one of the **Set Exposure**, **Live**, **Continuous** or **Snap** buttons to start the scanning procedure to acquire an image.
- Scanned images are shown in separate windows.
- Click on the **Stop** button to stop the current scan procedure if necessary.



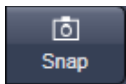
Select **Set Exposure** for automatic pre-adjustment of detector gain and offset.



Select **Live** for continuous fast scanning – useful for finding and changing the focus.



Select **Continuous** for continuous scanning with the selected scan speed.



Select **Snap** for recording a single image.



Select **Stop** for stopping the current scan procedure.

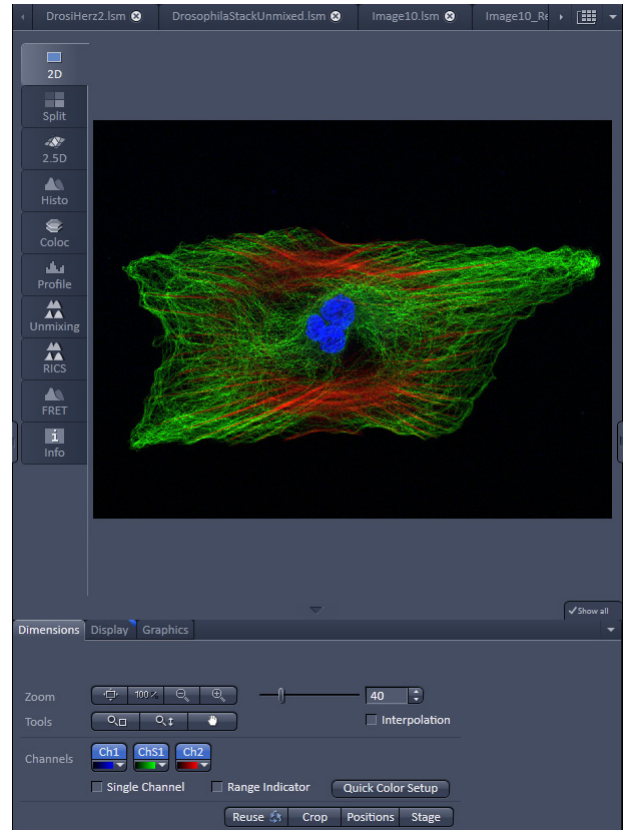



Fig. 22 Image Display

## Image optimization

### Choosing Range Indicator

- In the **View – Dimensions** View Option Control Block, activate the **Range indicator** check box (Fig. 23).

Clicking on the right hand side of the  button leads to a list of colors.

