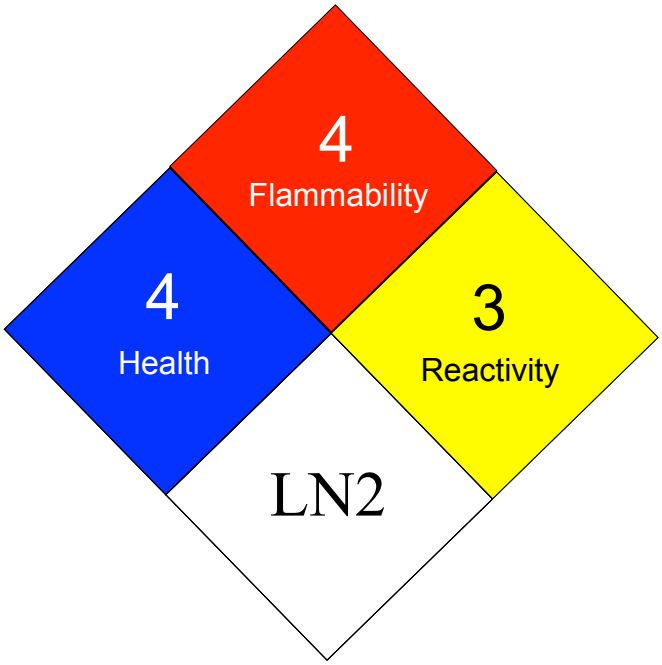
WVU Share Research Facilities

BNRF Lab
Chemical Hygiene Plan

380 Chemistry Research Laboratory



Emergency Contact:

Huiyuan Li, Ph.D.
Office: 380 Chemistry Research Laboratory
Cell Phone: (304) 293-0747

Huiyuan Li
3/26/2014

SECTION 1: NFPA CHEMICAL HAZARD LABEL

NFPA Rating - The National Fire Protection Association (NFPA) has developed a standard system (ANSI/NFPA 704) for indicating the health, flammability, and reactivity hazards of chemicals. In addition, a special precaution symbol may be used where necessary.

This system of identifying hazards associated with various materials was developed primarily for fire protection and emergency personnel but can be useful to anyone who needs to handle potentially hazardous material. As stated in NFPA 704, "This standard provides a simple system of readily recognizable and easily understood markings, which will give at a glance a general idea of the inherent hazards of any material and the order of severity of these hazards as they relate to fire prevention, exposure, and control."

General Rating Summary:

Health (Blue)

0- Hazard no greater than ordinary material
1- May cause irritation; minimal residual injury
2- Intense or prolonged exposure may cause incapacitation: residual injury may occur if not treated
3- Exposure could cause serious injury even if treated
4- Exposure may cause death

Flammability (Red)

0- Will not burn
1- Must be preheated for ignition, flashpoint above 93°C (200°F)
2- Must be moderately heated for ignition flashpoint above 83°C (100°F)
3- Ignition may occur under most ambient conditions, flashpoint below 83°C (100°F)
4- Extremely flammable and will readily disperse through air under standard conditions, flashpoint below 83°C (100°F)

Instability (Yellow)

0- Stable
1- May become unstable at elevated temperatures and pressure, may be mildly water reactive
2- Unstable; may undergo violent decomposition, but will not detonate. May form explosive mixtures with water
3- Detonates with strong ignition source
4- Readily detonates

Special Symbols (White)

There are only two NFPA approved symbols although other symbols are also used to identify hazards:

OX- oxidizer
~~W~~- Water reactive, use no water
ACID - Indicates the presence of an acid in the lab – corrosive material with pH lower than 7.0
ALK - Denotes an alkaline material, also known called base. The caustic material has a pH greater than 7.0
COR - Corrosive material – it can be either an acid or a base.
LN2 - Liquid Nitrogen.

SECTION 1.1: EMERGENCY CONTACTS

In case of FIRE, INJURY, or EMERGENCY ASSISTANCE, contact in the following order:

9-911 from any campus phone
or
Campus Security
Phone: **(304) 293-3136 (293-COPS)**

Then Call

Huiyuan Li, BioNano Research Facility Manager
Cell Phone: **(304) 906-5368**
Room: **380 Chemistry Research Laboratory (Downtown Campus) AND G75D Engineering Sciences Building (Evansdale Campus)**

Or

Kolin Brown
Cell Phone: **(304) 366-6551**
Office Phone: **(304) 293-9683**
Room: **G75D Engineering Sciences Building (Evansdale Campus)**

Marcela Redigolo
Cell phone: **(304)680-3007**
Office phone: **(304)293-9973**
Room: **G75D ESB**

For non-emergency assistance please contact:

Huiyuan Li, BioNano Research Facility Manager
Cell Phone: **(304) 906-5368**
Room: **380 Chemistry Research Laboratory (Downtown Campus)**

SECTION 1.2: EMERGENCY EVACUATION PROCEDURES

In case of Fire or Chemical Spill all users should evacuate the laboratory immediately!

- Use the shortest, unobstructed path to the exterior of the building (Figure 1).

