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Work Instructions for Confocal Laser Scanning Microscopy

Overview of Confocal Laser Scanning Microscope

The present version of Confocal Laser Scanning Microscope includes the following,

1. Laser Source

- (a) Multi Argon (458 nm, 488 nm, 515 nm)
- (b) He-Ne Green (543 nm)
- (c) He-Ne Red (633 nm)
- (d) Infra red (700-900 nm)

2. Scanning Unit

- (a) Fluoview 300 (FV300)
- (b) Fluoview 500 (FV500)

3. Optical Microscope

- (a) Olympus BX 61 (Upright Microscope)
- (b) Olympus IX 81 (Inverted Microscope)

With the existing setup as on 04 June 2004 at Sophisticated Analytical Instrumentation Centre, Indian Institute of Technology Bombay, Confocal Laser Scanning Microscopy can be carried out in two different modes, namely

- Single Photon Mode
- Multi Photon Mode

FV300 can be operated only in single photon mode, whereas, both modes are operable in FV500.

0.1 To be Verified Before Switch ON

1. Ensure that appropriate Air Condition is switched ON (Note: A/C numbered One must be kept ON on ODD numbered date and A/C numbered Two must be kept ON on EVEN numbered date).
2. Make sure that both blowers (fitted at top right side of the room) are ON.
3. Confirm that exhaust present at the bottom is ON.
4. Assure that instrument was in operating condition by verifying the INSTRUMENT / USER log book details.

0.2 Switch ON Protocol-Single Photon Mode Confocal Laser Scanning Microscope

1. Switch ON the Laser source (For He-Ne Green, He-Ne Red, turn the KEY to I position; For Multi Argon, turn the KEY to ON position, and press the black button (AC power) to I position) except Infra Red.
2. Duration of minimum 10 minutes must be allowed after switch ON before the sample can be scanned with Multi Argon Laser.
3. Switch ON the Power Supply Unit (PSU) and Turn the KEY to ON position.
4. Switch ON the Microscope Controller Box (BX-UCB or IX-UCB).

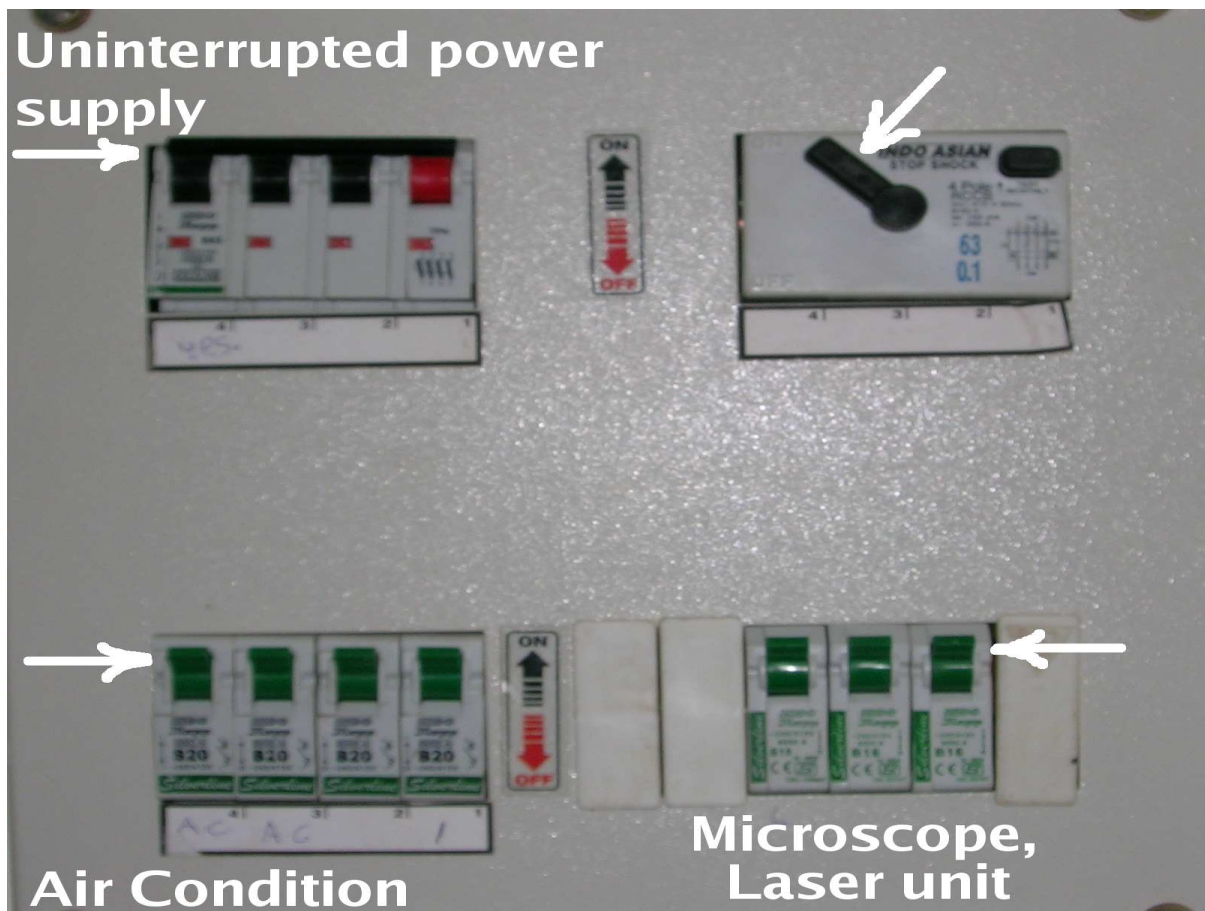


Figure 1: Switch board of CLSM room. Default position of switches as indicated by arrow.

