Phase contrast imaging

Midterm

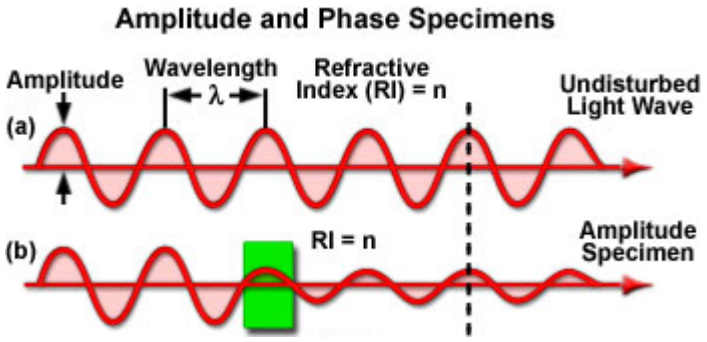
- 1 week from today
- In this class
- No Matlab
- You can create 1 side of 8.5x11” paper with anything you want
- Office hours on M 1 – 2, Th 10-11

- Last class
  - Light sources
- This class
  - Traditional tissue imaging
  - Phase contrast imaging

Light-matter interactions

- Refraction – changes speed, induces path length differences
- Absorption – reduces intensity
- Diffraction – Spreads out waves (spherical pattern)
- Scattering – Spreads out waves (randomly)

Detectors only see intensity

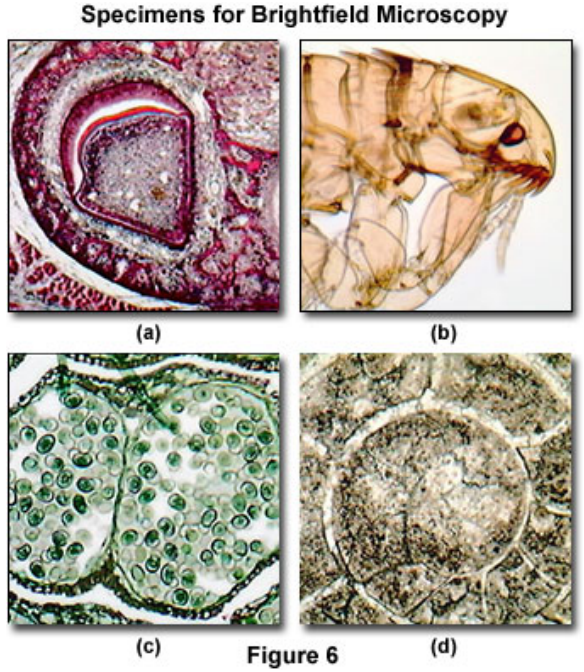


$$C = \left[\frac{Ib - Is}{Ib}\right] * 100\%$$

The problem is I_s is very low in cells.

We can see contrast in cells by **absorption** and **scattering**
Both properties reduce the amplitude of light

Biological samples have very small amplitude deviations, so there is very little contrast



