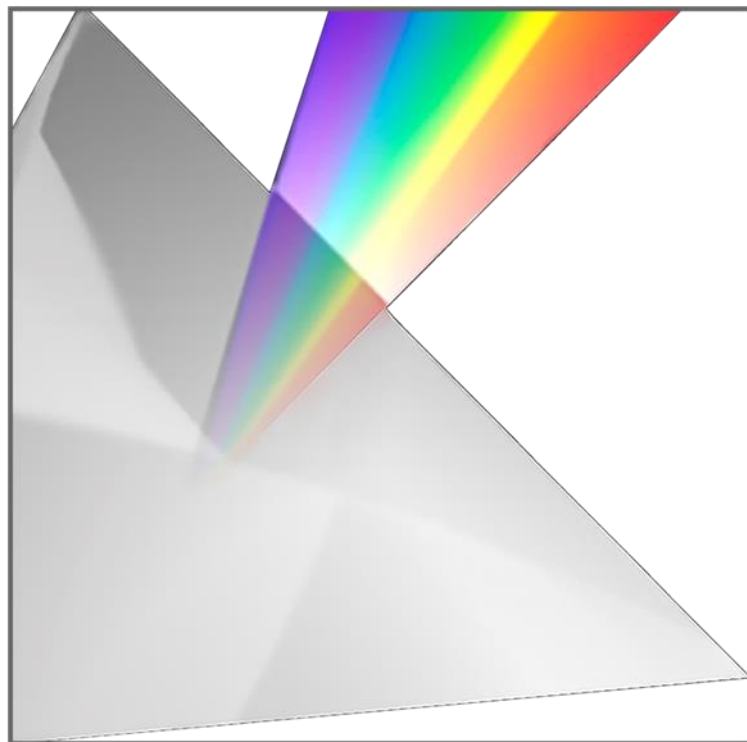


BASIC MICROSCOPE PROCEDURE

Laboratory of Imaging Tissue Structure and
Function



29.05.2017

Laboratory of Imaging Tissue Structure and
Function
Neurobiology Center

Table of Contents

Confocal Microscope Zeiss LSM 800 with AiryScan2

Confocal Microscope Zeiss LSM 7804

Confocal Laser Scanning Microscope Leica Sp8.....6

Confocal Microscope Leica SP 5.....9

Zeiss Spinning Disc – short manual11

Microscope Leica DMI6000 with simple confocal device ANDOR DSD213

MultiPhoton Confocal Zeiss MP InVivo15

MultiPhoton Confocal Zeiss MP Photomanipulation16

Confocal Microscope Zeiss LSM 800 with AiryScan

ATTENTION! IN CASE OF ANY DOUBTS PLEASE DO NOT HESITATE TO CONTACT THE STAFF OF LABORATORY OF IMAGING TISSUE STRUCTURE AND FUNCTION (PHONE 306 OR 502).

Turning on the system

1. Carefully remove the microscope cover.
2. Turn on buttons located on control power bellow computer: firstly **SYSTEMS**, then **COMPONENTS**.
1. Turn the key clockwise from 0 to I.
2. Turn on power strip located near to fluorescence lamp.
3. Turn on computer. Log into User account.
4. Start ZEN software, than choose **START SYSTEM**.
5. Skip calibration

Objectives

1. The objectives are switched automatically.
2. Before changing the slide and after ending the work **ALWAYS** lower the objective.
3. Apply a drop of immersion on the objective. For the oil objective (OIL) it should be regular immersion oil. For the water objectives (WATER) it should be water immersion oil (cap with the white sticker).



1. Oil immersion oil



2. Water immersion oil

After finishing your work

1. Save your date in folder USERS on the D drive. If you want to copy your date on some drive, first scan it with antivirus software “Bitdefender” available on second working station.
2. Clean immersion objectives. Use special lens papers. **NOTE: using other materials may damage the objective.** Wipe all immersion liquid out of the objective with dry lens paper. Than repeat this action with lens paper damped with objective cleaning liquid which is available near the microscope. Finally repeat the action with dry lens paper. **NOTE: leaving immersion liquid on the objective or cleaning the objective in different way than mentioned above may result in objective damage.**
3. If you want to clean your samples, use regular tissues and window cleaner which are available near the microscope.
4. Fill out the “LOG BOOK”

Turning off the system

If you are the last user this day you should completely turn off the system:

1. Close Zen program. Wait until microscope icon disappear form taskbar
2. Turn off computer
3. Turn the key from I to 0.
4. Switch off buttons located on control power bellow computer: firstly **COMPONENTS**, then **SYSTEMS**.
5. Switch off power strip located near to the fluorescence lamp.
6. Gently put the blue dust cover on the microscope.

