Imaging Methods: Breath Patterns

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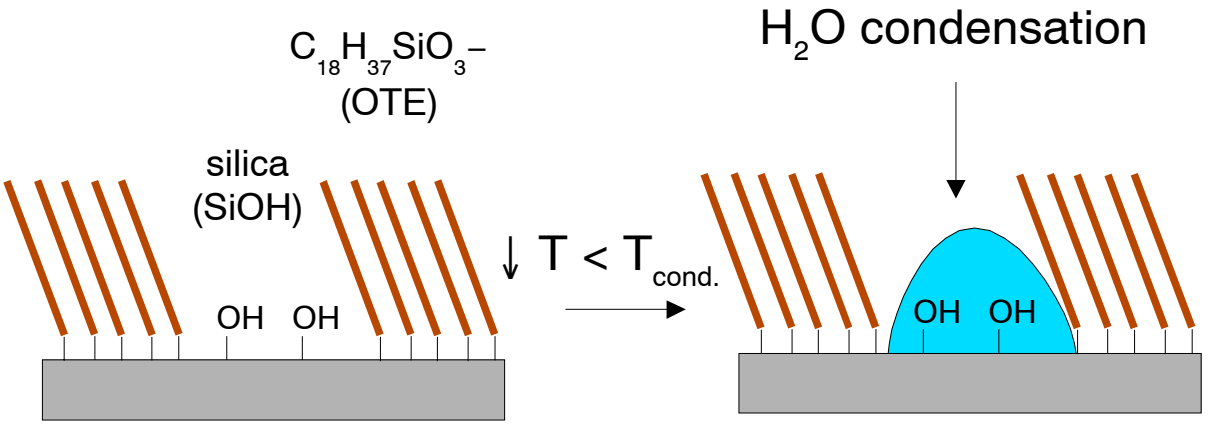
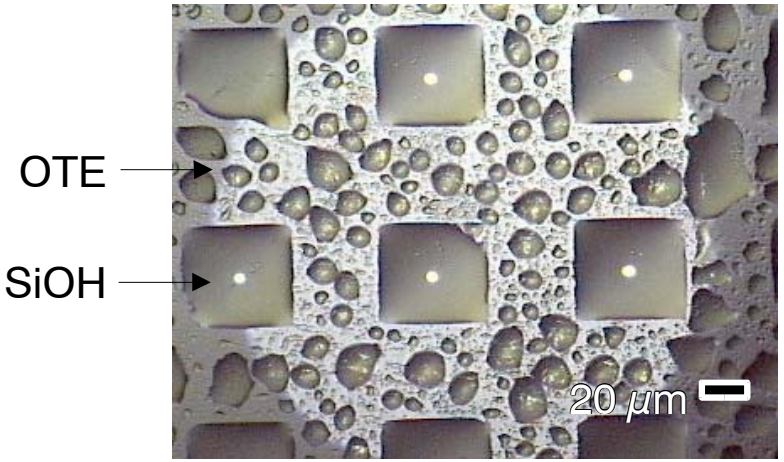
Breath / **condensation pattern**: By cooling a substrate below the condensation temperature H_2O will condense in different rates on the substrate with the nucleation rate of condensation depending on the surface topography and chemical composition / materials properties.

The resulting condensation pattern (with latteral dimensions above the wavelength of light) allows observation of differences in chemical surface composition (often vertically in molecular dimensions) by optical microscopy or even with the unequipped eye.



