



Two-Photon Imaging Facility

Operator and User regulations

1. Introduction

The IZKF Core Facility “Two-Photon Imaging” is equipped with both expensive and potentially very dangerous optical machinery (high-power, near infrared “class 4” lasers). Moreover, the equipment is vulnerable to malfunction due to incorrect usage. To maintain high quality standards, the Core Facility “Two-Photon Imaging” implements regulations on how to work safely with the equipment and collegial behavior in the facility laboratories which are located on the second floor, corridor 46, room 9 and 10 (Two-Photon Imaging) and corridor 47, room 10 (In-vivo Imaging).

Since the Core Facility laboratory is located within the Laboratory Animal Science Institute, the rules and regulations of the Laboratory Animal Science Institute also apply to the Core Facility “Two-Photon Imaging”.

A list of equipment available at the Core Facility “Two-Photon Imaging” can be found in appendix 1. Contact details of the Supervisory Board and the Core Facility Director are listed in appendix 2.

2. User Regulations

A. Basic Core Facility regulations

- No experiments may be conducted without the authorization of the Director of the Core Facility and/or other Core Facility supervisors. Authorization of experiments is only granted after the planned experiment has been explained and the potential and possibilities of the study discussed.
- Requests for experiments and equipment reservation may be made by either sending an email to tplsm@ukaachen.de, by contacting the Director of the Facility (Appendix 2) or by entering a booking into the booking calendar (for trained users).
- Direct “keycard” access to the two-photon microscope room (corridor 46, room 9) is only granted to Core Facility staff and trained users (as determined by the Core Facility Director). The same regulations apply to the usage of the in-vivo imaging systems (corridor 47, room 10).
- The prerequisite for using the Core Facility is acknowledgment of facility guidelines including imaging fees and the acknowledgements/co-authorship policy (please sign the “User and PI Agreement”).
- Access to the two-photon microscope room is only permitted after instruction on S1 laboratory guidelines and laser safety rules has been provided by a responsible person (laser safety officer). The user must sign a declaration (appendix 3) confirming personal

awareness of the risks involved in working with class 4 laser light sources and his/her understanding of S1 laboratory guidelines. Copies of the S1 security/laser safety statement form can be obtained from the Core Facility Director. These copies can be attained only after an introduction to S1 lab security/laser safety and regulations as currently applicable within the two-photon Core Facility.

- The facility two-photon microscope room is equipped with laser warning lights next to the entrance indicating that one or both class 4 laser are in use. When warning lights are on, the doors are automatically locked and access is only granted to persons with “keycard” access, or if permission is granted by the persons currently using the two-photon microscope(s), after simply knocking on the door. Leaving the room is also only possible using a “keycard”.
- Access to in-vivo imaging systems is only permitted after an introduction to S1 laboratory guidelines (optionally radiation protection guidelines) has been provided by a responsible person. The MIS Lumina XR meets the requirements for a full-protection X-ray device. Nevertheless, users must sign a declaration confirming personal awareness of the risks involved in working with X-ray sources and the radiation protection guidelines on using the X-ray module of the MIS Lumina XR.
- X-ray imaging of live animals is only granted to persons with sufficient qualifications according to RWTH guidelines for X-rays in laboratory animal science (“Röntgenverordnung – RöV”).
- The responsible persons are to follow laser/X-ray safety instructions at all times!
- In case of emergency such as fire, standard safety regulations and guidelines as currently implemented on UKA premises apply.
- Trained users will receive access to separate booking calendars (MS Outlook) for the two-photon microscopes (and evaluation computer) or the in-vivo imaging systems.
- All work should be performed between 8:00 a.m. and 5:00 p.m. Working in the facility at other times is only possible with permission of the Core Facility Director and only for those users who have had extensive training in laser safety and correct usage of the imaging systems.
- The users of the Core Facility “Two-Photon Imaging” are divided into three categories:
 - (1) Internal users:
 - Members of the medical faculty of RWTH Aachen
 - (2) RWTH Aachen users:
 - Members of RWTH Aachen
 - (3) External users:
 - a. Other universities or academic partners
 - b. Industrial partners
- All users have equal rights and responsibilities during their work in the facility. The user groups reflect the fees for usage of the Core Facility “Two-Photon Imaging”. The fees cover

imaging costs, assistance and basic consumables and will be modified at regular intervals according to general price changes.

Please note:

IZKF Core Facility users are obliged to refer to the support provided by the Facility as an acknowledgement in their publications. Please use the following wording:

This work was supported by the Core Facility "Two-Photon Imaging", a Core Facility of the Interdisciplinary Center for Clinical Research (IZKF) Aachen within the Faculty of Medicine at RWTH Aachen University.

