Confocal Laser Scanning Microscope Instructions
(Updated Version, May 2013)

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Confocal Laser Scanning Microscope
- Leica TCS SP2 -

1. Filter-free SP head: a spectrophotometer for each detector channel - design your own filters, maximize sensitivity, minimize crosstalk, record emission spectra

   Lenses: 10x, 40x (Oil, DIC), 63x (Oil, DIC)

3. Laser system:
   Ar (50mW): 458 nm, 488 nm, 514 nm
   GreenHeNe (1.2 mW): 543 nm

4. Two detection channels for fluorescence

5. Transmitted light detection

6. Leica Confocal Software (LCS, version 2.61) for 2D and 3D imaging, ROI scanning, time lapse imaging, quantification, FRAP and FRET applications

7. Simulator workstation for processing images with LCS

Contact:
Dr. Michaela Strüder-Kypke
Office: Science Complex, room 1253
Tel: (519) 824-4120 ext. 52737
E-mail: confocal@uoguelph.ca
User Guidelines

1. When you begin to use these facilities please provide a Billing Information Sheet including the supervisor’s signature and the Trust Fund Account number.

2. Before any unsupervised access is granted, users must enroll in supervised training sessions during which they will review with the confocal manager how to operate the equipment properly and safely. The time required for the training sessions will vary depending upon the user’s demonstrated competency with the equipment. Billing will be at the “supervised” fee rate.

3. When using these facilities you must clearly write in each sign-up book the date, your name, department, log on and log off time, and total number of hours you used the equipment.

4. For any planned after hours use of the confocal system, please make arrangements with the confocal manager for access to the hallways.

5. Users are expected to bring all their own supplies including pipets, slides, coverslips, computer disks, etc. However, the facility will provide immersion oil and lens paper.

6. Files saved to computer hard drives must be removed as soon as possible. All computer hard drives will be cleared on a regular basis - it is the users’ responsibility to manage their own image files.

7. Please notify the confocal manager immediately of any problems that you encounter with the equipment - it is essential that we work together in taking care of the facility. Improper care of the equipment will result in rejection of access to the facility.

Laser Safety

Please be aware that the confocal microscope is Class 3B laser equipment! This laser equipment may be operated only by persons who have been trained in the use of the system and the potential dangers of laser radiation.

As it is not possible to anticipate every potential hazard, please take care and apply common sense to the operation of the confocal microscope.

- Observe all safety precautions relevant to Class 3b lasers.
- Avoid exposing eyes or skin to direct radiation!
- Do not deviate from the operating instructions provided.

The failure to observe these instructions shall be exclusively at the user’s own risk!
**Confocal Basics**

Basic principle of a confocal microscope

The principle of confocal imaging was advanced by Marvin Minsky and patented in 1957, and is employed in all modern confocal microscopes.

In a conventional widefield microscope, the entire specimen is bathed in light from a mercury or xenon source, and the image can be viewed directly by eye or an image can be taken. The significant amount of fluorescence emission that occurs at points above and below the objective focal plane is not confocal with the pinhole and forms extended Airy disks in the aperture plane, usually experienced as a blurry image.

In contrast, the illumination in a confocal laser scanning microscope is achieved by scanning one or more focused laser beams across the specimen. The emitted light only from the focal plane in the specimen is detected by a photomultiplier tube (PMT) through a detection pinhole, and transformed into electrical signals which are converted to images by the specific software and displayed on the computer screen.

The detection pinhole suppresses signal from structures that are out of focus.

The depth of the focal plane is determined by the excitation wavelength, the numerical aperture of the objective, and the diameter of the detection pinhole.

Although unstained specimens can be viewed using light reflected back from the specimen, they usually are labeled with one or more fluorescent probes.

