

Mission of the Advanced Light and Fluorescence Microscopy (ALFM) facility at CSSB Hamburg

The ALFM facility aims to provide researchers with the most efficient support for light microscopy and correlative light and electron microscopy projects and offers them state-of-the-art imaging equipment. All users will receive advanced training on the systems. Services include troubleshooting, advice on experimental design, support with data analysis, and advice on grant applications. Research collaborations are possible and welcome.

If you have questions, please contact the head of the ALFM facility via email (roland.thuenauer@cssb-hamburg.de) or via phone (040 8998 – 87730).

The terms of use of the ALFM core facility comply with the quality standards for access to research infrastructures (RIs) established by the European Science Foundation (ESF Member Organization Forum on Research Infrastructures, issued 04/2011) and the DFG specifications for “Gerätezentren”

(http://www.dfg.de/download/pdf/foerderung/programme/wgi/basic_requirements_research_infrastructures.pdf)

(http://www.dfg.de/formulare/55_04/55_04_de.pdf)

Usage rules

1. ALFM facility access

Our devices and equipment are accessible for work groups at CSSB and CSSB partners. Access can also be granted to academic researchers and research groups from outside of CSSB, if there is sufficient equipment capacity. Access is also possible for non-academic researchers or research groups, but usage conditions including fees have to be discussed and agreed on beforehand.

The initial registration and booking of devices is organized via an online booking system (<https://ppms.eu/cssb-hamburg/login/?pf=2>). Physical access to the facility requires a CSSB transponder. External users will receive a CSSB guest transponder dependent on availability. The ALFM is accessible 24 hours/7 days for trained and experienced users (‘Tier 2 users’). For other registered users (‘Tier 1 users’), accessibility is restricted to Mo - Fri from 9:00 – 17:00, when ALFM staff is usually available for support. Please refer to paragraph 5 for a definition of the tier system at the ALFM facility.

2. Equipment and techniques

The available devices are described on the website of the ALFM facility (https://www.cssb-hamburg.de/facilities/alfm/index_eng.html).

3. User support

The ALFM facility head and ALFM staff will advise users in their specific technical, experimental or analysis questions before and during the application period. Upon request, support will be also available during ongoing projects. The head of the ALFM facility can be directly contacted via email (roland.thuenauer@cssb-hamburg.de) or via phone (040 8998 – 87730).

4. Steps required to use the ALFM facility

Step 1: Online registration

The initial registration for the ALFM facility should be done online (direct link to user registration: <https://ppms.eu/cssb-hamburg/areq/?pf=2>). Users have to fill a form containing the following items:

- General personal information about the user
- General information about the research group and the research institution the user belongs to

These data will be processed via the ALFM online booking system.

After filling and submitting this form, the ALFM staff will contact the researcher to arrange further steps.

Step 2: Initial meeting

The first step will be an initial meeting with the ALFM head or its representative. The purpose of this meeting, which can involve the PI or the project leader of the respective project, is to determine the best way in which the ALFM facility can support the researcher and if there are prerequisites that have to be clarified beforehand, such as biological or chemical safety considerations.

Step 3: Basic training and clarification of administrative requirements

Before access to the facility can be granted, the following mandatory steps are required:

- Registration form: Correctly and completely filled registration form.
- S2 biosafety training certificate: Training in S2 biosafety is required since all microscopes of the ALFM facility are in an S2 area. The training needs to be verified by signature.
- The document stating the usage rules of the ALFM facility (this document) needs to be signed by the user and the responsible PI or project leader.

After that, the transponder of the applicant will be unlocked for the ALFM facility. External users will receive a CSSB guest transponder dependent on availability.

Only for S2 Projects:

In case a project falls under biological safety level S2, the researcher/research group has to apply for an extension of the S2 registration for the S2 organism(s) involved. Work at the ALFM facility can only start after official permission is granted and the S2 work is discussed with the responsible BBS (Christoph Hagen) and the ALFM facility head (Roland Thüner). A separate document detailing S2 standard operating procedures (SOPs) is available.