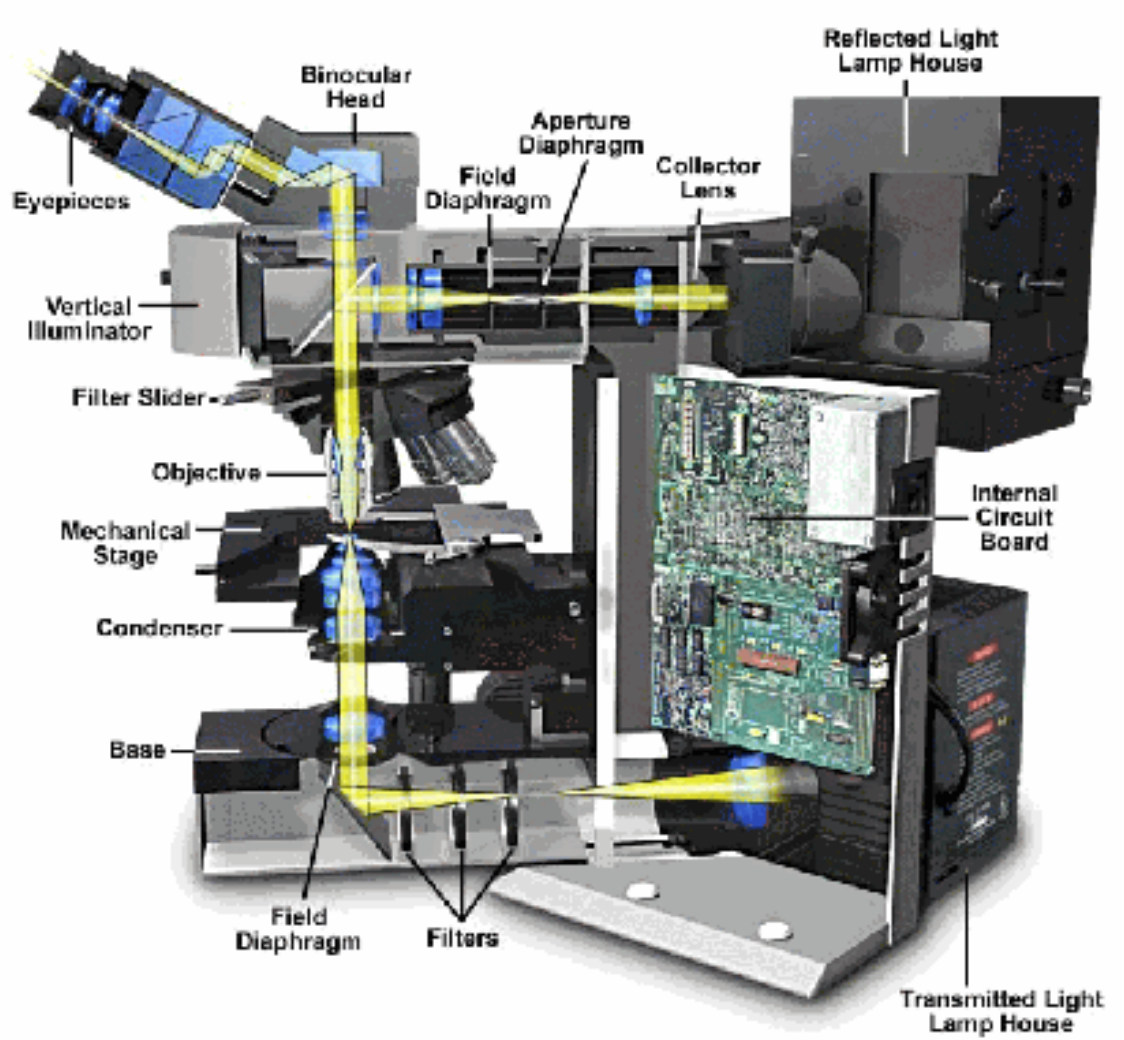


optical microscope image after H_2O condensation (octadecylsilane surface with silica squares)

The contrast can often be increased by temperature cycling above and below the condensation temperature.

Imaging Methods: Optical Microscopy

visual inspection / magnification of an object by the use of light, resolution around half the wavelength of light (practically around 0.5 μm for separated points)



two modes of illumination:

1) transmission: light source is on opposite side of specimen with respect to eyepieces (partially transparent samples required)
2) reflection: illumination from the same side as eyepieces, reflected light is observed (non–transparent samples can be investigated)

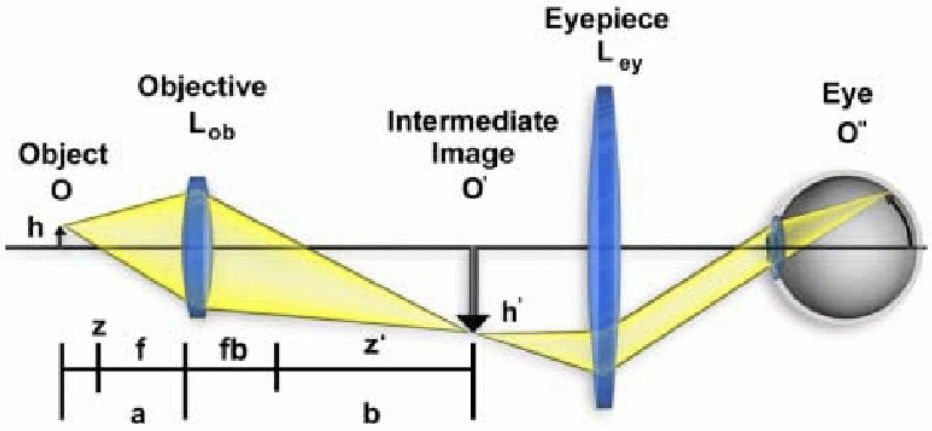
numerical aperture (NA):

NA = r / f → radius of objective r divided by the distance objective–specimen f (~ focal distance of objective)

Michael W. Davidson and Mortimer Abramowitz "Optical Microscopy": http://micro.magnet.fsu.edu/primer/opticalmicroscopy.html

