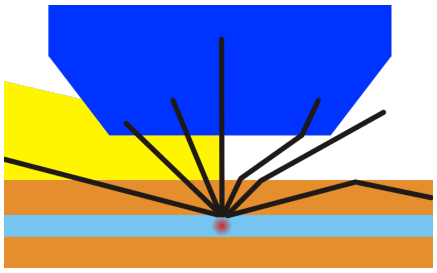
Oil immersion

In light microscopy, **oil immersion** is a technique used to increase the resolution of a microscope. This is achieved by immersing both the objective lens and the specimen in a transparent oil of high refractive index, thereby increasing the numerical aperture of the objective lens.

Immersion oils are transparent oils that have specific optical and viscosity characteristics necessary for use in microscopy. An **oil immersion objective** is an objective lens specially designed to be used in this way. Many condensers also give optimal resolution when the condenser lens is immersed in oil.



Principle of immersion microscopy. Path of rays with immersion medium (yellow) (left half) and without (right half). Rays (black) coming from the object (red) at a certain angle and going through the coverslip (orange, as the slide at the bottom) can enter the objective (dark blue) only when immersion is used. Otherwise, the refraction at the coverslip - air interface causes the ray to miss the objective and its information is lost.

Theoretical background

The resolution of a microscope is defined as the minimum separation needed between two objects under examination in order for the microscope to discern them as separate objects. This minimum distance is labeled δ. If two objects are separated by a distance shorter than δ, they will appear as a single object in the microscope.

A measure of the resolving power of a lens is given by its numerical aperture, NA:

$$\delta = \frac{\lambda}{NA}$$

where λ is the wavelength of light. From this it is clear that a good resolution (small δ) is connected with a high numerical aperture.

The numerical aperture of a lens is defined as

$$NA = nsin\alpha_0$$

where α_0 is the angle spanned by the objective lens seen from the sample, and *n* is the refractive index of the medium between the lens and specimen (≈1 for air).

State of the art objectives can have a numerical aperture of up to 0.95. Because sin α_0 is always less than or equal to unity, the numerical aperture can never be greater than unity for an objective lens in air. If the space between the objective lens and the specimen is filled with oil however, the numerical aperture can obtain values greater than unity. This is because oil has a refractive index greater than 1.

Oil immersion objectives

From the above it is understood that oil between the specimen and the objective lens improves the resolving power by a factor 1/*n*. Objectives specifically designed for this purpose are known as oil immersion objectives.

Oil immersion objectives are used only at very large magnifications that require high resolving power. Objectives with high power magnification have short focal lengths, facilitating the use of oil. The oil is applied to the specimen (conventional microscope), and the stage is raised, immersing the objective in oil. (In inverted microscopes the oil is applied to the objective).

The refractive indices of the oil and of the glass in the first lens element are nearly the same, which means that the refraction of light will be small upon entering the lens (the oil and glass are optically very similar). Oil immersion objectives are designed with this in mind, and do not perform well without oil, because in this case there will be much reflection of light at the glass/air interface. Using the objective without oil invalidates the corrections, which

assume the presence of oil. Nevertheless, in situations in which not maximal clarity but a closer general view is preferred (for instance, where magnifications must be changed back and forth during the observation of a delicately mounted slide), the oil can be skipped at the cost of image sharpness.

One usually employs oil immersion only on fixed specimens. While it is possible to use the oiled objective on temporary mounts, any motion of the slide relative to the objective typically moves the cover slip and disturbs the objects being observed.

Immersion oil

Cedar tree oil has an index of refraction of approximately 1.516. The numerical aperture of cedar tree oil objectives is generally around 1.3. In modern microscopy, synthetic immersion oils are more commonly used. NA values of 1.6 can be achieved with different oils.

See also

- Water immersion objective
- Index-matching material
- Solid immersion lens

References

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External links

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- "Immersion Oil Microscopy" [2] by David B. Fankhauser, *Biology at University of Cincinnati, Clermont College* (website), December 30, 2004.
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References

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[2] http://biology.clc.uc.edu/fankhauser/Labs/Microscope/Oil_Immersion.htm
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