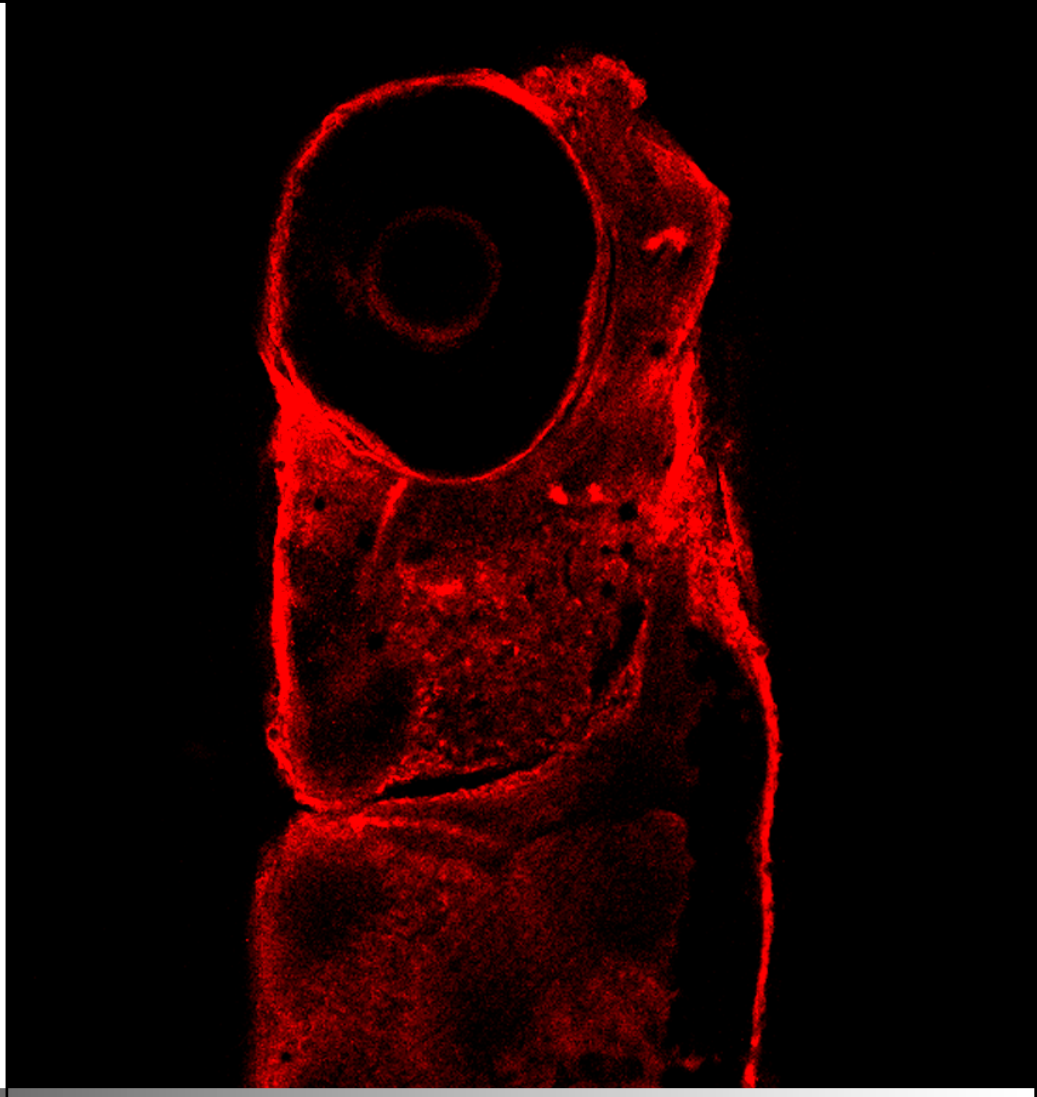
CB&IACore Facility



The University of Ottawa
CB&IA**Core Facility**

uOttawa

Zeiss LSM 510/ AxioImager.M1 Confocal Microscope

Objectives

Zeiss LSM 510 Axio Imager M1 Microscope Objectives

Room: 4502

	1	2	3
	EC Plan-Neofluar 10x 0.3	**Plan-Apochromat 20x 0.8**	**EC Plan-Neofluar 40x 1.3 Oil**
Magnification	10x	20x	40x
Numerical Aperture	0.3	0.8	1.3
Working Distance (mm)	5.2	0.55	0.21
Coverglass (mm)	0.17	0.17	0.17
Immersion	None	None	Oil
Optical System	ICS	ICS	ICS
Flatness	****	*****	****
Color Correction	****	*****	****
UV Transmission	****	***	****
IR Transmission	***	****	***
DIC	(not equipped) ****	(DIC II) *****	DIC III
Catalog #	420340-9901	420650-9901	420460-9900

	4	5	6
	Plan-Apochromat 63x 1.4 Oil	**Achroplan 40x 0.8 W**	**W Plan Apochromat 63x 1.0 VIS-IR**
Magnification	63x	40x	63x
Numerical Aperture	1.4	0.8	1
Working Distance (mm)	0.19	2.1	2.1
Coverglass (mm)	0.17	0	0
Immersion	Oil	Water	Water
Optical System	ICS	ICS	ICS
Flatness	*****	**	*****
Color Correction	*****	***	*****
UV Transmission	***	**	***
IR Transmission	****	***	****
DIC	DIC III	(DIC III) ***	(DIC III) *****
Catalog #	420780-9900	440090-9901	421480-9900

