Immersion Oil and the Microscope

John J. Cargille
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Since the microscopist's major field of interest is the application of microscopes and related equipment, the fields in which the instruments are used are, in a sense, secondary. However, many scientists, having selected a field, find the use of a microscope a necessity but secondary to their major field of interest. Therefore, the microscope is thrust upon them as an essential tool. Often, basic background necessary for proper use of the "tool" is lacking or inadequate, having been picked up on the job so they can "get by."

Considering the number of microscopes being used in all types of laboratories and the number of scientists and technicians using these instruments, from reports and requests we gather that they have learned to use them in what might be referred to as "on the job" training to the "get by" level of proficiency.

This paper will attempt to broaden the understanding of the "business area" of the microscope, between the condenser and the objective, as it is affected by the use of oil immersion objectives, and also expand on properties of immersion oils and how they can be more fully utilized.

THE FUNCTION OF IMMERSION OIL

Immersion Oil contributes to two characteristics of the image viewed through the microscope: finer resolution and brightness. These characteristics are most critical under high magnification; so it is only the higher power, short focus, objectives that are usually designed for oil immersion.

Oil immersion objectives are generally available from 40 to 120x. These must not be confused with "high dry" objectives or water immersion objectives that are also made in this range. Just as an "oil" immersion objective must be used with oil to get a usable image, a "water" immersion objective must be used with water and a "dry" must be used dry. The use of oil on a high dry will destroy the image by negating corrections for spherical and chromatic aberration.

For any given lens there is a fixed focal length. With the objective in focus there is a cone of light extending from a point on the specimen to the full diameter of the objective lens. The angle formed by this cone is the angular aperture (A.A.), shown diagrammatically in Figure 1. It may vary from 10° for low power dry (long focus), to 140° for high power oil (short focus). the greatest theoretical angular aperture is, of course, 180° with zero focal length.

Below the specimen is a second, matching, cone of light, the base of the cone being the top surface of the condenser and the apex at a point on the specimen. Theoretical illumination, then, provides a straight line path for each ray from condenser to objective lens.

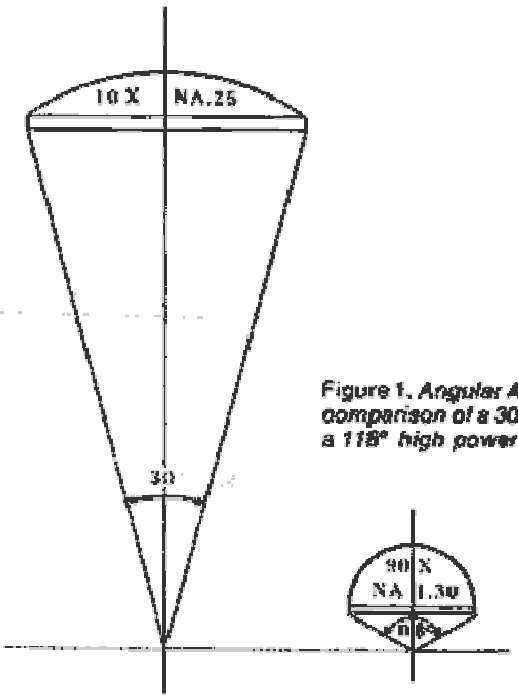

Figure 1. *Angular Aperture. Schematic shows comparison of a 30° low power objective with a 118° high power immersion objective.*