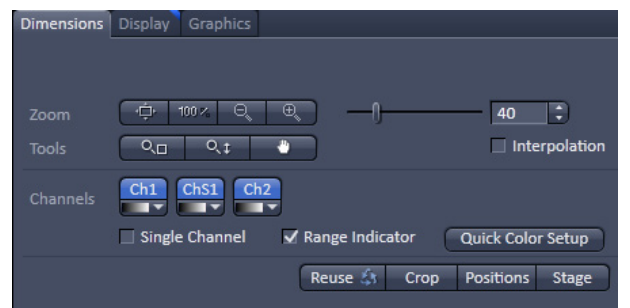


Fig. 23 View Dimensions Control Block

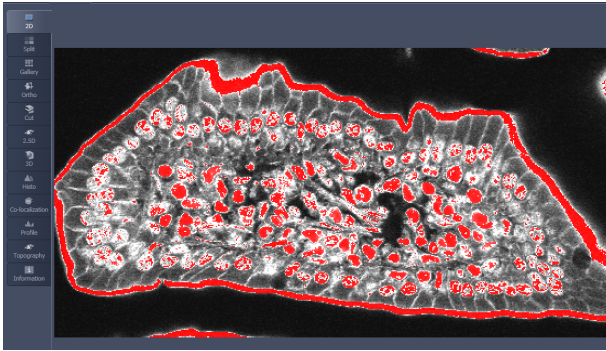


Fig. 24 Image Display

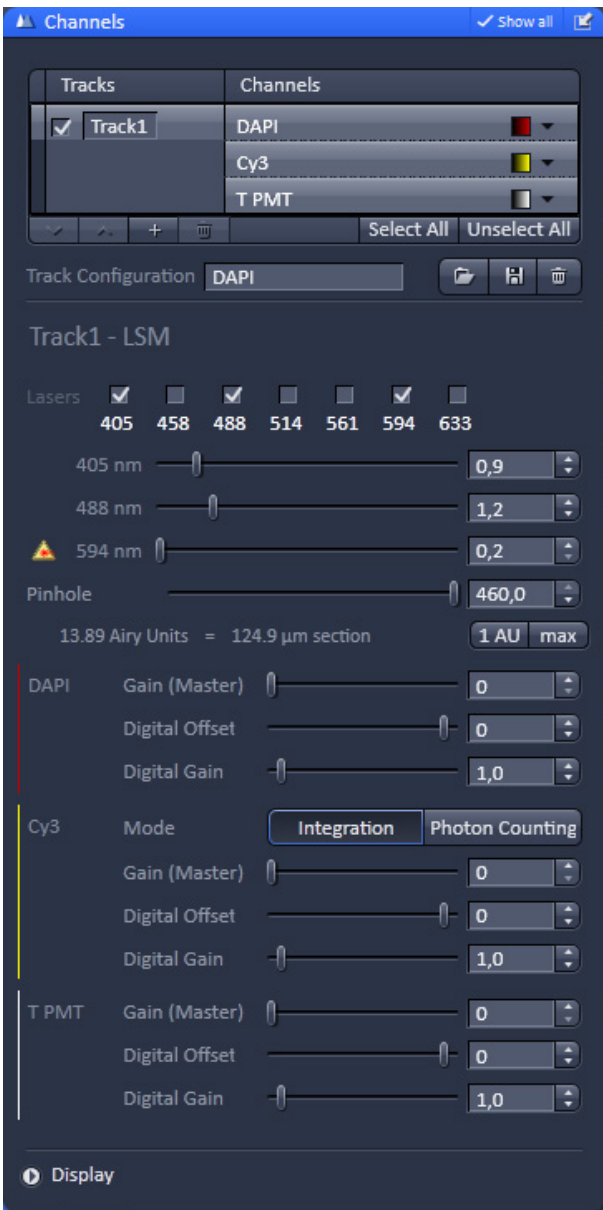


Fig. 25 Channels tool

The scanned image appears in a false-color presentation (Fig. 24).

If the image is too bright, it appears red on the screen. Red = saturation (maximum).

If the image is not bright enough, it appears blue on the screen. Blue = zero (minimum).

### Adjusting the laser intensity

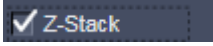
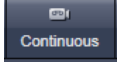
- Set the **Pinhole** to **1** Airy Unit (Fig. 25).
- Set the **Gain (Master)** high.
- When the image is saturated, reduce AOTF transmission in the **Laser** control section of the **Channels Tool** (Fig. 25) using the slider to reduce the intensity of the laser light to the specimen.

### Adjusting gain and offset


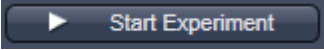

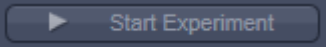
- Increase the **Digital Offset** until all blue pixels disappear, and then make it slightly positive (Fig. 25).
- Reduce the **Gain (Master)** until the red pixels only just disappear.



## Scanning a Z-Stack

- Select **Z-Stack**  in the main tools area.
- Open the **Z Stack** tool in the **Left Tool Area**.
- Select **Mode First/Last** on the top of the **Z-Stack** tool.
- Click on the  button in the **Action** button area.

A continuous XY-scan of the set focus position will be performed.

- Use the focus drive of the microscope to focus on the upper position of the specimen area where the Z-Stack is to start.
  - Click on the **Set First** button to set the upper position of the Z-Stack.
  - Then focus on the lower specimen area where the recording of the Z-Stack is to end.
  - Click on the **Set Last** button to set this lower position.
  - Click on the  button to set number of slices to match the optimal Z-interval for the given stack size, objective lens, and the pinhole diameter.
  - Click on the  **Start Experiment** button to start the recording of the Z-Stack.
-  When a multi-dimensional acquisition tool is not selected, the respective tool and its set parameters are not included in the multidimensional image acquisition. If no multidimensional tool is activated, the  **Start Experiment** button is grayed out and only single images can be scanned.