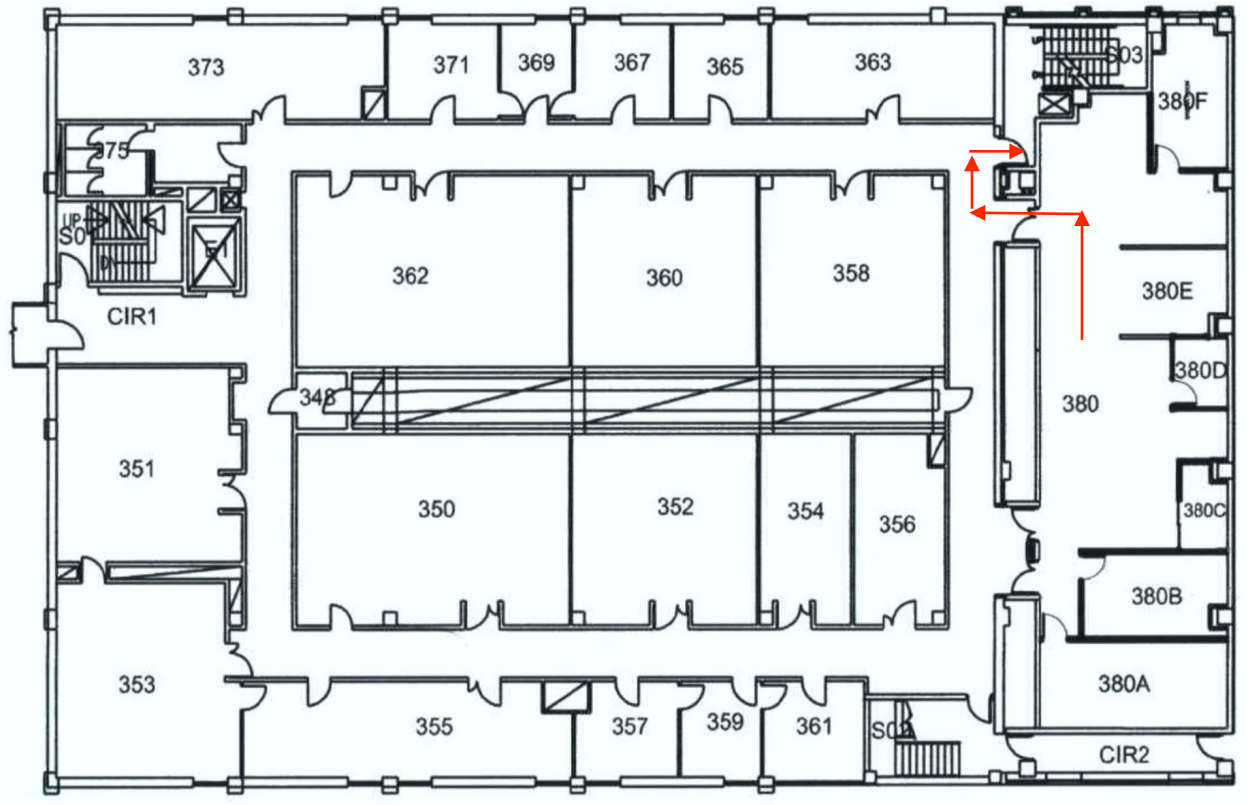


Figure 1: BNRF Lab Evacuation Plan for room 380 on the third floor of the Chemistry Research Laboratory Building. Closest exits marked with red arrows.

- Evacuate the building by pulling the fire alarm pull station when exiting the building. All basement exits have a fire alarm pull station.
  Do not wait next to the building. Move across the street to a safe distance.
- Call the emergency contacts from a safe place.

SECTION 1.3: SHUTDOWN PROCEDURES

If a dangerous situation is evident (smoke, fire, sparks, etc.), ONLY if it is safe to do so, should a user attempt to shut down a system. The user should then notify all other persons in the lab to evacuate immediately. After evacuation, a user should contact proper emergency personnel from a safe place.

If no one is available and a machine is not acting as expected then the user should attempt to put the machine in its default mode; do not leave the machine running in an abnormal state! If the machine cannot be placed in its default mode, the user should stay by the tool and contact one of the WVU Shared Facilities Staff Members. If it becomes necessary to leave the tool then

the user should leave a large, legible note on the machine stating that the tool is down, and the user's contact number.

Listed below are the procedures to place the BNRF CRL equipment into a safe default mode, or to shut down. Shutdown procedures may also be found on the cover of each instrument's logbook or in the Standard Operating Procedures found in Appendix A.

To place the Biosafety Cabinet in default mode:

- **Lower the sash to close the laminar flow hood and turn off the light.**

To place the LC-MS/MS in default mode:

- **In the "Tune" software program, select "standby" from the drop down menu in the upper left corner. If the instrument is unresponsive in this program (i.e.: instrument controlled by Xcaliber software) then stop the running method, and close the Xcaliber, then select "standby" from the Tune software.**
- **DO NOT shutoff the mass spec power button unless absolutely necessary.**

To place the Autoclave in default mode:

- **Press the "stop" button on the front panel (Figure 2). The current run will stop immediately, however you will not be able to open the loading door until the temperature and pressure reach near ambient levels.**
- **If the run is finished, switch off the power button (green button on the bottom right).**

Figure 2. Part of front panel of autoclave.

