**Leica Thunder microscope**

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**Specifications about Thunder:**

The Thunder microscope can be used for making pictures of fluorescence and histological staining’s. Due to 2 camera’s, one for fluorescence and one for colour. Thunder uses a quadroblock that selects which wave length is providing the excitation of fluorescent dyes. This block turns and moves so that it only lets the specified wavelength through (395, 470, 550, 640). The emitted light is filtered with a filter that is specified for emission wave-lengths (440-510-590-700). In comparison to the Leica Confocal Microscope, Thunder is much faster, due to taking one shot instead of scanning per pixel (confocal). And compared to a ‘normal’ fluorescence microscope, the Thunder results is sharper and better images. Thunder has algorithms to correct blurred spots, and Thunder can make Z-stack pictures.

**Important notes before using Thunder:**

* Book the E4.32 Thunder via Supersaas (max. 2 sessions of 4 hours per week)
* Do not image on the same day as the staining
* Know which dye is in what excitation-emission spectrum
* When booking is cancelled, inform the WhatsApp-group

**Troubleshoot:**

Console not working: Make sure the green light inside the microscope (lower left) is stable, and not flashing. If this light is flashing, push this button once.

Black through ocular: Make sure that you selected ‘Live’, and the eye is selected on the touchscreen. And don’t use far-red when looking through the ocular, the human eye is not capable of seeing far-red light.

Error in software: Make screenshot of the error, and place this screenshot in the folder ‘Error’. Most of the times the error resolves when restarting the LAS X program.

63x lens is ‘too low’: The 63x lens has a cleaning mode, meaning it is a bit lowered. This is not needed, but sometimes someone uses it. To resolve this, push the lens, and turn. The lens should now go back up.

Zstack doesn’t work: Check the Z-step size, this needs to be at least the given Z-step size in ‘objective configuration’. Otherwise the Z-steps are too small to make for the objective.

**Protocol**

**Starting up:**

1. Write name and additional information in the log-book
2. Turn on the computer and the ‘master-switch’ (green button behind microscope)
3. Open LAS X Software
   1. Use standard configuration, click ‘OK’
4. Open project
   1. Make ‘new project’, give name according to ‘yearmonthday’ (for example ‘20231115’)
5. Go to tab ‘Configuration’:
   1. Check ‘Camera preferences’, select needed camera (fluorescence or colour)
   2. Set digitation: 16 bits
   3. Image flip: vertical
6. Check ‘objective configuration’:
   1. Check the available objectives
7. Go to tab ‘Acquire’:
   1. Click on ‘+’ for each used dye, this adds new fCr
   2. Choose colour for the dye (can be to own preference)
   3. Rename the fCr to the name of your dye
   4. Click on dropdown menu and select light-source, for brightfield use ‘TL-BF’, for fluorescence use ‘FLUO’.
8. Select the added dye, and select the correct excitation colour, and the correct emission filter, according to following table.

|  |  |  |
| --- | --- | --- |
| Colour | Excitation | Emission |
| Blue | 395 | 440 |
| Green | 470 | 510 |
| Red | 550 | 590 |
| Far-red | 640 | 700 |

1. Select objective you want to use. (When using 63x use oil!!)

**Microscope:**

1. Turn the light in the microscope on, button on right top
2. Take the insert and slide the insert into the microscope (right to left)
3. Place sample in the insert (cover-glass down)
   1. Align sample above the lens of the microscope.
   2. Click on green light to fix the table, the green light should be stable (when green light flashes, you can move the table by hand, and the console won’t work!)
4. Turn of the light in the microscope to prevent bleaching of your dye’s.
5. Select the first dye, and click on ‘Live’.
   1. On the touchscreen of the microscope click on the eye, to see through the microscope.
   2. Look through the ocular of the microscope, use the black round button to focus on the sample.
   3. Use the black console to find an interesting position in your sample
   4. Click ‘Stop’ to prevent bleaching.

