

# BIOL227

## Course introduction & microscopy

# BIOL 227 Learning Outcomes

Most of your learning for 200-level courses is at the knowledge and comprehension-level

As you progress toward the 400-level you will build evaluation and synthesis-level cognitive skills.

Professors/researchers continue to hone their synthesis-level skills.

How good you are at the application to synthesis level, depends on how solid your foundation is at the knowledge and comprehension level.

Cognitive Skills	Active Verbs That Describe Observable and Measurable Learning		
<b>Knowledge Level:</b> The successful student will recognize or recall learned information.	list state name tell recall label	record define relate recall repeat select	underline arrange describe memorize recognize reproduce
<b>Comprehension Level:</b> The successful student will restate or interpret information in their own words.	explain translate identify restate discuss tell reference	describe express classify locate review critique interpret	report summarize discuss compare illustrate estimate reiterate
<b>Application Level:</b> The successful student will use or apply the learned information.	apply use practice demonstrate complete	sketch solve construct conduct dramatize	perform respond role-play execute employ
<b>Analysis Level:</b> The successful student will examine the learned information critically.	analyze distinguish differentiate appraise calculate experiment	inspect categorize catalogue quantify measure relate	test critique diagnose extrapolate theorize debate
<b>Evaluation Level:</b> The successful student will assess or judge the value of learned information.	review justify assess defend report on investigate	appraise argue rate score select measure	choose conclude compare evaluate interpret support
<b>Synthesis Level:</b> The successful student will create new models using the learned information.	develop plan build create design organize	revise formulate propose establish integrate modify	compose collect construct prepare devise manage

1. List unique features (at the organismal, cellular and subcellular level) of a particular taxon
2. Identify/label these features (e.g. cell morphology, cell walls, food/contractile vacuoles, vasculature in plants, etc.)
3. Describe these features and explain why they are important. What advantage do they confer to the organism? What information does it give you in terms of the organism's environment?
4. Recognize the diversity of organisms and their unique features across various levels of the taxonomic hierarchy.
5. Compare taxa within a taxon.

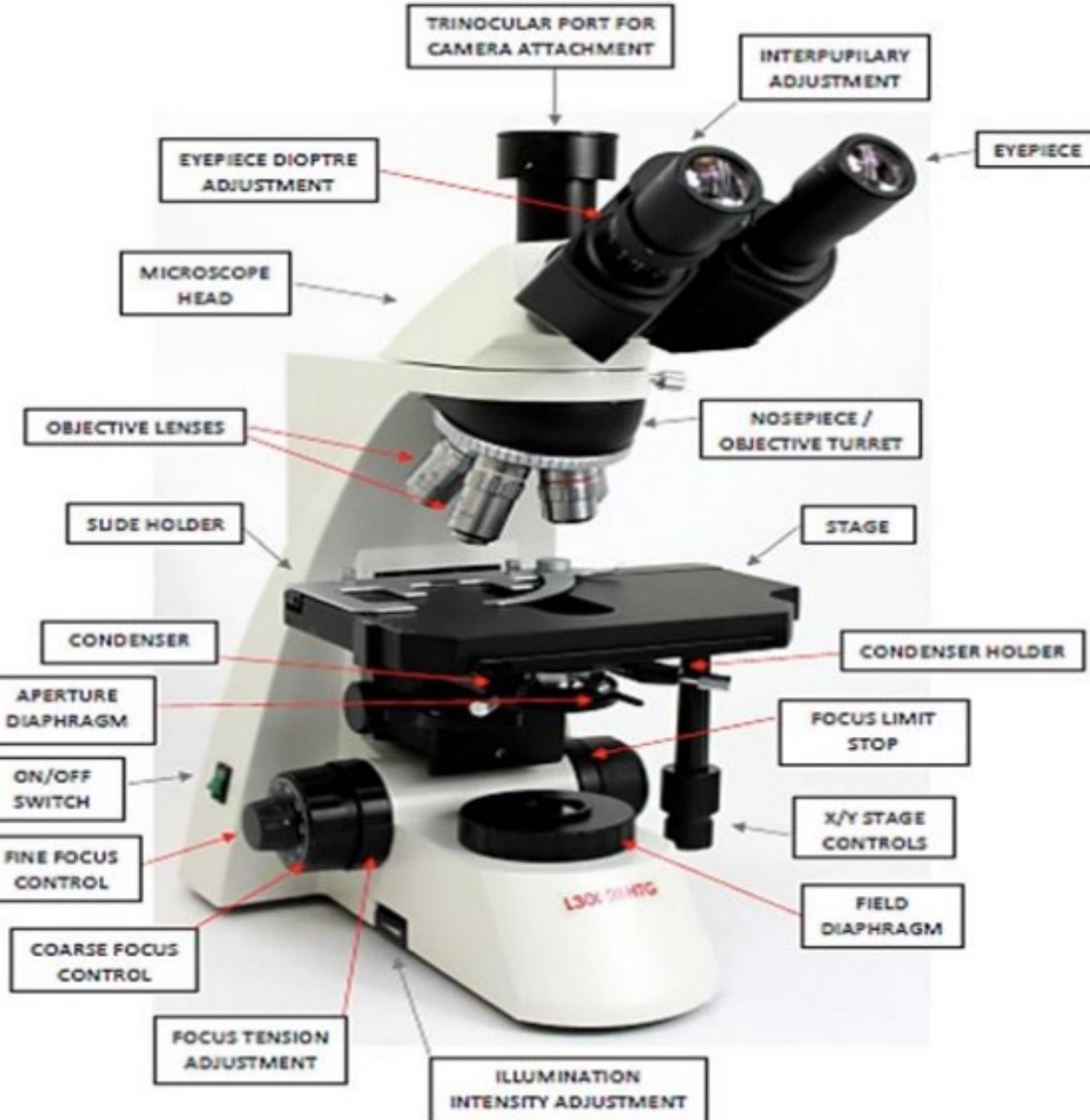
# Why do these skills, in this course, matter?

