



## User Registration Form CMCB Technology Platform



### User information:

Full Name:	
Position:	
Group Leader/ Principal Investigator:	
Institution:	
Phone Number:	
E-mail Address:	

### Sample Information:

→ I certify that the samples contain no infectious or hazardous material, both for mice and man.

Does your sample contain <u>GVOs</u> ? (genetically modified organism, by German "Gentechnik-Gesetz" law)	No <input type="checkbox"/>	Yes (specify approval #, project leader): <input type="checkbox"/>	
If yes, where do you dispose the GVO material?	Facility <input type="checkbox"/>	Home Lab <input type="checkbox"/>	
Cell type/ Cell line and species:	Primary human <input type="checkbox"/>	Primary mouse <input type="checkbox"/>	Other (specify) <input type="checkbox"/>
	Human (line) <input type="checkbox"/>	Mouse (line) <input type="checkbox"/>	
If primary human, were the donors screened for blood-borne pathogens?	No <input type="checkbox"/>	Yes <input type="checkbox"/>	Unknown <input type="checkbox"/>
Are your samples fixed? (e.g.: EtOH, Formaldehyde, ...)	No <input type="checkbox"/>	Yes (specify): <input type="checkbox"/>	
List potentially harmful chemicals or toxins that you use	Propidium iodid <input type="checkbox"/>	Trizol <input type="checkbox"/>	Other (specify): <input type="checkbox"/>
Short description of the project, including facility devices used:			
User signature	Group leader signature	Date	



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### Safety instruction:

➔ I confirm that I am receiving safety instructions on a yearly basis by my host institution or have been instructed by the Facility staff for following topics:

General work and lab safety	Yes <input type="checkbox"/>	Instructed by the Facility (specify date): <input type="checkbox"/> <input type="text"/>	n.a. <input type="checkbox"/>
Biological safety (S1)	Yes <input type="checkbox"/>	Instructed by the Facility (specify date): <input type="checkbox"/> <input type="text"/>	n.a. <input type="checkbox"/>
Biological safety (S2)	Yes <input type="checkbox"/>	Instructed by the Facility (specify date): <input type="checkbox"/> <input type="text"/>	n.a. <input type="checkbox"/>
Laser safety	Yes <input type="checkbox"/>	Instructed by the Facility (specify date): <input type="checkbox"/> <input type="text"/>	n.a. <input type="checkbox"/>
Radiation safety	Yes <input type="checkbox"/>	Instructed by the Facility (specify date): <input type="checkbox"/> <input type="text"/>	n.a. <input type="checkbox"/>
User signature	Instructor signature (if applicable)	Date	

# CMCB Light Microscopy Facility (LMF)

Joint facility of BIOTEC/CRTD/BCUBE  
(valid from 15.07.2020)



LMF-rules, to be signed by every LMF user:

1. **Laser safety** instructions
  2. **Mercury lamps safety** instruction
  3. **Biological safety**
  4. Booking System use, "SIFFY"-emails
  5. Booking restrictions at LSMs
  6. Data storage and automated deletion procedure
  7. LMF Acknowledgement
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## 1. Laser safety instructions

(LSMs, TIRF, microdissection, ultramicroscope, as well for all LMF-rooms)

When using microscopes equipped with lasers, special care has to be taken to avoid laser radiation getting into your eyes or onto your skin. Especially, laser radiation can irreversibly harm your eyes. This relates to all laser scanning microscopes (LSMs), but also to wide-field microscopes that are equipped with laser illumination (Live Cell/TIRF AF6000, WF Laser Microdissection, Ultramicroscope).

