

STANDARD OPERATING PROCEDURE			
Zeiss LSM 980-I-NLO			
Supersedes Document/ Revision No: NA	SOP-4-001	Revision Date:	31.10.2022

Item: Zeiss Microscope LSM 980 Airy Scan  
SIP: 2842000262  
Equi-Nr.: 1061250  
UKL-Anlagen-Nr.:

This SOP describes startup and shutdown of the LSM 980

Usage of SOP: When needed.

**Usage of this microscope is only allowed after training by the Core Facility!**

## Turning on

- **If needed, switch 2 Photon laser on a minimum of 30 min before starting the session by turning the key from pos. Standby to On (must be in horizontal position).**

- Start Microscope main switch (power supply) **#NLO1**
- Start computer **#NLO2**  
Login: *IMT-ZEISS-04*  
PW: *imtzeiss!22*



- Accept the opening of the "Fill out usage time" form and fill it in (except for stopping time). **For overnight experiments, select "live cell imaging" instead of "Zeiss LSM 980."**
- Start "Zen blue" software
- Click "Start System"
- Wait for Zen to boot
- Allow stage calibration [(no sample should be present and lowest magnification objective (10x) should be used)]
- Turn on the lasers you will need. Alternatively, load saved experiment template, which will also activate all necessary lasers.
- Place the slide on the stage. Using the joystick, make sure that the stage is centered, and that the objective is far away from the edges of the stage insert.

## On LSM software

- On the "Locate" tab., use the pre-defined buttons (DAPI, GFP/RFP, TL) to inspect your sample

### At microscope control panel:

Press "Objectives" and select appropriate objective. 40x and 63x are selected AiryScan objectives.

- 10x (air) N.A. 0.45
- 20x (air) N.A. 0.8
- 40x (water) N.A. 1.0 (ddH<sub>2</sub>O at room temperature, Immersol W for 37°C)
- 63x LD (oil) N.A. 1.2 (use RT oil for room temperature, 37°C oil for 37°C)
- 63x (oil) N.A. 1.4 (use RT oil for room temperature, 37°C oil for 37°C)

### On Microscope:

- Select the appropriate insert for the stage.
- Apply 1 drop of immersion liquid, if needed, onto the objective (water/oil) or directly onto the coverslip of your slide (oil). *Adding a drop on the coverslip is preferable.*
- Use the joystick to position the objective under the area of interest.
- Focus objective upwards (turning the fine focus adjustment knob on the right hand side of LSM in a clockwise direction) until the liquid just comes into contact with the coverslip (or for dry objectives when the objective is very close to it).

*At microscope control panel:*

- Select "Control" then select "Reflector" to choose correct filter cube
- GFP/DsRed, DAPI, TL (transmitted light),

*In Zen software:*

- Click "Locate/Microscope Control" and open the shutter by simply clicking on the pre-defined buttons (see above)

Note: buttons are designed so that the emitted light is reflected towards the oculars.

*At Microscope:*

- Turn LED on/off by pressing large button sitting on table.
- Use as low a percentage of light intensity (5% is the minimum) as possible to avoid bleaching your sample.
- Focus on the sample by using looking through the ocular.
- Remember to *turn off LED* by pushing on the manual shutter again.

### Image Setup

*In Zen software:*

- Close shutter and switch reflector back to "NoneLSM"
- Click "Acquisition" button
- From "Experiment Manager" above select: "Channel"

Within "Imaging Setup:"

- Select appropriate beam splitters (MBS) according to laser lines required.
- Avoid allowing the borders of the detectors to overlap in wavelength.

*Check your settings (standard confocal):*

- Use 1 track at a time if necessary.
- Avoid hardware shifts between tracks if possible. This is much faster and will allow you to scan between lines, if desired. If hardware shifts are unavoidable, use between Z-stacks as the setting.
- Under "Channels": Select correct track with checkmark and highlight name of track
- Pinhole should be set to 1 Airy Unit for standard confocal measurements.
- Click "Live" button: Adjust focus, laser power and gain. To avoid bleaching your sample use as low a laser level as possible. For standard detectors the gain can be increased up to ~1000v if needed, otherwise you may need to increase the laser power.
- Use "Range Indicator" button on lower display to check for saturated pixels. Red pixels are oversaturated and should be avoided. Blue pixels have a value of zero.
- Repeat this process for each additional channel and track.
- You can balance between scan speed and averaging (line) to get best signal. You can try an average of "4" to start with.

