

# Basic Zeiss LSM 510 Meta User Guide

## ZEN Software

**1. Start-up** – Follow the start-up instructions.

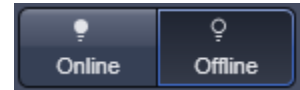
- 1 – Mercury Lamp
- 2 – Main Switch (left of microscope)
- 3 – Computer

**2. ZEN 2009 icon** – double click on this icon to start the software.

**3. Start System**

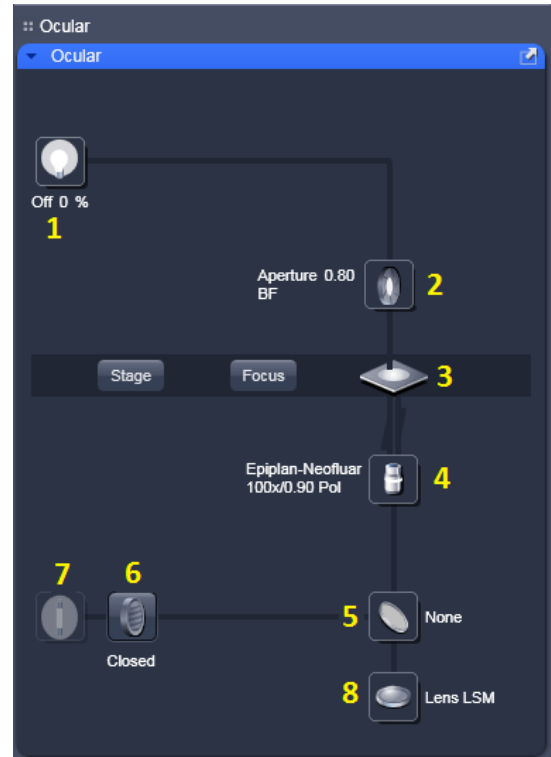
**4. The Ocular Tab** will be selected as it opens

- Pressing the **Online** button will allow you to view your sample through the oculars
- While in the **Offline** position the image can be viewed on the computer screen



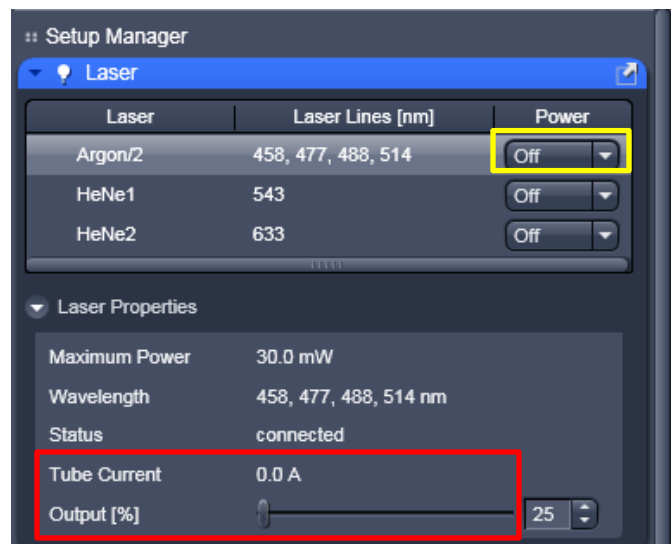
**5. In the Ocular drop menu** you will find the microscope positions.

- 1. Halogen Bulb
  - You may select your desired objective here.
- 2. Aperture
- 3. Specimen/Stage
- 4. Objective
  - Once you've selected **Online**, you may select the filter that corresponds to the color you'd like to view through the oculars.
    - DAPI
    - FITC
    - Rhodamine
    - Bright Field Settings
- 6. Hg Bulb shutter
- 7. Hg Bulb
- 8. Lens



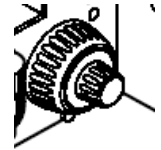
**6. Select the Acquisition Tab**

- Under the **Setup Manager** select the **Laser** drop menu as well as the **Laser Properties** drop menu.
  - Argon/2-Select Standby from the drop menu to warm up the laser. The Status will read "Ready" once it's done and you can then choose On from the drop menu. Adjust the Output [%] slider so that the Tube Current reads 6.1 A.
  - HeNe1 & HeNe2-Select On
- *Note: The only lasers necessary to turn on are the ones you are using. You may leave any unnecessary lasers in the Off position.*



**7. Load Specimen** – The 10x objective uses **NO** oil or water. There is a 40x oil, 63x oil, and 100x oil objective which all require a single drop of oil on the lens before loading a specimen.

- Place your slide upside down into the stage – the width of the holder can be adjusted manually.
  - *Please ask the light microscopy technician for other stage holders if you have a specimen that is not prepared on a slide*
- If using the 10x objective start it at the lowest (bottom) position. For the 40x oil, 63x oil, and 100x oil objectives raise the objective (by moving the coarse focus knob clockwise) until the oil just touches your slide.



### 8. Focus while viewing through the oculars

- Turn on light with the closest buttons to you on the right side of the microscope
  - Mercury (fluorescence)-Top (FL on/off)
  - Halogen (bright-field)-Bottom (HAL on/off)
- Focus on your specimen and turn the light off.

