

Two-Photon Imaging Facility

Operator and User Regulations

1. Introduction

The IZKF Aachen 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 Optical 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 manager are listed in appendix 2.

2. User Regulations

A. Basic Core Facility regulations

- No experiments may be conducted without the authorization of the Manager 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 manager 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 manager). 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", Appendix 4).

B. Access to the Core Facility

- 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 manager. 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 IVIS 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 IVIS 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 manager and only for those users who have had extensive training in laser safety and correct usage of the imaging systems.

C. Support for users

- Prior to the use of the equipment, the Facility manager or staff will advise the user in their specific questions and also regarding sample preparation, image analysis, choice of the microscope and the imaging technique to be used.
- Upon request and consultation, the user will be individually trained on the microscope by the Facility manager or staff.
- Facility manager or staff are always available to discuss progress, imaging data obtained and possible new experiments.
- In case of problems or questions during image acquisition, the user can contact the Facility manager or staff for help/assistance.
- The Facility manager or staff also supports interested users in establishing new imaging techniques.
- The Facility manager or staff supports and advises on the application and acquisition of new microscopy systems within the framework of large-scale research equipment applications.
- The Facility manager or staff supports the users in writing the method section for publications.
- Contact details of the Facility manager and staff can be found in the Appendix 2 or on the IZKF webpage:
<https://www.medizin.rwth-aachen.de/cms/Medizin/Die-Fakultaet/Einrichtungen/IZKF-Aachen/Core-Facilities/Two-Photon-Imaging-Facility/-pnnd/Team/>

D. Booking and use of the equipment

- After consultation of the Facility manager or staff and successful completion of the training, an e-mail with the login data to the outlook booking calendar will be send to the user. Additionally the user is added to the e-mail distributor of the Facility.
- The user has to enter the name, e-mail address and phone number in the online booking calendar when booking a time slot for a certain equipment.
- The booked time in the outlook calendar are the basis of calculation of the fees.
- These data are only used to generate invoices and anonymous usage statistics.
- At the end of the project, the user has to inform the Facility manager or staff and has to remove their data from the computer.

E. Regulation of usage priorities

- All users have the same priorities in terms of booking the equipment.
- In case the equipment is fully utilized, the Facility manager or staff may decide on assignment and/or access to equipment. Decision-making will take into account the urgency of the project and the nature of the samples.

F. User fees

- The users of the Core Facility "Two-Photon Imaging" are divided into three categories according to the guidelines of the IZKF Aachen.
 - (1) Internal users – Medical Faculty
 - (2) Member of the RWTH Aachen
 - (3) External users - other universities, academic partners or Industrial partners

Microscope System	Internal User	RWTH	External User
Olympus FV1000MPE	25 €/h	25 €/h +19%VAT	80 €/h +19%VAT
Leica Stellaris8 DIVE FALCON	35 €/h	35 €/h +19%VAT	110 €/h +19%VAT
Image Processing Computer	Internal User	RWTH	External User
Datec & Fujitsu R940	4 €/h	4 €/h +19%VAT	15 €/h +19%VAT
Service	Internal User	RWTH	External User
Staff support	15 €/h	15 €/h +19%VAT	48 €/h +19%VAT

- The fees will be modified at regular intervals according to general price changes.
- User fees will be charged on the base of microscope usage time and invoices are issued semi-annually.
- The invoice will be sent to the responsible principal investigator (PI) of the working group, who also signed the "User and PI agreement" (appendix 4).
- Introductory consultations will not be invoiced.

- The fees for usage hours in the core facility can be financed by research grants of DFG (including Collaborative Research Centers (SFB) or other scientific consortia), Emmy Noether or Heisenberg programmes, *Bundesministerium für Bildung und Forschung* (BMBF), European Union (EU) or other grant agencies.
- Further information can be found on the DFG website:
https://www.dfg.de/formulare/52_01/52_01_de.pdf
https://www.dfg.de/formulare/52_01/52_01_en.pdf
https://www.dfg.de/formulare/55_04/55_04_de.pdf
- The core facility offers assistance to all users in preparing the specific imaging section of their grant application.
- The money collected through user fees will be used for specific equipment repairs, replacement and additional equipment for existing microscopes (objectives etc.) and specific user support by the staff as well as supporting material for Facility users (e.g. antibodies and dyes for testing/pilot experiments). This is in line with DFG rules for equipment user fees and will maintain a high level of infrastructure at the Facility.

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

"Gefördert mit Mitteln des Interdisziplinären Zentrums für Klinische Forschung in der Medizinischen Fakultät der RWTH Aachen"

G. 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 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 manager 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. 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 time limited storage space on the "IZKF Cloud" server for imaging data. The server can be used for data transfer via web-access or network connection (Hausnetz).
- The Core Facility manager reserves the right to delete old data or data without any reference to a user.

H. 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.
- Work of biological safety level S2 is not permitted.
- Wear lab coats or red 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 manager.
- 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 manager about offenses against the working guidelines.

I. Regulations regarding collegial behavior:

- Avoid disturbing other users/experiments working in the 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).

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- In case of multiple users, update each other regularly regarding the conducted experiments and requirements.
- Keep the level of noise as low as possible.
- Treat each person's working space with care and respect.
- Please contact the Core Facility manager immediately if there are complaints regarding the Facility in general, system functionality, planning or (anti)social behavior. Complaints regarding the Core Facility manager should be directed to (one of) the Core Facility supervisors.