ICS=Infinity Color Corrrected System

Zeiss LSM510 AxioImager.M1 Microscope Filter Sets

Room 4502

1. **Transmitted Light**

2. **Zeiss Filter Set 02 (DAPI)**

Excitation:	G	365
Beam Splitter:	FT	395
Emission:	LP	420

3. **Chroma Filter Set 49001 (CFP)**

Excitation:	BP	436/20
Beam Splitter:	FT	455
Emission:	BP	480/40

Similar to Zeiss Filter Set 47

4. **Chroma Filter Set 49002 (GFP)**

Excitation:	BP	470/40
Beam Splitter:	FT	495
Emission:	BP	525/50

Similar to Zeiss Filter Set 38

5. **Chroma Filter Set 49003 (YFP)**

Excitation:	BP	500/20
Beam Splitter:	FT	515
Emission:	BP	535/30

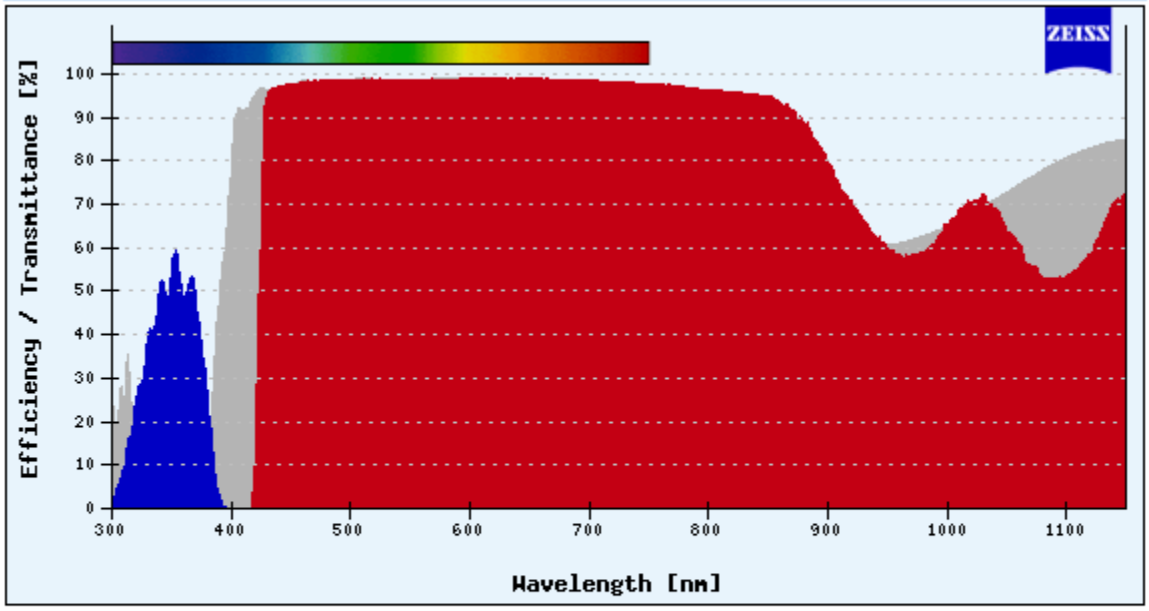
Similar to Zeiss Filter Set 46

6. **Zeiss Filter Set 45 (Cy3.5/mcherry)**

Excitation:	BP	560/40
Beam Splitter:	FT	585
Emission:	BP	630/75

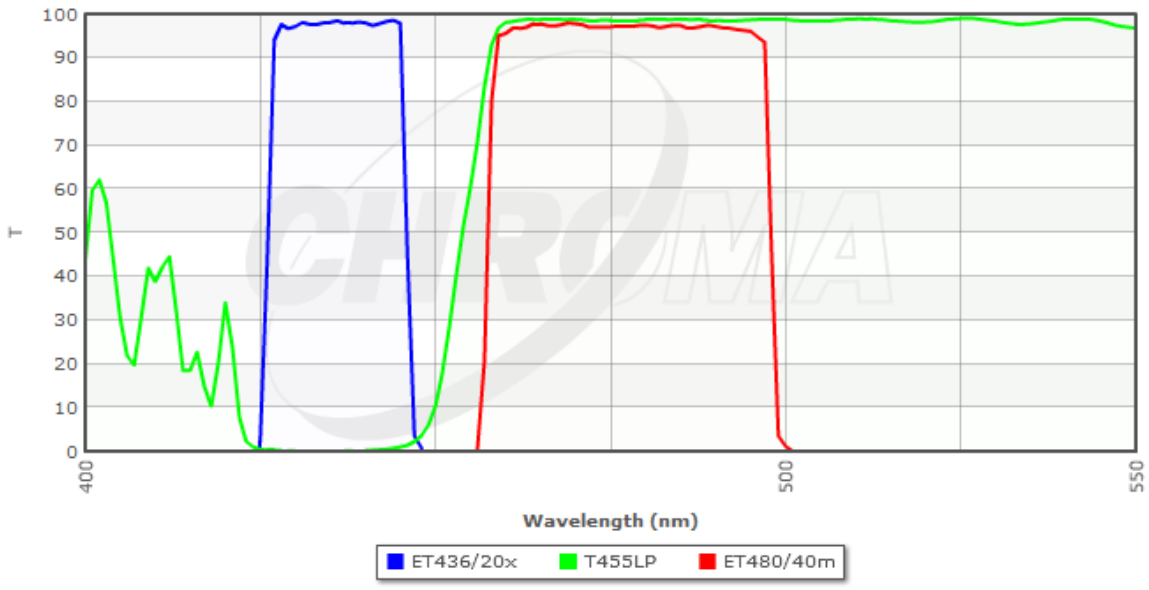
Filter Sets

Filter Set 02 (488002-9901-000)

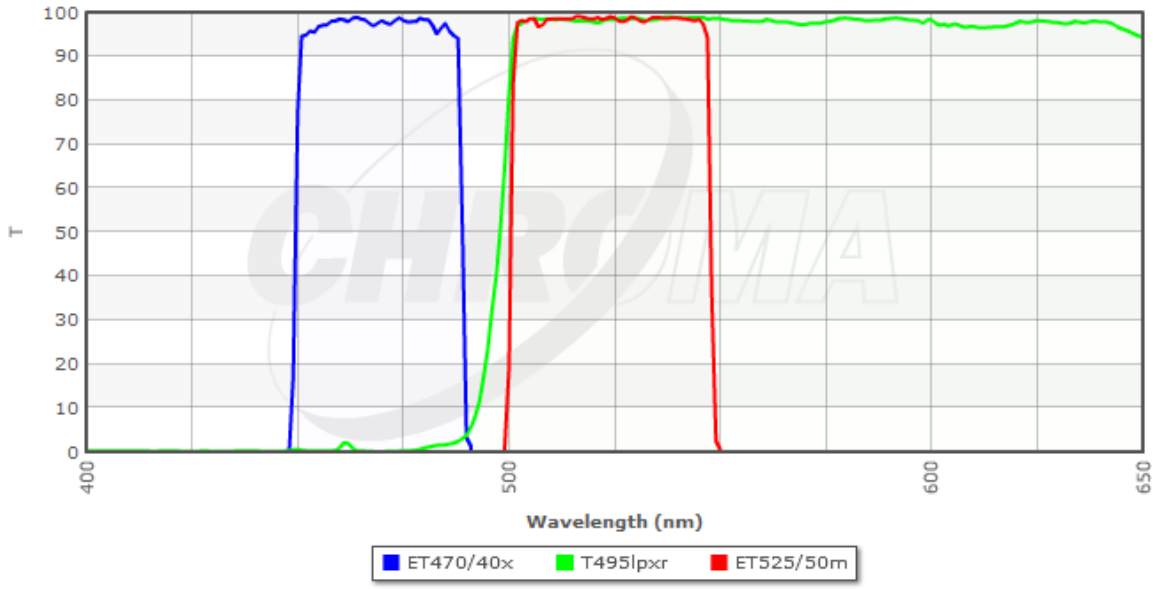


Filter Set	02
Excitation	G 365
Beam Splitter	FT 395
Emission	LP 420

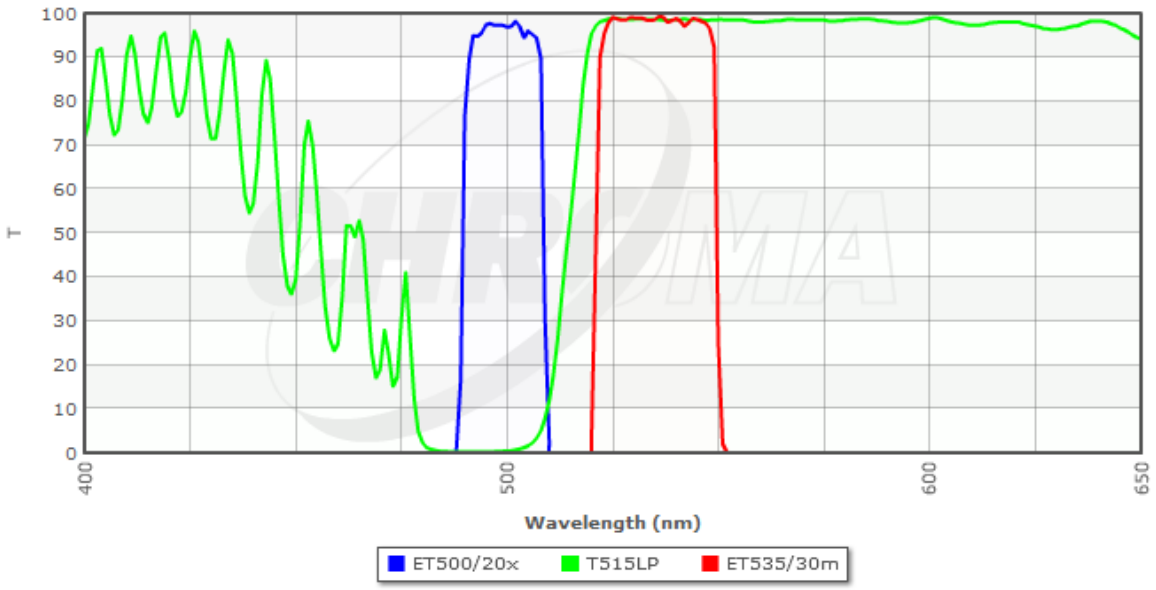
49001 ET - CFP



49002 ET - GFP (FITC/Cy2)

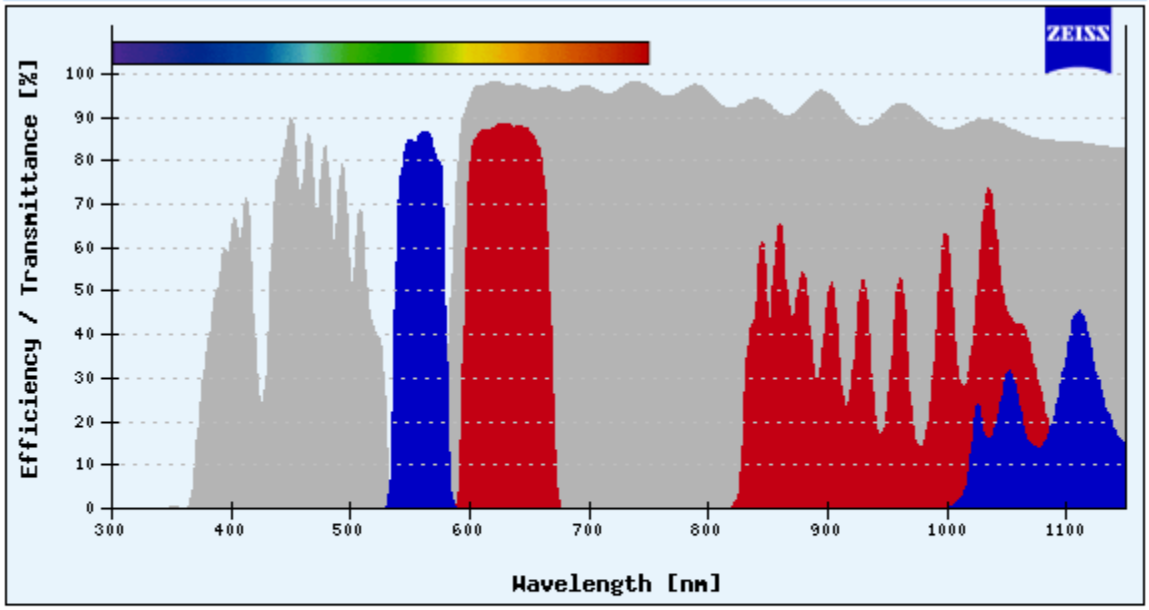


49003 ET - EYFP



Filter Sets

Filter Set 45 (000000-1114-462)



	Filter Set	45
☑	Excitation	BP 560/40
☑	Beam Splitter	FT 585
☑	Emission	BP 630/75

Zeiss LSM 510 AxioImager.M1 Microscope Guide (ZEN)

