BIH Cytometry Core

User Guidelines

General rules:

- All users of the BIH Cytometry Core must complete and sign one of the registration forms (see website).
- An initial project meeting is scheduled to discuss the user's needs in instrumentation, service and training (service request via iris.charite.de).
- The user account is user specific and password protected. It is against the Core Facility policies to make reservations for anyone other than yourself.
- Only the material that has been registered with the Core Facility can be handled in the lab. All new material has to be registered with and approved by the Core Facility beforehand (safety regulations).
- Users must wear labcoats and gloves at any time at the instruments. Do NOT wear gloves when using the phone and when touching the door handles.
- Keep the bench clean and ordered. Empty the small waste bags into the big one and do not leave anything on the bench or in the fridge after your experiment (e.g. tubes, caps, tips, paper, etc.).
- If you want to bring students or guests please contact the Core Facility staff in advance. There might be restrictions on the number of people you can bring due to space limitations.
- Follow appropriate procedures regarding handling of biohazardous materials and clean up any spills.
- Analysis of L2/S2 samples is restricted to the MACSQuant "Erato" in room 0.0083 (S2 lab). All projects must be registered with the appropriate authorities.

- The safety of data on the computers at the instruments or on the server "P-CU3-Nutzer" will not be guaranteed by the Core Facility. It is the responsibility of all users to save their data by transfering them to their own storage media via their account on the server.
- Do not use the computers at the instruments for any other purpose than Flow Cytometry acquisition and analysis. Any user that violates this rule will have to pay for the cost of repair and the downtime of the respective instrument.

- Any formal presentations or publications resulting from work performed at or from service provided by the Flow & Mass Cytometry Core Facility has to be acknowledged. The following statement is suggested: "**We would like to acknowledge the assistance of the BIH Cytometry Core**" In case of extended advise on experimental design or data analysis co-authorship is suggested.

Failure to follow the BIH Cytometry Core's User Guidelines will result in one warning and then in deactivation of the user's account.

Access can be restored after an additional project meeting (charges apply), additional training might be required. Persistent offenders (more than three violations in a calendar year) will be required to undergo training and additional requirements might apply (e.g. assisted instrument usage) as determined by the Core Facility staff.

For questions contact the head of the BIH Cytometry Core - phone 450 639446 - room 1.0070

Flow Cytometry - Analysers

- All new users of the analysers, regardless of previous flow cytometry experience, must take training on the proper use of the instruments and and of our Quality Assurance (QA) protocols.
- A basic training is provided by the Core Facility and is obligatory before instruments can be used unassisted. The basic training course consists of 4 sessions (hands-on, one-by-one training).
- Depending on the progress of the training, additional training sessions might be required before instruments can be used unassisted.
- Usage of LSRII is reserved for experiments that require > 8 fluorescence parameters or usage of the 561nm laser.

- To assure constant high level quality of the instruments, all users have to follow the QA protocol each time when using the instruments (see detailed protocol at the analysers). Please take into account the additional time this will take when booking the instruments (~ 15-30min).
- Filtration of all samples is mandatory for use of the LSRII (35µm filter, each sample directly before measurement). On the other instruments filtration is necessary if cells do not stay in single cell suspension during the measurement.

Data management:

LSRII and CantoII:

- Do not leave any data ("experiments") in the Diva database. Remaining data in the database will lead to slow down of the computer and eventually to a crash and will therefore be deleted without further notice (service charges may apply).
- Data may be housed on the computers in the user's folder to a certain amount (CantoII: 4GB/user; LSRII: 10GB/user). For transfer of data the account on the server system "P-CU3-Nutzer" has to be used. No USB-keys or external hard drives allowed!

MACSQuant Erato and Euterpe

- Do not leave any .mqd or .fcs-files on the computers. For transfer of data the account on the server system "P-CU3-Nutzer" has to be used. Any remaining data will be deleted without further notice (service charges may apply).