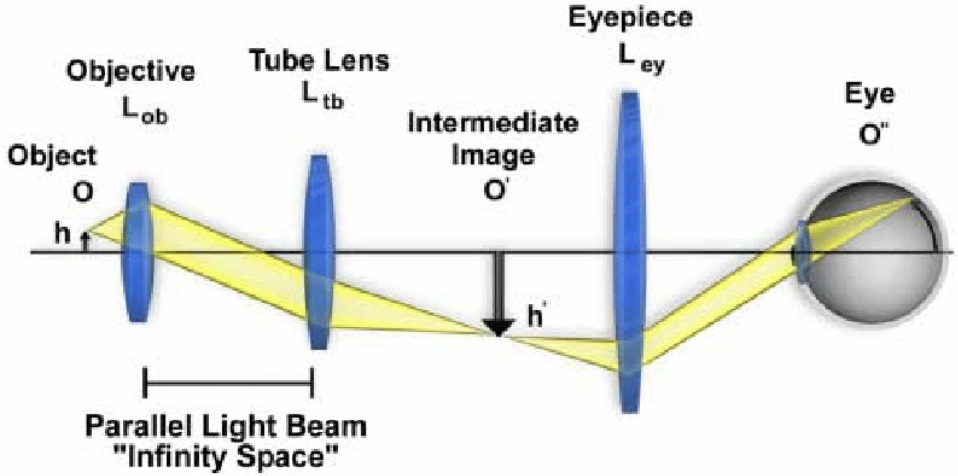
Light Paths in Optical Microscopy

Finite-Tube Length Microscope Ray Paths



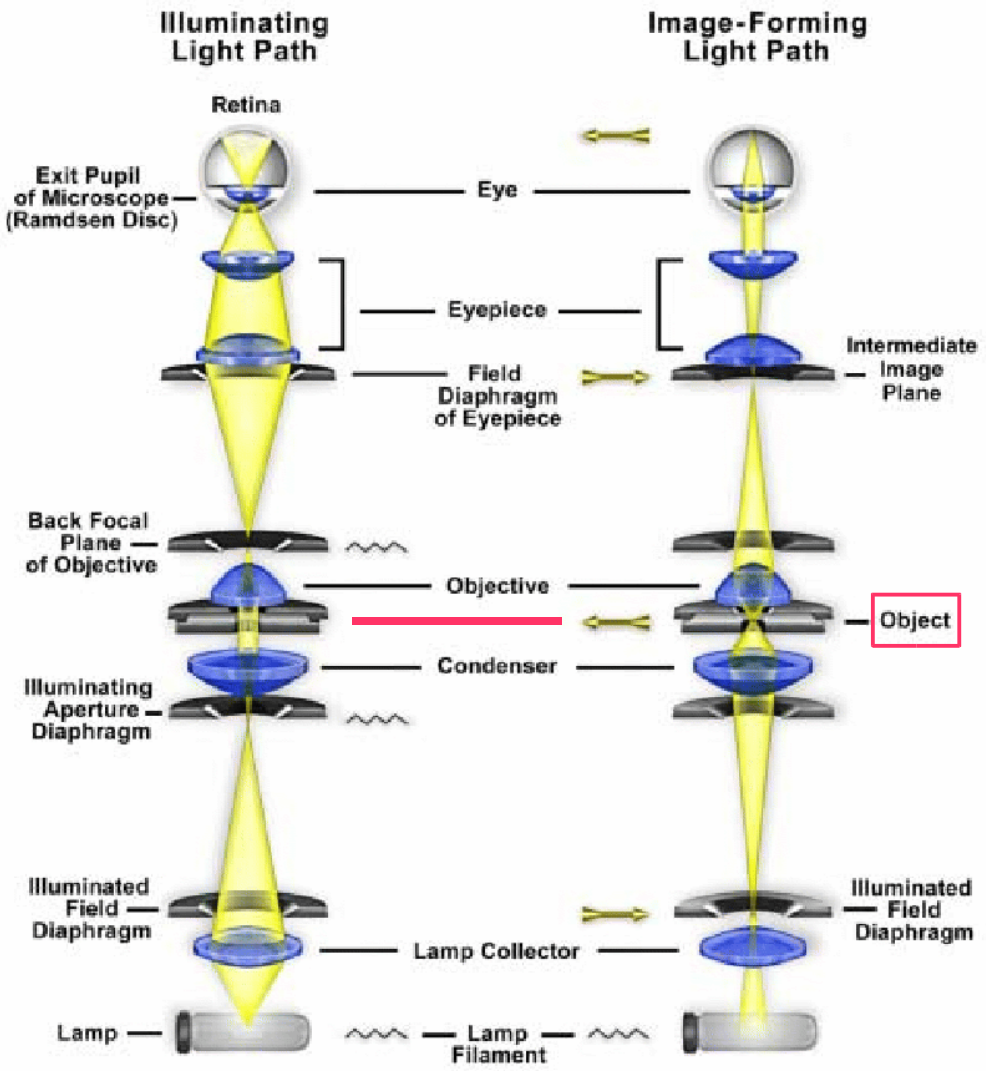
→ traditional microscopy design

Infinity-Corrected Microscope Ray Paths



→ modern design: manipulation of light in "infinite space" region, simpler, less distortion

Light Paths in Köhler Illumination



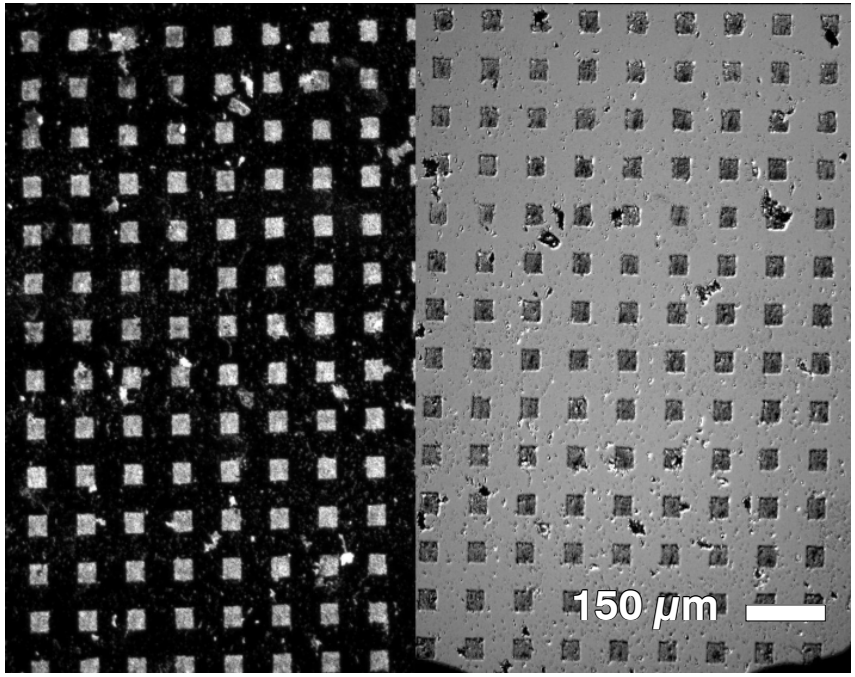
Köhler illumination provides uniform brightness over whole field of view free from glare