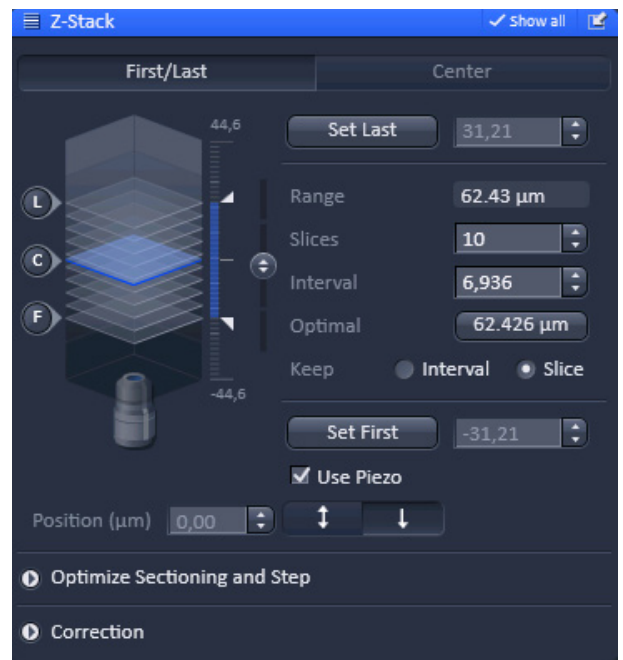


Fig. 26 Z-Stack tool

## Storing and exporting image data

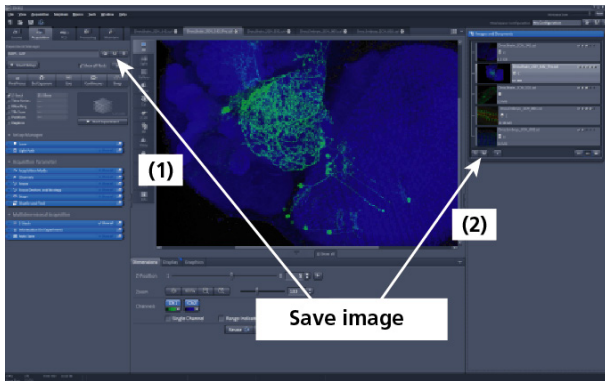


Fig. 27 Save Image buttons in ZEN

- To save your acquired or processed images, click on the **Save** or **Save As** button in **File** menu, or click the  button in the main toolbar (Fig. 27/1), or click on the  button at the bottom of the **File Handling Area** (Fig. 27/2).

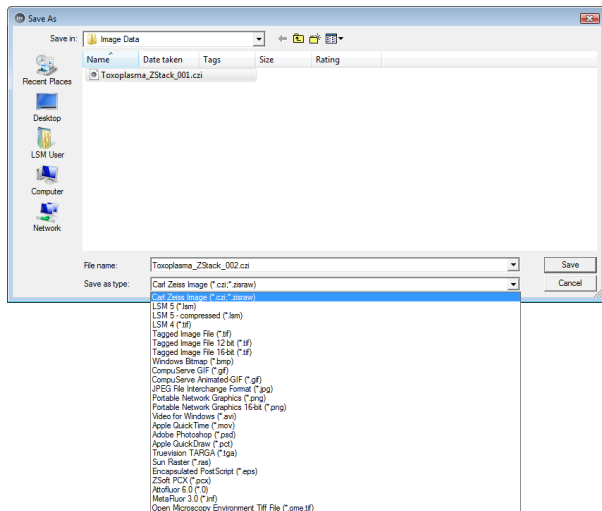


Fig. 28 Save as window

- The WINDOWS **Save As** window appears.
- Enter a file name and choose the appropriate image format. Note: the LSM 5 format is the native Carl Zeiss LSM image data format and contains all available extra information and hardware settings of your experiment.
- Click on the **Save** button.

If you close an image which has not been saved, a pop-up window will ask you if you want to save it. Choosing **yes** will lead you to the WINDOWS **Save As** window.

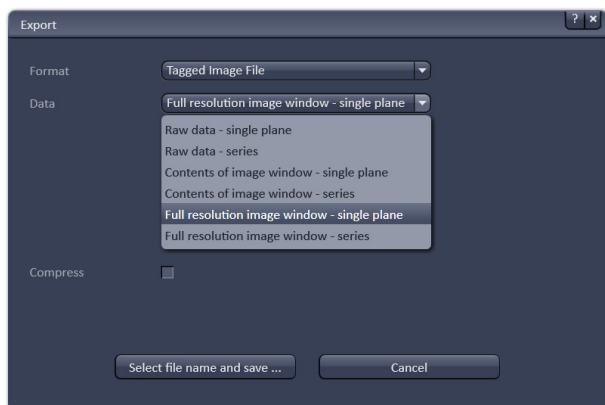
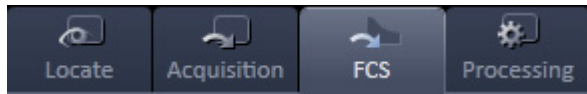


Fig. 29 Export window

To export image display data, a single optical section in raw data format or the contents of the image display window including analysis and overlays, choose **Export** from the **File** menu. In the **Export** window you can select from a number of options and proceed to the WINDOWS **Save As** window to save the exported data to disk.

## Using FCS



- Click the **FCS** button for Fluorescence Correlation Spectroscopy.
- Use the **FCS** tool groups (**Setup**, **Navigation**, **Multidimensional Acquisition**) in the **Left Tool Area** to acquire and analyze **FCS** data.

