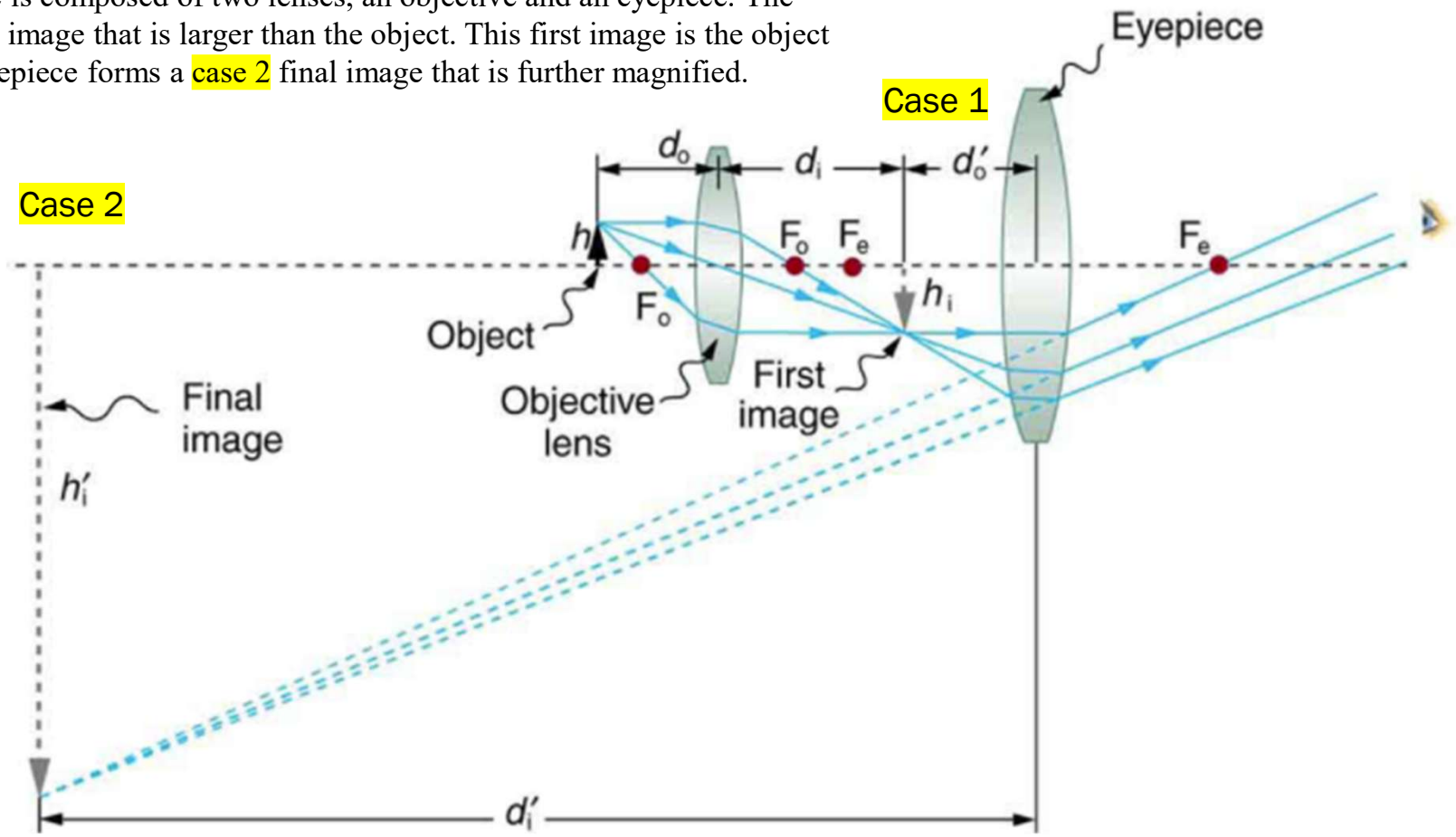
Lecture-15

CSO202: Atoms, Photons & Molecules

Debabrata Goswami

SCHEMATICALLY...

a compound microscope is composed of two lenses, an objective and an eyepiece. The objective forms a case 1 image that is larger than the object. This first image is the object for the eyepiece. The eyepiece forms a case 2 final image that is further magnified.



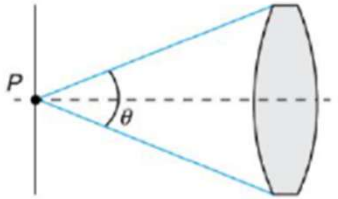
Since each lens produces a magnification that multiplies the height of the image, it is apparent that the overall magnification 'm' is the product of the individual magnifications: $m = m_o m_e$, where m_o is the magnification of the objective and m_e is the magnification of the eyepiece. This equation can be generalized for any combination of thin lenses and mirrors that obey the thin lens equations.