Room 4502

Start Up Procedure

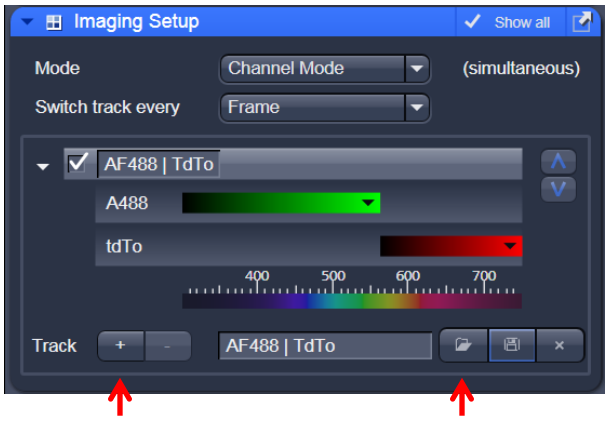
1. Remove **dust cover** from microscope
2. Toggle **X-Cite Lamp** switch **ON**
3. On Remote Control Pad, toggle the **System/PC** switch and the **Components** switch **ON**
4. Always leave **Power Supply 231** switch **ON position**
5. Turn **Computer ON**
6. User name: **New User** *(no password)*
7. Open program: **ZEN 2009**
8. Press **Start System** button to initialize hardware

Lasers

1. Click **Acquisition** tab. Under **Setup Manager** heading, click on **Lasers** to open the lasers tool
2. Turn on the required laser(s) for your study:
   - To turn on the **Argon/2** laser select **Standby** first. Click on **laser properties** at bottom of the Laser Tool. After about 1 minute of warming up, status will change from *warming up* to *ready*. Change **Output (%)** to 50. Select **On**.
   - The **HeNe543** and **HeNe633** lasers can be turned on directly by selecting **On**

Configuring Microscope Mode

1. You can load pre-configured tracks in the **Imaging Setup Tool**. Click on the **open** icon to select an optical track. To add another track, click the **+** button to add a new empty track, and then use the **open** icon to select a pre-configured track to replace the empty track.



2. Open the **Light Path Tool**. You can toggle between the different tracks found in the **Imaging Setup Tool** to view the respective optical configuration in the **Light Path Tool**
3. For each track, click on **Lasers button** found in the **Light Path Tool**, and set the desired **%Transmission** for each Laser. Recommended:
   - Argon/2: 5-10%
   - HeNe633: 5-10%
   - HeNe543: 50-80%

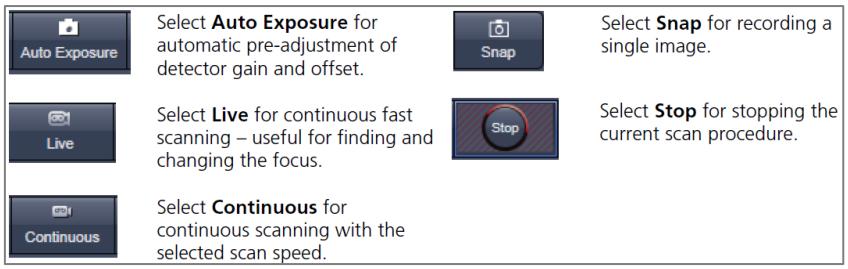
Configuring Scan Parameters

1. Open the **Acquisition Mode Tool** located under the Online Acquisition heading
2. Select the appropriate **objective** from the drop down list
3. Select the appropriate **Frame Size** ie 1024x1024
    - OR click **Optimal** button to get a frame size appropriate for Nyquist Sampling
4. Select the appropriate **Scan Speed** ie 5 – 9
5. For **Averaging** (to increase Signal/Noise ratio):
    - **Number**: 2 or 4
    - **Mode**: Line
    - **Method**: Mean
    - **Bit Depth**: Set as desired
    - **Direction**: →

Set Sample on Stage and Focus

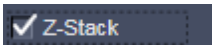
1. To be able to view specimen down ocular, need to first click **Occular Tab**, then **Online**
2. Lightly wipe your slide (on both sides) with a kimwipe and a small amount of 70% ethanol to clean. If using an oil lens, add a very small drop of immersion oil
3. Set slide on stage, bring specimen into focus and select desired area of interest
4. Click on **Acquisition tab**

Adjusting Channels and Capturing an Image

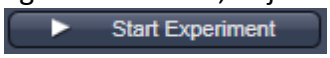


1. Open the **Channels Tool** found under the Online Acquisition heading
2. For each channel, set the **pinhole** size to correspond to **1 Airy Unit** as a starting point
3. Adjust the pinhole sizes to get the same resulting optical section thickness across all channels
4. Perform the following for **each individual channel** (temporarily deactivate other channels):
    - Click **Live** button to get a preview image
    - For the **Range Indicator**, click on the **Channel Color**, found under your image window 
        - Red = saturated, Blue = zero pixels
    - Adjust your **Gain (Master)** such that a bit of red (overexposure) is seen, and then adjust the **Digital Offset** so that minimal blue (zero pixels) is obtained
    - Click **Stop** button
5. Once all channels have been adjusted, **re-activate** all of your channels/tracks
6. Click **Snap** to capture your final image

To Capture a Z-stack

1. Check the **Z-stack option** in the main tools area 

2. Open the **Z stack Tool** found under the Multidimensional Acquisition heading
3. Make sure that **First/Last** mode is selected
4. Click on **Live button** to get a preview image (with only one active track)
5. Turn **Fine Focus** knob CW to an optical plane for starting Z-stack capturing, and click **Set First** button
6. Turn **Fine Focus** knob CCW to an optical plane to be the last optical section of the Z-stack and click **Set Last** button
7. Click **Stop** button
8. Re-activate all of your channels
9. Click on the 

button to set number of slices to match the optimal Z-interval for a given stack size, objective lens, and pinhole diameter
10. Click on the 

button to start acquiring your Z-stack

To Save an Image

1. To save an image, click **File**, **Save as** or click the  button in the **Main Toolbar**, or the  button in the **File Handling Area**.
2. Enter a file name, select the LSM 5 (.lsm) format, and press **Save**

Reuse Button

1. To capture a new image using the exact acquisition parameters (pinhole diameter, Gain (Master), Digital Offset, excitation, beam path, scan mode, frame size, speed, data depth, scan direction, average, zoom) of an existing image, click **Reuse** button on the bottom panel of an opened image
2. The acquisition parameters of a displayed image can be viewed by clicking on **Info** button on the left of the image

Shut Down Procedure

1. If used oil lens, thoroughly **remove oil** using dry Lens Paper
2. Put **10x objective** in place
3. Put **Argon laser** on **standby**, but leave other lasers on

Continue if last user:

4. From the Lasers Tool, turn **lasers OFF**
5. **Close** the **ZEN** program
6. **Shut down computer**. Wait for computer to turn off
7. Always leave **Power Supply 231** Box switch **ON**
8. On Remote Control Pad, toggle the **System/PC** switch and the **Components** switch **OFF**
9. Toggle **X-Cite Lamp** switch **OFF**
10. **Cover microscope** with dust cover

Quick Guide

LSM 5 MP, LSM 510 and LSM 510 META

Laser Scanning Microscopes

LSM Software ZEN 2009

October 2009

ZEISS

We make it visible.

Contents

Page

Introduction

This LSM 510 / LSM 510 META / LSM 510 NLO Quick Guide describes the basic operation of the LSM 510 / LSM 510 META / LSM 510 NLO Laser Scanning microscope with the ZEN 2009 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, Axioskop 2 FS MOT). 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 510 / LSM 510 META / LSM 510 NLO 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

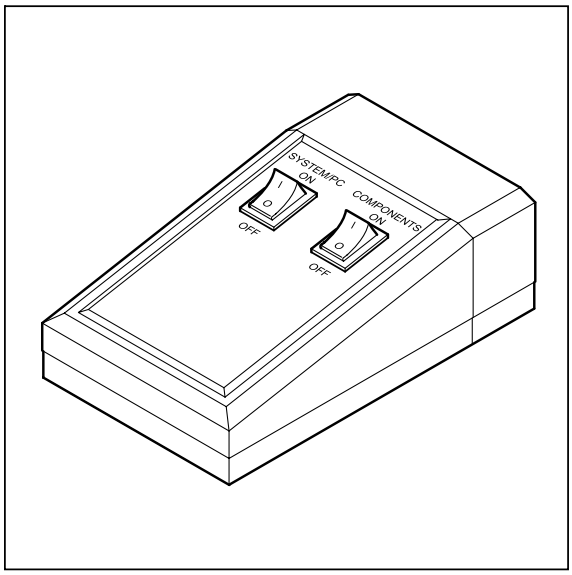


Fig. 1 **REMOTE CONTROL switch**

Switching on the LSM system

- When set to **ON** the REMOTE CONTROL switch labeled **System/PC** provides power to the computer. This allows use of the computer and ZEN software offline (Fig. 1).
- 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.

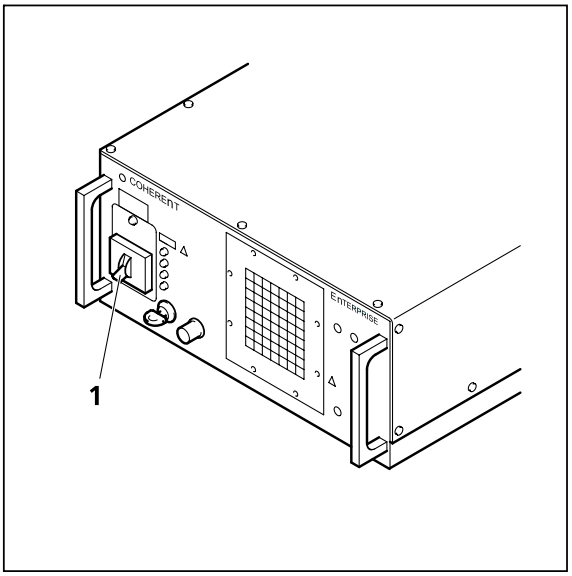


Fig. 2 **Power supply of UV-Ar laser**

Switching on the Enterprise UV-Ar Laser

- If the UV laser is required, switch it on via the toggle switch (Fig. 2/**1**) on the power supply.
  - It will be ready for operation after a few seconds.