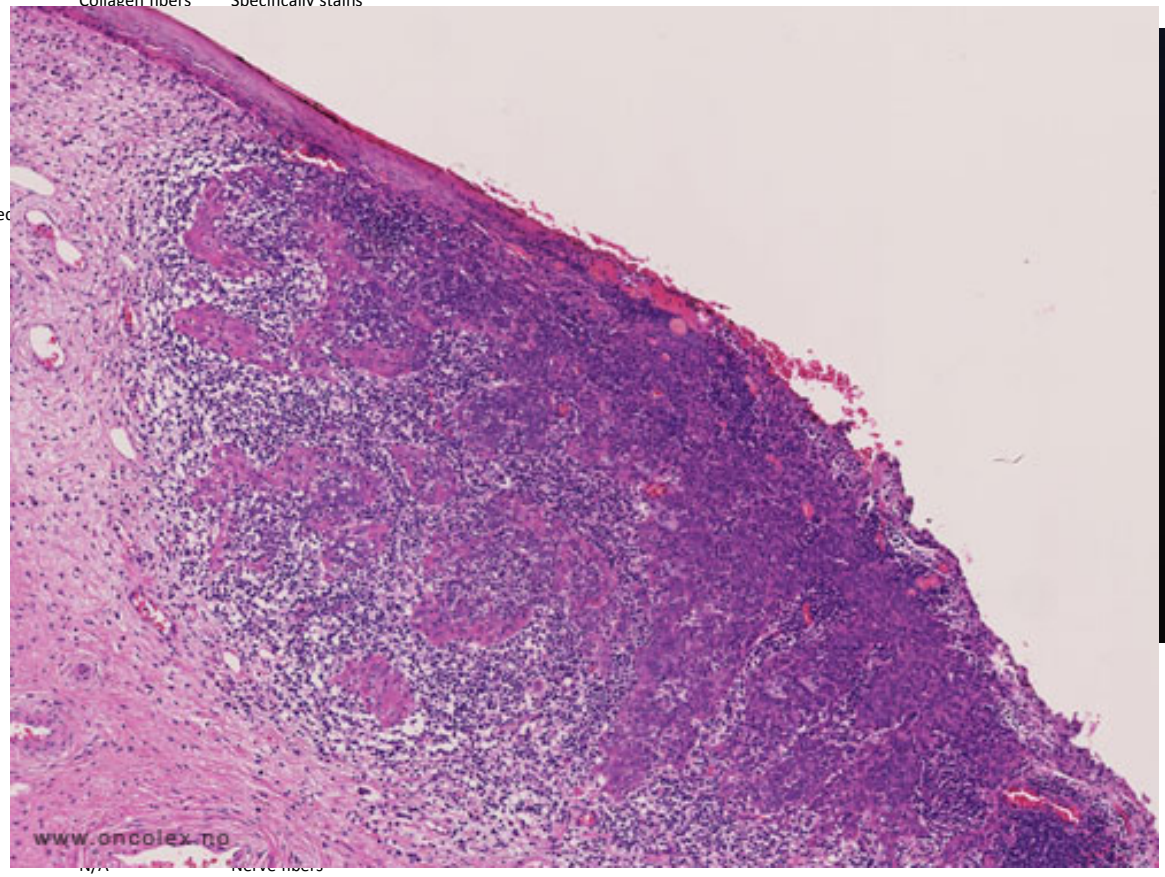
Histological staining

Increase the amplitude by applying something that absorbs visible light

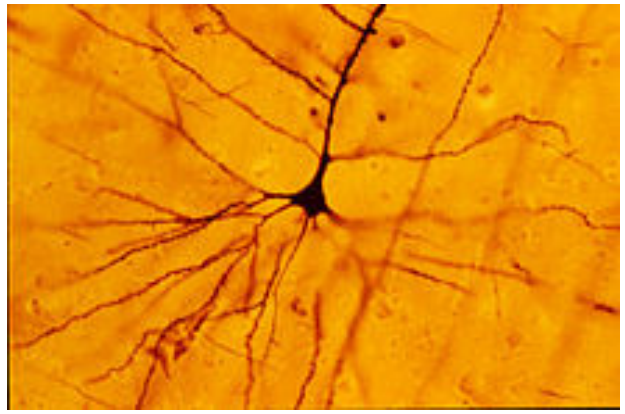
Useful only if chromophore is selectively bound by the sample

