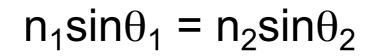
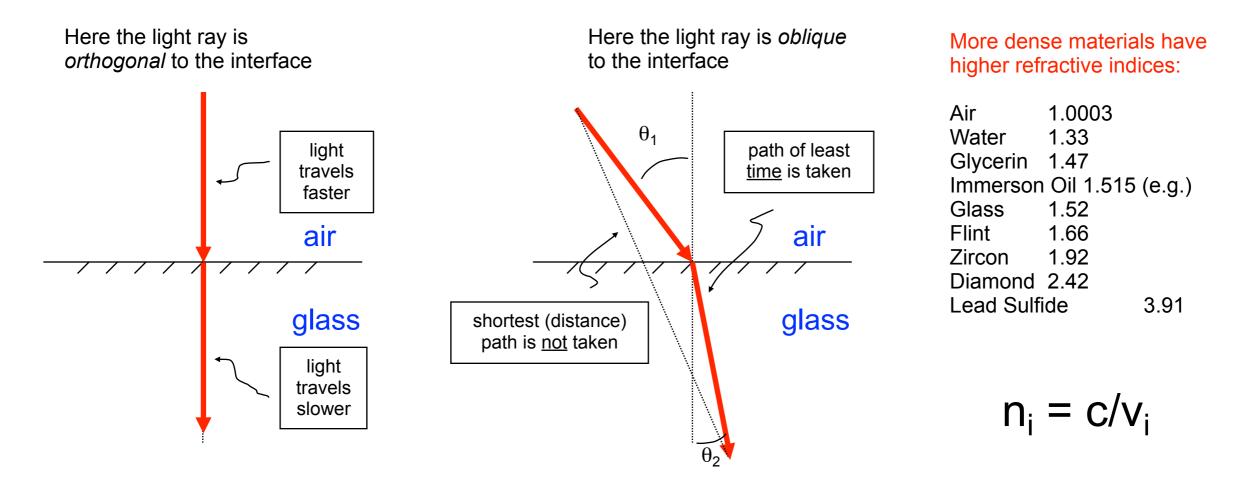
ONBI Recap slides

Ian Dobbie 12/1/2015 ian.dobbie@bioch.ox.ac.uk

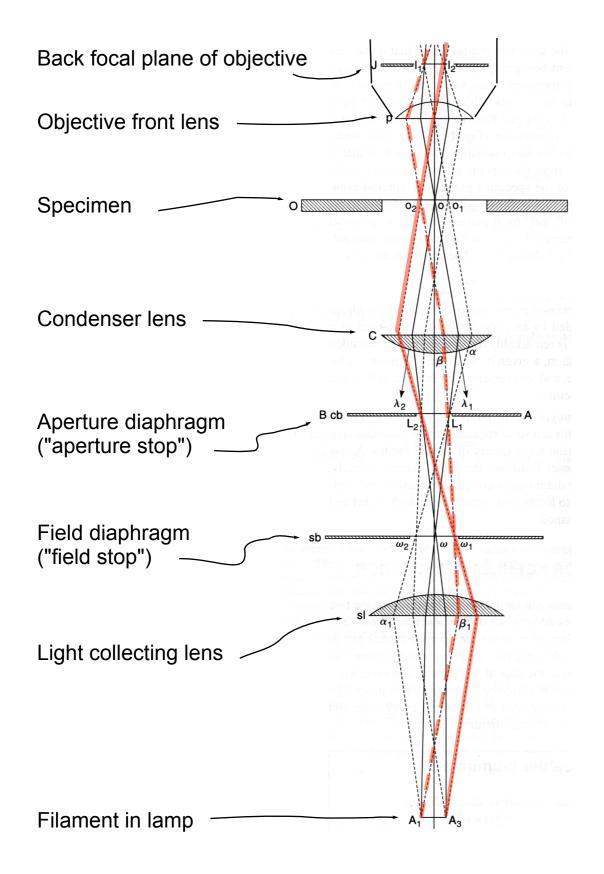
How lenses work

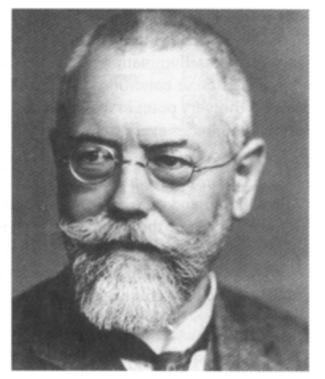
- Refraction--the "bending", or change in the <u>direction</u>, of light
- Explaining refraction doesn't require the "wave" formalism, just the rays
- The speed of light depends on the medium through which light is propagating
- Refraction occurs when light rays travelling through one type of medium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





Koehler illumination emphasises the difference between imaging planes and illumination planes