Starting the ZEN software

- Double click the ZEN 2009 icon on the WINDOWS desktop to start the Carl Zeiss LSM software.

The **ZEN Main Application** window and the **LSM 510 Startup** window appear on the screen (Fig. 3)

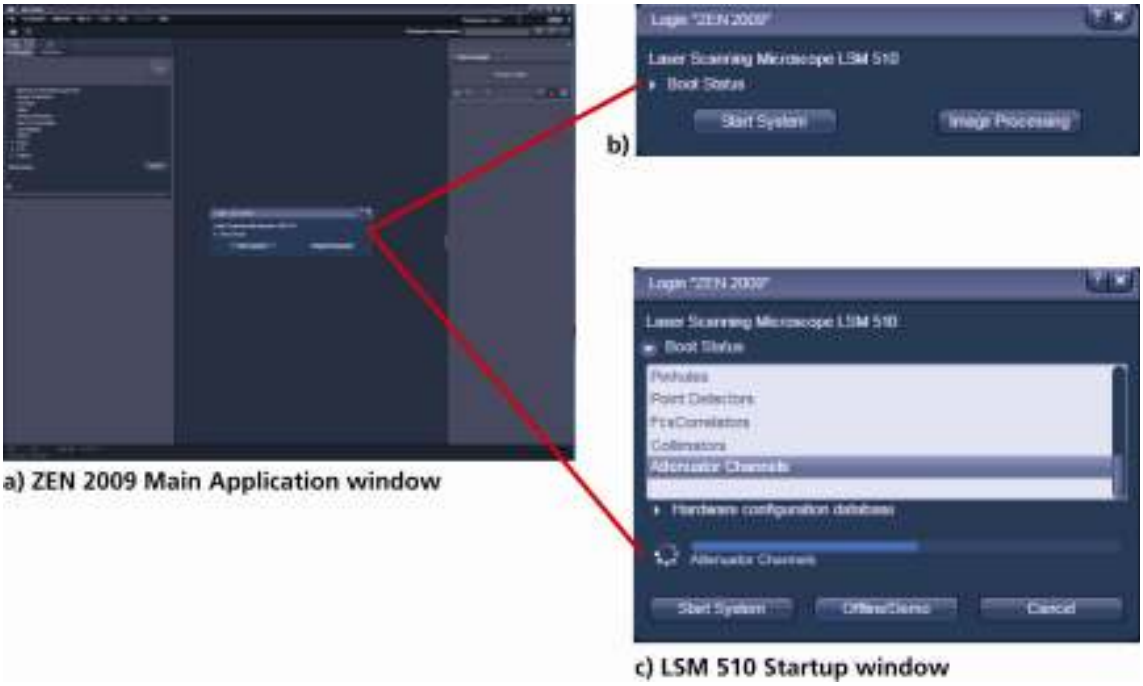


Fig. 3 **ZEN Main Application Window at Startup (a) and the LSM 510 Startup Window (b and c)**

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.

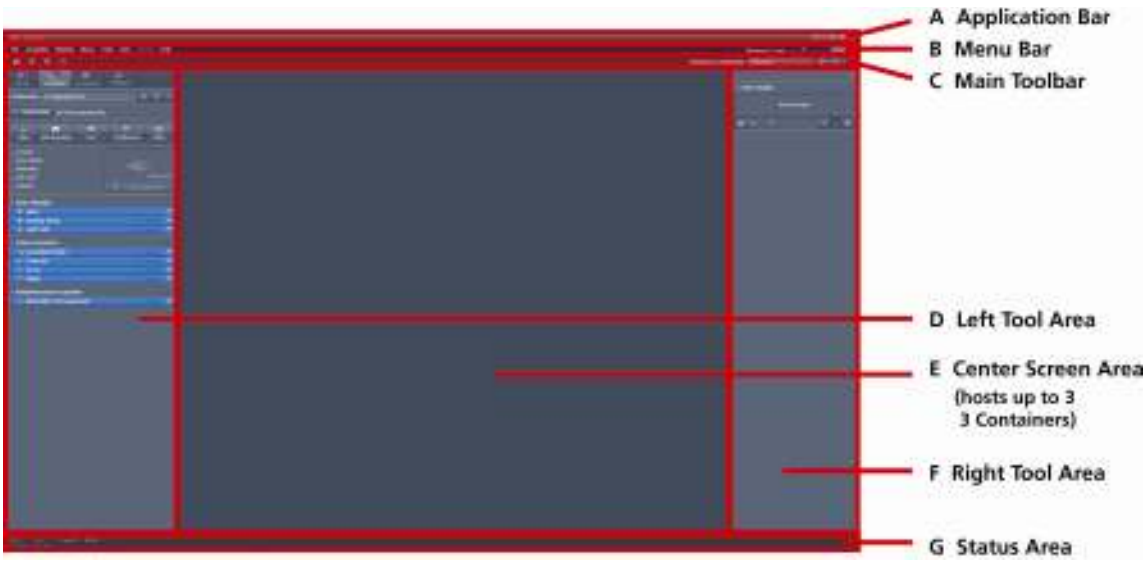


Fig. 4 ZEN Main Application window after Startup with empty image container

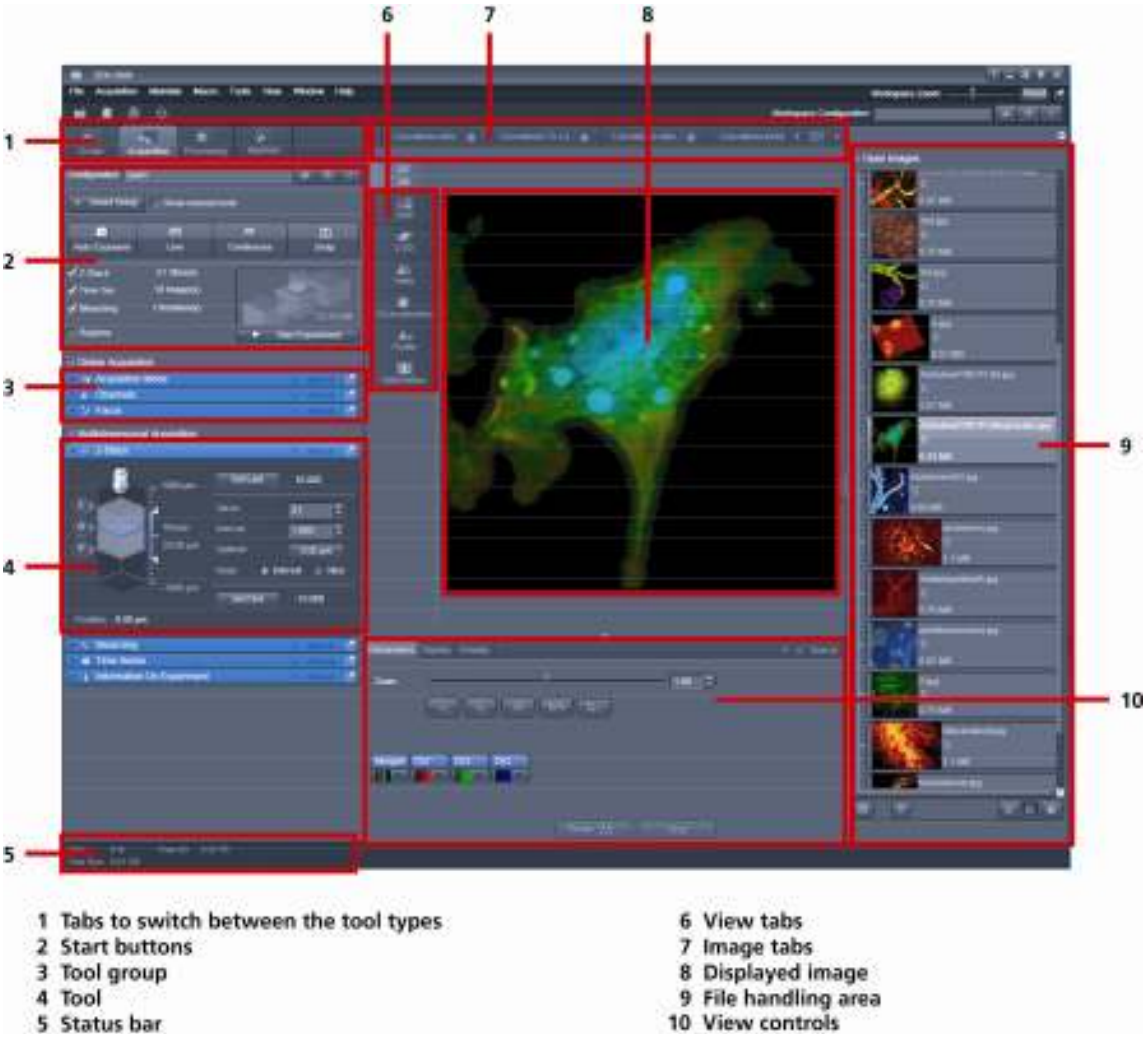


Fig. 5 ZEN Main Application window after Startup with several images loaded

Introduction to ZEN – Efficient Navigation

The **ZEN 2009** 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.

