Contrast-enhancing techniques in light microscopy

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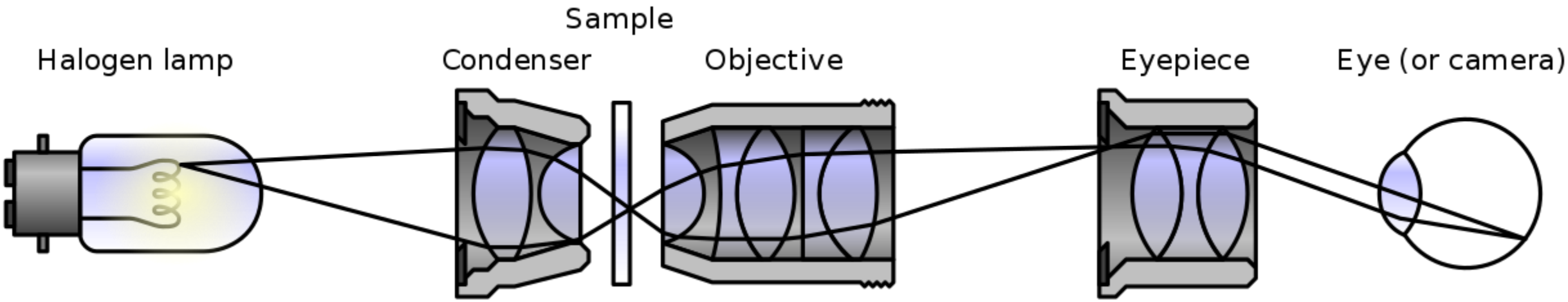
Light Microscopy

IMG CAS

Köhler illumination

Critical illumination in transmission light microscopy



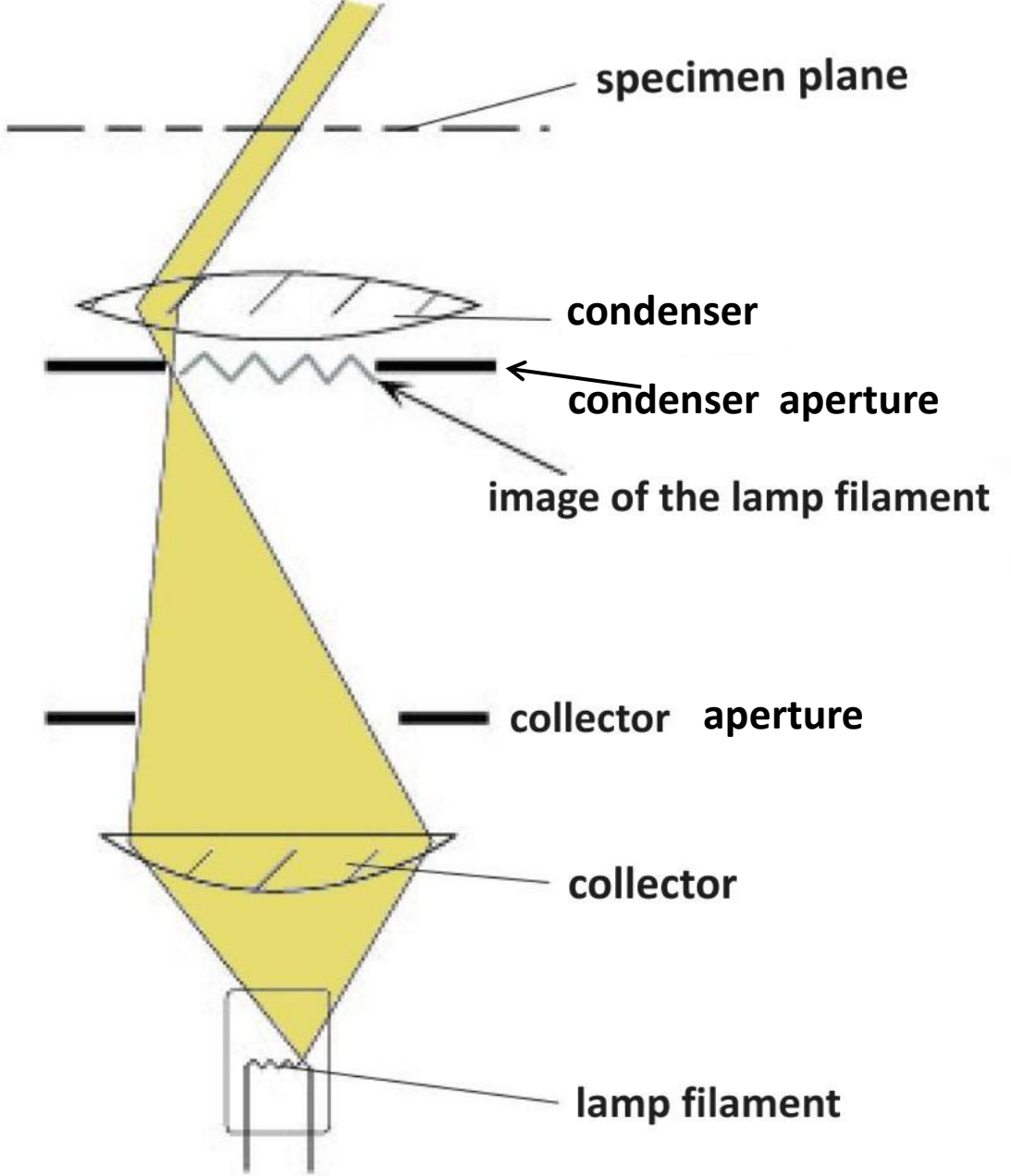
- Inhomogeneous illumination
- Lamp filament visible in the picture of specimen

https://en.wikipedia.org/wiki/Critical_illumination

Köhler Illumination

- **Even illumination** of the sample.
- **Image of the illumination source (e.g., a halogen lamp filament) is not visible in the resulting image**.
- It requires additional optical elements.
- **Predominant technique for sample illumination in modern scientific light microscopy**.