To shut down the Millipore water purification system and water supply:

- **On the right side the LED screen, press ⬇ button to highlight "Standby" and ➡ press button to confirm the selection.**

- **During emergency, such as water flooding or the instrument electronic error, the complete shut-down is necessary. Unplug the Millipore water system. Switch OFF the water supply at the wall to the left side of the Millipore.**

SECTION 1.4: EMERGENCY RESPONSE EQUIPMENT

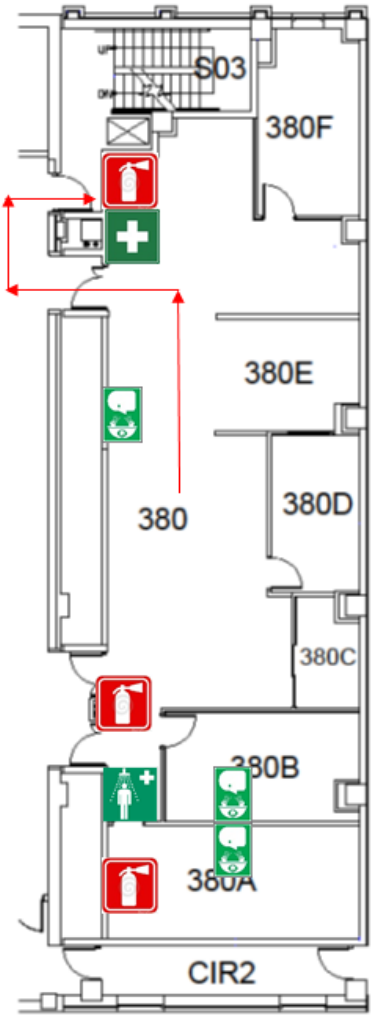


Figure 3: Locations of the safety shower ( ), eyewash stations ( ), first aid kit ( ) and fire extinguishers ( ) at 380 CRL.

First Aid kit is located inside the lab, **380 CRL**, on the top of N2 generator by the entrance door (in figure 3). A **safety shower** is located inside 380 CRL room, in front of the door of 380A (

 in figure 3), and **eyewash stations** are located in the rooms, 380A, 380B and 380 over the sinks ( in figure 3).

The **Chemical Hygiene Plan** and **SDS**s file folders are located on the bookshelf in 380E. Fire extinguishers, type ABC, are located in the rooms 380A and 380, indicated in figure 3 by the  symbol.

SECTION 2: LAB OVERVIEW

The Bio-Nano Research Facility (BNRF) Laboratory in the Chemistry Research Laboratory (CRL) Building is one of two BNRF labs. The other is located in the Engineering Sciences Building (ESB) on the Evansdale campus. The BNRF is one of the four WVU Shared Research Facilities. As part of the WVU Shared Research Facilities, the BNRF lab provides student, postdoctoral and faculty researchers with the opportunity to learn how to use cutting-edge cell culture, imaging, and biosample identification and quantification equipment. The facility is open to all researchers, including researchers at University, government laboratories and industries.

SECTION 2.1: FACILITY DESCRIPTION

The BNRF lab in the CRL is located in the main room 380 CRL and contains five small rooms within its space, rooms 380A, 380B, 380C, 380D, and 380F.

Room 380A is the room for the cell culture.
Room 380B is the room for fluorescence microscope, spectrofluorometer, and profiler.
Room 380C is the room for one chemical hood. Currently the room is not in use.
Room 380D is the room for Raman Spectroscope, under responsibility of the WVUSRF Material Characterization facility (MCF).
Room 380F is the office room.
This chemical hygiene plan describes hazards and procedures that are valid for all labs within 380 CRL, except when stated otherwise. Specific hazards related only to room 380D will be described clearly in a separate section of this document.

SECTION 2.1: LABORATORY ACCESS

Access to the WVU Shared Research Facilities is controlled through the WVU ONITY Lock system. The door to the BNRF main lab, 380 CRL , and 380D CRL, has two ONITY locks.

The BNRF Onity lock is subject to the CEMR lock policy. A version of the CEMR Electronic Lock policy may be found on the website http://www.its.cemr.wvu.edu/cardlocks/index.php or a full, written version may be obtained upon request to the Dean's office.

The following persons are the official operators of this lock:

- Kolin S. Brown
- Harley Hart
- Lisa Sharpe

In addition, the WVU Lock Shop and WVU Card Services also have capabilities to add students, staff and faculty to this lock.

BNRF users are only given access to the BNRF laboratory during normal working hours after they have completed all required safety trainings and have a signed user agreement on file with the SRF BNRF Facility Manager.

The normal working hours of the BNRF lab are 8 AM to 5 PM, Monday to Friday. After-hours are 5 PM to 8 AM, Monday to Friday, and full time during weekends and holidays.

SECTION 2.2: REQUIRED SAFETY TRAINING

All BNRF users must complete the following three safety and equipment trainings to receive access to the facility during normal working hours.

- SRF General Lab Safety Training
- SRF Chemical Safety Training
- Biosafety Training

Copies of the safety presentation slides are located on the Shared Research Facilities website: http://sharedresearchfacilities.wvu.edu/info/safety/

SECTION 2.3: USER AGREEMENTS

All BNRF users must have a user agreement on file with the WVU SRF BNRF Manager. User agreements must be signed by the user, the user's advisor/supervisor and the user's department chair, when appropriate.

