Nano-Structural Imaging Lab

Safety Guides

General Laboratory Safety

Lab Policies

1. Follow all instructions given by the lab manager and/or supervisors.
2. Only trained users are allowed to operate the instruments.
3. Visitors need permission from the Director to enter the lab.
4. All research projects must be under the direct supervision of a BCA science faculty who is responsible for the biological and/or chemical safety requirements of each project and implementing adequate safety guidelines for each student.
5. The instruments are to be used for training of BCA students and for research purposes. No personal use of the instruments is allowed.
6. Only the supervisors are allowed to change detectors and other microscope accessories.
7. Running unauthorized programs or changing preferences on the microscope computers is not allowed. Laptops are provided for personal use and internet access. **Do not plug memory sticks in the microscope computers**; ask a supervisor for your files.
8. Lab users are responsible for cleaning up their own work areas.

Safety Instructions

1. Food and beverages are **not** allowed in the lab and are not to be stored in the lab refrigerator.
2. Wear protective laboratory equipment (gloves, lab coat, goggles, etc.) when necessary.
    a. Latex gloves are used in the lab; notify a supervisor if you have a latex allergy.
3. In the event of any emergency such as injury, spill, accident, fire or equipment failure, notify a lab supervisor **immediately**.
4. Hazardous materials are not allowed in the instruments. This includes: (a) infectious biological materials, (b) toxic chemicals, (c) radioactive materials, and (d) unknown substances that are potentially hazardous. Please check with the

lab manager before introducing an object of unknown composition into any of the microscopes.

5. Read all labels and safety instructions carefully before performing any lab tasks.
6. Any protocol requiring hazardous chemicals is to be performed under the chemical fume hood, by trained laboratory personnel.
7. Keep long hair tied back.
8. Never leave a hot plate that is in use unattended; assume the hot plate is hot and never touch the heating surface.
9. Do not handle hot glassware with bare hands.
10. No open flames are permitted in the lab.
11. Students are not permitted to work alone in the laboratory.
12. Be prepared for an experiment or preparation by reading protocols before coming to the lab.
13. If any part of an experiment, preparation or equipment use is unclear, ask questions. Do not attempt to perform a task you do not understand.
14. Do not leave an on-going experiment or preparation unattended.
15. Keep aisles clear and store backpacks and jackets underneath tables to prevent tripping.
16. No rough-housing or fooling around is allowed in the lab.
17. Any item that has come in contact with a toxic chemical or biological contaminant must be disposed of in the container labeled “Hazardous Waste” found in the chemical fume hood.
18. Wash hands thoroughly when leaving the lab, especially before eating.

Emergency Procedures

1. *In case of a fire drill*, exit the lab, turn right and leave the building immediately through the doors at the end of the hall. Move a safe distance from the building.
    a. Lab supervisors will be responsible for locking or shutting down the instruments and safely storing any samples that are in the process of being prepared.
    b. A map of the evacuation route is located on the wall next to each door.
2. *In the event of a fire*, notify the lab supervisor and exit the building, moving a safe distance away. Pull the fire alarm next to the exit doors to alert the rest of the building.
    a. Lab supervisors will be responsible for extinguishing the fire, if possible.
    b. There is a fire extinguisher located next to door 2.
        i. To operate the fire extinguisher, remove it from the wall, pull the pin and aim at the base of the fire, standing 8 feet back.
3. *In case of a flood or activation of the sprinkler system,* exit the lab immediately and move to an unaffected area.
    a. Lab supervisors will shut down the equipment, if safe to do so.
        i. SEM – power switches located in the upper, right corner of the electronics box.
        ii. Confocal – power switches located on the front panel.
        iii. TEM – EM Stop button located in the bottom left panel.
    b. Do not attempt to unplug any electrical devices that are wet.
4. *In case of a power failur*e, stay calm and remain in the lab until power is restored unless otherwise directed by school officials.
5. *In case of a building lockdown*, lab supervisors will lower and close all blinds and lock the doors. Stop all experiments or preparations and remain in the lab until the “All Clear” signal is given.
6. *In case of broken glass*, pieces can be swept up and placed in the container labeled, “Broken Glass” on the central bench-top work area.
7. *For minor cuts or burns*, a first-aid kit is available on top of the chemical storage cabinet.

Chemical Safety

Read these instructions carefully before using any chemicals. If you are unsure of how to handle or dispose of any chemical, consult a lab supervisor or the MSDS. Always exercise caution when handling chemicals.

