Lighthouse Core Facility

Zentrum für Translationale Zellforschung (ZTZ)
Breisacher Straße 115
79106 Freiburg

Bio-Imaging Light Microscopy Core Facility (BiMiC)

Institute for Disease Modeling and Targeted Medicine (IMITATE)
Breisacher Straße 113
79106 Freiburg

User Guidelines

The Lighthouse Core Facility located in the Zentrum für Translationale Zellforschung (ZTZ) and the Light Microscopy Core Facility of the IMITATE (IMT) are jointly-operated, shared resource laboratories of the Medical Center - University of Freiburg (Universitätsklinikum Freiburg). The ZTZ houses the research laboratories of the Department of Medicine I (Hematology, Oncology, and Stem-Cell Transplantation) (Med. I), and the Center for Chronic Immunodeficiency (CCI), and laboratories from the DKTK/Comprehensive Cancer Center Freiburg (CCCF). The above institutes, along with the Medical Faculty of the University of Freiburg, are the main sponsors of the facility. However, facility resources and support are accessible to all research laboratories of the Medical Center, as well as from the University of Freiburg. The primary services of the facility include: Flow Cytometry and Cell Sorting, Confocal and Multiphoton Microscopy, HCS/Automated Microscopy/Image Cytometry, Widefield/Fluorescence Microscopy, Quantitative PCR, and Digital PCR.

Contact Persons:

Dr. Marie Follo, Head of Facility	Telephone: 0761 270 77697 e-mail: marie.follo@uniklinik-freiburg.de
Jan Bodinek-Wersing	Telephone: 0761 270 77680 e-mail: jan.bodinek@uniklinik-freiburg.de
Dipl. Biol. Dieter Herchenbach	Telephone: 0761 270 63770 e-mail: dieter.herchenbach@uniklinik-freiburg.de
Urmila Gopakumar	Telephone: 0761 270 77680 e-mail: urmila.gopakumar@uniklinik-freiburg.de
Elitsa Bodurova	Telephone: 0761 270 77697 e-mail: elitsa.bodurova-spassova@uniklinik-freiburg.de
Dr. Michael Selle	Telephone: 0761 270 63770 e-mail: michael.selle@uniklinik-freiburg.de
PD Dr. Franck Ditengou – BiMiC (Light Microscopy IMITATE)	Telephone: 0761 270 63213 e-mail: franck.ditengou@uniklinik-freiburg.de

Facility Website:

www.core-facility.de/

https://www.uniklinik-freiburg.de/medizin1/forschung/lighthouse-core-facility.html

Lighthouse Core Facility - Equipment Overview

Flow Cytometry and Cell Sorting

High Speed Cell Sorters (ZTZ):

- Beckman Coulter MoFlo Astrios, 14 color (Lasers: 405 nm, 488 nm, 561 nm, 640 nm)
- Becton Dickinson FACSAria III, 17 color (Lasers: 375/405 nm, 488 nm, 561 nm, 633 nm)
- Becton Dickinson FACSAria Fusion, 16 color (Lasers: 405 nm, 488 nm, 561 nm, 640 nm)
- Beckman Coulter Cytoflex SRT, 15 color (Lasers: 405 nm, 488 nm, 561 nm, 640 nm)

FACS Analyzers (ZTZ):

- BD LSR Fortessa (5 machines), 14-17 colors, multiple laser wavelengths available, depending on system used (355 nm, 405nm, 488 nm, 561 nm, 640 nm), plate loaders on two systems
- Sony ID7000C 5-laser, full spectral analyzer, spectral unmixing, 40+ colors, advanced plate loader (96- and 384-well) and multitube rack (355 nm, 405 nm, 488 nm, 561 nm, 638 nm)
- Sony SP6800 ZE Spectral Analyzer, 32 channel spectral detector, spectral unmixing, 18 colors, (488 nm and colinear 405 nm/638 nm)
- Beckman Coulter CytoFlex LX 4-laser, 14-color (UV, 405 nm, 488 nm, 640 nm)

Microscopy and Imaging

Confocal and Lightsheet Microscopes:

- Zeiss LSM 980 AiryScan 2-Photon Confocal Microscope: AiryScan 2, Quasar spectral detector, photoablation, photoactivation, integrated climate chamber, 4 lasers (405 nm, 488, 561 nm, 633 nm), multiphoton laser (tunable 700-1300 nm; stable 1400 nm) - IMITATE
- Zeiss Lightsheet 7.1 microscope: For imaging, entire living model organisms, or fixed and cleared tissues. Integrated climate chamber, 4 lasers (405 nm, 488, 561 nm, 633 nm) - IMITATE
- Zeiss LSM 880 AiryScan Confocal Microscope: AiryScanFast detector, Quasar spectral detector, integrated climate chamber, 4 lasers (405 nm, 458 nm, 488 nm, 514 nm, 561 nm, 633 nm) - ZTZ
- Zeiss Celldiscover 7 (CD7): automated system with advanced widefield *and* confocal modes, LSM 900 scanhead with AiryScan 2. Fully integrated climate chamber, 4 lasers (405 nm, 488, 561 nm, 633 nm) -IMITATE
- Zeiss LSM 710 Confocal Microscope: Quasar spectral detector, integrated climate chamber, 4 lasers (405 nm, 458 nm, 488 nm, 514 nm, 561 nm, 633 nm). Camera for widefield imaging – ZTZ

High Content and Automated Microscopes:

- Olympus scanR High Content Screening Station: inverted widefield microscope, HCS/image cytometry, quantitative imaging, integrated climate chamber, multiple filters available, spectral range from DAPI to Cy7, advanced analysis possibilities, kinetic analysis, AI/deep learning - ZTZ
- Akoya Fusion Phenocycler/CODEX – Brightfield and fluorescence imaging, highly multiplexed imaging. Up to four slides as standard scanner, single slides for high parameter analysis. Phenocycler uses barcoded antibodies for imaging 40+ markers. Samples are on standard slides. For tissue sections, tissue microarrays, or fixed samples on slides - ZTZ
- Zeiss Axioscan: Brightfield and fluorescence slide scanner. X-Cite LED for illumination, standard fluorescence and additional OPAL dye filter set - IMITATE
- Sartorius IncuCyte® S3: widefield system located within a cell culture incubator (37°, 5% CO2), long term live cell imaging, independent imaging and analysis of up to 6 plates simultaneously, 2 color fluorescence (GFP, mCherry) and brightfield - ZTZ

Standard Widefield Systems:

- Zeiss Axio Observer 1: inverted widefield microscope for transmitted light and fluorescence, Apotome, small, on-stage climate chamber with CO2 - ZTZ
- Zeiss Axio Observer 2: inverted widefield microscope for transmitted light and fluorescence, Apotome, small heated stage (without CO2) - ZTZ
- Zeiss Axio Imager: upright widefield microscope for IHC, transmitted light and fluorescence - ZTZ
- Zeiss SteREO Discovery.v8: stereo microscope, transmitted light, fluorescence (GFP/RFP) - ZTZ

Quantitative PCR (QPCR) and Digital PCR (dPCR) (ZTZ)

- Roche LightCycler® 480 (2 machines), Real-Time PCR Cycler, 384-well and 96-well blocks
- Stilla Naica Crystal Digital™ PCR System (Naica Crystal Geode (x2), Naica Crystal Reader), up to 6 colors (FAM, HEX, Atto 550, ROX, Cy5, Atto 700).

Additional Systems (ZTZ)

- Formulatrix Mantis – liquid dispenser/miniature pipetting robot, low dead volume
- OLS Curiox Laminar Flow HT2000 washing system – 96-well plate format

Analysis Workstations

There are several dedicated analysis workstations (ZTZ and IMITATE) equipped with offline versions of all manufacturer/machine specific softwares, including the following programs:

Flow Cytometry:

- FlowJo X (2 licenses)
- BC Kaluza
- Sony Amateras (spectral analyzer)
- BD FCAP Array (Bead array analysis)
- Verity Software ModFit LT
- CytoBank (Cloud Software)
- Fluorofinder Plus (Online License)

Microscopy:

- Akoya InForm and PhenoChart software
- Olympus HCS scanR Analysis, incl. AI/deep learning, kinetics modules
- Zeiss Zen Blue / Zen Black offline (multiple licenses)
- Bitplane Imaris – 3D reconstruction and analysis
- QuPath, CYTOMap
- Media Cybernetics ImagePro Plus, incl. AutoQuant Deconvolution
- CellProfiler / CellProfiler Analyst
- ImageJ / Fiji

Nucleic Acid Analysis:

- Premier Biosoft BeaconDesigner 8.1
- Roche LightCycler 480 software offline
- Stilla Crystal Reader/Crystal Miner
- Qiagen QIAcuity digital PCR system

Data Storage

All systems allow data to be saved either directly to the Lighthouse Z:pool data transfer system (max. storage time 28 days). All facility analysis computers are also connected to the Z:pool. USB sticks or portable hard drives are not allowed to be connected to any of the facility systems. For external users who cannot access the Z:pool, please contact one of the staff members for alternative ways to move your data.