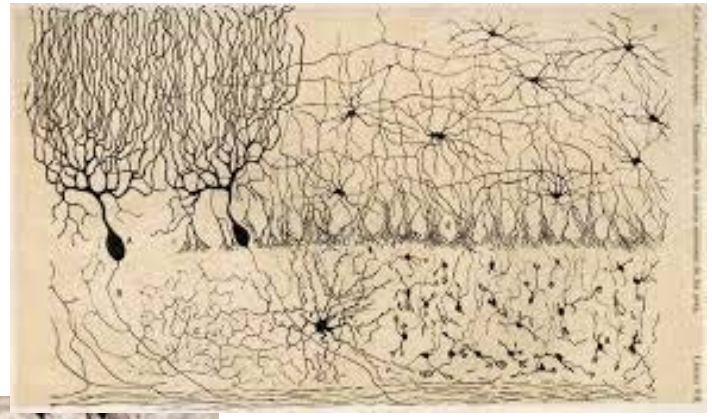
Only works on dead samples

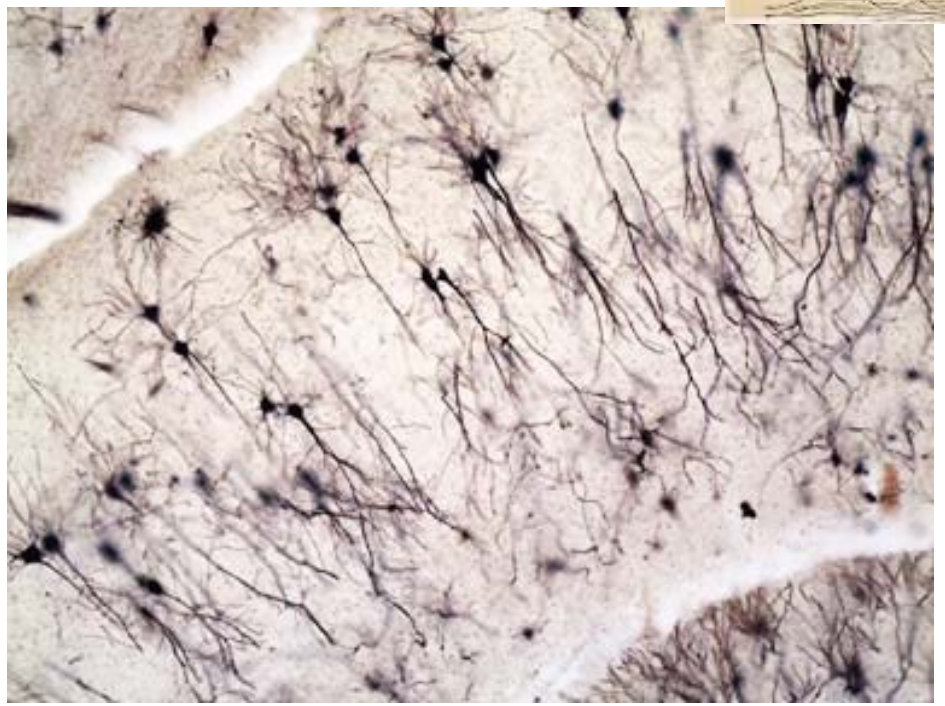
Stain	Common use	Nucleus	Cytoplasms	Red blood cell (RBC)	Collagen fibers	Specifically stains
Haematoxylin	General staining when paired with eosin (i.e. H&E)	Orange, Cyan Blue or Green	Blue/Brown/Black	N/A		
Eosin	General staining when paired with haematoxylin (i.e. H&E)	N/A	Pink	Orange/red		
Toluidine blue	General staining	Blue	Blue	Blue		
Masson's trichrome stain	Connective tissue	Black	Red/pink	Red		
Mallory's trichrome stain	Connective tissue	Red	Pale red	Orange		
Weigert's elastic stain	Elastic fibers	Blue/black	N/A	N/A		
Heidenhain's AZAN trichrome stain	Distinguishing cells from extracellular components	Red/purple	Pink	Red		
Silver stain	Reticular fibers, nerve fibers, fungi	N/A	N/A	N/A	N/A	Nerve fibers brown/black Fungi—black