Generally, all commercially bought microscope systems are typically equipped with electronic shutters that block lasers if the user is in risk of getting exposed to laser radiation (as for example if the transmitted light arm is pushed back). Still, the microscopes are categorized as class 3 laser devices (355 nm – 639 nm lasers), the SP8 MP, LSM 980 MP and the Ultramicroscope are even laser class 4 (680 nm – 1300 nm and 410-2400 nm, respectively). Laser class 4 means potentially harmful. If not operated properly, the risk of getting exposed to laser light (visible, and invisible; direct, and diffuse reflexes) is still there (e.g. if reflecting samples are tilted while image acquisition, or if objective lenses are removed and laser light can come out of the microscope stand collimated).

Therefore, all users need to be very careful when using microscopes equipped with lasers, as well already when working in LMF-rooms containing microscopes with lasers:

- Users are not allowed to remove objective lenses or other parts from the microscope system.
- The microscopes of the LMF are all intended to be used with biological samples. Any other types of samples (i.e. reflective samples in material sciences) are not allowed to be used without prior consultation of the LMF staff.
- Don't touch, tilt or exchange the sample during image acquisition. Don't put your hand into the laser beam. Image acquisition needs to be stopped before the sample can be touched, or removed, or exchanged to another sample.

- Don't bring any reflective items into the laser beam (tools, mirrors, wristwatch, jewelry).
- Avoid looking into the microscope from a direction towards the objective lens, where the laser could emerge from.
- Use minimal suitable laser power for your measurements.
- Alcohol and drugs can retard the blink reflex; don't work with laser devices in this case.
- Users are not allowed to bring colleagues /students etc. to the LMF rooms unless they signed these rules.
- Don't enter LMF-rooms when laser service is in progress (a warning sign will be at the door).

At the **Leica AF6000-TIRF** microscope, there is an obvious alignment procedure which involves that laser light is visible outside of the microscope. This TIRF-alignment is necessary before starting to measure in TIRF-mode. During the alignment, collimated laser light is released from the microscope towards the room ceiling. This laser light can in principle harm your eyes, and exposure to your eyes should be avoided in all cases. When the user is asked in the software to perform the TIRF alignment, be aware not to look from the top into the microscope. Also, don't bring any reflecting materials (like the glass surface of your watch or a ring on your finger) into the laser beam, when you push back the transmitted light arm. When adjusting the laser with the "Smartmove"-Joystick, keep seated in front of the computer and only look at the laser light spot which will be visible at the ceiling of the room. If you cannot see a laser spot at the ceiling, you can also use a tissue and look at the laser spot on that tissue from below. The laser power during the alignment is well below 1 mW (like a typical laser pointer), hence your eyelid would most likely close before laser light can harm your eye. The same applies if you accidentally forget to stop the image acquisition and push back the transmitted light arm, also in this case laser light will be released towards the ceiling. Hence, at the TIRF-setup, always pay attention and consider this laser light potentially harmful.

Additionally, the system technically allows to switch to the "Ocular"-mode when acquiring TIRF-images. The problem: when using the QAX-Filtercube (which has no emission filter that would block the laser stray light), the user will notice remaining scattered, non-collimated laser light visible through the ocular. This is another obvious situation where laser light is not confined to the microscope system, but emerges towards the user. The power is below 1  $\mu$ W and can therefore not harm your eyes, but still avoid this situation by not looking through eyepieces during any image acquisition involving lasers. Generally, switching to "Ocular"-mode is not allowed when using the QAX-cube, neither in TIRF-mode nor in FLUO-mode. In the latter case, the scattered excitation light from the HXP-lamp including UV-light would emerge towards the ocular. Hence, you are only allowed to switch to "Ocular"-mode in bright-field-mode and in FLUO-mode using all FLUO-filter-cubes but the QAX-filter-cube.