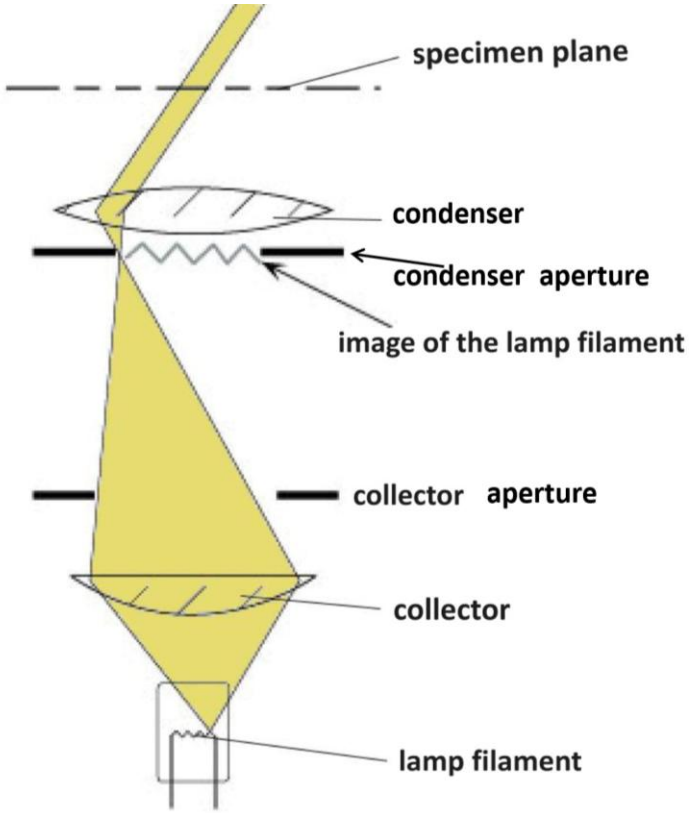
- **Reduces image artifacts** and provides **high sample contrast**.
- Even illumination of the sample is also important for advanced illumination techniques such as **Phase Contrast,** or **DarkField** microscopy.

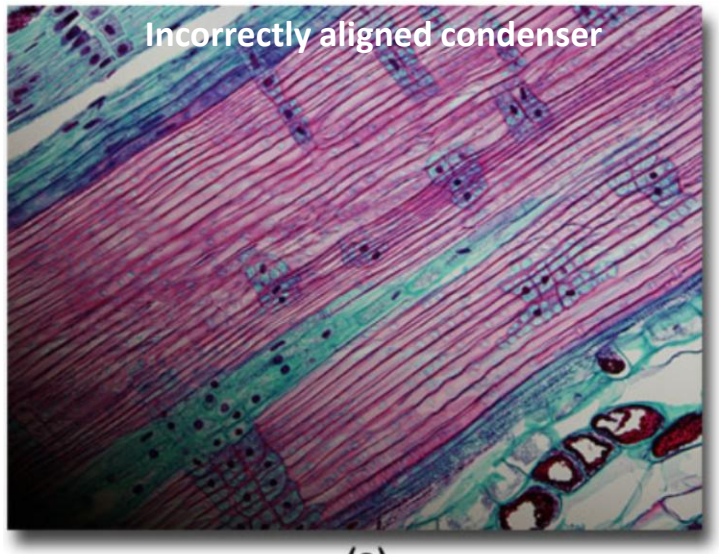


https://en.wikipedia.org/wiki/Kohler_illumination

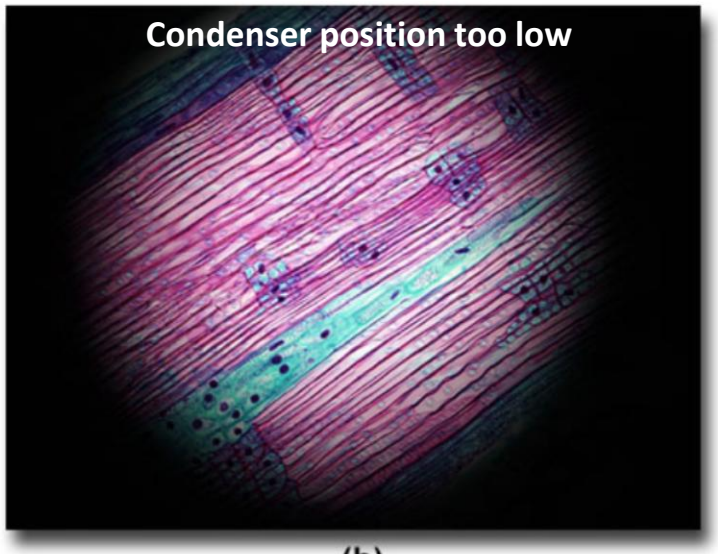
Pokorný P.: LIGHT MICROSCOPY AND ITS APPLICATIONS, DP, 2010.

Artifacts in Köhler illumination

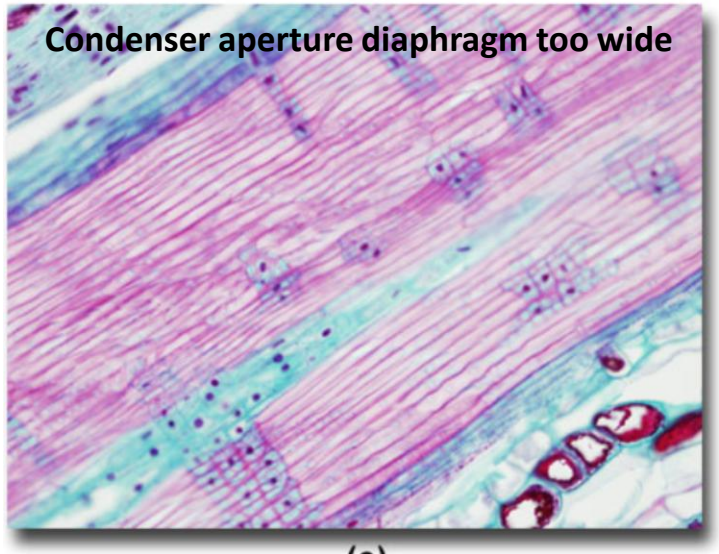




(a)