Golgi method





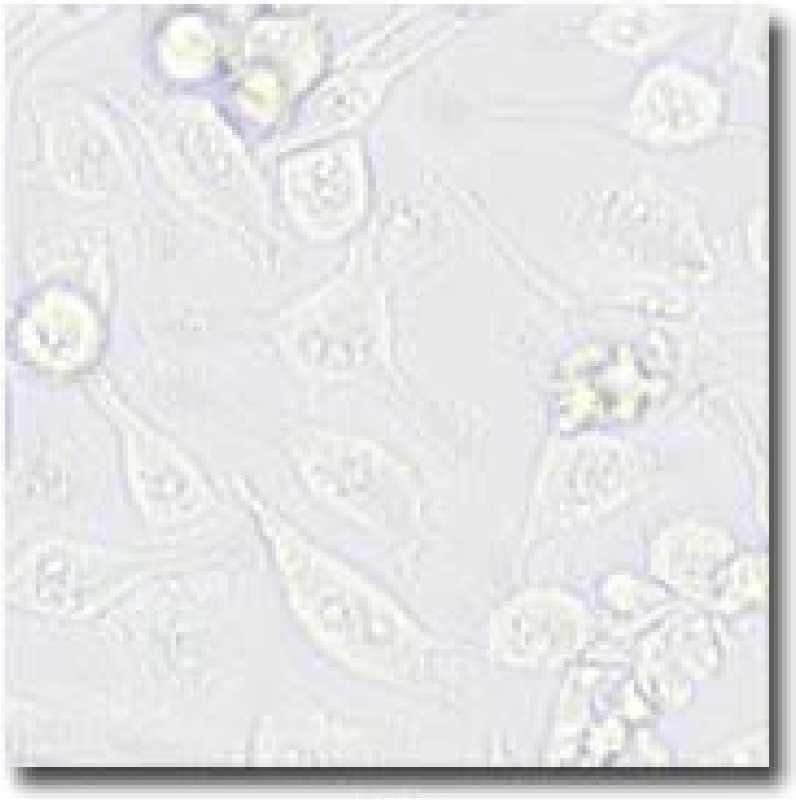


1.Immerse a block (approx. 10x5 mm) of formol-fixed (or paraformaldehyde- glutaraldehyde-perfused) brain tissue into a 2% aqueous solution of potassium dichromate for 2 days
2.Dry the block shortly with filter paper.
3.Immerse the block into a 2% aqueous solution of silver nitrate for another 2 days.
4.Cut sections approx. 20-100 µm thick.
5.Dehydrate quickly in ethanol, clear and mount (e.g., into Depex or Enthalan).
This technique has since been refined to substitute the silver precipitate with gold by immersing the sample in gold chloride then oxalic acid, followed by removal of the silver by sodium thiosulphate.

Why phase contrast?

$$y(x,t) = A\cos(kx - \omega t + \varphi)$$

- Sensitivity only to electric field intensity
- Traditional brightfield has very low contrast
- Interference of waves is very low with cells, so not much shows up.