Optical Microscopy: Bright– and Darkfield

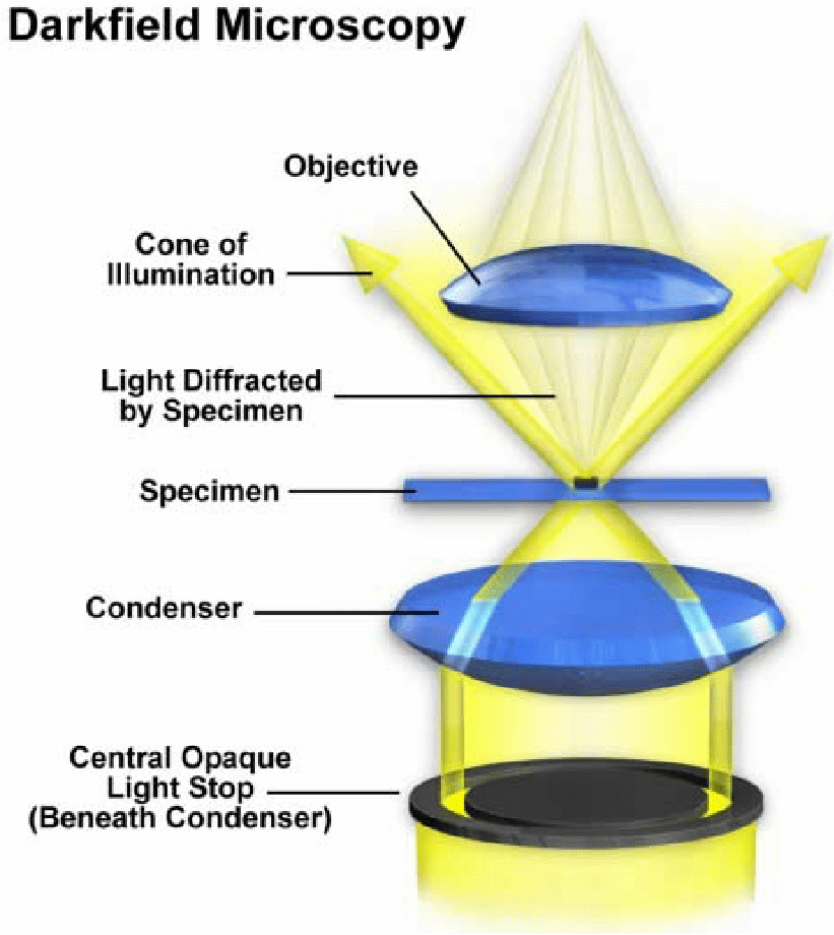
brightfield: conventional illumination with direct observation of light **absorption** / diffraction / reflection variations in specimen; often requires staining of sample

darkfield: striking illumination of specimen under oblique angle and observation of diffracted / reflected / refracted (scattered) light by specimen

thin film of polymer colloid particles (in squares) on OTE / SiOH (squares) pattern

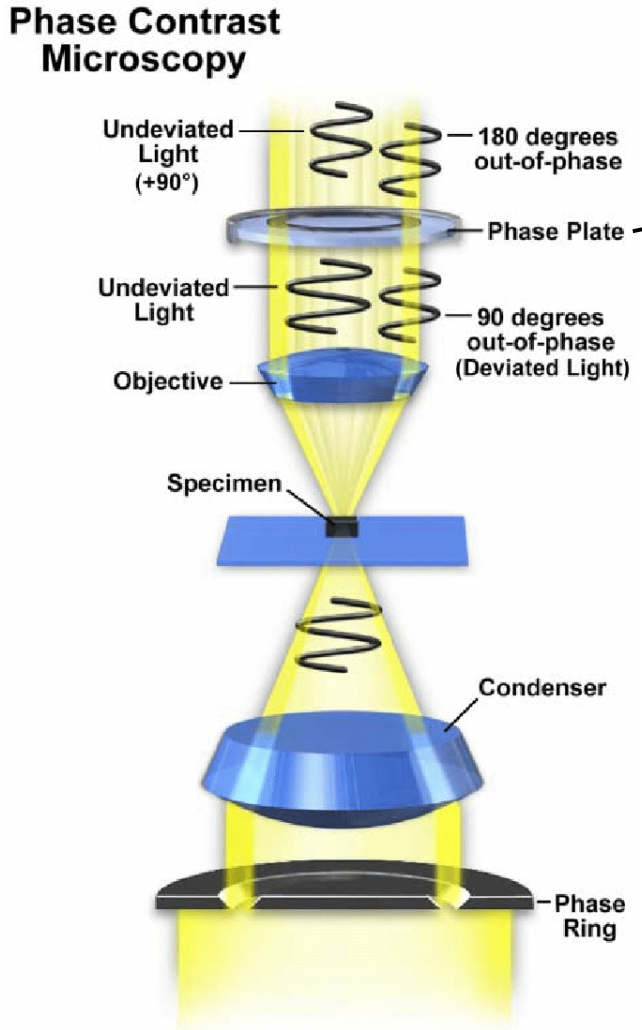


darkfield brightfield (DIC)