Confocal Microscope Zeiss LSM 780

ATTENTION! IN CASE OF ANY DOUBTS PLEASE DO NOT HESITATE TO CONTACT THE STUFF OF LABORATORY OF IMAGING TISSUE STRUCTURE AND FUNCTION (PHONE 306 OR 502).

Turning on the system

3. Turn on buttons located on control panel located on the left side of the microscope firstly **MAIN SWITCH**, then **SYSTEMS/PC** finally **COMPONENTS**.
4. Turn on computer. Log into LSM User account.(no password).
5. If you need to visualize greenish stains turn on ARGON LASER. Laser is located in room in the back of microscope. Turn on button located on laser control panel from OFF to ON, now wait 5 minutes for laser to hit up. After 5 minutes turn the key clockwise from 0 to 1, wait 2 minutes. Turn switch from “idle power” to “laser run”, then wait until green. diode turns on The diode should turn on in few minutes.
If you want to use laser 355 please contact laboratory staff.
6. Start ZEN software, than choose **START SYSTEM**.
7. Turn on fluorescence lamp.

Incubation

If your sample need special temperature or CO2, turn on hitting and CO2 in Zen program (bar INCUBATION) or on display panel (bar INCUBATION) . **After using of glass frame for CO2, please lay it on the bottom of environmental chamber.**

If you need to change stage insert please contact with laboratory staff.

Objectives

4. The objectives are switched automatically. **NOTE: please check currently used objective, because the microscope is equipped with objectives with the same magnification but differing in immersion liquid that should be used.**
5. Before changing the slide and after ending the work **ALWAYS** lower the objective.
6. Apply a drop of immersion on the objective. For the oil objective (OIL) it should be regular immersion oil. For the water objectives (WATER) it should be water immersion oil (cap with the white sticker).



1. Oil immersion oil



2. Water immersion oil

After finishing your work

5. Save your data in folder USERS on the D drive. If you want to copy your data on some drive, first scan it with antivirus software “Bitdefender” available on laptop placed near the entrance of laboratory
6. Clean immersion objectives. Use special lens papers. **NOTE: using other materials may damage the objective.** Wipe all immersion liquid out of the objective with dry lens paper. Then repeat this action with lens paper dampened with objective cleaning liquid which is available near the microscope. Finally repeat the action with dry lens paper. **NOTE: leaving immersion liquid on the objective or cleaning the objective in different way than mentioned above may result in objective damage.**
7. If you want to clean your samples, use regular tissues and window cleaner which are available near the microscope.
8. Fill out the “LOG BOOK”

Turning off the system

If you are the last user this day you should completely turn off the system:

7. Close Zen program.
8. If information about active lasers occur switch off active lasers.
9. Turn off computer
10. Turn off fluorescence lamp.
11. Switch of Argon laser. Turn off switch from “**laser run**” to “**idle power**”, wait 2 minutes. Then switch key from 1 to 0, next wait 10 minutes.
Waiting 10 minutes is very important, this allow laser to cool down. Switching off laser without waiting 10 minutes shortens laser lifespan.
After 10 minutes switch off power button from “ON” to “OFF”.
12. Switch off buttons located on control power near microscope: firstly **SYSTEMS/PC**, then **COMPONENTS** finally **MAIN SWITCH**.

Confocal Laser Scanning Microscope Leica Sp8

ATTENTION! IN CASE OF ANY DOUBTS PLEASE DO NOT HESITATE TO CONTACT THE STAFF OF LABORATORY OF IMAGING TISSUE STRUCTURE AND FUNCTION (PHONE 306 OR 502).

