Status: 01.05.2025

Concept and Terms of the Flow **Cy**tometry Units at the School of Medicine and Health of the **TU**-**M**unich (CyTUM)

Munich site (CyTUM MIH)

at the Institute for Medical Microbiology, Immunology and Hygiene (MIH)

Weihenstephan site (CyTUM LTI)

at the Department of Animal Physiology and Immunology (LTI)

Concept

Contact details

Location Munich - CyTUM-MIH

TU Munich
School of Medicine and Health
Department of Medicine and Health
Flow Cytometry Unit (CyTUM) of the
Institute for Medical Microbiology, Immunology and Hygiene (MIH)
Trogerstrasse 30
81675 Munich

Director of the Institute: Prof. Dr. Dirk H. Busch

Tel.: 089 – 4140 – 4120
Fax: 089 – 4140 – 4868
E-Mail: dirk.busch@tum.de

Head of the Flow Cytometry Unit:

Dipl-Ing (FH) Thomas Pohl
Tel. (Sort room): 089 – 4140 – 7450
Fax: 089 – 4140 – 4131
E-Mail: th.pohl@tum.de

Staff of CyTUM-MIH
Tel.: 089 – 4140 – 7450

Corinne Angerpointner
E-Mail: Corinne.angerpointner@tum.de

Status: 01.05.2025

Location Weihenstephan - CyTUM-LTI

TU Munich
School of Life Sciences Weihenstephan
Flow Cytometry Unit of the Technical University of Munich (CyTUM) at the Chair of Animal Physiology and Immunology (LTI)
Weihenstephaner Berg 3
85354 Freising

Director of the Institute: Prof. Dr. Dietmar Zehn

Tel.: 08161 – 71 – 3508
E-Mail: dietmar.zehn@tum.de

Head of the Flow Cytometry Unit (currently):

Prof. Dr. Dietmar Zehn

Tel. (Office): 08161 – 71 – 3508
Tel. (Sort room): 08161 – 71 – 5559
E-Mail: dietmar.zehn@tum.de

Staff of the CyTUM, Location Weihenstephan

Inge Celler

Tel. (Lab): 08161 – 71 – 3510
E-Mail: celler@tum.de

Cross-campus Cytometry Platform of TUM – CyTUM

Modern technologies, such as Fluorescence Activated Cell Sorting (FACS) and multi color flow cytometry analysis are increasingly influencing the status of scientific research as an essential resource.

A core facility for cell flow cytometry was created in 1999 at the institute of Medical Microbiology, Immunology and Hygiene (MIH), under former director Prof. H. Wagner and initially supervised by Dr. rer.nat. Matthias Schiemann.

In the wake of Dietmar Zehn's appointment to the Science Center in Weihenstephan, there was an urgent desire to make competitive resources in the field of flow cytometry available to the existing broad user community at this campus as well

With support from the TUM and the DFG, a "state of the art" analytical center for flow cytometry was realized at the Chair of Animal Physiology and Immunology (LTI).

Subsequently, the intention to organize these locations in a higher-level structured cytometry platform at the TUM (CyTUM) arose combining synergy as shown below. The unit at MIH, now referred to as "CyTUM MIH" and the unit at Weihenstephan as "CyTUM LTI".

By bundling those locations together, CyTUM now provides a wide variety of flow cytometry cell analyzers and cell sorters to users across the bigger area of Munich. During the last decade, CyTUM also steadily established a cross-collaboration with the “Immune Monitoring, Technische Universität München, Munich, Germany" as well as the Core Facility Cell Analysis at the TranslaTUM, Munich.

A conceptual agreement for handling equipment, respectively organizational procedures was initialized between those partners, one being a part of this Concept and Terms of use.

Specific informations of CyTUM-MIH

The structure of the Core Unit Flow Cytometry at the Institute of Medical Microbiology, Immunology and Hygiene provides access to well established and innovative methods with state of the art instruments for the individual scientific questions of all scientists across the campus rechts der Isar. In addition, the Core Facility can also provide services to projects funded by public research or industrial companies. This broadly diversified offer to various clients leads to a reputation for methodological and scientific expertise in the field of flow cytometry.