**Major improvements of a confocal microscope over a conventional microscope**

1. Signal not originating from the focal plane will not be captured.
2. Optical sectioning: Change of the focal plane does not create blurring, but gradually cuts out parts of the object as they move away from the focal plane. Thus, these parts become darker and eventually disappear.
3. Three-dimensional data sets can be recorded.
4. Scanning the object in x/y-direction as well as in z-direction (along the optical axis) allows viewing the objects from all sides.
5. Due to the small dimension of the illuminating light spot in the focal plane, stray light is minimized.
6. Image processing allows superimposition of many optical slices, giving an extended focus image which can only be achieved in conventional microscopy by reduction of the aperture and thus sacrificing resolution.
**Leica DM IRE Microscope: General Information & Care**

### Lenses

<table>
<thead>
<tr>
<th>Lenses</th>
<th>Immersion</th>
<th>Coverslip µm (#)</th>
<th>Bright Field</th>
<th>DIC</th>
<th>Step Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 x</td>
<td>-</td>
<td>with &amp; without</td>
<td>BF</td>
<td>BF</td>
<td>-</td>
</tr>
<tr>
<td>40 x</td>
<td>OIL</td>
<td>0.17 (1.5)</td>
<td>BF</td>
<td>BF</td>
<td>C 40</td>
</tr>
<tr>
<td>63 x</td>
<td>OIL</td>
<td>0.17 (1.5)</td>
<td>BF</td>
<td>BF</td>
<td>E 63</td>
</tr>
</tbody>
</table>

**Immersion oil**

Use only the immersion oil provided by the facility. Do not mix the immersion oil with other substances including immersion oil from another source. If you used any other immersion oil during previous observations with a different microscope, remove all traces with Windex.

**Cover slip**

Sealing the coverslip completely with nail polish or other sealing materials is highly recommended. This prevents mixing of immersion oil and embedding materials, movement of the coverslip when moving the stage, and moving embedded materials during z-sectioning. Be sure that any nail polish is completely dried before you mount the slide onto the stage.

**Lens care**

After use, clean the lenses with lens paper only. Do not use Kim Wipes, cloth, etc. Fold the paper, and hold both sides of the paper keeping the folded edge straight. Draw the folded edge back and forth over the lens surface. Change the place on the paper and continue until there are no more traces of immersion oil on the paper.
Confocal Laser Scanning Microscope Instructions

Startup

Start-up in order
1. UV lamp: **ON** (warm up for 10 min)*
2. Microscope, Main Switch: **ON**
3. Laser fan button: **ON** (check that you hear the cooling fan)
4. Scanner power button: **ON** (wait for 20 sec before starting the software)
5. PC power button: **ON** - start-up computer separately
6. Ar/ArKr laser key: **ON** (warm up for 30 min, best after 1 hour)*
7. Green HeNe laser key: **ON** (warm up for 15 min)*
8. Windows login**
9. Leica Confocal Software (LCS): **Start** - Personal Profile

Additional Information:
* You can use the UV lamp and the lasers immediately after you’ve switched them on, but they will not be at their optimal performance
** Each laboratory will have its own user name and password
Automated stage

A: stepwidth at fine focusing (0= 0.05 µm, 1=0.1 µm, 2=0.7 µm, 3=1.5 µm)
B: motor drive - UP to upper threshold
C: motor drive - DOWN
D: focus wheel
E: upper threshold
F: lower threshold

Carry out the following tasks …
- Set and delete a threshold: press and sustain the E or F key
- Override an upper threshold: use the focus wheel
- Switch between fine and coarse focus: press B and C keys simultaneously

IMPORTANT!
- Do not use the automated stage without upper threshold setting: the stage travels to the mechanical end-switch position, which damages the condenser, the objectives and the specimen.
- The upper threshold is important for proper application of the software.
Important Controls for Microscope Settings

1) Switch Port
2) Transmitted Laser Detector Knob
3) Epifluorescent Light Pin
4) Reflector Turret
5) Turret for Wallaston Prisms
6) Condenser turret
7) Polarizer
8) Analyzer
9) Illumination Intensity Control
10) Neutral Density (ND) Filter
11) Aperture Diaphragm
12) Field Diaphragm
13) Focus Wheel for Condenser
14) Centering Screws for Condenser
Microscope settings for:

