1. Purpose

To set-up laboratory microscope, prepare microscopic slides and view them under microscope

2. Background Information

Microscopy is the study of minute objects by way of magnification allowing us to see objects not seen by the naked eye. The microscope was first invented in 1590 and since then has been perfected and redeveloped into a sophisticated, useful piece of machinery.

The invention of the very first microscope is credited to Hans, and his son, Zacharias Janssen in approximately 1590. The first microscope consisted of a simple tube with lenses at either end. Total magnification was between 3x and 9x. Robert Hooke then improved on this creating a more sophisticated compound microscope in 1660. Hooke coined the term “cell” from observing cork under the microscope. Anton van Leeuwenhoek (1632-1723) was credited with creating the best microscopes of the time period. He was the first to describe bacteria (teeth scrapings), protozoans from pond water and helped to prove theory of blood circulation.

There are four types of microscopes split into two categories; light microscopes which consist of the compound and dissection microscopes and electron microscopes which include the scanning electron microscope and transmission electron microscope. The most commonly used microscope is the compound microscope.
Parts of microscope

- Ocular lens or eye pieces
- Head
- Objective lenses
- Arm
- Stage
- Stage control
- Nose piece or turret
- Iris diaphragm and condensor
- Light source
- Brightness adjustment
- Base
- Course focus
- Fine focus
- Light switch
3. Safety Information

4. Materials/Equipment

Microscope
Microscope slide

5. Procedure

Set-up and pre-use checks of the microscope

- Carefully unpack the microscope from its carrying box or remove the dust cover.
- Place in a suitable position on the bench at the correct working height for the user. Do not place too close to the edge of the bench.
- Check the power cord is in good order and plug it in if not already plugged into a power socket.
- Switch on the power at the socket and on the microscope, check the light bulb is operating.
- Rotate the objective lens turret to place the 4x objective lens in position over the light source.
- Clean all refracting surfaces to remove dust and grease. See the ‘Section-Care of an Optical Microscope’ for more information.
- Fill in the equipment log with your name, date, time you used the instrument and note any comments on its performance.

Care of an optical microscope

- Location for use: avoid the following conditions - dust, vibration and exposure to high temperature, moisture or direct sunlight.
- Use the coarse adjustment only with the low power objective.
- Clean all oculars and objectives with lens paper and 70% ethanol after each use. Use a soft brush to remove dust.
Move or transport the microscope with one hand under the base and the other hand gripping the arm
Avoid jarring or bumping the microscope
Use oil each time the oil immersion lens is used. Use immersion oil with the oil immersion objective only
Store the covered microscope in a protected area. Cover with the vinyl cover and store in a place free from moisture, dust and fungus
Since bulbs are expensive and have a limited life, turn the illuminator off when you are done. When replacing a bulb or fuse be sure to turn off the power switch and disconnect the power source cord from the socket
Never attempt to dismantle the microscope, to avoid the possibility of impairing the operational efficiency and accuracy
To maintain the performance of the instrument have the microscope serviced regularly, eg annually
If a microscope is unserviceable, attach an 'Unserviceable' tag to the arm of the microscope and disconnect the power lead. Fill out the Equipment Log and advise the trainer

Alignment of microscope using Kohler Illumination

Switch on the light source and make sure that light is coming through the field diaphragm at the base of the microscope stand. It may help to place a piece of paper over the field diaphragm to see the light
Place your specimen on the stage and turn the nosepiece (which holds the objective lenses) to the 10X lens. Open the field diaphragm as far as it will go
Notice whether or not your specimen is illuminated. It will help to place a piece of paper over the top of the specimen to see if light is getting through to it. Open the aperture diaphragm (on the condenser) fully to give the maximum illumination
Now bring your specimen into focus with the focussing knobs. If the light is too bright, reduce it with the rheostat/dimmer on the light source
When the specimen is in focus, close the field diaphragm and begin to carefully move the condenser up and down with the condenser focussing knobs. Look for a sharp image of the edge of the field diaphragm
When the edge of the field diaphragm silhouette is sharply defined, centre it with the two knobs coming out diagonally from the condenser (or the field diaphragm on some microscopes). When it is centred, open the field diaphragm until its edge is outside the field
Now open up the field diaphragm until the edge of the diaphragm silhouette is outside the field of view. You should also now be able to turn up the light at the dimmer.

