

1. Biological agents

- Risk classification
- Rules of conduct
- Proper disposal
- Hygienic measures

2. Handling of hazardous substances

3. Actions in case of an accident in the lab

4. Working with lasers

5. General behavior in case of emergency

When handling biological agents in the laboratory, special rules of conduct and compliance with certain protective regulations must be observed.



Symbol for biohazard

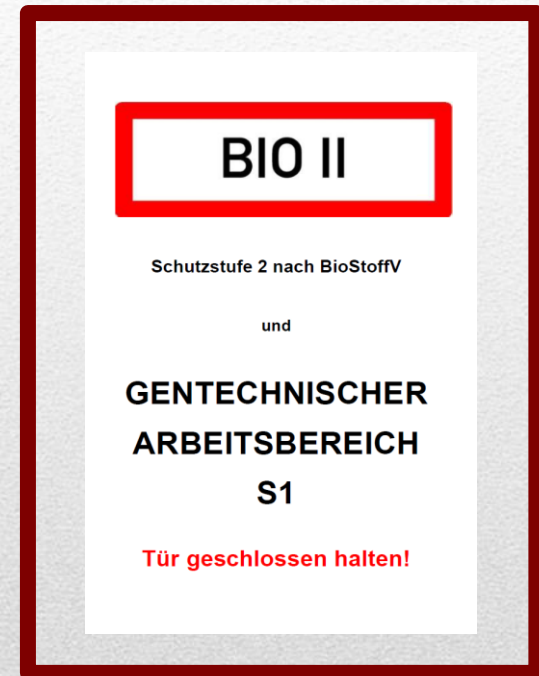
Area of application

- cell culture lab (r. 117)
- storage room (r. 116 + ante-room)
- MRE / CLSM lab (r. 115)
- AFM labs (r. 131 a/b + ante-room)
- SpiDi lab (r. 132)
- BONG lab (r. 309)
- chemistry lab (r. 310)
- OS lab (r. 311)

These rooms are labelled as

“BIO II” and / or

“Gentechnischer Arbeitsbereich S1”



Responsible persons

phone

responsible for genetic and bio labs: Prof. Käs

0341/9732470

responsible for biological safety: Dr. Weizenmann

0341/9432592

emergency call / technical service:

0341/9734333

medical office:

0341/9938-00

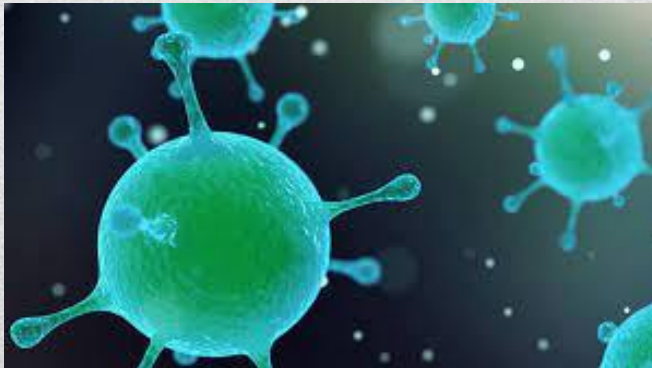
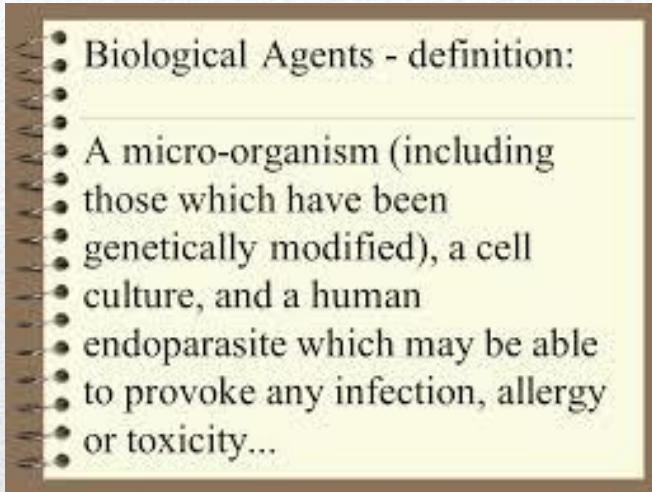
regulatory authority:

Sächsisches Staatsministerium für Energie, Klimaschutz,
Umwelt und Landwirtschaft (SMEKUL)
(Saxon State Ministry for Energy, Climate Protection,
Environment and Agriculture)

see
operating
instruction
(page 2)

