

# BIH Cytometry Core Facility (BIH CCF) User Guidelines

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# BIH Cytometry Core Facility (BIH CCF)

## User Guidelines

### General rules

- The BIH Cytometry Core Facility (BIH CCF) uses OpenIris (iris.charite.de) for scheduling of instruments and services.
- All users must complete and sign one of the registration forms (see website or OpenIris) before instruments or service can be used.
- First time users need to request an initial project meeting via OpenIris to discuss the requirements in instrumentation, service, and training.
- Your account at the core facility is user specific. 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 must be registered with and approved by the core facility beforehand (safety regulations). Use the registration form to register new biological material.
- For S2 material an official announcement to LAGeSo is necessary and a project meeting is required each time before new sample material can be used (update on risk assessment and 'Hygienemaßnahmen').
- Users must wear lab coats and gloves at any time at the instruments. Do NOT wear gloves when using the phones and when touching the door handles.
- Lab rules according to the "lab safety instructions" and the "Betriebsanweisungen" must be respected at any time.
- Any formal presentations or publications resulting from work performed at or from service provided by the BIH CCF must be acknowledged. The following statement is suggested: **"We would like to acknowledge the technical support of the BIH Cytometry Core Facility"**.

In case of extended advise on experimental design or data analysis co-authorship is suggested.

# BIH Cytometry Core Facility (BIH CCF) User Guidelines

Failure to follow the BIH CCF's User Guidelines and the protocols for quality management at the instruments 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 BIH CCF Team:

phone: 450 639446 / 539445 (CVK) 539488 (CCM)

email: [cytometry-core@bih-charite.de](mailto:cytometry-core@bih-charite.de)

### Flow Cytometry - Analyzers

- All new users of the analyzers, regardless of previous flow cytometry experience, must take training on the proper use of the instruments and on our quality assurance (QA) protocols.
- This 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.
- To assure constant high-level quality of the instruments, all users must follow the QA protocol each time when using the instruments (see detailed protocol at the analyzers). Please consider the additional time this will take when booking the instruments (~ 15-30min).
- Filtration of all samples is mandatory (35µm filter, each sample directly before measurement), unless otherwise discussed.

### Access to the analyzers

- Analyzers can be booked up to 14 days ahead with the online scheduler OpenIris ([iris.charite.de](http://iris.charite.de)). If you plan a longitudinal experiment, contact the BIH CCF staff for options of booking >14 days ahead before you start your experiment.
- 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.
- 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 and biosafety concerns.

# BIH Cytometry Core Facility (BIH CCF)

## User Guidelines

### Data management

#### *Diva-instruments (Cantoll and Symphony A3)*

- 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 computer's hard drive in the user's data folder for up to 10GB/user. For transfer of data, you can use the core facility data server or your own Charité server account.

#### *Cytek Aurora*

- After transfer of the 'Exported Experiments' please delete them from the hard drive of the computer.
- Do not leave >40GB of data in the "My Experiment" folder on the hard drive of the computer.

#### *General policies*

- No USB-keys or external hard drives allowed!
- The safety of data on the computers at the instruments or on the core facility data server will not be guaranteed by the core facility. It is the responsibility of all users to save their data by transferring 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. You may also open the pdf-documents provided by the core facility on the computer's desktops. Usage of the internet is strictly forbidden. Any user that violates this rule will have to pay for any resulting costs of repair and downtime of the respective instrument.

## Flow Cytometry - Cell Sorting

### General information

- Cell sorting is offered as a service, the cell sorters are run by core facility staff. Any exceptions must be discussed with the head of the core facility with sufficient advance notice.
- 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 and biosafety concerns.

### Sample preparation for cell sorting

- Bring your sample(s) in a buffer like PBS/BSA or PBS/FCS.
- We recommend using EDTA, Universal Nuclease 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 (see table on next page). However, the minimum volume should not be less than 300µl.
- Note that while it is always possible to dilute the cells if they are too concentrated, they cannot be concentrated if they are too diluted. Sort time will be longer if the sample is too diluted.

### Collection of sorted fractions

- Bring sufficient numbers of clearly labeled collection tubes (see table on next page).
- We recommend coating the tubes from the inside with buffer containing protein (FCS, BSA) and to sort into tubes already containing buffer/medium (at least 1/10 of the tube volume).
- Collection options:
  - 1.5ml tubes: 6-way sorting on Aurora CS / 4-way sorting on Arial/Fusion
  - 5ml tubes: 4-way sorting
  - 15ml: 2-way sorting
  - 96-well plate: 1-way sorting
- Single Cell Sorting (also as Index Sorting) is available. Contact us for more options.