The scientific expertise and excellence is reflected in the large number of publications in collaboration with the Core Facility.

Until December 31st 2010, the financing of cell sorting was ensured by funds of the Z-project of SFB 456 and especially by institute funds. Funding by the TRR179 was added on July 1st 2016.

Constantly high operating and consumables costs, reagents for quality assurance, renewal of instrumentation and last but not least personnel costs especially for the execution of cell sorting made it necessary to establish a new concept reallocating some of those costs to users of the Core Facility..

Specific informations of CyTUM-LTI

This functional unit, with a high end sorter and analyzer at the Weihenstephan Science Center was part of TUM's strategy to support the establishment of new research foci and technologies at the site of the life science campus Weihenstephan, which would lead to a sustainable strengthening of basic research and at the same time enable new approaches in translational research. In case of Prof. Dr. Zehn, this is the establishment of an immunological/infectiological focus and the future consistent combination of small animal model-oriented basic research with application-oriented research in large animal models. This should lead to new solutions to combat problematic infections in humans and animals.

The main reasons for creating the unit in Weihenstephan were the close proximity to other experimental facilities at the Weihenstephan campus. Transporting samples to MIH would involve travel times of 1 hour in each direction. Furthermore, due to legal regulations it would not be possible to transport the majority of samples originating from infected individuals by public transport.

Performance potential of CyTUM

As mentioned before, the sites at MIH and LTI were designed to provide cross-institutional and cross-campus scientists access to established and innovative methods and instruments for their scientific questions. The combination of a wide variety of constantly renewed instruments and equipment available to researchers led to a high scientific value.

The demand by users shows in the electronically documented usage data of the “Booked” scheduler. Since its beginning in 2006, more than 1000 users registered in the online booking system and thereby gained access to instrument reservations for their experiments. After appropriate instructions, they can directly schedule a flow cytometry analyzer or arrange an individual appointment for cell sorting with the FACS operators. These measures support third-party funded projects by taking advantage of the infrastructure created by CyTUM.

For this scientific input and our expertise at the CyTUM, work done in collaboration with the Core facility, is reflected in acknowledgements or co-authorship. This applies equally to the work published in recent years by the heads of the institutes overseeing CyTUM, D.H. Busch and D. Zehn, in which cytometry analyses and sorting form the decisive technological basis.

CyTUM Structure: Overview of Members and Chair of the CyTUM Steering Committee.

The structures are built in a way that further partners can be equally integrated into the existing organization.

The decision-making body for the overall structure of the CyTUM is the Steering Committee with the members listed below and led by the respective heads of the institutes where the units are located.

Tasks of the participating institute managements:

- Appointment of responsible persons for the individual locations
- Representation of the interests of the institutions towards the respective faculties and the university management

Tasks of the heads of the sites:

- Ensuring and monitoring the ongoing operation of the units
- Admission and training of new users
- Routine activities necessary to maintain operations

Steering Committee Responsibilities:

- Coordinate cost and user fees
- Joint consultation on new acquisitions
- Supervision of the operation of the facility

Members of the Steering Committee CyTUM:

- Prof. Dr. Dirk Busch, TUM, MIH
- Prof. Dr. Dietmar Zehn, TUM, LTI

Spokesperson of the Steering Committee CyTUM:

- Dipl.-Ing Thomas Pohl

Overview and goal of CyTUM

The main goal of CyTUM is to provide access to state of the art instruments in flow cytometry as well as support all scientists and their respective questions with our scientific expertise. Therefore, CyTUM is open for use to scientists and those interested. Connecting functional units at the MIH and LTI should provide synergies concerning:

- Usage of resources
- Transfer of technologies
- Stratification regarding complementing but not identical instruments
- Efficient and balanced quality management
- Access to alternative instruments in case of Instrument failure during experiments that cannot be postponed
- Procurement and securing of central university resources

The CyTUM network should primarily fulfill the requirements of service across campus, local utilization and proximity to work being a priority. Through “remote control” options, operators of the respective units should be able to provide help (e.g. with instrument settings) even from a distance.

Service for industrial areas is possible in all units, although it should remain in close relation to active collaborations.