<https://www.smekul.sachsen.de/>

Biological agents in general:



picture: <https://www.svlfg.de/biologische-arbeitsstoffe>

Categorisation into different risk groups

- ➔ **risk group 1**
(e.g. bacteria, yeast)
- ➔ **risk group 2**
(e.g. salmonella, hepatitis A)
- ➔ **risk group 3**
(e.g. HIV, SARS-CoV-2)
- ➔ **risk group 4**
(e.g. variola, Ebola)

Genetically modified organisms (GMOs):



organism whose genetic material has been altered in a way that does not occur naturally (crossing or natural recombination) and is capable of replication or of transferring genetic material

picture: <https://microbenotes.com/genetically-modified-organism/>

Risk classification

Bio-substances / GMOs are classified into one of the following risk groups according to the risk of infection they pose, based on the state of scientific knowledge:

Risk group 1: they are unlikely to cause disease

Risk group 2: they can cause a human disease and pose a risk to workers; they are unlikely to spread to the population; effective prevention or treatment is possible

Risk group 3: they can cause a serious human disease and pose a serious risk to workers ... effective prevention or treatment possible

Risk group 4: they can cause a serious human disease and pose a serious risk to workers ... effective prevention or treatment **not** possible

1. Biological agents – Risk classification

Biological agents in our group are:

**cell lines and
genetically modified organisms
(GMOs)**

→ risk group 1

**→ safety level S1 accordingly
Genetic Engineering Safety
Regulations (GenTSV)**

- commercially acquired cell lines
- already genetically modified cell lines from other laboratories
- cells, transfected in our cell culture lab

**non tested primary tissue
samples from university
hospital**

→ risk group 3**

**→ safety level S2 accordingly
Regulation on Biological
Agents (BioStoffV)**

- human tissue samples directly obtained after surgery

1. Biological agents – Risk classification

do not confuse risk group 3 and risk group 3**

risk group 3** means a non-targeted activity:

- activities are not directly related to one or more biological agents
- biological agents are not known at least by species

human primary tissue could contain viruses, bacteria,
parasites which can causes infections and diseases



**safety level S2 accordingly Regulation on Biological Agents
(BioStoffV)**

1. Biological agents – Risk classification

Before starting work, a risk assessment (determination of the hazard potential) must be carried out for the planned work, from which the safety classification of the work results.

In the case of new obtainment of biological agents (cell lines, GMOs, primary tissue etc.), **you have to inform the responsible of the lab about the planned work in order to classify the potential risk.**

Responsible of the lab: Undine Dietrich, room 315, tel. 32472
(dietrich@physik.uni-leipzig.de)

Likewise the Biosafety Officer (BBS) has to be informed.

BBS of the faculty: Nicole Weizenmann, room 423, tel. 32520
(weizenmann@uni-leipzig.de)

1. Biological agents – Rules of conduct

Before starting work:

- receive the safety instruction
- get briefing from a trained employee
- inform yourself about potential danger (see also chapter 2 and 3)



Access to laboratories only after safety briefing

and

for visitors only in the presence of a trained employee.

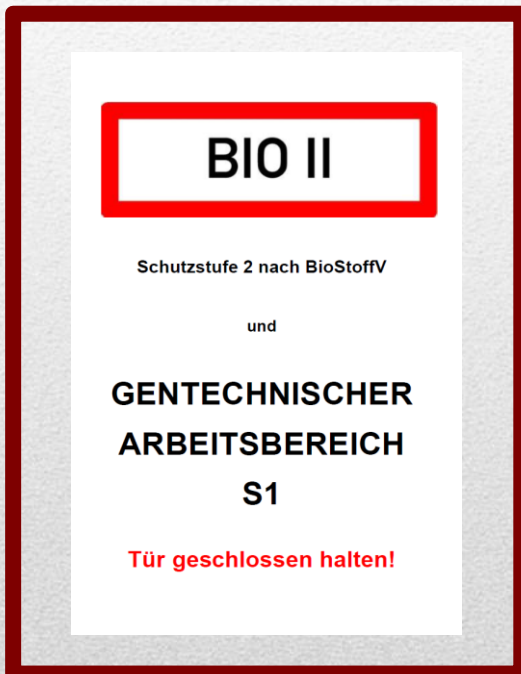
picture: <https://microbiozindia.com/lab-safety-rules-in-pharmaceuticals-all-you-need-to-know/>

Before starting work:

- inform yourself about
 - operating instruction (Betriebsanweisung)
 - skin protection plan (Hautschutzplan)
 - hygiene plan (Hygieneplan)
 - emergency plan (Notfallplan)

(displayed in each lab and on our PWM website
→ → → internal – lab safety)
- take the necessary protective measures

Work with bio-substances of **risk group 3**** may only be carried out in laboratories of **safety level S2**.