1. Students are **NOT** permitted to handle any chemicals that are hazardous or toxic, at any time. Any protocol, or part of a protocol, that requires such chemicals will be carried out by a lab supervisor. These chemicals include, but are not limited to:
    a. glutaraldehyde
    b. osmium tetroxide
    c. formaldehyde
    d. paraformaldehyde
    e. propylene oxide
    f. sodium cacodylate buffer
    g. liquid carbon dioxide
    h. liquid nitrogen
    i. liquid ethane
    j. liquid propane
2. Hazardous chemicals are stored in a locked chemical storage cabinet that is rated for flammable materials. Hazardous chemicals that need to be kept cold are stored in the locked lab refrigerator.
3. Cryogenic liquids can cause frostbite. They are only to be used by lab supervisors wearing thermal gloves and goggles or a face shield.
4. Never pipette any liquids by mouth; use a pipette pump.
5. Wear protective laboratory equipment (gloves, lab coat, goggles, etc.) when necessary.

6. Any mixtures or solutions that are prepared by students or staff **MUST** be clearly labeled with:
    a. All contents and concentrations.
    b. Any warnings or precautions for use or storage.
    c. Initials of the preparer.
    d. Date prepared.
    e. Expiration date (if known).
7. Read all labels and warnings carefully before using any chemical. For further information, refer to the MSDS found in the binder on top of the chemical storage cabinet. Be sure to ask a lab supervisor if you have any questions about how to handle or dispose of a chemical.
8. Treat all chemicals as if they were dangerous; do not taste, touch or smell anything.
9. Hazardous and/or volatile chemicals must be used under the chemical fume hood.
10. Never assume that a clear liquid is water.
11. Use the minimum amount of a chemical that is required to minimize waste production.
12. Substitute less hazardous chemicals whenever possible.
13. Carry reagent bottles and beakers with two hands to avoid spilling or dropping them.
14. Avoid transporting open containers from one lab to another.
15. Do not return unused reagent to the original container to avoid contamination.
16. Do not leave containers and bottles open for extended periods of time. Close caps and lids tightly when not in use.
17. Always return chemicals where you found them when you are finished.
18. Dilute solutions of alcohol, acetone or salts can be flushed down a drain fitted with a chemical trap with plenty of water. Any solution containing resin, fixative or any other toxic chemical must be disposed of in the amber bottle labeled “Hazardous Waste” found in the chemical fume hood.

Emergency Procedures

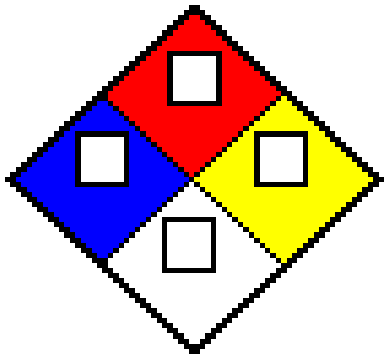
Always consult the MSDS to determine any special procedures or instructions for a particular chemical.

8. *In case of a chemical splash*, an eyewash station is available on the pillar behind the SEM.
    a. Press the paddle to begin the flow of water.
    b. Place eyes in the flow, keeping them open; use fingers to hold eyelids open if necessary.
    c. Keep rinsing until the flow stops after 15 minutes.
    d. If vision is still impaired, seek immediate medical attention.
9. *In case of a spill:*
    a. If the spilled material is non-toxic (i.e. acetone, alcohol) wipe up excess material immediately and rinse the area with water.
    b. If the spilled material is toxic (i.e. glutaraldehyde, osmium tetroxide) do not attempt to clean it up yourself; notify a supervisor who will clean the area with a spill kit. Dispose of any waste in the hazardous waste container.
    c. If the spilled material is biological (i.e. bacterial or cell culture), wearing gloves, wipe up the excess material and clean the area with 10% bleach; dispose of any waste in the hazardous waste container.
10. *In case of skin or clothing contact:*
    a. Immediately wash the affected area with soap and plenty of water. Seek medical attention if necessary.
    b. If an extensive area is affected, use the emergency shower.
11. *In case of ingestion:*
    a. Consult the MSDS for instructions and seek medical attention.
12. *In case of inhalation:*
    a. Move to an area of fresh air.
    b. If breathing difficulties occur, seek medical attention.

Understanding Chemical Labels

The National Fire Protection Association (NFPA 704) system uses a diamond-shaped diagram of symbols and numbers to indicate the degree of hazard associated with a particular chemical or material. These diamond- shaped symbols are placed on containers of chemicals or materials to identify the degree of hazard associated with the chemical or material.