OR

Diese Arbeit wurde unterstützt durch Two-Photon Imaging Facility, eine Core Facility des Interdisziplinären Zentrums für Klinische Forschung (IZKF) Aachen der Medizinischen Fakultät der RWTH.

B. Regulations regarding the usage of imaging systems in the facility

- Do not change the settings of the imaging systems if you are not sure what the consequences are! It should always be possible to contact somebody with appropriate knowledge for support.
- In case of potential damage to the systems due to inappropriate use (i.e. chemical or mechanical damage to the optics or other parts of the system), the Core Facility staff are obliged to intervene and stop the experimental session(s) immediately.
- After usage, all equipment must be cleaned, fully functional, and brought back to its original state, i.e. ready for use by the next user for the next experiment.
- Malfunctioning equipment, due to technical problems or incorrect usage, or software errors/viruses should be reported immediately to the Core Facility staff.
- Adaptations to the setup or technical repairs/improvements on the imaging systems should only be performed by qualified personnel, i.e. the Core Facility Director and personnel from the corresponding companies. If work on one of the systems is required, the laboratory cannot be used for any other experiments because of safety and practical reasons.
- The Core Facility is not responsible for storage of the acquired data.

Two-Photon Imaging: All users should protect their own data by transferring it to an external storage device that is free of viruses directly after facility usage. In-Vivo Imaging: Data transfer is allowed only via network connection.

The Core Facility offers short-term storage space on the "IZKF Cloud" server for imaging data. The server can be used for data transfer via web-access or network connection (Forschungsnetz).

- The Core Facility Director reserves the right to delete old data or data without reference to a user.

C. Regulations regarding S1 laboratory guidelines

- All rooms of the Core Facility are classified as S1 laboratories and are subject to the official security regulations. Follow the S1 working guidelines during the use of the Core Facility.
- Wear lab coats or blue scrubs at all times during your work in the facility (also during work at the evaluation computer).
- It is strictly forbidden to bring food or drinks into the room.
- Coats and bags must be stored in the library at the end of corridor 46.
- All bottles, falcons, eppis, opened flasks etc. containing lab material, chemicals and solutions must be labeled with name of the content, date and name of the user. All unlabeled or old material will be thrown away during routine controls.
- Facility users should bring their own consumables such as syringes, fluorescent dyes, etc. needed for experiments. In case of regular usage of the facility, storage of consumables at a specified location within the facility can be discussed with the Core Facility Director.
- Experiments at the microscopes with toxic or infectious material (e. g. blood) are permitted only with special care and with additional measures to protect the microscope. Appropriate cleaning after experiments is obligatory.
- Keep your personal belongings and paperwork organized. Reduce paperwork to a minimum. Put your papers, notes and belongings in cupboards or drawers after work.
- Keep the workspace, sink and fridge/freezer clean.
- Inform the Core Facility Director about offenses against the working guidelines.

D. Regulations regarding collegial behavior:

- Avoid disturbing other users/experiments working in the two-photon facility.
- Inform other users if you are running ongoing, longer experiments with a sign on the door (outside).
- Only turn off/on any light sources in the two-photon microscope room after consulting with the other users in the room (check for running experiments).
- In case of multiple users, update each other regularly regarding the conducted experiments and requirements.
- Keep the level of noise as low as possible. Music is allowed but only if other users present within the facility approve.
- Treat each person's working space with care and respect.
- Please contact the Core Facility Director immediately if there are complaints regarding the facility in general, system functionality, planning or (anti)social behavior. Complaints regarding the Core Facility Director should be directed to (one of) the Core Facility supervisors.

- In case of system double bookings, the Core Facility manager may bring users into contact with each other in order to resolve planning problems. If any issues cannot be solved in a reasonable way, the Core Facility Director will make a decision which is likely to be based on the “first-in, first-out” principle.

Appendix 1:

Equipment in the IZKF Core Facility "Two-Photon Imaging"

The Core Facility "Two-Photon Imaging" is equipped with two two-photon microscope systems:

- (1) LaVision BioTec TriMScope I
 - (2) Olympus FV1000MPE
- and two in-vivo imaging systems:
- (3) Perkin Elmer MIS Lumina XR
 - (4) Berthold NightOWL

(1) LaVision BioTec TriMScope I:

