

## AMBIO User Guidelines and Rules

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## MISSION

The Advanced Medical BioImaging Core Facility (AMBIO) of the Charité has been established to provide access to advanced fluorescence microscopy systems and the necessary expertise in how to perform advanced imaging experiments on the molecular, cellular and tissue level. The general goal of AMBIO is to offer researchers from various backgrounds support during complex bioimaging workflows.

The AMBIO services include individual user training and assistance, and if required, supervised image acquisition and guidance for data analysis. Additionally, AMBIO staff members will offer advice on project specific issues including imaging strategy, sample preparation and instrument choice. If the capacities allow, AMBIO staff may further offer assistance during quantitative image analysis by suggesting image processing and analysis strategies or by offering access to specialized image processing software.

AMBIO services are available to all workgroups of the Charité and associated academic institutions (i.e. FU Berlin, HU Berlin, MDC/BIH, FMP Berlin). Providing sufficient capacity, AMBIO also offers its services to external users from both academic and commercial institutions.

## AMBIO SYSTEMS

Currently AMBIO offers access to 6 advanced imaging systems (Nikon) and 2 Analysis Workstations:

- |                                     |                 |             |
|-------------------------------------|-----------------|-------------|
| • System 1 – Wide-Field/TIRF/STORM  | (N-STORM)       | Room 01-306 |
| • System 2 – Scanning Confocal      | (Nikon A1Rsi)   | Room 01-306 |
| • System 3 – Spinning Disk Confocal | (Nikon CSU-X)   | Room 01-306 |
| • System 4 – Multi Photon Confocal  | (Nikon A1RMP)   | Room 02-117 |
| • System 5 – Wide-Field             | (Nikon Ti2)     | Room 01-306 |
| • System 6 – Upright Spinning Disk  | (Nikon Eclipse) | Room 06-315 |
| • System 7 – Analysis Workstation1  | (Nikon/HP)      | Room 01-307 |
| • System 8 – Analysis Workstation2  | (Charité)       | Room 01-307 |

Detailed technical information about each system is available within the IRIS system (<https://iris.charite.de>) and on the AMBIO website (<http://ambio.charite.de>).

## USER GUIDELINES and RULES

The following AMBIO User Guidelines and Rules are formulated in accordance to the “Basic Requirements for Research Infrastructures in Europe” (European Science Foundation; published 04/2011) as well as the Guidelines for Research Facilities of the DFG2 (published 11/2011). The head of AMBIO, in consultation and agreement with the AMBIO steering committee, can make changes to the user guidelines and rules at any time. AMBIO users will be notified about updates and changes to the user guidelines and rules. Disregard of the following guidelines and rules by a user can result in access to the AMBIO systems being revoked temporarily or permanently.

## REGISTRATION, CONSULTATION, TRAINING, BOOKING, CANCELLATION, USAGE

In order to utilize AMBIO services, all new users must be registered and trained as detailed below.

**REGISTRATION:** All new users are required to first register for a Charité IRIS profile on the IRIS webpage followed by submitting an AMBIO Project and Training request. Detailed information can be found here: <https://tinyurl.com/2dyupwye>.

To complete registration all new users and their principal investigator (PI) are required to agree to these **AMBIO User Guidelines and Rules** by signing the **AMBIO Declaration** that can be found here: <https://tinyurl.com/4w5zecd2>. All users are required to inform AMBIO staff when they no longer require account access due to their contract ending or if their affiliation with their workgroup changes or their proposed project ends in order to update their IRIS account and/or their AMBIO access can be changed or terminated accordingly. Registered AMBIO users will automatically receive an e-mail from the IRIS system to renew their account access after 1 year. If the user fails to prolong the project, the account will be inactivated automatically, but can be reactivated by AMBIO staff. For internal users of the Charité the registration requires details of the groups 8-digit account number (Kostenstelle), which allows AMBIO to charge fees via the ILV (Interne Leistungsverrechnung) system.

**PROJECT CONSULTATION:** All new users are required to make an appointment for an initial discussion of their imaging project (max. 1 h, no charge) with AMBIO ([ambio@charite.de](mailto:ambio@charite.de)). This consultation is mandatory to optimize the imaging strategy, sample preparation and microscopy technique. After determining the complexity of the project and the experience of the user, AMBIO staff will then make an appointment for a project specific training session.

