Light Microscopy Facts

1. Chromatic aberration is caused by a lens having a different refractive index for different wavelengths of light (the dispersion of the lens). Since the **focal length f** of a lens is dependent on the refractive index n, different wavelengths of light will be focused on different positions.

2. N**umerical aperture** (**NA**) of an optical system is a dimensionless number that characterizes the range of angles over which the system can accept or emit light.

$$\mathrm{NA} = n \sin\theta$$

where n is the index of refraction of the medium in which the lens is working (1.0 for air, 1.33 for pure water, and up to 1.56 for oils), and θ is the half-angle of the maximum cone of light that can enter or exit the lens.

In microscopy, NA is important because it indicates the resolving power of a lens. The size of the finest detail that can be resolved is proportional to λ/NA, where λ is the wavelength of the light. A lens with a larger numerical aperture will be able to visualize finer details than a lens with a smaller numerical aperture. Lenses with larger numerical apertures also collect more light and will generally provide a brighter image.

3. Optical resolution describes the ability of an imaging system to resolve detail in the object that is being imaged. The ability of a lens to resolve detail is usually determined by the quality of the lens but is ultimately limited by diffraction. 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.

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

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.

4. Depth of Field

In optics, particularly as it relates to film and photography, the **depth of field** (DOF) is the portion of a scene that appears sharp in the image. The DOF is determined by the subject distance (that is, the distance to the plane that is perfectly in focus), the lens focal length, and the lens f-number (relative aperture).

5. Magnification is the process of enlarging something only in appearance, not in physical size. Magnification is also a number describing by which factor an object was magnified. When this number is less than one it refers to a reduction in size, sometimes called **minification**.

6. A **real image** is a representation of an actual object (source) formed by rays of light passing through the image. If a screen is placed in the plane of a real image the image will generally become visible. Examples of real images include the image seen on a cinema screen, the image produced on a detector in the rear of a camera, and the image produced on a human retina. Real images can be produced by concave mirrors and converging lenses.

7. A **virtual image** is an image in which the outgoing rays from a point on the object never actually intersect at a point. A simple example is a flat mirror where the image of oneself is perceived at twice the distance from yourself to the mirror. That is, if you are half a meter in front of the mirror, your image will appear at a distance of half a meter inside or behind the mirror.

8. Oil Immersion Objective

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. 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 In addition to improving resolution, the use of oil is also advantageous in that it reduces the reflective losses as light enters the lens (again because the oil and glass are optically alike).