Copies of the User Agreements are located on the Shared Research Facilities website: http://sharedresearchfacilities.wvu.edu/r/download/160912

SECTION 2.4: DRESS CODE

The following dress code is required for all users entering the BNRF Lab:

- No sandals or open toed shoes, feet must be fully covered
- No shorts, legs must be fully covered

- Long hair should be tied back

Users operating cell culture and sample preparations must wear proper protection gear and adhere by accepted safety protocols:

- Nitrile gloves
- Lab coat
- Goggles when necessary

Users operating autoclave must wear proper protection gear and adhere by accepted safety protocols:

- Heat insulated gloves

Users working with cryogenics must wear proper protection gear and adhere by accepted safety protocols:

- Cold Insulated gloves
- Full face shield if working with liquid nitrogen

SECTION 2.5: AFTER HOUR ACCESS

After hour access to the BNRF Lab is given upon request by the WVU Shared Research Facilities BNRF and/or MCF Manager. The manager will use his/her own discretion to grant access when a user has demonstrated that he or she can work alone safely and handle an emergency.

SECTION 2.6: TEMPORARY USER SUPERVISION

Temporary users in the BNRF lab must be accompanied by a trained BNRF user or staff member at all times. Temporary users may include summer research participants, visitors or class participants.

There is a chemical inventory list of chemicals that are found in the BNRF in this Chemical Hygiene Plan. The inventory is updated monthly.

SECTION 3: LABORATORY HAZARDS

Users should be aware of the following safety hazards when working in the BNRF lab:

1. Biohazards
2. Thermal burns
3. UV light exposure
4. Cryogenics
5. Chemical hazards
6. Mechanical hazards
7. High Voltage hazards
8. Vacuum hazards

SECTION 3.1: BIOHAZARDS

All biohazards brought into the BNRF must be approved by the facility manager before arrival.

NOTE: This manual describes biological safety cabinet safety and disposal of biohazards. All persons entering this lab must have completed Biosafety Training in compliance with WVU EH&S and CDC guidelines. This DOES NOT cover best biological practices and protocols.

Any areas, equipment and materials (including wastes) that present a biohazard must be clearly identified by an appropriate label or sign. An example biohazard sign is shown in Figure 4.

Figure 4: Example biohazard label

Biological safety cabinets (BSCs) are primary devices intended to contain and minimize exposure when working with biohazardous materials. BSCs are designed to protect laboratory personnel against exposure during experimental procedures as well as protect experimental materials from contamination. BSCs utilize vertical laminar airflow to achieve a barrier of protection against airborne contaminants. The laminar airflow reduces turbulence in the work area and minimizes cross contamination. The BSCs in the BNRF are Thermo Scientific 1300 Series A2 units and contain HEPA (High Efficiency Particulate Air) filters to create a near sterile working environment. The BSCs are also equipped with ultraviolet (UV) for germicidal applications. Care must be used to always have the sash down while the UV light is on to

prevent exposure to UV radiation. BSCs are inspected and certified annually for proper airflow and filter integrity to ensure they are functioning properly.

Using the BSC is required in BNRF when handling the following materials or procedures:

- Handling cells (including seeding cells, changing media, splitting cells, and performing assays, etc).
- Preparing culture media or other liquids used for cell culture.
- Removing caps from tubes after centrifugation, vortexing open tubes, aspirating with syringe, etc.

Use of a BSC must be used in combination with aseptic technique (such as decontamination with 70% ethanol, and material sterilization with autoclave, etc) to ensure proper protection from exposure to infections materials.

All refrigerators and freezers within the lab are only for the storage of samples and non-explosive chemicals used for cell culture. DO NOT store any food or drinks in any of the refrigerators or freezers in the laboratory. (Food and drinks are not permitted in the lab at any time!)

SECTION 3.1.1: BIOLOGICAL WASTE DISPOSAL

The following wastes generated in the lab are considered biological waste:

- Cell culture waste liquid
- All sharps
- Plastic serological pipettes, tips and cell culture vessels

The following procedures must be used for disposal of:

1. Liquids — Waste cell culture media and buffer solutions (see BNRF cell culture media formulation and additives/ buffers in Appendix D) are aspirated into the vacuum flasks containing bleach. When the flask is half full, add more bleach. After sitting 30 min with bleach, pour down the drain. Wash the flask with tap water. Then spray the outside surface with 70% ethanol to decontaminate and wipe with paper towel. Discard the paper towel as regular trash. The Waste Tissue Culture Media Guidance and Used and Leftover buffers Guidance are attached in Appendix C.
2. Sharps — Sharps (needles, glass Pasteur pipettes, etc.) should be disposed in the sharp container located on the workbench. SRF staff will seal the sharp container and autoclave it and then dispose as regular trash.
3. Lab vessels — Plastic serological pipettes, tips, cell culture vessels should be disposed in the biohazard bag in the drum under the biosafety cabinet. SRF staff will autoclave the biohazard trash when it reaches 2/3 full. Then it will be sealed and disposed as regular trash.

SECTION 3.1.2: BIOLOGICAL LIQUID SPILL

The following procedure should be followed when the biological liquid spill happens in BNRF lab.

1. Spills on the lab floor or bench:

- Alert all personnel in the vicinity to leave the immediate area.
- Wear personal protective equipment (gloves, goggles, and lab coat).
- Cover an area twice the size of the spill with disinfectant (i.e. 10% bleach) soaked paper towels.
- Pour additional disinfectant solution onto the spill. Avoid splashing.
- Allow 20 minutes contact period.
- Wipe down any contaminated stationary equipment or furniture twice with disinfectant.
- Use forceps to remove broken glass and other sharp items, and place in sharps container.
- Remove towels and re-clean area with disinfectant solution.
- Collect and dispose the paper towel in regular trash container.
- Decontaminate (autoclave, or use a chemical disinfectant) reusable clean-up items and other permanent equipment.
- Inform laboratory personnel when the clean-up is complete.

