Safety Assessment and Rules for the MBI Microscopy Core

The microscopy safety schedule extends the general safety regulation for NUS rooms and labs. Fire safety, handling of chemical, electrical devices and outlets are to be handled and respected as in all other labs. The items addressed here are in addition to all these preexisting measures.

This safety schedule does not comprise the protection of the instruments and the investment in the microscopy facility. Anyone who wishes to enter the microscopy rooms and use an instrument is required to get trained and certified on that instrument. The below outlined safety schedule is however part of that training.

The schedule for personal safety in the microscopy labs is divided in the following categories sorted by risk (risk = hazard x likelihood to happen):

i) Laser Safety
ii) Non-laser radiation
iii) Fire and suffocation
iv) Sharps
v) Explosion and shrapnel
vi) Chemicals and poisons
vii) Burns
viii) Mechanical injuries

Laser Safety

By far the greatest risk in the microscopy core are eye injuries due to direct exposure to powerful laser beams. All laser light used in the MBI with the exception of the optical traps (1064nm), and the 3 UV lines and the pulsed laser in TOYAMA's lab are visible. Visible laser light will trigger an immediate lid reflex and pupil contraction effectively limiting the exposure time to 50ms or less. This is however too long to prevent damage for any laser source stronger than 2mW at the eye. As all our laser sources are strong than this, it is *strictly prohibited for users* to **open laser combiners** (the boxes that host the lasers and feed the light into fibers) or to **unplug laser fibers**. The laser power at the lens is much weaker and less bundled than at the source or the fiber and hence the risk of using an intact instrument is always much lower than a partially disassembled one. As a trivial secondary rule it follows: never use a laser based system if you discover a damage to the fiber, casing or optics. Please stop using the instrument immediately and report of condition to the administrator of the system.

Laser license: If a system operates on a clear chamber or has more than 5mW power (at the laser), the user will still need a laser license! The core helps you to obtain the license but it is your responsibility to have a valid laser license.

The **risk of laser light** – if visible – comes exclusively from the fact that it can be focused onto a diffraction limited spot (usually less than a square micron) and that hence the power density can reach 500kW/square centimeter per mW laser power. No other light source has a comparable property and while Xenon and Mercury lamps if unprotected, can inure the eye, the damage is usually mild and temporary. Any measure that destroys the coherence of the laser light will also

eliminate this harmful feature of laser light. Diffusing material in the light path or defocus of the eye will disarm the laser light. Even a thin sheet of frosted glass or paper will prevent injury. If you are unsure about the state a microscope or its optics are in, overing the lens output with a sheet of paper will render the laser light harmless while simultaneously tell you were the light does travel. If you set up a system anew, it is hence always ways to initially keep the specimen covered.

Goggles. You can use laser goggles and we provide them as per regulation. Keep however in mind that on some TIRF systems and the SIM, the laser beam is stronger than the breakdown power on the goggle and hence you can sustain injury through the goggle! Also a tiny air gap between your skin and the goggle will be sufficient for injury. Most importantly, with the goggles on, you will not see the beam and hence perhaps be unaware of the laser exposure.
Goggles attenuate (weaken) the laser light very strongly but they do not remove the coherence and hence the focusing property!
Keep in mind that laser light is bad for your skin, not just your eyes. It can cause minor burns and if shorter than 460nm local DNA damage and cytotoxicity. Try not to get any laser light on your skin even though the damage thresholds are at least 5 order of magnitude higher than for your eyes. We are very skeptical about laser goggles because they make you unaware of laser exposure on your skin.

Systems where the beam widens very strongly after the lens, defocus the laser light and are hence without risk of injury if the operator keeps a distance of more than 10cm from the lens and does not insert reflecting materials into the beam path. This includes all **confocal systems** or **widefield systems**. Do not wear jewelry at any time when handling the running instrument or cover it with gloves. Jewelry is unacceptable in any microscopy room with a laser source independent of the laser power and the instrument type. Do not hold or tilt the slide while the scope is operating as the beam can reflect of the glass surface if the immersion breaks down.
If the above distance and reflective material rules are observed, confocal instruments do not require the use of laser goggles at any time of the operation.