**TRAINING:** Due to the complexity of the available imaging modalities and the desired applications, users must receive **project specific training** on AMBIO systems **for every new imaging project**. To allow for time-efficient user training (max. 3 h in one session) the **user should supply an appropriate test sample** for the training session so that imaging parameters and conditions can directly be identified. For further imaging projects that require a different imaging system or mode (i.e. wide-field versus TIRF) the user will need to request additional training on the new system, the length of training will be determined on the user's experience. A successful training status is reached when both AMBIO staff and the user feel comfortable with the user performing non-supervised imaging experiments. After successful training the user is entitled to use the system without direct supervision by AMBIO staff (autonomous). **Laser safety** instructions (if applicable) are given within the training.

**BOOKING:** After a validation of the training by the head of AMBIO, the user is enabled to reserve slots via the online IRIS booking system on a first-come-first-serve basis (<https://iris.charite.de>). The shortest bookable time slot is 30 min. To efficiently use the bookable time, users should avoid 'Swiss cheese booking' (multiple short time slots with breaks). Please, be considerate and book reasonable time slots, leaving enough time for others in the morning or afternoon. AMBIO will take the right to intervene if inappropriate time slots (i.e. too long or too scattered) are booked. If the AMBIO imaging systems are not available or only partially functional due to technical issues, the users will be either notified directly or the issue will be marked in the scheduler in IRIS. Specific technical issues with the systems will be communicated by e-mail or telephone to affected users. Users are encouraged to communicate any technical issues immediately to AMBIO staff ([ambio@charite.de](mailto:ambio@charite.de)).

**CANCELLATION POLICY:** If required (change in experimental schedule, sickness) the user can cancel a session at any time within the IRIS system. To allow efficient usage of AMBIO time slots (i.e. rebooking by other users after cancellation), a booked session can be canceled free of charge **until 48 h before the start**

**of the session.** In case of a **short-term cancellation** (less than 48 h ahead) the user is charged **50%** of the fees unless another user takes the slot, in which case the cancellation charge is waived. The same applies if a user **cancels the remaining imaging session ad hoc** within IRIS. Users can shift the imaging session within the same day without additional charges. **‘No-show’ or non-cancelled slots will be charged 100% of the fee.** Users can choose to receive **automated alerts for cancellations** of a specific system within the IRIS system.

**ASSISTANCE:** Depending on the level of experience, care and performance of the user, AMBIO staff can recommend or require the user to book imaging sessions *with* AMBIO staff assistance. Users can also request assistance by AMBIO staff at any time by communicating with AMBIO staff accordingly ([ambio@charite.de](mailto:ambio@charite.de)). AMBIO staff members are usually available during the core working hours (weekdays 9 am – 5 pm).

**LIVE-CELL INCUBATION:** For experiments that require incubation (i.e. 37°C) the user should mark ‘Incubator’ under ‘Setup’ in the IRIS booking GUI. IRIS automatically adds 1 h before and 30 min after the booking slot for **heat-up and cool-down phase** during which time room temperature experiments cannot be booked. Regular usage charges apply for both times. If the next user uses the incubator, too, the cool-down phase can be booked as imaging time. The user is responsible to turn ON and OFF the incubator system before/after usage. Please, check whether the next user uses incubation or not, and leave the incubator ON accordingly. We recommend switching the incubator ON about 1 h before the start of the experiment to allow full thermal equilibration (minimal sample drift).

**ACCESS:** Autonomous users may receive **transponder access** to AMBIO rooms in agreement with the AMBIO head (signature required on Charité transponder form). AMBIO also provides guest transponders to external groups if needed. The guest transponder must not be passed on to third parties or novice users, but only to autonomous users within the same group (same PI). In the absence of AMBIO staff (off peak hours), the advanced user is responsible for the equipment and ensures that no unauthorized persons have access to AMBIO. To prevent theft or damage by unauthorized persons the users are obliged to **lock the AMBIO door** to the corridor, if they are the last to leave the facility (also short-term).

**MAINTENANCE:** In the event of acute technical issues on the AMBIO systems already booked imaging sessions may be over-written/cancelled (charges waived) by AMBIO staff to allow repair or maintenance of the systems. Affected users will be notified accordingly and AMBIO staff will assist the users to find alternate imaging sessions.