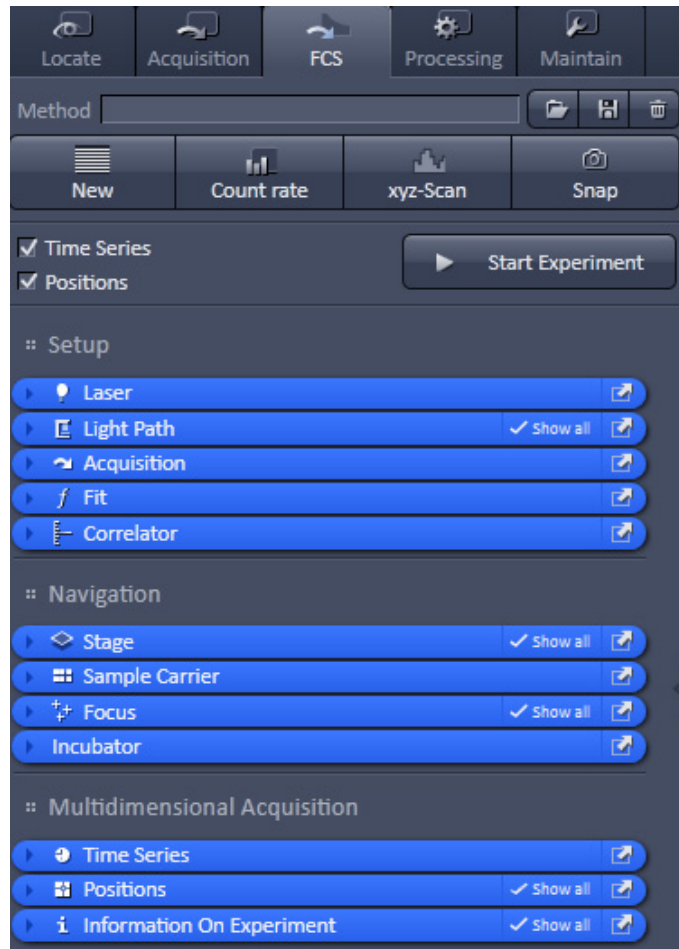


Fig. 30 FCS Panel

## Setting a configuration



- Open the **Light Path** tool to define the light path, laser lines and pinhole position.

Depending on the system configuration, the **Light Path** displays the selected track, which is used for the FCS procedure. The pinhole position and size can be set by pressing the **Adjust pinhole...** button.

The laser line and power can be set in the **Visible light / Invisible light** drop down menu by pressing the respective laser icon (see Fig. 31).