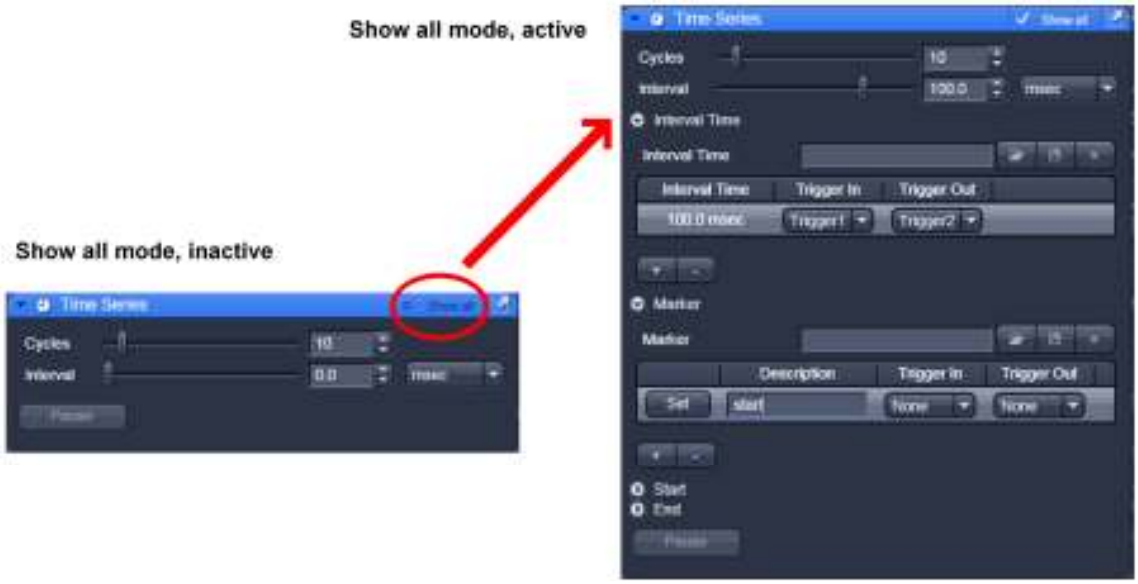


Fig. 6 **Basic and Pro Mode**

A focus in the development of **ZEN 2009** 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** de-activated, 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).

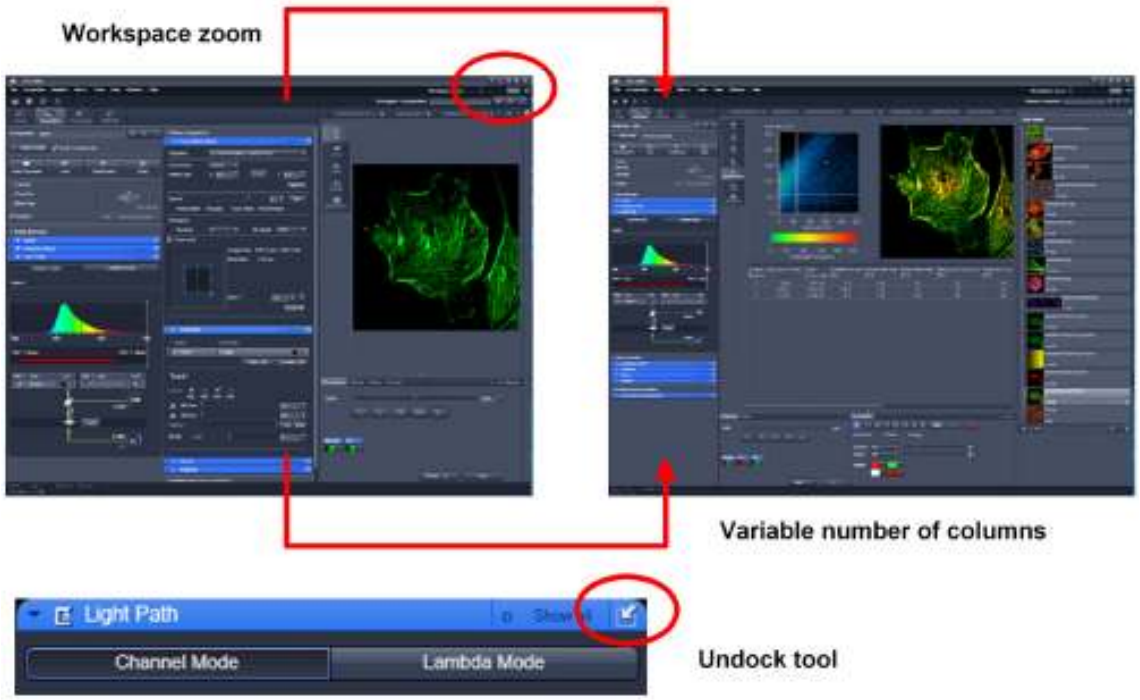


Fig. 7 **ZEN Window Layout configuration**

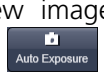
More features of **ZEN 2009** include:

- 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., "Online Acquisition") to the right and a new tool column automatically opens. Alternatively use the context menu "move toolgroup 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 2009** 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 2009** 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 **Auto Exposure** 

button. For an empty image document press the **New** 

button.

The new document is immediately presented in the **Open Images 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.

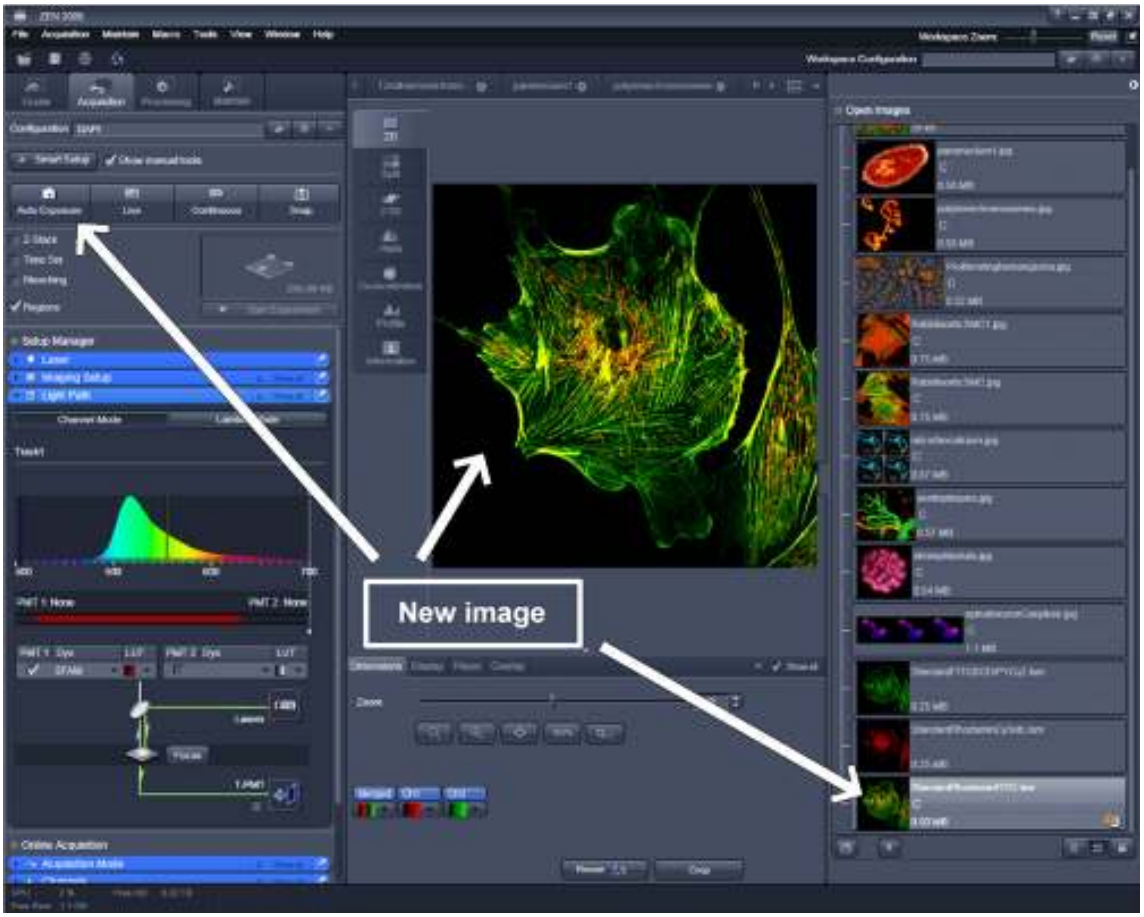


Fig. 8 **New image document in the Open Images Ares**

Advanced data browsing is available through the **ZEN File Browser** (Ctrl F or from the **File** menu). The File Browser can be used like the WINDOWS program file browser. Images can be opened by a 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.

