Carl Zeiss LSM 700 Standard Operation Protocol
Basic Operation

Please make sure that the COMPRESSED AIR has been TURNED ON prior to the use of the equipment. Kindly inform the administrator if the gauge displays LOW level of compressed air.

1. Switch on the Mains (labeled ① and ②) mounted on the wall.
2. Switch on the Fluorescent Lamp (labeled ③).
3. Turn the Laser Key (labeled ④) 90° clockwise for power supply.
4. Press ON/OFF (labeled ⑤) to switch on the computer system.
5. Click to log into LSM User at the startup screen.

Launching the ZEN 2010 Software

6. Double click ZEN 2010 software icon on the desktop of WINDOWS to start the software programs, if necessary.

7. Select ‘Start System’ to initiate the confocal component.

8. Secure the slide/dish on the platform with cover slip(s) facing downwards.

9. Click ‘Ocular’ then ‘Online’ to enable the specimen to be viewed through the binocular, and select appropriate objective to examine the slide/dish.

Please refer to the table on P.2 for detailing info of each objective.
<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
<th>Magnification</th>
<th>NA</th>
<th>Medium</th>
<th>Phase Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EC Plan-Neofluar 10x/0.30 Ph1 M27</td>
<td>10x</td>
<td>0.3</td>
<td>Air</td>
<td>Ph1</td>
</tr>
<tr>
<td>2</td>
<td>EC Plan-Neofluar 20x/0.50 M27</td>
<td>20x</td>
<td>0.5</td>
<td>Air</td>
<td>HF</td>
</tr>
<tr>
<td>3</td>
<td>Plan-Apochromat 40x/1.3 Oil Ph3 M27</td>
<td>40x</td>
<td>1.3</td>
<td>Oil</td>
<td>Ph3</td>
</tr>
<tr>
<td>4</td>
<td>Plan-Apochromat 63x/1.40 Oil Ph3 M27</td>
<td>63x</td>
<td>1.4</td>
<td>Oil</td>
<td>Ph3</td>
</tr>
<tr>
<td>5*</td>
<td>EC Plan-Neofluar 5x/0.16</td>
<td>5x</td>
<td>0.16</td>
<td>Air</td>
<td>HF</td>
</tr>
<tr>
<td>6*</td>
<td>Plan-Apochromat 20x/0.8 Ph2 M27</td>
<td>20x</td>
<td>0.8</td>
<td>Air</td>
<td>Ph2</td>
</tr>
</tbody>
</table>

*Whilst Position 1-4 are fixed, lenses fitted onto the other two slots are subjected to change. Please consult administrator if necessary.

# Do not change from oil lens to air lens UNTIL LENS OIL HAS BEEN COMPLETELY REMOVED.

10. Match the designated filter (if phase contrast is to be included in image acquisition).

11. To view the sample with epi-fluorescence, assign the option accordingly (Note that the corresponding fluorescent filter will change automatically).

**Hint:** Try to use DAPI fluorescence (if available) for the ease of focus.
To enable viewing the specimen through binoculars, **Lambda** setting on the *fluorescent lamp remote control panel* must match with the *fluorescence channel* on *Zen 2010*.

![Image of Lambda settings on remote control panel]

Make sure that the *remote control panel* is switched ON (one of the 4 Lambda bars will be displayed in a *SOLID COLOUR*). To update these settings on the panel, **press each black node once to switch to its corresponding channel**.

<table>
<thead>
<tr>
<th>Name</th>
<th>λ₁</th>
<th>λ₂</th>
<th>λ₃</th>
<th>λ₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation (nm)</td>
<td>400 nm</td>
<td>440 nm</td>
<td>470 nm</td>
<td>535 nm</td>
</tr>
<tr>
<td>Dye(s) Used</td>
<td>DAPI</td>
<td>CFP</td>
<td>FITC / GFP</td>
<td>Rh</td>
</tr>
</tbody>
</table>

* Although turning the wheel CLOCKWISE shall raise the fluorescence intensity (Online ONLY), this has no impact to the signal strength under the *Acquisition Mode*.

12. Locate the sample of interest through the eyepiece, and optimize the region of view.

13. Click ‘*Offline*’ before move to ‘*Acquisition*’ mode.
14. Click ‘**Load Acquisition Configuration**’ to select a defined configuration from the drop-down list.

Note that this configuration serves as a template for the most commonly used fluorescence(s). To avoid sample bleaching and to reduce acquisition time, either **deselect undesired track(s)** OR **highlight these track(s) and click ‘−’**.
The table below demonstrates all configurations available for simultaneous acquisition of numerous common dyes.

<table>
<thead>
<tr>
<th>Configuration Name</th>
<th>Track 1</th>
<th>Track 2</th>
<th>Track 3</th>
<th>Track 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue_Green</td>
<td>DAPI</td>
<td>405 nm</td>
<td>A488</td>
<td>488 nm</td>
</tr>
<tr>
<td>Blue_Red</td>
<td>DAPI</td>
<td>405 nm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Green_Red</td>
<td>A488</td>
<td>488 nm</td>
<td>mChe</td>
<td>555 nm</td>
</tr>
<tr>
<td>Blue_Green_Red</td>
<td>DAPI</td>
<td>405 nm</td>
<td>FITC</td>
<td>488 nm</td>
</tr>
<tr>
<td>DAPI_A488_A555_A633</td>
<td>DAPI</td>
<td>405 nm</td>
<td>A488</td>
<td>488 nm</td>
</tr>
</tbody>
</table>

15. Alternatively, select ‘Smart Setup’ at ‘Acquisition’ mode.

16. Set up the experiment as instructed below, and click ‘Apply’.

- **Assign different colour to different dye used**
- **Choose the channels only if you have included such fluorescence in your sample**
- **Select:**
  - Fastest – to acquire signal within the shortest period of time
  - Best Signal – to acquire signal of the best possible quality
  - Best Compromise – to acquire signal of reasonable balance between both speed and best signal
  - **Linear Unmixing** – this optional is **NOT RECOMMENDED**, as the system will be set in lambda mode automatically
17. Check the box for ‘T-PMT’, if BRIGHT FIELD is to be included in image acquisition.