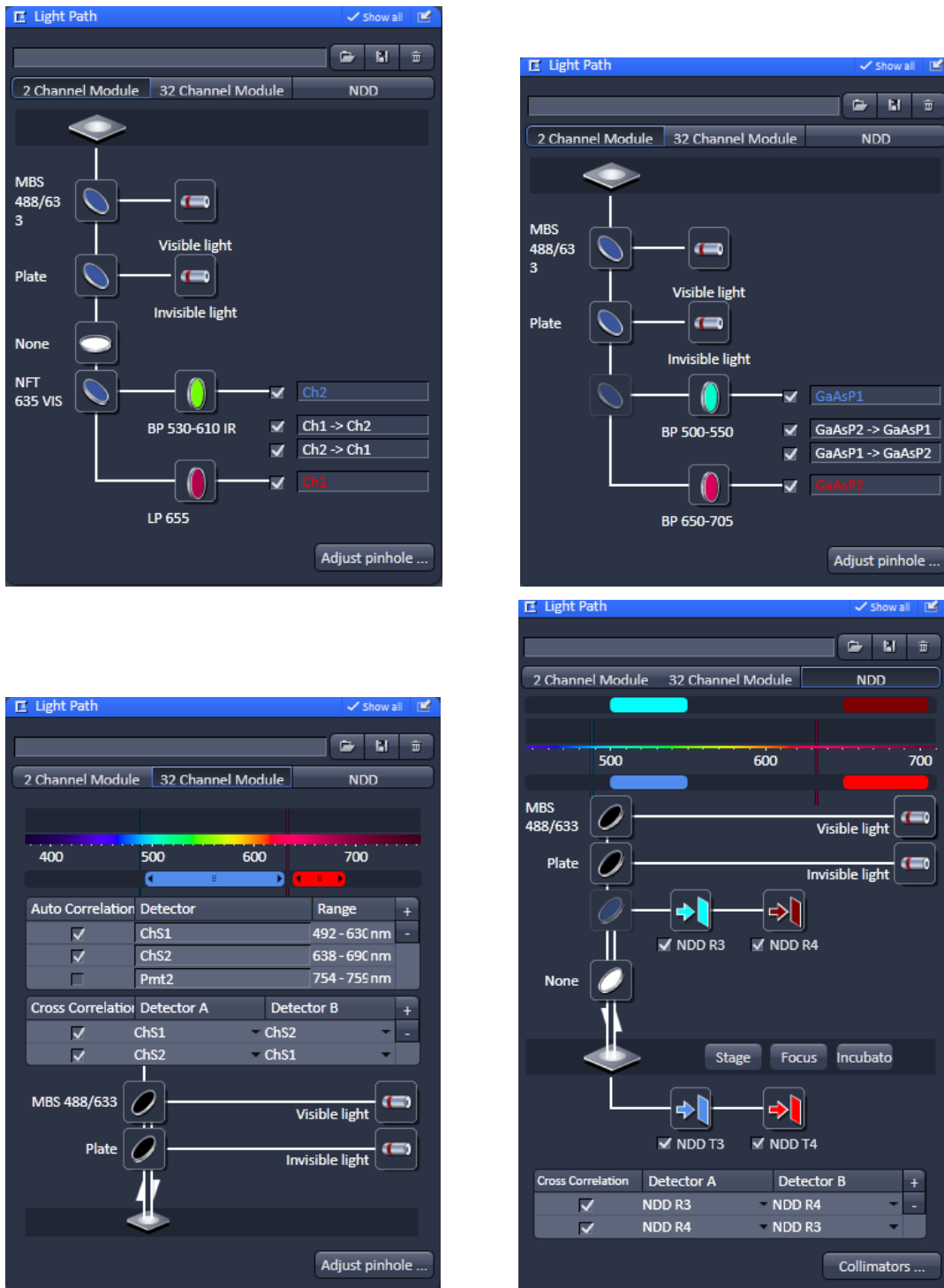


Fig. 31 Light path tool of a LSM 780 system with the 2 Channel mode ConfoCor 3 (above left), 2 Channel mode BiG (above right), 32 channel mode Quasar (below left) and Channel mode NDD (below right)

You can change the settings of this panel using the following function elements:



Activation / deactivation of the excitation wavelengths (check box) and setting of excitation intensities (slider). Open the **Laser Control** tool by clicking the **Laser** icon.



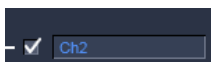
Selection of the main dichroic beam splitter (MBS) or secondary dichroic beam splitter (SBS) position (ConfoCor 3 only) through selection from the relevant list box.



Selection of a block filter (ConfoCor 3 only) through selection from the relevant list box.

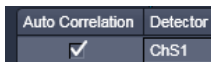


Selection of an emission filter (ConfoCor 3 only) through selection from the relevant list box.

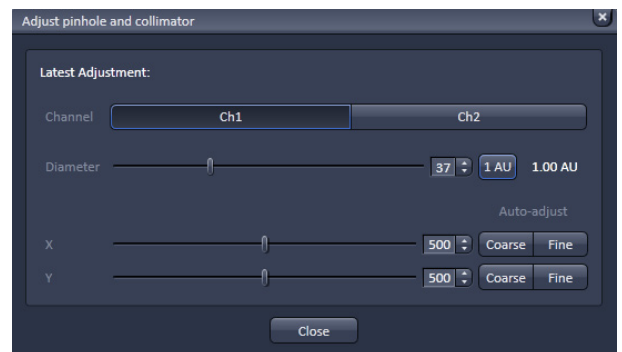


Activation / deactivation (via check box) of the selected channel (shown for the ConfoCor 3 and Quasar))

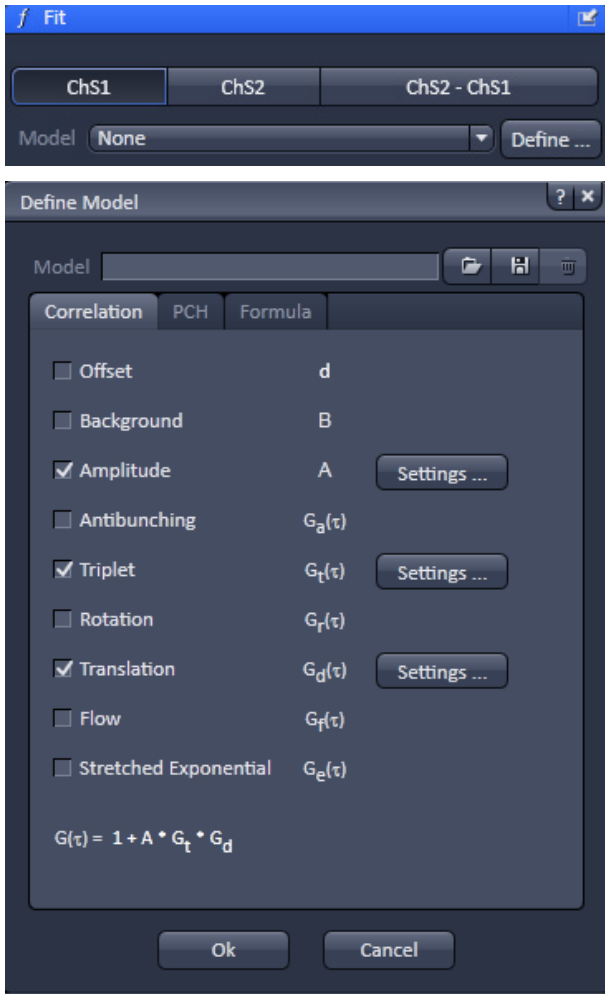
or



- Set the pinhole **Diameter** (via slider or input box; not available for **NDD**).
- Press the **Count rate** button to open the Real Time display window for the detector **Count rate** in all active channels. Adjust the laser power in the **Laser Control** panel to obtain a satisfactory count rate.
- Press **Auto-adjust** buttons to align the pinhole for each newly defined beam path. After adjusting the sample carrier, align the pinhole automatically in **X** and **Y** by conducting a coarse first and then a fine alignment.



**Fig. 32 Pinhole adjustment**



Use the **Fit** tool to define model equations to which measured data can be fitted by pressing the **Define** button.