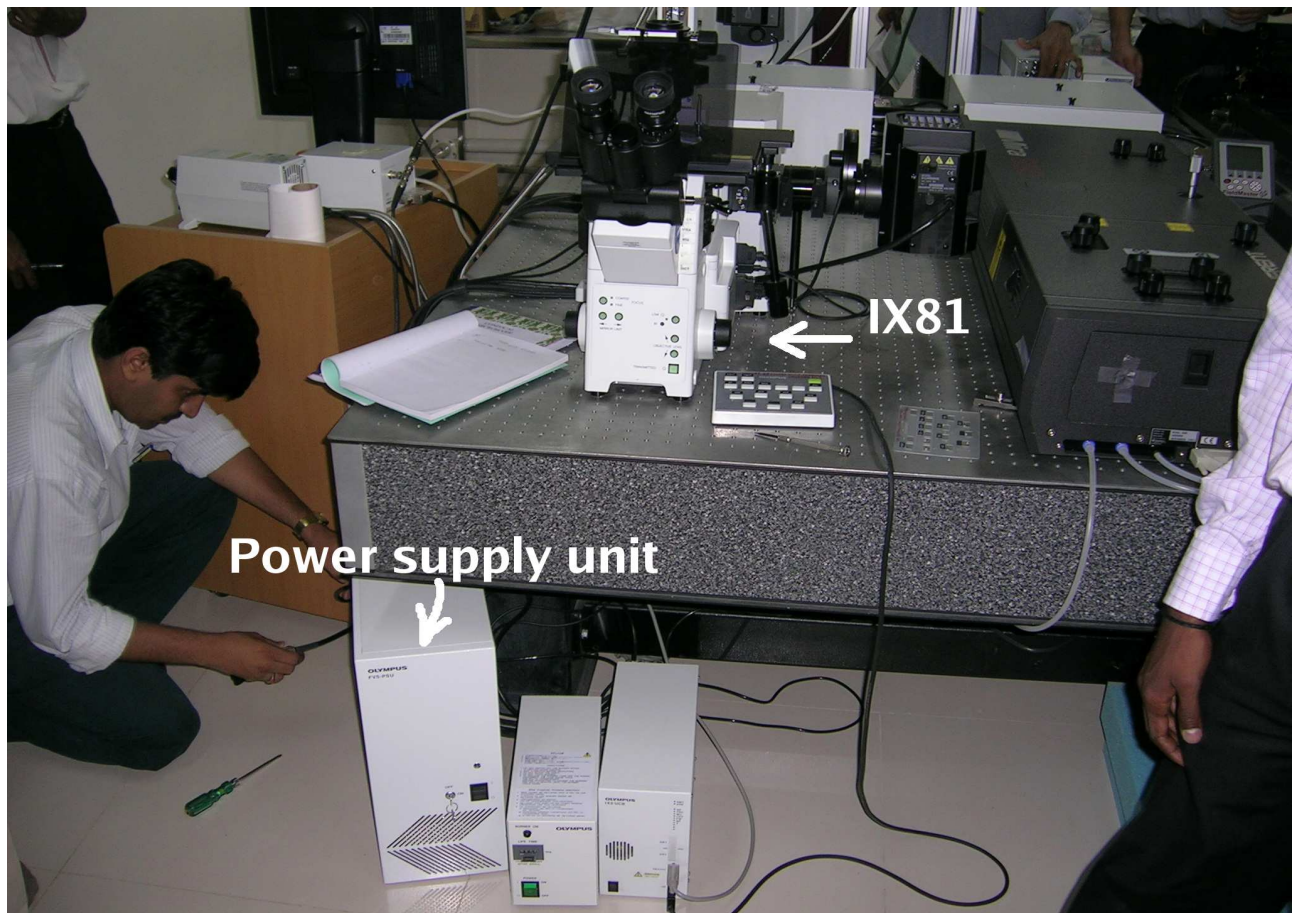


Figure 2: Confocal Laser Scanning Microscope FV500 attached with IX81 inverted microscope.

5. Switch ON the Transmitted Light Power Supply (LG-PS2) and if needed The Mercury Burner too. (Note: Wait for minimum 15 minutes before switch OFF after Switch ON. Duration of 3 minutes must be maintained between switch OFF and switch ON of the Mercury burner) **Overriding this condition will lead to Mercury Burner damage.**)
6. Switch ON the the Computer Power Unit.