Step 4: Training at microscopes

Before using a specific microscope, the researcher needs to get trained directly at the setup ('microscope usage training').

Normally, the researcher will start as tier 1 user and is able to use the microscope according to the rules for tier 1 users (see the next paragraph for a definition of the tier system at the ALFM facility).

5. Tier system

The ALFM facility has several advanced setups that require intense training and experience to be operated. Therefore, we implemented a tier system that ensures sufficient training of all users.

Tier 1:

Tier 1 user refers to users who have had basic trainings (biosafety) and microscope usage training(s) at specific microscope(s). In case further support or supervision is required, it can be always requested via the online booking system or via contacting the ALFM facility head

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(roland.thuenauer@cssb-hamburg.de, 040 8998 – 87730). At advanced setups, tier 1 users are allowed to book and work independently only Mo.-Fr. From 9:00 – 17:00. A few special devices are accessible for tier 1 users only under supervision from ALFM staff. Please see table 1 for a detailed list which tier access rules apply to which instruments.

Tier 2:

Tier 2 users are independent users who have demonstrated sound knowledge of the procedures and a level of expertise through working responsibly at the microscopes. After they have been assessed for their proficiency, they will be able to book and work independently at the microscopes 24/7.

microscope	tier access rules
Leica SP8 confocal	Tier 1: independent use only Mo.-Fr., 9:00-17:00
FALCON FLIM unit for Leica SP8	Tier 1: independent use only Mo.-Fr., 9:00-17:00
Leica cryoCLEM widefield	Tier 1: only under supervision
Leica cryoCLEM confocal	Tier 1: only under supervision
Leica DMI8 inverse live cell wide field #1	-
Primo Alveole micropatterning unit for Leica DMI8 inverse live cell wide field #1	Tier 1: independent use only Mo.-Fr., 9:00-17:00
Leica DMI8 inverse live cell wide field #2	-
Leica DM6 B upright wide field #1	-
Leica DM6 B upright wide field #2	-
Abbelight SAFe super-resolution	Tier 1: independent use only Mo.-Fr., 9:00-17:00
NikonTi2 spinning disc with FRAP unit	Tier 1: independent use only Mo.-Fr., 9:00-17:00
Nikon SIM microscope	Tier 1: independent use only Mo.-Fr., 9:00-17:00
Nikon Ti2 spinning disc with SoRa unit	Tier 1: independent use only Mo.-Fr., 9:00-17:00
Zeiss LSM880 Airyscan	Tier 1: independent use only Mo.-Fr., 9:00-17:00
Zeiss Elyra P1	Tier 1: independent use only Mo.-Fr., 9:00-17:00
Zeiss Lattice Light Sheet	Tier 1: independent use only Mo.-Fr., 9:00-17:00
Analysis computer and HIVE data storage and analysis server	-

Table 1: Tier access rules

6. Booking system

The booking of the devices and services (training, data analysis,...) available at the ALFM facility is organized via an online booking system (<https://ppms.eu/cssb-hamburg/login/?pf=2>). In dependence of the availability of the systems and of her/his tier status, the user will be able to book devices and services.

Using a specific device always requires a login at the respective computer. Logging in is only allowed if a valid booking was done beforehand.