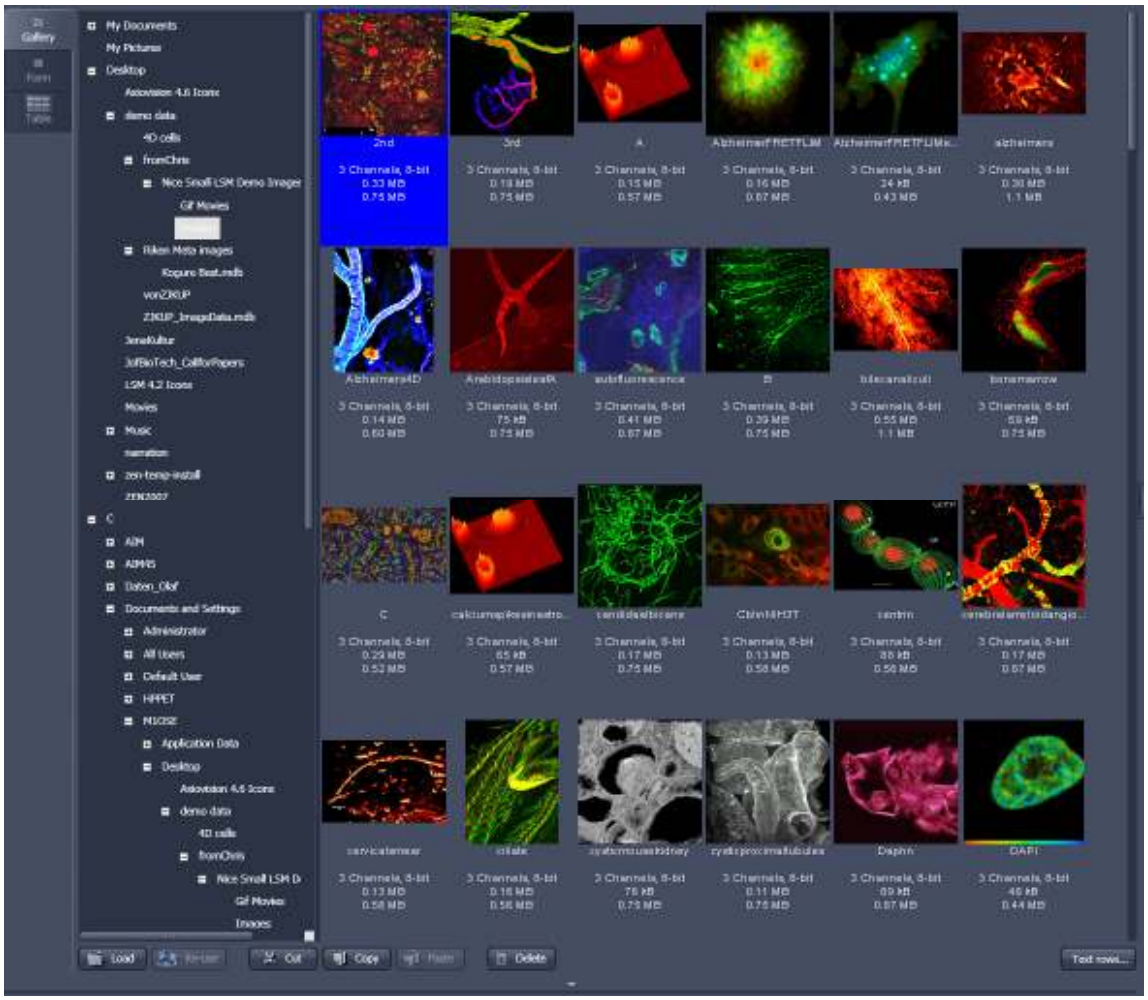


Fig. 9 **File Browser**

Turning on the lasers

- To manually switch lasers on or off open the **Laser** tool.
- All available lasers can be operated within this tool (Fig. 10).

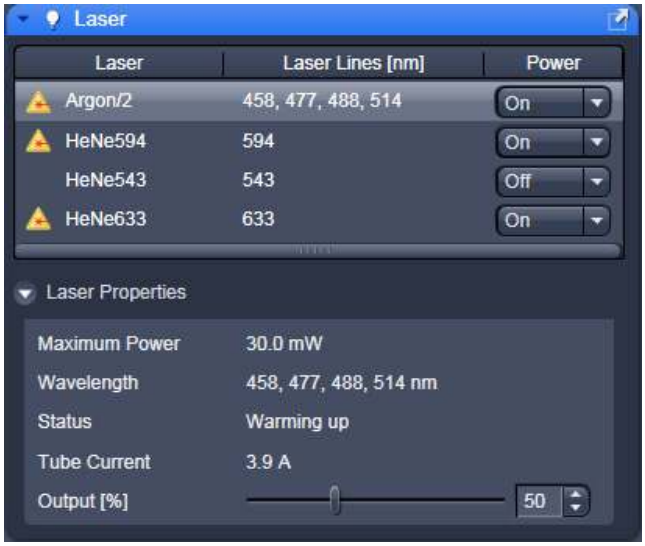


Fig. 10 **Laser Control tool**

Setting up the microscope

Changing between direct observation, camera detection and laser scanning mode

The **Ocular**, **Camera** and **LSM Acquisition** buttons switch between the use of the LSM and the microscope the beam path and indicate which beam path is currently in use for the microscope:

- Click on the **Ocular** button to change open the controls for the microscope beam path and for direct observation via the eyepieces of the binocular tube, lasers are blocked.
- To set the hardware in position for using the microscope, click **Online** if not yet active.
- To close the light shutters on the microscope click **Offline**.
- Click on the **LSM Acquisition** button to move back to the LSM system.

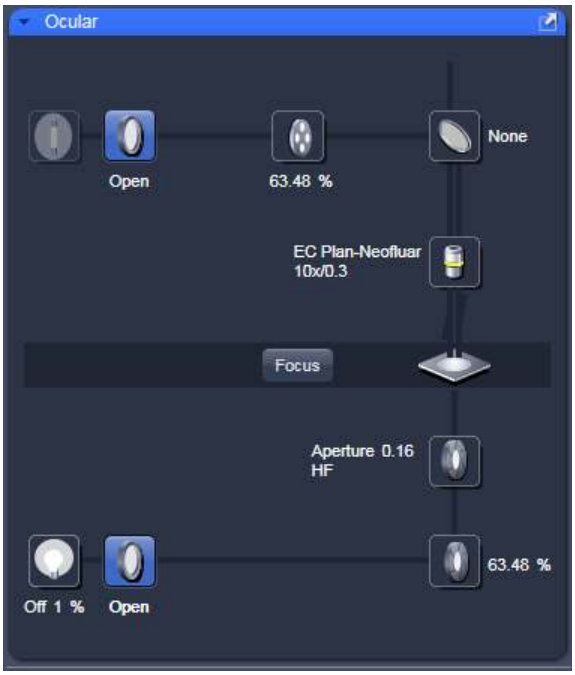


Fig. 11 **Microscope Control window, e.g.: Axio Imager.Z1**

Setting up the microscope and storing settings

Click on the **Ocular** tab for direct observation; press the **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.

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.

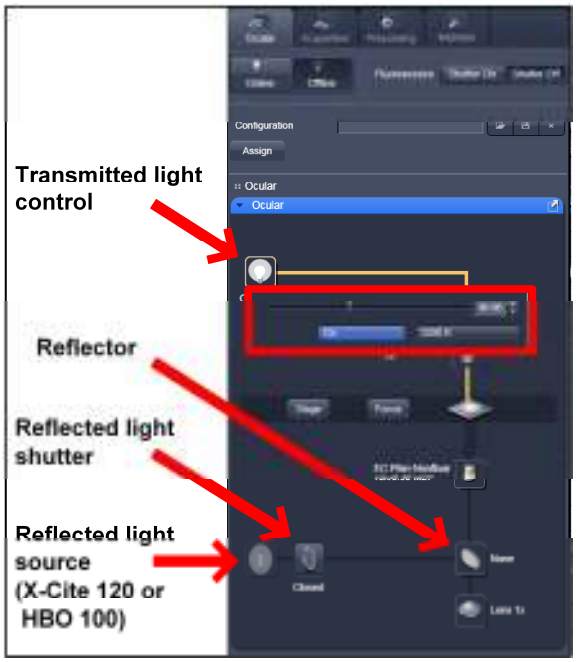


Fig. 12 **Microscope Control window with Transmitted Light pop-up menu**

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.

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

Fig. 13 **Configuration panel**

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

Configuring the beam path and lasers

- Click on the **Acquisition** button.

Setting up a configuration

Simultaneous scanning of single, double and triple labeling:

- Advantage: faster image acquisition
- Disadvantage: Eventual 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 **Imaging Setup** and the **Light Path** tool in the **Setup Manager** Tool group to access the hardware control window to set-up the beam path.

The open **Light Path** is shown in Fig. 14.

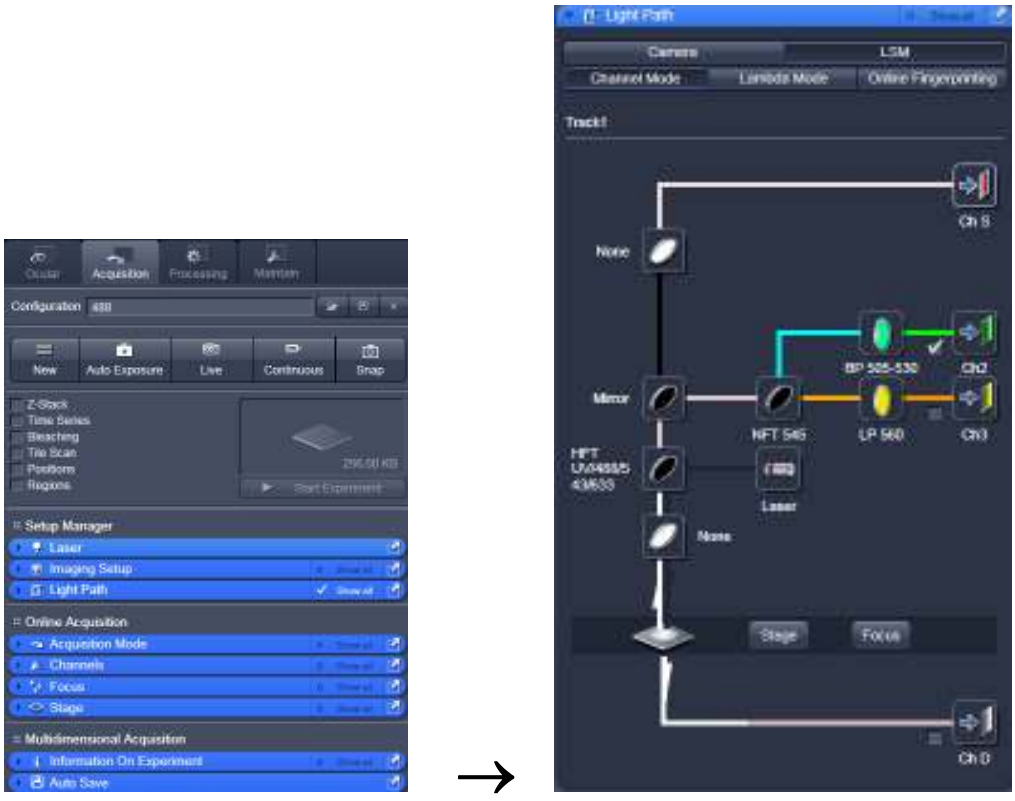


Fig. 14 **Light Path tool for a single track (LSM)**

Settings for track configuration in Channel Mode

- Select **Channel Mode** if necessary (Fig. 15).

