BIOLOGICAL IMAGING DEVELOPMENT COLAB

UCSF University of California San Francisco

# Leica SP5

Laser Scanning Confocal Microscope

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## Contents

# 1 Introduction

The Leica Sp5 is an inverted laser-scanning confocal microscope with nine laser lines and five PMT detectors (photo- multiplier tubes). The system is enclosed with an incubation unit to regulate experimental temperature and air gas (5% CO2) for live cell imaging.

The objective turret houses six lenses including 10x Air, 20x Air, 20x Oil, 40x Oil, 63x Oil, and 63x Water. The stage is motorized for large tiled stitching or marking and storing positions.

Please sign up for time using the MyCores microscope scheduler. Reservations can be made up to 4 weeks in advance for owner's consortium and beginning on Friday for the following week for non-owners.

# 2 Hardware Start-Up

1. Turn on the three green power switches from left to right. See Figure:1.
   (a) The first powers the PC and the Microscope.
   (b) The second powers the Scanner.
   (c) The third powers the Laser.
   (d) Once the three power switches are On, turn On the Laser Emission with the Key.


Figure 1: The power supply switches located at the right side of the computer desk.

2. Turn on the fluorescent lamp source See Figure:2.


Figure 2: Fluorescent Lamp located to the right of the microscope.

3. Note: the Leica CTR6500 Control Box, located under the fluorescent lamp source, should always remain powered On, do NOT turn off.

# 3 Logging In to the Computer

1. Sign in to the computer with your UCSF account. These are the same credentials as your UCSF MyAccess login.

2. Check the stage and objectives for stray (not cleaned up) oil. Contact the BIDC if you find oil on non-oil objectives, or if you notice excessive oil. There should never be oil on the stage, or on non-oil objectives. Oil on non-oil objectives will permanently damage objectives. Improper use of immersion oil will not be tolerated.

3. Before starting the software, make sure the arm of the microscope (where the brightfield illumination source and transmitted detector are located) is down in its resting position over the stage. Additionally, no sample should be in the sample holder. See Figure:3

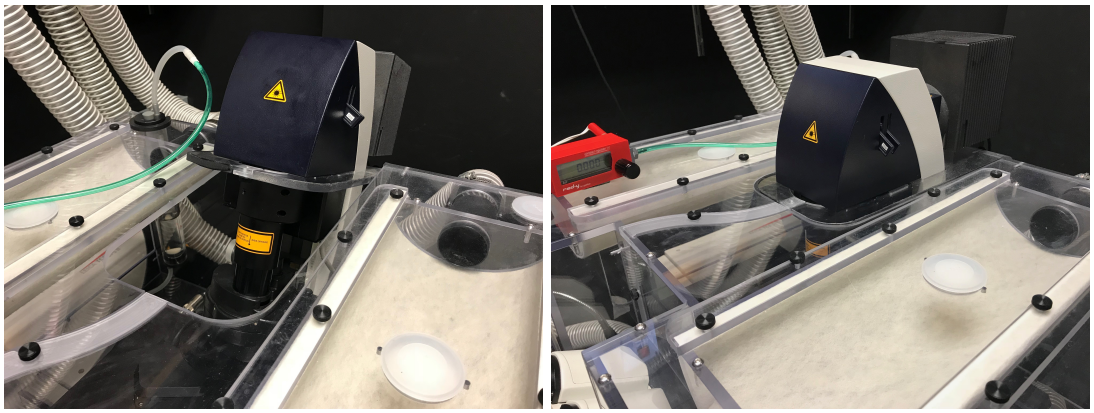

Figure 3: The image to the left shows the arm of the microscope lifted. The image on the right shows the arm of the microscope down in its resting postion.

# 4 Starting the Software

1. Start the LAS AF Software
2. The software will pause, continue by clicking the Ok button.
3. A window will appear asking to initialize the stage. You may initialize the stage if you wish to do tiled images. If you select Yes, be sure that your sample is not on the stage, and that the objective turret is all the way down (Press and hold Z Down arrow button on the right-hand side of the microscope body). Damage to the objective or your sample may result if these precautions are not followed.