These laboratories comply with safety level S2:

- cell culture lab (r. 117)
- storage room (r. 116 + ante-room)
- MRE / CLSM lab (r. 115)
- AFM labs (r. 131 a/b + ante-room)
- SpiDi lab (r. 132)
- OS lab (r. 311)

There is an obligation to keep records for work with GMOs and bio-substances.

**GENTECHNISCHER
ARBEITSBEREICH
S1**

Tür geschlossen halten!

These laboratories comply with safety level S1:

- BONG lab (r. 309)
- chemistry lab (r. 310)

**There is an obligation to keep records
for work with GMOs and bio-substances.**

1. Biological agents – Rules of conduct

University of Leipzig, Faculty of Physics and Earth Science, Dept. of Soft Matter Physics
and Dept. of Molecular Biophysics

OPERATING INSTRUCTIONS

ACCORDING TO

§12 GENTECHNIK-SICHERHEITSVERORDNUNG
FÜR GENTECHNISCHE LABORBEREICHE DER
SICHERHEITSSTUFE S1

**There is an obligation
of the compliance of
the operating
instruction.**

Stand: 03.08.2018

The operating instruction according to

“Biostoffverordnung” (BioStoffV)
→ Ordinance on Biological Agents

correspond to those for

“Gentechnik-sicherheitsverordnung”
(GenTSV)
→ Genetic Engineering Safety
Regulations

are displayed both in the detail and in
summary form in each laboratory

... and on our **PWM website**.

→ → → internal – lab safety

Wear protective equipment:



- closed lab coat
- disposable protective gloves
- sturdy and closed footwear
- protective eyewear if necessary (liquid nitrogen)

**Remove protective clothing before leaving the laboratory.
Do not wear protective gloves outside the laboratory!**

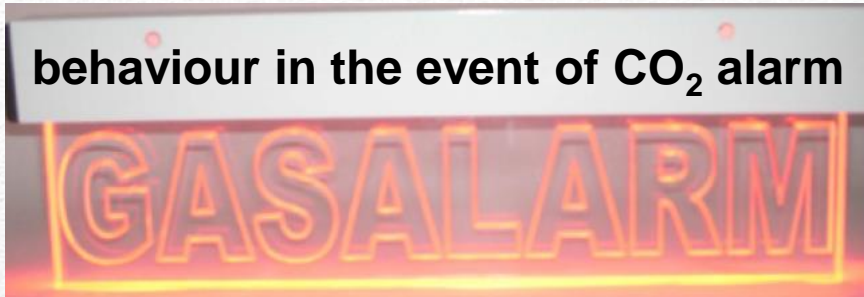


1. Biological agents – Rules of conduct

- doors and windows must be closed during work
- keep laboratories clean and tidy
- keep order and cleanliness at the workplace
- clearly identifiable labelling of samples and cell culture bottles
- wash and disinfect hands after finishing work or before leaving the laboratory (see skin protection and hygiene plan)
- eating, drinking, smoking and storing food is prohibited
- knowledge of operating instructions, hygiene and skin protection plan

1. Biological agents – Rules of conduct

behaviour in the event of CO₂ alarm



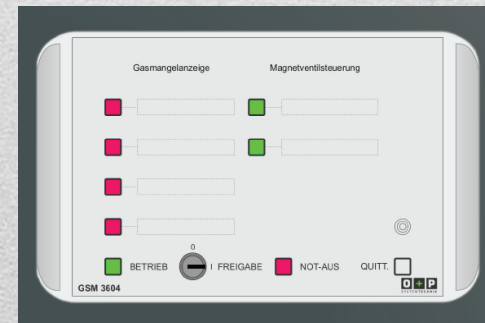
room 115 (CLSM/MRE lab) and room 117 (CC lab) are equipped with a CO₂ warning system



the CO₂ sensor is positioned near the floor (CO₂ accumulates on the ground)

the alarm is triggered at 0,5 vol% → acoustical „BEEP“
AND → visual „GASALARM“

Not to be confused with the control of the gas tapping point!!!



behaviour in the event of CO₂ alarm



in case of an alarm:

- if possibly open the window and push the emergency stop (r. 115)
- leave the lab immediately
- keep the door open
- inform the dispatcher (34333)

no "storage" of packaging material or other empty containers in the laboratories, each employee has to ensure immediately disposal:

- polystyrol boxes in the **yellow container** for plastic waste in the courtyard
- empty ethanol containers in the **yellow container** as well
- cardboard boxes in the **blue container** for paper in the courtyard
- empty glass bottles or glass breakage in the container for glass in the courtyard - **no glass breakage in normal waste**

1. Biological agents – Rules of conduct

- use syringes, cannulas, scalpels only if absolutely necessary, disposal in puncture resistant, autoclavable container
- do not replace the protective cap after using cannulas, syringes, scalpels – **risk of injury** - but dispose immediately in container