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:

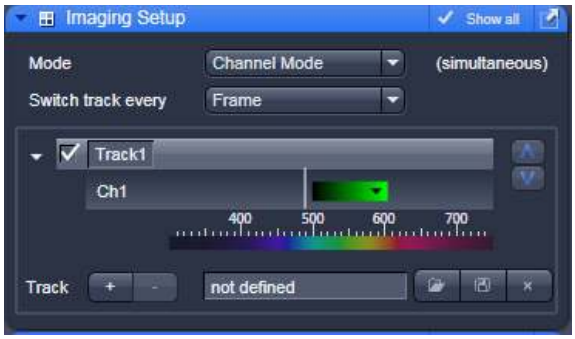


Fig. 15 **Imaging Setup tool for a single track (LSM)**

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

Selection of the main dichroic beam splitter (HFT) or secondary dichroic beam splitter (NFT) position through selection 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, META detectors ChS1-8, transmission ChD) for the scanning procedure and assigning a color to the channel.

- Select the appropriate filters and activate the channels.
- Click the **Laser** icon to select the laser lines and set the attenuation values (transmission in %) in the displayed window.
- 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.
- In the **Imaging Setup** tool the Detection Bands & Laser Lines are displayed in a spectral panel (Fig. 16) to visualize the activated laser lines for excitation (vertical lines) and activated detection channels (colored horizontal bars).

Fig. 16 **Detection Bands & Laser Lines display**

Fig. 17 **Track Configurations window**

- For storing a new configuration click and enter a desired name in the first line of the list box (Fig. 17), 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**.

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 **List of Tracks** panel in the **Imaging Setup Tool** (Fig. 15, Fig. 16 and Fig. 17).

Switch track every

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**.

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.

A click on this arrow button will move the selected track (highlighted in light grey) one position upwards in the list box.

A click on this arrow button will move the selected track (highlighted in light grey) one position downwards in the list box.

Scanning an image

Setting the parameters for scanning

- Select the **Acquisition Mode** tool from the **Left Tool Area** (Fig. 18).
- 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 λ.

The number of pixels influences the image resolution!

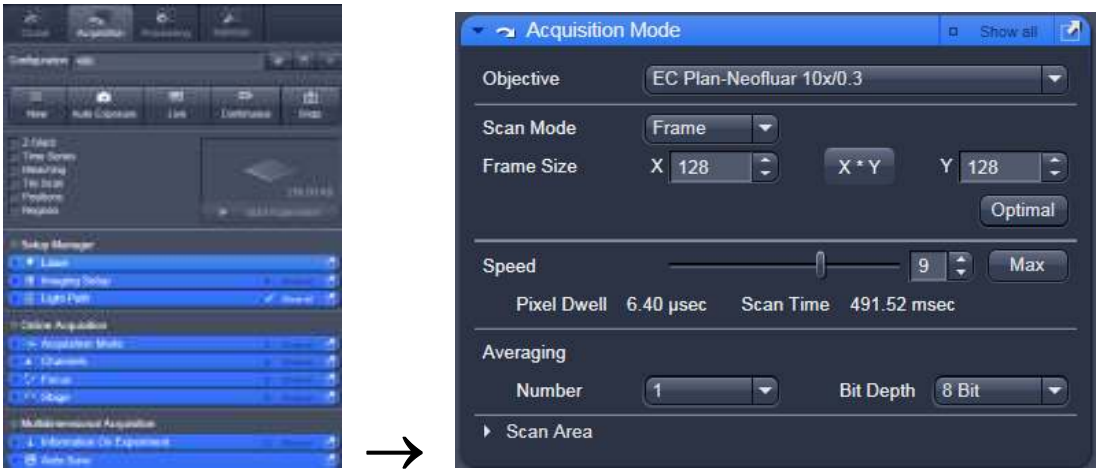


Fig. 18 **Acquisition Mode tool**

Adjusting scan speed

- Use the **Scan Speed** slider in the **Acquisition Mode** tool (Fig. 18) 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. 18).

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.

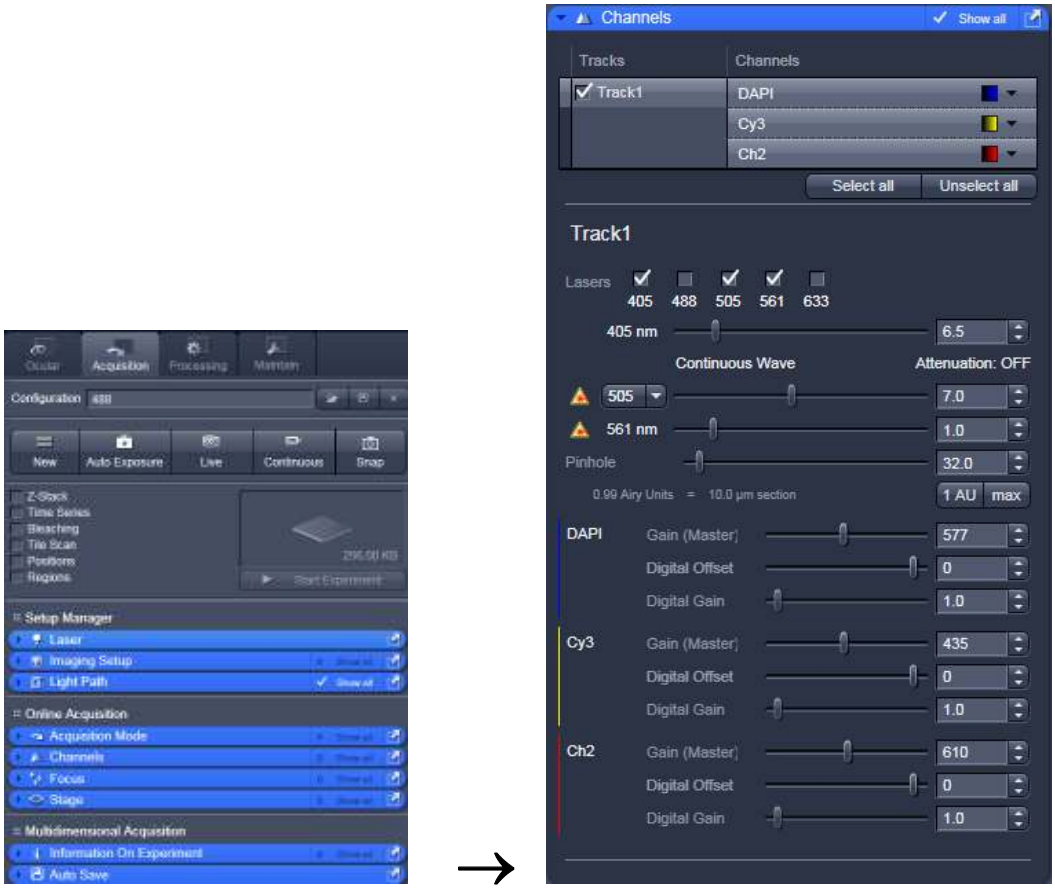
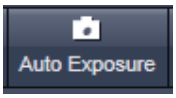


Fig. 19 **Channels tool**

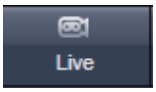
Image acquisition

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

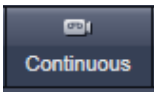
- Use one of the **Auto Exposure**, **Live**, **Continuous** or **Snap** buttons to start the scanning procedure to acquire an image.
- Scanned images are shown in separate windows.
- Click on the **Stop** button to stop the current scan procedure if necessary.



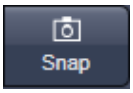
Select **Auto Exposure** for automatic pre-adjustment of detector gain and offset.



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.

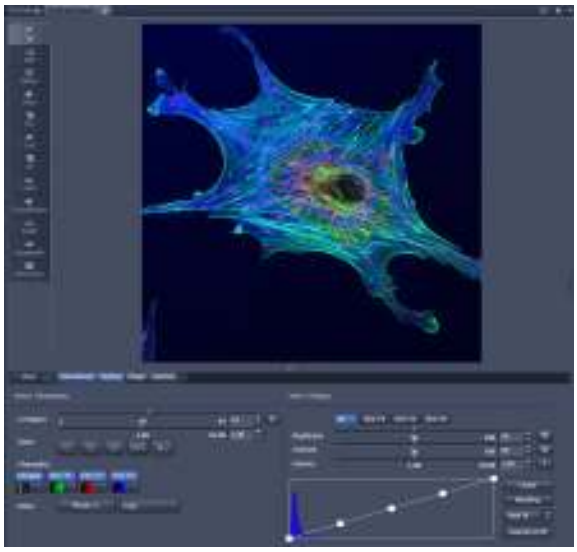


Fig. 20 **Image Display**

Image optimization

Choosing Range Indicator

- In the **View – Dimensions** View Option Control Block, click inside the color field in the  button under the channel button (Fig. 21).

