



## **KACEY™**

1854 A Hendersonville Road

Box#112

Asheville, NC 28803

Phone (828) 685.3569 Fax (828) 685.3571

Website: [www.kaceydiagnostics.com](http://www.kaceydiagnostics.com) Email: [sales@kaceyinc.org](mailto:sales@kaceyinc.org)

### **Microscopy with Oil Immersion**

When light passes from a material of one refractive index to material of another, as from glass to air or from air to glass, it bends. Light of different wavelengths bends at different angles, so that as objects are magnified the images become less and less distinct. With "dry" objective lenses this loss of resolution prevents using magnifications of above 400x or so. In fact, as you will see later, even at 400x the images of very small objects are badly distorted.

Oil immersion microscopy is essential to any microbiology lab. Stained smears of **mixed bacteria** are recommended for practice.

#### **Principle**

Placing a drop of oil with the same refractive index as glass between the cover slip and objective lens eliminates two refractive surfaces, so that magnifications of 1000x or greater can be achieved while still preserving good resolution.

#### **Using immersion oil**

The objective lens must be designed specifically for oil immersion microscopy. Attempting to use immersion oil with a "dry" objective will only foul the lens. To use an oil immersion lens, first focus on the area of specimen to be observed with the high dry (400x) lens. Place a drop of immersion oil on the cover slip over that area, and very carefully swing the oil immersion lens into place. Focus carefully, preferably by observing the lens itself while bringing it as close to the cover slip as possible, then focusing by moving the lens away from the specimen. When in focus the lens nearly touches the cover slip. The focal plane is so narrow that it is very easy to focus right past it. If you are focusing toward the specimen, you can drive the lens right into it.

#### **When to use an oil immersion lens**

Use an oil immersion lens when you have a fixed (dead - not moving) specimen that is no thicker than a few micrometers. Even then, use it only when the structures you wish to view are quite small - one or two micrometers in dimension. Oil immersion is essential for viewing individual bacteria or details of the striations of skeletal muscle. It is nearly impossible to view living, motile protists at a magnification of 1000x, except for the very smallest and slowest.

A disadvantage of oil immersion viewing is that the oil must stay in contact, and oil is viscous. A wet mount must be very secure to use oil. Oil immersion lenses are used only with oil, and oil can't be used with dry lenses, such as your 400x lens. Lenses of high magnification must be brought very close to the specimen to focus and the focal plane is very shallow, so focusing can be difficult. Oil distorts images seen with dry lenses, so once you place oil on a slide it must be cleaned off thoroughly before using the high dry lens again.

NOTE: Oil on non-oil lenses will distort viewing and possibly damage the coatings.