These skills allow us

- to make better arguments for the role biodiversity plays on our planet.
- to formulate and defend hypotheses about a taxon's evolutionary history.
- to pick better model organisms for research (i.e. create/develop better research questions)
- ...

# The compound light microscope

1. Refraction
2. Lenses
3. Resolution: Abbé equation



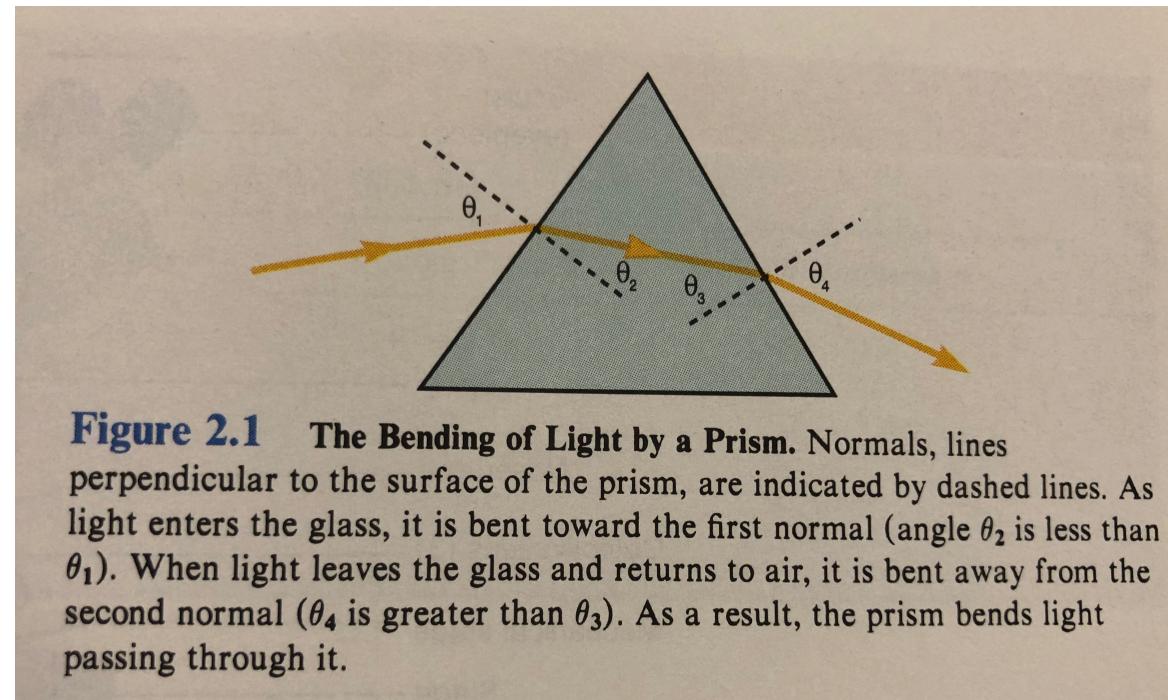
When light passes from one medium to another, refraction occurs.