18. Uncheck ‘Track 2’ and highlight ‘Track 1’.

19. Click ‘Live’ to examine the sample

20. Adjust the pinhole to ‘1 AU’.
21. Adjust **laser power** and **master gain** to obtain an image of interest with adequate intensity

*User is advised NOT to use too extreme settings on laser power and master gain (as too high laser power will induce sample bleaching; and too high master gain (> 800 mV) will cause significant noise and shorten the shelf life of the PMT).*

22. **Fine tune the focus** to obtain the brightest view; and, if signal is saturated, re-adjust the laser power and master gain.

23. Reduce the **digital offset** to introduce a blue-coloured area (see above) on which the signal is absent and the background noise is minimal.

24. Examine the individual channel(s) **ONE AT A TIME** to ensure that the signal intensity and the background noise on the area of interest is **appropriate**.

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**Note** the level of **background noise and the signal** on areas of interest.
25. Click ‘Stop’ to complete the preview of the sample.

26. Repeat Steps 18-25 for various dyes on other tracks. (No adjustment needed for pinhole and laser power for T-PMT)

27. Set up the ‘Acquisition Mode’ as follows:

![Acquisition Mode](image)

The table below summarises the setting recommended for image acquisition.

<table>
<thead>
<tr>
<th>Scan Mode</th>
<th>Frame Size</th>
<th>Line Step</th>
<th>Speed</th>
<th>Averaging Number</th>
<th>Bit Depth</th>
<th>Averaging Mode</th>
<th>Direction</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frame</td>
<td>1024 x 1024</td>
<td>1</td>
<td>6 or 7</td>
<td>1</td>
<td>12 Bit</td>
<td>Line</td>
<td>- - &gt;</td>
<td>Mean</td>
</tr>
</tbody>
</table>

The Speed and the Averaging Number of acquisition depends upon the strength of signals. Weak signal shall require slower acquisition with an increased number for averaging.

28. Check all tracks, before ‘Snap’ to capture the image with the setting.

![Acquisition Mode](image)

If necessary, repeat the steps with different regions of interest.

The two figures above show a sampler image during (above left) and acquired from (above right) an experiment.
Steps 29-35 below are to demonstrate the setup on an acquisition of image with ‘THICKNESS’ resulted from an overlapping sum of numerous scans.

29. Select the ‘Z-Stack’ option.

30. Click ‘Live’ to examine the sample.

31. Turn the focus ring VERY GENTLY to CLOCKWISE direction to locate the starting slide, and ‘Set First’.

32. Then turn the focus ring VERY GENTLY to ANTI-CLOCKWISE direction to locate the final slide, and ‘Set Last’.

33. Click ‘Stop’ to complete the preview of the sample.
34. Click ‘Optimal’ to change to recommended interval value determined by Zen 2010.

![Image of Z-Stack interface](image)

This digit suggests the distance between each slide. Hence the smaller the value within a given range the more slides required per acquisition (and the better retention of details but with a larger file size), and vice versa. To manually input the interval value, opt between optimal value and the value at μm section of 1 Airy Unit. i.e. in this case, $0.99 \mu m < x < 2.00 \mu m$

35. **Check all tracks**, before ‘Start Experiment’.

![Image of channels and tracks](image)

36. To cancel the ‘Z-Stack’ experiment in the midst of procedure (IF NECESSARY), click ‘Finish Current Step’.

![Image of stop button](image)

37. When finished, click ‘Save’ icon to save the image data.

![Image of save icon](image)

* indicates that the file has yet to be saved. The logo will disappear once the file has been safely into a designated folder.
On the ‘Save As’ prompt, assign an appropriate destination folder (if necessary) and click ‘Save’.

38. Upon completion, go to ‘File > Exit’ to quit the Zen Software.

39. Oil lens (i.e. 40x/63x), IF USED, must be thoroughly cleaned using lens cleaning tissues (i.e. NOT KIMWIPES).
   a. Oil residue from the objective lens should firstly be removed using a DRY lens cleaning tissue.
   b. The surface is then wiped with another lens cleaning tissue, moistened with 100% absolute ethanol.
   c. Objective lens is subsequently wiped dry with lens cleaning tissue.

40. Check if the equipment will be used by other users.

In cases where others have reserved the next session(s), proceed to Step 40.

Proceed to the following steps IF THE FACILITY IS IDLE WITHIN THE NEXT HOUR.

   i. Shut down the Windows system to turn off the computer.
   ii. Turn the Laser Key (labeled ④) 90° anti-clockwise to power off.
   iii. Switch off the Fluorescent Lamp (labeled ③).
   iv. Switch off the Mains (labeled ② and ①) mounted on the wall.
   v. Ensure that the COMPRESSED AIR has been TURNED OFF after the use of the equipment. Kindly inform the administrator if the gauge displays LOW level of compressed air.

41. Users are reminded to sign on the log-in sheet before departure.