ONBI Recap slides

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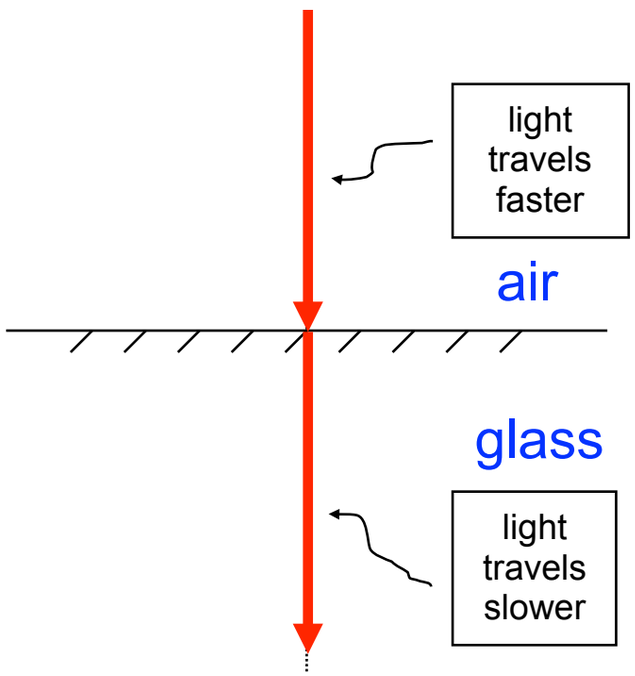
How lenses work

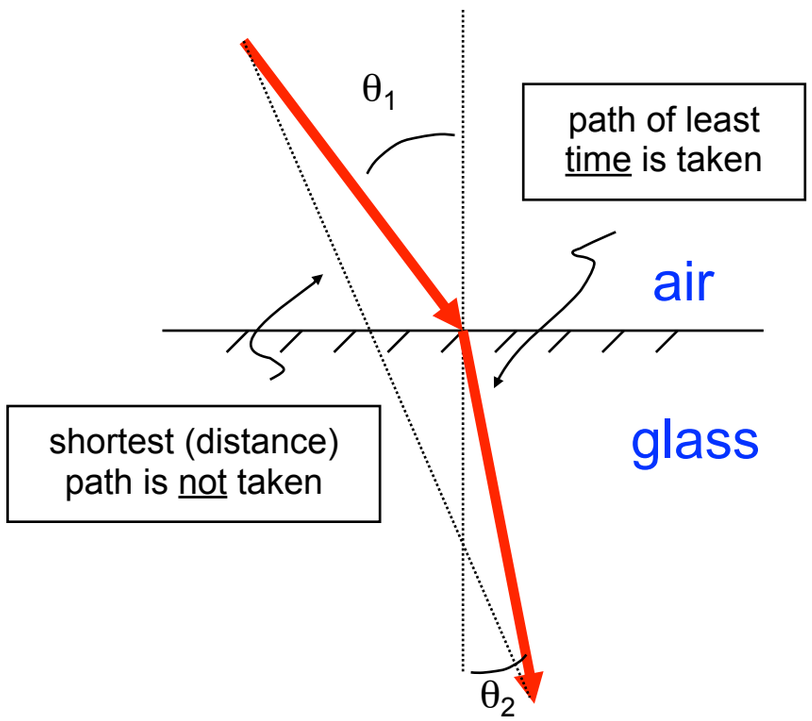
- Refraction--the "bending", or change in the direction, of light
- Explaining refraction doesn't require the "wave" formalism, just the rays
- The speed of light depends on the medium through which light is propagating
- Refraction occurs when light rays travelling through one type of medium meet an interface with another type of medium
- The extent of refraction depends on the angle of incidence (Snell's law)