Medium can be air, glass or oil

Refraction index= a measure of how much a medium slows the velocity of light

- Refractive index of air=1
- Refractive index of glass=1.52
- Refractive index of immersion oil=1.52

The direction and magnitude of the bend is a function of the refractive indices at the interface of the two media



**Figure 2.1 The Bending of Light by a Prism.** Normals, lines perpendicular to the surface of the prism, are indicated by dashed lines. As light enters the glass, it is bent toward the first normal (angle  $\theta_2$  is less than  $\theta_1$ ). When light leaves the glass and returns to air, it is bent away from the second normal ( $\theta_4$  is greater than  $\theta_3$ ). As a result, the prism bends light passing through it.

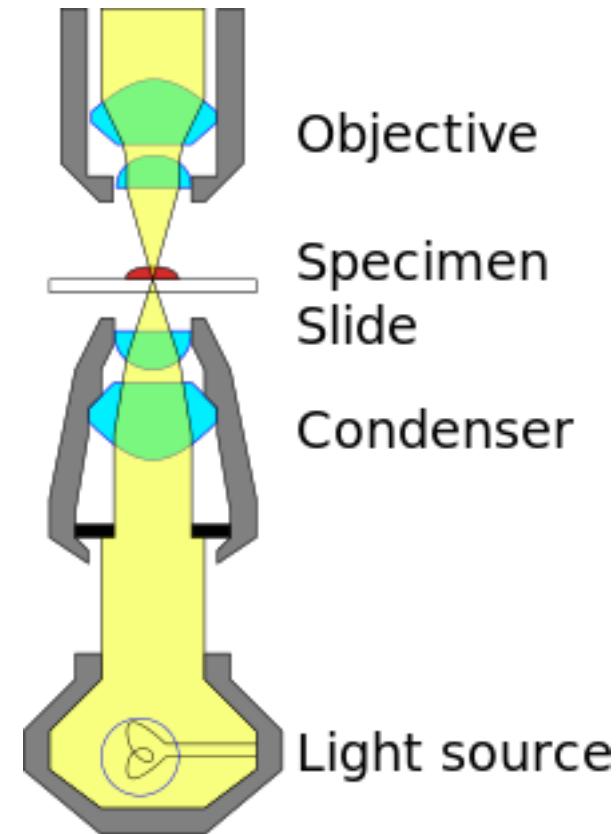
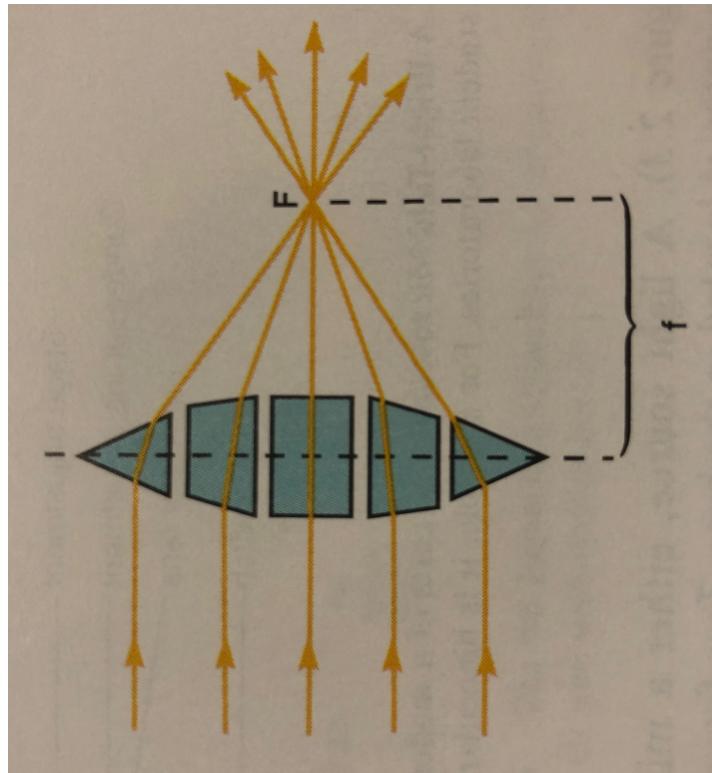
# Lenses – a microscope's jewels.

