Light Microscopy Facility (LMF)

joint facility CMCB (BIOTEC/CRTD/BCUBE)

(valid from 1.11.2017)

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. Special booking restrictions at LSMs
6. Data storage and automated deletion procedure
7. Acknowledgement of LMF usage and support

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 SP5-MP and macro-SPIM are even laser class 4 (710 nm – 990 nm and 410-2400 nm, respectively), and by that they are classified as potential 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 things into the laser beam (tools, mirrors, wristwatch, jewellery).
- 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 µW 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 devices of the LMF. 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. 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 in BIOTEC and CRTD 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. The 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, “SIFFY”-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 make new bookings or extend their booking any time (unless start time has not elapsed). The bookings can **at maximum be 14 days (2 weeks) in the future.**
Users can **delete** or **shorten** their booking up to **24 hours** before the respective slot starts, without getting charged for it.

Less than 24 hours before a slot starts, **bookings are binding** and users are **NOT allowed** to cancel or shorten it, although the booking system allows it (all booking modifications are sent to LMF staff by the software). 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 **SIFFY** (**S**ystem **I**s **F**ree **F**or **Y**ou) email which works the following:

1. Send an email to imaging-all-user@lists.biotec.tu-dresden.de. It only needs to contain the following info in the subject field: SIFFY, name of system, time of offered slot
(e.g. SIFFY SP5-I today 10-11 am)

2. **Responsibility if nobody replies**: It is still the **responsibility** of the user who booked the slot originally to switch off the system, if no one else takes it. If you are not around to turn off the system, ask an LMF trained colleague of you or the LMF staff.

3. **Take an offered slot**: if a slot is offered in the SIFFY emails and you would like to take it: Reply to the list with “Taken from (time)”
(e.g. subject: SIFFY SP5-I today 10-11 am
text field: TAKEN from 10:30-11 am)
Answer the SIFFY email within the text field (don't change the subject field). The new user should not change the original booking, as the old user will be charged for the slot. The new user will become responsible for the system (see point 4.).

4. **Responsibility if somebody replies:** By officially taking the slot, the new user takes over the responsibility to switch off the system (if he is the last person of the day).

5. Special booking restrictions at LSMs

5.A. Booking restrictions for LSMs (except for LSM780/FCS)

Additional rules concerning the booking times of the highly used confocals (LSM) are:

- max. duration: 3 hours during the core time (9 am - 4 pm weekdays)
- by booking on the day before after 5pm you are allowed to book as you like. The rule above doesn't apply in this case.

These rules are not implemented in the booking system software yet and, if not absolutely necessary, we would like to keep it that way, to be able to respond more flexible to your imaging needs.

Longer booking times are still no problem outside the core time. If you need more time during the core time please consult the imaging facility. And most importantly, talk to each other if a bottleneck should occur. In the booking system you can easily see who booked a system and when (email-address, telephone number and room number is shown) and. Therefore, it should not be a problem to contact people.

5.B. Booking time restrictions for LSM780/FCS microscope

Bookings can only be done within fixed shifts:

Weekdays:
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

Weekends:
1.) morning shift: as early as wanted – 2 pm
2.) afternoon shift: 2 pm – as late as wanted

Additional comments to this shift system:

- Each user can only book within one shift per day
- Shorter bookings than the shift length are allowed, of course. Please stick to start or end time of shift.
- If you cannot book in advance what you need, try to contact the most booked user, or the LMF.
- From the day before after 5pm, you can book remaining time slots. All rules above don't apply in this case.

- If you want to let the other users of this machine know which booking preferences and wishes you have, feel free to describe your experiments and booking needs to the list email address: imaging-FCS-user@lists.biotec.tu-dresden.de (and make sure that you are subscribed to this list).