Microscope table:	Anti-vibration table (active dampening) Newport ST-series
Laser:	Spectra Physics MaiTai HP (serial #: 3261)
Laser modulation:	LaVision BioTec TriMScope I with fixed pre-chirp, optical beam multiplier and non-resonance mirror scan head
Microscope stand:	BX61WIF (motorized upright microscope) (serial #: 8A21020)
Objectives:	XLUMPLAN20x → 20x0.95WD2.0 XLPLN25xWMP-SP → 25x1.05WD2.0 LUMFI60x → 60x1.10WD2.0 UMPLFLN 10XW → 10x0.30WD3.5
Detection:	Single beam mode: filter based detection system; imaging with up to six detectors is possible (three direct PMT detectors (d=25 mm) at the normal PMT port and three GaAsP detectors (Hamamatsu H 7422-40) at the ultrasensitive PMT port with optional exchange of detectors between the ports). One detector port in transmission below the sample stage (usually not occupied). Spectral filtering is performed by manual selection of dichroic mirrors and bandwidth filters in front of each PMT. Multi-beam mode: Two highly sensitive Hamamatsu C9100-02 EMCCD cameras (serial #: 220400) with motorized filter wheel (LaVision BioTec FW8-0036) for spectral filtering. Fluorescent filters available from 380-670 nm in approx. 20 nm and 50 nm bandwidth steps (including corresponding dichroic mirrors).
Microscope stage:	Motorized LaVision BioTec intravital X/Y stage.

Incubation system: Life imaging services incubation system CUBE&BOX. Sensor: Pt-100 thin-layer RTD temperature range: +5° C to 50°C Stability: ± 0.1°C at sensor location, Precision: ± 0.3°C at sensor location

Image acquisition computer: Windows 7 Prof. SP1 64Bit system; Intel i7-3770 CPU @3,40/3,41 GHz, 3,19 GB RAM, Nvidia Quadro 410, 30-inch flat screen, LaVision TriMScope control/trigger board, second AD-Board and Hamamatsu C9100 control board.

Image acquisition software: LaVision BioTec Inspector Pro

Others: Classic HBO fluorescent light source (X-Cite120) including light guide, four fluorescent filter cubes, bright field trans-illumination light source (TH4-200) with an aplanatic achromatic 1.4 condenser. Laser safety and IR-viewer.
glasses

(2) Olympus FV1000MPE: (serial number for Olympus-manufactured system parts: FV10M-348-162)

Microscope table: Anti-vibration table (active dampening) (Newport RS2000-series; MG-FV-AV 7)

Laser: Spectra physics MaiTai DeepSee HPDS-OL with wavelength adjustable pre-chirp (serial #: 4532)
Argon laser diode (458/488/515 nm), including a multi-laser combiner unit.

Laser beam modulation: Olympus Fluoview FV10 single beam scanner optimized for NIR wavelengths.

Microscope stand: BX61WIF (motorized upright microscope; serial #: 8K17109)

Objectives: XLPLN25xWMP-SP → 25x1.05WD2.0
LUMFI60x → 60x1.10WD2.0
UMPLFLN 10XW → 10x0.30WD3.5

Detection: Filter-based detection system; three internal non-directly scanned PMT detectors (d=25mm). Bandwidth selection of emitted light by filters in motorized filter wheel is in front of each PMT (confocal detectors). Four ultra-sensitive external non-descanned PMT detectors (d=32mm) for direct detection of emitted light. Bandwidth selection by spectral filters and corresponding dichroic mirrors (in a filter cube) in front of each PMT. Internal optical zoom unit.

Microscope stage: LaVision BioTec intravital stage (manual).

Image acquisition computer: Windows 7 Prof. 64bit system; Intel Xeon CPU E5-1620-0 @ 3,60GHz/3,60GHz, 8 GB RAM with FluoView interface board, 30 inch TFT-monitor.

Image acquisition software: Fluoview FV10 2.0

Others: Classic HBO fluorescent light source including light guide, two fluorescent filter cubes.

with CCD camera Olympus XM10 (12 bit monochrome camera, serial #: 51000191)
Cell-P acquisition software.

Laser safety glasses and IR-viewer.

Other equipment (two-photon microscope room):

- Small animal surgery station including stereomicroscope and basic equipment for preparation of chemicals.
- Trigger box for the transformation of external signals (ECG and respiration) into trigger pulses for imaging.
- 1st image processing computer: "Fujitsu" Siemens computer (16GB RAM)
- 2nd image processing computer: "Datec 1" computer (64GB RAM)
- 3rd image processing computer: "Datec 2" computer (192GB RAM)
- Image processing software:
- ImagePro Analyzer 3D 7.0
- Bitplane Imaris 83 (MeasurementPro, Track, XT)
- Fiji (ImageJ)
- Autoquant X3
- XuvStitch
- Olympus Fluoview FV10 2.0 viewer
- LaVision Biotec Inspector Pro

(3) MS Lumina XR:

Light source: Halogen Tungsten Lampe (150 W), LED lamps for photographic images.

Detection: -90°C cooled back-thinned, back-illuminated grade 1 CCD camera, 1024x1024 pixel, 13x13mm, (16Bit).