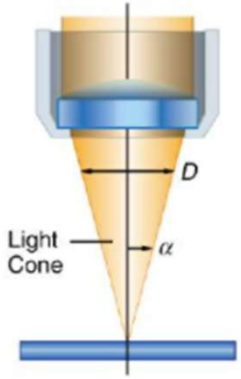
CRITICAL ELEMENTS OF A MICROSCOPE

Normal optical microscopes can magnify up to 1500× with a theoretical resolution of −0.2 μm. The lenses can be quite complicated and are composed of multiple elements to reduce aberrations. Microscope objective lenses are particularly important as they primarily gather light from the specimen. Three parameters describe microscope objectives: the **numerical aperture** (*NA*), the **magnification** (*m*), and the **working distance**. The *NA* is related to the light gathering ability of a lens and is obtained using the angle of acceptance θ formed by the maximum cone of rays focusing on the specimen (see Figure below) and is given by: $NA = n \sin\alpha$, where n is the refractive index of the medium between the lens and the specimen and $\alpha = \theta/2$. As the angle of acceptance given by θ increases, *NA* becomes larger, and more light is gathered from a smaller focal region giving higher resolution. A 0.75 *NA* objective gives more detail than a 0.10 *NA* objective.

While the numerical aperture can be used to compare resolutions of various objectives, it does not indicate how far the lens could be from the specimen. This is specified by the "working distance" which is the distance (in mm usually) from the front lens element of the objective to the specimen, or cover glass. The higher the *NA* the closer the lens will be to the specimen and the more chances there are of breaking the cover slip and damaging both the specimen and the lens. The focal length of an objective lens is different than the working distance. This is because objective lenses are made of a combination of lenses and the focal length is measured from inside the barrel. The working distance is a parameter that microscopists can use more readily as it is measured from the outermost lens. The working distance decreases as the *NA* and magnification both increase. Immersion techniques are often used to improve the light gathering ability of microscopes. The specimen is illuminated by transmitted, scattered or reflected light though a condenser.

The $f/\#$ (f-number) describes the light gathering ability of a lens. It is given by $f/\# = \frac{f}{D} \approx \frac{1}{2NA}$





The numerical aperture (*NA*) of a microscope objective lens refers to the light-gathering ability of the lens and is calculated using half the angle of acceptance θ. Note, α is half the acceptance angle for light rays from a specimen entering a camera lens, and D is the diameter of the aperture that controls the light entering the lens.

As the f-number decreases, the camera is able to gather light from a larger angle, giving wide-angle photography. As usual there is a trade-off. A greater f/# means less light reaches the image plane. A setting of f/16 usually allows one to take pictures in bright sunlight as the aperture diameter is small.

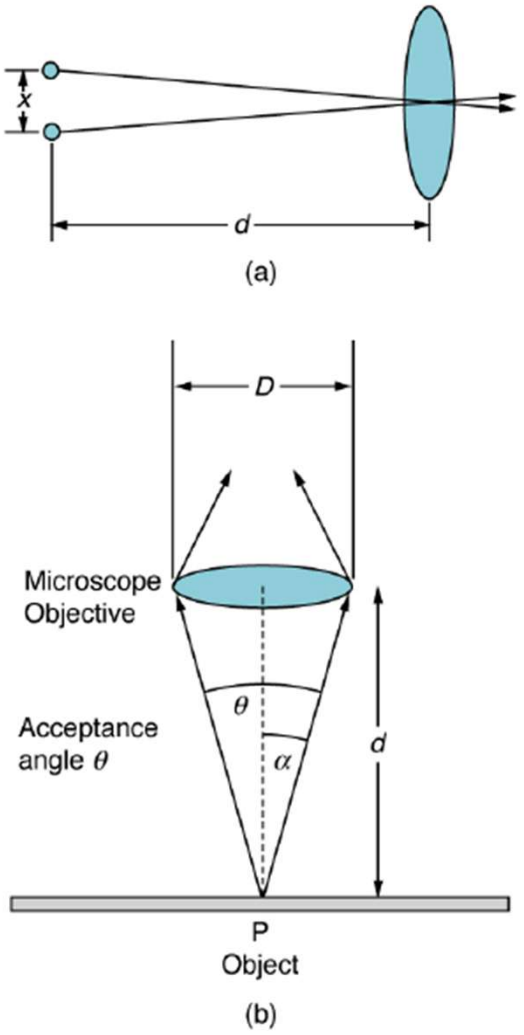
RESOLUTION OF A MICROSCOPE

- In most biology laboratories, resolution is presented when the use of the microscope is introduced.
- The ability of a lens to produce sharp images of two closely spaced point objects is called resolution.
- The smaller the distance by which two objects can be separated and still be seen as distinct, the greater the resolution. The resolving power of a lens is defined as that distance x.
- An expression for resolving power is obtained from the Rayleigh criterion.
- As shown in (a) of the figure on right, we have two point-objects separated by a distance x. According to the Rayleigh criterion, resolution is possible when the minimum angular separation is:

$$\theta = 1.22\frac{\lambda}{D} = \frac{x}{d}$$

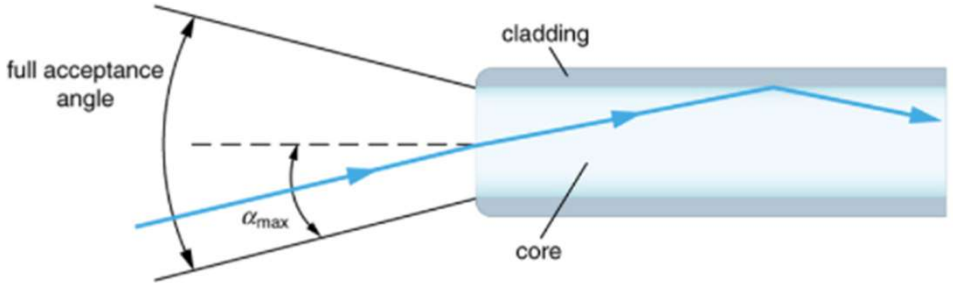
where is the distance between the specimen and the objective lens, and we have used the small angle approximation (i.e., we have assumed that x is much smaller than d), so that $\tan\vartheta \approx \sin\vartheta \approx \vartheta$. Thus, the resolving power is:

$$x = 1.22\frac{\lambda d}{D}$$



RESOLUTION (CONTD.)

Another way to look at resolving power is to use the concept of Numerical Aperture (*NA*), which is a measure of the maximum **acceptance angle** for the objective. For an optical fiber based system, it is a measure of the maximum **acceptance angle** at which the fiber will take light and still contain it within the fiber.



Can the NA be larger than 1.00? The answer is 'yes' if we use immersion lenses in which a medium such as oil, glycerin or water is placed between the objective and the microscope cover slip. This minimizes the mismatch in refractive indices as light rays go through different media, generally providing a greater light-gathering ability and an increase in resolution.

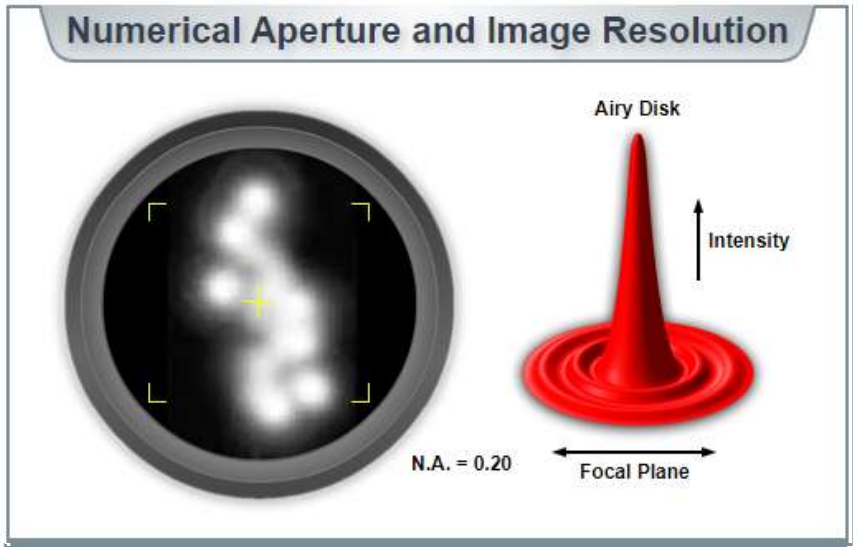
Thus, in general, *NA* is a measure of the ability of the lens to gather light and resolve fine detail. The angle subtended by the lens at its focus is defined to be $\theta = 2\alpha$ (from the figure). Using small angle approximation, we can write:

$$\sin\alpha = \frac{D/2}{d} = \frac{D}{2d}$$

Since the *NA* for a lens (discussed 2 slides back) is $NA = n\ \sin\alpha$, where n is the index of refraction of the medium between the objective lens and the object, we get:

$$x = 1.22\frac{\lambda d}{D} = 1.22\frac{\lambda}{2\sin\alpha} = 0.61\frac{\lambda n}{NA}$$

Thus, *NA* is important because it relates to the resolving power of a lens. A lens with a large *NA* resolves finer details.


Numerical Aperture and Image Resolution
Airy Disk
Intensity
N.A. = 0.20
Focal Plane

Movie