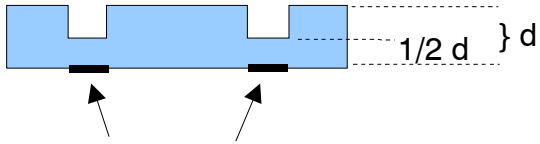


Optical Microscopy: Phase Contrast

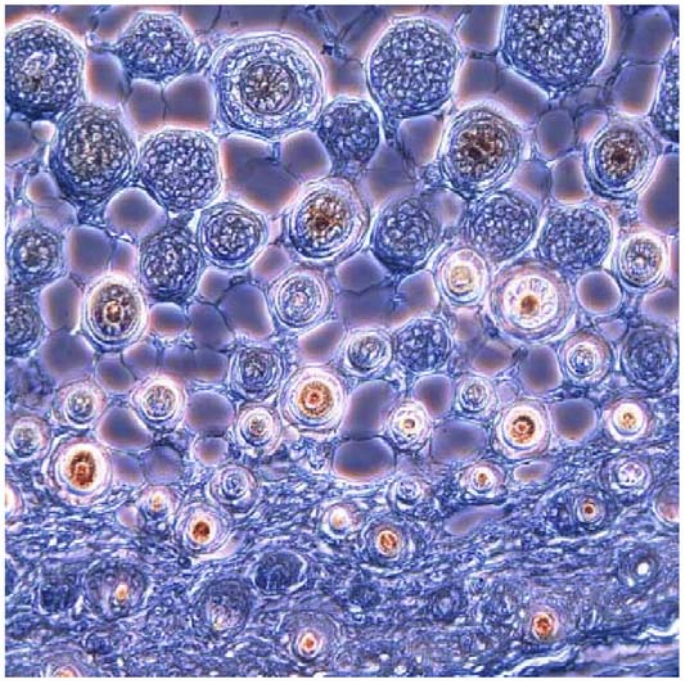
Variations of refractive index (or thickness) in the specimen cause different light velocities and phase differences compared to undeviated (zeroth order) light around the sample. Image contrast is obtained by interference of deviated and zeroth order light (phase lag 1/2 λ) at the eyepiece.



cross section through
phase plate (transparent !)

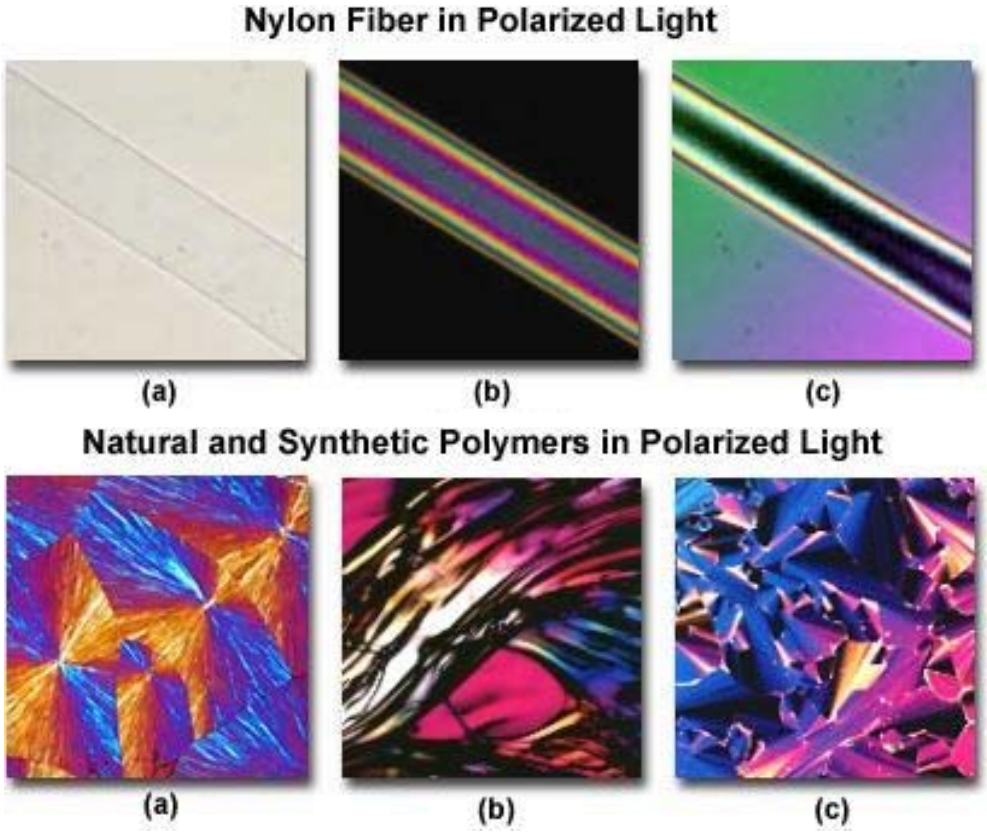


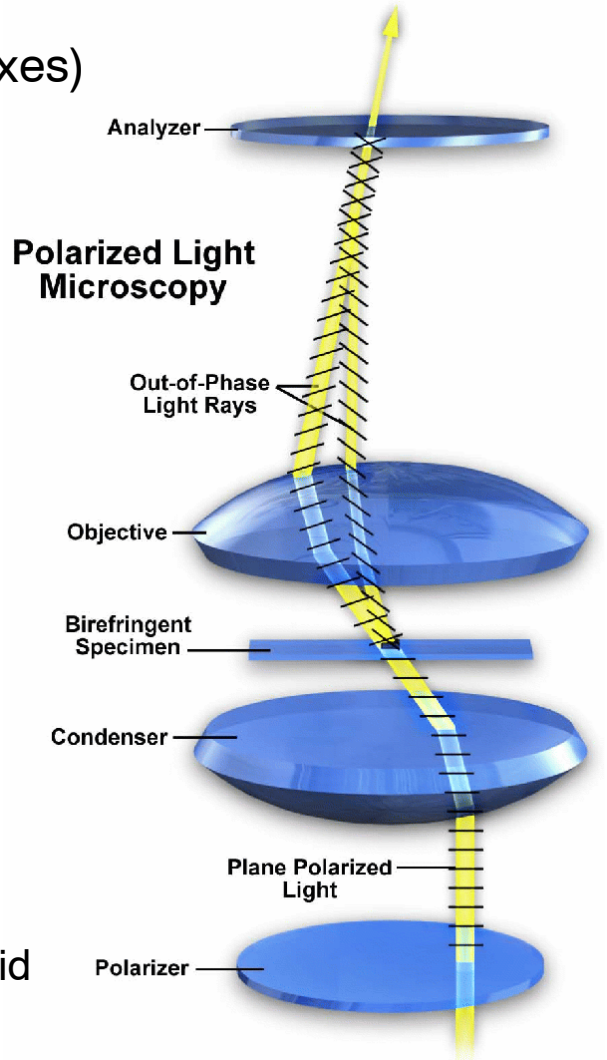
photomicrograph of hair cross section from a fetal mouse taken using phase contrast optics and a 20x objective