Turning on the microscope

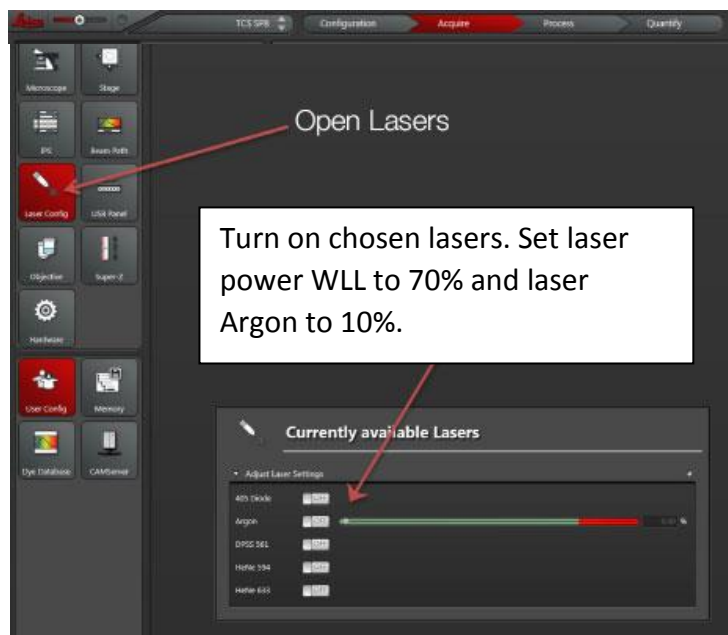
1. Turn on buttons located on control panel below the desk firstly PC Microscope, Scanner Power, Laser Power. Switch key clockwise from OFF to ON.



2. If you need laser 405 nm turn on power stripe (with sticker "laser 405 nm") located in left side of the microscope.
3. Turn on power stripe located in right side of the microscope.
4. Turn on fluorescence lamp.
5. Choose profile **LSM User** (without password), next start program LAS AF (ikon in the middle of monitor). When starting window occur - choose „**OK**”.
6. After few minutes window Microscope Stand will appear. If you want to make TileScan or Position click YES if not choose NO. If you choose yes stage will move and calibrate.



- In the configuration tab select the laser window, turn on lasers which you will use.



Incubation

If your sample need special temperature or CO₂, turn on temperature controller (blue box) and CO₂ controller (red box). Both boxes are located on the right side of the microscope. **If you need to change stage insert please contact with laboratory staff.**

Objectives

- The objectives are switched automatically. **NOTE: please check currently used objective, because the microscope is equipped with objectives with the same magnification but differing in immersion liquid that should be used.**
- Before changing the slide and after ending the work **ALWAYS** lower the objective with the button on the microscope stand.
- Apply a drop of immersion on the objective. For the oil objective (OIL) it should be regular immersion oil. For the water objectives (WATER) it should be water immersion oil (cap with the white sticker).



1 Zwykły olej immersyjny.



2. Olejek do immersji wodnej.

After finishing your work

1. Save your data in folder USERS on the D drive. If you want to copy your data on some drive, first scan it with antivirus software "Bitdefender" available on laptop placed near the entrance of laboratory.
2. Clean immersion objectives. Use special lens papers. **NOTE: using other materials may damage the objective.** Wipe all immersion liquid out of the objective with dry lens paper. Then repeat this action with lens paper dampened with objective cleaning liquid which is available near the microscope. Finally repeat the action with dry lens paper. **NOTE: leaving immersion liquid on the objective or cleaning the objective in different way than mentioned above may result in objective damage.**
3. If you want to clean your samples, use regular tissues and window cleaner which are available near the microscope.
4. Fill out the "LOG BOOK"

Turning off the system

If you are the last user this day you should completely turn off the system

1. In the configuration tab select the laser window, turn off all working lasers.
2. Close the LAS program.
3. If both computers are working, firstly turn off computer combined with right monitor, then quickly move a computer mouse to the left monitor. Close an Input Director program.
4. Turn off second computer.
5. Turn off fluorescence lamp.
6. Switch off powerbar located on the right side of the microscope. **(IF NIGHT HEATING IS NEEDED DON'T DO IT)**
7. Turn key EMISSION from ON to OFF, now wait 10 minutes, before switching off LASER POWER button.
Waiting 10 minutes is very important, this allow laser to cool down. Switching off laser power without waiting shortens laser lifespan.
8. Switch off powerbars located on the left side of the microscope. **ATTENTION: make sure that SPAD detectors are switch off, if not switch them off with button near the detector display.**
9. Turn off buttons located on control panel below the desk firstly PC Microscope then Scanner Power, finally Laser Power.