# 5 Turning on the Lasers

1. Go into Configuration tab, select Laser icon
2. Turn on desired lasers by clicking checkboxes. Note that the Argon additionally needs to be turned on to some percentage. It is recommended that you start at 20%. See Figure:4

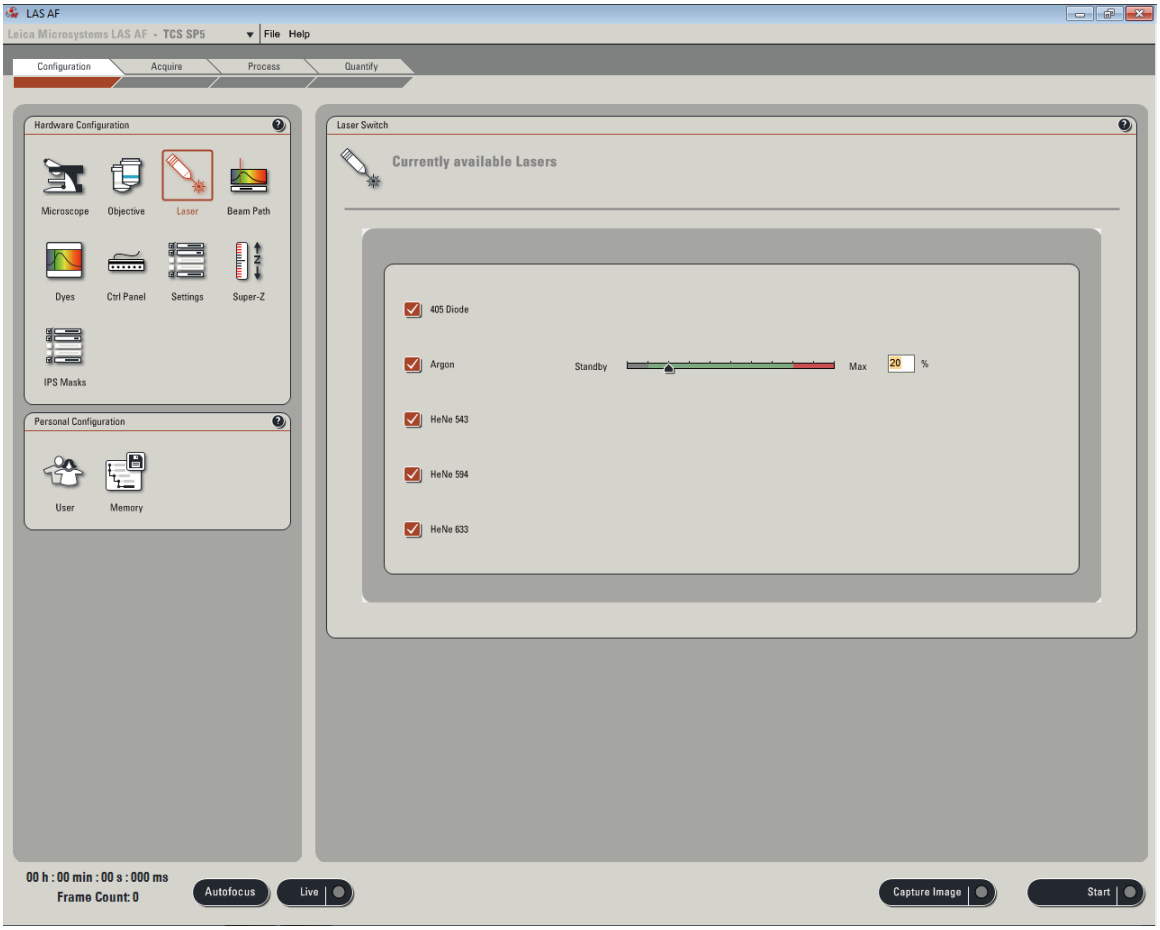


Figure 4: Laser settings under the configuration tab.

