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# Microscope care and handling

**General guidelines for use and handling of the microscope**

1. When you move your microscope, you should always use two hands. Place one hand around the arm, lift the scope, and then put your other hand under the base of the scope for support.

2. The work table should be large, flat, and stable.

3. Never touch the eyepiece or objective lens with your fingers because the oil on your skin will soil the lens.

4. Focus using the lowest power of magnification first, then increase magnification.

5. Know which way the focusing knob turns to raise and lower the stage, as it is important not to smash your sample into an objective. A good rule of thumb until familiar with the microscope is to set the minimum separation between the objective and the slide by external inspection and then focus by lowering the stage away from the objective.

> Note: The objectives in most modern microscopes are parfocaled. After the specimen is clearly focused by means of the 4X objective, the 10X, 40X, and 100X objectives can usually be swivelled directly into the light path and only require a slight adjustment of the fine focus knob to achieve sharp focus.

6. Dust is an enemy to your lenses, hence when finished; the microscope must be covered with a plastic cover again to keep out dust.

7. To preserve the lamps always turn the microscope on, then increase the intensity to the desired level. When finished first decrease lamp intensity, then turn the power off.

**Microscope cleaning**

**Caveats**

- Do not use dry lens tissue on a lens.
- Avoid touching lenses. Even light fingerprints, especially on objectives, can seriously degrade image quality.
- Lint free lens cleaning tissues should be used. Use a fresh portion of the lens paper frequently so that you don't transfer dust from one lens to another. Do not touch the part of the tissue that will be applied to the lens; excessive touching transfers natural oils from the fingers to the lens tissue
- Never use facial tissues to clean lenses. Such tissues are made of ground up wood fibres and may contain glass filaments, which can scratch lenses.
- Follow the manufacturer's recommendation for using cleaning solvents other than distilled water. Xylene used sparingly is generally acceptable for serious stains, such as residual oils.
- The first step in keeping your scope clean is to help it to not get too dirty. Although some dust is inevitable, always keep your scope covered with the dust cover when it is not in use. Never leave microscope tubes open. Always keep them closed with dust plug, eyepiece, or objective, as appropriate.

- Do not attempt to take optics apart for cleaning. Internal optics should not need routine cleaning and should be professionally serviced if needed.
- Use proper immersion liquids on immersion objectives as specified by the manufacturer. Avoid getting immersion liquid on non-immersion objectives; it can damage the lens mounting glue.

*Three basic principles underlie cleaning.*

1. Remove particulates and grit

2. Dissolve “grime” in minimal solvent

3. Remove by absorbing and or moving residue away by progressive changes of tissue

Begin by blowing off dust or loose material with a pressurized optical duster (sometimes called canned air or “Air Duster” (filtered dry nitrogen) or by using a bellows ball. Dust areas of the condenser lens *prior* to objectives in view of minute glass fragments that may be present from broken or chipped slides.

Careful fogging of the lens with breath can assist in removing water-soluble residue, although caution should be exhibited in regard to *excluding* saliva. Alternatively a small amount of distilled water may be used.

If still necessary, apply a small amount of lens cleaning solution to the lens tissue. Wipe the lens very lightly to remove gross dirt that was not blown away by the rubber bulb. If necessary, repeat the cleaning process with a new piece of lens tissue and with more pressure to remove oily or greasy residue.

**Cleaning Oil Immersion (O.I) Lens**

Oil immersion lens are generally wiped clean after use, initially removing oil from the outer edges, but if a lens does need more thorough cleaning, it may necessitate use of a solvent.

Consult the MSDS (Material Safety Data Sheets) information that is supplied with the immersion oil to see what solvents are recommended and cross reference this with the microscope manufacturer’s recommendations.
For the Cargille Type A or B immersion oil (most widely used), you can use naphtha, xylene, or turpentine (use very small amounts on lens tissue). Do not use water, alcohol or acetone, as the oil is insoluble to these solvents.

**Table 1**: Cargille Oils

| OIL TYPE | SOLUBLE | INSOLUBLE |
|---|---|---|
| TYPE A; B; 30 TYPE 37; NVH; OVH | CARBON TETRACHLORIDE; ETHYL ETHER; FREON FF HEPTANE; METHYLENE CHLORIDE; NAPTHA; TOLUENE; TURPENTINE; XYLENE | ACETONE; ETHANOL; WATER |
| TYPE DF | ACETONE;CARBON TETRACHLORIDE; ETHANOL; ETHYL ETHER; FREON TF; HEPTANE; METHYLENE CHLORIDE; NAPTHA; TOLUENE; TURPENTINE; XYLENE | WATER |
| TYPE FF | ; CARBON TETRACHLORIDE; ETHYL ETHER; FREON FF HEPTANE; METHYLENE CHLORIDE; NAPTHA; TOLUENE; TURPENTINE; XYLENE | (PARTLY SOLUBLE ACETONE) ETHANOL WATER |

Note that if too much solvent is applied, this may affect the integrity of the mountant e.g. cement or balsam in particular that was used to mount the objective lens (most important in older microscopes). In view of this, minimal application of a high vapour solvent is used in order to limit contact time with surfaces and cements. The lens should also be kept upright, and only allow the tissue to touch the base of the objective, which should help to prevent the solvent running into cements or other substances holding the lens in place.