**Bright Field (BF) and Differential Interference Contrast (DIC)**

<table>
<thead>
<tr>
<th>Port/Setting</th>
<th>BF</th>
<th>DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ports</td>
<td>VIS</td>
<td></td>
</tr>
<tr>
<td>2. Transmitted Laser Detector Knob</td>
<td>VIS</td>
<td></td>
</tr>
<tr>
<td>3. Fluorescent Light Pin</td>
<td>PUSH IN</td>
<td></td>
</tr>
<tr>
<td>4. Reflector Turret (Fluorescent Filters)</td>
<td>SCAN</td>
<td></td>
</tr>
<tr>
<td>5. Turret for Wallaston Prism</td>
<td>BF</td>
<td>C or E</td>
</tr>
<tr>
<td>6. Condenser Turret</td>
<td>BF</td>
<td>40 or 63</td>
</tr>
<tr>
<td>7. Polarizer</td>
<td>PULL OUT</td>
<td>PUSH IN</td>
</tr>
<tr>
<td>8. Analyzer</td>
<td>PULL OUT</td>
<td>PUSH IN</td>
</tr>
<tr>
<td>9. Fine Adjustment for Wallaston Prism</td>
<td>-</td>
<td>ADJUST</td>
</tr>
<tr>
<td>10. Light Intensity</td>
<td>ADJUST</td>
<td></td>
</tr>
<tr>
<td>11. ND (Neutral Density) Filter</td>
<td>OUT</td>
<td></td>
</tr>
</tbody>
</table>

**Köhler Illumination Setting:**

1. Focus the image
2. Close the field diaphragm (12)
3. Adjust the aperture diaphragm (11) if necessary
4. Adjust the condenser height with the focus wheel (13) until the edge of the field diaphragm is in focus
5. Centre the field diaphragm with the screws on the condenser (14)
6. Open the field diaphragm (12) until the edge just disappears from the field of view
7. Adjust the aperture diaphragm (11) so that it is two-thirds open
### Microscope settings for:

**Epifluorescence**

<table>
<thead>
<tr>
<th></th>
<th>VIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ports</td>
</tr>
<tr>
<td>2</td>
<td>Transmitted Laser Detector Knob</td>
</tr>
<tr>
<td>3</td>
<td>Fluorescent Light Pin</td>
</tr>
<tr>
<td>4</td>
<td>Reflector Turret (Fluorescent Filters)</td>
</tr>
<tr>
<td>5</td>
<td>Turret for Wallaston Prism</td>
</tr>
<tr>
<td>6</td>
<td>Condenser Turret</td>
</tr>
<tr>
<td>7</td>
<td>Polarizer</td>
</tr>
<tr>
<td>8</td>
<td>Analyzer</td>
</tr>
<tr>
<td>9</td>
<td>Fine Adjustment for Wallaston Prism</td>
</tr>
<tr>
<td>10</td>
<td>Light Intensity</td>
</tr>
<tr>
<td>11</td>
<td>ND (Neutral Density) Filter</td>
</tr>
</tbody>
</table>

### Microscope settings for:

**Laser Scanning**

<table>
<thead>
<tr>
<th></th>
<th>BF</th>
<th>DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ports</td>
<td>SIDE</td>
</tr>
<tr>
<td>2</td>
<td>Transmitted Laser Detector Knob</td>
<td>SCAN</td>
</tr>
<tr>
<td>3</td>
<td>Fluorescent Light Pin</td>
<td>PUSH IN</td>
</tr>
<tr>
<td>4</td>
<td>Reflector Turret (Fluorescent Filters)</td>
<td>SCAN</td>
</tr>
<tr>
<td>5</td>
<td>Turret for Wallaston Prism</td>
<td>BF</td>
</tr>
<tr>
<td>6</td>
<td>Condenser Turret</td>
<td>BF</td>
</tr>
<tr>
<td>7</td>
<td>Polarizer</td>
<td>PULL OUT</td>
</tr>
<tr>
<td>8</td>
<td>Analyzer</td>
<td>PULL OUT</td>
</tr>
<tr>
<td>9</td>
<td>Fine Adjustment for Wallaston Prism</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Light Intensity</td>
<td>MINIMIZE</td>
</tr>
<tr>
<td>11</td>
<td>ND (Neutral Density) Filter</td>
<td>OUT</td>
</tr>
</tbody>
</table>

* The turret might need to be switched to BF if scanning with 63x and high zoom!
Leica Confocal Software operation

F Focus your sample after the software opens

Acquisition

1. Press the “Beam” button and select a setting in the open “Beam Path Window”.

   Choose from the Leica prestored settings the one that matches your preparation most. They store the excitation wavelength and laser strength, the emission spectrum, detection window and colour, as well as the beam splitter. It is always possible to change those settings or to define and store your own settings in addition to the factory-set methods.