$$n_1 \sin\theta_1 = n_2 \sin\theta_2$$

Here the light ray is *orthogonal* to the interface



Here the light ray is *oblique* to the interface

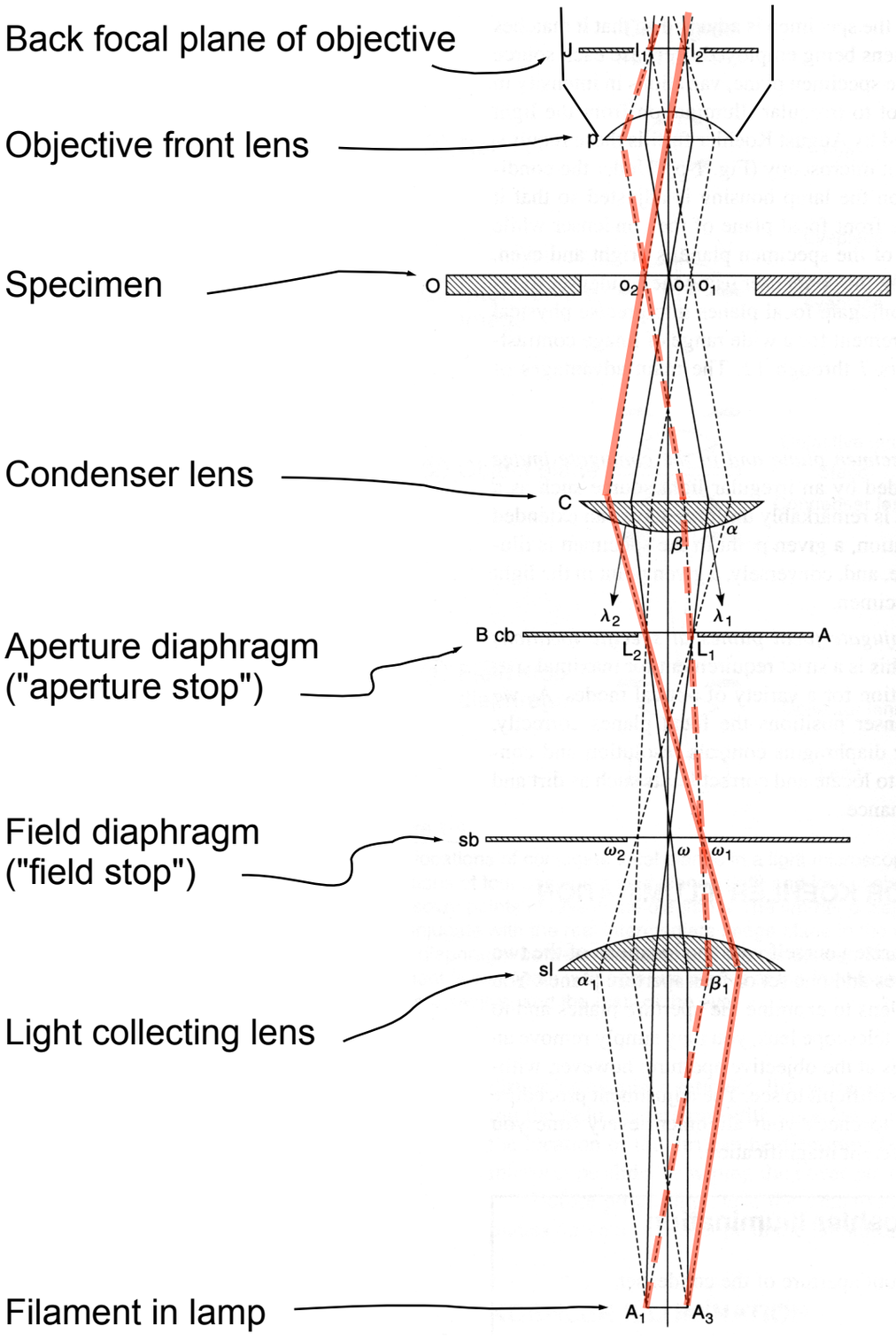


More dense materials have higher refractive indices:

Air	1.0003
Water	1.33
Glycerin	1.47
Immerson Oil	1.515 (e.g.)
Glass	1.52
Flint	1.66
Zircon	1.92
Diamond	2.42
Lead Sulfide	3.91

$$n_i = c/v_i$$

Koehler illumination emphasises the difference between imaging planes and illumination planes