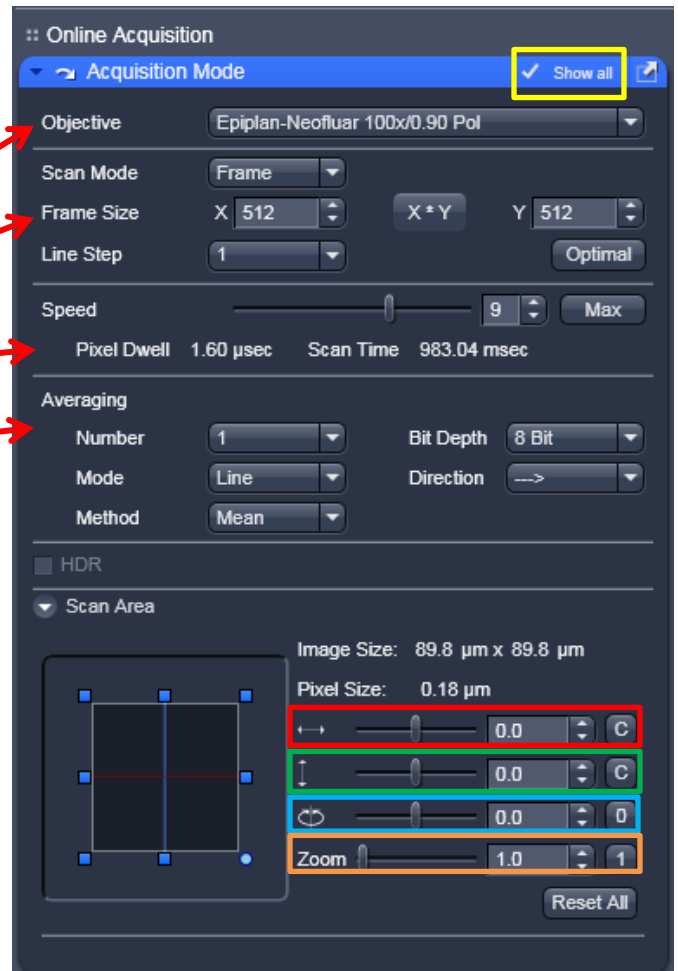
### 9. Set up/Select your Dye Configurations

- Open the **Imaging Setup** drop menu from the **Setup Manager** list. Check the **Show all** box on the right side of the menu.
  - You may either choose to set up your own track from scratch using the **Imaging Setup** and **Light Path** drop menus (See Setting Up a New Configuration guide)
  - Or you can load a previously saved track by opening the desired configuration and selecting **Apply**
- Check the configuration set up to make sure you will be able to see the desired dyes by opening the **Light Path** drop menu from the **Setup Manager** list to see which filters and dichroics each track is using.
  - Note: The light path is highlighted by the software. *In this picture the light is being directed towards ChS with no light going to Ch2 or Ch3.*

The screenshot displays two software panels. The top panel, 'Imaging Setup', has a 'Show all' checkbox checked. It shows 'Track1' selected with 'Ch2' as the channel. A wavelength scale from 500 to 700 nm is visible. Below it, 'CY 2 (Alexa 488, FITC)' is selected, and a red box highlights the 'Apply' button. The bottom panel, 'Light Path', shows a schematic of the optical path. A yellow line indicates the light path starting from the 'None' filter, passing through a 'None' dichroic, a 'None' filter, a 'None' filter, and a 'None' filter, then reflecting off a 'Mirror' to a 'Laser', and finally through 'LP 475' and 'LP 530' filters to 'Ch S'. 'Ch2' and 'Ch3' are also shown but are not highlighted.

## 10. Choose Image Parameters

- Under the **Online Acquisition** list open the **Acquisition Mode** drop box and check the **Show all** box on the right side of the menu.
  - The objective should show the objective you've selected
  - Frame size indicates how many pixels in each direction you want your image to hold
    - For publication images we recommend using 1024 x 1024
  - Select desired speed
  - Averaging
    - Number = how many times the image will be scanned. By increasing this number you can clean up noisy images
  - Under the **Scan Area** drop menu you'll find information on zoom.
    - Moves image along **X plane**
    - Moves image along **Y plane**
    - Allows a **rotation** of the zoomed image
    - Zoom** magnification
    - Each scroll bar has a zeroing button after it to reset the position back to the original starting point. There is also a **Reset All** button beneath the **Zoom** magnification scroll bar.