The diagram identifies three color-coded categories of hazard for each material:



- **Health Hazard (blue section)**
- **Flammability (red section)**
- **Reactivity (yellow section)**
- **Other Hazard Information (white section)**

Each category is divided in five levels of hazard potential with:

- **zero (0)** used to indicate no special hazards and
- **four (4)** for severe or extreme hazard potential.

The degrees of hazard in each of these categories are given as follows:

Health - The degree of health hazard of a chemical or material is based on the form or condition of the material, as well as its inherent properties**.** The degree of health hazard of a material should indicate the degree of personal protective equipment required for working safety with the material. **A rating of 1** is for **slightly hazardous (toxic) material** which require only minimal protection (for example, safety glasses and gloves) in addition to normal work clothing to work with safely. **A rating of 2** is for **moderately toxic or hazardous material** which requires additional PPE or equipment (e.g. chemical goggles, lab/work smock, local ventilation) in addition to that required for less toxic material. Consult the MSDS for specific health hazard and proper PPE to use with this material. **A rating of 3 or 4** is for **highly to extremely toxic (deadly) material (and any carcinogen, mutagen, or teratogen)**. These materials will require specialized equipment (e.g. respirator (or exhaust hood), full face shield, rubber apron, specialized glove, handling tongs, etc) beyond that required for moderately toxic material. You must consult the MSDS and/or other safety information

to determine the hazard (acute or chronic) and the proper PPE and engineering controls to safely use of this material.

Flammability - The flammability hazards deal with the degree of susceptibility of the material to ignite and burn. The form or condition of the materials, as well as their properties, affects the extent of the hazard. Many hazardous materials such as acetone and gasoline, have a flash point (ignition temperature) far below freezing and will readily ignite with a spark if the vapor concentration is sufficient. A low rating of **1** is for material with a flash point **above 200F** while more hazardous ratings of **2, 3, and 4** are for materials with **respective flash point below 200, 100 and 73 F**.

Reactivity - The reactivity hazards deal with the potential of a material or chemical to release energy. Some materials are capable of rapid release of energy without any catalyst, while others can undergo violent eruptive or explosive reactions if they come in contact with water or other materials. Generally this rating is used to indicate the potential to reactive if the material is heated, jarred, or shocked. A low rating of **1** indicates a **material that is normally stable but may be reactive if heated**. The more hazardous ratings of **2, 3, and 4** indicate a **material is capable of violent reaction, shock/rapid heating and detonation respectively.**

Other Hazard Information - An open space at the bottom of the NFPA diagram can be used to indicate additional information about the chemical or material. This information may include the chemical or material's radioactivity, proper fire extinguishing agent, skin hazard, its use in pressurized containers, protective equipment required, or unusual reactivity with water. For example, the usual signal to indicate unusual reactivity with water is the letter "**W**" with a long line through the center. Similarly, the words **ACID, COR** (corrosive), **RAD** (radiation), **OXY** (oxidizer), **Rad** (radioactive), **CARC** (carcinogen) or other abbreviations may be used.

Understanding Safety Symbols:

Poisonous
Self explanatory. Whereas most chemicals are fairly dangerous if ingested or inhaled, many of these are dangerous even on contact.

Environmental hazard
Relatively rare with laboratory chemicals (most of which pose some environmental hazard if not gotten rid of correctly), these require *particular* care to be taken on disposal.

Corrosive
Avoid contact with the skin. Bear in mind that these can (under some circumstances) rust chemical cupboards.

Explosive
Again, fairly self-explanatory, though fairly seldom seen in the average lab. Bear in mind that noise and movement can also trigger explosion (not just sparks/flames!).

Flammable or extremely flammable
Chemicals to be stored in flame-resistant cupboards. Volatile solvents can be a particular problem as they are prone to spread around from unsealed containers. This also covers pyrophoric materials (that catch fire spontaneously on exposure to air).

Irritant or Harmful
This symbol covers a wide range of (sometimes relatively minor) hazards - with precautions such as avoid contact with the skin, do not breathe, etc. - best to refer to relevant data sheet for details.

Oxidising chemical
Oxidising chemicals are materials that spontaneously evolve oxygen at room temperature or with slight heating, or that promote combustion. To be kept away from flammable chemicals at all costs!

Stow away from foodstuffs
Harmful material to be kept away from edible material.

Dangerous when wet
This generally means that it will react fairly violently with water.

Flammable Gas
Safety symbol used for the transport or storage of a flammable gas.

Non flammable gas
Safety symbol used in the transport of non flammable (and hence often non hazardous, at least out in the open) gases.

Organic Peroxide
Chemical safety symbol used in the transport and storage of organic peroxides.

Corrosive
Transport of corrosive materials - again, avoid contact with the skin.

Inhalation Hazard
Inhalation hazard transport/storage symbol.