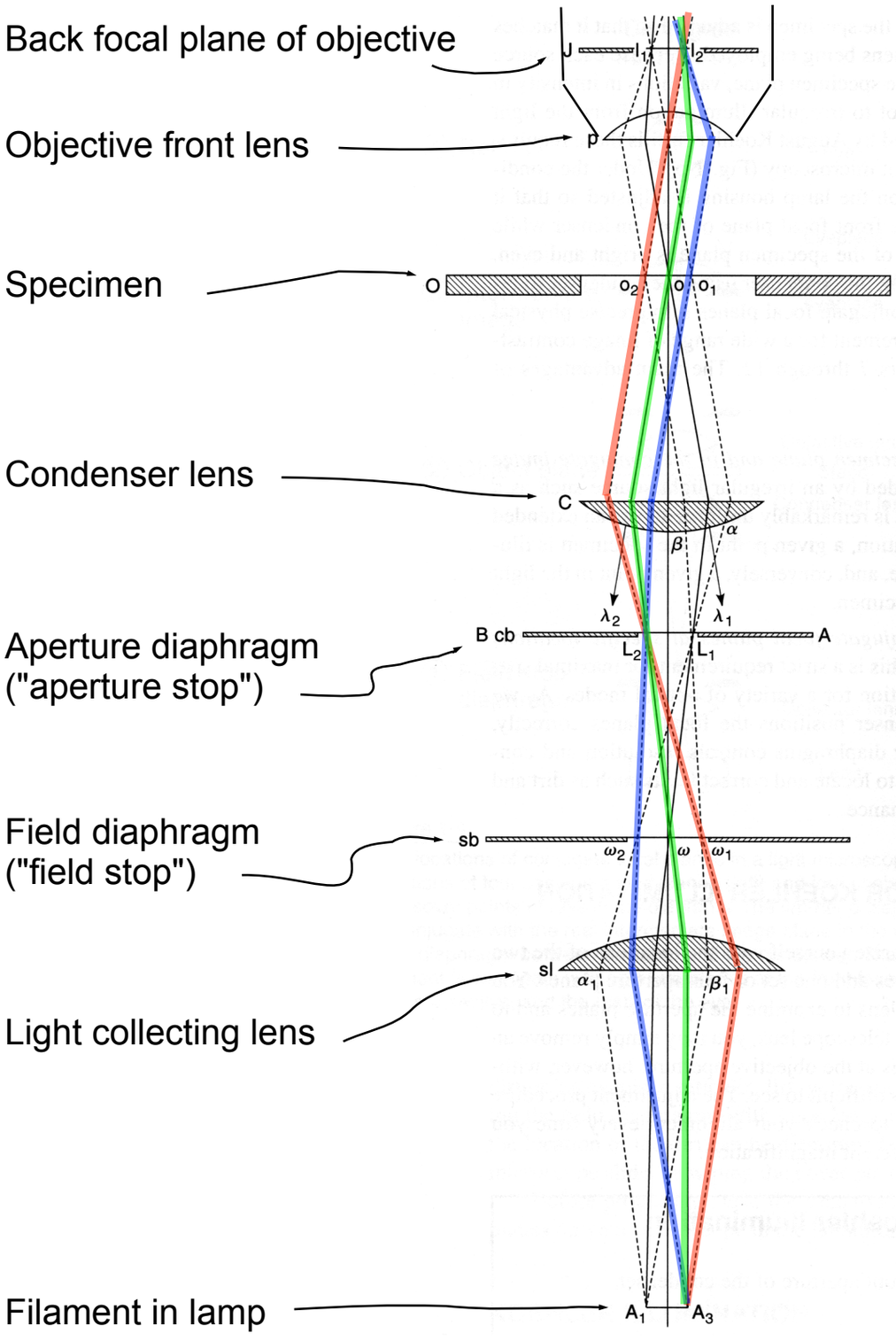
August Kohler
1866-1948

•To reduce artefacts, Koehler introduced the light collecting lens and adjusted the condenser position such that the lamp filament is maximally out-of-focus at the specimen plane.

•This innovation is essential to all modern microscopy--the main adjustment we make with transmitted light microscopy is to "Koehler" the microscope by focussing the condenser.

•Koehler illumination highlights a special relationship between two sets of planes in the microscope light path.

Koehler illumination emphasizes the difference between imaging planes and illumination planes



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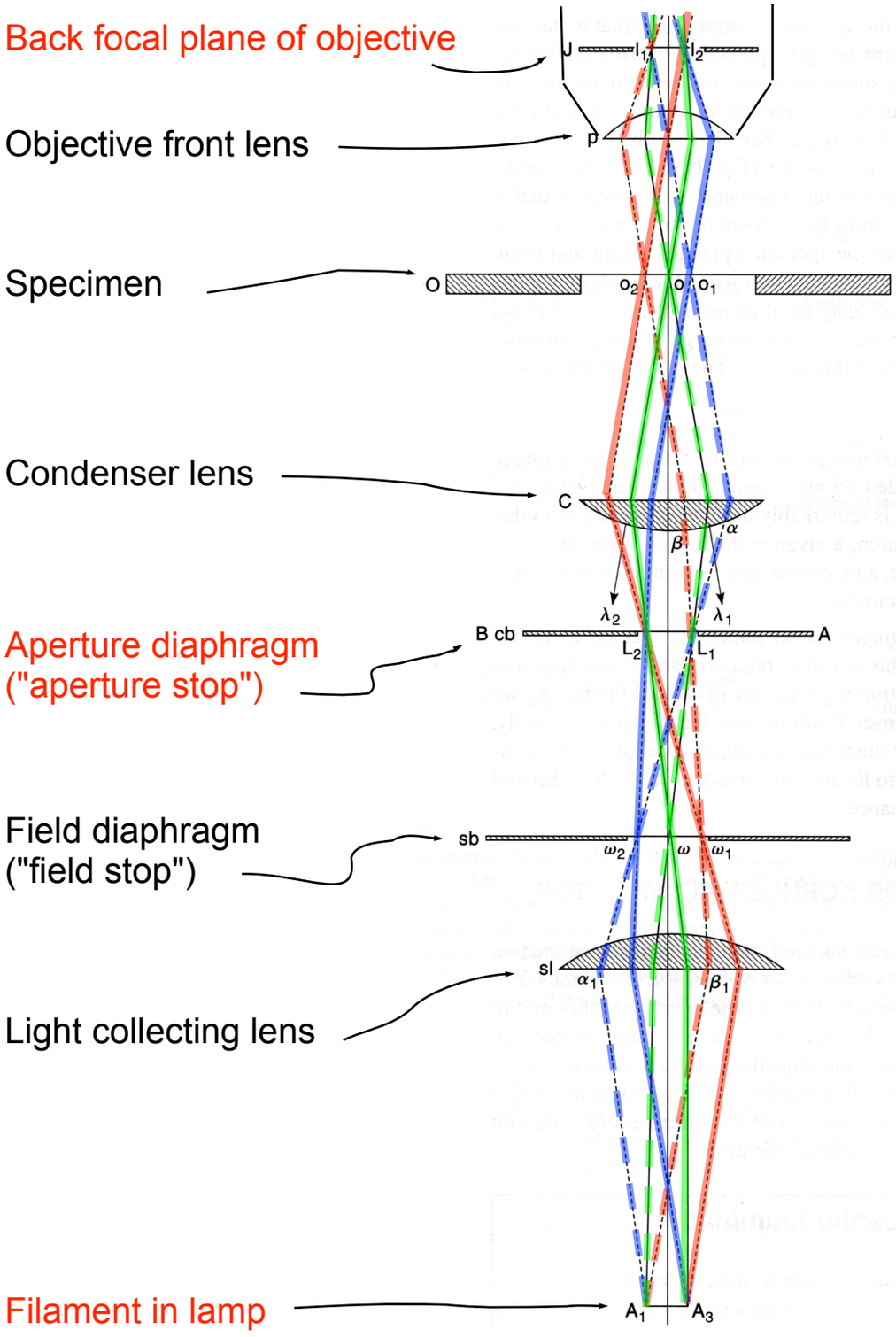