There are three options to define a model:

- **Correlation:** assemble a correlation model from predefined equations, which will be fitted analytically
- **PCH:** assemble a photon counting histogram model, which will be fitted numerically
- **Formula:** program a user defined model equation, which can be fitted analytically

**Fig. 33** Fit tool (above) and Define Model (below) windows

## Starting a measurement

- Open the **Acquisition** tool.

The panel of the **Acquisition** tool displays the selected measurement conditions used for the FCS experiment.

Enter the, **Measure Time**, **Repetitions** and **Bleach Time** into the corresponding input boxes and select the colors for the correlation functions.

Activate the required lasers and set the laser power.

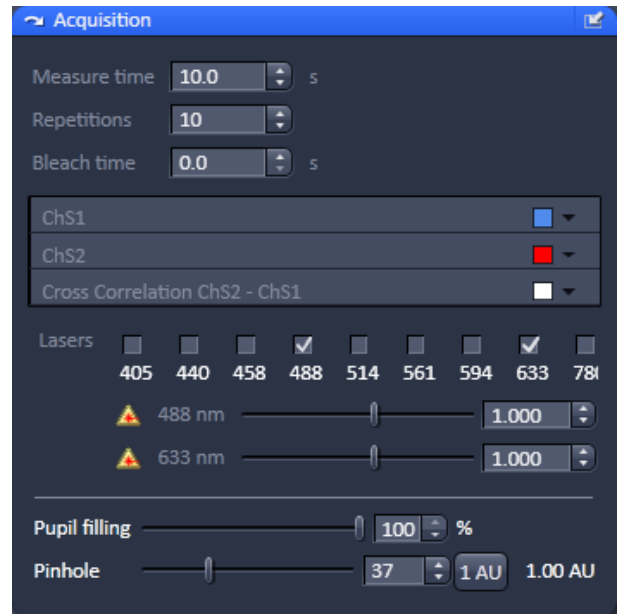


Fig. 34 Acquisition tool

- Open the **Time Series** tool.

Define a kinetic procedure by entering the cycle **Number**, the **Point to point time** distance between measurements, and the **Shape** in the corresponding input boxes.

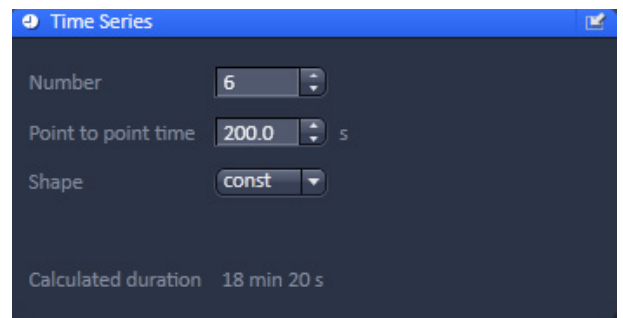


Fig. 35 Time Series tool

- Open the **Positions** tool.

Select the carrier and the sample or laser position by the stage or by the scanners.

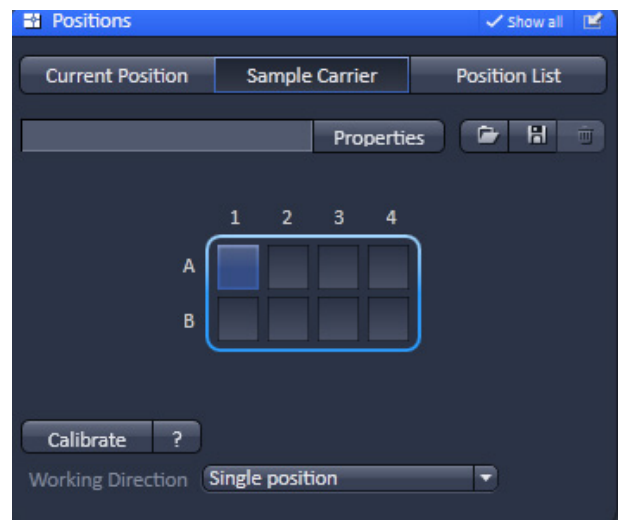
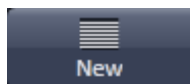


Fig. 36 Positions tool

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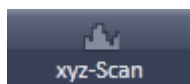
Start and monitor the experiment with the **Action** buttons.



Press the **New** button to open a new **FCS diagram** into an image container. If a measurement is triggered, all data are displayed in that window if highlighted.



Press the **Count rate** button to open the real time display window for the detector **Count rate** in all active channels. This allows you to optimize your experiment by changing the laser power and the pinhole size while monitoring the count rate.



Press the **xyz-Scan** button to display the current coordinates. You can define boundaries where a scan is performed with simultaneous acquisition of the count rate. This allows you, for example, to identify labeled molecules accumulated in the membrane.



Press the **Snap** button to trigger one measurement at the highlighted or first defined position.

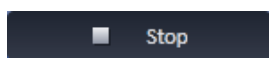


Press the **Start ConfoCor Experiment** button to trigger a measurement. All defined positions will be approached consecutively.