Confocal Microscope Leica SP 5

ATTENTION!

IN CASE OF ANY DOUBTS PLEASE DO NOT HESITATE TO CONTACT THE STUFF OF LABORATORY OF IMAGING TISSUE STRUCTURE AND FUNCTION (PHONE 306 OR 502).

TURNING ON THE SYSTEM

1. Turn on Leica CTR6500 (white box located on left side of the microscope)
2. Switch on button PC/Microscope on the TCS control panel. The computer will start automatically.
3. Turn on buttons Scanner Power, Laser Power.
4. Turn the key clockwise from OFF to ON (Laser Emission)



5. Turn on the fluorescence lamp (located under the microscope). The green power light and the shutter will come on. Once turned on, the lamp should remain on for a minimum of 30 minutes.



6. Log into Windows, choose „USER” account. Start LAS program.
7. Microscope stand: In order to calibrate the stage click „YES”. If you choose „NO” stage calibration step will be omitted.
8. Turn on needed lasers in „CONFIGURATION” tab. Argon laser should be set to approx. 10%.

Incubation

1. If your sample needs to stay in particular environment (temperature, CO₂ concentration), turn on the temperature controller (blue box located on the left to the microscope) and CO₂ controller (red box located right to the screen) **NOTE: Changing of the sample holder should be performed by Laboratory staff only.**

Objectives

1. The objectives are switched automatically. **NOTE: please check currently used objective, because the microscope is equipped with objectives with the same magnification but differing in immersion liquid that should be used.**
2. Before changing the slide and after ending the work **ALWAYS** lower the objective with the button on the microscope stand.
3. Apply a drop of immersion on the objective. For the oil objective (OIL) it should be regular immersion oil. For the water objectives (WATER) it should be water immersion oil (cap with the white sticker).



1. Oil immersion oil



2. Water immersion oil

After finishing your work

1. Save your date in folder USERS on the D drive. If you want to copy your date on some drive, first scan it with antivirus software available on second working station.
2. Clean immersion objectives. Use special lens papers. **NOTE: using other materials may damage the objective.** Wipe all immersion liquid out of the objective with dry lens paper. Then repeat this action with lens paper damped with ethanol which is available near the microscope. Finally repeat the action with dry lens paper. **NOTE: leaving immersion liquid on the objective or cleaning the objective in different way than mentioned above may result in objective damage.**
3. If you want to clean your samples, use regular tissues and window cleaner which are available near the microscope.

End your session

If you are the last user this day you should completely turn off the system:

1. In "ACQUIRE" tab Set all lasers to 0%
2. Open the „CONFIGURATION" tab, set Argon laser power to minimum and then set all lasers to the OFF position.
3. Exit LAS software and turn off the computer from Start menu.
4. Turn the „Laser emission" key counter-clockwise to OFF. Wait at least 15 minutes. **This keeps the fan running which helps cool the Argon laser and will maximize its useful life.**
5. Turn off SCANNER POWER, PC/MICROSCOPE POWER and LASER POWER.

Zeiss Spinning Disc – short manual

ATTENTION!

IN CASE OF ANY DOUBTS PLEASE DO NOT HESITATE TO CONTACT THE STUFF OF LABORATORY OF IMAGING TISSUE STRUCTURE AND FUNCTION (PHONE 306 OR 502).

Turning on the microscope

1. Turn on the 2 power bars in the room behind the microscope.
2. Turn on the power bar located next to the microscope stand.
3. Turn on the power bar located below the desk.
4. Turn on the lasers you would like to use by turning on the switches next to the microscope stand.
5. Turn on the fluorescent lamp.
6. Turn on the computer and the software (Zen, in the center of the desktop).