Systems where the *beam is collimated* pose a much greater risk or injury as distance from the lens does not diminish the laser energy proportionally. All **TIRF microscopes** collimate light when used in TIRF mode. To make matters worse, they generally use much more laser power than confocal systems. All our TIRF systems are inverted which makes it easy for the laser beam to travel upward to the users eye. The risk of injury is hence substantial and laser safety has to be observed at all times when using TIRF.
The first and basic rule is: *never operate the system without immersion* and a specimen on it. If the beam mis-adjusted, specimen and immersion will force the beam upward, reducing the risk to reach your eye. Stay away at all times from the space above the specimen – never enter half- space of about 50 degrees half angle from the vertical line above the specimen.
When entering the TIRF angle, the laser beam 'flies' very above the stage. Without a specimen, it will exit horizontally above the stage. In a non-damaged TIRF system the laser beam only travels to the left and the right of the user. It

will not come forward toward the user. Hence rule #2 – never enter the space to the left or right of the chamber with your eyes at any time during operation.
The biggest danger when operating a TIRF scope are during setup if the system sustained damage previously. If the collimator has been bumped or deformed, laser light might travel forward during adjustment. We recommend to cover the test or calibration specimen with a diffuse material such a thin paper upon turning on the lasers, this way you will see immediately if the beam is aligned properly. If the beam comes forward do not use the system under any circumstance – cut the laser light and call the administrator.
During operation, please use a blackened chamber or cover. This way laser injury is all but impossible during the longest duration of the experiment.

The by far most dangerous scope is the SIM. Laser light can travel in all directions from the stage during the setup period and when adjustment screws are turned. Hence never power up the scope without the calibration specimen in it and always start with a covered specimen or the covered insert. If imaging is insufficient and you need to check calibration or light output, always cover your specimen before re-engaging the lasers.
The laser beams on the SIM are very powerful (~100mW per wavelength) and well collimated. Do not make assumptions or take any risk whatsoever with the SIM.
Never let untrained companions or people without a laser license touch any of the controls and do not access the adjustments without a fully covered specimen.
The microscopy core provides **full calibration** of the system by a trained specialist (LIU Jun), try to avoid grating calibration by yourself!
TIRF and SIM have unacceptable risk levels without precautions. Do not operate these scopes without understanding <u>all</u> risks involved.

	Likelihood	Degree of Injury
Confocal	Very low	Negligible (strong irritation)
Widefield	Low	Substantial long lasting visual impairment
TIRF	Average-Low	High (local retinal damage)
SIM	Average	Very High (permanent retinal damage)

Measures:
All users of lasers must be licensed. Users must obey all laser safety rules at all times and have to demonstrate their handling skills to gain access rights (all implemented).
On systems where the specimen doesn't need to be visible during operation we use non-transparent chambers with laser safety doors entirely eliminating laser injuries to the user (implemented where applicable – ELYRA and ILAS2).
Confocal training and safety is sufficient as hazard is low.
Recommend: additional signs for all TIRF systems. Make frosted glass covers available. Provide more laser goggles. Provide fluorescent sheets so laser light can be seen despite the goggles.
Consider: front shield with attenuation coating for the SIM. Have frosted boxes manufacture for TIRF and SIM so users can safely check direction of laser light.
Install speckle detector warning system which alerts users to stray laser light.