Optical Microscopy: Polarized Light

Birefringent or optically anisotropic samples (e.g. minerals, liquid crystals, oriented polymers) can be observed between two crossed plane polarizers. Variation in intensity and color occur due to different light velocities for differently oriented polarization vectors in the specimen plane, leading to the rotation of the polarization axes.
(birefringence → correlates to orientation of anisotropic crystal axes)



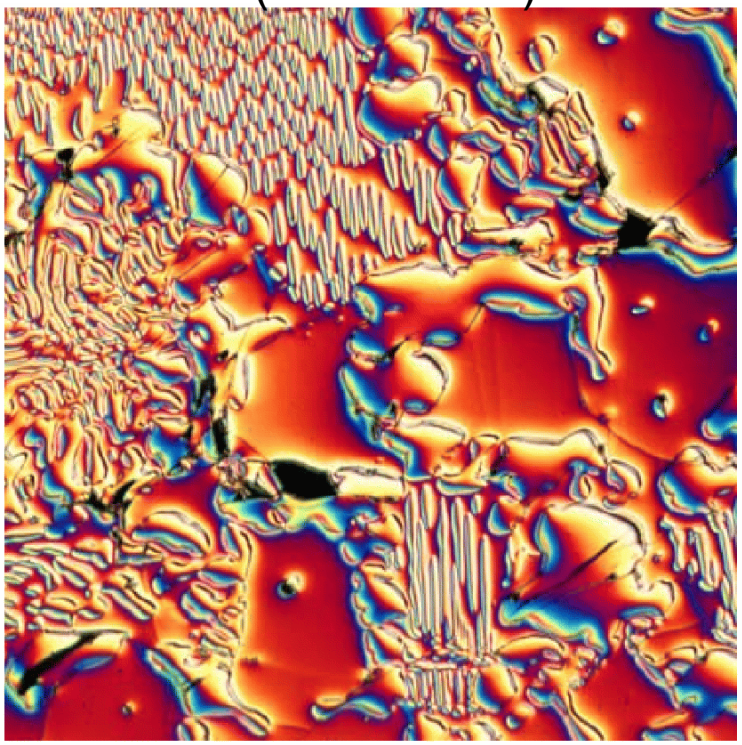


a) spherulite crystallization, b) polycarbonate without crystallization, c) liquid crystalline phase of DNA

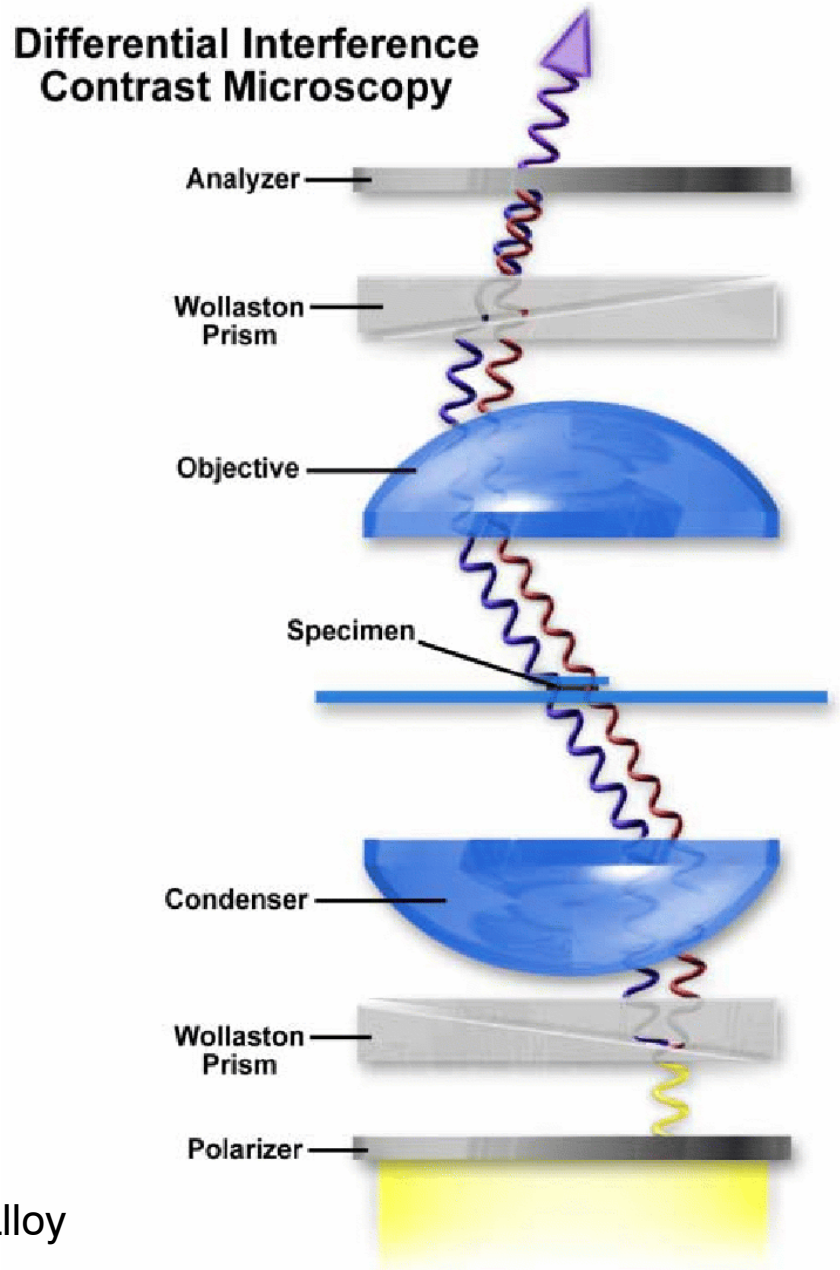
Optical Microscopy: Differential Interference Contrast (DIC)