Access to the analysers:

- Analysers can be booked up to 14 days ahead with the online scheduler OpenIris (iris.charite.de).
- It is against Core Facility policies to make reservations for anyone other than yourself.
- For use of the instruments a booking in OpenIris, covering the complete time the instrument is used, is mandatory.
- The last user of the day is fully responsible for shutting down the instrument. Users must be aware that even if their reservation is not the last slot of the day, it may become so if subsequent slots of other users are deleted meanwhile. Therefore, every user must check the reservation calendar before leaving, to ensure he/she has not become the last user of the day.

Flow Cytometry - Cell Sorting

General Information:

- Cell sorting is offered as a service, the AriaII cell sorter is exclusively run by Core Facility staff.
- First time users must request a project meeting at least 5 days ahead of the desired date for cell sorting (use OpenIris - iris.charite.de - to request an initial project meeting). We recommend to discuss all new sort protocols in advance, this can shorten the time for setup of the new experiment at the time of sorting.

Sample preparation for Cell Sorting:

- Bring appropriate controls for each staining panel (unlabeled cells, fully stained sample and single stainings of the cells or compensation beads - include unstained compensation beads in this case). Contact us for help with the choise of appropriate controls.
- We recommend to use EDTA, DNase or other reagents to prevent cell aggregation of adherent or otherwise sticky cells.
- Filtration of cells directly before cell sorting in our lab is mandatory. Sterile 35µm filters and tubes will be provided.
- Cell concentration should be adjusted to the nozzle size used for sorting (see table) to achieve optimal efficiency. However, the volume should not be <300µl.
- Bring sufficient amout of clearly labeled collection tubes. We recommend to coat the tubes from the inside with buffer containing protein (FCS, BSA) and to sort into tubes already containing buffer/medium (appr. 1/10 of the tube volume). Cells can be sorted into 5ml tubes and 1.5ml tubes (4-way sorting), 15ml (2-way sorting) or into 96-well/384-well plates (1-way sorting). Single Cell Sorting (also as Index Sorting) is available. Contact us for other options.

Quality control (QC) and data management:

- A QC (8-peak beads) is done with each experiment and saved with the data.
- Sort checks of the sorted fractions will be performed at the end of the sort.
- Sort reports and .fcs-files of sample and sort checks will be moved to your account on the server system "P-CU3-Nutzer". Please secure the data on your own storage device afterwards. Experiments including QC will be saved on the data server of the Core Facility and can be provided upon request.

Booking of Cell Sorting:

- Cells sorting service can be booked up to 14 days ahead with the online scheduler OpenIris (iris.charite.de).
- We have extended hours for cell sorting on tuesday, wednesday and thursday (until 9pm). Refer to OpenIris for details on operating hours. Sorting outside these hours can be arranged upon request by way of exception.
- For the calculation of the time required to run your sample(s) you can use the table below (approximate values for good quality samples, bulk sort). The sorting of one 96well plate will take around 5min if the target population is > 5% of your total cells.
- If there is more than one sample to sort and/or a new setup with single stainings needed, extra time needs to be booked for that. Contact us for help with time management.
- An extra 30min per sorting slot has to be booked for sorter setup, Quality Assurance and data management.

nozzle size*	70 µm	85µm	100µm and 130µm
cell concentration, sample for sorting	$5x10^7$ cells /ml	$3x10^7$ cells/ml	please inquire
max. number of events per hour**	$8x10^7$	$4x10^7$	
max. cell number per collection tube			
15ml tube	$10x10^6$	$5x10^6$	
5ml FACS tube	$3x10^6$	$1.5x10^6$	
1,5 ml Eppi	$1x10^6$	$0.5x10^6$	

*will be decided upon during the initial sort discussion

**events include debris, doublets, etc

Mass Cytometry - CyTOF2^{Helios} 'Tangerine'

General Information:

- The CyTOF2^{Helios} is staff operated.
- Users must register every project by completing a registration form that can be downloaded from our website. It will ask for a project name and information relevant to the mass cytometry aspect of your experiment.
- First time users must request a project meeting (use OpenIris to request an initial project meeting) at least 3 months before the first samples are supposed to be analysed.

  We recommend to schedule this meeting before samples are collected and stored to make sure sample quality is optimal for mass cytometry.
- Schedule a project meeting for additional projects at least 6 weeks before the desired date for sample acquisition.