0.3 Switch ON Protocol - Multi Photon Mode Confocal Laser Scanning Microscope

1. Switch ON the Laser source (Infra Red)

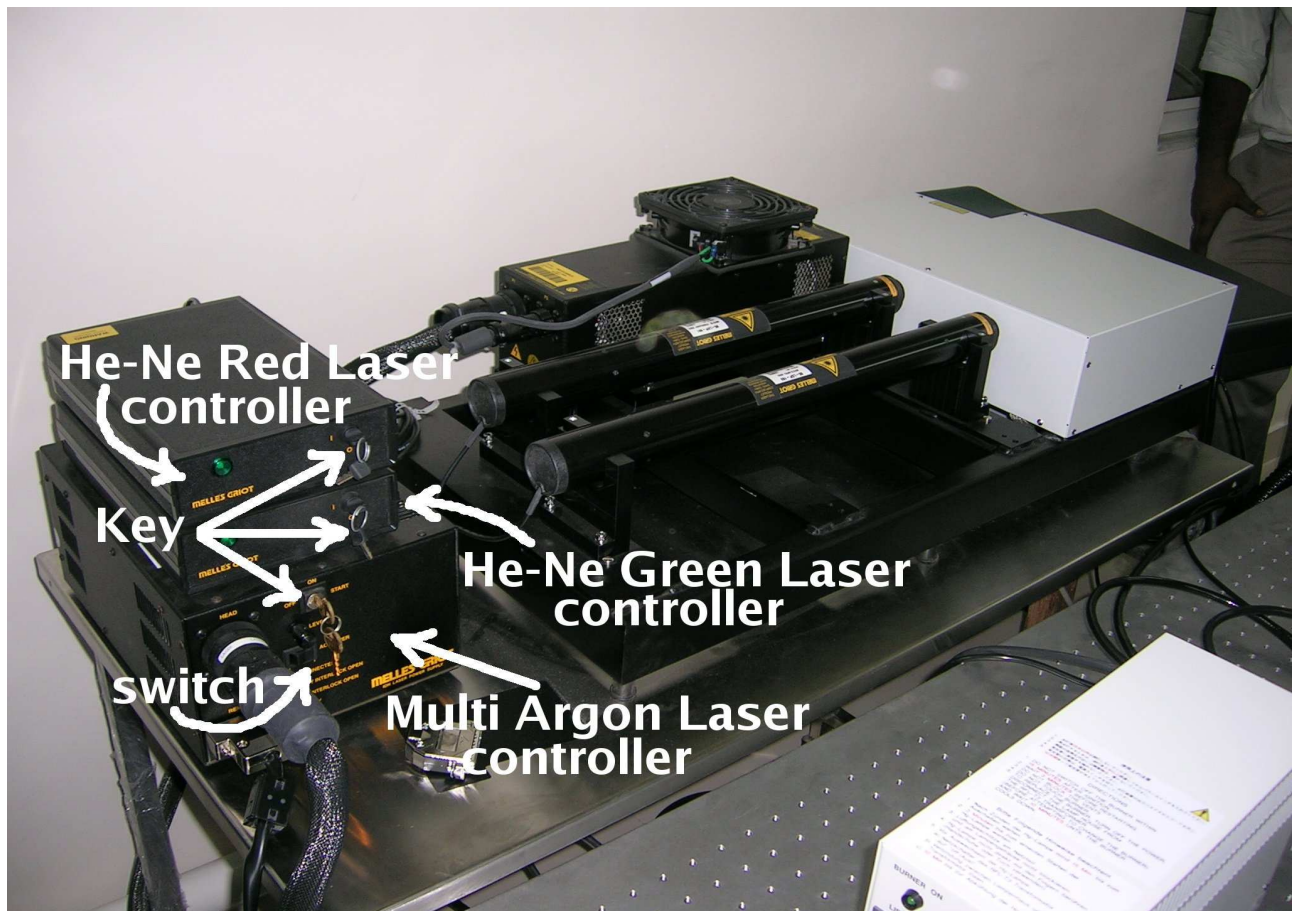


Figure 3: Laser unit comprising Multi Argon, He-Ne Green and He-Ne Red.