Poisonous Gas
Used for transport of a poisonous gas - on gas cylinders, or sometimes as an indicator on vehicles.

Marine Pollutant
Marine pollutant - do not dispose of in sewer system.

Miscellaneous danger
Catch-all symbol for all other dangers (usually specified in the space).

Explosive
Used in the transport of explosive materials.

Poison
More general symbol for the transport of poisonous materials (not necessarily a gas).

Spontaneously Combustible
Spontaneously combustible material (treat with great caution!).

Flammable Solid
Flammable solid.

Flammable Liquid
Used in the transport of flammable liquids.

Biohazard

Laser Radiation

Radioactive

Materials that Require Special Precautions

Flammables and Combustibles: Flammable/combustible materials are materials which under standard conditions can generate sufficient vapor to cause a fire in the presence of an ignition source. Flammable materials can generate sufficient vapors at temperatures below 100°F (38°C); combustibles, at temperatures at or above 100°F (38°C) and below 140°F (60°C). The vapors of these materials are invisible, and a vapor trail to an ignition source away from the immediate area can result in a flashback. Flammables are more hazardous at elevated temperatures due to more rapid vaporization. In addition, flammable and combustible materials react with oxidizers which can result in a fire.

Cryogens: Some of the hazards associated with cryogens (fluids used to maintain extremely low temperatures) are fire, pressure, embrittlement of materials, and skin or eye burns upon contact with the liquid. Cryogens can condense nearly pure liquid oxygen from the air, creating a severe fire risk. A pressure hazard exists because of the large expansion ratio from liquid to gas, causing pressure build up in containers. Many materials become brittle at extreme low temperatures. Brief contact with materials at extreme low temperatures can cause burns similar to thermal burns.

Compressed Gases: Special systems are needed for handling materials under pressure. Toxic and corrosive gases present special problems in designing engineering controls. The physical and health hazards of any material are typically compounded by the pressure hazard.

Corrosives: Corrosives are materials which can react with the skin causing burns similar to thermal burns, and/or which can react with metal causing deterioration of the metal surface. Acids and bases are corrosives.

Light-Sensitive Materials: Light-sensitive materials are unstable with respect to light energy. They tend to degrade in the presence of light, forming new compounds which can be hazardous, or resulting in conditions such as pressure build-up inside a container which may be hazardous.

Peroxidizables: Peroxidizables are substances or mixtures which react with oxygen to form peroxides. Some peroxides can explode with impact, heat, or friction such as that caused by removing a lid. Peroxides form inside the containers of some materials even if they have not been opened. Examples include ethyl ether, tetrahydrofuran, liquid paraffins (alkanes), and olefins (alkenes). See Appendix C for additional materials which may form peroxides.

Allergens: The term allergen describes a wide variety of substances that can produce skin and lung hypersensitivity. Examples include diazomethane, chromium, nickel bichromates, formaldehyde, isocyanates, and certain phenols. Wear suitable gloves to prevent hand contact with allergens or substances of unknown allergenic activity. Conduct aerosol producing procedures in a fume hood.

Radiation Safety

Sources of Radiation: Laser Radiation

The laser scanning confocal microscope uses three visible wavelength lasers, class 3B, as the source of illumination for fluorescent confocal imaging:

- Blue multi-line Argon (Ar) 100 mW Laser for excitation wavelengths 458 nm, 476 nm, 488 nm and 514 nm.
- Green Helium/Neon (He/Ne) 1 mW Laser for excitation wavelength 543 nm.
- Red Helium/Neon (He/Ne) 10 mW Laser for excitation wavelength 633 nm.

Protection Measures in Place: Laser Radiation

There are several safety measures and protection devices built into the microscope to protect the user from exposure to laser radiation:

- A laser radiation symbol on areas where it is emitted. 
- A safety beam guide and beam stop located between the condenser base and the transmitted-light detector. These serve as protection against laser radiation by blocking the laser beam.
- Fiber optic cabling shields the laser beam until it leaves the microscope objective and passes through the specimen.

Users are given a safety instruction sheet prior to use of the instrument. Observance of the following safety rules will protect the user from any exposure to laser radiation:

- Do not look into the eyepiece while the scanner is in use.
- Do not look directly into the laser beam at any time.
- Do not place any reflective objects into the beam path.

Sources of Radiation: X-ray Radiation

Electron microscopes are known to produce small amounts of ionizing, or X-ray, radiation. We are exposed to this type of radiation on a daily basis, from natural sources, such as the sun and naturally occurring radioactive elements, and human sources such as getting an X-ray, MRI or CT scan. The Nano-Structural Imaging Lab has two electron microscopes – the 30 kV scanning electron microscope (SEM) and the 200 kV transmission electron microscope (TEM). X-rays are only produced by these instruments inside the electron column while the electron beam is being generated, or while the microscope is in use.

