CONFOCAL LSM 5 PASCAL MICROSCOPE OPERATING MANUAL

Version: 2.0

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1.0 INTRODUCTION

1.1 Scope
These procedures apply to the Carl Zeiss CONFOCAL LSM 5 PASCAL MICROSCOPE. All maintenance should follow the procedures set forth in the manufacturer's maintenance and operations manuals. This document is for reference only. Personnel should be trained by authorized staff before operating this equipment.

1.2 Description
The Confocal LSM 5 Pascal microscope features both optical and laser scanning modes of operation with multiple objectives ranging from 2.5X to 100X. A number of custom filters and metrology functions can be used to analyze samples. Scanned images can be saved to be recalled and used later or captured as an image file for export.

1.3 Safety
1.3.1 This machine is connected to 110 VAC. Be very careful and aware of electrical hazards. If you encounter any electrical malfunctions, contact NanoFAB staff immediately.
1.3.2 This machine uses two lasers the first laser is a Class IIIb 450 – 530 nm laser emitting 200 mW maximum output. The second is a Class IIIa 543 nm laser emitting 5 mW of maximum output. Do not stare into the light beam or its reflection.
1.3.3 This machine has no EMO (Emergency Off); If electrical must be removed from the system it must be unplugged.
1.3.4 The microscope sample stage is capable of movement take care not to pinch your fingers or break your sample.
1.3.5 Read any posted NanoFAB Engineering Change Notices (ECN) for any hardware, process or safety changes before running the tool.

2.0 HARDWARE

2.1 LCD Touch screen
2.2 Class IIIa 453 nm Laser
2.3 Class IIIb 450 – 530 nm Laser
2.4 Motorized X, Y & Z axis sample stage
2.5 Motorized objective selector
3.0 REQUIREMENTS

3.1 Training
You must be a qualified user on the Zeiss Confocal microscope in order to use this system. Contact a staff member to schedule a training session for this system.

3.2 Restrictions
3.2.1 None.

4.0 OPERATING PROCEDURE

4.1 Powering ON the Confocal Microscope
4.1.1 Turn ON the (# 1) power strip next to the system computer first. Then turn ON the (# 2) power strip located underneath the microscope (Fig. 1). The computer should begin powering up.

(Fig. 1)

4.1.2 A windows login box will appear when the software loads. The username “Administrator” will be loaded, DO NOT enter a password just press the enter key to log into Windows.
4.1.3 Double click the **LSM 5 PASCAL** icon on the desktop of WINDOWS XP to start the Carl Zeiss microscope software. Once the **Switchboard** (Fig. 2) window appears on the screen ensure that “**ONLINE MODE**” is selected and then click the “**START**” button to begin.

***Note*** **OFFLINE MODE** is only used to examine saved database files without powering the microscope up. If **OFFLINE MODE** is left **ON** and the user wants to use the microscope then the microscope will not communicate with the computer and the Carl Zeiss software will not function properly.

(Fig. 2)

4.1.4 The LSM5 Pascal selection toolbar (Fig. 3) will load. Select the two buttons labeled “**Micro**” and “**Config**” from this window.
4.1.5 The following two windows will open, Microscope Control (Fig. 4) and Configuration Control (Fig. 5).

(Fig. 4)  (Fig. 5)

4.1.6 To use the Confocal microscope in optical mode you must select the CAMERA tab (Fig. 6) in the Configuration Control window. To begin using the microscope in Laser Scanning mode, leave the LSM 510 tab selected in Configuration Control and skip forward to Section 5.0.

(Fig. 6)
4.1.7 In both open windows select the “Reflector” button and then select Analyzer Module D (Fig. 7) from the drop down menu.

(Fig. 7)

4.1.8 In both of the open windows select the “Transmitted Light” button, turn ON the lamp and set the slider to 79% (Fig. 8).

(Fig. 8)
4.1.9 You will now utilize the **TOUCHSCREEN** (Fig. 9) located to the right of the Confocal Microscope. Press the **MICROSCOPE** button on the left hand side of the touch screen. Then press the **OPEN** button for the RL Shutter.

(Fig. 9)

4.1.10 Use the black **Focus Dial** at the right hand base of the microscope (Fig. 10) to focus your sample. To change objectives press the desired objective button on the **touch screen** and the microscope will rotate to that objective. For initial focus it is best to start out with a low 2.5X or 5X objective and work your way up from there.

(Fig. 10)
4.1.11 To capture an image file for analysis click on the **Scan** button in the **LSM 5 Pascal** toolbar (See Fig. 3) this will open the **Scan Control** window (Fig. 11). To find the proper contrast settings you may either select the “**FIND**” button on the right hand side and the contrast will be detected automatically. You may also set the contrast manually by selecting the “**FAST XY**” button and adjust the **Exposure Time** slider watching the image generated for optimum contrast.