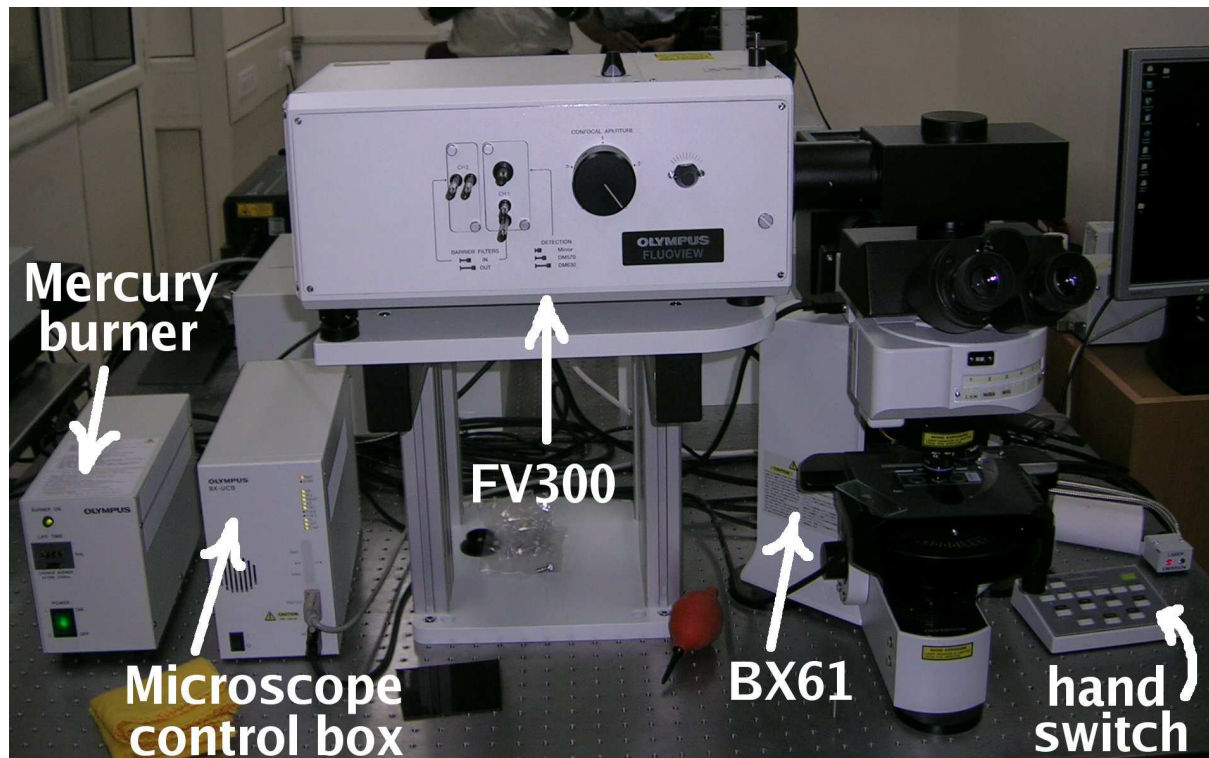


Figure 4: Confocal Laser Scanning Microscope FV300 attached with BX61 upright microscope.