1. Biological agents – Rules of conduct

- avoid formation of aerosols (e.g. during decanting, stirring, shaking, pipetting, centrifuging, working with ultrasound)
- for internal transport of GMOs or bio material, use tightly closed, break-proof, liquid-tight and autoclavable containers



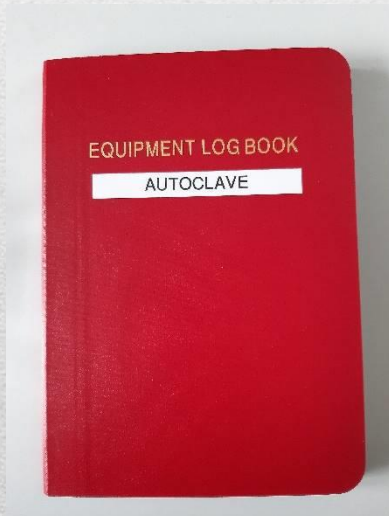
- storage of GMOs or primary samples in liquid nitrogen (r. 116)



1. Biological agents – Proper disposal

Solid waste that contains GMOs or bio material must be inactivated before disposal and then discard as residual waste.

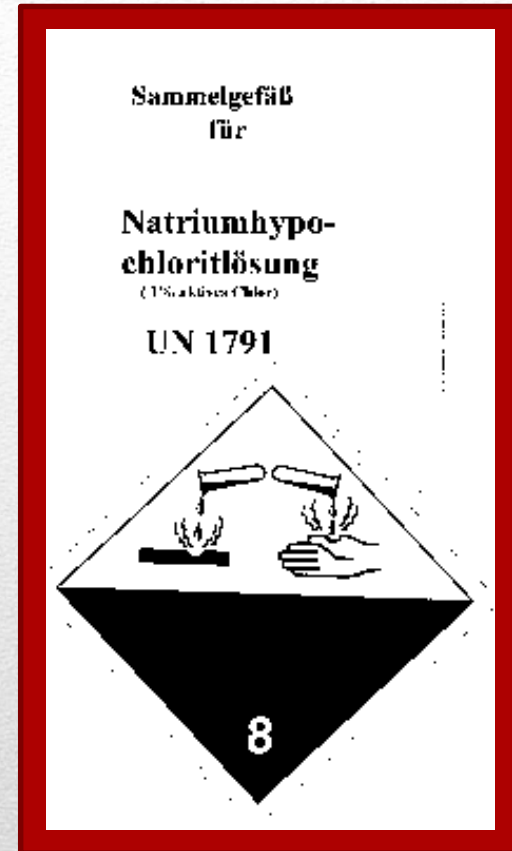
autoclav (r. 116, ante-room) 121 °C, 20 min, program P3



- get briefing from a trained employee
- every using of the autoclave must be documented in the equipment log book
- autoclaving should take place during the working day if possible

1. Biological agents – Proper disposal

Liquid wastes containing GMOs or bio material are inactivated with 1% Na-hypochlorite solution and collected in appropriately labelled containers.



Other hazardous substances in accordance with the **Material Safety Data Sheet (MSDS)** of the respective manufacturer (see also chapter 2. Handling of hazardous substances).

1. Biological agents – Hygienic measures

After finishing work:

- clean work surfaces, equipment and instrument (ethanol 70%, Bacillol AF)
- switch off all devices (in the hood, microscope, cell counter etc.)
- after cleaning, switch on UV lamp of the safety work benches
- clean your hands before leaving the laboratory (Sterillium, Baktolin, Baktolan ... **see skin protection and hygiene plan**)



by the way:

the weekly cleaning of the floor is done with Microbac forte, please follow the dosage of **20 ml to 1 l water**



What are hazardous substances?

Hazardous substances are substances and preparations which are very toxic, toxic, low toxic, corrosive, irritant, explosive, oxidising, highly flammable, highly flammable, carcinogenic, fruit-damaging, mutagenic and/or biohazardous,

or

which, when used, give rise to dangerous or explosive hazards substances or preparations are formed or released.

Resulting from this: ...

Inform about potential dangers **before** starting work and take the necessary protective measures.

2. Handling of hazardous substances

First information are on the packages / bottles in form of pictograms:



picture: <https://chemicals.ie/msds/>

2. Handling of hazardous substances

Meaning of pictograms



harmful



toxic / very toxic



corrosive



highly flammable / inflammable



fire accelerating



endangering the environment

For detailed information see the corresponding Material Safety Data Sheet available at the internet site of the manufacturer.