Three lenses:

- Condenser lens
- Objective lens
- Ocular lens

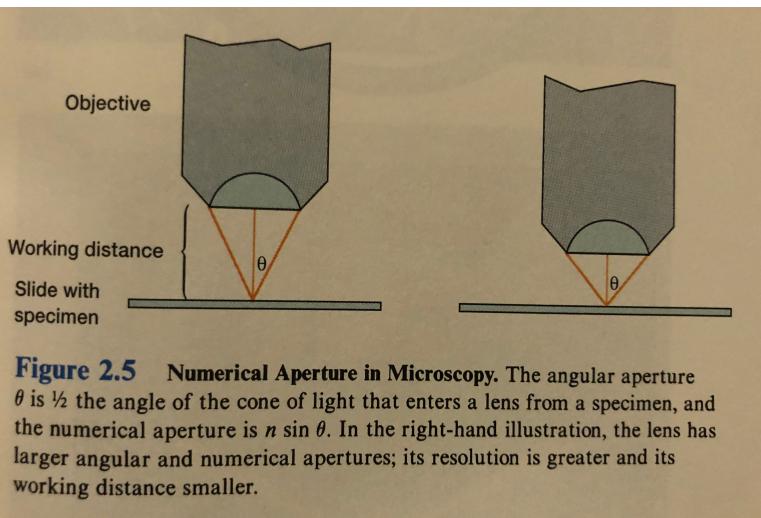
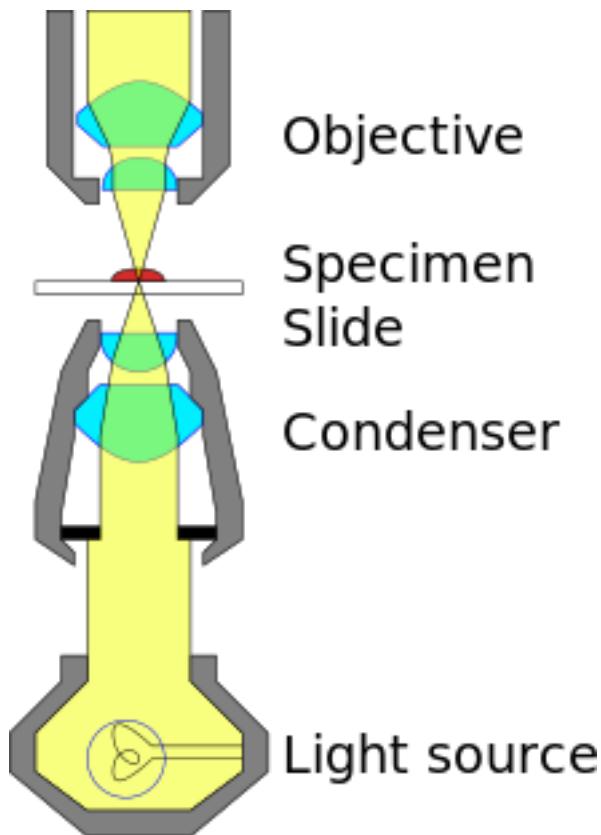
The objective and ocular lenses magnify the image. Hence why we call it the compound microscope.

The condenser lens (a collection of prisms) converges rays of light to a focal point (F).

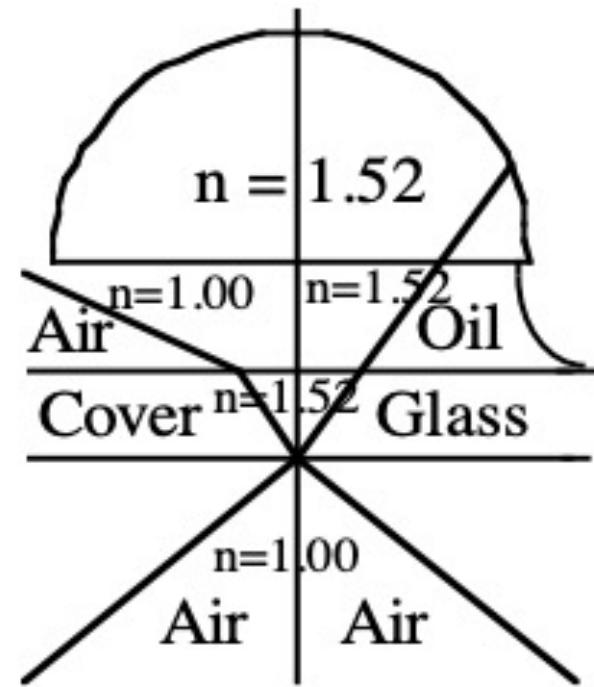
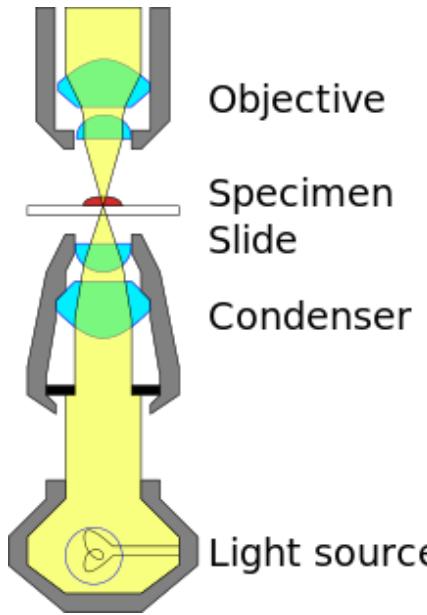