contrast enhancement of non–absorbing specimen due to variations in thickness / slope / refractive index

→ light is split into two perpendicular polarizations with minute horizontal separation (below resolution limit) and recombined (interference) after sample



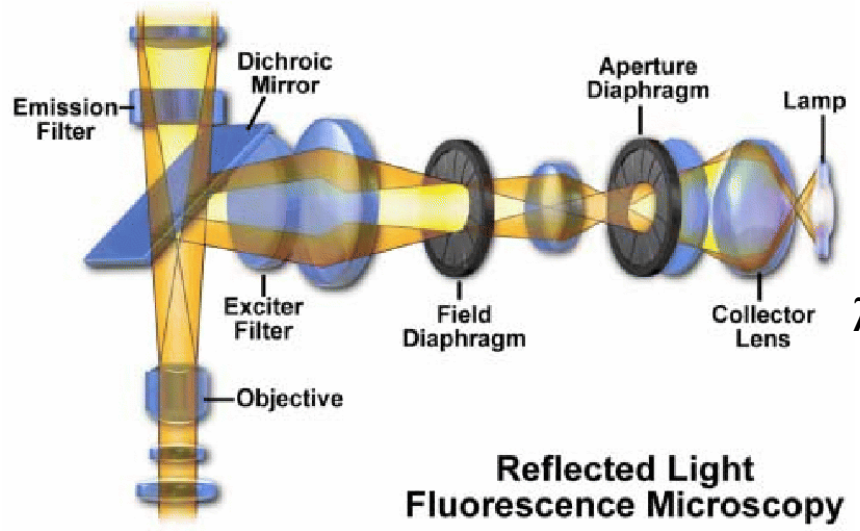
reflected light DIC of defects on the surface of a ferro–silicon alloy

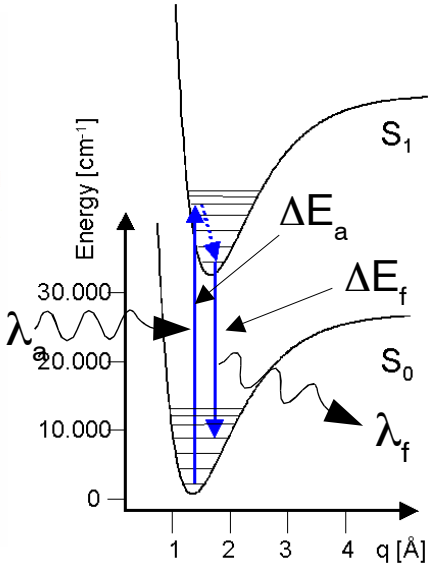


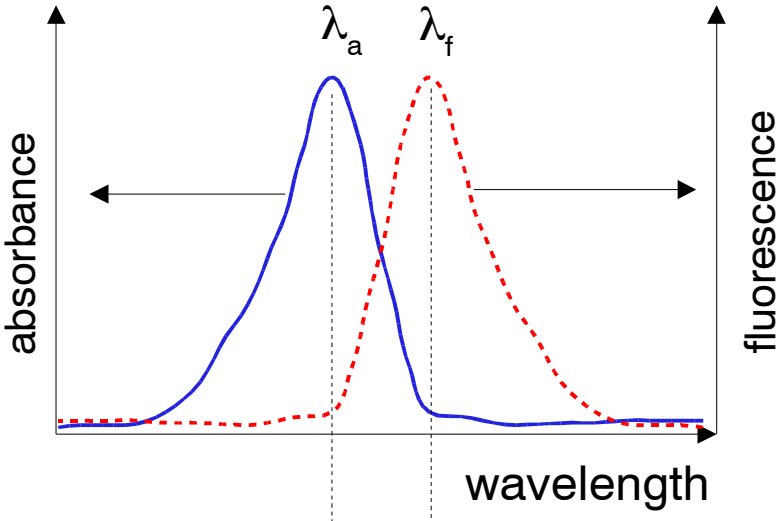
Optical Microscopy: Fluorescence

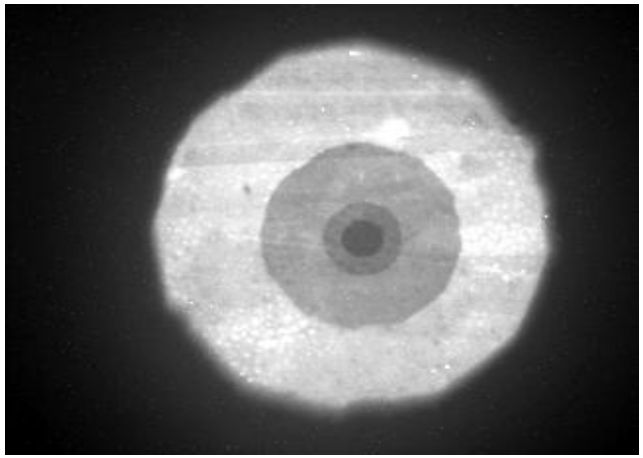
chemical surface functions (like $-NH_2$) can be specifically decorated with fluorescent labels