3. Click Settings icon, and adjust bit depth resolution if necessary (default is 8 bit, other options are 12 and 16 bit) If you are quantifying your image intensities, it is recommended to use 16 bit. See Figure:5

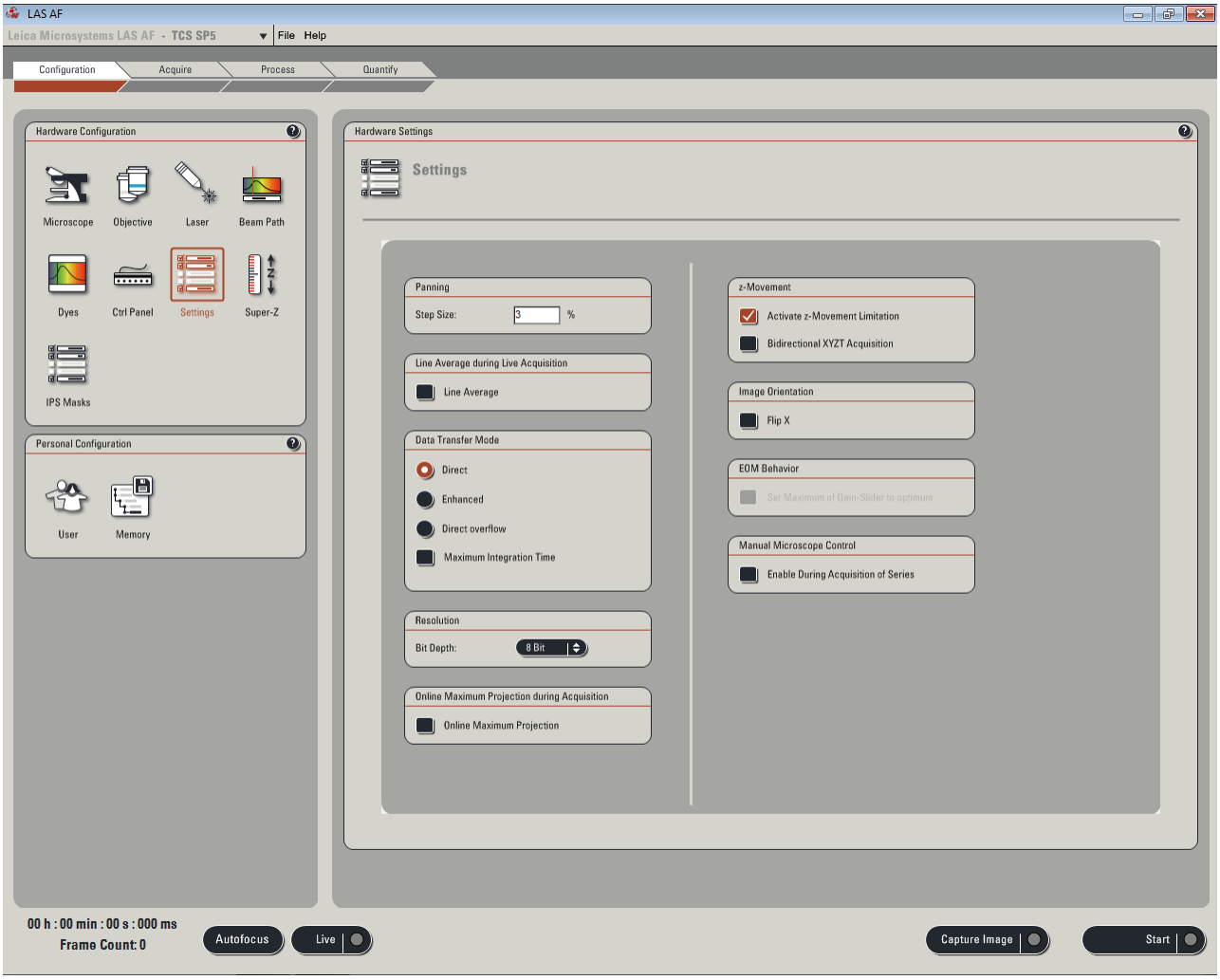


Figure 5: Bit depth settings.

4. It is not recommended to change any other settings.

# 6 Focusing Your Sample

1. Place your sample on the stage and find your sample through the eyepieces.
2. To focus on fluorescence, use the panel on the lower front of the microscope. See Figure:6. Select the desired filter from the list below and press the shutter button.