2. Press the “Obj” button in the open “Beam Path Window” and check that the correct lens is selected.

   Check objective lens every time you switch objectives

3. Press the “Mode” button in the open “Beam Path Window” to select the scan mode.

   xyz, xzy: spatial scan mode
   xt, xyt, xzt, xzyt: time scan mode
   xyλ, xzλ: spectral scan mode

4. Press the “Format” button in the open “Beam Path Window” to select the scan format.

   Besides the numerical aperture of the objective and the excitation wavelength, the scan format, together with the electronic zoom, determines the spatial resolution of the recorded data. A structure can only be scanned without information loss if the smallest optically resolvable distance (= lateral resolution) is scanned with about 2 to 3 raster points.

5. Press the “Speed” button in the open “Beam Path Window” to select the scan speed.

   The higher the set scan speed, the shorter the dwell time of the laser point. The higher the scan format at a constant speed, the shorter the dwell time of the laser point over a sampling point.
   Using a lower scan speed results in a better signal-noise ratio, but relatively long impact of the light on the specimen can bleach it photochemically.
6. Press the “Pinh” button in the open “Beam Path Window” to set the detection pinhole. Click the “Airy 1” button to set the pinhole size automatically to the optimal value of 1 Airy unit, depending on the objective in use.

In addition to the numerical aperture of the objective and the wavelength of the light, the detection pinhole also determines the thickness of the optical sections. The wider the diameter of the pinhole, the more light reaches the detector. The image becomes bright but signal from structures outside the actual focal plane will also appear in the image. Increasing the diameter of the pinhole above 1 Airy unit is recommended only for the detection of very weak signals.

7. Assign functions to the dials.

The function of the the dials on the Panel Box can be defined by the software. Just do a right mouse click on each of the 7 boxes, select “Configuration” and choose the function. The sensitivity of the dials can be set by right mouse click - “Sensitivity” (especially recommended for offset function). The dial #7 should remain as “Z-Position”.

In the beginning it is recommended to assign the functions as shown below:

**Working with one dye in the lower spectrum only:** \( PMT_1 \) and \( PMT\ trans \)

**Dual staining:** \( PMT_1, PMT_2 \) and \( PMT\ trans \)

keep Z-position always here!
8. Press the “Continuous” button to start the continuous scan.

The continuous scan is used to find the area of interest and zoom into the region, if desired, and to set gain and offset for each detection channel.

9. Set Gain and Offset for each PMT

While scanning, click the “QLUT” button in the Viewer window. Then adjust the gain of the channel with the according dial.

**Blue** colour of a pixel indicates full saturation. The image should show some blue pixels, but never a continuous area of blue (=‘overexposure’). The offset of the channel has to be set as well.

**Green** pixels indicate zero intensity - green pixels should uniformly cover the background and they should be more numerous than blue pixels, but they should never form a continuous area (= ‘underexposure’).

If you work with two detectors, set the offset so that the green in both images is adjusted to be the same.

10. Stop the continuous scan by pressing the “Continuous” button.

11. Press the “Aver” button to define the number of frames sampled (frame average).

Averaging means that the image will be scanned several times and the intensity values of the pixels will be averaged. This removes the noise in your image. However, averaging also bleaches the sample - if your preparation is bleach sensitive, don’t use this function.

12. Press the “Single Scan” button to get a single optical slice image.

The single scan is used to take a single optical slice image. This image will be saved.
SERIAL SCAN

At the start of each session, you have to switch the z-scan mode. The default setting is “Z-Galvo”, which means that the galvo stage controls the vertical scanning. However, this system does not have a galvo stage. You need to switch to “Z-Wide”, which means that the microscope stage controls the vertical scanning.

Switching the z-scan mode:

Press the “Z-Scan” button and switch to “Z-Wide”

or

Press “Z Position” in the “Panel Box Setting” dialogue window to switch the z-scan mode.