  Every project needs to be discussed from a technical point of view before samples can be handled. Technical feasibility of the project, expected sample income, timing of sample measurements and instrument availability will be discussed.
- The Core Facility offers an antibody conjugation service for the labeling of purified antibodies with metal isotopes. Please contact us for details.

Sample preparation for mass cytometry:

- Samples have to be provided completely stained and washed. Please bring the samples as cell pellets, keep samples cool, avoid any shaking.
- Cell preparation protocols and staining protocols have to discussed with and to be approved by the BIH Cytometry Core to achieve the best possible sample quality and avoid damage to the detector by contaminating metals.
- We will filter (20µm filter, if not discussed otherwise) and count all samples prior to the run.
- "Low quality" samples (e.g. cells not staying in single cell suspension, improperly fixed material, residues of sticky material, etc.) may cause clogging. In this case the user will be informed of the possible risk of a completely blocked nebulizer and/or sample capillary and can decide if the sample should be run anyway. The resulting costs will then be charged to the user's account.

Booking of CyTOF2^{Helios}:

- Please check the CyTOF2^{Helios} - "Tangerine" scheduler for operation hours and free slots.
- Long term longitudinal experiments should be discussed in advance to guarantee availablity of the service.
- Use OpenIris to request 'mass cytometry - sample acquisition' (Services). Please place your order at least 5 working days in advance.
- There is no limit in advance booking. Slots booked more than 4 weeks in advance may be charged at 50% if canceled at short notice (less than two weeks before) and preventing other users from using the service.
- Indicate the date you want the samples to be run and need to upload a "sample template" (details on file names, metals, cell numbers to be acquired and number of samples). Please also indicate the earliest time you will be able to bring the samples at the requested date.
- Time for sample acquisition will be calculated based on your project details and sample template provided.
- You will get a status update on your order indicating date and starting time of the measurement if your date of choice is available.
- In case the date you requested for your samples is not available (e.g. because the time that is needed for these samples is not available at that date) the next possible date for the request will be suggested.

- Do not begin the experiment until a time for measurement is officially scheduled and confirmed.

Quality control (QC) and data management:

- The CyTOF2^{Helios} will be tuned (calibrated) and EQ calibration beads will be measured for QC for each project per day. Background contamination of the instrument will be documented before and after the measurement of samples.
- EQ calibration beads will be added to the sample for normalization to adjust for time dependent decrease of signal and day-to-day variations of the instrument.
- The use of 'anchor' samples with each run is highly recommended to control for batch effects (day to day variations in sample preparation and staining procedure).
- We will copy the normalized data (.fcs-files) to your account on the server "P-CU3-Nutzer". Please move them to your own storage medium.
- All data (.imd, .FCS, .fcs) will be moved to a hard drive. Be aware that mass cytometry raw data (.imd-files) are very large data files. You will need to provide the Core Facility with sufficient data storage capacity (hard drive or server space) for these data.

Imaging Mass Cytometry - IMC^{Hyperion}

General Information:

- The IMC can be used as service or independently. Instrument assembly and tuning will be performed by the Core Facility.
- Users must register every project by completing a registration form that can be downloaded from our website. It will ask for a project name and information relevant to the imaging mass cytometry aspect of your experiment.
- First time users must request a project meeting (use OpenIris to request an initial project meeting) at least 3 months before the first samples are supposed to be analysed.

  We recommend to schedule this meeting before samples are collected and stored to make sure tissue quality is optimal for imaging mass cytometry.
- Schedule a project meeting for additional projects at least 6 weeks before the desired date for sample acquisition.

  Every project needs to be discussed from a technical point of view before samples can be handled. Technical feasibility of the project, expected sample income, timing of sample measurements and instrument availability will be discussed.
- The Core Facility offers an antibody conjugation service for the labeling of purified antibodies with metal isotopes. Please contact us for details.