(b)



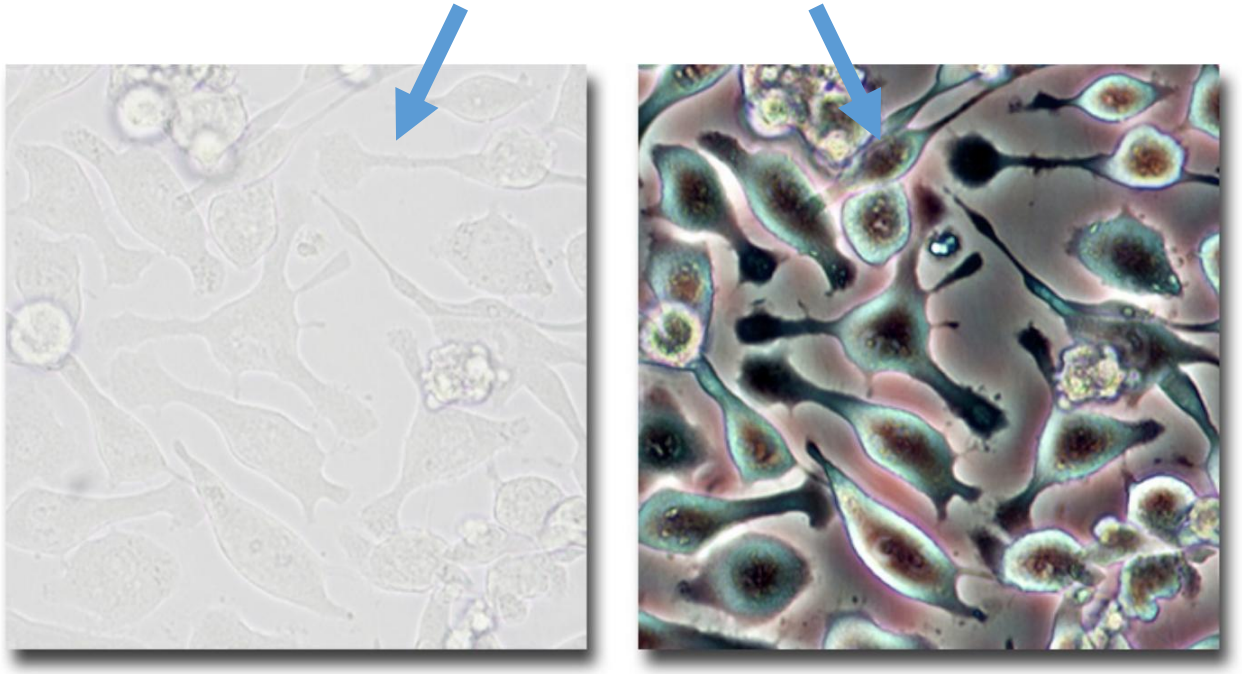
(c)

(d)

Phase Contrast

Brightfield vs. Phase Contrast Images

Living cells imaged in both brightfield and phase contrast illumination.



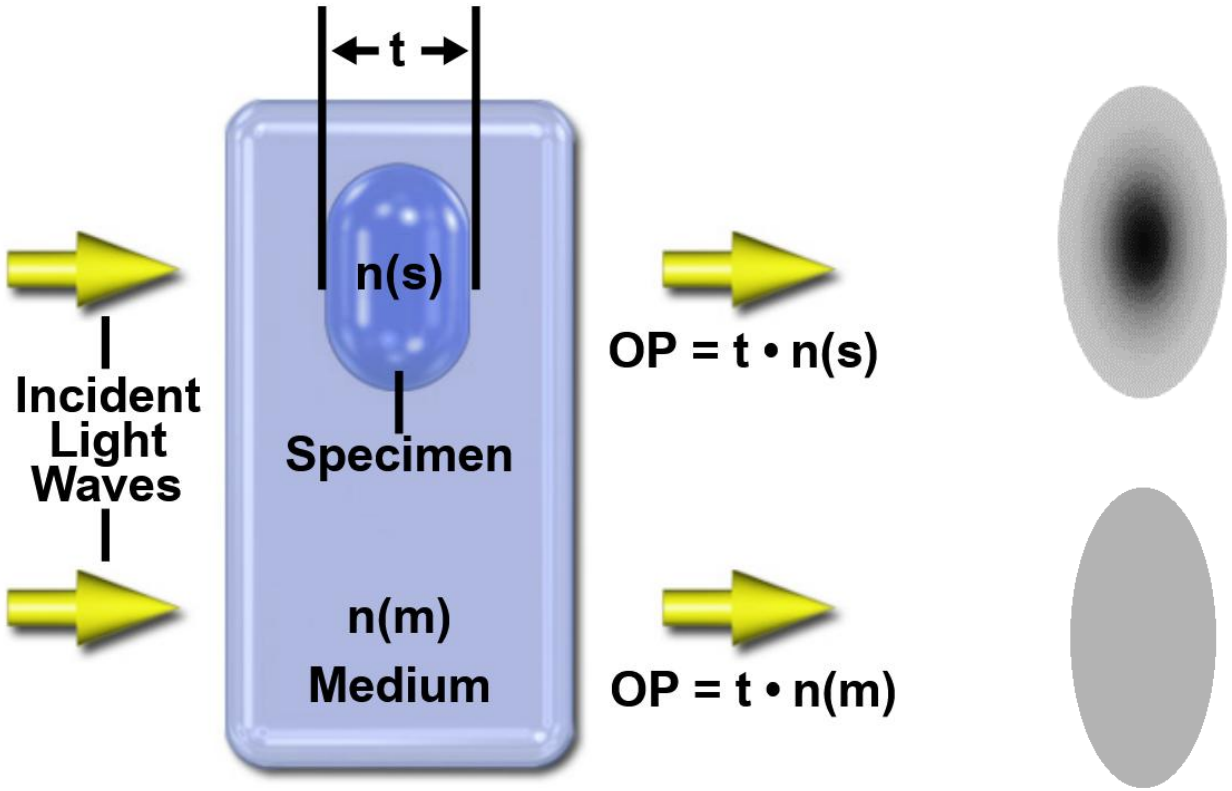
- **Problem**: Many living unstained samples are thin and optically transparent
  - Hard to see by Brightfield.
- **Solution**: Transmitted light-based techniques for improving contrast.
  - E.g., Phase Contrast, Darkfield, QPI.

https://www.**microscopyu.com**/techniques/phase-contrast/introduction-to-phase-contrast-microscopy

Physical Phenomena Causing Image Contrast

- Why do we see microscopic objects (in light microscopy)?
  - **light absorption**
  - **fluorescence**
  - **phase shifts of light waves**
- Our eyes are good at seeing differences in **amplitude (intensity)** and **wavelength (color)**, but ***not in phase***.