CyTUM – Instrument specifications of the locations

Location MIH:

Devices should foremost meet the requirements of scientific questions of work groups and clinical routine at the representative institute. For external user, depending on the equipment, it is possible to analyze and sort biological sample material up to biological safety level BSL2 according to §7 GenTG and in collaboration with the Virology department of Prof. Protzer up to biological safety level BSL3.

The CyTUM-MIH location has a variety of different flow cytometry analysis and cell sorting equipment at its disposal.

Cell sorter:

- MoFlo Astrios EQ BSL-1
- MoFlo Astrios EQ BSL-2
- FACS ARIA III
- Bigfoot Spectral Cell Sorter

Cell analyzer:

- CytoFlex
- CytoFlex S
- CytoFlex LX
- Cytek Aurora
- Navios
- CyAn Lx314

Configuration of the CyTUM MIH cell sorters:
Upfront the selection of the best fitting instrument for a specific cell sorting experiment should be coordinated with the CYTUM MIH staff. The devices offer unique features and different combinations of laser and filter setups. Electable excitation wavelengths include:

- UV 351 nm
- violet 405 nm
- blue 488 nm
- green 532 nm
- green/yellow 561 nm
- yellow 592 nm
- red 633 – 652 nm

with simultaneous detection of up to 30 parameters.

The staff of the flow cytometry unit routinely performs cell sorting. After consultation with the operator, the sample material should be prepared accordingly by the person conducting the experiment and brought on the scheduled time to the sorting facility, using appropriate transport measures.
Sorting of up to 6 populations from 50ml format down to 1536 well plates in bulk or single cell mode are eligible.
Any necessary modifications to the equipment for special experimental requirements must be discussed with the CYTUM-MIH staff before the experiments are carried out; hints for possible special modification can be found on the CyTUM homepage for the different locations.

Location LTI

The equipment is primarily intended to meet the requirements of scientific research groups at the Weihenstephan campus and to serve as an alternative for users at the other location. The sorting device FACS ARIA Fusion allows analysis and sorting of biological sample material up to safety level S2 according to §7 GenTG. According to the state of scientific knowledge, Safety level 2 is defined, as genetic engineering work with microorganisms and cell cultures for which a low risk to human health or the environment can be assumed.

CyTUM LTI has a variety of different flow cytometry analysis and cell sorting equipment.

Cell sorter:

- FACS Fusion (ARIA III sorter in L2/S2 safety cabinet), 4 way and plate sorting.

Cell analyzer:

- FACS Fortessa
- FACS Accuri
- CytoFlex LX

Configuration: 16 fluorescence detectors, one forward and one side scattered light detector

- blue 488 nm
- red 640 nm
- violet 405 nm
- yellow/green 561 nm

Requirements for using on-site equipment

Before using the analysis instruments, scientific personnel receives an instruction (so called "driving license") by the members of the Core facilities. This briefing is device-specific, intended to ensure proper handling of the equipment and usually carried out and documented by the head of the facility. After the briefing, the scientific employee, once registered in the booking system gets the permission to use devices and thus the authorization to access and manage own time slots. This instruction does not replace the offered internal trainings. Users of facility equipment agree on facility terms, among others regarding service billing and rules for authorship.

Registration with the online booking system

Instrument reservations for analyzers at the sites are done via the jointly provided internet booking system, the “Booked” scheduler:

https://tumemi0-booked.srv.mwn.de/

Requests for sort reservations are sent to cellsort.mih@mh.tum.de - Registration forms and biological safety statements are available for download on the CyTUM website.

Usage fees

Billing is based on the reservations entered in the online calendar in accordance with the recommendations of the DFG for facility services. The services and facility support for cell analysis and sorting varies as described bellow. Furthermore, we discriminate between two different types of users by their affiliation (Internal or External). Billing times are charged for every started half hour.

Cell analysis

Cell analysis instruments are divided into two instrument types:

- *Instruments type A* (instrumentation class I according to DFG form 55.04 - 11/21, cell analyzers with up to 3 lasers)
- *Instruments type B* (instrumentation class II according to DFG form 55.04 - 11/21, cell analyzers with more than 3 lasers).