6. Data storage and automated deletion procedure

During data acquisition at the LMF-system, data need to be saved on the local hard disk at D:/USER/<YOURNAME>. Any other location (especially directly storing on a remote path like the fileserver or a USB-devices) causes problems.
To avoid shortage of local disk space on the systems, the LMF deletes the folder D:/USER/* upon email notice every four months (Feb/June/Oct).
Users need to copy their data to a safe storage medium. Users are free to choose this medium, e.g. the user's fileserver-space (for internal users, daily backup by the IT-department), a fileserver of the user's institute (for external users) or a personal USB-device. The LMF advices the users to make the copy as soon as possible after the data acquisition, as sudden data losses at the LMF-systems can happen.
For any connection to fileserver, it is the responsibility of the user to make sure to properly close the connection after copying the data. The user can simply try to connect again and make sure that the system asks again for a password. Never store any passwords on LMF-devices.
User can bring their own laptops if they want to work at their own computers while acquiring images. For users belonging to BIOTEC, CRTD or B-Cube, personal laptops can be connected to available network sockets in the LMF rooms. Users from other institutes are not allowed to use these network sockets.

7. Acknowledgement of LMF usage

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, 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 for excellent support."

The LMF will appreciate receiving publications acquired with LMF support or instrumentation.

I hereby confirm that I have read and understood the points above.

(Your signature will be collected on a copy of this document during your first introduction.)

Introduction for devices of the LMF at the BIOTEC/CRTD

To be filled by the user:

Name of user:_____ Group:_____

I confirm that my group member will get an introduction for the needed LMF devices. The staff costs for the introduction will be charged according to the current price list. Any costs resulting from his/her faulty operation of the equipment, i.e. repair of any damage caused by the user, will be charged to my group's account

Dresden, _____
groupleader

To be filled by LMF staff:

I confirm that I have introduced the user to the following equipment:

☐	ApoTome1 2nd floor CRTD	☐	ApoTome1 3rd floor CRTD
☐	ApoTome1 BIOTEC	☐	ApoTome2 CRTD
☐	Image Processing PC, BIOTEC	☐	Image Processing PC, CRTD
☐	Image Processing PC II, CRTD		
☐	Live Cell 4D BIOTEC	☐	Live Cell AF6000 CRTD
☐	Live Cell/TIRF AF6000 BIOTEC		
☐	LSI Macro Confocal CRTD	☐	Ultramicroscope CRTD
☐	LSM 700 inverse BIOTEC	☐	LSM 780 upright CRTD
☐	LSM 780/FCS inverse BIOTEC	☐	LSM 780/FLIM inverse CRTD
☐	SP5 I upright BIOTEC	☐	SP5 II upright CRTD
☐	SP5 MP inverse CRTD		
☐	WF Zeiss Laser microdissection CRTD	☐	WF Olympus upright BIOTEC
☐	WF Leica inverse CRTD	☐	WF Leica upright BIOTEC
☐	WF Screening System BIOTEC	☐	WF Slide scanner Axioscan CRTD
☐	SteMi Discovery CRTD		

Dresden, _____
facility staff

User Registration Form
BIOTEC/CRTD 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 *GVOs*? (genetically modified organism, by German "Gentechnik-Gesetz" law)	No ☐	Yes (specify approval #, project leader): ☐	
If yes, where do you dispose the GVO material?	Facility ☐	Home Lab ☐	
Cell type/ Cell line and species:	Primary human ☐	Primary mouse ☐	Other (specify ☐
	Human (line) ☐	Mouse (line) ☐	
If primary human, were the donors screened for blood-borne pathogens?	No ☐	Yes ☐	Unknown ☐
Are your samples fixed? (e.g.: EtOH, Formaldehyde, …)	No ☐	Yes (specify): ☐	
List potentially harmful cemicals or toxins that you use	Propidium iodid ☐	Trizol ☐	Other (specify): ☐
Short description of the project, including facility devices used:			

User signature	Group leader signature	Date

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 ☐	Instructed by the Facility (specify date): ☐	n.a. ☐
Biological safety (S1)	Yes ☐	Instructed by the Facility (specify date): ☐	n.a. ☐
Biological safety (S2)	Yes ☐	Instructed by the Facility (specify date): ☐	n.a. ☐
Laser safety	Yes ☐	Instructed by the Facility (specify date): ☐	n.a. ☐
Radiation safety	Yes ☐	Instructed by the Facility (specify date): ☐	n.a. ☐

User signature	Instructor signature (if applicable)	Date