User Guide to Confocal Microscope:

Zeiss LSM Pascal
This guide does not cover all the details in documentation that comes with the Zeiss microscope. Please do find time to go through the original documentation once. This document should be referred for quick review.

You should be trained before you use this machine.

Please email ftsalles@stanford.edu for training information.

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Starting the microscope
Remove the blue cover from the microscope. Check if the system is already turned on. The LED display on the microscope tells you if the microscope is switched on. Proceed to check the laser if already on. Turn the microscope by turning a black switch on the left of the microscope, just below the air-table. Thus should turn all the systems except the laser on (That needs to be switched on separately) Check if the computer is already on, turn the computer monitors on.
Note :: Remember to turn the microscope on before running the Zeiss software in the online mode for imaging. See switching software on the computer below.

Switch Lasers "on"
To switch on the laser, turn the key on the laser power source to your left. To actually use the laser for scanning a sample, you would have to activate it in the Zeiss control software.

Start Zeiss Software
Double click on LSM 5 Pascal icon to start confocal operating software. The operating mode window will appear. The control software checks for operating microscope.

If the microscope is not turned on an error window appears. Choose offline or online mode as per use. This brings up the start menu for the microscope.
Choose acquire mode from the tab.

**Configuring the laser**
Click on the acquire button in the main window and choose Laser sub window.
To switch on the laser, choose the laser you want turned on and click on the on tab.
To set the required laser path settings, choose "Config" button from the main menu.

**Configuring microscope mode**
To set the microscope, hit "Acquire" and choose "Micro".
Click on the objective lens icon to choose an appropriate lens.

**Set sample on the stage and adjust focus manually**
Remember this is an inverted microscope, simple to set an experiment and be able to image in real time.
Be very careful not to touch the lens below the stage. Do not drop anything on the lens.
For transmitted light, click "transmitted light" button at the top of the axiovert control window. Switch the halogen light (HAL) on, and vary transmitted light intensity by the scroll bar next to it. Use the manual focus wheel to raise the objective lens until the specimen is in focus.
Configure scanning parameters

Push the bottom slider on the microscope front to right, to prepare microscope for scanning mode. Click "Acquire", and choose "Scan" from the menu.

For a fast scan, click "Fast XY". Choose "channels" sub window.

"Detector gain" sets the gain on the photo-detector;
"Amplifier Offset" sets the background;
"Amplifier gain" alters amplification factor.

Check the pin-hole factor in the channel window. The microscope can automatically set the pin-hole size for you based on the objective lens being used.

For choosing the right settings of detector gain and laser power to be used for a sample, click on "pallet" button on the scanning image window and choose red and blue range indicator. Now adjust the detector gain and laser power settings such that you avoid under-saturation and over saturation of your imaging photo-detector. Over saturation is shown by red color on the image, while under saturation is shown by blue color. Optimize such that both these colors are minimized.

To improve image quality, you can choose the appropriate frame size. (For high quality scan choose 1024*1024, for a low quality scan 512*512 scans are good). You can also adjust the scan speed(5 or 6 for low scan). Magnify, move, rotate the image if needed using buttons at the bottom.

Click 'Stop' if the image has been optimized. You can click on single to get a 'single' image of the sample. Click 'Save as' to save the image to the da-
tabase. If you are using fluorescence in your images work quickly cause you will be bleaching your sample quickly.

**Set Z slice parameters**
For taking images with different Z slices, the slice parameter needs to be set. This way you can create true 3-D images of your sample. Click on 'Z-stack' button in the scan control window (this will open Z settings panel). Choose 'Fast XY'. Choose the focus wheel to set the start of z-stack. Click 'Mark first' in the panel. Now change the focus to go to bottom of the stack and click 'mark last'. Click 'start' to start scanning for different z stacks.


**Creating 2.5D topological images**
To create a 2.5D topological view of the image, click 'Topo'. This should show the view where all the z slices are shown together, with a z scale. You can choose a noise filter from the window panel on the right, choose either "none", "median" or an "FFT". The intensity threshold can also be set from the panel. A useful function is to fill holes in the 3D data by "fill holes". Various display options are also available.

**Creating 3D images, storing 3D data**
The Zeiss software creates a point cloud representation of the Z slices created by the scanning of the sample. This can be used to create a rendered 3D image of the sample. Various options for displaying the surface of the objects can be used. Click on "3D" button to open the 3D image mode. You can choose surface type form basic, advance and full resolu-
tion. Also the 3D image can be rotated to an view. A movie of the 3D rendered views can be saved in the software too.

An example 3D view of the imaged somata is shown here.


To save the image in the data base, and the 3D data, click 'Save as'. Choose the location of the .mdb file and choose the database you want to save the images in. The 3D data can be saved as a .txt file which gives the point cloud of the 3D image.

**Switching off the system**

If you are the last user of the day, you need to switch the laser off and cover the microscope. Firstly exit from the control program. Then shut down the microscope from the switch on the left side of the microscope. In the end, switch the laser off from the key button on the left side, down below.

Do not leave the laser on if nobody is using the microscope after you. The laser has a finite life time and replacing the laser is an expensive affair. Finally cover the microscope with the Zeiss cover. Clean up the table after use, and keep any slides back on the rack.