The following rules apply for booking and logging:

- For each booking, a project needs to be specified in the PPMS booking system. New projects can be generated by clicking on 'Make a new request: request/start a new project' in PPMS. The project will become usable after verification by ALFM staff.
- Booking is possible not earlier than 3 weeks before.
- Per week, maximally two half days or a whole day may be booked by a user and/or individual project at one microscope during normal working hours (Mo.-Fr., 9:00 - 17:00). In case longer bookings are required for specific experiments, please contact the ALFM facility head before (roland.thuenauer@cssb-hamburg.de).
- Cancellation of a booking is regularly possible only until 24 hours before the start time. For later cancellations the user can take advantage of the possibility to offer the booked time to other users via the online booking system. Only when a replacement is found, the cancellation is free of charge.
- When devices are not used within 1 hour of the beginning of a reservation, others can use them. However, the original booking will be charged.
- If a booking immediately follows, the user has to finish the measurement in time. In case no booking follows immediately, the session can be extended until the beginning of the next booking.
- Since frequent switching on and off of lasers and light sources significantly shortens their lifetime, it is not possible to book microscopes shorter than 1 hour.
- If the brake until the next booking is longer than 2 hours, the user has to switch off the system including the laser(s) and light sources. Otherwise, the complete system must not be switched off.

If deviations from these rules are required to carry out specific experiments, please contact the ALFM facility head (roland.thuenauer@cssb-hamburg.de).

For necessary deviations from this regulation due to experimental needs and whenever other booking problems occur, the ALFM facility head or their representatives decide on the assignment and/or the access to the workplaces. The ALFM facility reserves the right to shift or cancel individual appointments due to technical or organizational reasons after consultation with the affected user(s). These cases do not warrant compensation claims to the ALFM facility.

7. Data storage

On each microscope computer a designed drive ('data storage drive') exists. Please use only this drive for saving data that is generated during a session.

Please remove the data from the data storage drives within one week, or immediately upon request of ALFM facility staff, to ensure that the drives have enough free memory. Older data will be regularly deleted.

ALFM users can use the HIVE data storage and analysis server of the ALFM facility. Please contact ALFM staff or the facility head to get an access and an account for the HIVE data storage and analysis server. The HIVE data storage and analysis server is meant to be used for intermediate data storage while data analysis and data processing is carried out. All users must remove their data from ALFM systems afterwards and are responsible themselves for long-term storage.

Without explicit allowance of the ALFM facility head, it is not allowed to use external storage devices (USB sticks, external discs,...) for data transfer. It is not allowed to copy any data that was not generated with ALFM microscopes or the ALFM analysis computer to the microscope computers.

8. Usage fees

Hourly fees for usage of the instruments and software usage are charged; the minimal unit is 1 h. The fees for academic users from CSSB and from CSSB partners are listed in table 2. Different usage fees apply for users from external academic institutions and for users from industry; they are available upon request.

Since some experiments, especially live-cell imaging experiments, require long imaging periods, the following rebates apply for long continuous bookings or usage times:

- Bookings/usages times longer than 12 h: 50% reduction after the 12th hour
- Bookings/usages times longer than 24 h: 100% reduction after the 24th hour

However, please be reminded that such long bookings need to be agreed with the core facility head beforehand.

Instrument/Software	Usage fee per hour in €
Leica SP8 confocal with FALCON FLIM unit	15
Leica cryoCLEM widefield	15
Leica cryoCLEM Stellaris 8 confocal	15
Leica DMI8 inverse live cell wide field #1 with Primo Alveole	15
Leica DMI8 inverse live cell wide field #2 with Thunder	15
Leica DM6 B upright wide field #1	10
Leica DM6 B upright wide field #2	10
Abbelight SAFe 360 super-resolution microscope	15
Nikon Ti2 spinning disc with FRAP unit	15
Nikon SIM microscope	15
Nikon Ti2 spinning disc with SoRa unit	15
Zeiss LSM880 Airyscan	15
Zeiss Elyra P1	15
Zeiss Lattice Light Sheet	15
Laminar flow hood	2
Analysis computer	2
AIVA via HIVE	2
ARIVIS via HIVE	2
Imaris via HIVE	2
NIS Elements via HIVE	2

Table 2: Hourly usage fees for academic users from CSSB and from CSSB partners

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The billed usage time is calculated from the booked time plus eventual overtimes when the instrument was used longer than booked. The cancellation policies described in paragraph 6 of this document apply.

