## Mounting Matters

When light rays travel through a lens, the lens bends the ray paths in such a way that all rays originating from a point in the sample will again all cross at a point at the surface of the detector (such as your retina or a camera) to create an image. When the rays do not all cross together, the image will appear blurred. A lens causes bending to occur because the light's electric field interacts with electrons in the glass more strongly than it does with electrons in the surrounding air. The degree to which light interacts with a material, and thus bends, is termed the material's **refractive index** (RI).

In the case of biological microscopy, light does not simply travel through a lens. Instead, the sample is often bathed in an aqueous solution (RI=1.33) and attached to a flat, glass (RI=1.51) coverslip. After the coverslip, the light may further pass through an additional **immersion media** before reaching the objective lens. These other refractive index changes also cause the ray paths from the sample to bend. Microscope objectives are designed to compensate for these extra bends, based on specific assumptions about how much extra bending will occur. For example, all modern objectives used in biological microscopy assume that the coverslip has the refractive index of glass (1.51) and is precisely 170 um thick (gauge #1.5). Other assumptions are made about the RI of the sample and immersion media. If these assumptions are not met, all of the rays from a point in the sample will not re-cross at a point on the detector and the image will be blurred (often due to **spherical aberration**). **Be sure to use an objective whose design assumptions match your sample and mounting procedure, or else the image will be poor!** Some objectives include a 'correction collar' that allows the user to make fine adjustments in how the rays will bend within the objective in order to accommodate a wider range of refractive index changes. **If the objective has a correction collar, adjust it appropriately or the image will be poor!**

When imaging with multiple wavelengths (dyes), the above optical problems become even more complex, because different wavelengths of light bend somewhat differently, even in the same material, a property termed **dispersion**. Some objectives are designed to work with multiple wavelengths, but others are not. If a single wavelength objective is used to image multiple wavelengths, some of the wavelengths will be out of focus or focus to different locations, an artifact termed **chromatic aberration**.

Importantly, biological samples themselves can bend (as well as scatter) light, since cells are a mixture of water (RI=1.33) and curved lipid surfaces (RI=1.43). These sample effects cannot be incorporated into the assumptions of objective design, since every sample is different, and micro-scale optical properties are not predictable. Sample-induced aberrations often become significant when imaging >50 um past the coverslip, though some objective (especially high numerical aperture) and imaging techniques are far more sensitive. Light scatter causes a randomization in the direction of light rays that make forming a sharp image essentially impossible. Special techniques such as multi-photon microscopy are able to mitigate the impact of light scatter to some degree. For fixed samples, various '**clearing' solutions** are available that homogenize the sample's refractive index enough that imaging depth is almost unlimited by scatter and aberrations.

Please contact us (microscopy@umich.edu) to help you understand how to best mount your sample and then choose the best available objective for imaging. We look forward to working with you!

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