MICROSCOPE IMMERSION OIL

Robert Sacher, Cargille Labs

Microscope immersion oil is used in light microscopy to improve imaging. The use of microscope immersion oil as part of a microscope lens system will produce a brighter and sharper image than a similar design not using immersion oil. The reason is that this oil replaces the air gaps between the condenser and the bottom of the slide and between the top of the slide or cover glass and the objective lens with a medium that has a refractive index equal to the lowest refractive index of these glass components.

How does this work? There is a cone of light extending from a centered point on the specimen to the diameter of the objective lens. The angle of this cone is the Angular Aperture (A.A.). A.A. ranges from 10° for long focus low powered objectives to 140° for short focus high power objectives. Numerical Aperture (N.A.) is equal to n SIN(l/2 A.A.) where n is the lowest refractive index between the slide and the objective lens. The N.A. of an objective is often printed on the objective or can be obtained from the manufacturer. The higher the N.A., the brighter the image and the higher the resolution. This is because a higher N.A. allows light from more oblique, contrast enhancing angles. Objectives for oil immersion are usually marked as such by the manufacturer. Oil immersion objectives have approximately 50% increased resolution over dry objectives of the same focal length. Air has a refractive index of 1.00 [note that in this article refractive index is at 5461 Angstroms (green) at 23°C unless otherwise specified], so when the gap between the slide and the objective is air, the highest possible N.A. for the objective approaches 1.00. If, however, immersion oil with a refractive index of 1.518 (the same as the refractive index of the slide and cover glass) is used, the highest possible N.A. approaches 1.518 (the practical upper limit of N.A. is normally 1.4). Using a higher refractive index oil will not improve the N.A. of the objective.

You need to use an "oil immersion objective" to use immersion oil. Using immersion oil on a "dry" objective will destroy the image. Besides oil immersion objectives, there are objectives designed for immersion in water (refractive index 1.33). Water immersion is useful to view live material. Glycerin (refractive index 1.47) and silicone oil (refractive index 1.40) are sometimes used because, like water, they have very low fluorescence. Higher N.A. values can be achieved with objectives designed for use with bromonaphthalene (refractive index 1.67), methylene iodide (refractive index 1.75). A new Olympus objective can obtain N.A. 1.65 using Cargille Refractive Index Liquid Series M (refractive Index 1.780 at 5893 Angstroms and 25° C) as immersion oil. If the N.A. of the oil immersion objective lens is > 1, then the condenser beneath the slide, must also be oiled to fill the gap between the condenser and the slide in order to realize the full N.A. of the condenser and the objective. If the condenser is not oiled, the highest possible N.A. is 1. The N.A. of the completely oiled system is a function of the N.A. of the objective and the N.A. of the condenser. Many microscope users neglect to oil the condenser, because it is awkward and messy, and therefore do not get the advantage of oil immersion microscopy. In fluorescence microscopy where the objective also serves as a condenser (for excitation light), the substage condenser does not need to be oiled.

If the specimen is mounted in water on a slide with a cover glass, water (refractive index 1.33) becomes the limiting factor, with maximum N.A. approaching 1.33. The water should be re-

Continued on following page

MICROSCOPE IMMERSION OIL

Continued from preceding page

placed with a medium of higher refractive index (microscope immersion oil for example) for best results. Most mounting media have refractive indices above 1.518. You can dispense with the cover glass if the specimen is "fixed" to the slide and can come in direct contact with the immersion oil.

Historically, natural Cedarwood Oil (that had been thickened) was the immersion oil of choice because its refractive index and dispersion (the change of refractive index with wavelength) were close to those of the lowest refractive index glass being contacted in the condenser, slide, cover glass and the objective system. The disadvantages of cedarwood oil are: high absorption of blue and UV light and high background fluorescence, yellowing with age, a tendency to harden on lenses, acidity, changing viscosity and inconsistency of optical values. Another natural oil, sandalwood oil, was used for fluorescence microscopy, because its fluorescence is lower than cedarwood oil. It has higher fluorescence than modern immersion oils. In the 1940s, synthetic immersion oils were developed to improve the properties of natural oils and this led to universal standards, first, the German DIN 58~884 Standard and finally the ISO 8036-1 Standard. According to the ISO Standard, microscope immersion oil must have a refractive index (ne: refractive index at the green e line or 5461 Angstroms) of 1.5180 ± 0.0005 and Abbe Ve = 44 ± 3. Abbe Ve is a measure of dispersion defined as (ne - 1)/(nF' - nC'). Note that nF' is the refractive index at the blue 4800 Angstrom line and nC' is the refractive index at the red 6439 Angstrom line. It is essential to have the immersion oil match not only the desired refractive index, but also the desired dispersion, to cut down on chromatic aberration (rainbow effect). In addition, the ISO 8036-1 specifies acceptable percent transmittance, viscosity, and label information. There are, as mentioned above, microscopes designed to use non-standard immersion oil such as water, glycerin, etc. Immersion oils must be made of stable, nonvolatile, ingredients and must not be a skin irritant. Early synthetic immersion oils contained PCBs (polychlorinated biphenyls) but these were replaced by non-PCB formulas in the 1970s.

