

USER GUIDELINES

I. General Information

The Cytometry Core Facility (Cyto Kiel) is located on the second floor of the Institute of Immunology: Universitätsklinikum Schleswig-Holstein - Campus Kiel

Haus U30 - Lab 242 and 247.6 Michaelisstr. 5 24105 Kiel

Support by the Facility Management can be provided as followed:

Mon	Tue	Wed	Thu	Fr
08:00 - 17:00	08:00 - 17:00	08:00 - 13:00	08:00 - 17:00	08:00 - 17:00

The core lab is open for *trained registered users* with their own access to the Immunology building around the clock for measurement on the analyzers. Service and Sorting service is only provided during official working hours or after personal consultation with the Flow Core Staff.

The main contact person for technical or administrative questions is:

Christian Peters

Email: Cytometry.corefacility@uksh.de

Christian.Peters@uksh.de

Phone: +49 431 500 31037 (office); +49 0431 500 31042 (Flow lab)

For further information refer to the Cyto Kiel - Homepage

II. Equipment

The Flow Cytometry Core lab is equipped with different cell analyzers, cell sorters, and image flow cytometers:

Instrument	Laser (nm)	Detection channels	Operation mode
MACSQuant X (Miltenyi)	404 / 488 / 640 nm	FSC, SSC, 2 violet, 4 blue, 2 red	autonomous
FACSCanto (BD)	488 / 633 nm	FSC, SSC, 4 for blue, 2 red	autonomous
LSR Fortessa (BD)	405 / 488 / 561 / 640 nm	FSC, SSC, 6 violet, 2 blue, 5 yellow-green, 3 red	autonomous
Northern Lights (Cytek)	405 / 488 / 640 nm	38 channels – up to ca. 24 parameters	autonomous
Aurora (Cytek)	355 / 405 / 488 / 561 / 640 nm	67 channels – up to ca. 40 parameters	autonomous
FACS Aria III (BD)	405 /488 / 633 nm	FSC, SSC, 3 violet, 5 blue, 3 red	service semi-service
FACS Melody (BD)	404 /488 / 640 nm	FSC, SSC, 3 violet, 4 blue, 2 red	service semi-service
<u>ImageStream</u> (Merck Milipore)	405 / 488 / 561 / 592 / 642 / 762 nm	6 detection channels in the range of 435 – 780 nm SSC in 762/35 available	autonomous



III. Billing Policy/Practice

The instrument time and service are billed in 30 min increments based on the scheduled time and time used.

Internal users (Institute of Immunology) will be charged via "interne Leistungsverrechnung", other UKSH users will be charged by the Cytometry Facility via invoice. All charges are billed quarterly. For operator-controlled sorting services, an additional time of 1 h is needed for each booking for device startup, technical setup, data management, and shutdown. These costs are only charged by 50 % (=30 min regular price), the remaining 50 % is funded by the Cytometry Core Lab.

Tab. 1: Price list of facility services for UKSH and CAU related institutes

			Operating mode in Euro/ h		
Instrument class	Laser	Brand	autonomous	semi- autonomous	service
Cell analyzer	≤3 lasers	MACS Quant X FACS Canto	15,-	/	55,-
Cell analyzer	>3 lasers	LSR Fortessa Northern Lights Aurora	25,-	/	65,-
Cell sorter	3 lasers	FACS Aria	/	60,-	80,-
Cell sorter	3 lasers	FACS Melody	/	40,-	80,-
Image Flow Cytometer	8 lasers	ImageStream	40,-	/	80,-

Tab. 2: Price list for device specific training sessions

Instrument class	Initial training	Attendance	Costs in Euro/ h
Cell analyzer	2x 2 h	max 2 participants/ session	50,-
Cell sorter (FACS Aria)	2x 2 h	max 1 participants/ session	80,-

For users affiliated with CAU and UK-SH prices do not include 19 % VAT (Mehrwertsteuer). For the non-CAU/UK-SH academic users, 19 % VAT (Mehrwertsteuer) is added on top of the list prices. For non-academic commercial customers, 70 % is added on top of the list prices (19 % VAT + 51 % extra).

IV. User and Material Registration

To get permission for the use the cytometry facility services a user has to complete and sign the <u>User Guidelines Agreement</u> and <u>Registration Form</u>

An initial project meeting should be scheduled for the discussion of requirements regarding instrumentation, training, and service.