2. Spills inside the biological safety cabinet (BSC)

- Keep the cabinet running.
- Clean up the spill area with disinfectant (i.e. 70% ethanol or 10% bleach) soaked paper towels similar as the description above.
- If the material has spilled on the pipette tips, wipe the tips with disinfectant soaked paper towel and disposal the tips in the sharp container.
- If the material has spilled on the small instrument, such as the pipetter, wipe twice with disinfectant soaked paper towel. And leave it in BSC.
- If the material has spilled in the grill panel, notify the lab manager, and the trained technician will handle it during service.
- Wipe down working surface, side wall, and items inside the BSC once more with towels and disinfectant.
- Remove all used paper towels into regular trash container.
- Place all the re-useable items in autoclave pan. BNRF staff will autoclave items.
- Place all the broken sharps in sharp container.
- The BSC must run for at least ten minutes after cleanup before being used for experiments.
- Report the spill incident to BNRF staff.

SECTION 3.2: THERMAL BURNS

Thermal burns can occur within the BNRF when working with the following equipment:

- Autoclave
- Heat plate
- Mass spectrometer
- Dry heat block

All lab users are given proper instruction on equipment operation before given authorization to use the tool. Users must follow all operational procedures outlined in the training and in the Standard Operating Procedures located in Appendix B.

Users must wear heat-insulated gloves to operate the autoclave. It is also suggested that the heated insulted gloves be worn when operating hot plates or the dry heat block with high temperature. Do not touch the ion-source when the mass spectrometer is running. Put the mass spectrometer in "standby" status, wait it to cool down, and then process any process related to the ion source.

If you are burned, you should seek medical treatment immediately. Severe burns to the face, third-degree burns, or burns over large areas of the body should be treated as emergencies. Report the burn to our staff and your supervisor as an occupational injury.

Minor burns should be treated by using first aid procedures. Immerse the burn in cool water immediately. Remove clothing from the burn area, and keep the injured area cool for at least five minutes.

SECTION 3.3 UV LIGHT EXPOSURE

UV light exposure can occur from the following equipment:

- Biosafety cabinet
- Fluorescence microscopy

All lab users are given proper instruction on equipment operation before given authorization to use the tool. Users must follow all operational procedures outlined in the training and in the Standard Operating Procedures located in Appendix B.

UV light sources are used in the biological safety cabinet and fluorescence microscope. There are three ranges of UV radiation. UV-A (320-400 nm) has lowest hazard potential. UV-B (290-320 nm) has mid to high hazard potential, and can cause skin and eye burns. UV-C (190-290 nm) has the strongest hazard potential causing severe burns. The severity of the UV hazard is

elevated by the fact that UV radiation symptoms of overexposure are not immediately felt. Therefore special care should be taken for UV exposure.

UV-C (254 nm) is commonly used in Laminar air flow cabinet (biosafety cabinet). This should be treated with extreme caution. DO NOT expose yourself to BSC UV light. **The UV lamp should never be left on when the hood is open**. Even a small opening at the bottom of the cabinet can exceed occupational exposure standard several feet away.

UV light usage in biosafety cabinet:

- Before starting experiment, keep the sash off, TURN ON the UV light, and keep it on for 15 minutes. Then TURN OFF UV light. Open the sash. Clean the surface of the BSC. Then start the experiment.
- When experiment is done, clean the work space inside of the BSC. Close the front window of the BSC. Then TURN ON the UV light. The UV light will be off automatically after one hour.

Fluorescence microscopy uses UV light source. The UV beam is sent through the objective to the sample. The emission light is fluorescent light, not the UV light. This is considered relative safe. However, when you position you sample slides, you might look directly at UV light range. It is mandatory to wear UV protection goggles while working with the Fluorescent microscope. The goggles can be found by the side of the microscope. So when the user adjusts sample slide position, the following safe procedure is required.

- Always start with “bright field” of the light source. Place the sample slide on sample stage and adjust to the interested view position using the mechanical stage control knob.
- Wear the safety glass on and then switch to UV light range. Use the correct UV light filter to view the sample slide.
- Keep your safety glass on until your experiment is done.
- **SWITCH THE LIGHT SOURCE BACK TO BRIGHT FIELD**. Then turn off the instrument.

Acute effects include redness or ulceration of the skin. The long-term large cumulative chronic exposure includes premature aging of the skin, wrinkles, and skin cancer. The eyes are also susceptible to UV damage. The danger to the eyes is enhanced by the fact that light can enter from all angles around the eye and not only in the direction where one is looking. Chronic exposure to UV-A radiation can lead to the formation of cataracts. When such incidents of overexposure occur, it is important to identify the causes and adjust your practice to prevent future incident, and seek medical treatment. In case of exposure, seek medical attention immediately.

SECTION 3.4: CRYOGENIC HANDLING

Liquid nitrogen (LN2) is inert, colorless, odorless, non-corrosive, non-flammable, tasteless, extremely cold, and has no warning properties. Special care must be taken by personnel who handle or work in areas where liquid nitrogen is used. The hazards associated with LN2 include:

- Over-pressurization and explosion due to LN2 vaporizing to nitrogen gas (700x expansion ratio) in unvented containers.
- Severe burns caused by exposure to cold temperatures.
- Asphyxiation due to displacement of oxygen in the air in confined work areas.