*Check your settings (Airy Scanning):*

- Avoid saturated pixels (also those due to background noise). It may be necessary to lower gain and increase laser slightly to get signal out of background noise, in particular when using fast scanning mode.
- Airy Units should be set at ~2.5 or slightly higher.
- There is no need to average with Airy Scanning. Averaging should be set to "1".
- Try scanning without multi-band filters first. Using laser excitation alone may be enough to separate your fluorophores. This will save you unnecessary hardware shifts. If you need to use filters, set for scanning between Z-stacks to avoid unnecessary shifting.
- The filters present here are less precise than with the standard confocal mode. Be aware of crosstalk (spectral overlapping) between the fluorophores/lasers. If the crosstalk is too high, it may be necessary to use standard confocal imaging (including the possibility of spectral unmixing) instead of Airy Scanning for your sample.

Z-Stacks:

- Activate "Z-Stack" in Experimental Manager.
- Focus down through sample (turning focus counterclockwise) and set "First"
- Focus up through sample (turning focus clockwise) and set "Last"
- For deconvolution or 3D reconstruction you can select "optimal" spacing interval, otherwise select slice number or spacing interval of choice.
- Select "optimal" frame size.
- Select "Start Experiment."

### Tiles:

- For tiling your samples be sure that you allow an overlap of 5 to 10% to allow better results when the tiles are then stitched together.

### Saving Images:

- Images and stacks will appear in the small window on the upper right side.
- Images should be saved directly the PC (Q:\IMT-A-CoreFacility). **No saving on the desktop!**

### Switching off:

- Check online calendar.
  - If next user is scheduled more than 1h later, switch off all lasers.
  - Exit Zen software.
  - *Allow 1 minute for cooling after laser shutdown*
  - Meanwhile:
    - Remove slide / specimen.
    - Clean objective: If water was used, take fresh, gray Kimwipe to absorb liquid. Do not wipe.
    - If oil or Immersol was used, use fresh, gray Kimwipe to gently remove the bulk of the liquid (**do not wipe the lens**). Then take a cotton applicator soaked in ethanol to clean the objective. While rolling the applicator, gently move it in small circles starting at the center and moving it gradually to the outside. Do not wipe. Repeat with a fresh cotton applicator.
- Note.** Do not use the ready to use cotton applicator; instead roll a cotton on the naked side of the stick. Discard the used (oiled) cotton and keep the stick, as it can be re-used.

### At control panel:

- Press "Objective/10x" to switch back to the 10x objective
- Pull upper part of LSM back down and close all doors of the chamber.
- Enter stopping time, click on check mark
- Shut down PC
- Switch off the Main switch (power supply) **only after computer has finished shutting down**
- **Turn off the key of the 2P laser (!).**

### End of the session

- Remove all of your waste such as samples, slides, coverslips or paper, throw it in the appropriate waste bin. GMOs belong in the special autoclave waste bin!
- **If last user of the day, verify 2P laser is off (key in vertical orientation). If not, turn it off).**
- Leave the Microscope Room and lock the door!

## Booking System:

To make an appointment, please use our booking system under <http://booking.core-facility.de> use the "Booking System" Shortcut on the Desktop

- Please write your name, phone number and your work group in the reservation.
- The appointments are binding.
- If you have to cancel an appointment, please delete it from the calendar.
- Please inform the next user when you are delayed.

## Live Cell Imaging:

Allow the chamber and insert to warm up at least 2 hours prior to imaging!

Go to software right panel and select "Incubation"

Turn on (at 37°C) (appearing in this order, left to right):

- *H Insert P*, as needed (for large, thick insert on right)
- *H Insert XL*, always (for entire chamber)
- *H Insert P*, as needed (for small insert on left)
- *H Dev Humid*, always (check water level in bottle - refill with ddH<sub>2</sub>O to level between red lines)

Place chamber lid over installed insert and dummy slide or dish on stage.

Close all climate chamber doors and sliders.

After 2 hours warm up time, at control panel:

CO<sub>2</sub> *Small V* - turn on at 5%, and also turn on at wall outlet (rotate knob from red to green)

Insert samples into chamber and secure them so they are snug within the holder.

Allow samples to equilibrate to the chamber temperature for 20 to 30 minutes before starting a time series.

Activate Automatic file save and enter file name and storage location on D:\Images.

Set up and start Time Series using Experiment Manager in Zen.

After Imaging is completed -

To shut down the incubation system:

At Control Panel / Incubation:

Shut down all active systems.

Turn off CO<sub>2</sub> at wall outlet (rotate knob from green to red).

Open climate chamber and push back microscope neck.

Remove insert lid

Reinstall standard insert by holding the *inner* screws.

Close climate chamber.



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### **Maintenance:**

Regelmäßige Wartung gemäß Wartungsvertrag sowie Reparaturen und Justierungen führt der Servicespezialist der Herstellerfirma Zeiss durch.

Ansprechpartner: Hr. Timo Walch  
Service-Hotline: 0180 333 633 5

## Daten der ersten und nachfolgenden Bearbeitungen (mind. jährlich)

Rev.-#	Datum	Beschreibung	Verfasst von
001	31.10.2022	1st	F. Ditengou