Non-laser Radiation

We have several short arc lamps in operation: xenon burners (XBOs) and mercury burners (HBOs) pose a moderate radiation risk as their light emitting region is very small (usually shorter than 0.3mm) and their radiation output is high (up to 20W) which when focused does injure the human retina. Their spectra cover however the entire visible range and in turn they trigger a very strong lid reflex and pupil contraction so that permanent damage rarely occurs even though the exposure can trigger temporary blindness and intense pain.
XBOs emit a substantial amount of UV radiation and HBO a very large amount of UV and deep UV. HBO radiation is immediately harmful to all uncovered skin and one should never risk exposure. All MBI HBO bulbs are coated against hard UV and should not emit light shorter than 360nm. Failure of the coating can be immediately noticed by strong ozone small emitted from the lamp casing. If you smell ozone, please immediatel shut down the arc lamp and alert the staff.
All our arc lamps are encased which makes direct exposure unlikely. The light is fed into the illuminator of the scope or a multimode fiber. If you see any white light from an arc lamp emanating from the scope casing or the fiber casing (i.e. not from the illuminator lens or the objective), please also shut down the arc lamp and alert the staff.
Unfiltered light from either XBO and HBO is too intense even at the fiber or the casing to be safe for human eyes. It also contains UV in the 360nm to 400nm range at normal operating conditions.
If you use a filter selector for UV dyes (especially DAPI), be alert that now the light cannot be seen anymore. Keep hands away from the illuminated area and to not come close to the objective with your eyes. Normal coated glasses will shield your eyes from this kind of UV radiation. Lab goggles usually will not, so observe the distance.

	Likelihood	Degree of Injury
Incandescent	high	None, warm soft light
Visible LED	high	None, intense white or red light
Solid state source	Very low	Negligible, strong irritation
XBO	Very low	Very low, pain and temporary blindness
HBO	Very low	Moderate, UV stress, pain, and lasting irritation

Measures:
Radiation warnings for XBO and HBO are part of training schedule. Recommend to stress it in more detail in SOP. Signage makes no sense as the devices are safe as long as they are encased.
No action needed for incandescent and LED and solid state sources.

Fire and Suffocation

Fires pose a somewhat augmented risk in the microcopy rooms compared to the open labs. For one the user is trapped behind two doors (scope door and main door) and the rooms have a dedicated fire extinguishing system (FM200 from DuPont) which displaces some of the oxygen in the rooms.
The doors are arrange in such a way that if the pressure level outside of the rooms drops due to fire, the doors will swing open to the lower pressure area. All doors can be pushed open from the inside by human force. None of the doors is locked at any time. If the fire alarm sounds, determined push your door open and

leave. It is ok to close the room doors behind you to avoid fire, sot, and dust damage to the microscopes. The central microscopy door in level 9 is pressure neutral and slides sideways. Its motor can be overridden by manual force should the power suddenly fail. The doors to the individual rooms would suction lock if the fire broke out in the rooms. It is hence important to maintain a minimum amount of combustible liquids in the rooms. We limit the amount of immersion oil and cleaning alcohol in the rooms and ask everyone not to increase these amounts. Fires stared in the scope rooms are very unlikely due to the metal tables and the encased motors and drives of the scopes. If you add electronics and machines to the rooms please ensure they are certified and ground at all times. Also do not use drives with brushed motors or older fan motors (which can spark). All oil pumps must be metal encased and grounded. If you use high voltage equipment, please seek permission to do so prior to starting it.
The fire siren is audible in the individual rooms but attenuated strongly by the thick walls. *Do not wear headphones or operate any audio entertainment* when in the microscopy rooms at any time! You will not hear the file alarm anymore. We evaluated xenon flashlights in the rooms to alert the users to fire danger but they do ruin experiment recording and we have a large number of test and false alarms. We do hence rely on the acoustic signal, please make sure that you can hear it at all times!
Should smoke, heat, or fire reach the microscopy rooms, the central area will be flooded by FM200 gas. The system will issue a second horn alarm after which there will be a 30 second delay before the gas is released in very large quantities. FM200 is non-toxic and only mildly irritating. And while it does displace a large amount of air, it is not a suffocating gas. It fights fire by a competing low-energy reaction with the fire and not by oxygen displacement.
While you should be safe in an FM200 atmosphere, it is against local regulation to be there – you are urged to leave the room within the 30 second evacuation time and not enter any of the rooms again until the fire department has cleared the area and the normal atmosphere is restored.

