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| Fluorescent Microscope Operation |
| **Purpose** | This procedure provides instruction for FLUORESCENT MICROSCOPE OPERATION. |
| **Policy Statements** | This procedure applies to technical staff who perform fluorescent microscopy. |
| **Principle** | A high-pressure mercury bulb light source is used to irradiate a fluorochrome stained specimen with UV excitation light. Fluorescent compounds in the specimen absorb some of the excitation light and reemit (fluoresce) the remaining light at a lower wavelength. The emitted light and the reflected excitation light are collected by the objective and pass through a dichromatic mirror which removes the excitation light and allows the longer wavelength emitted light to form an image. The fluorescence microscope is equipped with filters (490nm/520nm band pass) suitable for use with fluorescein isothiocyanate, R-phycoerythrin, auramine rhodamine and acridine orange. |
| **Materials** | **Supplies** |
|  | * Lens cleaner
* Lens paper
* Immersion oil (Zeiss 1111806)
* Mercury Spill Kit # 23946, LabSafety Supply, Inc.-Located outside of the darkroom above the virology sink.
* Mercury Lamp, short arc HBO 50W/AC L2 (Osram) Alpha Source product # 69214-1 CHC#9878
* Phillips screwdriver
* Slotted screwdriver
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| **Special Safety Precautions** | 1. Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**
2. [*Biohazard Containment*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicrobiology%5CMC%20200%20%20%20%20Safety%5CMC%20201%20%20%20Biohazard%20Containment%20R.doc)
3. [*Safety in the Microbiology/Virology Laboratory*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicrobiology%5CMC%20200%20%20%20%20Safety%5CMC%20202%20Safety%20in%20the%20Microbiology%20Lab%20Policy%20R.docx)
* [*Biohazardous Spills*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicrobiology%5CMC%20200%20%20%20%20Safety%5CMC%20204%20Biohazardous%20Spills%20R.docx)
1. Physical Hazard:
* The mercury lamp emits UV light, which is damaging to the eyes and skin. **Do not look** **directly at the mercury arc**. The high luminance of the lamp can cause severe damage to the retina if the arc or the surface of the reflector is viewed directly. The barrier filters in the scope prevent UV light from entering the eye during routine use of the microscope.
* The mercury lamp is under high pressure when warm and can become unstable and explode .It is, however, located within a protective housing that will contain the explosion.
1. Chemical Hazard:
* If a lamp should explode, mercury vapor will escape. The vapor will condense and fall inside the housing. If a bulb explodes:
* All personnel should leave the immediate area at once so that no mercury vapor is inhaled.
* Close the curtain at the darkroom entrance.
* The area should be thoroughly ventilated for a minimum of 30 minutes.
* When the lamp housing has cooled, any mercury residue should be picked up with a special adsorptive agent contained in the Mercury Spill Kit. Call Security at 5-7777 and contact the Safety Specialist 6-6712 for disposal instructions. Wear gloves and handle material in a safe manner to prevent cuts from any glass shards which may be present.
* Contact BioMed (5-6383) after decontamination is complete to arrange for repair as the collector lens will likely have been damaged.
* Notify Lead MLS, Micro Supervisor and Lab Safety Officer.
* Complete a Laboratory Safety Report and submit it to the Laboratory Safety Officer for quality assurance
* Submit a copy of the report to the supervisor.