Characteristics of the different scan modes:

<table>
<thead>
<tr>
<th></th>
<th>Galvo</th>
<th>Z-Wide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum step width</td>
<td>0.04 µm</td>
<td>0.1 µm</td>
</tr>
<tr>
<td>Maximum drive distance</td>
<td>170 µm</td>
<td>max. drive distance of stage (cm)</td>
</tr>
<tr>
<td>Drive module</td>
<td>Galvo stage</td>
<td>Microscope stage</td>
</tr>
</tbody>
</table>

13. Press the “Continuous button.

14. Open the ‘Series” window.

15. Turn the “Z-Pos” Dial on the Panel Box to focus through your sample.

16. Press the “Begin” button to define the start position of the vertical series.

17. Press the “End” button to define the end position of the vertical series.
18. Adjust the intensity within the vertical series.
Bring the specimen to a position within the vertical scanning region that shows the strongest signal and adjust the intensity by pressing the “QLUT” button and adjusting the gain dial as described above.

19. Stop the continuous scan by pressing the “Continuous” button.

20. Press the “Sect” button to define the number of optical sections. Go to “Others” to define a certain customized step size or number of images.

<table>
<thead>
<tr>
<th>Image Dim. z/y (mm):</th>
<th>The height of the entire image stack between the beginning and end points of the image series.</th>
</tr>
</thead>
<tbody>
<tr>
<td># Sections:</td>
<td>The number of configured sections.</td>
</tr>
<tr>
<td>Step size (mm):</td>
<td>The step size, i.e. the distance the stage moves from one section to the next.</td>
</tr>
</tbody>
</table>

Calculate the number of sections / Unchanged height of the image stack
Enter the desired step size in the “Step Size” field.
Then click the “Calculate” button next to the Step Size filed.

Calculate the step width / Unchanged height of the image stack:
Enter the # of desired sections in the # Sections” field.
Then click the “Calculate” button next to the # Sections filed.

21. Press the “Series” button to create an image series.

<table>
<thead>
<tr>
<th>When working with specimens that are …</th>
<th>Low signal</th>
<th>Bleach sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detector Gain</td>
<td>Increase</td>
<td></td>
</tr>
<tr>
<td>Scan Time</td>
<td></td>
<td>Reduce</td>
</tr>
<tr>
<td>Laser Power</td>
<td>Increase</td>
<td>Reduce</td>
</tr>
<tr>
<td>Pinhole</td>
<td>Open</td>
<td></td>
</tr>
<tr>
<td>Averaging</td>
<td></td>
<td>Reduce or don’t use</td>
</tr>
<tr>
<td>Scan Speed</td>
<td>Slow (200)</td>
<td>Fast (800)</td>
</tr>
<tr>
<td>Scan Format</td>
<td>Avoid undersampling</td>
<td>Avoid oversampling</td>
</tr>
<tr>
<td>Zoom</td>
<td></td>
<td>Short interval</td>
</tr>
<tr>
<td>Beam expander</td>
<td>Small diameter (3)</td>
<td>Large diameter (6)</td>
</tr>
<tr>
<td>Accumulation</td>
<td>Useful</td>
<td>Can be useful</td>
</tr>
</tbody>
</table>
Viewing Options

**OVERLAY IMAGE**

1. Activate (button color becomes white) the channels for an overlay image.

   For example: When a transmitted image (here, Ch3) is not used for an overlay, inactivate the channel by clicking a button to make its color gray.

2. Click the button “Overlay”

   If you would like to change the way to overlay
   a. Go to Acquire
   b. Click an Overlay icon
   c. Choose one of three settings

3. Saving the Overlay image.
   a. Click the button “Display” and choose 1:1 (The image is displayed in its actual size).
   b. Click on the Overlay image with the right mouse button
   c. Choose “Send to”, then “Experiment”, then “Selection (Snapshot)”

   The overlay image will be saved as separate tiff file in your experiment.
   “Selection (all)” will save a screenshot of all activated channels.

**SINGLE IMAGE**

If you want to look at just one of the images:
   a. if you want to look only at the overlay image, activate the channels, select “Overlay” and select “Single”
   b. if you want to look at one of the channels, inactivate the other channels, or
   c. select the channel you wish to look at and select “Single”

Revert this by selecting “Tile”
**SCALE BARS**

1. Make sure you are still in “Acquire”. Click “Display” in the “View” option.

2. Check the box “Scale”.

3. If you want to modify the settings of the scale bar, select the button “Scale” and change the parameters.

**SAVE ANNOTATED IMAGES**

Overlay images, Images with scale bars, Profile, Graphs etc

1. Click the button “Display” in the Experiment window and select “1:1”.

2. Select the image you want to save. The image is surrounded by a dotted line, which means that the image is activated.

3. Right-click on the selected image. Then select “Send to” - “Experiment” - “Selection (Snapshot)”. The image is saved as TIFF file called ‘Snapshot’ in your experiment.
Processing