Humans cannot reliably detect the presence of nitrogen. Liquid nitrogen has a 700x expansion ratio which may create physical hazards and injuries from the explosion of unvented containers. Extensive tissue damage or burns can result from exposure to LN2 or cold nitrogen vapors.

Asphyxiation may result from the displacement of oxygen in the air with nitrogen to levels where there is insufficient oxygen to support life. Inhalation of oxygen deficient air can cause dizziness, nausea, vomiting, loss of consciousness, and death.

In addition, the BNRF has an ultralow temperature freezer for sample and reagent storage. Working with materials that have been stored in the freezer is dangerous and can cause cryogenic burns or damage to human skin.

SECTION 3.4.1: PERSONAL PROTECTIVE EQUIPMENT – LIQUID NITROGEN

The following personal protective equipment is required when handling or using LN2:

- **Water proof thermal insulated gloves** (e.g., cryo-gloves):- Hands must be protected with water proof thermal insulated gloves that can be quickly removed if LN2 is spilled on them. Insulated gloves are not intended for submersing hands into LN2. Users should use insulated gloves when working with LN2 and the ultralow freezer to prevent the occurrence of cryogenic burns.

- **Safety goggles**:- Eyes are most sensitive to the extreme cold of LN2 and its vapors. Over-pressurization may result in the explosion of improperly vented equipment. Chemical splash goggles must be utilized when handling LN2.

- **Full face shield**:- Face must be protected against splashes and spills of liquid nitrogen by a face shield.

SECTION 3.4.2: ACCIDENTAL EXPOSURE

Users are required to wear appropriate personal protective equipment (PPE) for their safety. If at any time a piece of PPE becomes damaged or torn, it should be replaced immediately. If a

user has been exposed to liquid nitrogen, the following procedures should be followed immediately.

For frostbite exposure:

1. Remove all contaminated clothing and gloves, and any clothing item that may restrict circulation to the frozen area.
2. Do not rub frozen parts, as tissue damage may result.
3. Place the affected area in a warm water bath that has a temperature not exceeding 105°F (40°C).
4. Never use dry heat!!

In case of MEDCIAL EMERGENCY contact:

9-911 from any campus phone
or
Campus Security
Phone: **(304) 293-3136 (293-COPS)**

If going to the hospital for medical assistance, make sure you:

- **Take a copy of the MSDS with you**
- Inform medical personnel you were exposed to liquid nitrogen.

SECTION 3.4.3: LIQUID NITROGEN SPILL RESPONSE

Personal protective equipment should be worn at all times. If a significant quantity of liquid nitrogen has escaped, or been spilt, the area affected will not contain adequate oxygen to support life. The area should be immediately evacuated and the following personnel should be contacted:

Environmental Health & Safety
Phone: **(304) 293-3792**

Then call,

Huiyuan Li, BioNano Research Facility Manager
Office Phone: **(304) 293-0747**
Cell Phone: **(304) 906-5368**
Room: **380 CRL**

Or

Kolin Brown, Cleanroom Manager
Cell Phone: **(304) 366-6551**
Office Phone: **(304) 293-9683**
Room: **G75D ESB**

If no one responds to any numbers above, then contact:

Barbara Foster
Office Phone: **(304) 293-2729**
Room**: 215 Clark Hall**

If a person is showing symptoms of mild or severe asphyxia, they should be moved to an area with fresh air. If they are not conscious, security should be called immediately and resuscitation started by a qualified first aid officer or physician.

Cold burns from liquid nitrogen should be immediately and continually flushed with tepid or unheated tap water.

Professional medical advice should always be sought urgently for significant cold burns and asphyxia. Persons affected may need urgent medical treatment at a hospital.

SECTION 3.5: CHEMICAL HAZARD

There are several chemicals that are used frequently for the Mass Spectrometer in the 380 CRL BNRF lab:

- Methanol
- Acetonitrile
- 2-Propanol
- Acetic acid
- Hydrochloric acid
- Phosphoric acid
- Trifluoroacetic acid

The chemical inventory can be found in APPENDIX A.

SECTION 3.5.1: CHEMICAL STORAGE

Chemicals for the Mass Spectrometer and other general purpose are stored in the chemical storage cabinets. The chemicals are categorized as acid, base, flammable, and solid. The chemicals in the same category are stored in one cabinet.

Other chemicals used are stored in 4 degree or -20 degree explosion-proof fridge/freezer as suggested by manufactures.

SECTION 3.5.2: AUTHORIZING CHEMICAL USAGE

The WVU Shared Research Facilities BNRF only purchases chemicals that are general use. Often, research projects require the use of chemicals that are specific to a project. Any BNRF user, who wishes to bring a new chemical into the BNRF, must first obtain permission before bringing a new chemical into the lab. The user must submit a signed material tracking form and a material safety data sheet(s) (MSDS) for each chemical container to the BNRF manager for approval. A copy of this form can be found on the shared research facilities website (http://sharedresearchfacilities.wvu.edu/r/download/154420). This form identifies the chemical, proper storage and proper disposal methods. Material tracking forms are kept in a binder in the BNRF manager's office. The MSDS are added to the MSDS files kept in 380 CRL.

All containers must be brought to a SRF staff member to receive an approved chemical label, which is signed and dated by the SRF staff member. The chemical container must be appropriate for the chemical and it must be labeled appropriately with the full chemical name to receive an approved chemical label. Only containers with approved chemical labels may be taken into the BNRF.