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| **Procedure** | Operation1. Keep a log of the hours the lamp is in use. The bulbs have an expected life of 180-200 hours. Bulbs exceeding 200 hours have a much greater risk of instability and explosion.
2. Avoid switching the microscope on and off frequently, as this can impair the stability of the lamp and shorten its life. The bulb must cool down for **one hour** prior to being reignited. Reigniting a warm bulb increases the chance of explosion.
3. Allow the bulb to warm up for 5-10 minutes before use to attain its maximum brightness.
4. Close the shutter in the light path when the slide on the stage is not being viewed to avoid quenching of the fluorescence.
5. Mounting media (buffered glycerol) of a pH appropriate for the stain must be used for coverslipping FA slides to achieve adequate fluorescence.
6. Store stained slides in the dark at 2-8°C or colder to preserve fluorescence.
7. After each session, remove oil or mounting fluid from the objectives, condenser, and stage using lens cleaner and lens paper. Clean the dry objectives first before cleaning the oil immersion objectives.
8. Avoid dragging the high dry objective through oil on the slide.
9. Organic solvents such as alcohols and acetone should not be used on the lenses because the solvent may dissolve the optical mounting cement.
10. The stage should be cleaned regularly and any spilled immersion oil and mounting fluid must be removed or slides will stick as they are moved across the stage.
11. Annually, the microscope is to be cleaned and adjusted professionally.
12. Bulb Replacement - The mercury bulb should be replaced as soon as the bulb shows advanced blackening causing decreased brightness or has been in use for 200 hours. The risk of the bulb bursting is greatly increased with advanced blackening.
13. Wait for lamp housing to cool down before opening (at least 30 minutes).
14. Unplug the power unit from the wall.

1. Loosen Phillips screws on right and left side of housing front.
2. Pull housing front up using knob.
3. Loosen screws **#4** and **#6** on socket.
4. Pull socket out.
5. Loosen Phillips screw **#1** on heat sink and pull heat sink off.
6. Loosen setscrew on base of lamp and pull bulb out.
7. Put used bulb in zip lock bag, seal, label with Hazardous Waste label and take to Hazardous Waste Room located in basement for disposal.
8. Never touch glass parts of the bulb with your hands. When inadvertently touched, it should be degreased immediately with alcohol and a soft, lint free cloth. Wipe dry. Insert new bulb with “UP” on top. Position bulb so seal point is at the side not in the light path. Tighten setscrew and reattach heat sink.
9. Slide socket back in.
10. Tighten screws **#4** and **#6** on socket.
11. Close housing front and tighten screws on housing.
12. Turn scope on.
13. Allow bulb to warm up for 5-10 minutes and perform alignment as soon as bulb warms up.
14. Alignment

1. Remove an objective and set aside. Place a white card on the stage.
2. Open the light path so it shines on the card.
3. Focus the filament image with knob **#6** (collector).
4. Separate the real image and reflected (mirror) image using horizontal adjustment **#5**.
5. Focus the mirror image using adjustment **#3**.
6. Center the real image using adjustment **#1** (vertical) and **#5** (horizontal).
7. Align mirror image over real image using adjustment **#4**.
8. Replace objective and check field brightness using a control slide.
9. Adjust the field brightness using the focus knob **#6**.

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| **References** | 1. Versalovic, James ., et al, *Manual of Clinical Microbiology*, 10th edition, 2011, ASM press, American Society for Microbiology, Washington, D.C.
2. Leica DMLS Microscope Instruction Manual, 1995, Leica Mikroskopie, Wetzlar, Germany.
3. Osram Mercury Lamp package insert, 06/13.
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| **Training Plan/ Competency Assessment** | **Training Plan** |  **Competency Assessment** |
| 1. Employee must read the procedure
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation
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| **Historical Record** |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** |
| 1 | Becky Carlson | 07/11/1993 |
| 1.2 | Helen Stefan | 08/03/2007 Formatting change ; added bulb change procedure |
| 1.3 | Helen Stefan | 09/11/2011 Added order information for mercury spill kit and FITC filter specifications. |
| 1.4 | Tina Gronquist | 08/11/2014 Reformatted to CMS version |
| 2 | Becky Carlson Helen Stefan | 4/4/2015 Re-numbered from MC 810Added close darkroom curtain in the event of bulb breakage, use of buffered glycerol mounting media in operation section, CHC order number for mercury bulb and updated references. |
| 3 | Helen Stefan | 7/25/17 Updated logo and lamp housing diagram in replacement and alignment section. Added R-phycoerythrin |
| **Archived by:** |  | **Archived Date:** |
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