	Likelihood	Degree of Injury
Fire in scope room	Extremely low	High – evacuation will be impeded
Fire in main room	Very low	Low – evacuation will be possible
FM200 discharge	moderate	Very low – danger from fire only

Measures:
Fire alarm: microscopy core has been certified and is hence fire safety compliant.
Recommend: put signs up not to use audio equipment or headphones inside the rooms.
We evaluated optical warning signs and would like to have them but only if non-interference with experiments can be assured. So far, no suitable product has been found.
FM200: technically no action required. We could put up signs explaining the timing of the FM200 release if users forgot about it.
Unsure: shall we install foam fire extinguishers in each room?

Sharps

The two most common sharps inside the microscope rooms are cover slips and injector or pulling needles.
Cover slips are not sharps per se but they are very thin (.17mm) glass plates which easy break and then expose cutting surfaces. Due to their limited strength degrees of injuries to hands is limited to minor cuts. The risks are mainly in the shards not being easily visible and possibly contaminated by specimen.
The microscopy core owns a HEPA filtered vacuum cleaner and no attempt should be made to collect glass fragments by hand. Used cover slips can be left glued to the slides and disposed as a whole. We recommend not to re-use cover slips. If you have to clean and re-use expensive sapphire slip, we recommend to use the sonicator or spin cleaner with no manual interaction in the cleaning process.
Microinjector needles are usually made from pulled glas and very pointy once mounted, they should only be moved by the actuators and not by hand. The specimen should also not be touched by hand during the operation. The needle tips are virtually invisible (only on phase microscopy) and paired with the injection mechanism are reasonable safety risk. Sharps containers must be used for disposed needles.
Pulling and force transduction **needles or cantilevers** are made of any material. We do currently not provide such equipment and hence have no safety schedule for them. Please obey your lab's guidelines when using them.
We do currently not operate any core **microtomes** and hence glass blades are not in use at the MBI Microscopy Core.
Syringes used for flow chambers are blunt compared to medical injection syringes and hence skin penetration requires a substantial force to occur.

	Likelihood	Degree of Injury
Cover slip cut	High	Minimal and local
Microinjector prick	Moderate	Low without injection flow
Syringe prick	Very low	Skin injury unlikely

Measures:
Needles and sharp pipettes: users are instructed during lab safety training. Labeling the injectors might be sensible.
Cover slips: users are made aware of during normal microscopy training. General sign in the microscopy room could be useful. Also to tell users where to find the vacuum cleaner.

Explosions and Shrapnel

While we do store open Ethanol in all rooms for lens cleaning. The dispensers limit the amount to a small squirt per push. Forced airflow in the rooms is high and hence flammable aerosol-air mixtures are considered impossible to build up. No flammable gasses are currently provided by us and hence no such mixtures need to be considered.
HBO bulbs, especially when still warm are under considerable gas pressure (same for the closely related HID bulbs) and they can fragment when touched or dropped. While the blast energy is too low to inflict considerable injury, the shrapnel from the bulbs is considered dangerous for skin and eyes in particular. The shards are also contaminated by mercury and hence to be considered mildly toxic. MBI does not use the common radioactive bulbs. XBO bulb shatter even

more violently but only upon heat-up and hence the fragments are contained by the lamp house.
Our rule is that users are not allowed to swap HBO and HID bulbs. Only the administrator can do so and only when the bulbs are cold.

	Likelihood	Degree of Injury
HBO burst	Moderate	Substantial
Aerosol explosion	Impossible	High – blast injuries

Measures:
HBO bulbs: users are told during training not to access bulbs. Recommend to put labels on the HBO/HID sources.
Aerosols: no action to be taken as we close microscopy room when airflow is halted.