A chemical must be approved before being brought or stored into BNRF, even if the chemical is only going to be used one time.

SECTION 3.5.3: CHEMICAL HANDLING

All wet chemical processing or any work with open chemical vessels must be performed in a chemical hood. The BNRF has 2 chemical hoods. Users are required to wear personal protective equipment (PPE), such as lab coat, goggles, and nitrile gloves when working at chemical hoods.

When working in a chemical hood:

- Use the appropriate PPE and type of gloves for chemical usage.
- Keep the work area inside the hood clean and free from obstructions.
- Do not store items in the hood.
- Do not leave unlabeled chemicals unattended in a hood.

SECTION 3.5.4: ACCIDENTAL CHEMICAL EXPOSURE

Users are required to wear appropriate personal protective equipment (PPE) for their safety, if at any piece of PPE becomes damaged or torn it should be replaced immediately. If a user has been exposed to a chemical, the following procedures should be followed immediately.

For eye exposure:

- Remove all contaminated clothing and gloves.
- Initiate water flow at eyewash station by either pressing on the pedal or the hand lever.
- Hold eyes open with fingers and lower face into eyewash bowl, so that water is rinsing the eyes.
- Keep eyes open and rotate.
- Flush eyes for a total of 15 minutes.
- Seek emergency medical attention.

For exposure to any part of the body:

WARNING: Do not wipe off the chemical; you will only increase area of contact! Increasing area of contact will increase absorption through the skin and may result in faster or more sever reaction or poisoning.

- Remove all contaminated clothing and gloves.
- Initiate water flow at safety show by pulling down ring.
- Flush contaminated area for a total of 15 minutes.
- Seek emergency medical attention

In case of MEDCIAL EMERGENCY contact:

9-911 from any campus phone
or
Campus Security
Phone: **(304) 293-3136 (293-COPS)**

If going to the hospital for medical assistance, make sure you:

- **Take a copy of the MSDS with you**

SECTION 3.5.5: CHEMICAL SPILL RESPONSE

If at any time a user is unsure of how to clean up a spill or is uncomfortable in trying to clean up the spill they should immediately evacuate the area and seek assistance. Users should attempt

to only contain the spill and then seek assistance from the SRF staff. Personal protective equipment should be worn at all times.

If a chemical has been spilled:

A spill kit is kept in the BNRF lab beside the chemical hoods. Users should only attempt to contain the spill if it is less than one gallon.

To contain the spill:

1. Open the spill kit by pressing the red lever with the thumb and rotating the lid counter clockwise.
2. Put on the green gloves inside the spill kit.
3. Remove a pink absorbent pad and place over the spill area. Use as many pads as necessary to contain the spill.
4. Seek SRF staff assistance.
5. With staff assistance, place all pads in the garbage bag.
6. Remove any remaining contents from the spill kit and place the garbage bag inside the pail.
7. Close the spill kit lid.
8. Fill out the USED Spill Kit label and attach to outside of the kit.
9. The BNRF staff will contact EH&S for pickup.

In case of an ACUTELY HAZARDOUS SPILL contact in the following order:

Environmental Health & Safety
Phone: **(304)293-3792**

Then Call,

Huiyuan Li, BNRF Manager
Cell phone: **(304)906-5368**
Office phone: **(304)293-0747**
Room: **380 CRL**

Kolin Brown, Cleanroom Manager
Cell Phone: **(304)366-6551**
Office Phone: **(304)293-9683**
Room: **G75D ESB**

or
Marcela Redigolo, Electron Microscopy Facility Manager

Cell Phone: **(214)766-2904**

If no one responds to any numbers above, then contact:

Barbara Foster, Safety director of C. Eugene Bennett Department of Chemistry
Office Phone: **(304) 293-2729**
Room**: 215 Clark Hall**

SECTION 3.5.6: CHEMICAL DISPOSAL

Used chemicals must be disposed into chemical waste jars. All chemical waste is disposed and stored in an appropriately chosen waste jar that is properly labeled with the word WASTE. The jar label should clearly list all contents and percentages of chemical waste in the jar. Ideally, the waste jar should be an empty original container of the same chemical being disposed of. Each chemical or mixture should have its own waste jar. Waste jars should be closed when not in use. Do not overfill the waste jar. The chemical waste labels are available on the top of bookshelf in room 380E.

A chemical disposal form is filled out and submitted to EH&S for pickup. A copy of the hazardous chemical disposal form can be found on the WVU EH&S website, http://ehs.wvu.edu/forms.

SECTION 3.6: MECHANICAL HAZARDS

BNRF 380 CRL has several centrifuges, mixers, and shakers for sample preparation. Some general safety guidelines must be followed:

- Do not wear loose fitting clothing or necklaces that could be drawn into a rotating assembly.
- Do not defeat interlocks on doors, access panels, etc.
- Do not stop moving parts by hand. Wait for motion to stop on its own.
- Be aware of assemblies that vibrate and could “walk” into other objects or fall off the bench.
- Safety glasses must always be worn around any power tool (such as centrifuges, mixers, and shakers) when operating.
- Always be familiar with the operating procedures writing by the manufacturer.
- When using the centrifuge, pay careful attention to the instructions on balancing samples. Check the condition of tubes and bottles. Make sure you have secured the lid to the rotor and the rotor to the centrifuge. **ALWAYS** use screw-capped tubes!