What Phase microscopy does…



… converts differences in the optical path to differences in the amplitude.

http://nic.ucsf.edu/dokuwiki/lib/exe/fetch.php?media=april_12_lecture-_oweiner.pdf

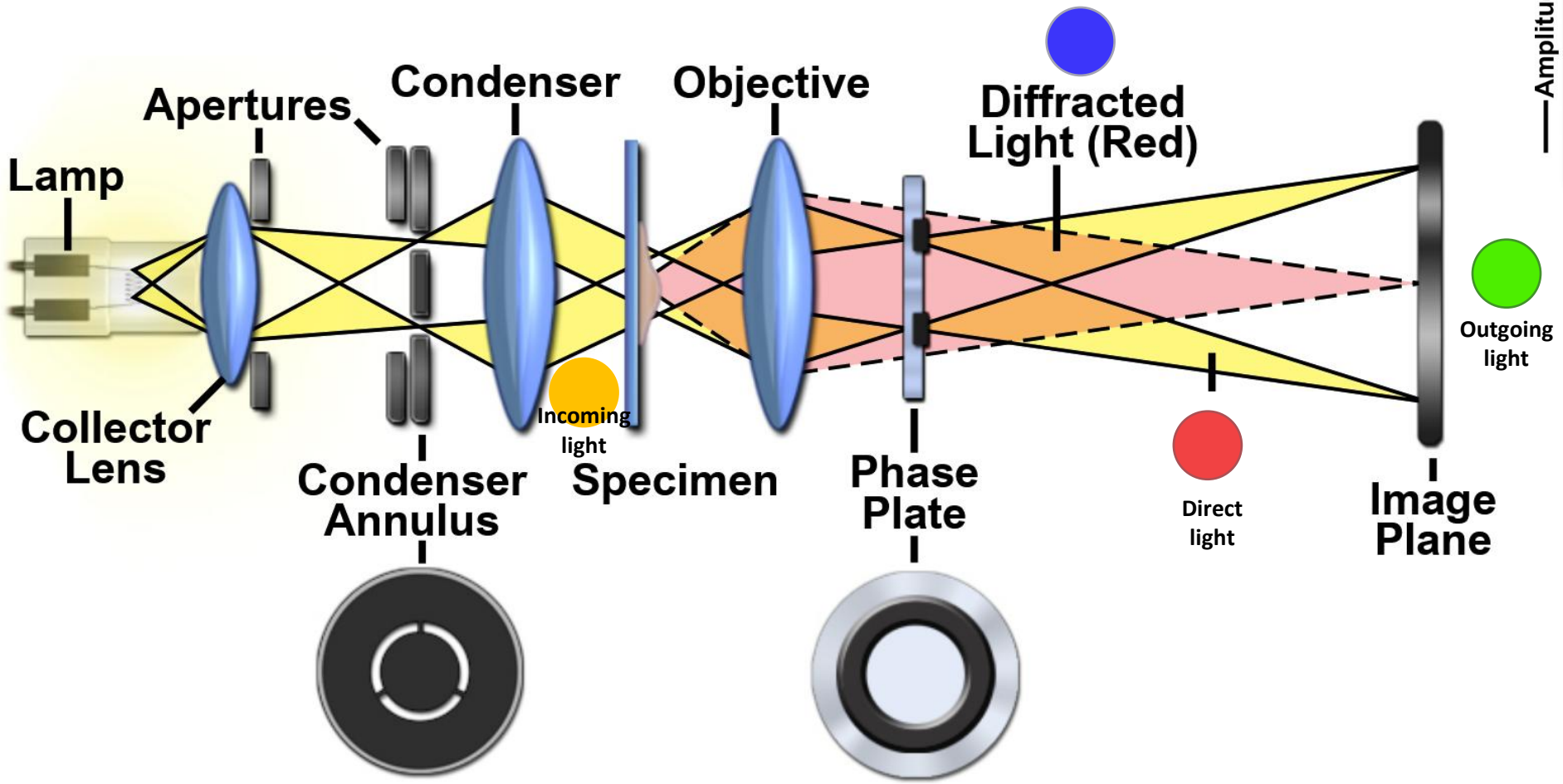
Solution – Phase Contrast microscopy

The Nobel Prize in Physics 1953

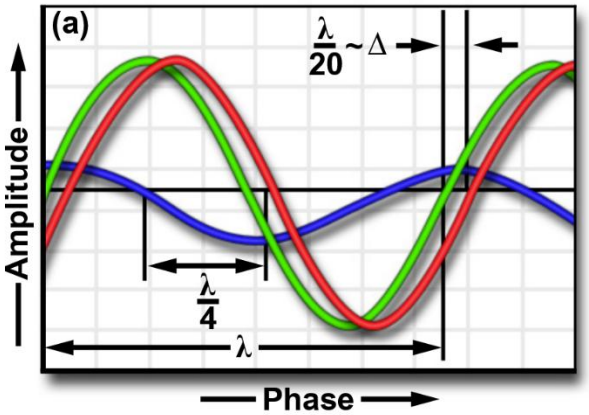
Frits Zernike, the Netherlands

„For his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope."