V. Booking

The online booking system is available under openiris.io: Provider: Cyto Kiel. The direct links for booking the respective machines can be found also on the https://example.com/homepage-of-the-Institute-of-Immunology.

+49 431 500 31037 (office C. Peters)

CYTO KIEL Cytometry Facility Kiel Institute of Immunology

Users need an introduction to the respective machine to be activated for booking. The permission to **book and use** devices applies only for the registered person and material. Reservations for analyzers can only be booked two weeks in advance.

A registered user is not allowed to book and measure samples for another person.

Service appointments for sorting or analyzer service have to be organized in agreement with core staff via email or personal communication (Contact: christian.peters@uksh.de; Phone: 500 31037).

When booking an analyzer, time for setup, QC measurement, cleaning procedures, and data backup has to be included.

If more time is needed to finalize the actual measurement, the user has to ask the next person in line for permission to finalize the experiment and has to update the online booking.

VI. Cancelation Policies

To avoid being charged users must delete their booking at least 24 h in advance. This can be done by deleting the booking from the online system. Within 24 h before the start, the booking can only be canceled. Thereby the time slot becomes available for other users, but canceled reservations are still registered and will be charged 40% of the original pricing. Bookings in progress cannot be canceled anymore. Moving a scheduled time slot to a later time point on the same day is possible.

VII. Biosafety Regulations

The user is obligated to follow the legal regulations of GefStoffV, BioStoffV, and GenTG. The general rules are summarized in the <u>Betriebsanweisung</u>.

Samples brought into and analyzed inside the Cytometry Facility have to comply with the risk and safety level S1 to S2 of the BioStoffV and of the GenTG. Before measuring samples of the Biosafety levels S2 or GenTG levels S1 or S2 Users have to notify the Core Facility to discuss and exemplify measures to be taken for sample handling. Otherwise, it is prohibited to bring such samples into the facility. If possible, such samples have to be inactivated and fixed in PFA-containing buffers to reduce the biological risk.

VIII. General Lab Rules

The user must wear personal protective equipment. It is mandatory to wear a lab coat, closed shoes all the time, and gloves for working on the instruments.

It is forbidden to wear gloves when touching the phone or door handles.

The user must keep the working bench and laboratory clean:

- empty small waste bags into large ones before leaving
- do not leave anything on the working bench or in the fridge e.g. sample tubes, plates

The user must follow appropriate procedures regarding the handling of potentially bio-hazardous material.

Only registered material can be handled inside the Flow Core (refer to V. User and material registration).

In case of problems with the devices, the facility staff has to be informed immediately.

If misuse leads to time delay and/or device malfunction the responsible user will be charged for incidental costs.

IX. Use of Instruments

Analyzers

All new analyzer users must perform initial training with regard to general laboratory introduction, proper instrument use and specific Quality Assurance (QA) protocols regardless of previous experience in flow cytometry.

USER GUIDELINES — Cytometry Core Facility Michaelisstr.5 | 24105 Kiel https://www.uksh.de/immunologie/Forschung/ Cyto+Kiel+_+Cytometry+Core+Facility.html



CYTO KIEL Cytometry Facility Kiel Institute of Immunology

This basic training, provided by the Core Facility, is obligatory prior to autonomous use of instruments and consists of two face-to-face sessions (2x 2 h) including hands-on time.

Depending on the training progress and previous experience additional training sessions might be scheduled to get permission for autonomous instrument use.

The use of the BD LSR Fortessa is restricted to applications that require >8 fluorescence parameters or the 561 nm laser.

The use of the MACSQuant X is restricted to applications that need the 405 nm laser and/ or plate measurement (96well, 384well plates).

Cell sorter

The use of the cell sorter is generally possible as full service after personal consultation with the Cytometry Facility Staff.

In exceptional cases, the measurements might be done in a semi-autonomous operational mode after the agreement of the Cytometry Facility Staff. In the semi-autonomous mode, the user is allowed to measure and sort cells. Device startup and all technical setup remain in the care of the Facility staff. In the case of semi-autonomous operation, the user must perform a basic training of two face-to-face sessions (2x 2 h) including hands-on time. Depending on the training progress additional training sessions might be scheduled to get permission for semi-autonomous instrument use.