1. Internal users
    a. Internal user (institution of the TU Munich) uses service in the sovereign area (e.g. teaching except further education and training, basic research):
        i. Self-reliant operation of *Instruments type A* / 15,00 € per hour.
        ii. Service operation of *Instruments type A* by a CyTUM employee / 55,00 € per hour.
        iii. Self-reliant operation of *Instruments type B* / 25,00 € per hour.
        iv. Service operation of *Instruments type B* by a CyTUM employee / 65,00 € per hour.

    It must be verifiably documented that this service is used for the sovereign area. Until further notice, no overhead transfer, no VAT

    b. Internal user (institution of the TU Munich for CyTUM) uses service in the economic area (e.g. contract research, routine activity):
        i. Self-reliant operation of *Instruments type A* / 24,00 € per hour.
        ii. Service operation of *Instruments type A* by a CyTUM employee / 68,00 € per hour.
        iii. Self-reliant operation of *Instruments type B* / 35,00 € per hour.
        iv. Service operation of *Instruments type B* by a CyTUM employee / 79,00 € per hour.

    Until further notice, no overhead transfer, no VAT

2. External users
    a. Institutions of the Free State of Bavaria
        i. Self-reliant operation of *Instruments type A* / 28,00 € per hour.
        ii. Service operation of *Instruments type A* by a CyTUM employee / 80,00 € per hour.
        iii. Self-reliant operation of *Instruments type B* / 41,00 € per hour.
        iv. Service operation of *Instruments type B* by a CyTUM employee / 92,00 € per hour.

    If it can be verifiably documented that the service is used for basic research, the following billing rates apply:

        i. Self-reliant operation of *Instruments type A* / 18,00 € per hour.
        ii. Service operation of *Instruments type A* by a CyTUM employee / 66,00 € per hour.
        iii. Self-reliant operation of *Instruments type B* / 30,00 € per hour.
        iv. Service operation of *Instruments type B* by a CyTUM employee / 78,00 € per hour.

    Overhead payment, subject to VAT.

    b. Other users:
        i. Self-reliant operation of *Instruments type A* / 38,00 € per hour.
        ii. Service operation of *Instruments type A* by a CyTUM employee / 108,00 € per hour.
        iii. Self-reliant operation of *Instruments type B* / 55,00 € per hour.
        iv. Service operation of *Instruments type B* by a CyTUM employee / 125,00 € per hour.

    Overhead payment, subject to VAT.

    .

Cell sort

Unless otherwise specified, these prices cover all preparatory and follow-up activities, including any necessary adjustment work for a sorting appointment. All sorting appointments are run by operators of the CyTUM facilities. There is no segregation in prices for instrumentation, only affiliation will be accounted for.

3. Internal users
    a. Internal user (institution of the TU Munich) uses service in the sovereign area (e.g. teaching except further education, basic research) by an employee of the CyTUM / 80,00 € per hour.

        It must be verifiably documented that this service is used for the sovereign area.

    b. Internal user (institution of the TU Munich) uses service in the economic area (e.g. contract research, routine activity) by an employee of the CyTUM / 107,00 € per hour.

        Until further notice, no overhead transfer, no VAT.

4. External users
    a. Institution of the Free State of Bavaria
        External user uses service by an employee of CyTUM / 125,00 € per hour

        If it can be verifiably documented that the service is used for basic research, a billing rate of 96.00 € per hour applies.

        Overhead payment, subject to VAT

    b. Other users:
        Other external user uses service by an employee of CyTUM / 170,00 € per hour.

        Overhead transfer, subject to VAT.

The usage fee is determined quarterly for each user and invoiced to the respective workgroup/project leader. The invoice amount is due within a month and must be transferred to the account specified within.

All necessary operating costs and consumable materials, reagents for quality assurance and personnel costs, in particular for the performance of cell sorting by the operators, are included in the fees.

By creating a reservation entry, the experimenter accepts the CyTUM usage regulations on his own responsibility. Violation of the Concept and Terms of use will be reprimanded by the head of the functional unit. In case of repetition, the head of the functional unit reserves the right to exclude the user from further handling of the equipment.