## USAGE FEES

AMBIO’s long-term existence depends on the income from usage fees (covered by 3<sup>rd</sup> Party Funds of the user labs). As of January 2022, AMBIO’s usage fees are as follows (9% increase compared to 2021):

• Imaging Project <b>Consultation</b> (max. 1 h)	free
• Imaging Project <b>Training</b> (excluding usage)	32,70 €/h
• <b>CORE hour Usage</b> for AMBIO systems 1-4, 6	16,35 €/h
• <b>CORE hour Usage</b> for AMBIO system 5	10,90 €/h
• <b>OFF hour Usage</b> for all AMBIO systems	50% of CORE price
• Image analysis workstation Usage (booking required)	free
• AMBIO Staff <b>Assistance</b> (in addition to usage)	32,70 €/h

CORE hours: Mo – Fr, 9am – 6pm  
OFF hours: Mo – Fr, 6pm – 9am, Sat/Sun all day (plus Berlin holidays)

Until further notice the above fees will be chargeable to the user accounts. Users will be notified if these fees are changed in the agreement with the AMBIO steering committee.

**Long-term continuous booking discount:** AMBIO offers a 50% discount on the above rates for continuous bookings of one system for longer than 24 h. Meaning, the first 24 h are charged with normal rates, thereafter the fees are charged at 50% of original rate (independent of the type of hours (core, off)).

**Technical issues:** If the user experiences unforeseen technical problems on the imaging system that do not allow meaningful data acquisition, and communicates that with the AMBIO staff, the charges for the session will be waived. AMBIO will not have to pay damages (e.g. costs for sample preparation) but will support the user in finding a replacement imaging slot for the cancelled session.

**CALCULATION and CHARGING of USER FEES, INVOICING:** Upon registration and signing the AMBIO declaration the user allows AMBIO to charge the above usage fees to the appropriate account (Kostenstelle) of the responsible PI. **INTERNAL** (Charité) users are responsible to register the **correct and active account number (Kostenstelle, 8 digits)** within the IRIS system to allow booking of the charges through the internal ILV ("Interne Leistungsverrechnung") system. **EXTERNAL users** need to register with their correct **billing address**. The IRIS 'Agent' installed on each imaging system will track the actual usage times. A combination of the booked time and actual used time from the IRIS 'Agent' will be used to calculate the usage fees according to the above fees and policies. For **INTERNAL** (Charité) users, prior to charging (quarterly), the PI's will receive an invoice that needs to be validated within 1 week. If there are no complaints within this week, AMBIO will charge the calculated amount to the respective account. Invoices for **EXTERNAL users** are prepared by the Charité financial department and need to be paid within 4 weeks. AMBIO reserves the right to restrict the use of the facilities in case of wrong account numbers or if the fees are not paid in time. **Please note:** For **EXTERNAL users** (i.e. HU-Berlin, FU-Berlin, MPI, etc.) **19% taxes ('Mehrwertsteuer')** will apply according to the law in addition to the above user fees.

## USER RESPONSIBILITIES

Due to the costs and nature of the high-end imaging equipment that is specialized for low light detection and fast image acquisition, special care during usage is required from all AMBIO users. Users are only allowed to operate AMBIO equipment **after sufficient training** by AMBIO staff and are obliged to strictly follow the explained and demonstrated operation procedures. Users should ask AMBIO staff when they are in doubt about any of the procedures. **Users must report problems** with the systems to AMBIO staff directly, by email or by creating incidents within the IRIS system for their session. Users are not allowed to make hardware changes to the systems (e.g. exchange objectives) unless they were trained and have the explicit permission by AMBIO staff. To avoid hazards for other users and damage to equipment, AMBIO staff must be informed immediately if solutions have been spilled or a contamination with chemical or biological agents has occurred on the imaging system. AMBIO equipment and working space must be left clean and tidy for the next user. Used samples, supplies, broken glass and any other waste must be removed according to the safety standards. **Principal investigators are responsible for the user** from their research group, concerning their conduct at the microscopes, their knowledge about basic experimental procedures as well as general safety instructions and issues regarding biological safety. Specific regulations regarding AMBIO lab space are communicated by AMBIO staff during the training.