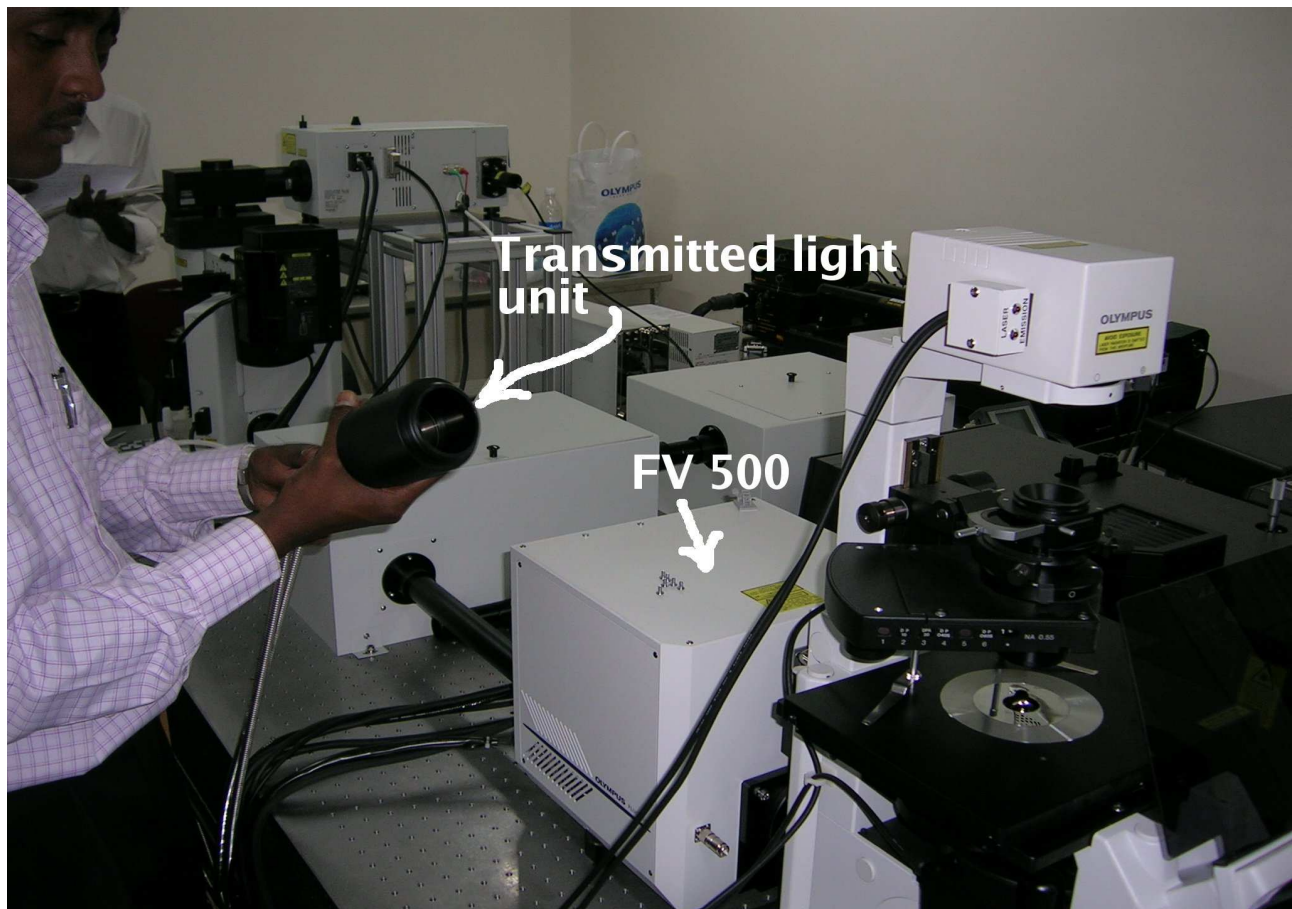


Figure 5: Transmitted light attachment for bright field transmission mode.

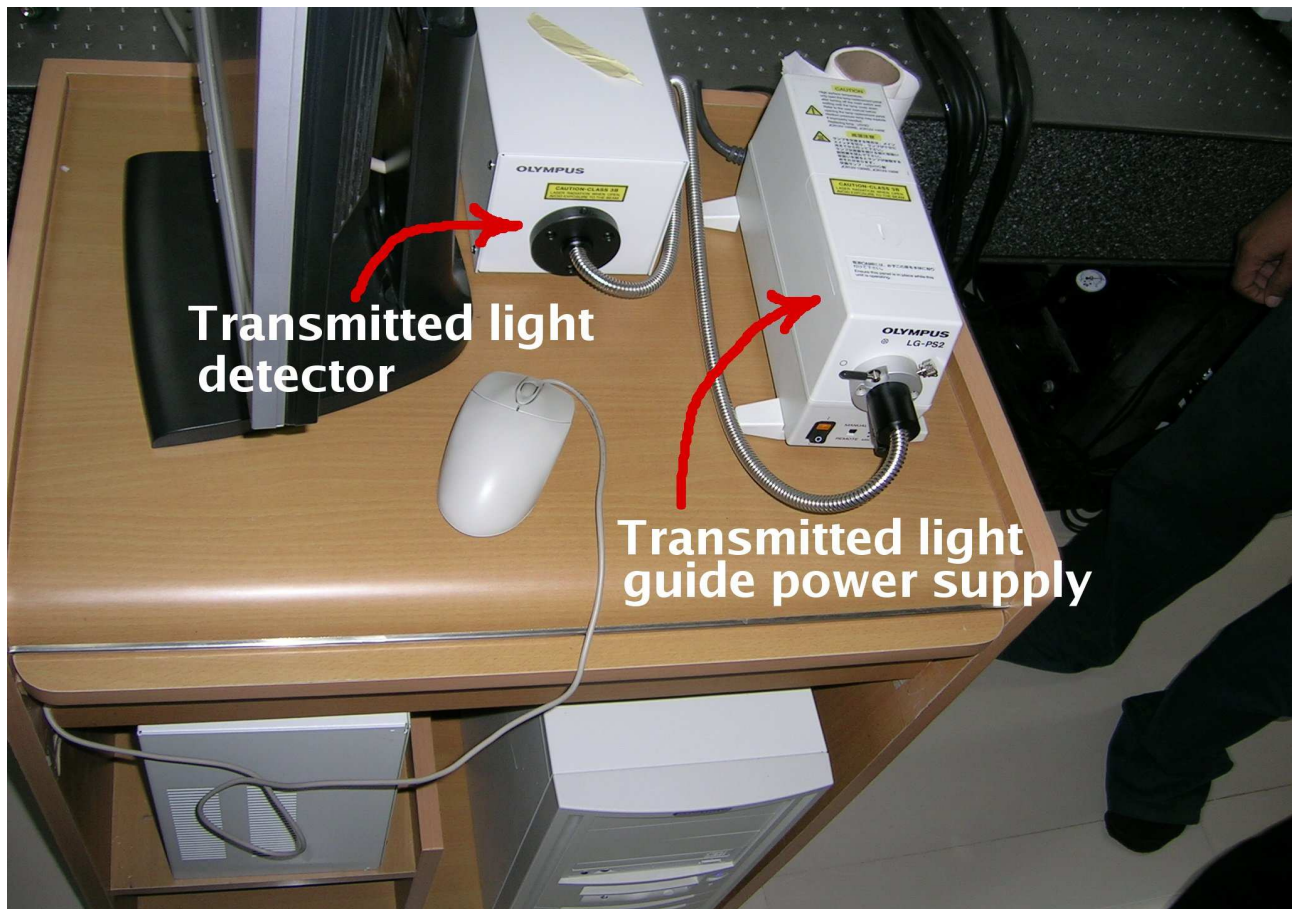


Figure 6: Transmitted light guide power supply and detector.