2. Handling of hazardous substances

MSDS contains information about:

- hazards identification
- composition
- first aid measures
- firefighting measures
- accidental release measures
- handling and storage
- exposure controls/personal protection
- physical and chemical properties
- stability and reactivity
- toxicological information
- ecological information
- disposal considerations



General rules of conduct:

- transport of glass bottles in appropriate transport containers (risk of breaking) - e.g. bucket or basket



... and never carry bottles on the neck

- mark vessels: name of the substance, name of the user, corresponding hazard symbols
- storage only in containers which are suitable for the hazardous substance (e.g. no plastic bottles for solvents)

General rules of conduct:

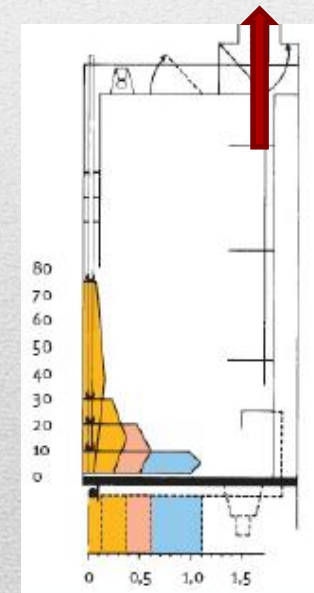
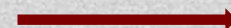
- keep very toxic, toxic, carcinogenic, fruit-damaging and mutagenic substances under lock and key
- when openly handling gaseous, dust-like or hazardous substances that have a high vapour pressure work always in a fume cupboard (chemical lab room 310)

when the sash is pulled down, the effect of the fume cupboard is at its best

sash gap (cm)



average air velocity in the sash gap (m/s)



- proper disposal (s. **MSDS**)

Liquid nitrogen (LN2):

- **has a boiling point of -196°C**
- **is heavier than air and accumulates on the ground**

Liquid nitrogen hazards:

- skin contact with liquid nitrogen can lead to cold burns and frostbite
- LN2 has a suffocating effect in high concentrations without noticeable signs, since oxygen in the breathing air is displaced by an accumulation of nitrogen
- depending on the duration of inhalation and the remaining oxygen concentration, drowsiness, malaise, blood pressure rise and shortness of breath occur
- plastic containers can become brittle in LN2, danger of splitting when material bursts

Protective measures and rules of conduct:

- always wear suitable protective gloves and eyewear
- clothing should cover all parts of the body (closed footwear!)
- do not use elevator together with LN2 container



Behaviour in case of danger:

Leakage of the bio-rack - the LED display of the level monitor lights up and a continuous signal sounds

- leave the room immediately or do not enter it
- leave the door open and come for a second person
- then enter the room only one at a time and ensure oxygen supply, open the window

First aid:

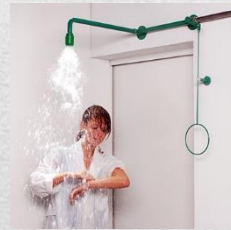
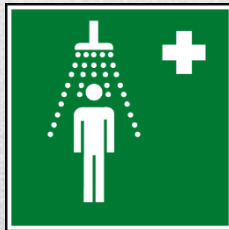
- skin contact: treat as burns or frostbite, do not rub, cover with sterile dressing
- eye contact: immediately rinse with water for at least 15 minutes
- inhalation: high concentrations may cause asphyxiation → fresh air supply, keep warm and calm, seek medical advice, if breathing stops artificial respiration

3. Actions in case of an accident in the lab

In order to avoid panic in case of emergency, everyone has to inform himself **before starting the work** about:



location of eye showers
in room 310 permanently installed,
otherwise flushing bottles



location of body shower (in room 310)



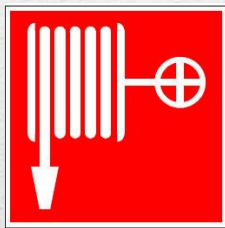
first aid facilities in room 310 and 116 (anteroom)
→ label at the lab door

3. Actions in case of an accident in the lab

In order to avoid panic in case of emergency, everyone has to inform himself **before starting the work** about:



function of disinfectants (Bacillol, Sterillium)