Incubation

If your sample need special temperature or CO₂, turn on hitting and CO₂ in Zen program (bar INCUBATION) or on display panel (bar INCUBATION) . **After using of glass frame for CO₂, please lay it on the bottom of environmental chamber.**

If you need to change stage insert please contact with laboratory staff.

Objectives

1. The objectives are switched automatically. **NOTE: please check currently used objective, because the microscope is equipped with objectives with the same magnification but differing in immersion liquid that should be used.**
2. Before changing the slide and after ending the work **ALWAYS** lower the objective.
3. Apply a drop of immersion on the objective. For the oil objective (OIL) it should be regular immersion oil. For the water objectives (WATER) it should be water immersion oil (cap with the white sticker).
4. **Clean immersion objectives. Use special lens papers. NOTE: using other materials may damage the objective. Wipe all immersion liquid out of the objective with dry lens paper. Than repeat this action with lens paper damped with cleaning mixture which is available near the microscope. Finally repeat the action with dry lens paper. NOTE: leaving immersion liquid on the objective or cleaning the objective in different way than mentioned above may result in objective damage.**



1. Oil immersion oil



2. Water immersion oil

Turning off the microscope

1. Clean all immersion lenses (objectives) with the cleaning mixture (little white bottle) and the lens cleaning paper. **IMPORTANT:** cleaning lenses with other papers/agents can lead to their damage.
2. Turn off the fluorescent lamp and the lasers.
3. Turn off the ZEN software and the computer.
4. Switch off the power bars under the desk and next to the microscope stand.
5. Switch off the 2 power bars in the room behind the microscope.
6. Fill out the log book.

Microscope Leica DMI6000 with confocal device ANDOR DSD2

ATTENTION! !!!IN CASE OF ANY DOUBTS PLEASE DO NOT HESITATE TO CONTACT THE STAFF OF LABORATORY OF IMAGING TISSUE STRUCTURE AND FUNCTION (PHONE 306 OR 502)

Turning on the System

1. Turn on two power stripes located on sides of microscope table.
2. Turn on the mercury lamp power source (located behind the monitor).

ATTENTION: Ensure that the lamp was turn off at least 30 minutes ago.

3. Turn on computer.
4. Start iQ3 ANDOR software.
5. Select DSD option from the 'REGISTRATION' window and click the OK button.
6. Before starting to use microscope calibrate ADNOR DSD 2.

ATTENTION: Lack of calibration may prevent from obtainment proper images.

7. Device setup → Camera → DSD Processing → Calibrate Alignment
8. Choose two corresponding dots on both windows, start calibration.
9. Wait until calibration is complete.
10. If calibration complete successfully you can start imaging. If calibration failed, repeat the calibration process.
11. Access to imaging options like Multi-snap, Snap, Z-scan, and Time Lapse is possible from main window.
12. Tile scan is available in ND Tree View.

Objectives

1. The objectives are switched automatically iQ3 ANDOR software.
2. Before changing the slide and after ending the work always lower the objective with the button on the microscope stand.
3. Apply a drop of immersion liquid on the objective. For the oil objective (OIL) it should be regular immersion oil (cap WITHOUT the white sticker). For the water objective (WATER) it should be water immersion oil (cap WITH the white sticker)

NOTE: Please always check currently used objective, because the microscope is equipped with objectives with the same magnification but differing in immersion liquid that should be used.

After finishing your work

1. Save your data. If you want to copy your data on some drive, first scan it with antivirus software available on the second working station in laboratory.
2. Clean immersion objectives. Use special lens papers.

NOTE: using other materials may damage the objective.

Wipe all the immersion liquid out of the objective with dry lens paper. Then repeat this action with lens paper damped with ethanol with is available near the microscope. Finally repeat the action with dry lens paper.

NOTE: leaving immersion liquid on the objective or cleaning the objective in different way than mentioned above may result in objective damage.

3. If you want to clean your samples, use regular tissues and window cleaner which are available near the microscope.

End your session

1. If you are the last user today, exit iQ3 ANDOR software.
2. Turn off computer.
3. Turn off fluorescent light source.
4. Turn off two power stripes located on sides of microscope table.
5. Wait 15 minutes, until microscope cool down.
6. Place the plastic cover over the microscope.
7. Fill out the log book.