- (a) Switch ON the Chiller by pressing the black button at the lower right side of the chiller container.(Note: Chiller temperature must be within 22° C.)
 - (b) Within 2 minutes, Switch ON the MIRA COHERENT 900 Controller by pressing the black button at the left end back side of the controller. (Note: Make sure that Controller is in Continuous Wave Mode (CW) and the slit is opened completely by rotating the Slit Adjustment Knob to maximum extent at the right side in clockwise direction).
 - (c) Switch ON the Power Meter.
 - (d) Switch ON the VERDI power supply by turning the KEY to ON position (AC power).
 - (e) Press the Shutter Open Push Button of VERDI power supply unit. (Note: LED glows in shutter open position).
 - (f) Duration of minimum 10 minutes must be allowed after switch ON before the sample can be scanned with Infra Red Laser.
 - (g) Make appropriate entries in the LOG BOOK meant for MIRA 900 Coherent Laser and VERDI pump Laser.
2. Switch ON the Laser source (For He-Ne Green, He-Ne Red, turn the KEY to I position; For Multi Argon, turn the KEY to ON position, and press the black button (AC power) to I position).
 3. Switch ON the Power Supply Unit (PSU) and Turn the KEY to ON position.
 4. Switch ON the Microscope Controller Box (BX-UCB or IX-UCB).
 5. Switch ON the Transmitted Light Power Supply (LG-PS2) and if needed The Mercury Burner too. (Note: Wait for minimum 15 minutes before switch OFF after Switch ON. Duration of 3 minutes must be maintained between switch OFF and switch ON of the Mercury burner. **Overriding this condition will lead to Mercury Burner damage.**)
 6. Switch ON the the Computer Power Unit.

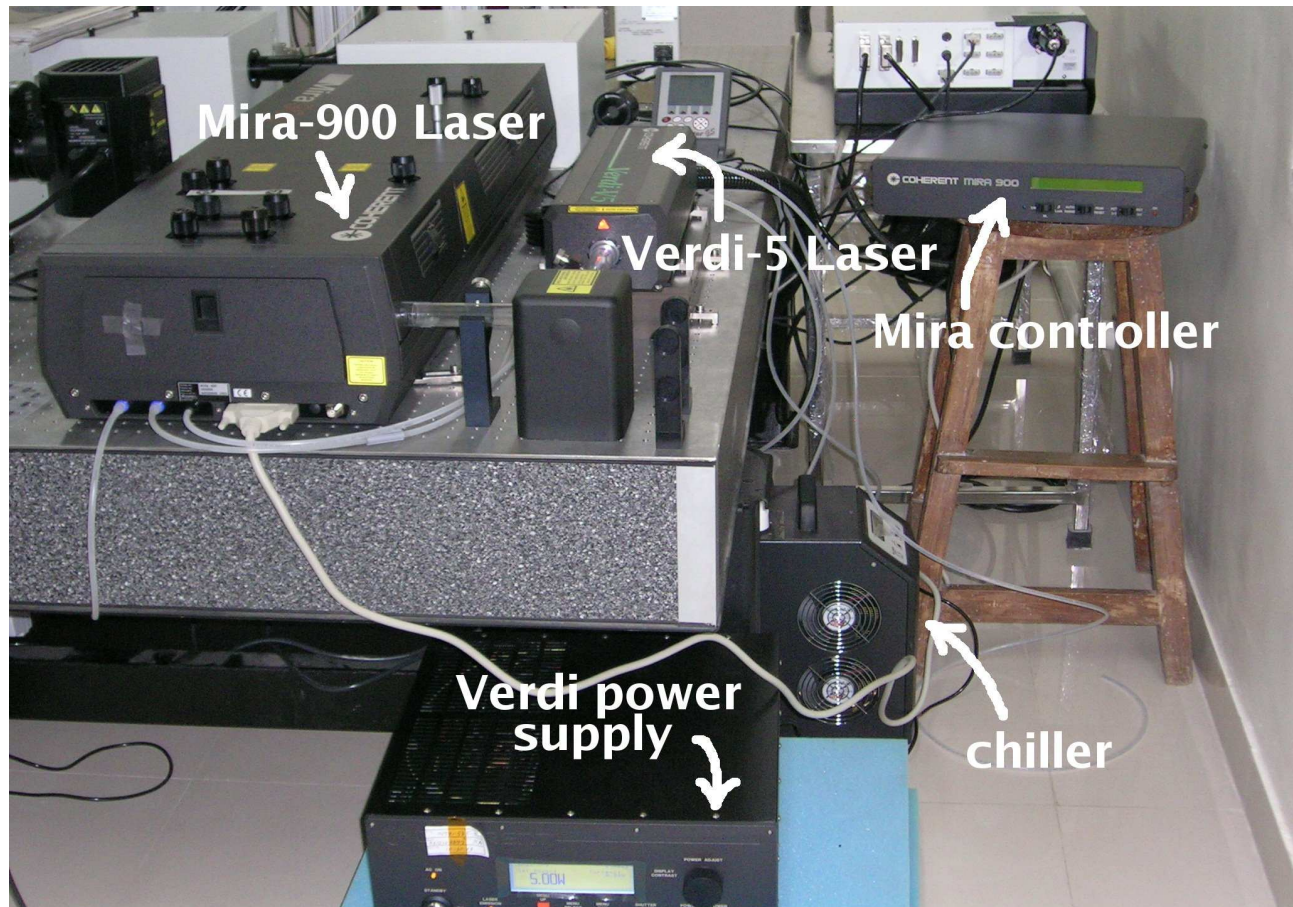


Figure 7: IR Laser unit for multiphoton confocal scanning microscopy comprising pump laser VERDI-5 and Main laser Mira 900.