Damage to the equipment, which is clearly caused by faulty behavior of the user, is to be compensated by the user according to the legal regulations. The costs of any repair work and any spare parts required for this are to be met by the respective user.

This Concept and Terms of use was developed in cooperation with the legal and financial department of the Technical University of Munich.

Status: 01.05.2025

Terms of use

Use of the analyzers

For new users, introductions to the analyzers ("driving license") are carried out regularly.

- Appointments should be planned realistically and, in the interest of all, times entered should be adhered to.
- If a booked appointment cannot be kept, the change in the online calendar should be made as soon as possible.
- A "warm-up" time of 15 minutes at the beginning of the day is mandatory for all equipment (especially if it is the first appointment of the day).
- For the FACS Fortessa and FACS Accuri, an additional cleaning time of at least 15 minutes must be included at the end of the session for each experiment.
- An entry of more than 3 consecutive hours in the core time of 10:00 - 16:00 is only allowed in exceptional cases and requires the agreement of the head at the CyTUM site.

Basic rules for the use of the analyzers

The following basic rules are binding, when using the online reservation system. These guidelines apply to flow cytometric analysis appointments:

- The execution of the switch-on and switch-off routines for the analyzer explained in the instruction is mandatory.
- The device is to be handed over directly to the following user or it is to be ensured, e.g. by telephone call, that the following analysis appointment also takes place. This is to prevent running times of the device over night or in extreme cases over the weekend.
- The device-specific instructions are bindingly accepted by using the device on one's own responsibility.
- Damage to the analyzer caused by the user's faulty behavior is to be covered in full by the user causing the damage.
- At the end of the measurements, a control measurement with water must be carried out (settings like duration, measurement speed and threshold according to the specification of the respective device) and documented by saving the analysis file.
- The analysis must be documented in the log book available at the device, as well as any error messages that occur and problems encountered while using the instrument. In addition, in case of malfunctions, a CyTUM employee is to be informed immediately.
- No samples containing radioactivity or potentially dangerous germs for humans may be measured on the analyzer. Fixed samples are considered inactive.
- All samples must be filtered before loading (nylon filter material - 100 µm)
- In addition, a visual inspection for clumps and aggregates must be performed. For analysis purposes, the cell density should not exceed a cell count of 1 x 10^8 cells/ml.
- Once done, the user is responsible for sheath fluids refilling and waste disposal. Any sample materials present must be disposed of and the workplace has to be cleaned with a wipe disinfection.

Use of Sorter

The use of the sorting devices (FACS Aria, FACS Fusion, Astrios and S3) is possible without instruction, whereby the actual device handling is the responsibility of the respective operator of the CyTUM. Appointments have to be requested per e-mail (cellsort.mih@mh.tum.de). Once approved, the CyTUM staff will then transfer the booking in the online reservation system. The filled out sort registration form (available as download on the CyTUM website) must be attached to the mail.

Basic rules for sorting

For flow cytometry sorting, please note the following:

- The "sin tempore" scheduled times are binding for the user and the CyTUM staff.
- Experimentally caused time deviations must be communicated to the respective operator by telephone as soon as possible. The user will also be immediately informed by the operator of any malfunctions of the equipment and the associated time differences. In both cases, an appropriate time solution will be found.
- No samples containing radioactivity may be measured on the FACS device.
- Samples of safety level S2 according to GenTG may only be purified in rooms of safety level S2. In the event of a clogging at the device (danger of aerosols), the user has to leave the sort room immediately and may only return after the decontamination measures have been completed by a CyTUM employee.
- The selection of the device and the settings of the fluidic system are the responsibility of the respective operator. As a rule of thumb, the cell diameter of the sample must not exceed 1/5 of the Nozzle diameter. By default, a 70 µm Nozzle is used and the sort sample is filtered through a 30 µm FILCON. Any necessary changes to the Nozzle size must be noted on the sort registration form. The cell density should ideally be 1 x 10^8 cells/ml for cell sorting.
- Changes in Nozzle size require adjustment times of the fluidic system. Increasing the sorting stream leads to a downtime of the instrument of 12 hours - if the sorting stream is reduced, the fluidic system is usually stable again after 2 hours.