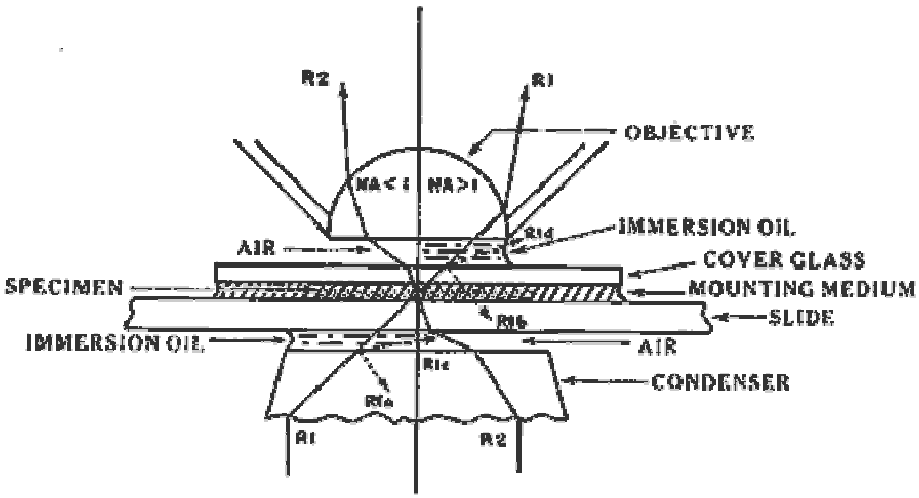

Figure 2. *Ray R1—Straight ray resulting from homogeneous path. (Optics oiled). Ray R2—Deviations in ray caused by air gaps. Notice R1 originates from a more oblique angle than R2 and forms a greater aperture angle at the specimen indicating more light reaches the objective and resolution will be greater. R1a and R1b show where reflection can take place, and R1c and R1d show where refraction can cause light loss, when optics are not oiled. Mounting medium is assumed to be the same index as the slide and the cover glass.*

This straight-line path is disturbed by any material of different refractive index. Ideally, Fig. 2, progressing upward from the condenser there is air (index 1.00), slide (approximately 1.515), mounting media, cover glass (approximately 1.515), air (1.00), and finally the objective. Since most condensers and objective lenses are

part of this discussion.

The resolution obtained is directly related to the angular aperture, the larger A.A. having a wider cone with more oblique rays. However, unless there is a homogeneous optical path, the most oblique Rays are lost by internal reflection within the slide or cover glass.

The oil immersion objective of medium angular aperture has more resolving power than a " dry" with larger angular aperture and the term, proposed by Ernst Abbe, "numerical aperture," (N .A.) must be considered. N.A. is equal to n x sin 1/2 A.A. where n is the lowest refractive index in the path. Therefore, the air gap between cover glass and objective gives a theoretical maximum N.A. of 1.00; water, 1.33; immersion oil, 1.515. Since other limitations permit only a practical N.A. of 1.40 with lens glass 1.515, the use of immersion oil permits full use of the resolving power of the objective. And, for a given angular aperture, immersion oil objectives increase the resolution by approximately 50% over dry objectives of equivalent focal length.

Just as immersion oil permits utilizing the full N.A. (resolving power) of the objective, it is also a necessity for obtaining the maximum N.A. of the condenser. With a condenser N.A. 1.40 and an air gap between the condenser and the bottom of the slide, the limiting value is N.A. 1. (air); The additional light being lost by internal reflection within the condenser.

Whereas an objective must be used "dry" or "oiled," depending on design, a condenser will work oiled or dry, but will be limited to a working value. Full resolution of a condenser, N.A. 1.25, can be obtained by "oiling" with water (1.33), or immersion oil (1.515). A condenser of N.A. 1.40 must have a media of 1.40 or greater, such as immersion oil 1.515, to utilize its designed N.A.

The third consideration is the mounting media itself. An "air mounted" specimen can receive a light cone of 1.0 N.A. The excess of light is lost by total reflection at the top surface of the slide. So, there is a second limiting or working N.A. value to be considered in determining the usable N.A. of the condenser.

The resolving power of an optical system is computed by averaging the N.A. value of the objective and the working N.A. of the condenser. The objective, as mentioned, must be used as designed, dry, watered or oiled. The condenser working N.A. is only equal to the designed N.A. when it is oiled to the slide, and a mounting media is used having an index greater than the condenser N.A.

IDEAL PROPERTIES OF IMMERSION OIL

Over the years there has been an increasing effort to standardize the optical values of immersion oil because it becomes an integral part of the optical system of the microscope. Obtaining the highest quality image requires that the microscope designer rely on consistent values of the immersion oil being used with the microscope. The first major step towards standardization of immersion oil was the German DIN (Deutsches Institute fur Normung) specifications 58-884 to which most European and American immersion oils, including Cargille Immersion Oils, currently comply. The United Nations' International Organization For Standardization (ISO) is currently in the final stages of writing a more international standard in basic accordance with the DIN specifications.

The refractive index of immersion oil is specified at one wavelength, generally the mercury e line (5461 A) because this wavelength is near the middle of the visible spectrum where the eye has the greatest sensitivity. The e line is specified by DIN /ISO; however, there are still some immersion oils calibrated at the sodium D line (5893 A) and the helium d line (5876 A). DIN/ISO specify that the refractive index of general purpose immersion oil at the e line at 23 C shall be 1.5180 +/- 0.0004 (DIN), +/- .0005 (ISO).