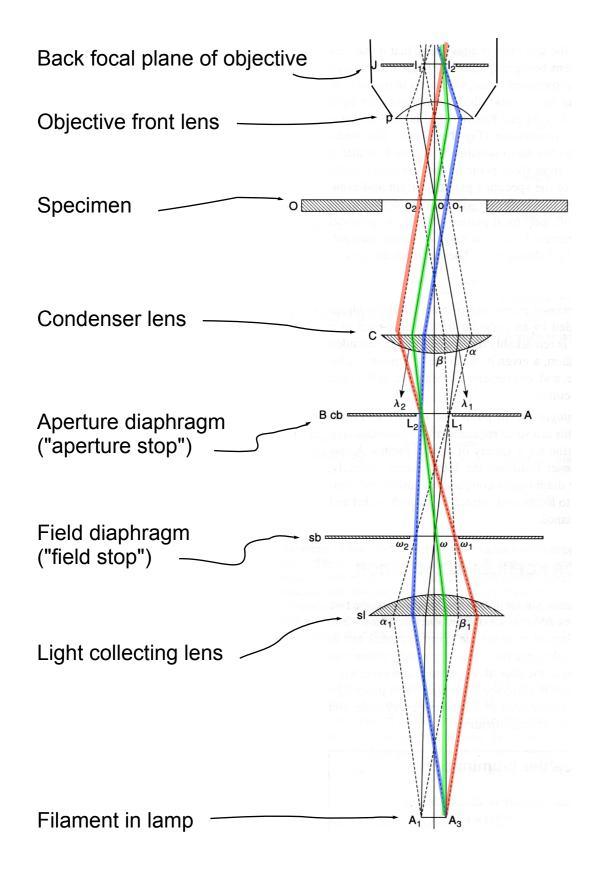
August Kohler 1866-1948

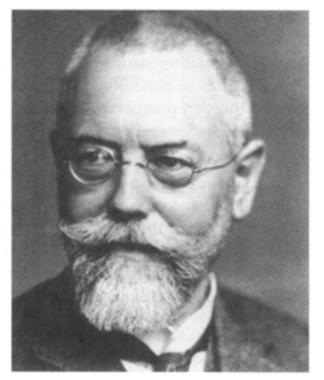
•To reduce artefacts, Koehler introduced the light collecting lens and adjusted the condenser position such that the lamp filament is maximally out-of-focus at the specimen plane.

•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





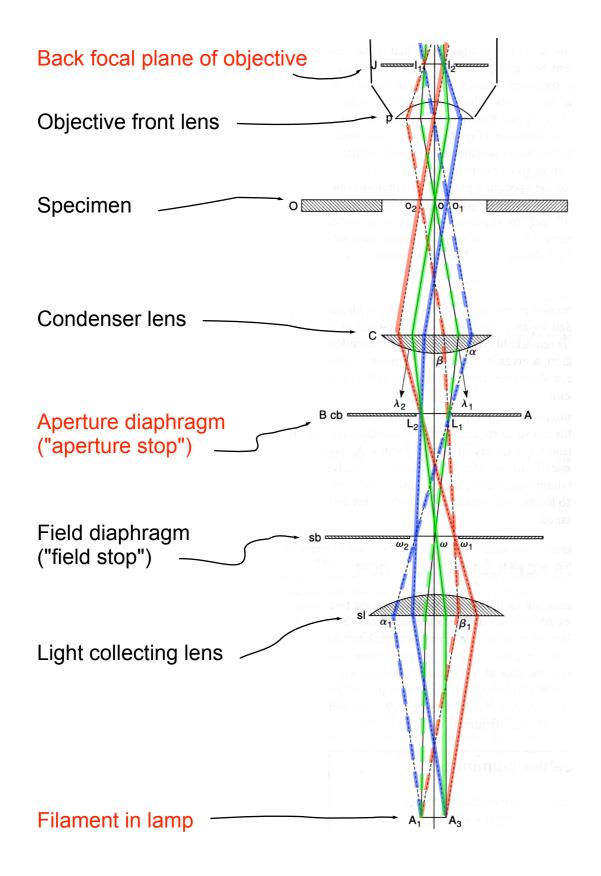
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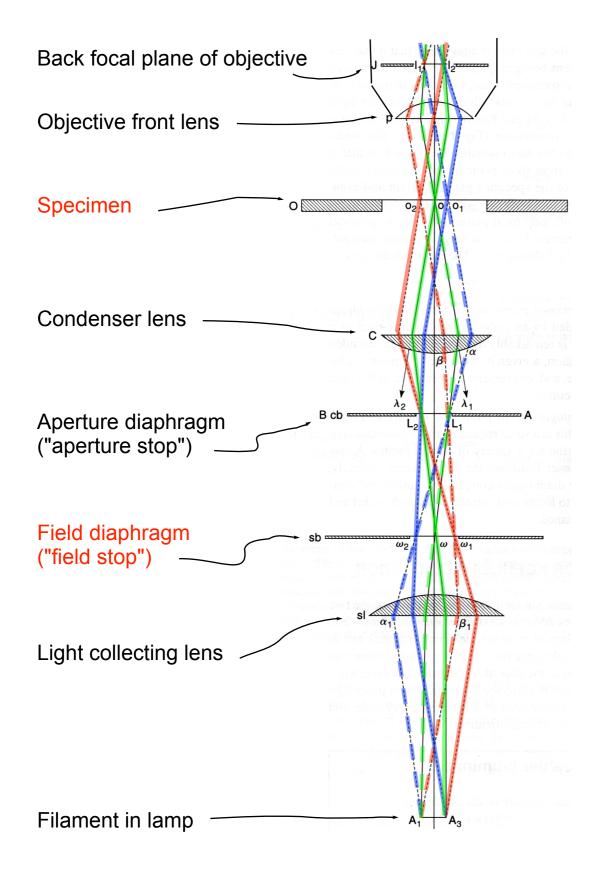
1866-1948