Filters: 10 position excitation filter wheels, equipped with 10 excitation filter (30nm bandwidth) in the range from 430-745 nm, 8 position emission filter wheel, equipped with 4 emission filters (GFP/DsRed/Cy5.5/ICG). Automated image integration to overlay with bioluminescence, fluorescence and photograph.

Imaging chamber: Light-sealed, heated stage to maintain optimum body temperature. Motor controlled stage, filter wheel, lens position, and f-stop.

X-Ray module:	Radiation shielded cabinet. Fast X-ray image acquisition times of 1-10 s reducing radiation exposure.
Additional accessories:	Optical zoom lens attachment for close up and high resolution X-ray images, optical X-FOV lens attachment for view field extension to 23x24 cm (5 mice). Gas anesthesia ports, 5 position manifold within imaging chamber. Syringe injection system
Anesthesia snit:	Xenogen XGI-8 gas anesthesia system for small animals (adapted to anesthetize up to five mice in parallel).
Software:	Living Image Software 4.4
(4) Berthold NightOWL:	
Light source:	Halogen 75 W tungsten lamp, ring-light fluorescent illumination.
Detection:	Andor Ikon, -70°C cooled back-thinned, back-illuminated grade 1 CCD camera, 1024x1024 pixel, 13x13mm, (16Bit).
Filters:	4 excitation filters per slide. 4 emission filters per wheel.
Imaging chamber:	Light-sealed cabinet with motor-driven camera. Sample size up to 250 mm. Temperature controlled animal tray
Anesthesia unit:	TEM gas anesthesia system for small animals (adapted to anesthetize up to five mice in parallel).
Software:	IndiGO™ software

Appendix 2:

Contact information

Core Facility "Two-Photon Imaging": tplsm@ukaachen.de

Director of the Core Facility (manager): Michael Vogt, PhD
mvogt@ukaachen.de , phone 37360 or 88603

Core Facility Scientific Supervisors: Univ.-Prof. Marc AMJ van Zandvoort, PhD
mamj.vanzandvoort@maastrichtuniversity.nl.
Prof. Christian Martin, PhD
chmartin@ukaachen.de , phone 89122

Core Facility Supervisors: Univ.-Prof. Rene Tolba, MD
rtolba@ukaachen.de , phone 80472

Laser safety officer: Michael Vogt, PhD
mvogt@ukaachen.de , phone 37360 or 88603

Deputy laser safety officer: Univ.-Prof. Rene Tolba, MD
rtolba@ukaachen.de , phone 80472

Appendix 3:

Declaration regarding S1 laboratory guidelines

The microscope room of the Core Facility is classified as a S1 laboratory and is subject to the official security regulations. I hereby declare that I will follow the S1 laboratory guidelines and the working regulations during the use of the Core Facility.

Declaration regarding laser safety

I hereby declare that I am familiar with the do's and don'ts concerning working with class 3B and 4 laser systems embedded in two-photon microscopic systems.

Furthermore, I state that I was instructed on the potential risks of working with class 3B and 4 lasers (see also "Betriebsanweisung Lasersicherheit").

Lastly, I state that I will strictly follow the instructions of the responsible laser safety officer and the house rules of the IZKF Core Facility "Two-Photon Imaging".

Date

City

Name

Signature

Dr. M. Vogt (Core Facility Director/ Laser safety officer)

Appendix 4:

Terms and conditions of use (Guidelines for the principal investigator)

- The use of the imaging systems will be invoiced on an hourly basis.
- The fee is based exclusively on equipment use, independent of staff support or scientific contribution of staff.
- You are obliged to inform the core facility "Two-Photon Imaging" about the submission of publications containing data generated in the Core Facility.
- Following the IZKF Aachen guidelines IZKF Core Facility users are obliged to refer to the support provided by the Facility as an acknowledgement in their publications. Please use the following wording:

This work was supported by the Core Facility "Two-Photon Imaging", a Core Facility of the Interdisciplinary Center for Clinical Research (IZKF) Aachen within the Faculty of Medicine at RWTH Aachen University.

- Send the imaging paragraph of the corresponding publication to the head of the Core Facility "Two-Photon Imaging" before submission. This regulation prevents possible errors in the methodological part of the paper and will improve the quality of the paper.
- Co-authorship of core-facility staff must be agreed upon following the guidelines of the medical faculty for the authorship of scientific publications, 26.05.2014.

Advice:

- The management of the Core Facility offers pre-experimental consultation to help with the experimental set-up (e.g. sample preparation/staining).
- During the experiments, we advise meeting on a regular basis to discuss progress, obtained imaging data, and possible new experiments. This consultation will help to generate high quality data, to prevent possible misinterpretations of imaging data, and to avoid unnecessary imaging time.
- To summarize: all this will help to improve the scientific outcome and reduce your imaging costs.

Date City

Name

Budget number

Signature