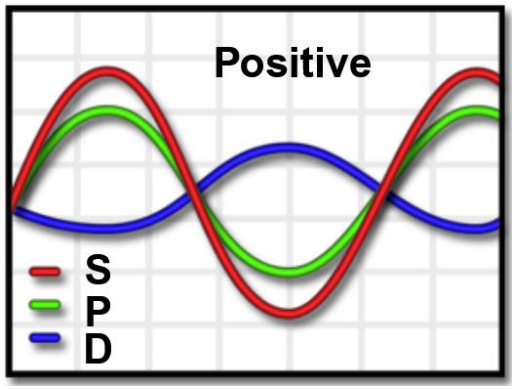
Phase Contrast + Köhler illumination



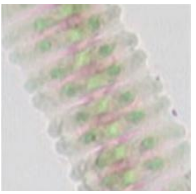
Brightfield



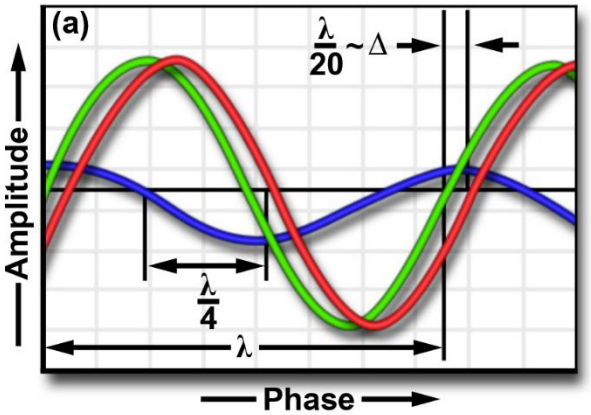
Phase Contrast

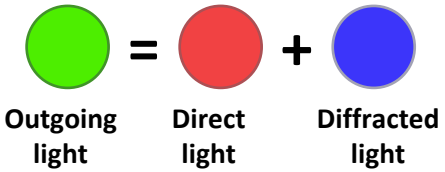


Positive and negative contrast phase plates

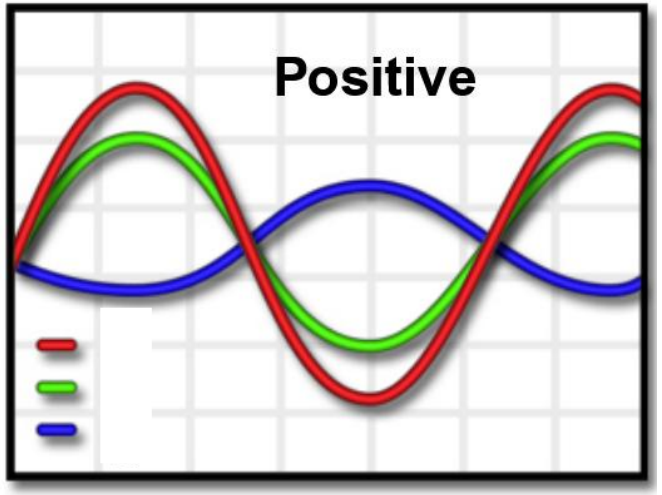


Brightfield: *Zygnema* filamentous algae