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

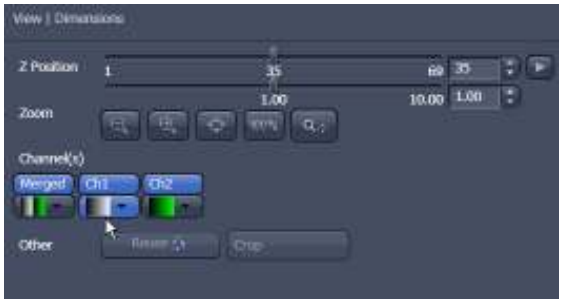


Fig. 21 **View Dimensions Control Block**

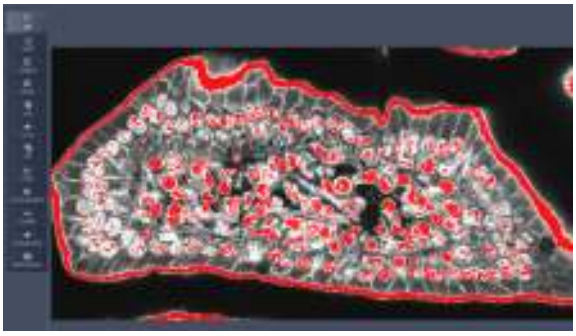


Fig. 22 **Image Display**

Fig. 23 **Channels tool**

The scanned image appears in a false-color presentation (Fig. 22).

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

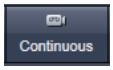
- Set the **Pinhole** to **1** Airy Unit (Fig. 23).
- Set the **Gain (Master)** high.
- When the image is saturated, reduce AOTF transmission in the **Laser** control section of the **Channels Tool** (Fig. 23) using the slider to reduce the intensity of the laser light to the specimen.

Adjusting gain and offset

- Increase the **Digital Offset** until all blue pixels disappear, and then make it slightly positive (Fig. 23).
- 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.

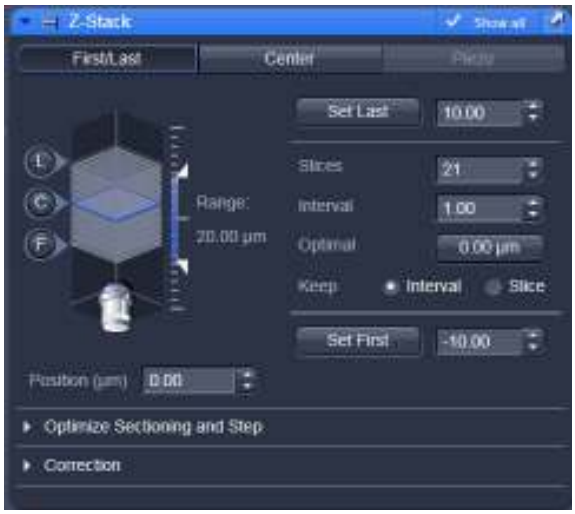
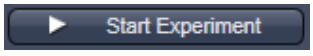
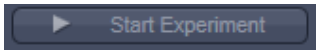


Fig. 24 **Z-Stack tool**

- 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.

Storing and exporting image data

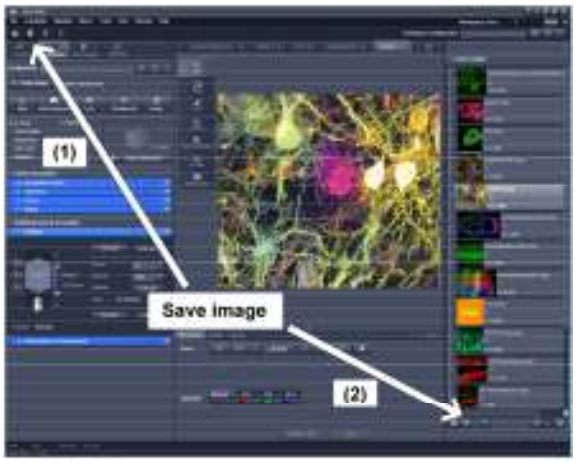


Fig. 25 **Save Image buttons in ZEN**

- 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. 25/**1**), or click on the  button at the bottom of the **File Handling Area** (Fig. 25/**2**).

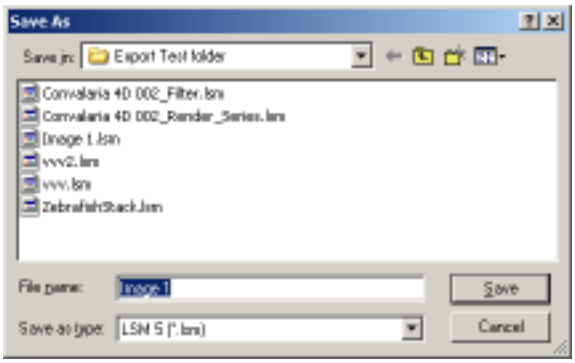


Fig. 26 **Save as window**

- 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. 27 **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.

Switching off the system

- Click on the **File** button in the **Main Menu** bar and then click on the **Exit** button to leave the **ZEN 2009** 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.
- **Wait until the fan of the Argon laser has switched off.**
- On the REMOTE CONTROL 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 UV-Ar laser of by the toggle switch on the power supply (Fig. 2).

Zeiss LSM 510 AxioImager.M1

Rm. 4502

Start Up Procedure

1. Remove **dust cover** from microscope
2. Toggle **X-Cite Lamp** switch **ON**
3. On Remote Control Pad, toggle the **System/PC** switch and the **Components** switch **ON**
4. Check if the **Power Supply 231** box switch is **ON**
5. Turn **Computer ON**
6. User name: **New User** *(no password)*
7. Open program: **ZEN 2009**
8. Press **Start System** button to initialize hardware

Shut Down Procedure

1. If used oil lens, thoroughly **remove oil** using dry Lens Paper
2. Put **10x objective** in place
3. Put **Argon laser** on **standby**, but leave other lasers on

Continue if last user:

4. From the Lasers Tool, turn **lasers OFF**
5. **Close** the **ZEN** program
6. **Shut down computer**. Wait for computer to turn off
7. Leave the **Power Supply 231** Box switch **ON.**
8. On Remote Control Pad, toggle the **System/PC** switch and the **Components** switch **OFF**
9. Toggle **X-Cite Lamp** switch **OFF**
10. **Cover microscope** with dust cover

CB&IA Core Facility

Attention Users!!

X-Cite Fluorescence Lamp:

The X-cite lamp must be on for at least 30 minutes before being turned off AND must not be turned on again within 1 hour of being turned off.

1. When starting, before you turn on the X-Cite lamp, make sure it has been OFF for at least 1 hour from previous use.
2. When finishing, before you turn off the X-Cite lamp, make sure it has been ON for at least 30 minutes.

Booking Time:

You are allowed to sign up for a maximum of **3 hours** per day. If no one signs up after you, you can continue to use the station until someone else needs it.

You are responsible for **showing up for your time booked**. A **$10.00 fee** applies if you did not show up for booked time.

Log Book:

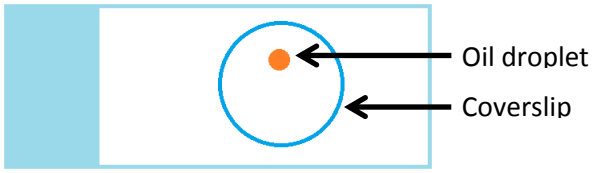
All users must log the time spent on the station as well as the time the fluorescence lamp was turned on/off in the **log book**.

NO FOOD OR DRINKS PERMITTED!

Attention Users!!

Immersion OIL Rules:

- Always keep immersion oil bottle **CLOSED**. This prevents water evaporation and maintains the integrity of the oil
- Keep oil bottle **CLEAN**. Oil should never be found along the sides of the bottle or around the neck
- Apply only a **SMALL AMOUNT** of oil to your coverslip



- Always load your sample on the microscope stage with the **STAGE LOWERED** to the lowest focus position
- Always **REMOVE OIL** using dry Lens Paper from the objective when changing slides. Re-apply new oil to the new slide
- If you are imaging a sample using an oil objective, **do not toggle between objectives**. Image with that objective only!
- When you are done your session, Wipe off oil objective using Lens Paper
- Do not get oil on any air objectives!

Your Data:

Save all of your work in **F:/ drive (Data), your lab-folder, your folder**. Files found on anywhere else will be deleted daily. You are **responsible for backing-up your own data** at all times. Do not rely on this computer as storage for your data. Once you have backed-up your files elsewhere, please delete your files from this computer.

How to connect to the CBIA CORE network

To connect to the shared folder CBIA CORE, click on this icon 

on the desktop, or click on start- all programs - Medtech and medmapdrives

You will be prompted with your UOttawa computer username and password. DO NOT put a check mark in Sauvegarde/Save!

Then go to My Computer, R: drive (Research), CBIA Core, your Lab Folder.

When you are done you HAVE to disconnect the drives. Go to my computer, right click on each of the three drives (Pool/ Research/Web) and select disconnect.

uOttawa
Faculté de médecine
Faculty of Medicine

RGN 2129 ● Téléphone : (613) 562-5648 ● medtech@uottawa.ca