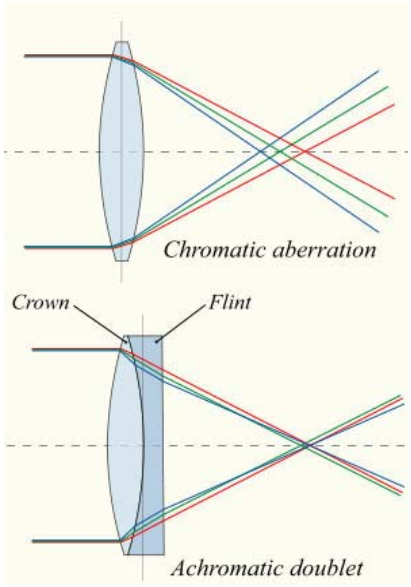
MICROSCOPY & STAINS



6/23/83, rvsd 7 July 1995, 5 July 2000, 5 July 2001, 8 Jan 02, 3 July 02, 12 July 04, 08July05, 31Mar06, 10July09, 12July10, 21Jan14, 17Sept15
Alcamo 5th: 68-81, TFC 7th: 56-74, 8th: 54-71, Black 6th: 52-71, Bauman 2nd, 93-112, 4th::94-121

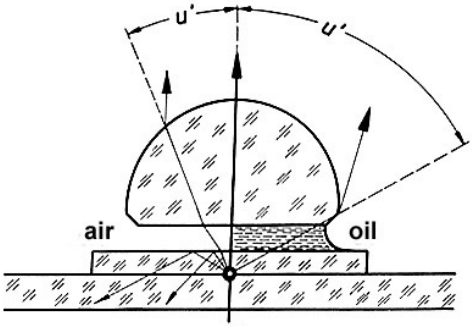
*Microscope: (*micro- tiny, *skopein:* Gk to see) Microorganisms usually transparent, often require staining
Metric system: meter (to measure), micrometer = µm= micron, (10^{-6}) and nanometer (nano- 10^{-9}) (p 94)
Bacteria usually 0.2 to 2 µm
Light microscopes resolve down to 0.3 µm (therefore, 2000xpower is the limit of resolution)

REFRACTIVE INDEX: ratio of speed of light through two media, usually =
(light speed through vacuum / light speed through medium)

(p 96) (Refractive index of immersion oil and glass: 1.5150)
$n_D^{25\,C}$ = 1.5150 D = D line of Na emission spectrum (specific yellow wavelength of light)

IMPROVEMENTS IN MICROSCOPES:

Chester Hall	(1730s)	correct chromatic aberration (blue refracts more than red) using flint glass and crown glass lenses produced first **achromatic** lenses
Joseph Jackson Lister	(1830)	(father of Joseph) corrected spherical aberration with multiple lense components
Ernst Karl Abbe	(1878)	**oil immersion** (increase cone of light, higher resolution, brighter) (p 99)
Ernst Karl Abbe	(1886)	**condenser** (focuses light on specimen)
Dark field microscopy		Dust in ray of sunlight: p 59. Req'd to see syphilis spirochete
Phase contrast microscopy		depends on minute differences in refractive index: see living cells without using stains.



PREPARATION OF SPECIMEN: (p 107)

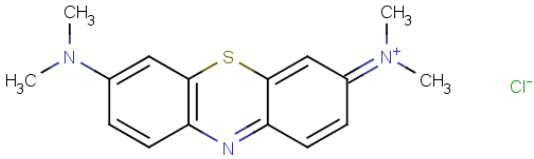
smear:	spread carefully, dry over flame to fix (coagulates proteins)
wet mount:	liquid suspension under cover slip
hanging drop:	drop on coverslip, Vaseline, invert on depression slide

STAINING: Salts of colored compounds, the ionized form either is **basic (positive)** or **acidic (negative)**:
Stains usually dissolved in an alcohol or water solution

SIMPLE STAINS

Basic dyes: are **positive when ionized, stain negatively charged bacteria**
examples: crystal violet (also Gentian violet, Hucker's stain)
methylene blue
safranin

methylene blue: (cation = blue)



Acid stains: are **negative when ionized**, stain positively charged materials
zB: stains background material such as glass, show capsules (p 110)
examples: eosin
nigrosin
India ink

Negative stain (p 110): use acid stain, stains the positive background. Unstainable capsules show up as halos

DIFFERENTIAL STAINS: (p. 104) Usually in four steps:

STEPS	**GRAM STAIN (p 109) Hans Christian Gram (1884)**	**ACID-FAST STAIN (p 110) (Ziehl-Neelsen)**	**ENDOSPORE STAIN (Schaeffer-Fulton) (p 110)**
1) primary stain	Hucker's stain	carbolfuchsin (a red dye)	malachite green
2) mordant	Iodine	steam specimen, several min	*steamed for five minutes*
3) decolorize	95% EtOH	acid-alcohol	wash 30 seconds with water
4) counter stain	safranin O	methylene blue	safranin
PURPOSE:	**Distinguish Gm +: purple fr Gm -: pink**	**Detect *Mycobacterium* sp (red rods)**	Detect spores (green) (*Bacillus* or *Clostridium*)

[**Fluorescence microscopy:** stain with fluorochromes:
auramine O glows yellow in UV, absorbed by *Mycobacterium tuberculosis*
fluorescein isothiocyanate apple green for *Bacillus anthracis*]