fire-extinguishing equipment



escape and rescue routes

Leakage or spillage of hazardous substances / GMOs / bio-substances

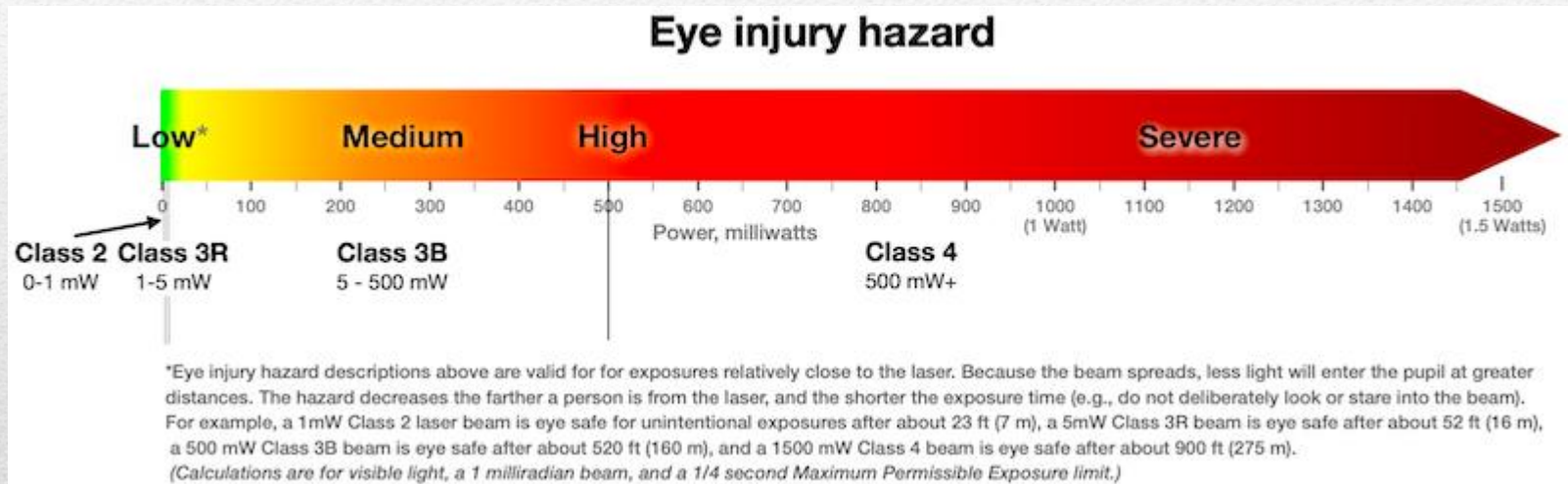
Put on gloves, secure affected area – take decontamination measures:

- **surfaces / devices:** collect material, dispose it properly or in case of biological agents take up material with paper towels or other autoclavable material and autoclave, then wipe disinfection
- **skin:** rinse off contaminated skin areas with plenty of water / disinfect
- **mucous membranes / eyes:** rinse off with plenty of water or take an eye shower
- **clothing:** take off and autoclave
- **inform laboratory manager / project manager,** consult doctor if necessary

4. Working with lasers



Lasers are classified based on their potential for causing injury — especially eye damage, since the eye is most susceptible to excess laser light.



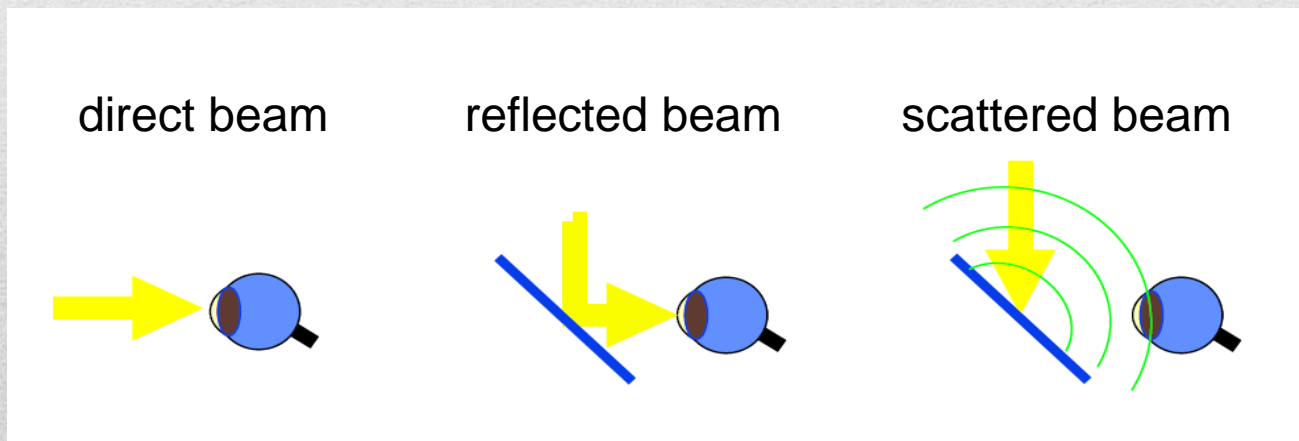
There are four main classes for visible-beam lasers: Class 2, Class 3R, Class 3B and Class 4. The first two are relatively safe for eye exposure; the last two are hazardous. The chart shows that the eye injury hazard increases as the laser's power increases.

picture: <https://www.laserpointersafety.com/laserclasses.html>

Hazard potential by laser:

- deposition of thermal energy in tissue
- tissue reactions or damage, dangerous for eye and skin (deep skin burn at IR wavelengths)
- local temperature rise, fire and explosion hazard

Damage can be caused by:



picture: www.cpg.uni-freiburg.de/fakultaet/wrkstatt/elektronikwerkstatt/documents/laser.pdf

In order to assess the risk potential, we have to distinguish between commercially purchased and own experimental setups.