   GFP

   RFP

   DAPI

3. Then hit the shutter button on the same panel to open the shutter.

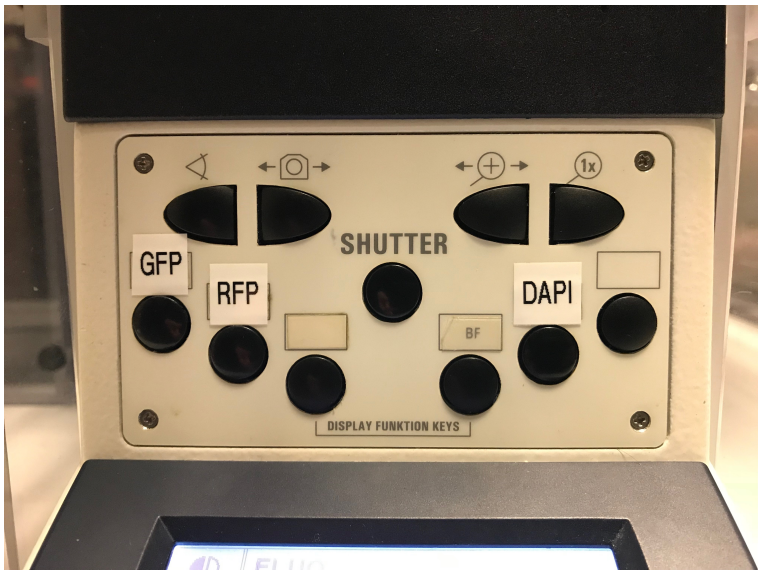


Figure 6: Panel at the front of the microscope for controlling filters for the eyes.

Note: if you notice that the light coming out of the objective appears dim and you are having a hard time finding your sample through the eyes, press the shutter button on the fluorescent light source to disengage the shutter and then once again to re-engage it. See Figure 2. You should now notice the light coming from the objective to be much brighter.

4. To focus on brightield see Figure:7:
   (a) Press the TL/IL button on the lower left-side of the microscope body to turn on the light source
   (b) Adjust the brightness using the INT button

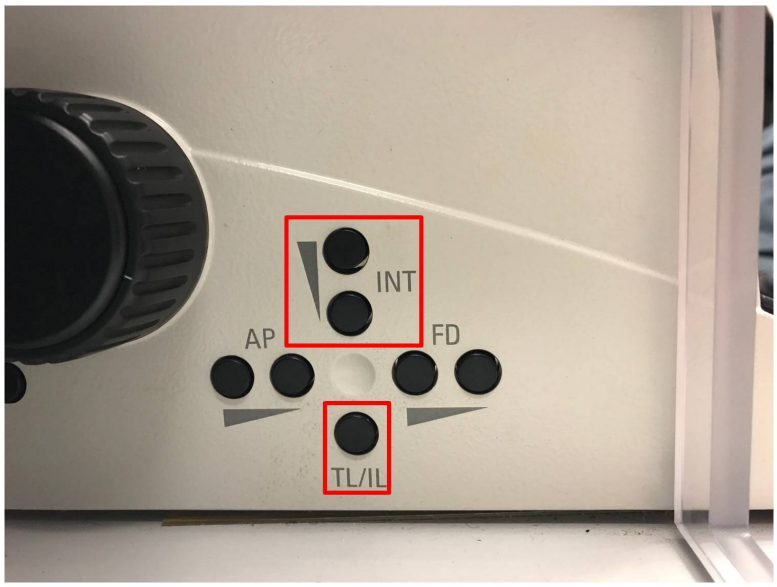


Figure 7: Brightfield controls located on left side of microscope.

5. Once your sample is in focus, go to the Acquire tab in software to begin imaging.

# 7 The Acquire Tab

1. Under Beam Path Settings, and the Load/Save single setting window, you may use the drop-down menu to find preset settings for common fluorophores, such as DAPI, GFP, or TRITC. See Figure:8