Resolution is the ability of a lens to separate or distinguish between small objects that are close together.

- Ernst Abbé (1870s) a physicist worked out the resolution of lenses mathematically.
- Abbé equation:  $d = \frac{0.5\lambda}{n \sin\theta}$ 
  - d= The resolving power of the objective lens. It gives the minimal distance between two points that can be resolved
  - n= refractive index of the medium in which the objective lens is working.
  - $\sin\theta = 1/2$  the angle of the cone of light entering the objective lens
  - Numerical aperture (N.A) =  $n \sin\theta$



**Figure 2.5 Numerical Aperture in Microscopy.** The angular aperture  $\theta$  is  $1/2$  the angle of the cone of light that enters a lens from a specimen, and the numerical aperture is  $n \sin \theta$ . In the right-hand illustration, the lens has larger angular and numerical apertures; its resolution is greater and its working distance smaller.



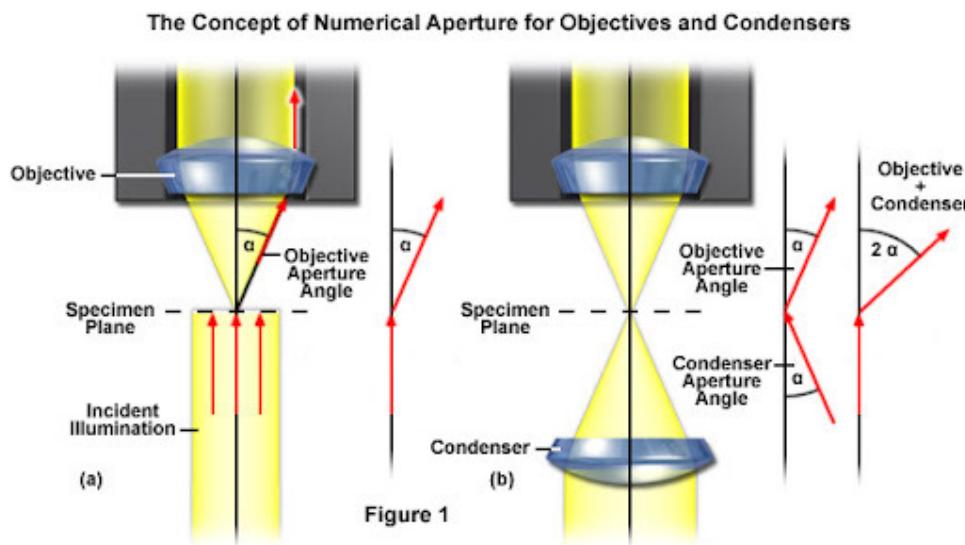
Property	Objective			
	Scanning	Low Power	High Power	Oil Immersion
Magnification	4×	10×	40–45×	90–100×
Numerical aperture	0.10	0.25	0.55–0.65	1.25–1.4
Approximate focal length ( <i>f</i> )	40 mm	16 mm	4 mm	1.8–2.0 mm
Working distance	17–20 mm	4–8 mm	0.5–0.7 mm	0.1 mm
Approximate resolving power with light of 450 nm (blue light)	2.3 μm	0.9 μm	0.35 μm	0.18 μm

# Aperture iris diaphragm

The condenser sits above the aperture iris diaphragm. The condenser aperture diaphragm is responsible for controlling the angle of the illuminating light cone and, consequently, the numerical aperture of the condenser.



Iris diaphragm



Always remember to do the Kohler illumination step!



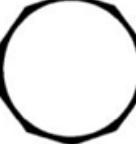
Field of view before field iris diaphragm adjustment



Field of view after field iris diaphragm adjustment



Field iris diaphragm partially closed



Field iris diaphragm fully open

Dark-field microscope is used for viewing live specimens. Less light pass through the specimen.

Background appears dark; only light reflected or refracted by the specimen enters the objective lens.