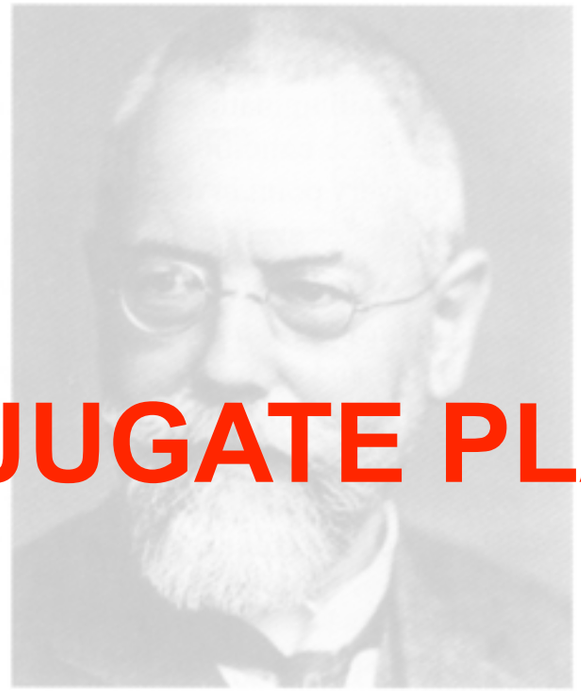

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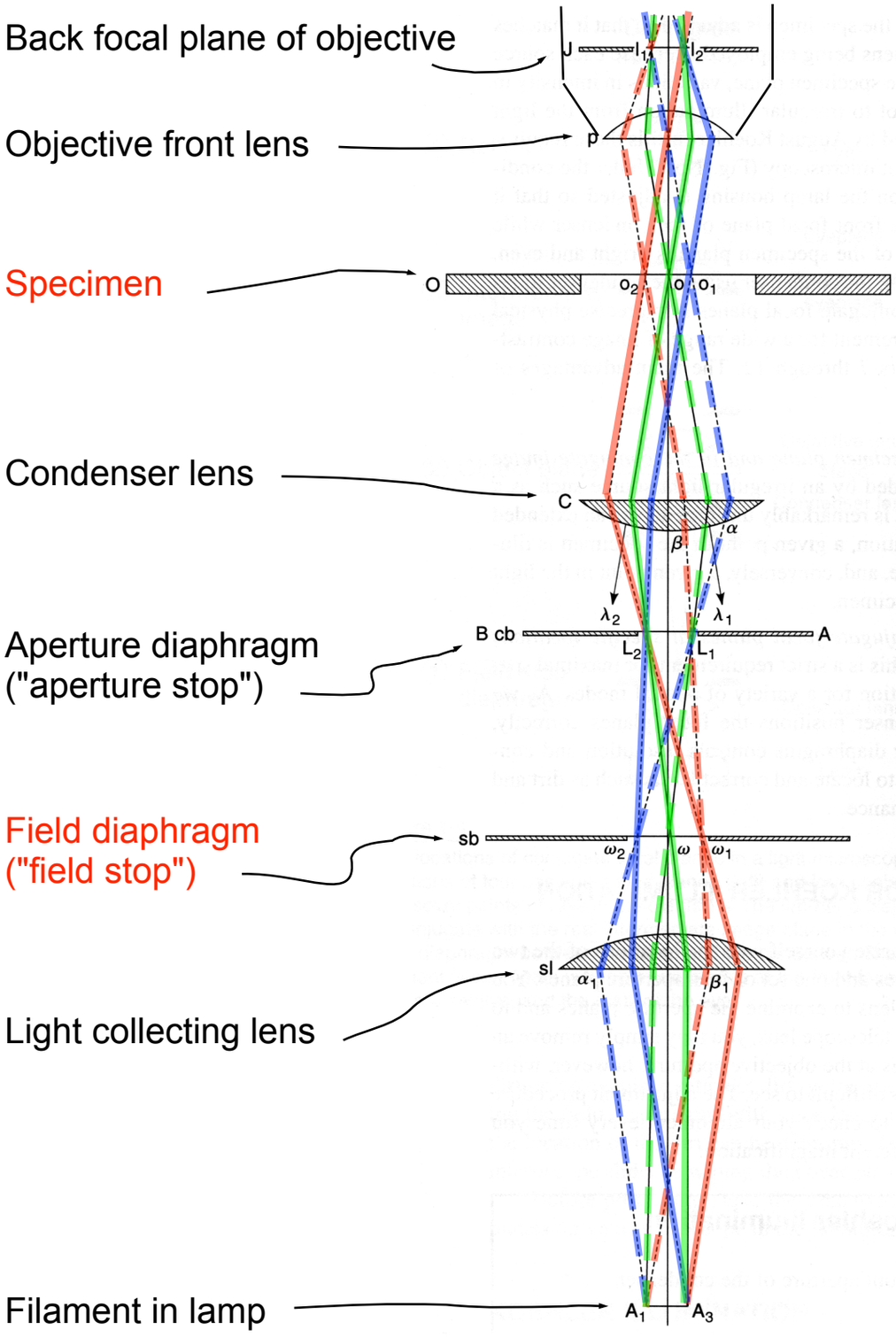