**Objectives:** Specifically, the immersion objectives and the microscope stage need to be cleaned as instructed with the correct cleaning tissue. Special care should be taken when moving the stage or focus knobs as under some circumstances the **objectives can be damaged by hitting the stage.**

**Hand over system:** If the next user is not present when your imaging session is finished, please **shut down the system completely.** If the next user is already present logging out of the Nikon Imaging Software (NIS) and the Windows user account is sufficient.

**IntensiLight:** The **fluorescence light source** (HG-lamp) should be switched OFF if not required for long-term experiments to save lifetime of the bulb (can be switched off while software is running). As a normal HG-Arc lamp it should not be switched ON for a minimum of 10 min after it has been switched OFF.

**Live-cell Imaging:** If incubation is ON and the next user also requires incubation, please, leave the incubation system ON.

Ask AMBIO staff if you have any doubt about the operation of the imaging system!

## DATA STORAGE/SAFETY and OMERO+

At present it is the responsibility of each user to ensure proper saving/storage of his/her experimental data. AMBIO provides data storage space for each user on the data storage drives on each imaging system. During image acquisition users should save their experimental data only to this **data storage drive** (i.e. D:\Users\Group\Name...). AMBIO does not take responsibility for data that is saved to the incorrect location on the computer (i.e. desktop, C:/) and accidental deletion from this incorrect storage location. AMBIO is also not responsible for long-term data backup or unauthorized people accessing experimental data. In order to prevent accidental removal of experimental data it is recommended that all users correctly label the user data folder with their user ID (name). AMBIO will not be held liable for damages resulting from viruses brought into the system by users. All users are required to back-up their experimental data on a regular basis in addition to the data storage drives on the imaging systems. If data needs to be deleted from the data storage drives on the imaging systems due to the drive being full, users will be notified by email about when the data will be deleted.

For **internal (Charité) users** there is a Charité-wide OMERO+ data repository system available for long-term storage of data and for data sharing and management. All AMBIO users are encouraged to request access to the OMERO+ system through the IRIS system (<https://iris.charite.de/service/?provider=p1976>). Further instructions are available at <https://ambio.charite.de/en/>.

**External users** may use virus free (scanned by AMBIO computer) external hard drives to transfer their data.

## ACKNOWLEDGEMENTs in GRANTS and PUBLICATIONS

**The long-term existence of AMBIO depends on its impact on research projects, which is mainly measured by the number of acknowledgements of AMBIO services in peer-reviewed publications or grant proposals.** These references are of utmost importance for AMBIO, as they demonstrate the effectiveness of the AMBIO staff in training the users and to justify the maintenance of such a core imaging facility. **Therefore, please ensure that AMBIO services are acknowledged** (instrument usage, custom software solutions for image or data acquisition or data analysis) in an appropriate way **in every**

**publication** that contains images and/or data recorded at AMBIO. Publications encompass, but are not limited to scientific papers, Bachelor-, Master- or doctoral theses as well as contributions to conferences.

Example text for acknowledgement: ***“We thank the Advanced Medical Bioimaging Core Facility of the Charité-Universitätsmedizin Berlin (AMBIO) for support in acquisition (and/or analysis) of the imaging data.”***

Please **report your publication** with AMBIO acknowledgment to the AMBIO staff either directly or by email ([ambio@charite.de](mailto:ambio@charite.de)). It is also encouraged to support AMBIO in poster sessions and talks you are giving. We are happy to hear about your successful projects and would be grateful for a PDF file of the final paper for AMBIO’s records.

**CO-AUTHORSHIPS:** If the AMBIO support required by the user to accomplish an imaging project goes well beyond the ‘routine’ service, in particular if AMBIO is heavily involved in project planning or assay development, AMBIO provides samples from own research work, or any other substantial intellectual or experimental contribution (i.e. super-resolution), the project should be conducted on a collaborative basis resulting in co-authorships of the involved academic AMBIO staff. Usage fees will incur nevertheless to cover the facilities’ running costs. The details of such an agreement should be discussed at an early stage between the respective project leader and the AMBIO head.