→ beaching with high intensitiy light provides contrast to unbleached regions at low fluorescence intensity









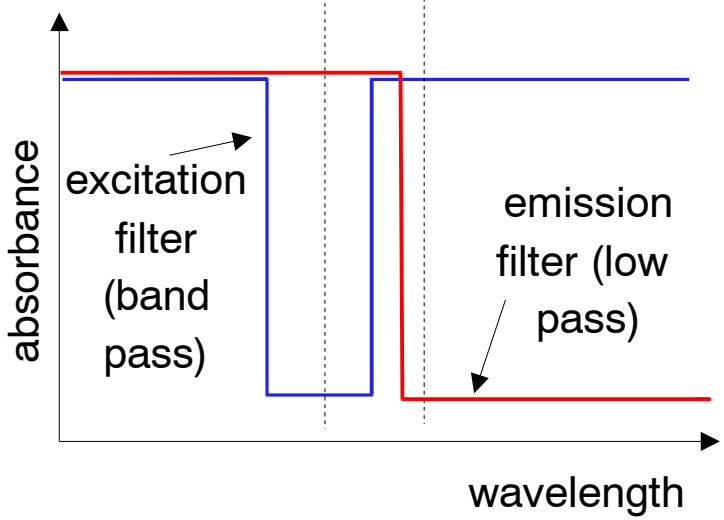
labeled and bleached amino–silane monolayer

$\Delta E_a > \Delta E_f$

$h\nu_a > h\nu_f$

$\nu = c / \lambda$

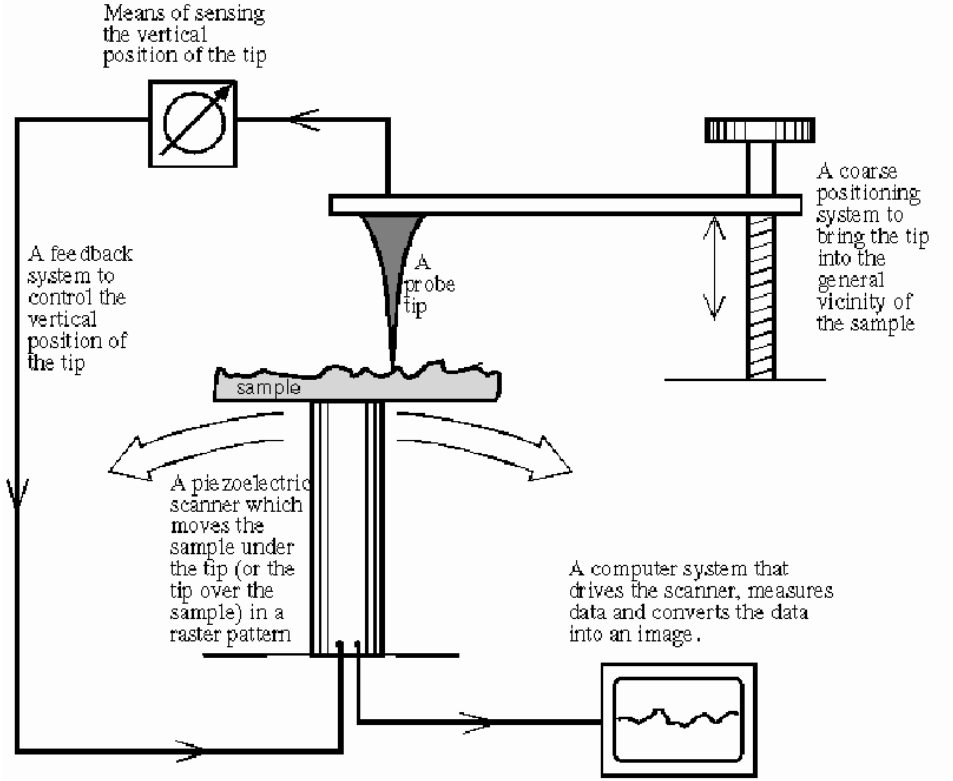
$\rightarrow \lambda_a < \lambda_f$



Imaging Methods: Scanning Probe Microscopy (SXM)

[...The scanning probe microscope is an imaging tool with a vast dynamic range, spanning the realms of optical and electron microscopes. It's also a profiler with unprecedented 3–D resolution. In some cases, scanning probe microscopes can measure physical properties such as surface conductivity, static charge distribution, localized friction, magnetic fields, and elastic moduli. As a result, applications of SPMs are very diverse. ...]

Rebecca Howland, Lisa Benatar, Jezz Leckenby "A Practical Guide to Scanning Probe Microscopy":
http://www.topometrix.com/spmguide/contents.htm



fundamental principle:

A probe **tip** is brought into close **proximity** / **contact** with a **specimen** which is **scanned** in the x–y–plane. The **interaction** of the probe tip with the surface is **recorded** with respect to the **x–y–position** of the sample and converted into a **3 D map** of the measured surface property (e.g. topography, conductivity, friction, mechanical module).