

Core Unit Cytometry

User Guidelines

Department of Immunology, University Medicine Greifswald

The Core Unit Cytometry (CU) is housed in the Department of Immunology, University Medicine Greifswald. Our stated goal is to offer all medical professionals and scientists an optimal platform for multiparameter flow cytometry for research purposes. The CU is available to all working groups of the University Medicine Greifswald as well as researchers from outside of the campus.

Services offered at the Core Unit

1. Flow cytometry

Flow cytometry is a powerful, high-throughput technology, and has become an indispensable instrument for analyzing multiple-characteristics of single cells within a population in a short period of time. This technology allows researchers to investigate the expression profile of surface as well as intracellular markers, cell cycle analysis, cellular proliferation, cell death (apoptosis or necrosis) etc.

BD LSR II

The BD LSR II is a flow cytometer that is offered by BD Biosciences. It is a bench top flow cytometer designed with 4 laser colors which are able to detect up to 14 colors or parameters at the same time. The BD LSR II has several significant features including an advanced fluidics system, high-performance data acquisition and analysis, and a unique optics system.

2. Chip cytometry

Next to flow cytometry, the CU will provide users with next-generation technology. Zellkraftwerk is an innovative tool which is based on the immobilization of cells on special slides referred as chips. With Chip cytometry, a researcher can analyze a virtually unlimited number of biomarkers of interest on a single tissue- or cell suspension sample. The advantage of Chip cytometry is that investigators can work at the single-cell level, with respect to morphology, expression profiling of biomarkers both at surface and intracellular levels. In addition, the chips can be preserved for up to two years after first use.

User duties and responsibilities

The instruments in the CU are common equipment and have to be treated with care so that we may operate them for a long time without incurring additional expense due to misuse. Therefore, we expect every user to follow the below stated rules and policies, in order to keep the facility a clean and reliable working space and useful analysis platform.

An introductory training and formal orientation by the CU-staff is mandatory for all users before using the facility. Users that are not introduced to the facility by CU staff will not be permitted to use the instruments.

All users are responsible for daily maintenance of the machine. Every user has to follow the Start-up, Cleaning and Shut-down Maintenance procedures. Users that do not follow the rules, will be excluded from using the instruments.

In case of malfunctions, please contact the technical staff immediately on the numbers listed below.

Biosafety

Flow cytometry core unit is a multi-user facility where many different samples from various sources that may contain known or unknown human pathogens are investigated. The safety of facility personnel and user is of ultimate concern. Information about the sample sources and potentially infectious agents is critical for effective biosafety measures.

- All samples with biological safety category 2 or higher that have no approval from Health and Safety for live measurements must be fixed prior to entering the facility and running the analysis.
- All biological or contaminated waste must be disposed of into the bottles provided by the facility. These bottles are meant for only the contaminated waste; all other waste can be disposed of in the regular waste.

Booking the instruments

- For planning and booking of the device, please contact the technical staff of the CU (Email/Telephone). A password-protected online calendar can be viewed by registered users. By booking an appointment, the user agrees to appear punctually on the agreed date. More frequent non-appearance without cancellation of the appointment may lead to exclusion. For foreseeable delays, the responsible operator should be informed. The employees of the CU are happy to advise you on the appropriate time schedule.

Usage Fees

The fees for running the LSR II flow cytometer follow the guidelines of DFG. As per the DFG guidelines, the costs for the use of the services offered by the CU depend on the type of service needed.

- Self-service (User = Operator): **25 € / h**
- Operation by personnel of the CU or training by personnel of the CU (CU = operator): **65 € / h** (up to 40 h refundable by DFG)

These fees include the basic reagents required for running the machines (e.g., sheath fluid) but no specific consumables such as antibodies, fluorophores or counting beads.

Data Management

- Please bring a USB stick with sufficient storage capacity. It is absolutely prohibited to connect your USB to the flow cytometer-computer. Please use the CU-provided USB stick to transfer the data from the flow cytometer-computer to the work-station computer. Our staff is happy to advise you. Please note that data older than 3 month will be removed from the central data store without warning.
- The facility takes no responsibility for the maintenance and physical integrity/storage/loss of any user's data.
- We strongly encourage users to back up their data immediately after analysis
- The data analysis software FlowJo is provided on a work-station computer. This computer can be booked via online calendar.

General issues

- The cells must be filtered through a 40 µm filter and provided in buffer which prevents clumping of the cells (e.g., PBS + EDTA). In general, cell densities for measurements should be 1×10^6 to 5×10^6 /ml for the LSR II. The CU employee can give you further information on this.
- Everybody takes care to ensure that tidiness and waste removal is performed without supervision by facility staff.
- The device configurations will only be changed by employees of the CU.

Scientific collaborations and acknowledgements

Any formal presentations or publications resulting from work performed in the CU should be acknowledged. Some instruments have been purchased from grants or other sources which also deserve acknowledgement.

We also encourage all users to acknowledge the facility on any papers that were generated with the help of instrumentation at the core unit.

Co-authorship should be credited to members of staff who have significantly contributed to the published data. For example: in-depth help in designing, testing/setting-up a staining panel, and helping with the analysis of the data. This constitutes scientific collaboration

For more information, please contact the Core Unit's staff:

Dr. VSN Murthy Darisipudi

Phone: 03834 86 5460

Email: venkata.darisipudi@uni-greifswald.de

Susanne Neumeister

Phone: 03834 86 5465

Email: susanne.neumeister@uni-greifswald.de