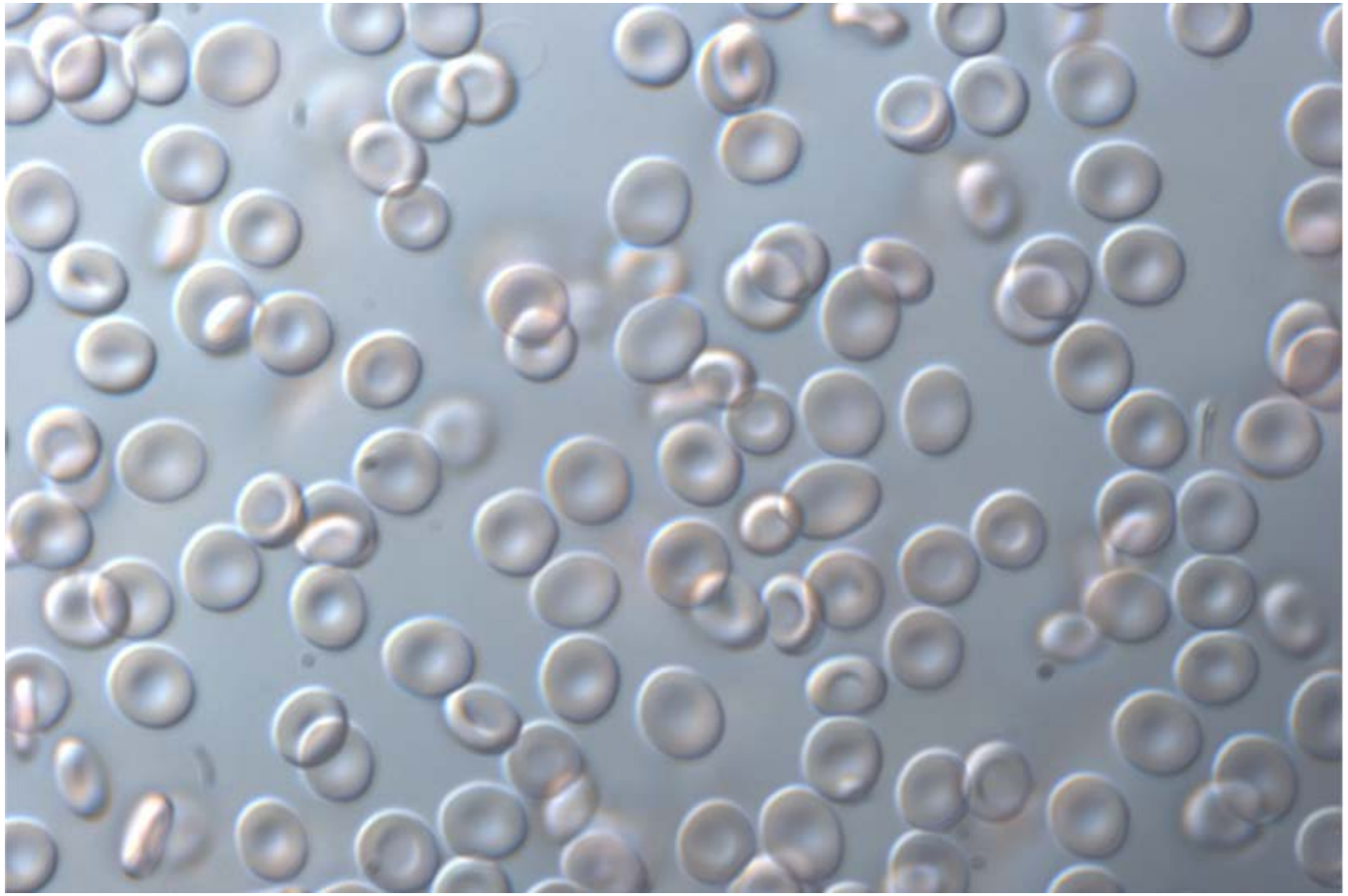


No chromophores in cell culture, so they don't show up as any colors naturally

Many layers of cells will scatter light, this is why you can't see through our skin. Trace amounts of pigments in the skin are what gives it color