## SAFETY

**LABORATORY SAFETY:** The user of AMBIO laboratory space must follow with the standard laboratory safety rules that are valid for any German biomedical laboratory. The users must be instructed according to these rules by their principle investigator or other responsible person (not by AMBIO staff) in how to properly use the laboratory space. Proper usage includes the proper use and disposal of hazardous substances (‘Gefahrstoffe’), the proper disposal of waste, the cleaning of all multi-user space after usage and the proper operation of all device. Specific operating instructions (‘Betriebsanweisung’) of the AMBIO laboratory space are displayed for all users at the entrance door of AMBIO. All users are asked to read and follow these instructions carefully. In case of any doubts the users are asked to contact the AMBIO staff.

**GVO – S1:** AMBIO laboratory space (see rooms below) is **certified as a S1 ‘Gentechnische Anlage’ (GVO Anlage 576/17)** lead by **Dr. Jan Schmoranzner**. The following rooms are part of Anlage 576/17:

- CCO Room 01-306 (Wide-Field, Confocal, CSU-X Spinning Disk, TIRF/STORM Systems)
- CCO Room 01-307 (Lattice light sheet, CSU-W Sora Spinning Disk, others)
- CCO Room 02-117 (Part in the back, Multiphoton System)

By signing the **AMBIO Declaration** you agree to the Safety Rules below:

***AMBIO users must not introduce any material, including genetically modified organisms (GVOs), classified as biological safety level higher than S1 into the AMBIO lab space or perform any experiments in AMBIO lab space that require a higher safety level than S1.*** Exceptions from S1 might be possible within the S2 laboratory space of AG Wolfgang Kübler (CCO, 06-315, Upright Spinning Disk Confocal) in agreement with Prof. Kübler.

**INTERNAL (Charité) Users must** provide the GVO information (GVO Anlage/Project Nr., GVO project leader) of their S1 experiment during registration in IRIS if they plan to **perform GVO projects (S1)** and

want to use the AMBIO GVO Anlage 576/17 for their project. The GVO project leader of the specific GVO Anlage will be responsible for the correct performance of any GVO projects by the registered user according to the §15 GenTSV. The **AMBIO GVO project leader (Jan Schmoranzer) should be** notified of any special requirements.

**EXTERNAL (non-Charité) Users** need to provide a list of all the GVOs (genetically modified organisms) for any GVO/S1 project that is performed at AMBIO and are requested to fill out the GVO table, which is part of the 'Formblatt Z' that lists information about the GVO used. The Formblatt Z can be downloaded at <https://ambio.charite.de/en/registration/>.

**LASERSAFETY:** During the training session, each user will receive instructions about laser safety, if applicable. The user must sign that he/she understood the risks of using the system and that he/she will ensure annual update on laser safety. Current AMBIO laser safety instructors are Jan Schmoranzer and Jutta Schüler. An annual refresher of these safety instructions is mandatory for the users working with hazardous laser systems.

## LIABILITY & INSURANCE

Maintenance of all AMBIO systems ('Medizintechnik') is normally covered by the Charité Facility Management (CFM). However, there is no further general insurance coverage for the AMBIO equipment. Each user is responsible for the equipment during the booked sessions and can be held liable by AMBIO for any damages due to misconduct. If AMBIO equipment is damaged due to user misconduct, the users Principal Investigator is liable for the damage. In the case of an accident on AMBIO premises and if no AMBIO staff is present, the user needs to ensure they call for the relevant help and assistance and also notify AMBIO.

## APPLYING for FUNDS

AMBIO encourages Principal Investigators to apply for funds that cover AMBIO service fees as part of 'supply funds' (i.e. Sachmittel) in grant proposals that include imaging experiments on AMBIO systems. Guidelines for requesting usage fees for core facilities have been published by the DFG ([https://www.dfg.de/formulare/55\\_04/55\\_04\\_de.pdf](https://www.dfg.de/formulare/55_04/55_04_de.pdf)). The AMBIO staff may consult PIs/users in applying for microscopy-related funds. Please notify AMBIO ([ambio@charite.de](mailto:ambio@charite.de)) if you apply for funding that includes imaging usage fees, so that we can list the proposal within the AMBIO reports. Further details can be found at the following link (<https://ambio.charite.de/en/>).



AMBIO thanks all users for their support and wishes great success and fun with your advanced imaging projects.

Best Regards,

Berlin, 04.01.2022



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