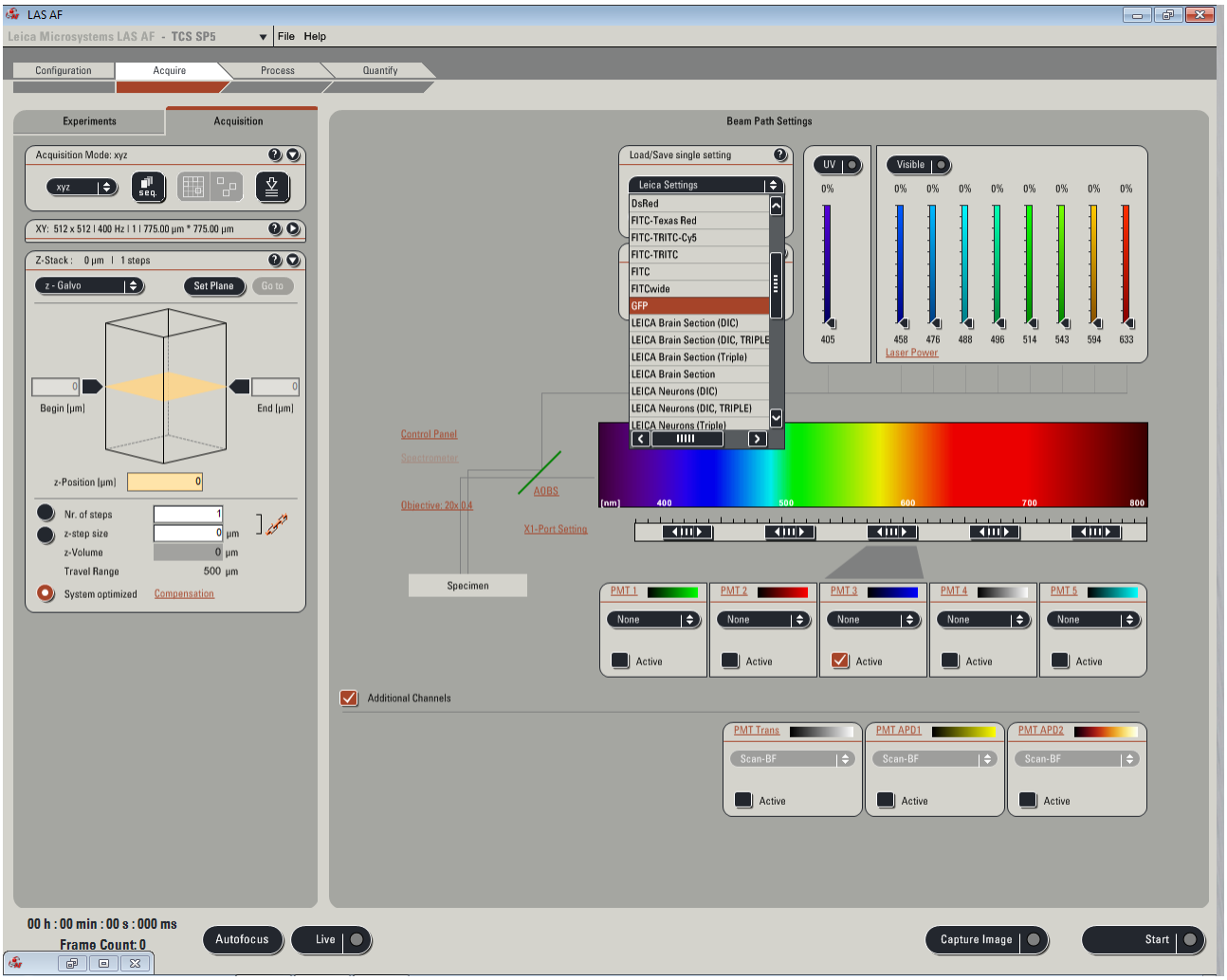


Figure 8: Selecting the GFP preset setting from the Load/Save Single Setting window.

2. Note: When creating your own custom acquisition settings, please refer to the Collection Settings PDF located on the BIDC website.