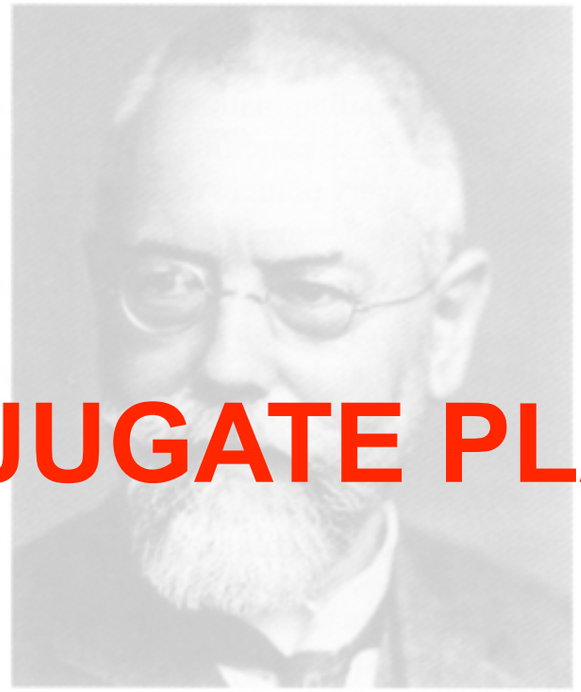

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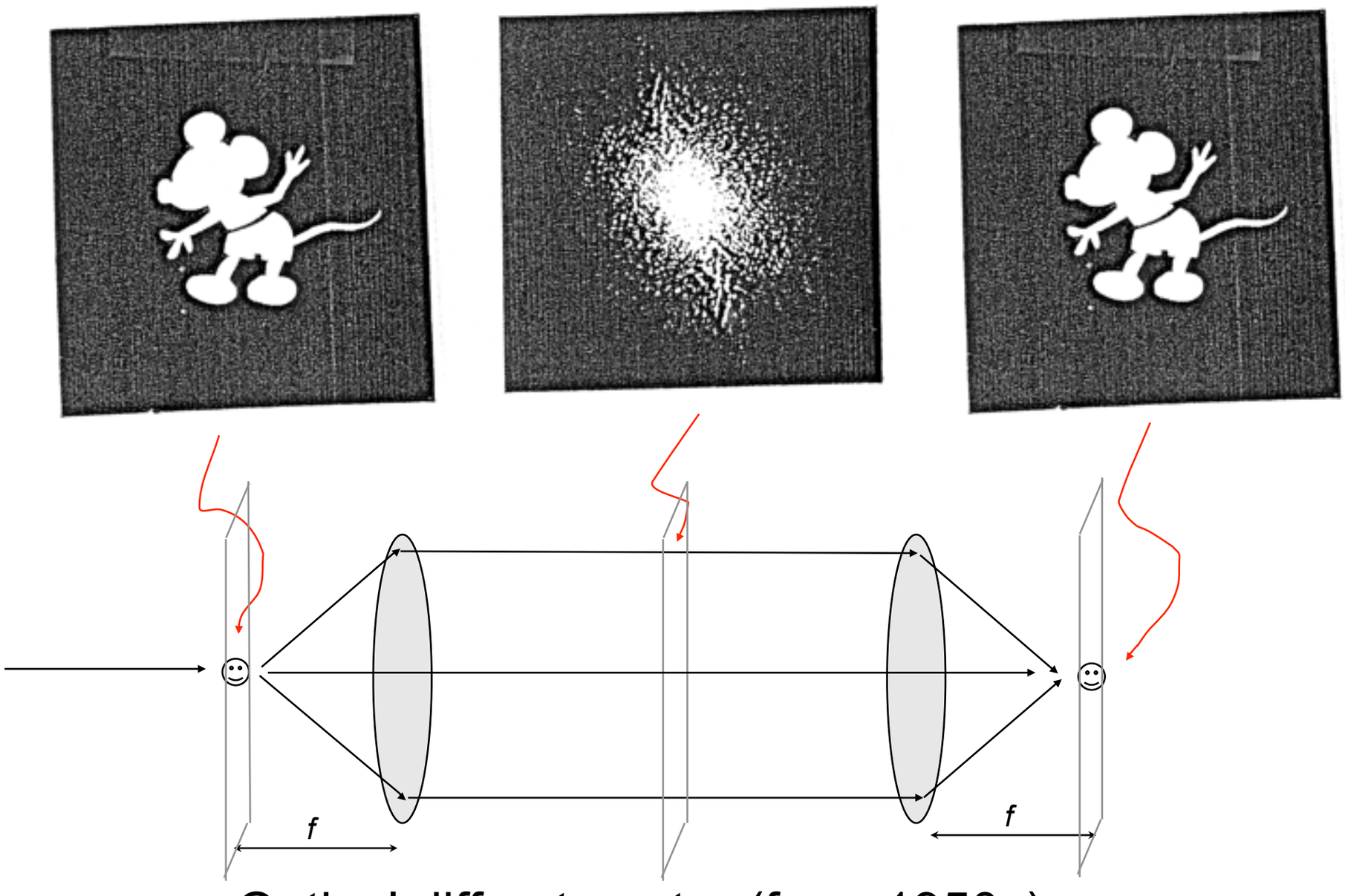
<u>Anything</u> can create a diffraction pattern

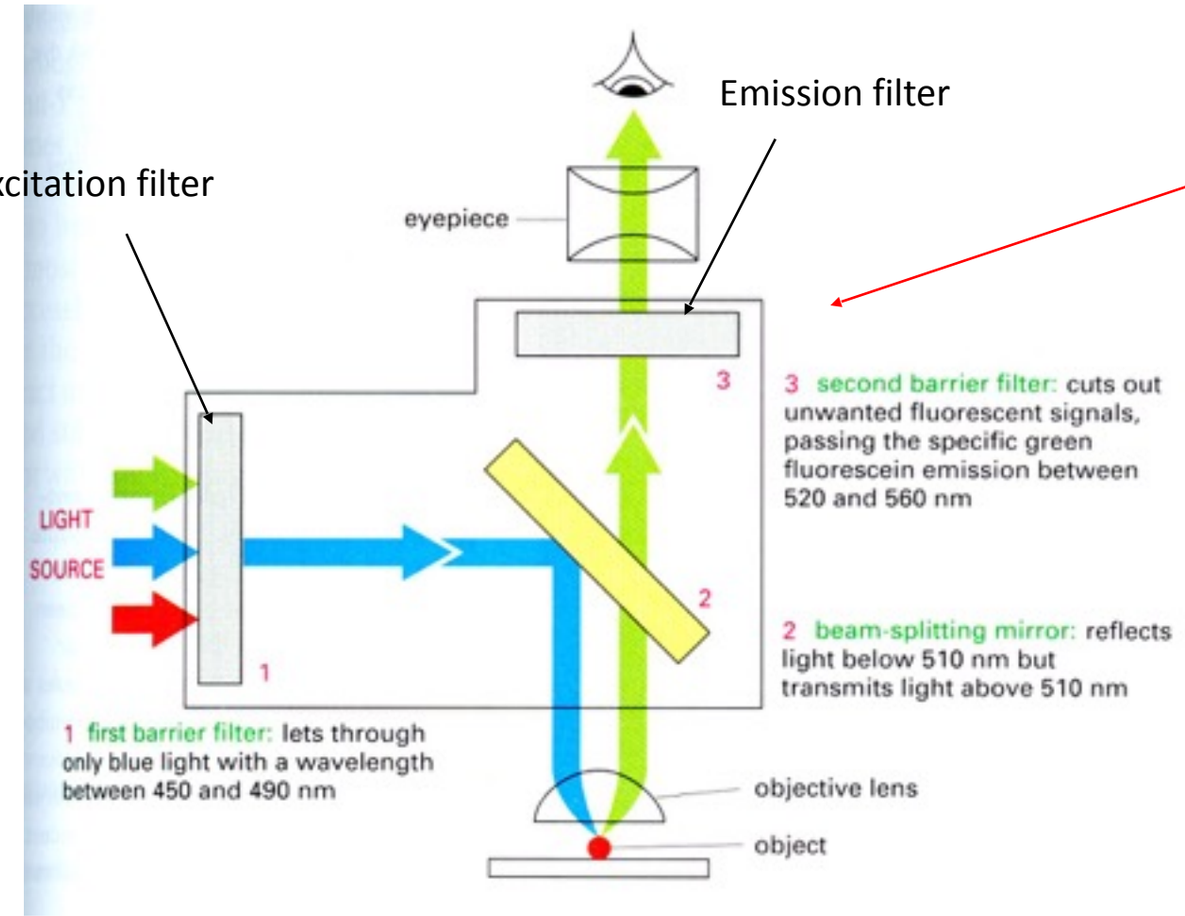
- The individual spots in diffraction patterns of protein crystals are particularly prominent because the protein crystals have the same structure repeated infinitely, but even individual objects generate diffraction patterns (which are even more complex)