In **purchased devices**, the lasers are encapsulated and existing safety devices prevent the unintentional escape of the laser beam. If used properly, there is no potential danger from laser beam.

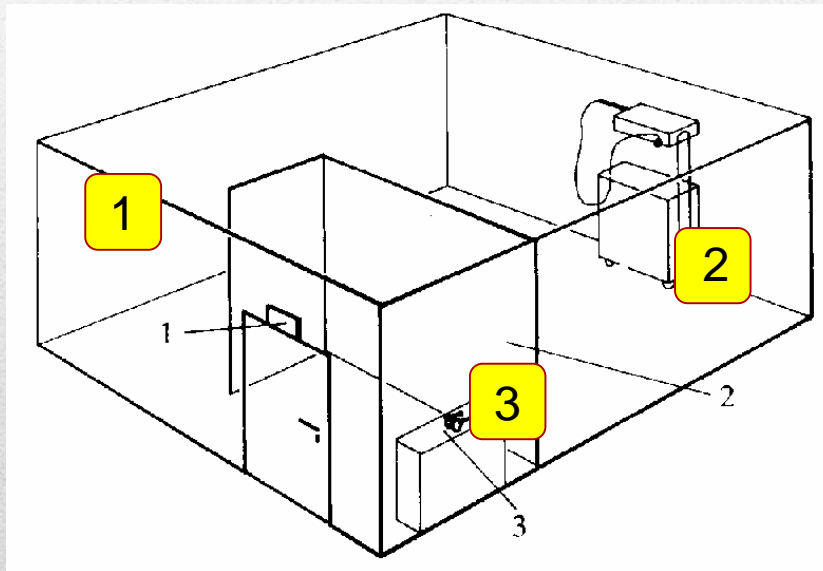
The devices concerned are:

- all AFMs from JPK (r. 131 / 1&2 and 309)
- SpiDi from Zeiss (r. 132)
- CLSM from Zeiss (r. 115)
- OS from RS Zelltechnik (r. 311)

4. Working with lasers

In our labs with self-designed experimental setups the following rules of conduct apply:

1. Protection against laser radiation



1. warning light in front of the entrance door
2. protective curtain separates input from the laser area
3. laser protective eyewear at the entrance range

**applies to room 309 - optical tweezer and
room 311 - optical stretcher**

2. Rules of conduct for persons who work on the laser

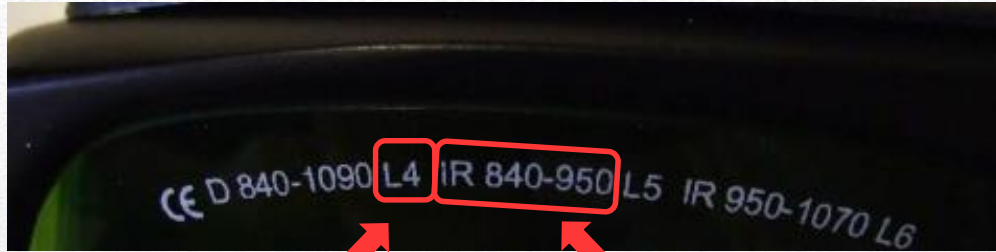
- switch on warning light - interlock
- warn all present persons before switching on lasers
- work on the laser or with the laser beam without arm jewellery such as watch, ring or bracelet (reflected, scattered beam possible)
- restrict laser beams as good as possible on the experimental area
- put on suitable laser protective eyewear!
- each setup and any modification of a setup must be approved by the person responsible for laser safety

responsible for laser safety in the lab: Bernd Kohlstrunk (phone: 32485)

3. Rules of conduct for persons who don't work on the laser

- if the warning light is switched on: before entering the laser lab, ask for sources of danger!
- wear suitable laser protective eyewear (available in the entrance area)
- do not bring head to beam height, i.e. be careful when bending down
- no unauthorized handling of optics or lasers!