Immersion oil contributes to the homogenous path of light between the condenser and objective by having the same index as the glasses in the system. "White" light, however, is composed of many wavelengths. For the refractive index of all wavelengths in the visible spectrum to match the glass optics, the dispersion value of

the oil must match those optics. Dispersion is generally indicated by nF- nC, or Abbe vD or Abbe ve computed from the expression:

$$\gamma_D = \frac{n_D - 1}{n_F - n_C}$$

DIN/ISO specify that immersion oil shall have a dispersion of ve = 44 +/- 5 at 23 C.

The major formulating problem is not so much matching index for the e line, since a number of materials can be formulated to accomplish this, but finding materials with suitable dispersion values, so that dispersion is closely matched across the visible spectrum. To the knowledge of the author, no immersion oil matches the optics of the microscope perfectly. One of the closest matches is thickened cedarwood oil which, for many years, was the most widely used, if not the only immersion oil available. The disadvantageous properties of cedarwood oil are: high absorption of blue and UV light, yellowing with age, a tendency to harden on lenses due to uneven volatility, acidity, and changing viscosity (diluting with solvent changes the index and dispersion).

The synthetic immersion oils eliminate many of the disadvantages cited above. Temperature is a factor often overlooked as it applies to immersion oil. Liquids and most solids change index in an inverse ratio to the change in temperature. The temperature for which the oil is adjusted should be stated on the bottle. Since a difference of 1°C in room temperature from the stated value causes a change in index of the oil of approximately 0.0004, summer weather or overheated rooms can affect the index match considerably. The temperature coefficients are usually stated in data sheets or available from the manufacturer. Most manufacturers of microscopes use oil standardized for 23°C (74°F) as specified by DIN/ISO. Some oils are made for use at 18°C, 20°C, and 37°C for special applications.

The selection of temperature of calibration is made to approximate the stage temperature condition under which the specific instruments are being used. The 23 C value for general purpose immersion oil is higher than the ideal room temperature of72°F (22.2°C) and allows for some increase in temperature for radiant heat from the microscope lamp and other sources. Often the simplest method of adjusting this temperature is increasing the room temperature slightly. The increase can usually be made more easily than cooling a room.

When working at wavelengths other than D line, i.e., Green line (5461 A), Blue line (4358 A), or in the near ultraviolet, the index can be brought to coincide with the glass by slightly changing the temperature in the area of the slide, oil and objective. The temperature change required can be approximated from dispersion values and the temperature coefficients.

It must be remembered that both the glass and the oil change index with temperature. However, the change in solids is generally so insignificant that it can be ignored.

Color is, of course, a consideration in formulating immersion oil because its presence denotes a loss of light through absorption. Just how critical color may be is an open question. That it is undesirable is accepted, but the presence of some color, if it cannot be a voided, ma y be more than offset by having other desirable specifications met.

The greatest problem created by color might be its creation of an absorption band at the wavelengths of the monochromatic illumination being used. Special oils have been formulated for work at lower wavelengths of approximately 3000-4000 A.

The acid value of immersion oil should be very low. The synthetics usually have acid values lower than cedarwood oil. High acidity can, in time, affect the condition of the metal parts of the objective, and possibly

more important, can cause deterioration of lens cements. The lens cement is also a seal that prevents oil from penetrating to the back of the lens. A crack or perforation in the cement draws oil by capillary attraction and a thin film of oil slowly creeps over the back of the objective lens; a poor image may develop without being immediately noticed. When the microscopist realizes the image has deteriorated, he may not realize the cause unless he has faced this problem before.

The defect in the lens cement should be repaired promptly by the manufacturer or a qualified repair service or the instrument will be used with inferior images and, when the oil spreads to cause an unusable image, time will be lost by successive cleanings. The cleaning of the back lens is awkward because of its relatively inaccessible position and the location of diaphragms which hinder access.

We have found that the manufacturers have placed objectives in an immersion oil and let them soak for 6 to 12 months before approving an oil for use with their instruments. The purpose is to be sure that the acidic, solvency, or any other properties will not injure their equipment in any way.

As each oil objective is made, it must be tested to insure the lens cement seals against entry of oil to the back of the lens. Immersion oil is used by some microscope manufacturers in this test work.

The synthetic oils should contain no volatiles, and be formulated from stable materials unaffected by oxidation, photo degradation or other forms of decomposition that might slowly change their properties. This stability means that the microscopist knows the material he is using and can rely on it having the same properties as when purchased initially. The stability is such that generally the quality control of the manufacturer has far more effect on the product than any shift in properties caused by age or exposure.

As pointed out earlier, the difference between the specification temperature and stage temperature causes changes in the optical properties. The deviations due to stage temperature can exceed, by far, any difference between batches of oil from the same manufacturer.

