Immersion Oils

Introduction

Compound microscope objectives in the 90X to 100X range require immersion oil. What does immersion oil do, and what is the proper technique for using it?

Concepts

- Microscopy
- Microbiology

Background

The upper limit of the resolving power of light microscopes is slightly above 1000X. Objectives of 90 to 100X, when coupled with a 10X eyepiece, approach the upper limit. Even in the range of 900 to 1000X, a clear image is only possible if every bit of available light is directed through the microscope optics to the viewer's eye. Immersion oils play an essential role in maximizing the amount of light producing the image the viewer sees.

In the airspace between the slide and the objective lens, light is refracted, scattered, and effectively lost. This happens because the refractive index of air (approximately 1.0) is very different from that of glass (approximately 1.5), and light passing through a glass/air interface is refracted (bent) to a large degree. By reducing the amount of refraction at this point, more of the light passing through the slide will be directed to the very narrow diameter lens of the high-power objective. The more light, the clearer the image. Placing a material with a refractive index equal to that of glass in the airspace between slide and objective directs more light through the objective and produces a clearer image. Immersion oils are formulated for just this purpose.

High-power objectives of 90X or higher are almost invariably intended for use with oil and will be engraved with the words, "oil," or "immersion," or "HI" (homogeneous immersion). These objectives are assembled with special sealants that prevent penetration of oil into the lens system. *Applying oil to an objective not designed for immersion will ruin the objective.*

Immersion oils are commonly available in two viscosities—low viscosity (Type A), and high viscosity (Type B)—and should be labeled with a refractive index of 1.515. The low viscosity oil is applied to the airspace between slide and objective, the high viscosity oil is (less commonly) applied between the condenser and the slide.

Safety Precautions

This laboratory activity is considered nonhazardous. Follow all normal laboratory safety guidelines.

Procedure

Low viscosity oil between slide and objective.

1. With low- or medium-power objective, locate a point or area of interest on the slide and center it in the image field.
2. Turn the revolving nosepiece so that the high power objective is just to one side of the slide. Place a single drop of immersion oil (low viscosity, Type A) on the slide (using the circle of light from below as a guide) and place a drop directly on the objective lens. Failure to apply oil to the objective will likely result in trapped air and reduced image quality.
3. Slowly rotate the high power objective into place and adjust the fine focus to fully resolve the image.

High viscosity oil between condenser and slide (optional).

Condensers with a numerical aperture (N.A.) of 1.0 and greater (usually engraved directly on the condenser) are also sealed to prevent oil penetration. Do not immerse condensers with an N.A. less than 1.0.

1. Before placing the slide on the microscope stage, rack the condenser down (using the condenser focusing mechanism) and apply a drop of oil (high viscosity, Type B) to the condenser lens.
2. Apply a drop of oil to the bottom of the slide directly below the specimen, and place the slide on the stage so that the drops will meet when the condenser is raised.
3. Raise the condenser until the drops converge. Follow the steps detailed above to oil the slide to the objective.

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Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Immersion oil should be cleaned from lens and slide surfaces when observations are complete. Oil left on lens surfaces will eventually dry and be very difficult to remove.

1. Carefully wipe oil from all glass surfaces with a folded piece of clean lens paper.
2. With a second piece of lens paper, moistened with a small amount of alcohol (ethyl or isopropyl), wipe glass surfaces to remove any streaks of residual oil.
3. To remove oil that has been allowed to dry on lens surfaces, moisten a folded piece of clean lens paper with a small amount of xylene. Gently wipe lens sufaces, giving the xylene a few moments to work. Xylene may soften cements used to assemble the objective—so wipe the surfaces again with clean lens paper moistened with dilute alcohol or distilled water.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Content Standards: Grades 5–8
Content Standard E: Science and Technology
Content Standards: Grades 9–12
Content Standard C: Life Science, the cell
Content Standard E: Science and Technology

References

Delly, J. G. Photography Through the Microscope; 9th ed., Eastman Kodak Co.: Rochester, NY, 1988; pp 18–19.

Leonard, W. H. A Practical Guide for Microscope Use and Care; Swift Instruments, Inc.: San Jose, CA, 1994; pp 15, 23.

Materials are available from Flinn Scientific, Inc.

Catalog No.	Description	
I0051	Immersion Oil, Low Viscosity	0.5 oz.
I0052	Immersion Oil, Low Viscosity	1.0 oz.
I0053	Immersion Oil, High Viscosity	0.5 oz.
I0054	Immersion Oil, High Viscosity	1.0 oz.
AB1175	Lens Paper, 50-sheet book	

Consult the Flinn Scientific website for current prices.