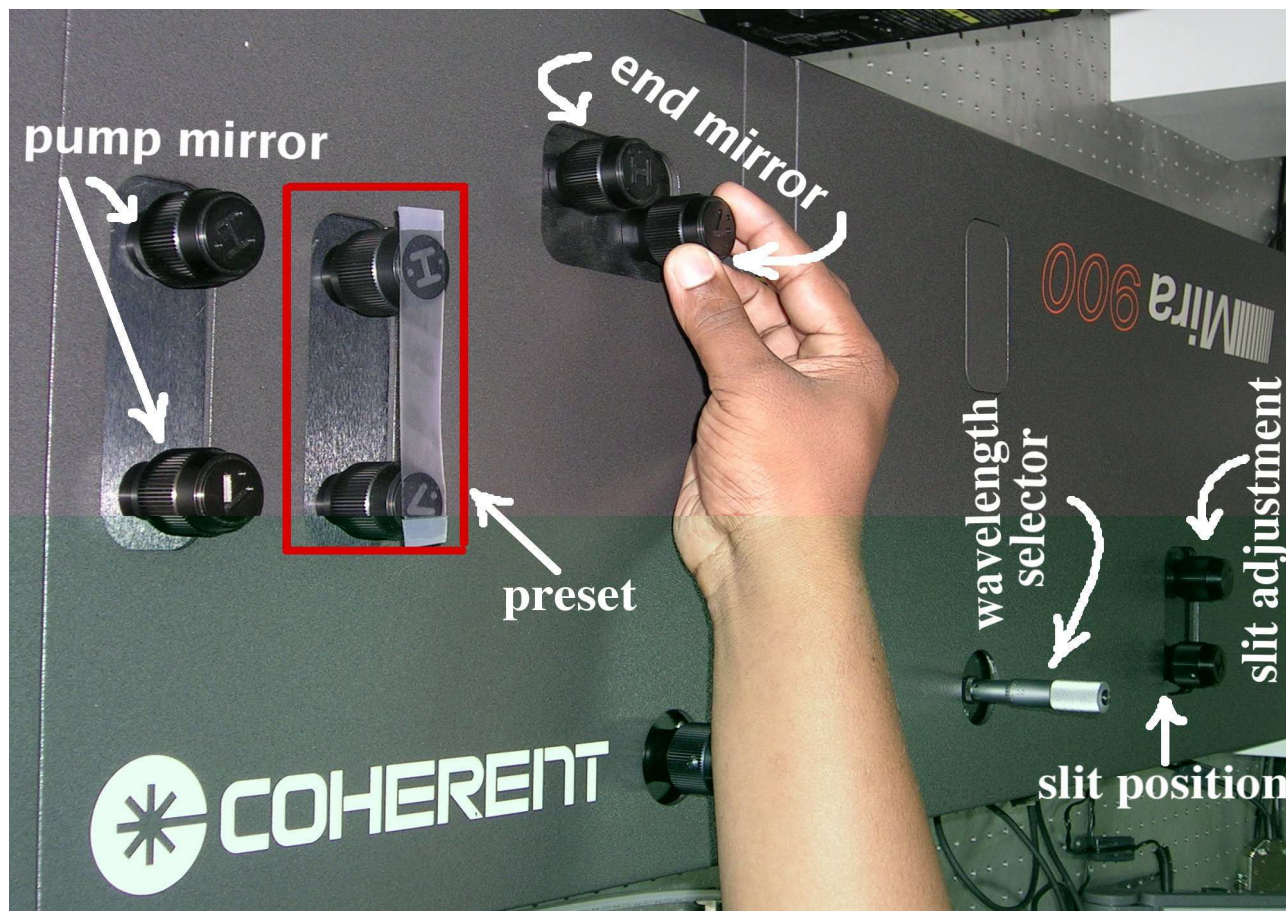


Figure 8: Mira 900 laser unit.

0.4 Verification Checks for Error Free Operation

0.4.1 Laser Unit

- Multi Argon

1. Yellow LED glows at the head of Multi Argon unit.

- Infra Red

1. MIRA Coherent 900 controller displays initially the increase in fluorescence and then the photodiode readings.
2. Check the VERDI power (5.00 W)
3. Check the CW power through Power meter which is kept at the exit of the MIRA 900.

0.4.2 Scanning Unit

- Fluoview 500 (FV500)

1. Green LED glows at the top of the FV500 scan unit.

0.5 Routine Shutdown Procedure - Confocal Laser Scanning Microscope

1. Remove the sample/glass slide from the sample holder. (Note: **Wipe out the immersion oil/water sticking to Objectives' lens part if the sample is viewed with immersion oil/water as medium between the objective lens and coverslip.**)
2. Engage the lowest magnification Objective in the optical path.
3. Switch OFF the the Computer Power Unit.