# BIH Cytometry Core Facility (BIH CCF)

## User Guidelines

### Experiment setup

- Bring appropriate controls for the initial setup of each staining panel.
  - unlabeled cells
  - fully stained sample
  - single stainings of the cells or compensation beads (include unstained compensation beads).
- It is recommended to book an appointment before your actual sort appointment for the initial experiment setup, if you have >8 parameters.
- If you have used your panel before, it is possible to use the last experiment as a template, as long as your reagents and staining conditions have not changed. Bring a fresh 'unstained' control each time for sorting on the Aurora CS.

### Quality control (QC), and data management

- A quality control (QC) with 8-peak beads is done with each experiment and saved with the data.
- The sorted fractions will be re-analyzed for purity (sort check) at the end of the sort. Apr. 1000 cells will be taken, the number will be adapted if sorted cells are  $< 10^5$ .
- Sort reports and .fcs-files of samples and sort checks will be moved to your account on the core facility data server. Please secure the data on your own storage device afterwards.
- Experiments including QC will also 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).
- For longitudinal experiments that require a booking further in the future contact the core facility.
- Sorting outside operating hours can be arranged upon request by way of exception.

### Ariall - CVK

- Appointments can be booked without restrictions for the blank slots via the OpenIris.
- Book the *late-night slots* (tue/wed/thu, 17.30-21.00, marked in the scheduler by ---) via OpenIris and wait for approval before starting your experiment.
- Send an email (cytometry-core@bih-charite.de) for *short notice bookings* (48h ahead) and *upon request slots* (marked in the scheduler), indicating details of your sort. Wait for confirmation before starting your experiment.

# BIH Cytometry Core Facility (BIH CCF)

## User Guidelines

### Aurora CS (Luke) - CVK and AriaFusion - CCM

- Send an email (cytometry-core@bih-charite.de), indicating details of your sort with sufficient advance notice. Wait for confirmation before starting your experiment.

### Required time for cell sorting

- An extra 30min per sorting slot must be booked for sorter setup, quality assurance protocols and data management.
- For S2-sorting, 30min disinfection time will be added to your booking by core facility staff.
- For the calculation of the time required to sort your cells you can use the table below (approximate values for good quality samples, bulk sort).
- Additional time is needed:
  - to load the samples and to adjust the gating for each sample
  - for the sort checks (control for purity), depending on the number of populations you are sorting
  - if a new cytometer setting with single stains is needed, or a template needs to be adapted (additional parameters, new gating strategy)
- The sorting of the minimum volume of 300µl (see sample preparation above) will take appr. 20min.
- The sorting of one 96well plate will take appr. 5min if the target population is > 5% of your total cells.

nozzle size*	70µm	85µm	100µm
cell concentration, sample for sorting	5 x 10 <sup>7</sup> cells /ml	3 x 10 <sup>7</sup> cells/ml	1,5 x 10 <sup>7</sup> cells/ml
appr. number of events per hour**	7x10 <sup>7</sup>	4x10 <sup>7</sup>	2x10 <sup>7</sup>
max. cell number per collection tube			
15ml tube	1 x 10 <sup>7</sup>	5 x 10 <sup>6</sup>	2 x 10 <sup>6</sup>
5ml tube	3 x 10 <sup>6</sup>	1.5 x 10 <sup>6</sup>	7 x 10 <sup>5</sup>
1,5ml Eppi	1 x 10 <sup>6</sup>	5 x 10 <sup>5</sup>	2 x 10 <sup>5</sup>

\*nozzle size will be decided during the project meeting

\*\* 'debris' is also part of the 'events per hour'

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<https://www.bihealth.org/de/forschung/core-facilities/cytometry/>



## Mass Cytometry - CyTOF2<sup>Helios</sup> 'Tangerine'

### General information

- The CyTOF2<sup>Helios</sup> is staff operated.
- Users must register every project by completing a registration form that can be downloaded from our website or in OpenIris. 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 handled.

We recommend scheduling this meeting before samples are collected and stored to make sure sample quality is optimal for mass cytometry.

Technical feasibility of the project, expected sample income, timing of sample measurements and instrument availability will be discussed.
- Schedule a project meeting for additional projects at least 4 weeks before the desired date for sample acquisition.
- 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

- Cell preparation protocols and staining protocols must be discussed with and to be approved by the core facility to achieve the best possible sample quality and avoid damage to the detector by contaminating metals.
- Samples must be provided completely stained.

Bring samples either washed 2x in water as a cell pellet or book washing service (Mini-1000) for the water washes and bring cells in 50µl cell staining buffer.

Additional cell preparation steps can be booked upon request (e.g. thawing of frozen samples).
- 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, even when run very diluted. 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 in case of blockage will then be charged to the user's account.

# BIH Cytometry Core Facility (BIH CCF)

## User Guidelines

### Booking of CyTOF2<sup>Helios</sup>

- Please check the CyTOF2<sup>Helios</sup> - "Tangerine" scheduler in OpenIris for operation hours and free slots. Long term longitudinal experiments should be discussed in advance to guarantee availability of the service.
- Use OpenIris to request 'mass cytometry - sample acquisition' (Services). Please place your request 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.
- In the request, indicate the date you want the samples to be run and upload the "sample acquisition 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 the sample acquisition template provided with the request.
- You will get a status update on your request 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, the next possible date for the request will be suggested.
- Do not begin to prepare the samples until a time for measurement is officially scheduled and confirmed!