(Fig. 11)

4.1.12 Once the proper contrast has been selected you will need to fine tune the focus of the microscope. The TV camera’s focus differs from that of the optical viewfinder, so getting the focus set up perfectly by looking directly through the optics will only give you a marginal image when switching to the TV camera. To set up the TV camera focus go to the **Scan Control** window once again (See Fig. 11) and press the “**FAST XY**” button, then use the focusing knob on the right hand base of the microscope (See Fig. 10) to focus the image as displayed on the computer monitor.
4.1.13 Once the contrast and focus of the image displayed on the monitor is correct you can now capture the image file by selecting the button titled SINGLE in the Scan Control window. A single frame image will be captured.

4.1.14 The captured image file will open in a separate window which has the metrology functionality available. Several options are there some of which include 2.5 D mapping, intensity mapping, point A to B measurement tools, etc.

4.1.15 The captured image can either be saved to the database where it can be accessed and analyzed in the future using the Carl Zeiss software. The image file may also be pasted into MS Paint for editing and then saved strictly as an image file with no database info attached.

4.1.16 Once you are finished viewing your samples with the ConFocal Microscope close the Scan Control window (See. Fig. 11).

4.1.17 In both the Configuration Control and Microscope Control windows (See Figs. 4 & 5) turn OFF the lamp and set the ANALYZER MODE back to NONE before closing both of these windows.

4.1.18 Close the LSM 5 Pascal window (See Fig. 3).

4.1.19 Select the EXIT button on the Carl Zeiss Switchboard window (See Fig. 2).

4.1.20 To power OFF the Confocal microscope skip forward to Section 6.0.
5.0 Using the LSM 5 Laser

5.1 Powering ON the LSM 5 Laser

5.1.1 In order to perform a laser scan and utilize 3D topography mapping the LSM 5 laser must be used. Turn the Laser ON by following steps 1 – 4 as shown below in Figure 12.

(Fig. 12)

5.1.2 Leave the laser operation mode switch set to the “Normal” position (See Fig. 12, Step 3) for at least 15 minutes to allow the tube sufficient time to warm-up before switching to “Reduced Lifetime” operation mode. The switch to “Reduced Lifetime” mode should only be made when you are ready to begin setting the contrast and scanning the sample. The Green LED will turn off and the Red LED will turn ON when reduced lifetime mode has been selected. Once the sample scan has finished set the operation mode switch back to Normal and verify the Green LED has turned on to ensure maximum laser tube life.
5.2 Laser Scanning a Sample

5.2.1 Go to the LSM 5 Pascal toolbar (See Fig. 3) and select the “Scan Control” button.

5.2.2 Once the Scan Control window opens you must select the “Mode” button. Then you will specify your scan parameters which can include, the Frame Size, Image Size, Data Depth, Scan Type, etc. The Z Stack parameters must be utilized and an Upper and Lower layer established if 3D topography mapping is desired.

5.2.3 Once the scan parameters have been established then you must set up the contrast. If you have not already done so set the Normal/Reduced Lifetime mode switch to Reduced Lifetime (See Fig. 12, Step 3). The Red LED above this switch should illuminate.

5.2.4 In the Scan Control window (See Fig. 13) select the “FIND” button to start the auto contrast routine.
5.2.5 You may now begin your scan by selecting the “Single” button from the Scan Control window (See Fig. 13). Remember that the length of time required to complete the scan is based on the factors you input. A scan can take from 30 seconds to 10 hours depending on the factors you input. If a scan is determined to be too long once it has started, then select the “STOP” button in the Scan Control window and lower your data requirements and select the “Single” button to start the scan again.

5.2.6 Once the scan completes the metrology functionality will be located in the same window that the scanned image is in. Functions such as, 3D topo, A to B point measurement, Height measurement, etc. are available.

5.2.7 The captured image can either be saved to the database where it can be accessed and analyzed in the future using the Carl Zeiss software. The image file may also be pasted into MS Paint for editing and then saved strictly as an image file with no database info attached.

5.2.8 Once you are finished viewing your samples with the Confocal LSM 5 Microscope, close the Scan Control window (See. Fig. 13).

5.2.9 Close both the Configuration Control and Microscope Control windows (See Figs. 4 & 5).

5.2.10 Close the LSM 5 Pascal window (See Fig. 3).

5.2.11 Select the EXIT button on the Carl Zeiss Switchboard window (See Fig. 2).
6.0 Turning Off the Confocal LSM 5 Microscope

6.1 Laser and Microscope Shutdown Procedures

6.1.1 If you were operating the Laser then the laser tube power supplies must be shut down first. If you were not using the laser skip forward to Step 6.0.5.

6.1.2 Ensure the run mode switch is in the “NORMAL” position the Green LED should be illuminated. Then turn the key switch on the lower supply to the “0” position.

(Fig. 14)
6.1.3 Wait approximately 5 minutes for the **FAN** located on top of the Laser tube (Fig. 15) to turn **OFF**.

(Fig. 15)

6.1.4 You can now turn **OFF** the POWER ENABLE rocker switch located on the **Lower** laser power supply (See Fig. 13). Then turn the **Upper** laser power supply key switch to the “0” position (See Fig. 13).
6.1.5 Turn OFF the power strips located underneath the microscope in the proper order shown below in Steps 1 & 2 (Fig. 16).

(Fig. 16)

6.1.6 The system is now secured and you may clean up the area and remove your samples.