3. Turn on the proper excitation source, either UV or Visible, by clicking the radio button. See Figure:9

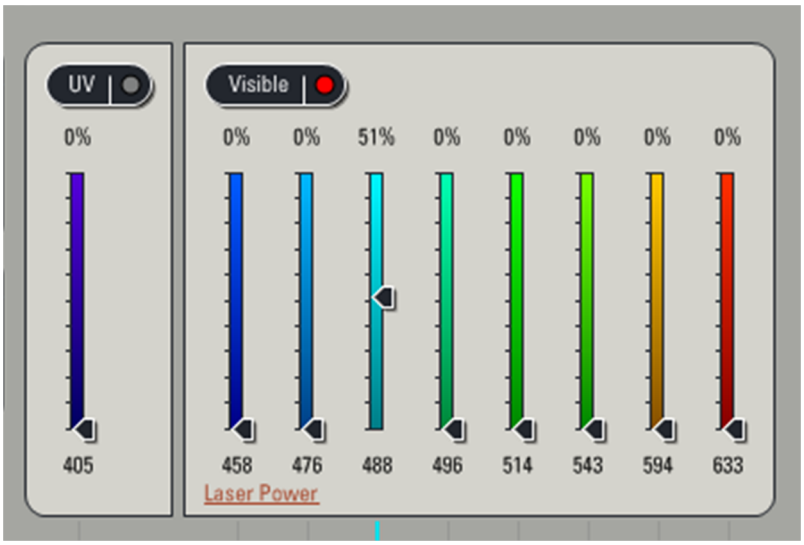


Figure 9: The figure above shows the visual laser radio button powered on.

4. Turn up laser to about 20-25%.
5. Once the laser line is selected and turned on, the appropriate PMT must be selected to capture the emission.Keep in mind, the minimum parameter for the PMT should be set at least 10 nm above the excitation wavelength.
6. Click 'Live' to view your sample on the computer and adjust the 'Smart Gain' between 700-800$V$ , although the lowest gain possible is recommended to reduce noise. See Figure:10
7. After setting the gain, adjust the Z Position (focus) using the dial on the smart panel, Not on the microscope. See Figure:10


Figure 10: The image above shows the smart board where the Smart Gain and Z- Position knobs are located.

8. Select Quick LUT on the upper lefthand side of the acquisition window, which will display all oversaturated pixels in blue, and all other gray values in shades of red/orange. Adjust gain and laser power appropriately in order to eliminate all oversaturated pixels. See Figure:11

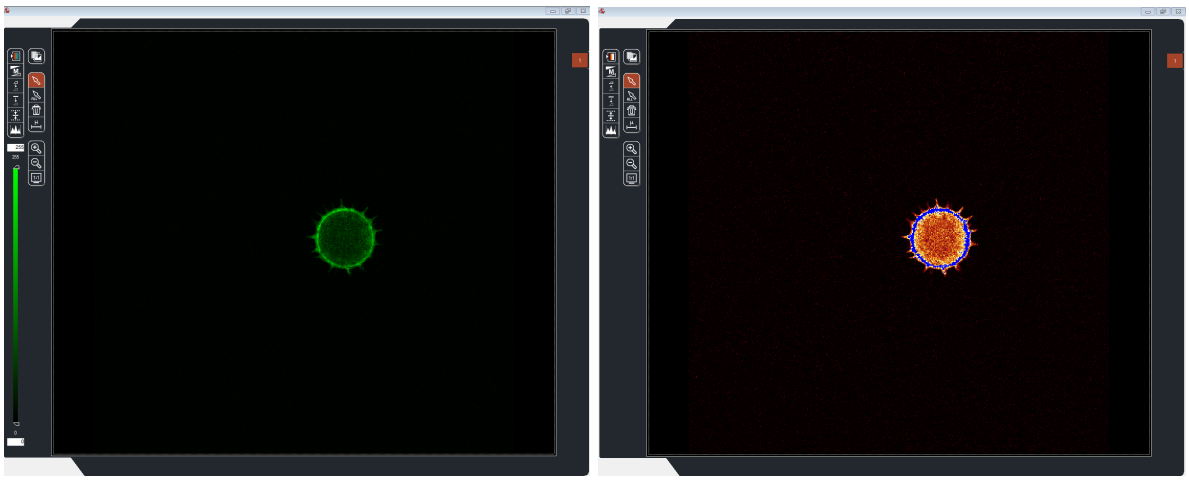

Figure 11: The look up table on the left shows pixels that are properly exposed. The image on the right shows pixels that are over-saturated.

9. Once your settings are satisfactory, you may save these personalized settings for future use using the Load/Save single settings window, by clicking the Save button. These will be stored for your personal account, and are not seen by other user accounts.

10. Repeat for each desired channel.

11. In the 'XY Panel', choose your pixel resolution. The default settings will be 500 x 500 but can be changed to a higher resolution such as 1024 x 1024 for imaging. Changing your scan speed will affect your field of view- scanning faster corresponds to a smaller field of view. The default scan speed setting is 400Hz but can be changed to 200Hz for imaging.