SECTION 3.7: HIGH VOLTAGE HAZARDS

The following instrument inside the BNRF uses high voltages:

- Mass Spectrometer

All Mass Spectrometer users are given proper instruction on equipment operation before given authorization to use these tools. Users must follow all operational procedures outlined in the Standard Operating Procedures located in Appendix B.

You must "STANDBY" the instrument when you do the following procedures:

- Open the ion-source to clean the HESI probe.
- Open the ion-source to change ion transfer tube.
- Open the ion-source to change to another ion-source.

You must completely shut down the instrument when you do the following procedures:

- Open the ion-source to clean the S-lens.
- Change the pump oil.

WARNING: Any attempt to bypass any safety interlocks on any piece of equipment in the BNRF may result in severe shock or electrocution.

SECTION 3.8: VACUUM HAZARDS

There are two instruments in BNRF using vacuum systems:

- Mass Spectrometer
- SpeedVac system

All Mass Spectrometer and SpeedVac users are given proper instruction on equipment operation before given authorization to use these tools. Users must follow all operational procedures outlined in the Standard Operating Procedures located in Appendix B.

A high vacuum system is required for the Mass Spectrometer. The following guidelines should be followed:

1. Never shut down the oil pump when the system is running.
2. Never try to open the ion-source when the system is running and vacuum is on.

Glass cold trap is used in SpeedVac to collect concentrates of the evaporated solvents. Some general guidelines should be followed:

- Only use the designated glass cold trap for this purpose.
- There are no defects on the glass cold trap, such as chips or cracks, that may compromise the integrity.
- Before you start the operation, make sure there is no leftover solvent in the cold trap. Otherwise remove the solvent to the designated chemical waste bottle with clear labels. Re-install the cold trap and make sure it seals well.

- Check if there is enough anti-freezing oil in the chamber. Add to the designated line if needed.
- Do not cryopump reactive materials. Only several types of solvent could be used in SpeedVac, such as H2O, Methanol, and Acetonitrile.

SECTION 4: GAS SAFETY

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The following gas cylinders are typically used by the cell culture incubators inside the cell culture room:

- Carbon Dioxide

Users should not have to open, close, or make adjustments with the regulators. Adjustments to regulators should ONLY be done by SRF BNRF staff.

All cylinders in use are mounted securely against the wall. All cylinders are transported using a cylinder hand truck. Eye protection or a face shield is to be worn when changing cylinders.

SECTION 5: VACUUM PUMP OIL

The Mass Spectrometer requires high vacuum. Oil is used in the instrument's vacuum pumps. Only authorized personnel are allowed to change the vacuum pumps oil. Due to contract at the present moment, only the engineers of the manufacturer of the Mass Spectrometer, Thermo Fisher, are authorized to do such procedure.

In case of maintenance, all used oil must be captured in an appropriately chosen waste jar, which is properly labeled with the words USED OIL. Once the service is completed, the waste is scheduled to dispose.

A hazardous chemical disposal form is filled out and submitted to EH&S for pickup. A copy of the hazardous chemical disposal form can be found on the WVU EH&S website, http://ehs.wvu.edu/forms.

APPENDIX A

CHEMICAL INVENTORY AND MATERIAL SAFETY DATA SHEET (MSDS)

BNRF LAB – room 380 CRL

APPENDIX B

STANDARD OPERATING PROCEDURES

BNRF LAB – room 380 CRL

APPENDIX C

EH&S Files
Waste Tissue Culture Media Guidance
Used and leftover buffers

BNRF LAB – room 380A CRL

APPENDIX D

BNRF cell culture media formulations and additives

BNRF buffers

BNRF LAB – room 380A CRL

BNRF cell culture media formulations and additives
BNRF buffers

TABLE 1 – Tissue Media Formulations

Dulbecco's Modified Eagle's Medium (DMEM)	ATCC 30-2002
Eagle's Minimum Essential Medium (EMEM)	ATCC 30-2003
F-12K Medium	ATCC 30-2004
Fetal Bovine Serum (FBS)	Sigma Aldrich 12003C

TABLE 2 – Additives/buffers

Dulbecco's Modified Eagle's Medium (DMEM)	ATCC 30-2002
Dulbecco's Phosphate Buffered Saline (DPBS)	Cellgro 20-030-CV
Hank's Balanced Salt Solution (HBSS)	Gibco 14025076
HEPES Buffered Saline Solution	Lonza CC-5022
HEPES Buffer Solution (1M)	Sigma Aldrich 59205C; CAS # 7365-45-9
Hydrochloric Acid Solution	Fisher SA54-1; CAS # 7647-01-0
L-Glutamine	Hyclone SH3003401; CAS # 58-85-9
Penicillin Streptomycin	Hyclone SV30010; CAS # 3810-74-0
Trypsin 0.25%	Thermo SH3023601; CAS # 9068-82-0
Trypsin 0.05%	Thermo SH3004201; CAS # 9068-82-0

TABLE 3 – Stains

Alexa Fluor 546 phalloidin	Invitrogen A22283
BODIPY 558/568 phalloidin	Invitrogen B3475
LysoTracker Deep Red	Life Technologies L12492; CAS # 200-664-3
CellMask Orange plasma membrane stain	Invitrogen C10045; CAS # 67-68-5
ProLong Gold antifade reagent	Invitrogen P36930
FM 4-64 membrane stain	Invitrogen F34653
Trypan Blue	Thermo SV3008401; CAS # 72-57-1