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) Olympus FV1000MPE
- (2) Leica Stellaris 8 DIVE FALCON

and an in-vivo optical imaging system:

- (3) Perkin Elmer IVIS Lumina XR

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

Microscope table: Anti-vibration table (active dampening) (Newport RS2000-series)

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

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

Objectives:	XLUMPLAN20x	20x	NA0.95	WD2.0
	XLPLN25xWMP-SP2	25x	NA1.05	WD2.0
	XLPLN10XSVMF	10x	NA0.6	WD8.0
	LUMFI60x	60x	NA1.10	WD2.0
	UMPLFLN10XW	10x	NA0.30	WD3.5

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: motorized PRIOR z-deck stage.

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.

Software: Fluoview FV10 2.0

Others: Classic HBO fluorescent light source including light guide, two fluorescent filter cubes. CCD camera Olympus XM10 (12 bit monochrome camera, serial #: 51000191) with Cell-P acquisition software.
Laser safety glasses and IR-viewer.

(2) Leica Stellaris 8 DIVE FALCON:

Microscope table:	Anti-vibration table (active dampening) Newport		
Laser:	Spectra Physics Insight X3 Dual (serial #: 2434)		
Laser modulation:	Integrated DeepSee dispersion pre-compensation		
Microscope stand:	Leica DM6 CFS (motorized upright microscope) (serial #:)		
Objectives:	FLUOTAR L 16x/0.6 IMM CORR VISIR	16x	WD 2.5
	HC PL APO 63x/1.2 W CORR CS2	63x	WD 0.3
	HC IRAPO L 25x / 1.00 W motCORR	25x	WD 2.6
Detection:	Confocal detectors: 2 HyD SP gated, 1 HyD S SP gated) (FLIM capable) 4TUNE DIVE (4-channel): 4X non-descanned 4Tune HyD RLD detectors (FLIM capable) with free tunable detection range SP8 FALCON (FAst Lifetime CONtrast) with LAS X Phasor FLIM Online Transmission detectors: 2 PMT with filter block		
Multi-beam mode:	Broad tuning range (680–1300 nm) for maximum flexibility Dual output at 1045 nm with optional pre-compensation for simultaneous two color excitation. Dual laser beam routing with VBE.		
Laser scanning:	TCS SP8 Scan head (resonant/non-resonant)		
Microscope stage:	Motorized Scientifica MMBP X/Y stage, Okolab laser security box (can be heated)		
Computer:	Expert CUDA Workstation, RAID High Power HPZ6G4 Workstation Windows 10 Professional (64 bit) Intel 6134 XEON3.2 8C, 192 GByte RAM, NVIDIA Quadro GP100 16GB, Pegasus3 RAID System (16 TB) 2 x 31,5 inch displays		
Software:	LAS X		
Others:	EL 6000 (LQ-HXP 120 LEJ) fluorescent light source, three fluorescent filter cubes (DAPI/GFP/RHOD). Laser safety glasses and IR-viewer.		

(3) IVIS 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 unit:	Xenogen XGI-8 gas anesthesia system for small animals (adapted to anesthetize up to five mice in parallel).
Software:	Living Image Software 4.4

Other equipment (two-photon microscope room):

- Small animal surgery station including stereomicroscope
- Basic lab equipment for preparation of chemicals and samples.
- Trigger box for the transformation of external signals (ECG and respiration) into trigger pulses for imaging.
- Isofluran anesthesia system for small animals
- Image processing computer: "Datec" computer (192GB RAM)
- Image processing computer: "Fujitsu R940" computer (192GB RAM)

Image processing software:

- ImagePro Analyzer 3D 7.0
- Bitplane Imaris / Stitcher
- Fiji (ImageJ)
- Autoquant X3
- XuvStitch
- Olympus Fluoview FV10 2.0 viewer
- Leica LasX

Appendix 2:

Contact information

Core Facility "Two-Photon Imaging":	tplsm@ukaachen.de
Manager of the Core Facility (manager):	Michael Vogt, PhD mvogt@ukaachen.de , phone 0241 80 37360 & 88603
Core Facility Scientific Supervisors:	Univ.-Prof. Marc AMJ van Zandvoort, PhD mamj.vanzandvoort@maastrichtuniversity.nl Prof. Christian Martin, PhD chmartin@ukaachen.de , phone 0241 80 89122
Core Facility Supervisor:	Univ.-Prof. Rene Tolba, MD rtolba@ukaachen.de , phone 0241 80 80472
Laser safety officer:	Michael Vogt, PhD mvogt@ukaachen.de , phone 0241 80 37360 or 88603
Deputy laser safety officer:	Univ.-Prof. Rene Tolba, MD rtolba@ukaachen.de , phone 0241 80 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 Manager/Laser safety officer)

Appendix 4:

User and PI Agreement

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

- The use of the imaging systems will be invoiced on an hourly basis for equipment use and staff support.
- The PI is obliged to inform the Core Facility "Two-Photon Imaging" about the submission of publications containing data generated in the Core Facility (publications are important to demonstrate the need and use of the core facility).
- Following the IZKF Aachen guidelines 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 by Core Facility staff is in accordance with the medical faculty guidelines for 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 (optional)

Signature