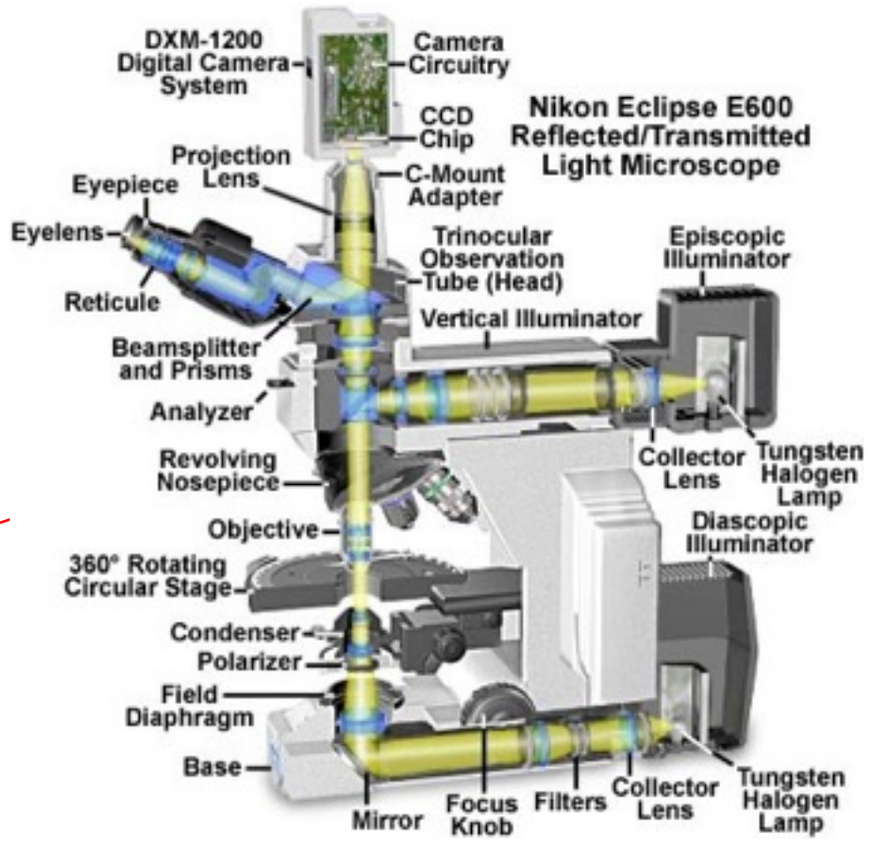


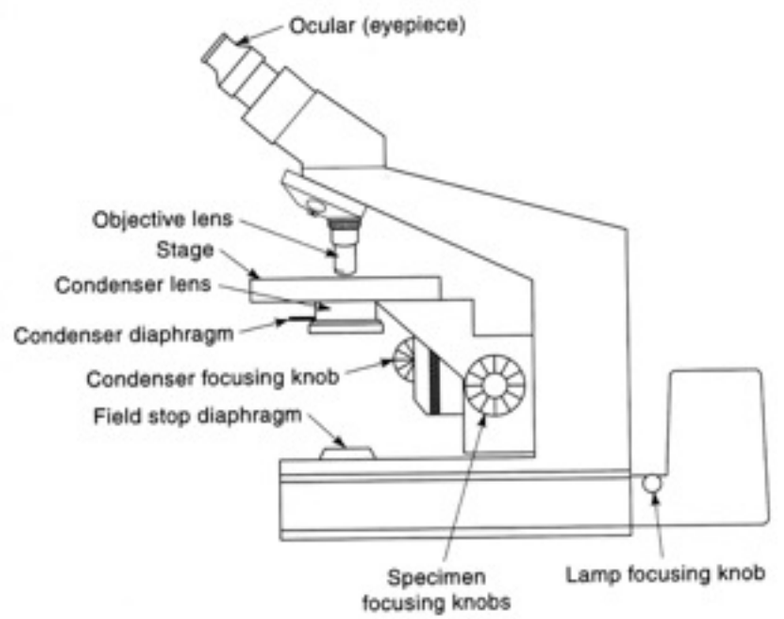
Optical diffractometer (from 1950s)