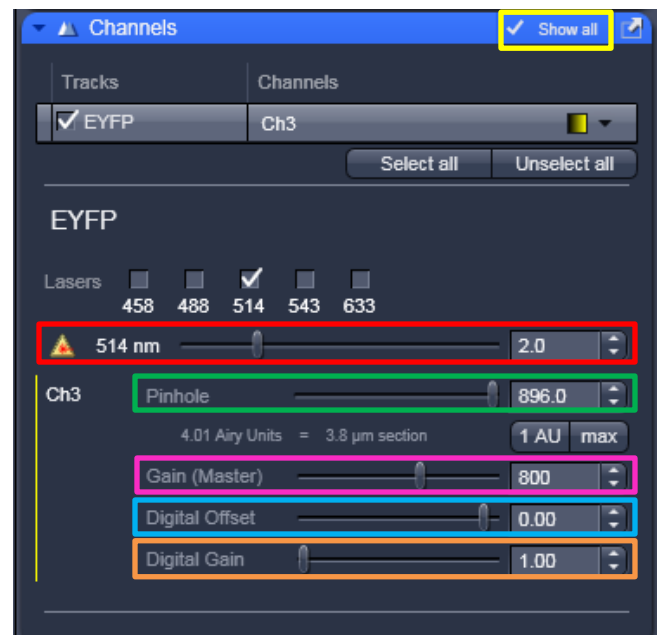
**11. Get Image on Computer Screen** by selecting the **Live** or **Continuous** button. Both will scan continuously until you select **Stop**

- Live**-Uses fastest parameters possible, not the ones you chose in the **Acquisition Mode** tab.
- Continuous**-Uses the parameters you selected in the **Acquisition Mode** tab.

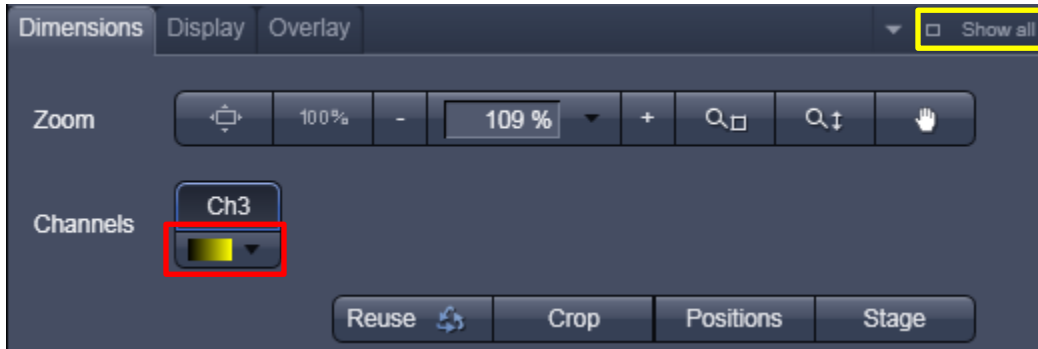


## 12. Adjusting PMTs

- Under the **Online Acquisition** list open the **Channels** drop box and check the **Show all** box on the right side of the menu.
  - You can adjust the **laser intensity** with the slide bar for each indicated laser
  - Pinhole**-typically start at 1Airy Unit. You can easily select this by pressing the **1 AU** button
  - Gain (Master)**-Adjusts both the foreground and the background
  - Digital Offset**-Adjusts the background
    - Note: If you remove too much background you will start to remove the foreground image as well
  - Digital Gain**-Adjusts the amplification value of the A/D converter



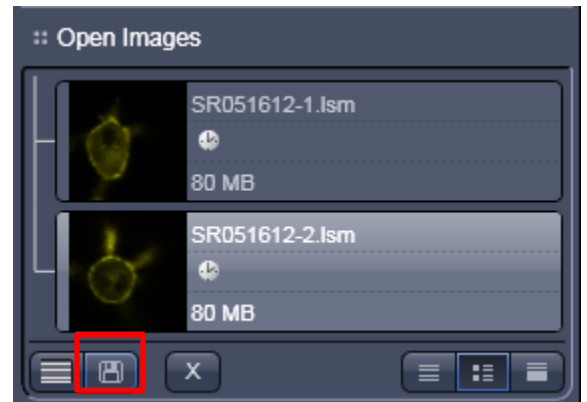
- To check your pixel saturation
  - Check the **Show all** box on the **Dimensions** tab located below your image
  - Select the **Range Indicator** option from the Channels drop menu (or click directly on the square indicating the color of your image)
    - Over saturated pixels will be **red**
    - Under saturated pixels will be **blue**
  - Adjust saturation with the PMT slide bars to the desired range.
    - *Note: You must either select Live or Continuous to see the changes as you make them.*



**13. Final Image** – Click the  button located near the top of the **Acquisition** tab.

**14. Saving your Image**

- Click **Save** from the **Open Images** toolbar on the right side of the program window.
  - All images will be saved in the D: drive under a folder with your name on it.
  - If you do not have a folder, create one.



**15. Shut Down** – If someone is signed up to use the scope within two hours after you are finished, leave the Argon laser on STANDBY and log-off – leaving everything else ON. If you are the last person using the microscope, exit the software (remember to shut the lasers OFF) and follow the shut-down procedure.

**3** – Click **Start** on the bottom left hand side of the computer and choose the **Shut Down** option. Wait until the screens go black and the fan for the Argon laser shuts off before proceeding to **2**.

**2** – Main Switch (left of microscope)

**1** – Mercury Lamp (underneath microscope and to the left)