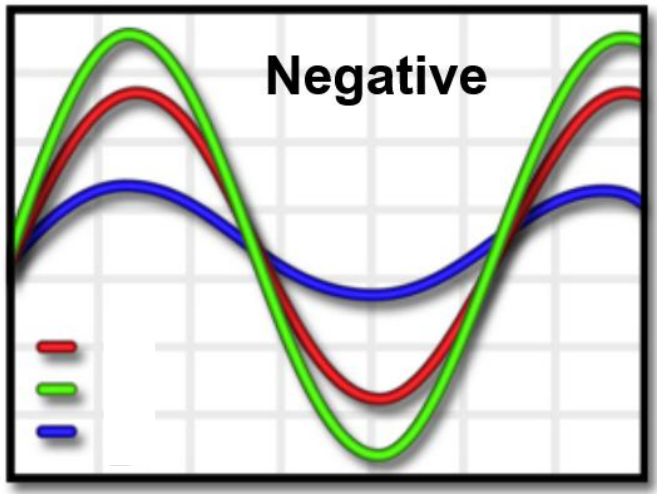




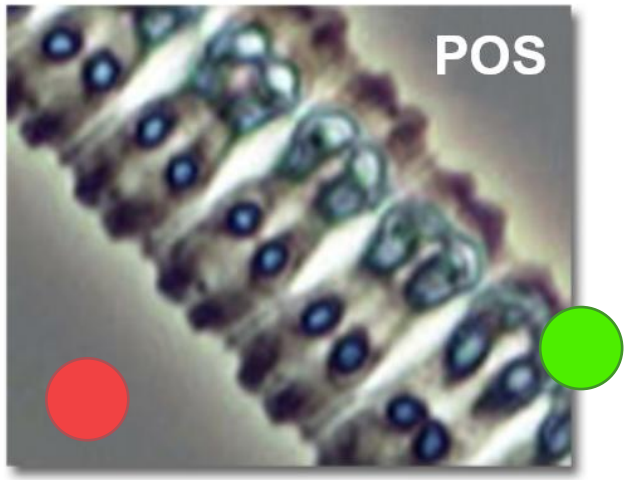
Phase Plates



Destructive interference

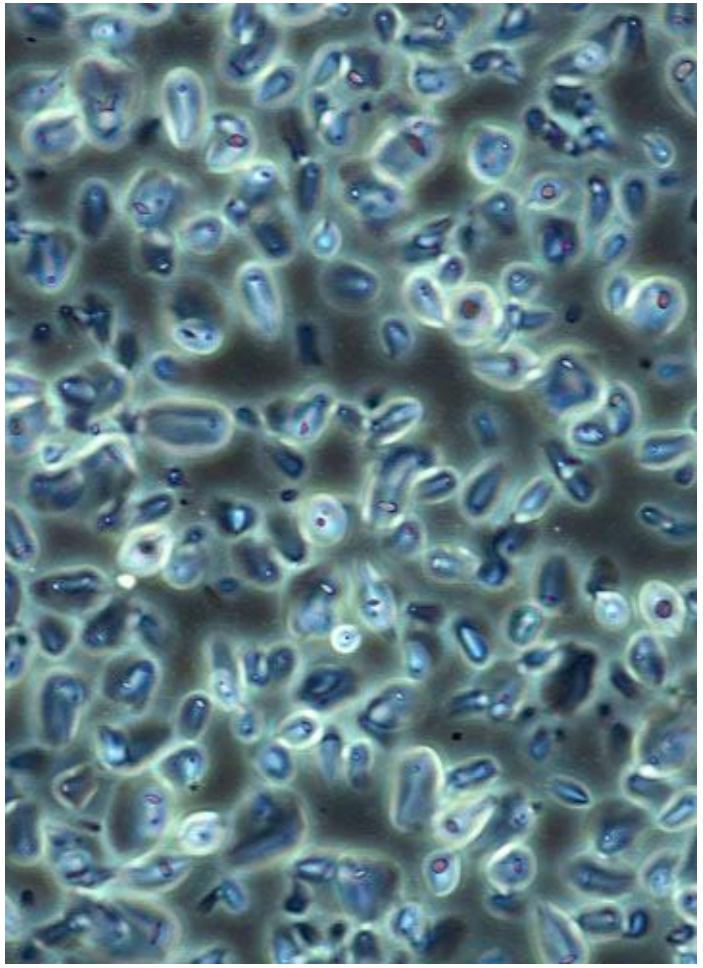


Constructive interference

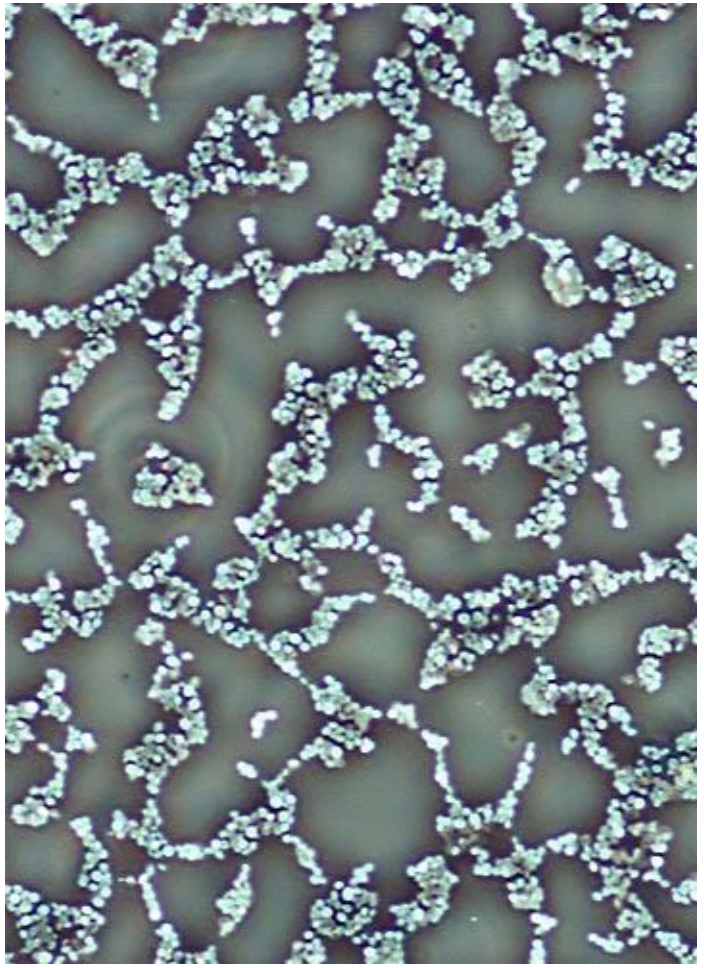


Phase Contrast images

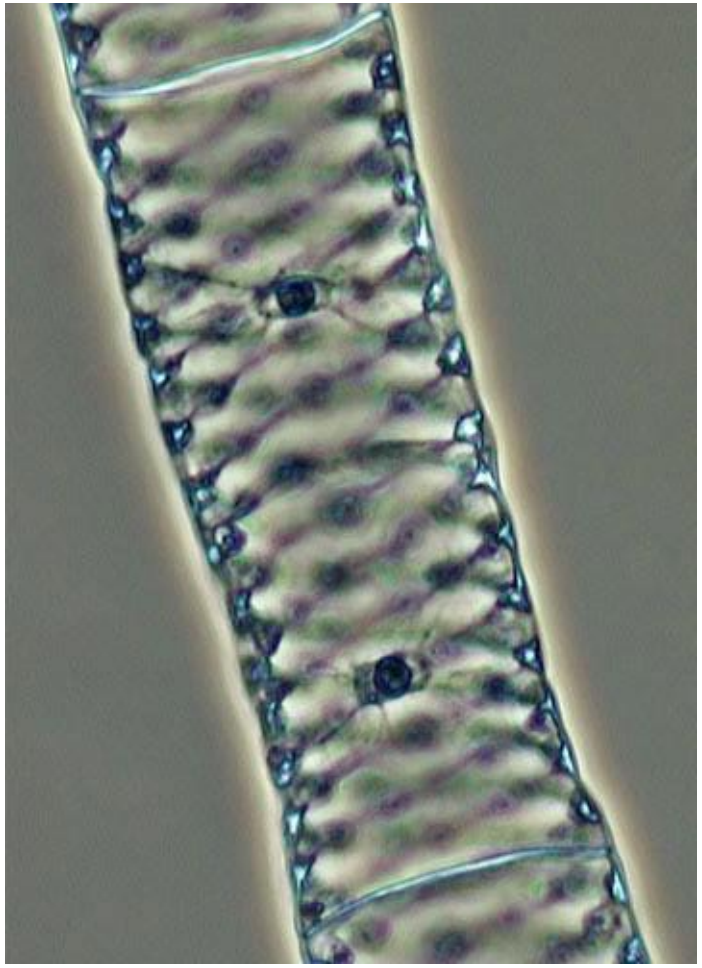
Halos in Phase Contrast



Arrowroot Starch Granules (positive)