For usage of cell lines with the cell sorters, a **negative test for mycoplasma** has to be provided before the sort.

Quality control and Cleaning procedure

To assure the constant quality of the flow cytometers all users have to follow the Facility QA procedures for each measurement session. Specific operational protocols and SOPs for instrument QC processes are available for each instrument.

Please consider by booking a device's time slot that an additional time (~15 min) is needed for this QA.

To check compliance with the cleaning steps and to ensure that the instruments are left in optimal condition, steps for the instrument cleaning procedure must be documented in a separate measurement file on the respective machines in the "Cleaning" user account. The cleaning step is recorded as a new sample. The exact cleaning instructions can be found in the experiment name in the respective Cleaning account or the short machine manual.

Compliance with the cleaning protocol must additionally be acknowledged in an on-site logbook. In addition, minor anomalies and problems will also be recorded in these logbooks. However, in case of problems that could affect the machine's performance the flow facility personnel must be informed immediately and directly. These problems have to be uploaded to the booking system as an "issue" (pencil icon in the device header in the scheduler tab in the booking system) and recorded in the logbook. Users who booked the respective machine in the following three days are informed automatically about newly created "issues". If the facility personnel can be informed the "issue" will be created for the users.

X. Data Handling

The safety of data on the instrument computers will not be guaranteed by the Core Facility. It is the user responsibility to backup and save their own data by transferring them to a storage media. The users are obligated to delete their own data files from the instrument computer after measurement on the same day.

The instrument computers will be cleaned up on a regular basis (weekly) by the Core Facility staff. The Core does not take responsibility for any data loss due to missed backup by the user.

USER GUIDELINES — Cytometry Core Facility
Michaelisstr.5 | 24105 Kiel
https://www.uksh.de/immunologie/Forschung/
Cyto+Kiel+_+Cytometry+Core+Facility.html

cytometry.corefacility@uksh.de

+49 431 500 31042 (facility lab)

+49 431 500 31037 (office C. Peters)



It is not allowed to use the instrument computers for any other purpose than data acquisition and analysis

XI. Acknowledgment

As Core Facility we provide access to state-of-the-art technology platforms as well as professional support and service to the immunologic community. Therefore, all users are obligated to acknowledge our contribution to data generated in this cytometry facility in any kind of formal publication and presentation. We suggest the following statement:

"We would like to acknowledge the support of the CYTO KIEL | Cytometry Facility of the UKSH"

When machines have been used, which are supported by DFG funding (Großgeräteförderung), the DFG-Project number of the respective funding has to be mentioned in the acknowledgment section of the publication. This applies to the following devices: [Cytek – Aurora: 509546995]. In case of extensive experimental or advisory support, we suggested a co-authorship.



USER REGISTRATION | SORT REQUEST - Cytometry Facility Kiel

Return to:		
Christian Peters I	nstitute	of Immunology Bld U30 Room 229
phone: +49 (0)431	500 310	037 email: christian.peters@uksh.de
Registration date		
User		
	Name	
Email a	ddress	
	Phone	
Institute/Depar	rtment	
Project Leader/ Bil	ling Add	dress*
	Name	
Email address		
Phone		
Institute/Department		
Billing A	ddress	
Biosafety – Please	provide	copy of individual Biosafety forms per material classification
· ·		d "Risikogruppe 2" or do they contain organisms classified as
"Risikogruppe 2"	accordir	ng to "BioStoffV" and "GenTV"?
□No		
☐Yes ("Risikogru	uppe 2"	– for work at S2 level project-specific permission by us mandatory!)
BioStoffV		
GenTV		



User Agreement

Return to:

Cytometry Core Facility / Christian Peters	
Institute of Immunology	email:
Bld. U30 Room 229	cytometry.corefacility@uksh.de
phone: +49 (0)431 500 31037	christian.peters@uksh.de

I have read and understood the above terms of use.		
I agree to comply with them and understand that any violation against these guidelines may lead to the withdrawal of permission of access to the Cytometry Facility Kiel (instruments and services).		
I acknowledge the general terms of use and fees for the use of the respective equipment and that additional costs caused by misconduct will be charged.		
Date		
User name		
User signature		
Group leader*		
Group leader signature*		





*required for billing authorization purposes