### Quality control (QC) and data management

- The CyTOF2<sup>Helios</sup> will be tuned (calibrated) before each run and in between if appropriate. EQ calibration beads will be measured as QC for each project per measurement 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 core facility data server. It is your responsibility to move them to your own storage medium and to delete data from the server to make space for new data.
- 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 for these data.

### Imaging Mass Cytometry - IMC<sup>Hyperion</sup>

#### General Information

- The IMC runs as a module that is attached to the CyTOF, so only one of the applications can be used at a time.
- Instrument assembly of the imaging module, tuning and laser power check will be performed by the core facility. Sample acquisition is offered as a full service or with assistance (project dependent).
- Users must register every project by completing a registration form that can be downloaded from our website or in OpenIris. 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 handled.

We recommend scheduling this meeting before samples are collected and stored to make sure tissue quality is optimal for imaging mass cytometry.

Technical feasibility of the project, expected sample income, timing of sample measurements and instrument availability will be discussed.

- Schedule a project meeting for additional projects at least 6 weeks before the desired date of first sample acquisition.
- 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 must 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 core facility 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. A small region of a representative tissue section will be ablated to test for contaminating metals in the tissue.

# BIH Cytometry Core Facility (BIH CCF)

## User Guidelines

### Booking of IMC<sup>Hyperion</sup>

- Use OpenIris to request 'imaging mass cytometry - sample acquisition' (Services).
- There is no limit in advance booking. Cancellations of a confirmed appointment may be charged at 50% if preventing other users from using the service.
- Information about the number of slides and tissue sections and the size of the regions to be ablated must be given with the booking. This information is used to plan the measurement.
- Indicate the date and time you will bring the samples fully stained to the core facility. You can also indicate a deadline for completion of the measurement that will be respected if possible.
- You will get a status update on your order indicating the date and approximate 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 (ROI) by the user on screenshots of the panorama or in the IMC software
  - the ablation of the sample
- The ablation process can be programmed and run unsupervised with an automatic shutdown at the end of the measurement. Long ablations will therefore be scheduled preferably over night and on weekends to reserve time for CyTOF measurements during working hours.
- Trained users may supervise their runs and change their slides in the absence of core facility staff (project dependent).

### Quality control (QC) and data management

- The IMC is tuned (calibrated) for each measurement.
- The use of 'anchor' samples on each slide is highly recommended to control for batch effects (day-to-day variations in sample preparation and staining procedure) and day-to-day variations of the instrument.
- A detailed documentation is provided with each measurement.
- We will move all data to your account on the core facility data server. It is your responsibility to move them to your own storage medium and to delete data from the server to make space for new data.

## Billing practices

### General information

- Charges cover the project specific costs for consumables and staff time. Rates for instrument usage and service are found on the BIH CCF website.
- Time for instrument usage and service will be billed quarterly based on the time booked and time used.
- Charges for BIH and Charité users will be booked on the accounts (Kostenstelle/Innenauftrag) used at time of booking. External users will be billed by "Rechnungslegung" (taxes may apply).

### CyTOF2<sup>Helios</sup>

- CyTOF service is charged by the hour. There is an extra cost per sample that covers consumable costs (cell strainers for filtering the cells, counting slides etc.).
- 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 caused by the sample (de-clogging, re-tuning, change of tubing, etc.) will be charged.

### IMC<sup>Hyperion</sup>

- IMC service is charged by the hour. Three different price types apply:
  - Experiment setup with instrument in standby mode, e.g. panorama, data management, etc. (charges contain staff time only)
  - Preparations with instrument in running mode, e.g. laser power test, tuning (charges contain staff time and instrument time)
  - Sample measurement (charges contain instrument time only)
- Time for sample measurement is calculated by the given ROI size in the request and the instrument time is dedicated accordingly. Reserved time may be fully charged (even if the final ROI size is smaller) if the dedicated time prevented measurements of other projects. If the final ROI size is bigger than originally requested the used time will be charged.

### Cancelation policies

#### *Flow Cytometry analyzers*

- 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.

#### *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.
- Long term bookings (reservation made by staff) may be charged 50% of the booked time if canceled less than 14 days in advance and no other user schedules at that time. The 48 hours lock (see above) also applies.
- Cancelations of bookings outside regular operating hours (reservations made by staff upon request) will be charged 100% of the booked time.

#### *CyTOF<sup>Helios</sup>*

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

#### *IMC<sup>Hyperion</sup>*

- 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. Cancelations of single sessions will result in the need for additional training sessions and charged as such.