Chemicals and Poisons

Most of the chemicals used inside the microscopy rooms are of low toxicity. One oddity is that one should not get immersion oils on the skin and wash or wipe it off immediately when getting into contact with it. While the standard calibration oil with a refractive index of 1.515 (from Olympus, Cargyle, Zeiss etc.) is considered only critical when ingested, higher refractive index oils are often mild contact poisons. Oils with RI above 1.6 also have toxic vapours and hence handling should be done at a distance from the face. We do not distribute such oil to the users. We do however have augmented RI oils for the PALM and the Deltavision microscope.
None of the chemicals should be ingested (that incudes the methanol enriched cleaning alcohol) and no drinks or foods must be consumed in any of the microscopy rooms).
We use concentrated acids for cover slip cleaning (especially for single molecule imaging), coatings for cover slips, and acetone suspension for sealing cover slips, and isopropanol for the high speed cleaner. But all these manipulations are performed outside of the microscopy rooms and hence no part of this schedule.

	Likelihood	Degree of Injury
Immersion oil	High	Negligible for short contact periods
Cleaning alcohol	Very high	None

Measures:
Oils: users instructed during training (implemented). To be added to SOP (not yet implemented).
Alcohol: no action to be taken.

Burns

Lamp houses get very hot during operation. Laser power supplies and encasings of gas lasers get hot.
Lamp house burns are unfortunately very common. Incandescent and HBO cases on illuminators are small and badly vented and hence get very hot. They feature grills which show that the light is on but they casing stays hot long after the light is switched off. HBO casings get even hotter than incandescent cases.

	Likelihood	Degree of Injury
Incandescent	high	Low – mild skin burn

HBO on scope	high	Medium low – light skin burns
XBO	moderate	Low – mild skin burn
Laser casing	low	None – retraction reflex fast enough
External HBO, HID, solid state	Very low	None

Measures:
Lamp houses: users are warned during training to stay clear of the encasing (implemented). 'Hot' lamps warning the users have been rejected because they may interfere with experiments.
All our newer external burners and arc lamp are forced cooled by fans and hence do not get critically hot.
No action needed regarding laser encasings as detrimental effects are too mild to be worried.
Ongoing: replacing HBOs with external HIDs and LED sources which also get hot but to a much lower degree.

Mechanical Injuries

While no person was ever injured at MBI this way, we experienced equipment damage by falling equipment from the elevated shelves and by motorized stages hitting lenses or other parts of the microscopes.
We have responded to the former thread by securing all elevated equipment with rubber feet or restraining the wiring so it is unlikely to be pulled. Most wiring is drawn to the backside of the microscope tables and this area is off limits for users. We do ask you to honour these zones and not run the risk of pulling down equipment by crossing the fences. All instrument panels are accessible from the front.
The motorized stages move with a lot of force and they can injured hands caught between moving parts of the stage. This is only a risk if the stages are operated automatically (i.e. when letting the joystick go will not stop them) and when traveling long distances simultaneously. Hence if you dry-run multi-well scan sequences, keep hands well clear of the stage. There is no emergency out for the stages and switching to manual mode will often be difficult. Hence if you get caught by a stage, it will take time to get freed.
Similar as for setting up laser illumination, we recommend to install the specimen, get clear of the scanning area and then engage stage and light sources. If you have to access the specimen "in experiment" make sure the scope is in manual mode again.

	Likelihood	Degree of Injury
Falling equipment	Extremely low	Potentially severe, 2kg box falling 1m
Trapped in stage	Very low	Minor – painful shear movement

Measures:
Falls: wiring moved to the back, backside fenced off. Signes installed that users shall keep clear (implemented).
Stages: follow protocol. If get trapped switch system to manual mode, free yourself. Keep calm while being caught (nothing implemented - to be added to SOP and training)

Contacts and resolution

Please forward complaints and suggestions or seek clarification for certain matters from these staff members in charge:

- rule resolution and administrative issues: Felix MARGADANT
- laser safety: LAU Wai Han
- bio hazard: CHIN Fei Li Jasmine
- Instrumentation: LIU Jun and MAK Kah Jun
- IT: Felix MARGADANT

If you wish changes to be implemented or if you are in disagreement with a staff member, please contact the microscopy head Felix MARGADANT. If you need to resolve dispute with the head, please contact any member of the microscopy committee (TOYAMA Yusuke, KANCHANAWONG Tony, YU Cheng Han, Jay GROVES, or its head G.V. SHIVASHENKAR)