Protection Measures in Place: X-ray Radiation

Both electron microscopes have extensive lead shielding built into the columns; on the SEM there is also shielding of the specimen chamber and on the TEM there is also shielding of the apertures, viewing window and camera port.

Other X-ray radiation protection measures include:

- Biannual inspections of each instrument for X-ray leaks by an independent company.
- X-ray badges for each instrument placed in the area where the user sits; badges are checked quarterly.
- Monthly inspection of the instruments with a Geiger counter by lab staff.
- Registration of both instruments with the New Jersey Bureau of Radiological Health.
- Yearly inspection by the New Jersey Bureau of Radiological Health.

*****IMPORTANT NOTE***** Neither instrument has EVER been shown to produce X-ray radiation greater than the normal background level.

http://www.state.nj.us/dep/rpp/njacdown.htm

Laser Scanning Confocal Microscope Safety Instructions

Note: The visible range lasers used by this microscope are fed through a fiber optic cable and completely shielded until they leave the microscope objective and reach the sample during scanning. The inverted design of the microscope provides additional protection from laser radiation. No ultraviolet or infrared lasers are used by this microscope. Exposure to laser radiation can cause serious damage to the eyes and skin. The microscope is equipped with several safety devices to minimize the emission of laser radiation. As long as the following instructions are observed, the user is safe and no eye protection is necessary.

1. Never touch any of the laser protection devices or the laser cabinet beneath the desk.
2. Do not look into the eyepiece of the microscope while the scanning head is operating or while switching the beam path.
3. Never look directly into a laser beam or a reflection of the laser beam; avoid all contact with the laser beam.
4. Do not place any reflective objects into the laser beam path.
5. Always keep eyes a safe distance of 20 cm from the opening of the objective.
6. Never change the following items during a scanning operation:
    a. Specimens
    b. Objectives
    c. Filter cubes
    d. Beam splitters
7. Do not use liquids near the microscope.
8. Never touch or disconnect any cables.
9. Do not attempt to shut down the microscope; it must be shut down in a specific manner, if there is a problem with the microscope that requires shut down or reboot, alert the lab supervisor.
10. Avoid manually changing objective lenses, use the software control instead.
11. Never use immersion oil on a dry lens.
12. Only change samples when the objective lens has been lowered to -4 mm.

SEM Safety Rules

1. The chemical fixation of biological specimens is to be done only under the direct supervision of a laboratory technician who is proficient in biological sample preparation for the EM.
2. Please read the Material Safety Data Sheet (MSDS) for :
    a) Platinum and gallium before using the FIB or the GIS;
    b) Liquid Nitrogen before using the X-ray detector.
    c) Glutaraldehyde, osmium tetroxide, liquid carbon dioxide and ethanol before preparing specimens.

    These can be found in the MSDS binder.
3. The microscope uses several high voltage wires that have the potential to cause electrical shock. No user should touch any external portion of the microscope with the exception of the stage adjustment knobs and the drawer handle. This includes all wires, external or internal detectors, the deposition system, Peltier chiller, liquid nitrogen Dewar and gaseous nitrogen tank / regulator.
4. Do not lean or rest objects on the microscope or microscope table.
5. Keep the area near the chamber free of dust, hair, and other clothing fibers.
6. Tilting of the stage is **not** allowed, except under the direction of a supervisor.
7. Do not **Stop** or **Shutdown** the xT microscope Server.
8. Always leave the microscope in High Vacuum.

TEM Safety Rules

1. The chemical fixation of biological specimens is to be done only under the direct supervision of a laboratory technician who is proficient in biological sample preparation for the EM.
2. Never go behind the microscope desk or touch any wires or cables coming from the microscope; it uses several high voltage wires that have the potential to cause electrical shock.
3. Do not lean on or touch **any** part of the microscope column. The only parts of the microscope a user should touch are the knob panels, trackball, mouse and keyboard.
4. Keep the area near the goniometer and specimen holder free of dust, hair, and other clothing fibers
5. Do not open any of the cabinets underneath the microscope table or shut off any of the computers.
6. No additional programs are to be installed or run on any of the microscope computers.
7. Do not close or alter any of the programs running on the microscope control computer unless directly instructed to do so by a supervisor; only use the second, Gatan computer for obtaining images.
8. Be aware of the foot pedals on the floor underneath the column; if pressed, the sample will be tilted.

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