1. Edit - Separating and Merging of Images

Select the arrow bar “Processing” and open the folder “Edit”
There you find the options “Separation” and “Merging”

1. You can use “Separation” to:
   • separate selected channels from a file that contains several channels
   • extract optical slices from a stack of images
   • crop images of channels

2. You can use “Merging” to:
   • merge two or more channels of images taken separately (the number of sections, the distance between the slices, and the format have to be the same in all the channels)
   • merge time series into a single file

Each editing creates a new file and will not alter your original file!
DATA MANAGEMENT

1. File types used in this application

Experiment (*.lei) A Leica-specific, binary data format. When the experiment is opened, both the image data and the experiment settings are loaded.

Annotation sheet (*.ano) A Leica-specific, binary data format. The elements on the annotation sheet, such as images, texts and graphic images, are each available as individual objects.

Tiff files (*.tif) These are Leica image files in single and multi Tiff format. Experimental files in RGB-Tiff format can be opened as well.

2. Experiment Overview Dialog Window

![Image of Experiment Overview Dialog Window]

IMPORTANT:

The Experiment Dialog Window shows files that are temporarily stored on RAM memory

Experiment: Click the “New” button to create a new experiment, which opens a new Viewer window. An experiment is a file that consists of one or more individual images or image series.

File name: Unsaved directories are named as “Experiment #”.

Active mark (red check): This mark shows the image that is currently in the viewing window.

Preview #: When you press “Cont.” button, an image file is created in a new experiment or a directory that contains an activated image or series file. When a “Preview file” is activated and you do a continuous scan, the preview file will be overwritten.

Image #: A new image file is created in a new experiment or a directory that contains an activated image each time you press the “Single Scan” button.

Series #: A new Series file is added in a new experiment or a directory that contains an activated image each time you press the “Series” button.
3. Export files in AVI format

1. Right click on a series of images or a 3D animation file in the Experiment Dialog window

2. Select “Export”
   a. Save in: select the folder in which you want to save the avi file
   b. Save as type: select “avi”
   c. File name: enter a name for the file
   d. Number of frames: depends on the number of your images
   e. Always save file uncompressed

3. Click the button “Save”

   • You will not see the avi file in the Experiment Dialog window.
   • Open an application software, to locate your file.
   • If a file contains 2 or more channels, these channels will be automatically overlayed in the avi file. If you do not want to overlay channels, you have to separate the channels (see below) and export each separation file as avi file.

4. Save Experiments

1. Activate the view you want to save.

2. Click the “Save” button to save the data of the current experiment (*.lei) or the current annotation (*.ano) sheet.

3. When saving an experiment, a folder is created at the file level with the name of the experiment. This folder then contains the description file (*.lei) for the experiment as well as the individual image files. The description file (*.lei) contains parameter settings and colour information for each image included in the experiment. All image files will have the experiment name plus their identifier.

4. When saving an experiment, the new folder contains all images included in the directory. Make sure to delete unnecessary images.

IMPORTANT: the original contents (*.lei, *.tif) in a folder need to be a set.
SHUTDOWN

Shut down in order:

1. Leica Confocal Software: close
2. Ar/ArKr laser key: OFF (start to count for 15 min)
3. GreenHeNe key: OFF
4. Scanner power button: OFF
5. Save your data onto the server or a CD/DVD
6. Windows: shut down
7. PC power button: OFF
8. Clean microscope lenses and bring down stage
9. Microscope: OFF
10. UV lamp: OFF
11. Wait for 15 min (after Ar key OFF): Laser button: OFF

IMPORTANT!

- If somebody will use the microscope within 2 hr after you, do not turn OFF the Ar/ArKr laser key (1), Laser button (10), and UV lamp. All the other units should be shut down.
- The Mercury lamp cannot be restarted within 30 min after use.
- Back up your files on Server/DVD/CD. Do not leave the files in the workstation for an extended period of time.
- Please cover the microscope if you are the last user for the day - but make sure that the mercury lamp has cooled off!