Epifluorescence microscope design

Epifluorescence microscopy uses illumination from above ("epi-") and a special cube containing two colored filters plus a special beam-splitting ("dichroic") mirror



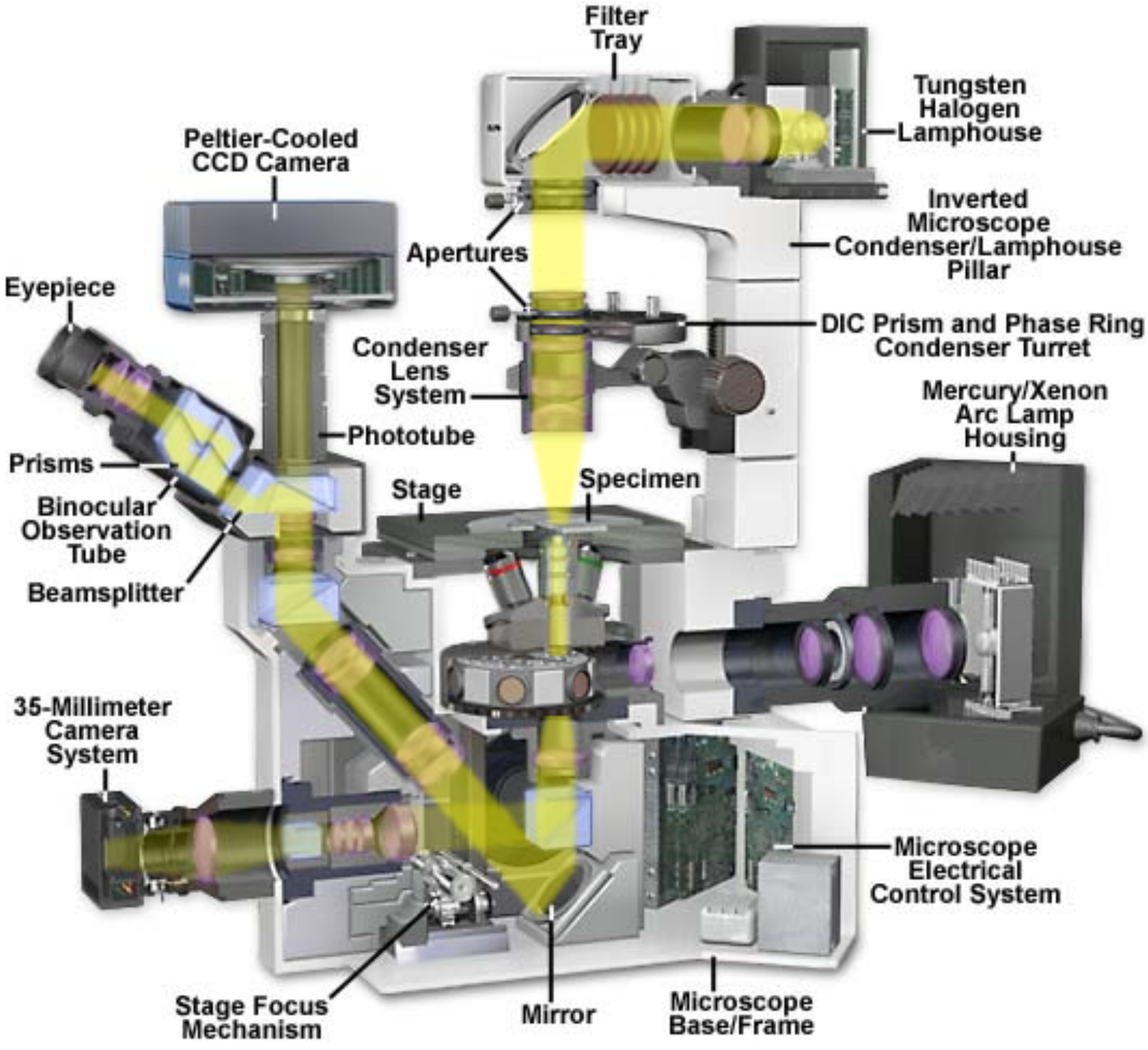




"Background" fluorescence is very dark!

Olympus IX70 Inverted Microscope Light Pathways

Interactive Java Tutorial



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