Red blood cells

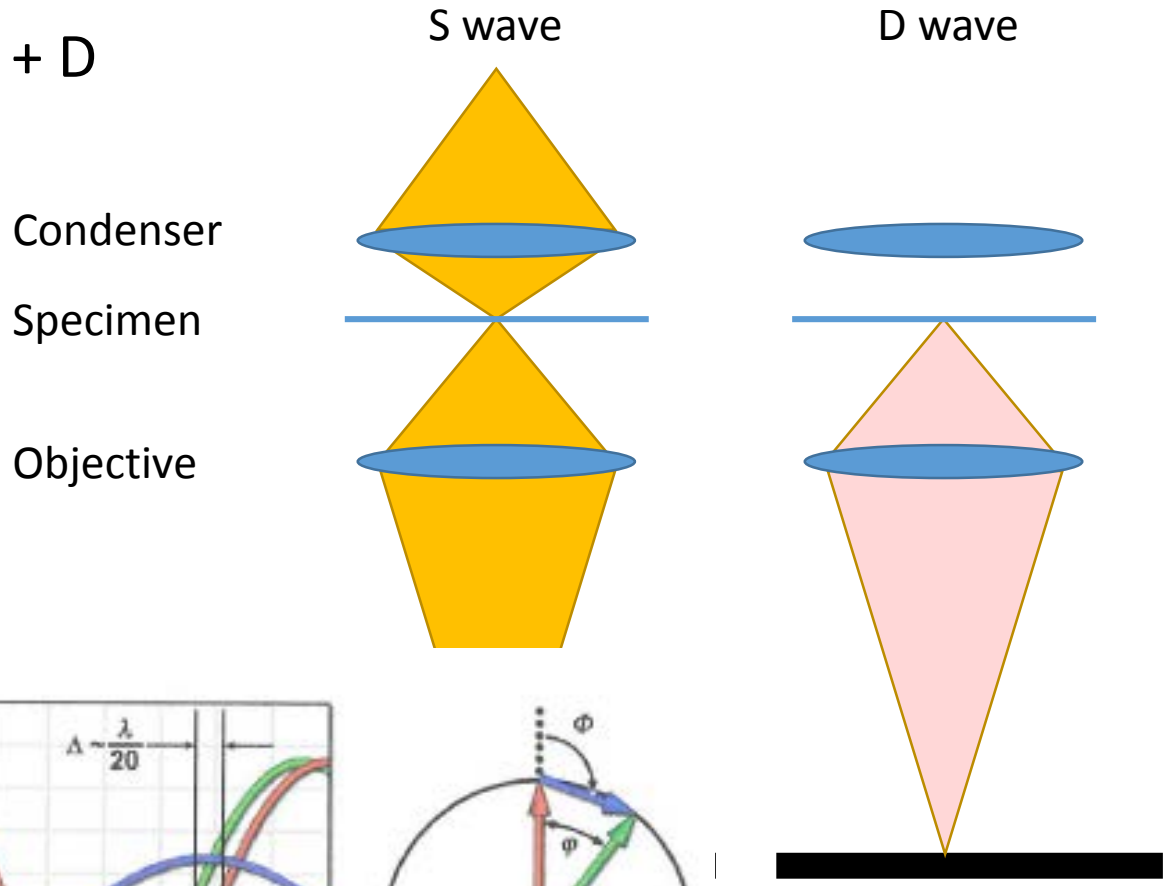
- Lots of pigment
- Still barely visible