**Imaging:**

1. Click on the camera in the touchscreen, now you are controlling from the computer.
2. Select ‘Image Format’, and set to ‘2x2 binning’ (When high resolution is needed, use ‘no binning’)
3. Click ‘Live’, your sample should now be visible on the screen.
   1. Click ‘autoscale’, to set the brightness automatically
   2. Use laser strength (max. 30%) and exposure time to get a nice picture
4. Check all dye’s and adjust their laser strength and exposure time
5. To capture an image use:
   1. Single image: 1 channel (1 dye colour)
   2. Capture image: All channels (all specified dye’s)
6. OPTIONAL: To improve quality, select Thunder
   1. Try out all 3 computational clearing algorithms, to your own preference choose one.
7. OPTIONAL: When you have dye’s that mark on different levels, use RFC:
   1. Select RFC in the top row.
   2. Click on ‘Live’
   3. Adjust the focus, so that the selected dye is sharp
   4. Click ‘Store Z position’
   5. Do step c and d for every dye.
   6. Click ‘Save’, and click ‘Apply RFC’

**Finishing:**

1. Save your project:
   1. Right mouse click on your project, and select ‘save as’.
   2. Navigate to your folder on the E: drive (when you are a student, create a folder in the folder of your supervisor), and click ‘save’.
2. Close the software with the ‘x’ in the top-right.
3. Remove your sample from the microscope
4. Lower the lens to the lowest position (When 63x is used, clean the oil!!)
5. Check when the next person is planned on the microscope. Next booking:
   1. Within 1 hour: Leave the microscope as it is now
   2. Longer than 1 hour: Shut down the computer, and turn off the ‘masterswitch’ behind the microscope

Download your images to an USB (Within 1 month)

**Additional protocols:**

**Z-stack:**

1. Make sure RFC is not selected
2. Go to Z-stack
3. Select dye of interest
   1. Zoom to the lowest part of the dye, click ‘begin’.
   2. Zoom to the highest part of the dye, click ‘end’.
4. Select ‘Zstep size’
   1. Check in ‘objective configuration’ what the minimum Zstep is of the used objective.
   2. Adjust the Zstep size to the minimum Zstep size of the used objective
5. Click on ‘start’
6. To create a 3D-model, click ‘3D’
   1. This shows a 3D model, to add axis select ‘axis scaling’.

**LAS X Navigator:**

1. Click on ‘LAS X Navigator’, in the top-bar (raster-logo)
2. Click on ‘Live’, you should now see the same as you saw in the smaller view tab.
3. Click on ‘Spiral’, LAS X Navigator is now making a spiral scanning around your region.
   1. If your Navigator region is big enough, click on ‘Stop’.
4. You can now select a region of interest, and go back to the previous viewer.
5. It is also possible to select **multiple regions**
   1. Click on the ‘+’ sign
   2. Select all regions you want to analyse.
   3. Click ‘Start’ to make pictures of all those regions
6. If the regions are next to each other, you can use Mosaiq Merge
   1. Click ‘Mosaiq Merge’, and then ‘Merge’. This results in 1 big picture of the pictures.
7. Another option is **focus map**
   1. Select a regions by dragging a square over your sample.
   2. Click on ‘+f’
   3. Select zigzag-wise positions
   4. For each region, adjust the Z to focus on the sample, than click on ‘set Z’ for each region
   5. Click ‘Start’, this will make a focus map

**Using and cleaning emersion oil for 63x lens**

1. Adding oil:
   1. Remove sample from the insert
   2. Turn lens down, and select 63x lens
   3. Add 1 droplet emersion oil on top of the lens
   4. Place your sample in the insert (cover-glass down)
   5. Turn the lens up, till the oil touched the glass of the sample
   6. Look through ocular to focus on sample
2. Cleaning oil
   1. Remove sample
   2. Take a tissue from the specific blue box
   3. Tap the tissue on the oil
   4. Add 70% ethanol on the tissue, now wipe the oil of the lens and your sample