## 2. Mercury lamps safety instruction for (HBO, HXP, X-Cite, ...)

On most of the microscope systems, there are mercury lamps installed for wide-field epi-fluorescence observation and detection ("fluorescence lamps", called HBO, HXP, X-cite or similar). For all these lamp types, there is the danger of a lamp breakage, which would result in mercury being released into the air. A lamp burst can occur in very rare cases, however has happened already at LMF devices. This potential risk therefore exists, hence the following safety instruction is given:

In the case of a mercury lamp burst, all personnel should leave the immediate area (the room) **at once**, so that no mercury vapor is inhaled. The burst of the lamp can be a noticeable loud explosion, however can also happen unnoticeable. Indeed, it may happen in between two imaging session, when the system was left on for the next user, or when a user has left the room during a long experiment. Therefore, whenever a user enters an LMF room with a running fluorescence lamp (there are typically more than one systems per room), the user has to verify that the lamps are working correctly. This is typically indicated by a green LED at the lamp house.

If any malfunction of a lamp is suspected, the user has to inform the LMF staff and enforce all people to leave the room immediately. If this happens off-time (in the evenings or on the weekend), clearly mark the door from the outside “Mercury lamp burst – do not enter this room!”, and put a tape around the door and door frame, so that nobody can enter the room, and report the issue to [imaging@biotec.tu-dresden.de](mailto:imaging@biotec.tu-dresden.de). LMF staff will then care for the room (The room will be ventilated thoroughly (at least 20 to 30 minutes, 2-3 air exchanges) and after the lamp housing has cooled, mercury residue will be picked up with a special adsorbent ). LMF-staff will announce when the room can be used again in such a case.

### 3. Biological safety (S1/S2, infectious material, chemicals ...)

All LMF rooms are at least classified as S1. The LMF-room BIOTEC-226 is classified as S2. Eating and drinking is not permitted in any LMF room and wearing of lab coats is required. All further instructions need to be obeyed as stated in the Genetic Engineering Laboratory Operating Procedures and Hygiene Plan, which are positioned at the respective rooms.

If gloves are worn by the user when handling the samples, these gloves need to be taken off after the sample has been put onto the microscope stage. In particular, no gloves are allowed to be worn when touching any controls of LMF equipment (for example microscope buttons) or computers. Parts of devices, which cannot be avoided to be touched with gloves (microscope transmitted light arm, stage insert clamps, incubator doors etc.) need to be carefully decontaminated by the user, in case they were contaminated with S1 or S2 material. This needs to be done right after contamination and at the end of the imaging session, according to the hygiene plan (with 80% ethanol for S1-contamination, and with Mikrozid AF liquid for S2-contamination).

S1-samples can be disposed at the respected LMF-bins. S2-samples need to be disposed in the user's home lab.

Each user has to fill out the “User registration form” for every new project. This is mandatory to keep track of all samples involving genetically modified organism (“GVO”, by German “Gentechnik-Gesetz” law) or any other potential hazards (pathogens, harmful chemicals, toxins etc.). By filling out the form, the user confirms that his samples contain no infectious or hazardous material, and that he receives yearly safety instructions by his home lab of his institution.

## 4. Bookings, modifications and cancellations, “SIFY”-emails

Users can book LMF equipment 24 hours 7 days a week. Support by the LMF staff team is available on weekdays during normal working hours. If users are working in the facility at “after office” hours, users have to ensure that they are not alone in case of an emergency and are able to call for help.

Users can create and extend bookings any time (unless start time has not elapsed). The bookings can **at maximum be done 14 days (2 weeks) in the future**.

Users can **delete** or **shorten** their booking up to **24 hours** before the respective slot starts.

Less than 24 hours before a slot starts, **bookings are binding** and users are **NOT allowed** to cancel or shorten it (LMF can check for short notice changes!). The user will be charged for the slot and stays responsible for the system especially if it is the last slot of the day. Furthermore, to make the system available to another user, the user **is required** to write a **SIFY** (System Is Free For You):

1. **Send an email to the all user list that you are member of:** [imaging-all-user@lists.biotec.tu-dresden.de](mailto:imaging-all-user@lists.biotec.tu-dresden.de). It only needs to contain the following info in the subject field:  
*SIFY system name time of offered slot, e.g. SIFY SP5-I today 10-11am*
2. **If nobody replies:** It is still your **responsibility** to switch off the system if no one else takes it. If you are not around, please ask the LMF or one of your LMF trained colleagues to switch off the system.
3. **Take an offered slot:** you can take an offered slot (free of charge!) by replying to a SIFY email to the mailing list. Please indicate the time you would like to use in the email text field, please **keep the subject**.  
e.g. subject: SIFY SP5-I today 10-11am – reply email text field: TAKEN from 10:30-11am  
The new user will become **responsible** for switching off the system which is especially important as last user of the day and before weekends.