4. Switch OFF the Transmitted Light Power Supply (LG-PS2) and Mercury Burner if it was switched ON earlier.(Note: Wait for minimum 15 minutes before switch OFF after Switch ON. Duration of 3 minutes must be maintained between switch OFF and switch ON of the Mercury burner) **Overriding this condition will lead to Mercury Burner damage.)**
5. Switch OFF the Microscope Controller Box (BX-UCB or IX-UCB).
6. Turn the KEY to OFF position and switch OFF the Power Supply Unit (PSU)
7. Procedure pertaining to Infra Red Laser in STANDBY Mode is applicable if Multi Photon Mode experiment was conducted, otherwise, Switch OFF procedure for He-Ne Green, He-Ne Red and Multi Argon Laser has to be followed.
8. Infra Red Laser in STANDBY Mode:
 - (a) Make appropriate entries in the LOG BOOK meant for MIRA 900 coherent Laser and VERDI pump Laser before switching the Infra Red Laser unit to STANDBY mode.
 - (b) Open the slit of MIRA 900 laser unit completely by rotating the Slit Adjustment Knob to maximum extent towards right side in clockwise direction.
 - (c) Switch OFF the MIRA COHERENT 900 controller by pressing the power supply button present at back side of the controller, after setting the mode to CW mode by pushing the mode change switch to CW.
 - (d) Switch OFF the Power Meter.
 - (e) Switch OFF the chiller by pressing the power supply button present on right hand side of the chiller.
 - (f) Close the laser shutter in VERDI power supply unit by pressing the shutter push button and then turn the KEY to STANDBY position.
9. Switch OFF: He-Ne Green, He-Ne Red and Multi Argon Laser

- (a) Switch OFF the He-Ne Green and He-Ne Red lasers by turning the KEY to O position.
- (b) Switch OFF the Multi Argon laser by turning the KEY to OFF position and wait for few minutes till the cooling fan stops rotating, then press the black button to O position (AC power).

0.6 Emergency Shut Down Procedure - Confocal Laser Scanning Microscope

1. Switch OFF: He-Ne Laser Unit

- (a) Switch OFF the He-Ne Green and He-Ne Red lasers by turning the KEY to O position.
- (b) Switch OFF the Multi Argon laser by turning the KEY to OFF position and wait for few minutes till the cooling fan stops rotating, then press the black button to O position (AC power).

2. Switch OFF: Infra Red Laser Unit

- (a) Before switch OFF, Open the slit of MIRA 900 laser unit completely by rotating the Slit Adjustment Knob to maximum extent towards right side in clockwise direction.
- (b) Switch OFF the MIRA COHERENT 900 controller by pressing the power supply button present at back side of the controller, after setting the mode to CW mode by pushing the mode change switch to CW.
- (c) Switch OFF the Power Meter.
- (d) Switch OFF the chiller by pressing the power supply button present on right hand side of the chiller.
- (e) Close the laser shutter in VERDI power supply unit by pressing the shutter push button and then turn the KEY to OFF position (AC power).

3. Scan Unit

- (a) Turn the KEY to OFF position and switch OFF the Power Supply Unit (PSU).
- (b) Switch OFF the Microscope Controller Box (BX-UCB or IX-UCB).
- (c) Switch OFF the Transmitted Light power supply (LG-PS2) and Mercury Burner if it was switched ON earlier.
- (d) Switch OFF the Computer Power Unit.

0.7 Shut Down Procedure for Infra Red Laser - Down Time greater than 3 months

1. Make appropriate entries in the LOG BOOK meant for MIRA 900 coherent Laser and VERDI pump Laser before Infra Red Laser unit is completely switched OFF.
2. Open the slit of MIRA 900 laser unit completely by rotating the Slit Adjustment Knob to maximum extent towards right side in clockwise direction.
3. Switch OFF the MIRA COHERENT 900 controller by pressing the power supply button present at back side of the controller, after setting the mode to CW mode by pushing the mode change switch to CW.
4. Switch OFF the Power Meter.
5. Switch OFF the chiller by pressing the power supply button present on right hand side of the chiller.
6. VERDI Power Supply Shut Down
 - (a) Close the shutter open button in VERDI power supply unit by pressing the shutter open push button.
 - (b) Press Menu button
 - (c) Enter into LBO settings

- (d) Enter into LBO cooling
- (e) Press Menu Select
- (f) Wait till temperature reaches 40°C
- (g) Turn the KEY to OFF position (AC power)
- (h) Press the switch present at back side of the VERDI power supply to completely switch OFF the Infra Red Laser.

0.8 To be Verified Before Leaving the Confocal Microscope Room

1. Ensure that appropriate Air Condition is switched ON (Note: A/C numbered One must be kept ON on ODD numbered date and A/C numbered Two must be kept ON on EVEN numbered date).
2. Make sure that both blowers (fitted at top right side of the room) are ON.
3. Confirm that exhaust present at the bottom is switched OFF if you leave after late office hours (after 1800 hrs).
4. Ensure that entries are made in the LOG book meant for Instrument and User details. Assure that Remarks regarding Instrument Malfunction and Difficulties Experienced are recorded in the LOG BOOK which will be useful for FOLLOW-UP and to keep track of Instrument's Efficiency.