Invoices for fees must be paid within 14 days.

All collected data are analyzed statistically.

Support with grant applications:

The fees for usage hours in the ALFM can be financed via DFG (research grants, scientific networks, Collaborative Research Centers (SFB), Emmy Noether or Heisenberg), BMBF or EU grant applications. More information you can find on the DFG web page at:

http://www.dfg.de/formulare/55_04/55_04_de.pdf or [55_04_en.pdf](http://www.dfg.de/formulare/55_04/55_04_en.pdf)

http://www.dfg.de/formulare/52_01/52_01_de.pdf or [52_01_en.pdf](http://www.dfg.de/formulare/52_01/52_01_en.pdf)

The usage rules of the ALFM are in accordance with DFG specifications for “Gerätezentren”. A most recent copy of this document should be sent together with your application. The ALFM will support all users in writing the specific imaging part of their grant application to achieve a high success rate.

9. User responsibilities

The users are obligated to operate the devices on the basis of the rules defined in this document and on the basis of the criteria communicated during their trainings.

Each user has the following responsibilities:

- The ALFM facility staff should always be contacted in case of any doubt concerning an operating step. In case a malfunction or untypical behavior of the system is observed, please immediately inform ALFM facility staff.
- To avoid harm to other users and equipment the ALFM facility staff has to be immediately notified when uncontrolled release of experimental solutions at the workplace or other chemical or biological contaminations occurs.
- During and after using the equipment, the instruments, and the workspace must be kept and left clean: clean objectives only with lens cleaning tissue, rinse containers for experimental solutions, no glass leftovers, put immersion medium dispensers in the designated trays.
- When the next booking is within 2 hours of the end of a session, the user has to quit the microscope software and log out, but the microscope itself including lasers and other light sources have to be left switched on. If the break is longer than 2 hours, the whole system has to be switched off.
- Work of biological safety level S1 and S2 is only permitted if the ALFM facility is informed about it.
- Work at the biological safety level S2 can only be started after an official permission is granted and the ALFM facility receives the permission. All rules stated in the separate S2 SOP document for the ALFM facility have to be respected.
- It is not allowed to book for other users or to give the login account data or transponders to others.
- The users have to inform the ALFM facility about end of the employment, change of the workgroup, or end of the scientific project at the ALFM facility. The data and user accounts of users that do not require access to the ALFM facility any more will be deleted within 2 months.
- Microscope computers should be used only for data recording. Data analysis should be carried out at different computers, such as the analysis computer of the ALFM facility.
- Microscopes and computers have to be operated without gloves.
- Only ALFM staff is allowed to carry out changes to the configurations of the microscopes, the incubation systems, and the computers, including software installations and software updates.

10. Acknowledgment of the ALFM facility in publications

The ALFM facility has to be acknowledged in each publication containing data generated or analyzed at the ALFM facility. Co-authorship on publications or patents is warranted when the ALFM contributed substantial scientific input. This should be discussed in advance between the ALFM facility head and the corresponding author of the publication. For any manuscript including data generated or analyzed at the ALFM facility, the ALFM facility staff will proof read the microscopy methods part before submission to ensure appropriate description of the methods and equipment.

Please send PDF files of all publications with ALFM involvement to the ALFM facility head (roland.thuenauer@cssb-hamburg.de) after acceptance. Publications can also be reported via the online booking system. This proof of excellence is extremely important for the ALFM facility, as these publications will be part of the evaluation process of the ALFM.

Please use this text as sample for acknowledgement:

We thank the Advanced Light and Fluorescence Microscopy (ALFM) facility at the Centre for Structural Systems Biology (CSSB), in particular XXX, for support with light microscopy and data analysis.

11. Signatures

User name

Signature

Date

Name of project leader/PI

Signature

Date

Roland Thünauer

ALFM facility head

Signature

Date