Your specimen should be properly illuminated and should give you a clear image. If it does not, check to make sure your lenses and other optical components are clean. Then re-check to see that you have followed each step properly.

Mounting slides

- Take plastic cover of microscope.
- Plug in power cord.
- Turn on light switch.
- Turn to lowest power objective lens – the "scanning lens".
- Mount the slide.
- Use the course adjustment knob to move stage to the topmost position.
- Adjust the focus with the course adjustment knob.
- Centre the object to view into the centre of focus.
- Move to the next highest power objective lens.
- Adjust only the fine adjustment knob to focus.
- If necessary centre the object to view into the centre of focus.
- Move to the next highest power objective lens.
- Adjust only the fine adjustment knob to focus.

Preparing wet mount slides - Solid samples

- Place a clean slide on a small piece of paper towel. Label one end of the slide with a pencil if the slide has a ground glass end or use an indelible pen.
- Prepare sample to be placed onto the microscope. This method will vary depending on the sample to be used. A small section of the solid sample is cut/
- Place the sample in the centre of the slide. For any tissue samples, ensure that they are flat against the slide, otherwise air bubbles may occur and these will affect the quality of the image.
- If preparing a slide with tissue, place a drop or 2 of water or stain onto the tissue.
- Place the cover slip over the sample by carefully placing one edge onto the slide and then lowering the rest of the cover slip. Allow the fluid to spread out between the slide and the cover slip, but do not add any pressure.
- If there is excess fluid coming from under the cover slip, carefully absorb it using a small amount of paper towel.
Preparing a wet mount slide – Fluid samples

- Place a clean slide on a small piece of paper towel. Label one end of the slide with a pencil if the slide has a ground glass end or use an indelible pen.
- With a disposable plastic pipette take up a small amount of liquid from the sample and place a drop on the middle of the slide.
- Holding the coverslip by the edges, place it at an angle of about 45° so that the angle includes the specimen being examined and the drop of water it is in. Gently lower the coverslip, which prevents bubbles (another type of artefact) from being trapped under the coverslip. If bubbles are present, tap the coverslip gently with a dissecting needle to move them to the edges of the coverslip and out from under it.
- The slide is now ready to be placed on a microscope and be examined for quality control.

Wet preparations will not last, slides will dry out quickly, so the observations must be made and recorded within the next half-hour.

Smear preparation

- Choose a clean and unscratched microscope slide (to reduce artefacts). Wipe the slide with a wet tissue to remove any residual oil or dust.
- Label the slide on the frosted end with the sample number and details and allow the slide to dry.
- Place a drop of distilled water on the middle of the slide.
- Using a sterile wire loop, transfer a small amount of bacteria to the drop of water (just touch the loop to the colony surface). Mix the loop in the water to ensure a homogenous suspension of bacteria in the water and spread the drop out over the surface of the slide (so that the smear is not too thick and the smear dries more quickly).
- Dry the smear by leaving the slide on the bench
- Be careful handling slides, any broken slides should be disposed of in the sharps bin as soon as possible.
- Always notify your trainer or supervisor if there is broken glass on the floor or benches.
- Always hold slides on the edges, this avoids smudges and fingerprints interfering with the image you see down the microscope.
- Observe your slide with the microscope, ensuring you locate a good region of the epidermis with the scanning (x4) lens before you increase your magnification to view the details of the cell structures.
6. Review

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Details</th>
<th>Approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>05-May-19</td>
<td>Original Issue</td>
<td>KR</td>
</tr>
</tbody>
</table>