Laser protective eyewear



$LX = 10^x$ x attenuation of the laser beam wavelength range

- must comply with DIN EN 207 and be clearly marked
- are always specially matched to a laser (wavelength)
- eyewears has to be completely intact

Laser alignment eyewear

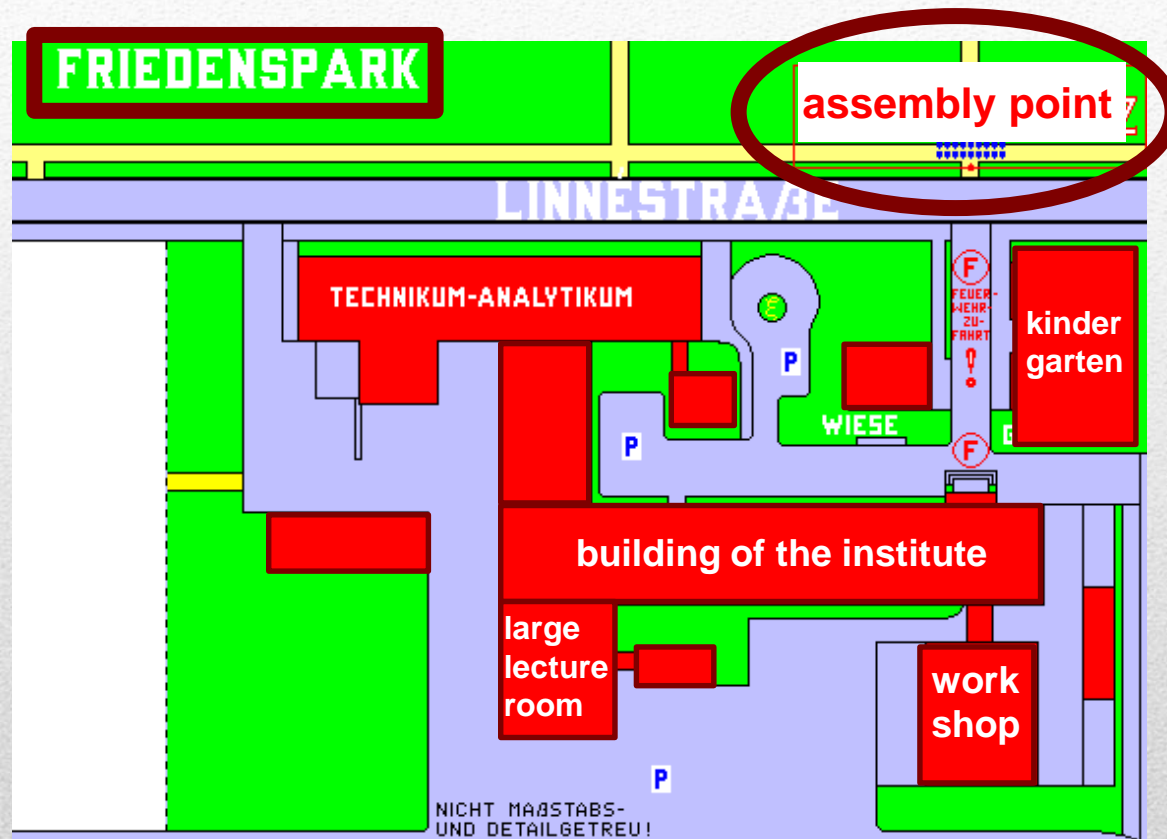
- may only be used for adjustment work on laser devices of classes 3B and 4 which emit in the visible spectral range between 400nm and 700nm
- must comply with DIN EN 208 and be clearly marked

5. General behavior in case of emergency

- give the alarm immediately
- phone dispatcher of the university (34333) or in case of fire not indicated by alarm system (acoustic signal) additionally the fire brigade 0-112
- keep calm - human lives before firefighting
- close windows and doors, do not lock doors
- switch off electrical appliances, except for room and staircase lighting
- leave the building immediately and go to the meeting place (Friedenspark)
- don't use elevators

5. General behavior in case of emergency

meeting place in case of fire alarm - Friedenspark



... and at the end an important, general note:

- please avoid to work outside the opening hours of the faculty
- if you are in the building after 19 o'clock, sign in the attendance book so that in case of emergency it is known that you are in the house (the book is in the main entrance beside the monitor)
- if you work alone, please inform an other person and keep in contact via phone
- in case of emergency call 34333 (Dispatcher)



Responsible persons:

project leader / group leader:

Prof. Josef Käs (32471), jkaes@physik.uni-leipzig.de

responsible for lab:

Dr. Undine Dietrich (32472), dietrich@physik.uni-leipzig.de

appointee for biological safety:

Dr. Nicole Weizenmann (32592), weizenmann@uni-leipzig.de

appointee for laser safety and responsible for electrical safety in the labs:

Dipl.-Phys. Bernd Kohlstrunk (32485), bkohl@physik.uni-leipzig.de