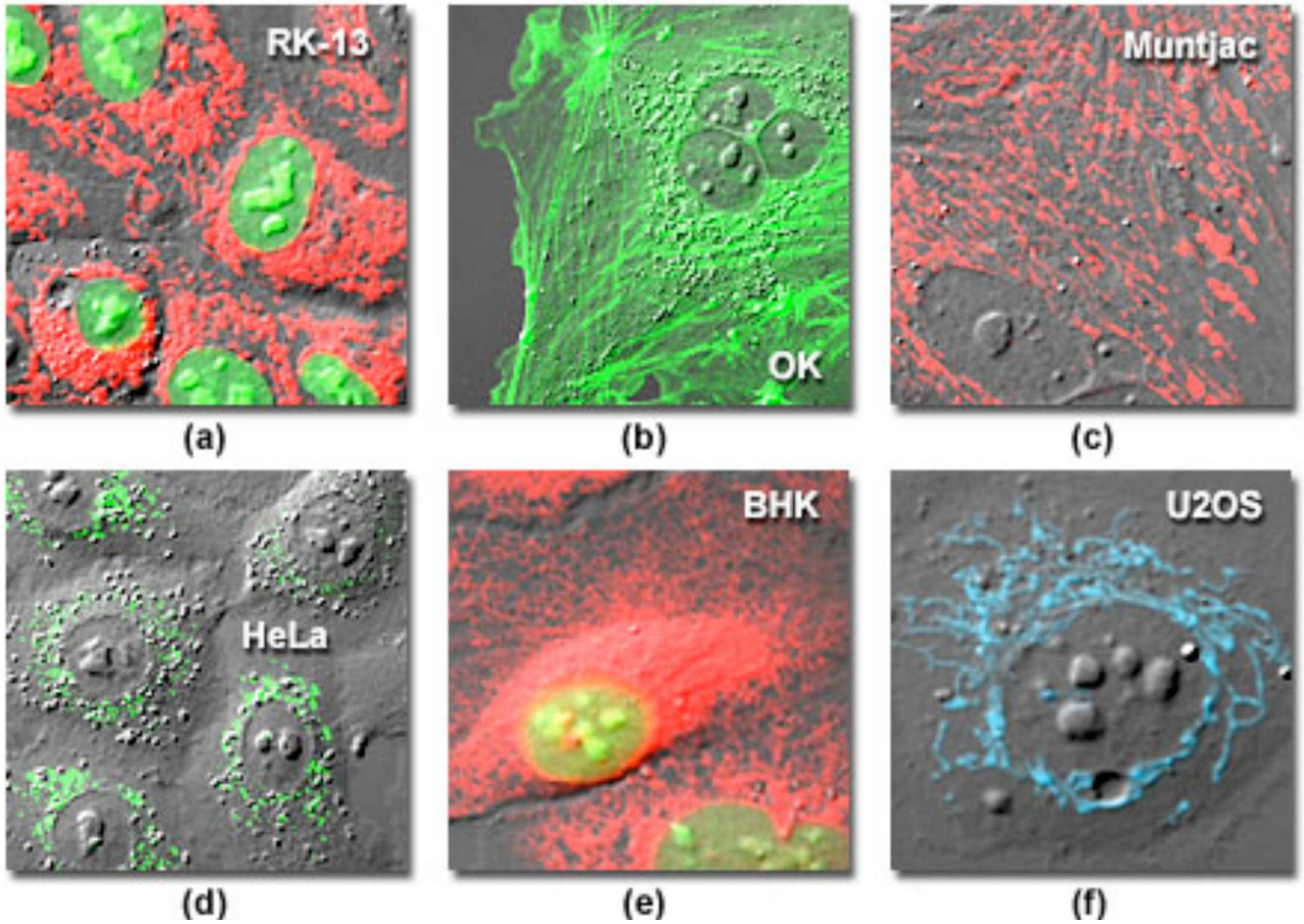


Yeast Cells (negative)



Spirogyra Filamentous Algae (positive)