Temperature is a factor often overlooked when using immersion oil. If you are using a microscope and immersion oil designed for the standard temperature of 23° C (73.4° F) but are using it at 37° C (98.6° F) the image will be noticeably poorer than at 23° C. This is because liquids in general, including immersion oils, change in refractive index with temperature at a rate 10 to 100 times greater than the glass optics. Fortunately, there is an oil specifically made to have the correct refractive index (1.518) a t 37° C: Cargille Immersion Oil Type 37. To work at temperatures between 37° and 23° C you can mix Type 37 with Cargille Immersion Oil Type B (a 23° C oil). You can attach a short thermometer, using sticky tape, to the side of your microscope close to the stage to monitor your temperature. To work at 23° C (73.4° F) you may need a room temperature of 20° C (68° F) to compensate for heat sources such as the light source and/or body heat.

If you are working at a single wavelength outside the center of the visible spectrum (5461-5893 Angstroms) such as blue or in the near ultraviolet, the refractive index of the oil at that single wavelength can be matched to the glass by adjusting refractive index by changing the temperature using the oil's temperature coefficient and dispersion. For example: if the wavelength is a blue light (4800 Angstroms), because the oil has higher dispersion than the glass objective at 23° C and the refractive index will go up with the decrease in wavelength (from the nominal 5461 to the blue 4800 Angstroms), one will need to raise the temperature a few degrees to lower the refractive index of the oil to match the glass and get a sharper image.

Color in immersion oil should be kept to a minimum because it represents selective absorption of light. A yellow oil means absorption of some blue light and probably more ultraviolet light. Such an oil when illuminated with ultraviolet will usually fluoresce. This "background fluorescence" can be so great as to obscure a faintly fluorescing specimen. In such a case for the lowest color, special low fluorescent immersion oils are available.

Acidity in immersion oils should be very low. High acidity can, in time, affect the metal parts of the objective and condenser, and can cause deterioration of the lens cement which will allow oil to get behind the lens and affect the image. Such deterioration of the image happens gradually over time. A lens with defective lens cement must be repaired by the manufacturer or a qualified repair service. Cleaning the back of a lens is awkward because of its relatively inaccessible position and the location of diaphragms which hinder access.

Viscosity of immersion oil is measured in centistokes (cSt), with low viscosity immersion oil (approximately 150 cSt) being like

the viscosity of maple syrup, medium viscosity immersion oil (approximately 300 cSt) being like 30W motor oil, high viscosity immersion oil (approximately 1259 cSt) being like the viscosity of corn syrup, and very high viscosity immersion oil (21,000 to 46,000 cSt) being thicker than honey (15,000 cSt). The viscosity selected for immersion oil is largely dependent on the preference of the user. Low viscosity immersion oil is similar to cedarwood oil and has less of a tendency to trap air bubbles than higher viscosity oils. Medium viscosity oil is particularly used for Automated Hematology Systems. High viscosity oil adheres better to the objective, so that a new drop of oil may not be needed when one slide is replaced with another. High viscosity oil will bridge a wider gap between the condenser or objective and the slide. Very high viscosity oils bridge even greater gaps and are useful where other oils would run, such as with inverted or inclined stages. When the different viscosity oils are soluble in each other, they can be blended for intermediate viscosities (consult the manufacturer).

Immersion oils come variously packaged. The most popular is an amber glass bottle with a glass dropper rod in the cap. The glass is inert and has been proved not to affect the oil over a long period of time. The amber color of the glass adds protection from light. With a little practice the dropper rod will deliver the exact amount of oil desired. The oil should be lightly touched to the slide or condenser rather than dabbed, which can entrap bubbles. As a practical matter, a glass bottle is heavy and less likely than plastic to be knocked over and spilled.

There are also a variety of plastic "squeeze" bottles. These bottles are inverted and squeezed to apply a drop of oil on the slide or condenser. When compared to glass bottles with dropper rods, the squeeze bottles require less practice, but there are problems with applying a uniform amount of oil, entrapping air bubbles, and messy, oily tips and bottles. There is also some question of the long term compatibility of the oil with plastic, especially with respect to slightly increasing the fluorescence of the oil.

Some microscopists will prefer to buy economically priced large 4 or 16 fl. oz. bottles and pour them into a smaller dispensing bottle. A balsam bottle with its glass dropper rod makes an ideal dispensing bottle.

Lens tissue should be used to remove the oil. Removal of oil will keep dust from gathering on the lens. The tissue can be dampened with a solvent, such as xylene or toluene. The solvent is mandatory if using drying type natural oils such as cedarwood or sandalwood oil in order to avoid a build up on the lens.

Microscope technology is one of the old scientific technologies but remains one of the most powerful, partially due to new innovations. Oil immersion microscopy is an old innovation that allows the microscope to perform at its peak. ■

1. W.C. McCrone, L.B. McCrone, and J.G. Delly, Polarized Light Microscopy, Microscope Publications, 1984, pp. 1-48

2. J.J. Cargille, Immersion Oil and the Microscope, Cargille Laboratories, 2d ed. 1985