12. Note: To Image a Z stack, Acquisition Mode must be in xyz. See Figure:12

    (a) In Z-Stack panel, set the beginning and end of your z-stack using the Z Position control knob on the smart panel, not the microscope, while in Live mode.

    (b) Once the limits are set, turn off Live mode to set the number of steps within the selected volume, or the z-step size to determine the number of steps.

    (c) There is also a "system optimized" option to use Nyquist sampling methods. Keep in mind that the optimized default setting oversamples in z and should not be used.

13. To make a multi-channel image, select the 'SEQ' button under Acquisition Mode module. See Figure:12

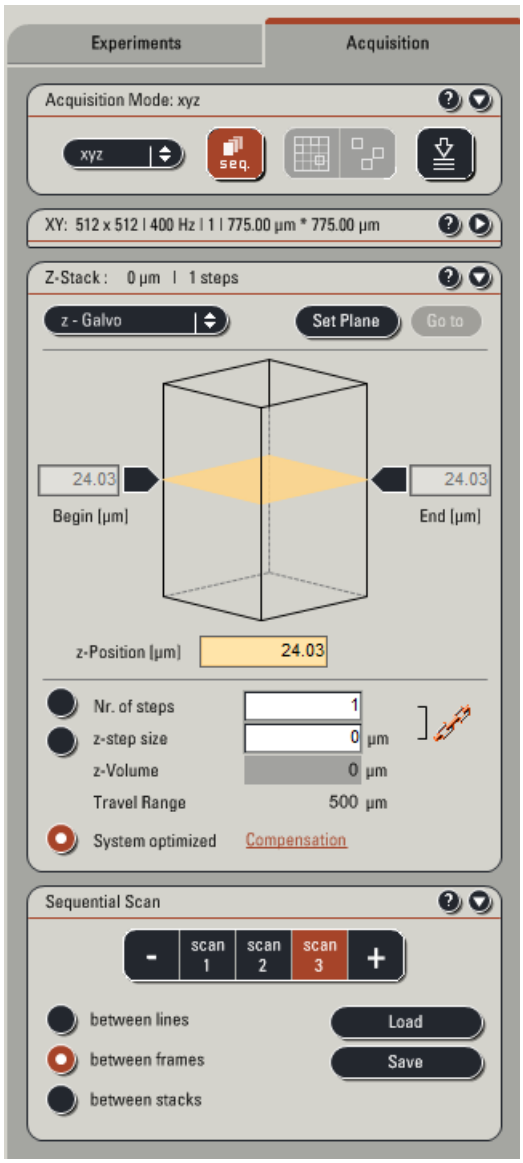


Figure 12: The Z-Stack and Sequencial Scan windows can be seen in the figure above.

(a) Assign Scan 1 to one of your saved acquisition settings to scan with the lowest energy laser line first (your 'reddest' channel).

(b) Click the + button in the Sequential Scan module and assign each channel a preset acquisition setting, going from lowest energy to highest energy (DAPI last)

(c) For imaging a single plane, choose to scan 'Between Frames'.

14. Click 'Start' to begin acquisition.

# 8 Saving Acquired Images

1. Click the 'Experiments' tab to view all images taken.
2. Delete or Rename images as needed.

3. Click 'Save All' to save images as a .lib file in your hard drive or on Box.
4. Please remove old files from the SP5 computer once they have been saved on your hard drive or Box.

# 9 Shut Down

1. After saving, close the LAS AF software.
2. Shut down the computer using Shut Down option in the Start Menu.
3. Remove samples and clean objectives as needed.
4. Shut off Leica shutter box.
   *Note: The shutter box must be left off for 20 minutes before turning on again.
5. Turn off PC/microscope power switch.
6. Turn off Scanner power switch
7. Turn emission key for laser to off. Leave the Laser Power switch on for 10-15 minutes. This will allow the fan to cool the laser.

# Contact the BIDC

The BIDC office is located in Medical Sciences Building Room S1109.
The BIDC office phone number is 415-476-4550.
If you need immediate assistance and no one is available in the office, or it is after business hours, please call the **BIDC Hotline** at 415-745-2432.