If needed, Lighthouse users can also access space on the Lighthouse CF IMT Data Server for ongoing and medium-term data analysis (i.e. for “hot” or “warm” data). Due to its large volume, HCS raw data should be stored directly to the CF IMT Data Server via the direct connection.

Due to space limitations and high costs, long-term storage and archival of data from Lighthouse systems is the responsibility of the individual researchers and group leaders. Contact us for information about alternative options.

User Fees

For current Lighthouse / BiMiC Core Facility user fees please see Appendix A.

Facility Access

Access to services and equipment of the facility can be granted only after a Lighthouse/IMITATE User Access Form has been signed and returned, stating which services are required and acknowledging that these user guidelines have been read and will be abided by.

Biosafety

Note: For experiments of Biosafety Levels 1 and 2 carried out within the lab space of the Lighthouse Core Facility, the respective group leader must themselves be listed as the responsible project leader within the respective rooms of the ZTZ and/or IMITATE buildings. The Lighthouse Facility will no longer be listed as the responsible party.

In the case of cell sorting, a Lighthouse/BiMiC Biosafety Form must be submitted before the start of every new scientific project.

Furthermore, a Lighthouse/BiMiC Biosafety Form must also be filled out for live cell imaging (LCI) experiments involving the use of samples classified as Level 2 through the Gentechnikgesetz or the Infektionsschutzgesetz.

It is the responsibility of the user and group leader to submit a new form when the project changes (i.e. addition of a new retroviral vector, etc.). This is necessary in order to determine the potential biohazard risks involved in the case of aerosol formation, and to protect the safety of the sort operators, staff and users of the facility.

Reservations

Appointments for all of the machines are available on a "first-come, first-served" basis. In the case of overbooking, preference may be given to groups belonging to the institutes of the ZTZ (Med. I, CCI, CCCF), and of the IMITATE, at the discretion of the head of the facilities.

With the exception of the cell sorters, users are allowed to reserve and use the machines, once they have been trained to use them. Training sessions for all machines occur regularly, and spots can be reserved by contacting the facility by e-mail or telephone.

Once a user has gained sufficient proficiency and is able to work independently on a machine, there is also the possibility to use the machines outside of normal operating hours. Trained users can gain access to the required area of the Lighthouse/IMATE Core Facilities through a special transponder.

User access to the Lighthouse/IMITATE Core Facilities service may be denied or rescinded:

- if the user does not fulfill his or her responsibilities regarding proper use of the equipment or the facility.
- if the capacity of the equipment or its operators is insufficient to fulfill the requested usage.
- if the operators, users or machines could be harmed through carrying out the planned experiment.

Online Calendar/Booking System

Our online calendar is available at https://ztz-buchung.uniklinik-freiburg.de and http://booking.core-facility.de as well. Calendar registration is linked to the Uniklinik LDAP system, but it is also possible for external users to access the system. With the exception of the high-speed cell sorters, each of the machines can be reserved via the online calendar, once a user has received proper training. Usage of the cell sorters requires the availability of an operator. Users of the cell sorting service may request cell sorting appointments via the online calendar. The appointments are then subject to approval by a sort operator. Appointments for the cell sorters can also be requested by directly contacting the facility via telephone (0761 270 77680) or e-mail (*facslab@uniklinik-freiburg.de*).

"No show" Policy

When possible, users should cancel their appointments at least 24 hours in advance of the scheduled start. "No shows" which have not cancelled their appointment or failed to have notified the Lighthouse Core Facility in some other way may be billed for their appointment as originally scheduled, at the discretion of the facility.