## 5. Booking restrictions at LSMs

### 5.1. General LSM booking restrictions

Only book maximum 3 hours LSM time during the core time (9am - 4pm on weekdays). If you need more time:

- Check the booking system on the **day before your booking after 5pm**: now you can freely extend your booking as needed.
- **Talk to each other** – you see names in the booking system, just get in touch with other users!
- Book longer slots outside the core time.
- Consult your LMF.



## 5.2. Booking time restrictions for LSM780/FCS microscope

FCS measurements might take longer, these fixed shifts will apply **in case of heavy usage**:

Weekdays	Weekends
1. Morning shift : as early as wanted – 12 pm 2. Midday shift: 12 pm – 4 pm 3. Evening shift: 4 pm – as late as wanted	1. Morning shift: as early as wanted – 2 pm 2. Afternoon shift: 2 pm – as late as wanted

Additional comments:

- Each user can only book one shift per day. Shorter bookings are possible, please stick to start or end time of the shift.
- As always: talk to each other to find solutions!
- From the **day before after 5pm**, you can book remaining time slots. All rules above don't apply in this case.

Communication within FCS user community is possible by using the list [imaging-FCS-user@lists.biotec.tu-dresden.de](mailto:imaging-FCS-user@lists.biotec.tu-dresden.de) (make sure to be subscribed to the list!).

## 6. Data storage and automated deletion procedure

During data acquisition save your data on a local hard drive, e.g. D:/USER/<YOURNAME>. Other locations, e.g. network or USB drives might cause problems.

The LMF is regularly cleaning up hard drives three times a year (Apr/Aug/Dec) after email notification via the imaging-all-user mailing list. **You are responsible for copying your data asap** to a safe storage medium, e.g. your fileserver space (within CMCB backed up daily by IT-department). Feel free to use the CMCB [owncloud](https://sharing.biotec.tu-dresden.de) (browse to “sharing.biotec.tu-dresden.de”) for data transfer (works with your booking system login). The **LMF cannot guarantee for your data** on LMF computers, hard drives can break! Make sure to close fileserver connections after data transfer! Do not save passwords on LMF computers.

You might bring your own laptop if you want to work while acquiring images. Users belonging to CMCB might connect personal laptops to available network sockets in the LMF rooms. Users from other institutes are not allowed to use these network sockets.

## 7. LMF Acknowledgement

Generally LMF users are obliged to acknowledge LMF usage and LMF support when

- **presenting data** as well as in
- **publications.**

Background is that the LMF needs to apply for funding of instruments and staff as well. Acknowledgements are the basis for these applications since it proofs the benefit created by the LMF.

Acknowledgment is possible in the material & methods part as well as in the acknowledgement. This could read like the following:

- **Material & methods:** “Confocal laser scanning was performed on an inverted Zeiss LSM 780 microscope of the CMCB light microscopy facility, a Core Facility of the CMCB Technology Platform at TU Dresden, using a Zeiss C-Apochromat 40x/1.2 water objective. Images were collected using 405, 488 and 561 nm laser lines for excitation and spectral detection bands ...“
- **Acknowledgements:** We thank the CMCB light microscopy facility, a Core Facility of the CMCB Technology Platform at TU Dresden for excellent support.”

**Please send your publications that benefitted from LMF usage to us**, we very much appreciate that!

You will need to confirm that you have read and understood the points above. Your signature will be collected on a copy of this document during your first introduction.