Sample preparation for imaging mass cytometry:

- Samples have to be provided completely stained. Samples can be stored at a dry and dust free place until time of measurement.
- Tissue sample preparation protocols and staining protocols have to be discussed with and to be approved by the BIH Cytometry Core to achieve the best possible sample quality and avoid damage to the detector by contaminating metals.
- A feasibility test will be performed for each project (one representative tissue section for H/E staining, one for IMC: DNA and 2-3 core markers, ablation of a small region).
- The Charité Core Facility 'iPATH.Berlin' offers services around sample preparation for imaging mass cytometry. Contact: Anja Kühl | anja.kuehl@charite.de | https://histopathologie.charite.de

Booking of IMC^{Hyperion} :

- Use OpenIris to request 'imaging mass cytometry - sample acquisition' (Services). Please place your order at least a week in advance.
- There is no limit in advance booking. Cancelations may be charged at 50% if preventing other users from using the service.
- Indicate the earliest date you can bring the samples (at least a day before desired date of measurement, if run as service) or the date you want to measure the samples yourself.
- You will get a status update on your order indicating the date and time of the measurement.

Sample Measurement

- Sample Measurement consists of creating a panorama view of the tissue sections, the choice of the region(s) of interest and the ablation of the sample.
- The ablation process can be programmed and runs unsupervised. The instrument can be programmed to shut down afterwards.

Quality control (QC) and data management:

- The IMC will be tuned (calibrated) for each project per day.
- The use of 'anchor' samples on each slide is highly recommended to to control for batch effects (day to day variations in sample preparation and staining procedure) and day-to-day variations of the instrument.
- We will move all data to your account on the server "P-CU3-Nutzer". Please move them to your own storage medium.

Billing practices

General information:

- Time for instrument usage and service will be billed based on the time booked and time used.
- Rates for instrument usage and service are found on the BIH Cytometry Core website.
- Charges are booked quarterly. Information on the charges that apply are visible to the group leader and group administrators in OpenIris.
- Charges for BIH/Charité users will be booked by "interne Leistungsverrechnung (ILV)" on the accounts used at time of booking. External users will be billed by the Charité via "Rechnungslegung".

CyTOF2^{Helios}:

- Time used for instrument tuning, washing, data normalization and data transfer to storage medium will be charged in addition to the running time of the sample.
- Time for troubleshooting of the instrument during sample measurement (de-clogging, re-tuning, change of tubing, etc.) will be charged.
- There is an extra cost per sample, that covers consumable costs (cell strainers for filtering the cells, counting slides etc.).

IMC^{Hyperion}

- Time used for instrument tuning, laser setup and data transfer to storage medium will be charged in addition to the running time of the sample.
- Different charges apply for supervised time (service) and unassisted time (independent usage and programmed laser ablation).

Cancelation policies

LSRII, CantoII and MACSQuant Euterpe:

- Users must cancel their time no less than 24 hours in advance of their scheduled appointment to avoid being charged. If the reservation is inside the 24 hours lock, users can cancel through OpenIris but will be charged if no other user schedules that time. Users may move their slot on the same day without being charged.

MACSQuant Erato (S2 Lab):

- Users must cancel their time no less than 48 hours in advance of their scheduled appointment to avoid being charged. If the reservation is inside the 48 hours lock, users can cancel through OpenIris but will be charged if no other user schedules that time. Users may move their slot on the same day without being charged.

Cell sorting service:

- Users must cancel their time no less than 48 hours in advance of their scheduled appointment to avoid being charged for the time. If the reservation is inside the 48 hours lock, users can cancel through OpenIris but will be charged if no other user schedules at that time. Users may move their slot on the same day without being charged.
- Long term bookings (reservation made by staff) will be charged 100% of the booked time if canceled less than 14 days in advance and no other user schedules at that time.
- Cancelations of bookings outside regular operating hours (reservations made by staff upon request) will be charged 50% of the booked time.

CyTOF^{Helios}

- Slots may be charged at 50% if cancelled at short notice (less than two weeks before) and preventing other users from using the service.

IMC^{Hyperion}

- Cancelations may be charged at 50% if preventing other users from using the service.

Staff assistance / Basic training course:

- Users must cancel staff assistance and training 7 days in advance of their scheduled appointment to avoid being charged. After that time 50% of the booked time will be charged.
- The basic training course is treated as one single appointment, starting with the first session.