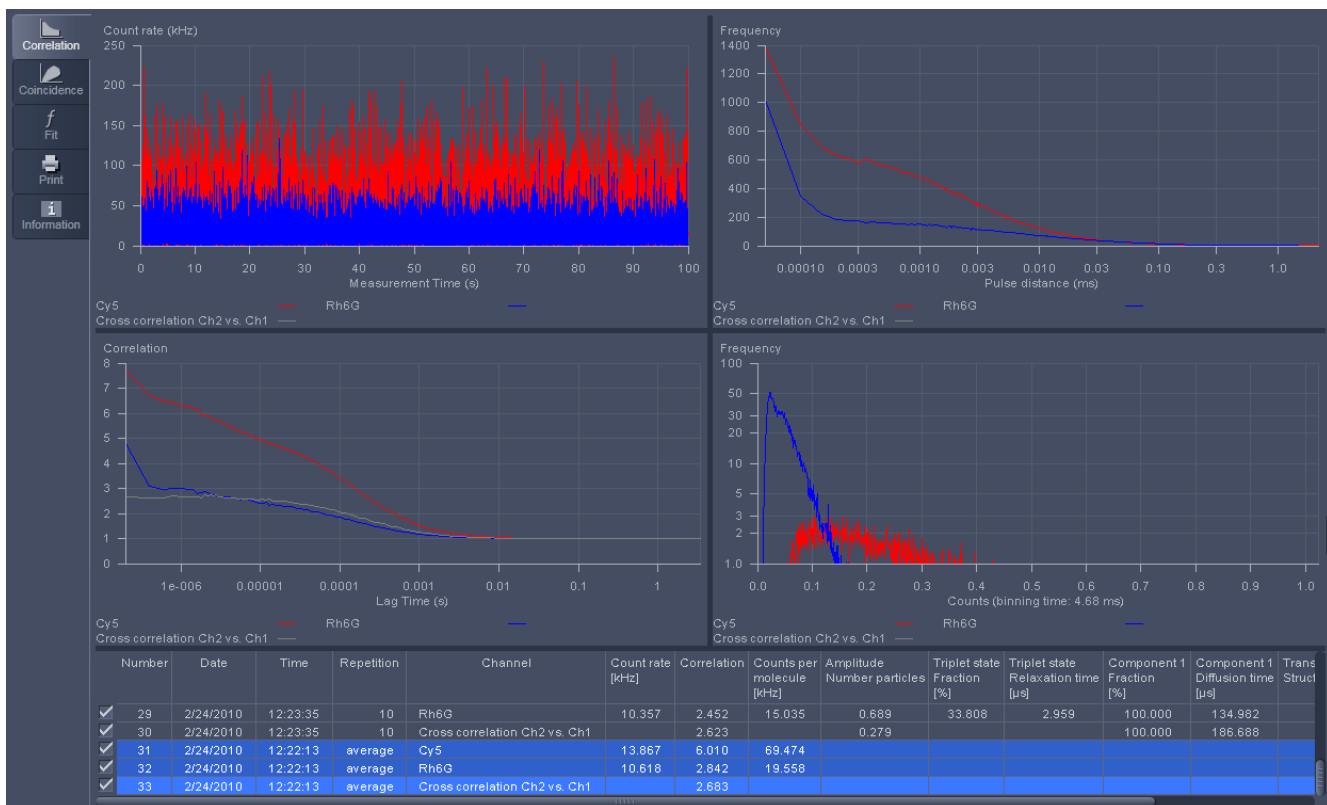
Press the **Stop** button to end a measurement. All data accumulated so far will be available and can be stored.

or



After completion of the measurement, the data will be displayed in the FCS Correlation diagram within an Image Container (see Fig. 37).





**Fig. 37 FCS Correlation diagram**

You have the following function elements:



Activate the **Correlation** panel to display measured data (Fig. 37).



Activate the **Coincidence** panel to display a scatter plot of the two channels (cross-correlation only).



Activate the **Fit** panel to select a model and fit the data to it.



Activate the **Print** panel to set up a connected printer and print a hardcopy of the data.