MultiPhoton Confocal Zeiss MP InVivo

ATTENTION! IN CASE OF ANY DOUBTS PLEASE DO NOT HESITATE TO CONTACT THE STAFF OF LABORATORY OF IMAGING TISSUE STRUCTURE AND FUNCTION (PHONE 306 OR 502).

Turning on the system

1. Turn on buttons on control panel located on the left side of the microscope, firstly **MAIN SWITCH**, then **SYSTEMS/PC**.
2. Turn on computer. Log into LSM User account.(no password).
3. Switch on microscope controller (white box) located bellow optical table. Wait for Zeiss touch panel to start.
4. Turn laser power key clockwise from 0 to 1. Laser controller is located on the floor behind the optical table
5. Turn on OPO module
6. Turn on “small computer” if you will use OPO, wait until Windows and OPO program start.
7. Turn on button COMPONENTS (located near SYSTEM/PC).
8. Turn on fluorescence lamp.
9. Start ZEN software, than choose **START SYSTEM**.
10. Wait with visualization until OK status occur on laser controller display.

Objectives

1. The objectives are switched manually.
2. Before changing the slide and after ending the work **ALWAYS** pick up the objective manually.
3. For immersion 20x objective use Milli-Q water. Lower down the objective, apply drop of water between cover glass and objective using a plastic pipette.

After finishing your work

1. Save your date in folder USERS on the D drive. If you want to copy your date on some drive, first scan it with antivirus software “Bitdefender” available on laptop placed near the entrance of laboratory
2. Clean immersion objectives. Use special lens papers. **NOTE: using other materials may damage the objective.**
3. If you want to clean your samples, use regular tissues and window cleaner which are available near the microscope.
4. Fill out the “LOG BOOK”

Turning off the system

LASER should be in work mode for at least 1 hour before switching of the key from 1 to 0.

If you are the last user this day you should completely turn off the system:

1. Close Zen program.
2. Switch off small computer, then main computer.
3. Turn off fluorescence lamp.
4. Switch off OPO module.
5. Turn laser key from 1 to 0.
6. Switch off microscope controller located bellow optical table.
7. Switch off buttons located on control power near microscope: firstly **COMPONENTS**, then **SYSTEM/PC** finally **MAIN SWITCH**.

MultiPhoton Confocal Zeiss MP Photomanipulation

ATTENTION! IN CASE OF ANY DOUBTS PLEASE DO NOT HESITATE TO CONTACT THE STUFF OF LABORATORY OF IMAGING TISSUE STRUCTURE AND FUNCTION (PHONE 306 OR 502).

Turning on the system

1. Turn laser power key clockwise from 0 to 1. Laser controller is located on the floor behind the optical table. Turn on only lasers that you want to use.
2. Turn on buttons on control panel located on the left side of the microscope firstly **MAIN SWITCH**, then **SYSTEMS/PC**, finally **COMPONENTS**.
3. Turn on computer. Log into LSM User account.(no password).
4. Turn on fluorescence lamp.
5. Start ZEN software, than choose **START SYSTEM**.
6. Wait with visualization until OK status occur on laser controller display.

Objectives

1. The objectives are switched manually.
2. Before changing the slide and after ending the work **ALWAYS** pick up the objective manually.
3. For immersion 20x objective use Milli-Q water. Lower down the objective, apply drop of water between cover glass and objective using a plastic pipette.

After finishing your work

1. Save your date in folder USERS on the D drive. If you want to copy your date on some drive, first scan it with antivirus software “Bitdefender” available on laptop placed near the laboratory entrance.
2. Clean immersion objectives. Use special lens papers and Milli-Q water. **NOTE: using other materials may damage the objective.**
3. If you want to clean your samples, use regular tissues and window cleaner which are available near the microscope.
4. Fill out the “LOG BOOK”

Turning off the system

LASER should be in work mode for at least 1 hour before switching of the key from 1 to 0.

If you are the last user this day you should completely turn off the system:

1. Close Zen program.
2. Turn off fluorescence lamp.
3. Turn laser key from 1 to 0.
4. Switch off microscope controller located bellow optical table.
5. Switch off buttons located on control power near microscope: firstly **COMPONENTS**, then **SYSTEM/PC** finally **MAIN SWITCH**.