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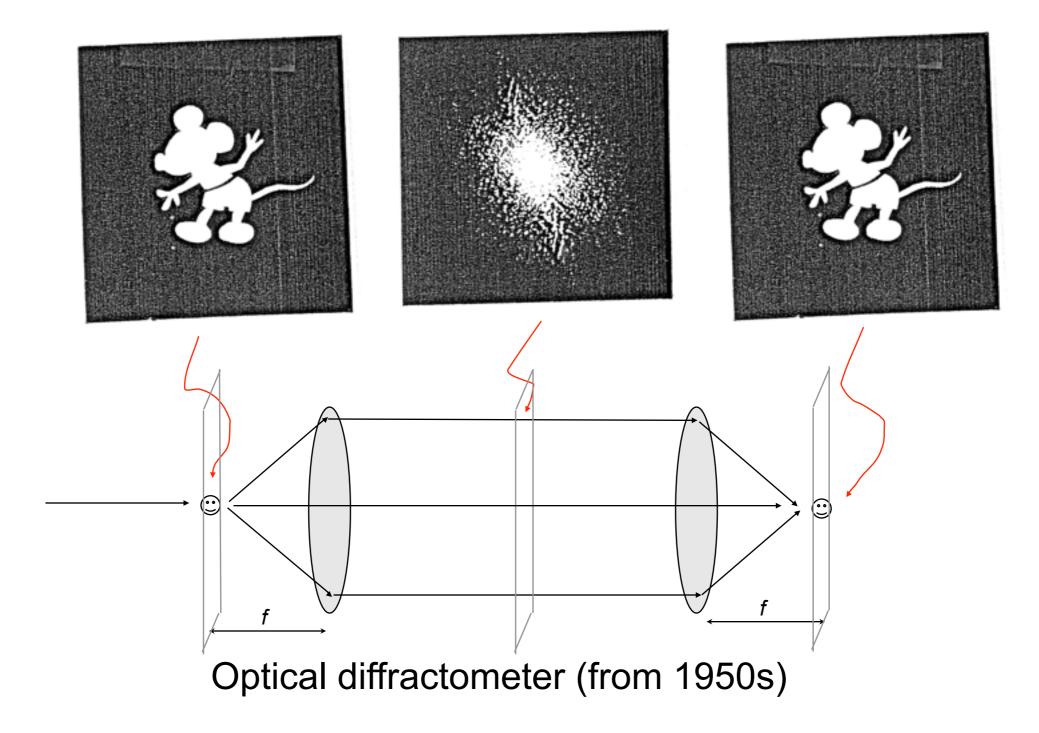
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Anything can create a diffraction pattern

• 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)



Epifluorescence microscope design

DXM-1200 Digital Camera-Camera Epifluorescence microscopy uses illumination from Circuitry System Nikon Eclipse E600 CCD above ("epi-") and a special cube containing two Reflected/Transmitted Chip Projection Light Microscope C-Mount Lens Evepiece colored filters plus a special beam-splitting Adapter Eyelens-Trinocular Episcopic Observation Tube (Head) Illuminator ("dichroic") mirror Reticule Vertical Illuminator Beamsplitter and Prisms Analyzer Collector Tungsten Lens Halogen Revolving **Emission filter** Nosepiec Lens Lamp Diascopic Objective 360° Rotating Circular Stage Excitation filter eyepiece Condenser Polarize Field Diaphragm Base-3 second barrier filter: cuts out Focus Filters Collector Tungsten Halogen unwanted fluorescent signals, Mirror Knob Lamp passing the specific green fluorescein emission between 520 and 560 nm LIGHT SOURC Ocular (eyepiece) 2 beam-splitting mirror: reflects light below 510 nm but transmits light above 510 nm 1 first barrier filter: lets through only blue light with a wavelength **Objective lens** objective lens between 450 and 490 nm Stage Condenser lens object Condenser diaphrage Condenser focusing knot Field stop diaphrag "Background" fluorescence is very dark!

> Specimen Lamp focusing knob focusing knobs

Filter Tray Tungsten Halogen Lamphouse Peltier-Cooled CCD Camera Inverted Microscope Condenser/Lamphouse Pillar Apertures Eyepiece DIC Prism and Phase Ring Condenser Turret Condenser Lens Mercury/Xenon Arc Lamp Housing System-Phototube Prisms ۰. Specimen Stage Binocular Observation Tube Beamsplitter 35-Millimeter Camera System Microscope Electrical Control System Microscope Base/Frame Stage Focus Mechanism Mirror

Olympus IX70 Inverted Microscope Light Pathways

Interactive Java Tutorial

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