Acknowledgements

Our user fees cover only a small fraction of our total running costs. In fact, most of the running costs are covered by our home institutes (Department of Medicine I, Center for Chronic Immunodeficiency, DKTK/Comprehensive Cancer Center Freiburg) and the Medical Faculty,- University of Freiburg, while the majority of our machines were financed with the assistance of public funding sources such as the DFG, Land Baden-Württemberg, or BMBF. We therefore depend on proper acknowledgements in publications and grants and proper listing of the project numbers below.

Specific Project Numbers

Our general support project number from the Medical Faculty (2023/B3-Fol) should *always* be listed, along with the specific instrument or service area project number(s) as listed below:

Service Area	**Project Number**
For *all* core facility services	Medical Faculty, University of Freiburg – **Project Number 2023/B3-Fol**
Akoya Fusion Phenocycler	Medical Faculty, University of Freiburg – **Project Number 2021/A2-Fol**

Cell Sorting (BC CytoFlex)	**DFG Project Number 450392965**
Zeiss LSM 980 MP (IMITATE)	**DFG Project Number 452929960**
Zeiss CellDiscoverer7 w/LSM900 (IMITATE)	**DFG Project Number 4529935043**
Zeiss AxioScan 7 Slidescanner (IMITATE)	**DFG Project Number 452933375**
Zeiss Lightsheet 8 (IMITATE)	**DFG Project Number 452932349**

Publications, posters or presentations that make use of Lighthouse/IMITATE services or equipment, or of data collected in the facility or by its staff should always list these project numbers in the respective acknowledgement/funding sections such as in the example below:

Lighthouse Core Facility is funded in part by the Medical Faculty, University of Freiburg (Project Numbers 2023/A2-Fol; 2021/B3-Fol) and the DFG (Project Number 450392965).

If you wish to in addition explicitly thank a member of the staff, just name the particular person(s) involved, here listed alphabetically: J. Bodinek-Wersing, E. Bodurova, FA Ditengou, M. Follo, D. Herchenbach, M. Selle, U. Jagadeshwaran.

Thank you.

Appendix A

Lighthouse Core Facility / BiMiC (IMT) User Fees

Fees for the use of Lighthouse services/equipment for *academic users* are described below. Non-academic will be charged VAT (MwSt.), in addition to the non-academic user fees. For information about fees for non-academic users, please contact us directly. All prices listed are for groups of the Medical Center – University of Freiburg and groups belonging to the Medical Faculty of the University of Freiburg.

Cell Sorting

- Standard fee 50 € / hour + 25 € setup fee

FACS Analysis (Minimum booking time is 30 min)

- 3 laser FACS analyzer 15 € / hour unassisted
- 4 laser FACS analyzer 20 € / hour unassisted
- 5 laser FACS analyzer 25 € / hour unassisted

Multiphoton, Confocal, Lightsheet Microscopes

- Zeiss LSM 980 NLO AiryScan 30 € / hour unassisted
- Zeiss Lightsheet 7.1 30 € / hour unassisted
- CellDiscoverer 7 (CD7) LSM 900 25 € / hour unassisted
- Zeiss LSM 880 AiryScanFast 25 € / hour unassisted
- Zeiss LSM 710 20 € / hour unassisted

High Content Screening, Slide Scanning, High Parameter Multiplexing

- Olympus ScanR HCS 12 € / hour unassisted
- Zeiss AxioScan 7 Slide Scanner 12 € / hour unassisted
- Akoya Fusion PhenoImager (OPAL) 10 € / hour unassisted
- Akoya Fusion PhenoCycler (CODEX) 20 € / hour unassisted

Note: With the exception of shared reagents required to operate the fluidics system (DMSO, Phenocycler buffer, etc.) consumables/reagents are *not* included in the above fees.

Widefield, long-term live cell imaging

- Zeiss Axio Imager 7 € / hour unassisted
- Zeiss Axio Observers I and II 7 € / hour unassisted
- Sartorius IncuCycte® 1 € / plate / hour

Quantitative PCR

- Roche LC480 I and II 10 € / standard run (2 hrs)

Digital PCR

- Stilla Naica 5 € / Geode / Run, 10 € / 2 Geodes
- Qiagen QIAcuity 10 € / Run

Note: consumables/reagents for dPCR are *not* included in the above fees.

For machines billed on an hourly basis, fees will be rounded up to the nearest quarter-hour.

Billing invoices will be sent out quarterly to the respective group leaders.