**Set-up and Illumination of the Compound Microscope**

1) Adjust the microscope for each eye.

One eyepiece, or the tube into which it fits, is usually adjustable. Place a specimen slide on the microscope stage, turn on the illumination, and focus at low magnification. Cover the eyepiece that has the focusing eye tube with a card and, with both eyes open, bring the specimen into focus for the other eye with the fine focus knob. It is important that vision be relaxed by looking up frequently to distant objects or to infinity by staring "through the wall." This will help prevent eyestrain caused by trying to "accommodate" the object, bringing it into focus with the eye at a point closer than infinity. When consistent sharp and relaxed focus has been obtained at one particular point on the slide, switch the card to cover the other eyepiece, but this time use the focusing ring on the open eyepiece to bring the same point on the slide into focus.

2) Set-up **Koehler illumination** (see below).

For optimum results with a compound light microscope, proper illumination of the specimen is essential. Koehler illumination is proper alignment of the incident or illuminating light for microscopy.
If you fail to do this, you will often have poor resolution, wacky contrast artefacts, and unevenly lit pictures.

There are several physical/mechanical requirements that will allow a microscope to be set-up with Koehler illumination. As a result, Koehler illumination may not be possible on less sophisticated microscopes. (For example, most "student" microscopes have fixed condensers and diaphragms, set to a compromise position). The sub-stage condenser must be capable of being focused up and down, the sub-stage condenser must be fitted with an aperture iris diaphragm and the lamp must be fitted with a condensing lens, a collector, and a field iris diaphragm. Hence there are two important adjustable iris diaphragms; the aperture diaphragm at the sub-stage condenser and the field diaphragm nearer to the lamp. The aperture iris diaphragm controls the angular aperture of the cone of light from the condenser. The field iris diaphragm controls the area of the circle of light illuminating the specimen.

*Step 1.* Open the *aperture* iris diaphragm wide and also open the *field* iris diaphragm wide. Turn on the lamp. Using a low power objective (10x or so) and a 10x eyepiece, slowly focus the specimen that has been placed on the microscope stage.

*Step 2.* Close the *field* iris diaphragm most of the way. Using the rack and pinion knob of the condenser, raise the condenser until the edges of the *field* iris diaphragm appear sharply focused. (The substage condenser is usually close to its highest position).

*Step 3.* If the *field* diaphragm does not appear centred in the field of view, use the substage condenser centering screws to center the *field* diaphragm. Then slowly open the *field* iris diaphragm until it just disappears from view. This step should be repeated each time a different objective is turned into place on the nosepiece.

*Step 4.* Now lift the eyepiece out of the body tube and look down the tube at the back lens of the fully-lighted objective. While looking down the microscope tube, slowly open and close the substage condenser *aperture* iris diaphragm. It will be seen that closing the *aperture* iris diaphragm "cuts into" the periphery of the back lens of the objective. For excellent illumination and contrast, approximately ¼-1/3 of the back lens should be occluded, thus leaving 2/3-3/4 of the back lens illuminated. Then replace the eyepiece in the tube. This step too should be repeated each time a different objective is turned into place on the nosepiece.

To approximate this setting without removing an eyepiece, open the substage diaphragm fully and gradually close it while looking through the microscope until the image gains a sudden increase in sharpness and detail. This should be close to the 2/3 open position; it can be achieved with a little practice and double checking initially by removing the eyepiece and looking down the tube.

Be careful when adjusting the condenser diaphragm, as closing the condenser diaphragm reduces resolution. To maximize both contrast and resolution, close the diaphragm just to the point where the image begins to get dark and no further.

The specimen should now be well-illuminated with even, glare-free light, giving good image contrast. If the lighting is too bright, use the rheostat, to turn it down, or add neutral density or other filters. Do not use the substage diaphragm to control brightness. Resolution will suffer if it is stopped down (closed) too far or opened too much. Although stopping down gives more contrast, it impairs resolution, and spurious details are formed by diffraction lines or fringes.

In short:

To set up a scope in **Koehler illumination**:

1. Rack the condenser up to its highest position.

2. Focus on a specimen with a 10X objective.

3. Close down the lamp field stop (the diaphragm in the base, also called the field diaphragm) while looking through the oculars. You should see a circle of light bordered by the diaphragm.

4. Lower the condenser slowly until the diaphragm is in sharp focus (it has numerous straight-sided edges).

5. Center the image of the diaphragm using the condenser centering screws.

6. Open the diaphragm until the light just fills the entire field of view. Fine focus again on the specimen, then open the diaphragm until it is just barely larger than the field of view.

7. Remove an ocular (eyepiece), and look through the tube. Adjust the condenser diaphragm (Not the one on the base) until about 75% of the visible opening is filled with light. Replace the ocular. You can make small additional adjustments with the condenser diaphragm to obtain optimum contrast, but remember that as contrast increases, resolution decreases.