Phase Contrast often used in conjunction with fluorescence microscopy



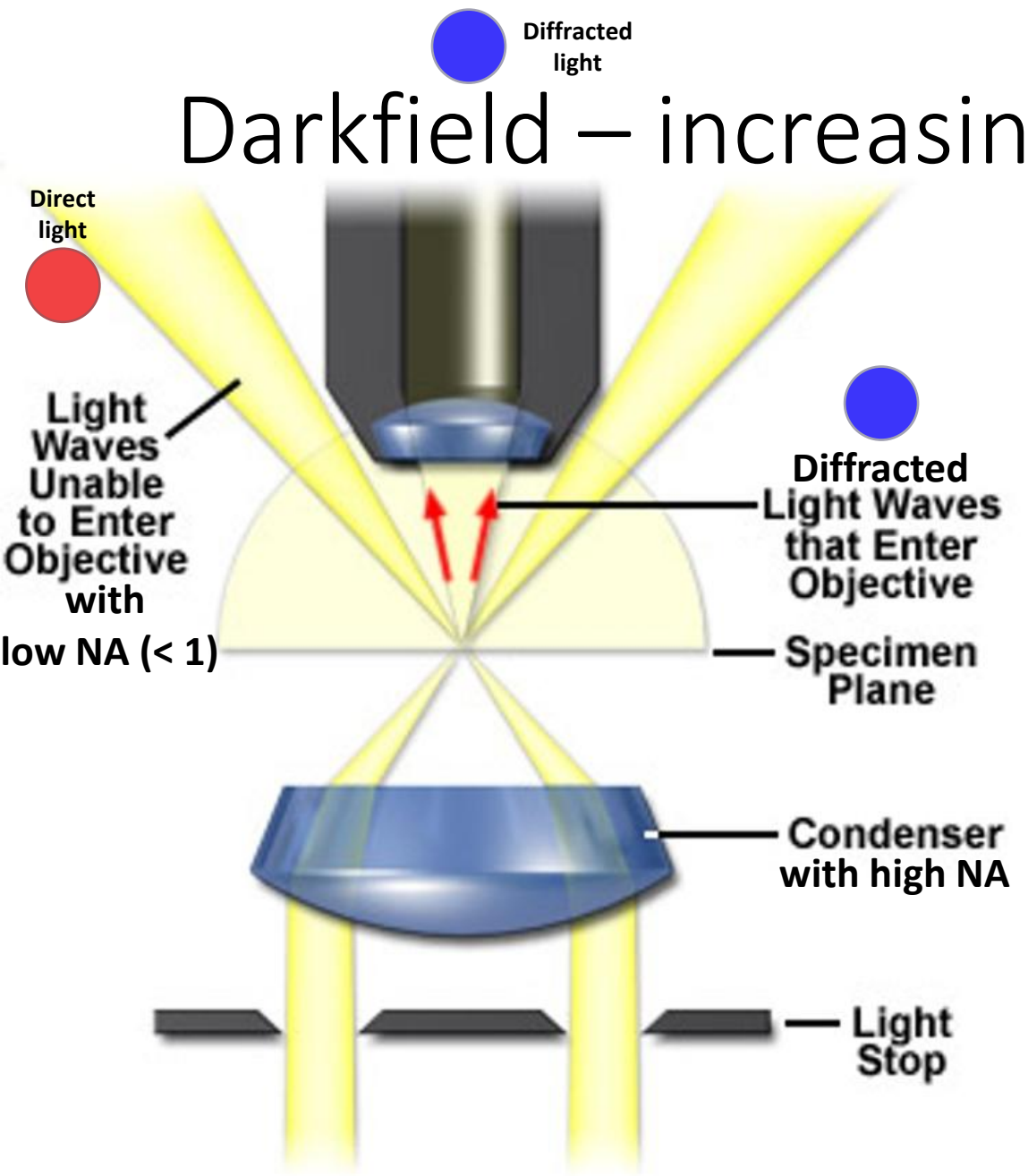
Fluorescent Proteins + PC

- To provide cellular or organismic reference.
- **Phase Contrast (PC) is much more general and less toxic detection tools than fluorescence.**

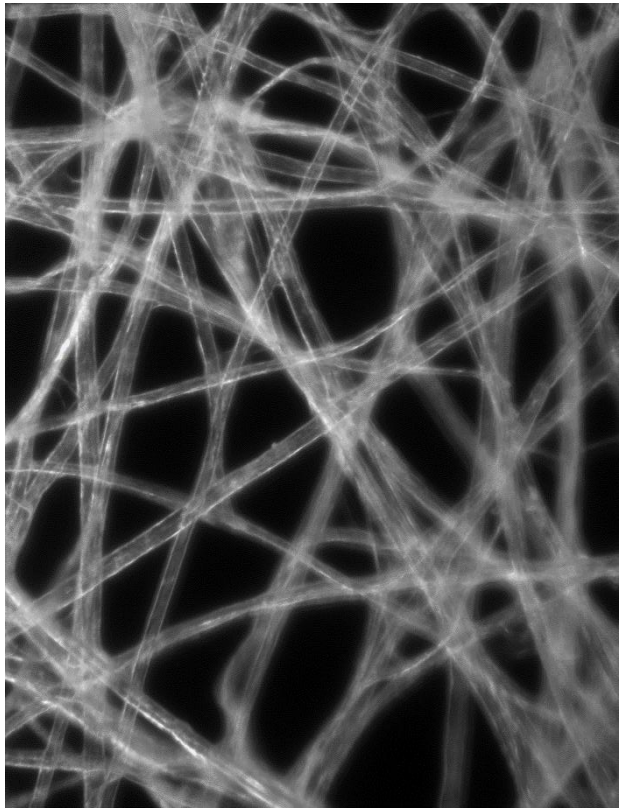
https://micro.magnet.fsu.edu/primer/techniques/

Darkfield

Darkfield – increasing contrast even more

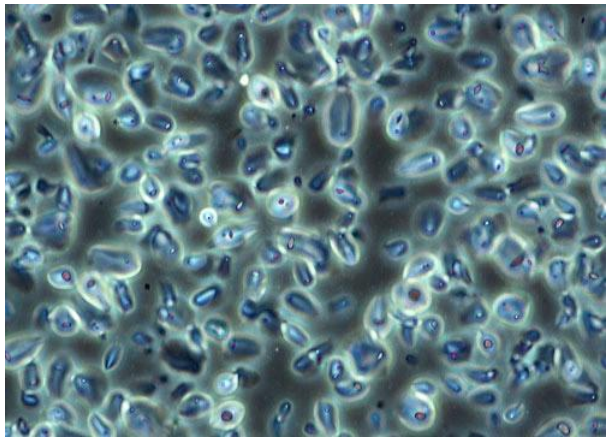


http://zeiss-campus.magnet.fsu.edu/articles/basics/contrast.html

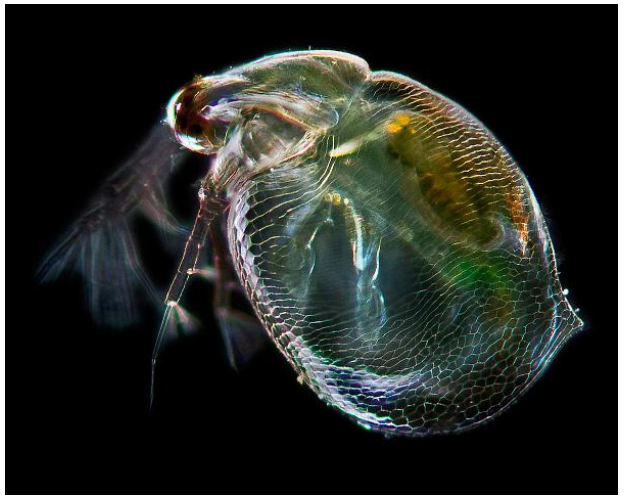


Summary

- **Phase Contrast** – converts optical path into contrast



- **Darkfield** – images only diffracted light



https://www.behance.net/gallery/2498099/Beyond-the-naked-eye