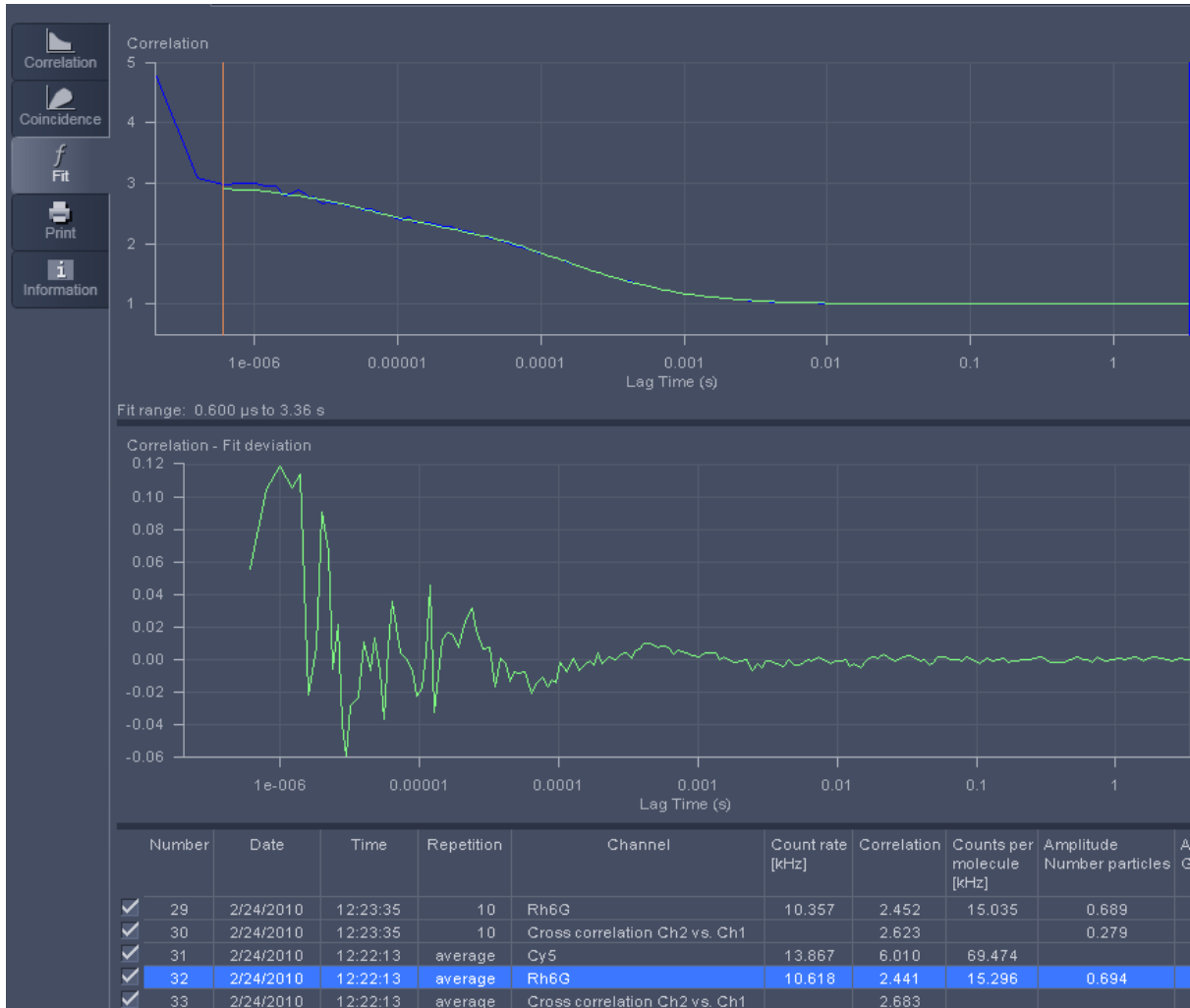


Activate the **Information** panel to give the data a name, input any comments and view the Meta data.

Save the data by clicking the **File** button in the menu bar.

## Analyzing the data

The acquired FCS data is analyzed in the fit display of the **FCS Fit** diagram (see Fig. 38).



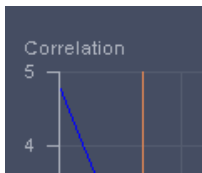
**Fig. 38** FCS Fit diagram

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You have the following options:



Activate the **Fit** panel to display fitted data (see Fig. 38).



Set the red and blue bars to define the start and end points of the curve fit window.



Load a predefined model from the **Model** drop-down menu. You can assemble a model by pressing the **Model** tool in the **FCS** tool group.



Define the conditions of the fit by activating / deactivating terms, setting the type of a parameter (fixed, free, or start value), defining limits and globally link parameters in the **Model** table.



Pressing the **Fit** button will fit the current loaded correlation functions to the defined model. The fitted data will be displayed in the **Model** and **Result** tables.



Pressing the **Fit all** button will take all ticked channels and fit them according to the selected model.



Pressing the **Undo** button will undo the last operation, or previous ones as well, if the button is pressed repeatedly.



Pressing **Redo** will redo the last cancelled operation, or previous ones, if the button is pressed repeatedly.



Pressing the **To Method** button will write back the settings to the method. If the method is stored, the settings will be active when the method is selected the next time.

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## Switching off the system

- Click on the **File** button in the menu bar and then click on the **Exit** button to leave the **ZEN 2012** software.
- If any lasers are still running you should shut them off now in the pop-up window indicating the lasers still in use.
- Shut down the computer.
- Switch off the Ar-ML laser with first the idle-run-switch (Fig. 2/3) and second the key switch (Fig. 2/1), then wait until the fan of the Argon laser has switched off. Don't switch off the main power yet.
- On the power remote switch turn off the **Components** switch and the **System/PC** switch (Fig. 1).
- Switch off the X-Cite 120 lamp or the HBO 100 mercury burner.
- Switch off the Ar-ML laser of by the main power switch on the power supply (Fig. 2/2).