Though generally known and understood, it should be pointed out that the resolution of an optical system increases as the wavelength of the illumination decreases. Therefore, microscopists tend to shift from white mean 5500 A to green (5461 A), to blue (4358 A) or to near ultraviolet (2750 A) illumination.

Standard optics can be used in the near ultraviolet, but a number of special optics are made, the finest of which are quartz which is a requirement for ultra violet. Work in the ultra violet and the use of quartz optics requires a good deal of experience to gain the best work the equipment is capable of producing.

The near ultraviolet and ultraviolet ranges fall into the category of fluorescent microscopy. Immersion oils for use in these wavelength ranges must have extremely low or no fluorescence. Any fluorescence only serves to "haze" the field and makes images indistinct. Sandalwood oil and several synthetic oils classified as "non-fluorescent," fluorescence-free, or very low fluorescent, are available.

Quartz optics generally have been used with a glycerin and water mixture, or to reduce evaporation, a solution of glycerin and sugar. Available from some manufacturers are stable non hygroscopic immersion oils especially blended for use with quartz optics.

Viscosity of immersion oil is mostly a matter of individual preference of the microscopist. Low viscosity oils are more likely to creep if applied over-abundantly. The low viscosity (150 cSt) is preferred by some when the distance from the cover glass to the objective is very small. Low viscosity oils are less apt to retain small bubbles.

Higher viscosities, (approximately 1250 cSt) have been found to be the most in demand and are supplied by more microscope manufacturers than any other. The higher viscosity oils fill the larger gaps more satisfactorily and are also reusable in that a second slide can be positioned and swung into place and contact made with the oil drop remaining on the objective lens. The 1250 cSt viscosity gives more latitude with oiled condensers since it will fill a larger gap without "breaking" and making re oiling unnecessary.

"Very high viscosity" (21,000 cSt) oil is particularly useful for wide condenser gaps, long focus (low power) oil immersion objectives, horizontal or sharply inclined instruments and some micro projectors. If the manufactured viscosity is not the most suitable, blends can be made. If a blend is desirable, an immersion oil manufacturer may mix a small special lot when the material is ordered as a percentage of different types of the regular line.

PACKAGING

Manufacturers generally package in small glass applicator bottles and larger stock bottles from which the applicator bottles can be refilled. The weight of glass containers tends to make them more stable than equal volume plastic containers. The larger stock bottles reduce the cost of the oil.

Immersion oil/Balsam bottles having a squat, very stable shape, an applicator rod, and a convenient glass drop on cover are available from some scientific supply houses. These bottles can be filled from the stock bottles in which the oil is purchased.

Microscope manufacturers use several types of packaging for the small oil bottle shipped as an accessory. The glass bottle with screw well-cap and glass applicator rod predominates. Polyethylene squeeze bottles or tubes are also supplied.

Plastic squeeze packages avoid breakage and mess if knocked over or dropped; however, the tips remain oily and capture dust unless completely cleaned after each use. The squeeze type are generally a little more awkward to use on a slide positioned on the microscope stage and a slight over-squeeze can put too much oil on the slide .

APPLYING IMMERSION OIL

The applicator rods with a taper-point or ball-end make it easy to apply oil to the positioned slide. The rods also have the advantage of measuring the drop by 'tipping-off' inside the neck of the immersion oil bottle to get the drop size experience indicates is right. Tipping-off inside the neck prevents creeping of oil to the outside of the bottle eliminating dust capture and oily fingers.

Avoid entraining air in the oil when applying it to the microscope. The bubbles, acting as lenses, destroy the image or reduce its clarity. The problem is usually avoided if the drop is touched to the slide or condenser and permitted to flow from the applicator. Dabbing increases the likelihood of bubbles.

The condenser should be oiled in a low position and, after positioning the slide, raised slowly to make contact. Oiling the objective is done by putting the drop on the slide and slowly lowering the objective into it, or with a parfocaled turret, slowly rotating the oil objective into the drop of oil. Once the optics are oiled and slides are changed, there may be enough oil on the objective to avoid reoiling.

Drying types, such as cedarwood, sandalwood, or other natural oils, should be removed immediately after use. If these oils harden they are difficult to remove, and lenses can be damaged. The non-drying synthetics can be left on lenses indefinitely since damage through hardening cannot occur; however, being oily, they will capture and hold dust. A time-honored solvent is Xylol. A small amount on lens tissue for a final wipe leaves optics in good order.

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