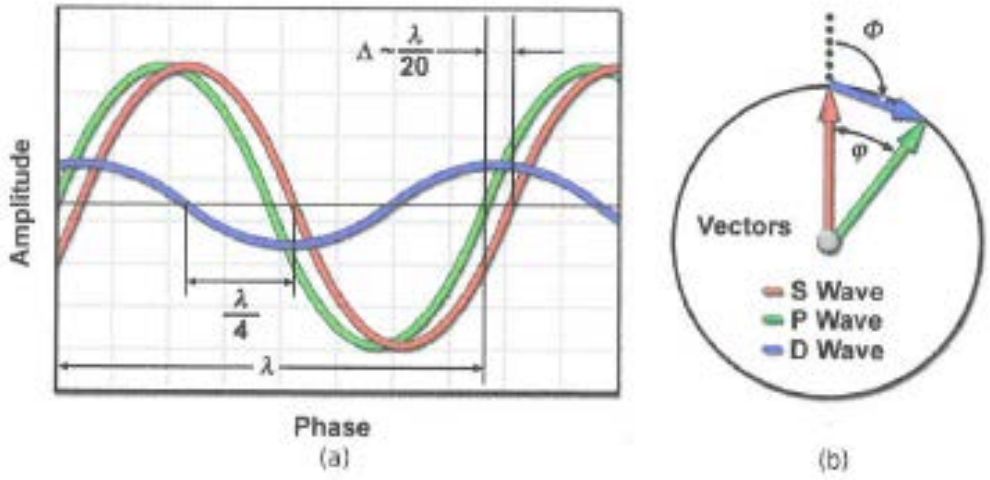


Phase contrast

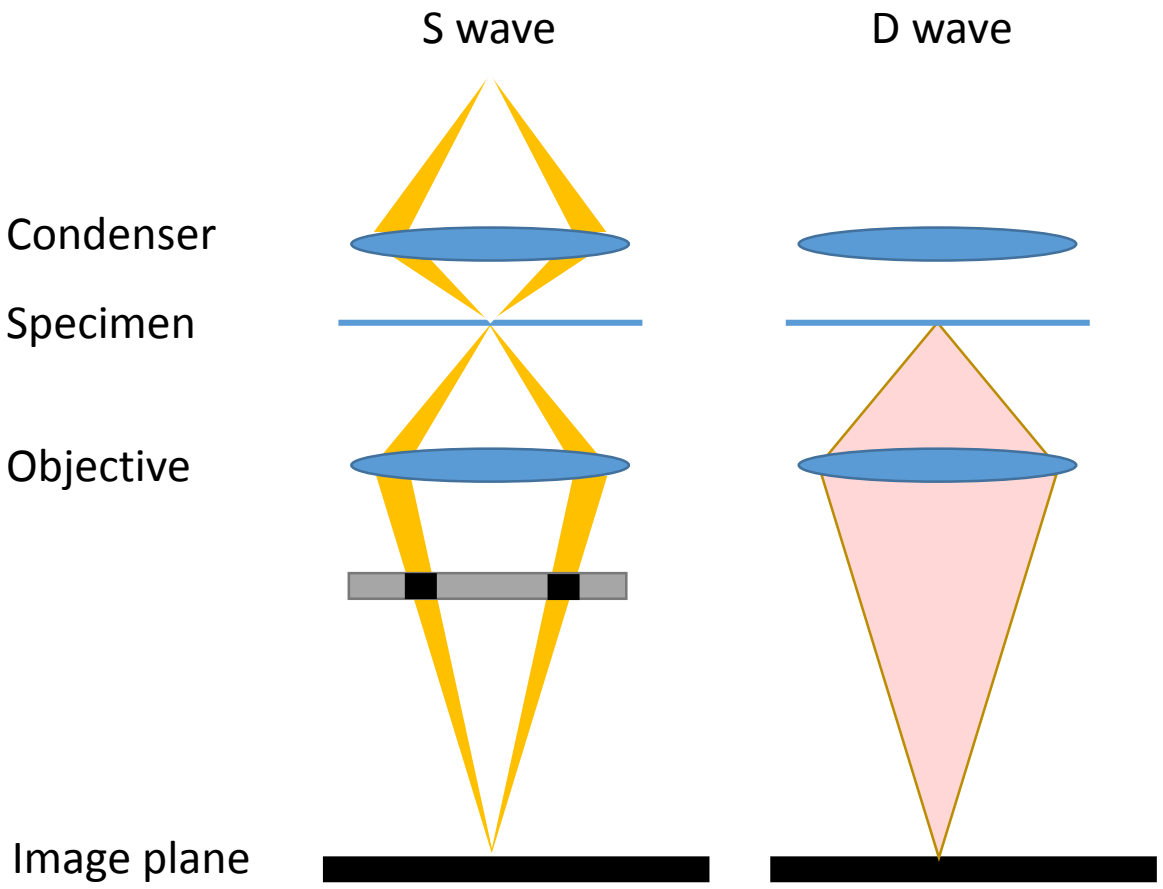
- Convert changes in phase to changes in amplitude
- Consider light impinging on a sample. The s (surround wave) goes on unchanged; it's the background. The d (diffracted wave) is the light diffracted by the sample. On the microscope, we detect the p (particle wave) as the interference between.
- D wave is usually phase shiftec by 90 degrees

P = S + D



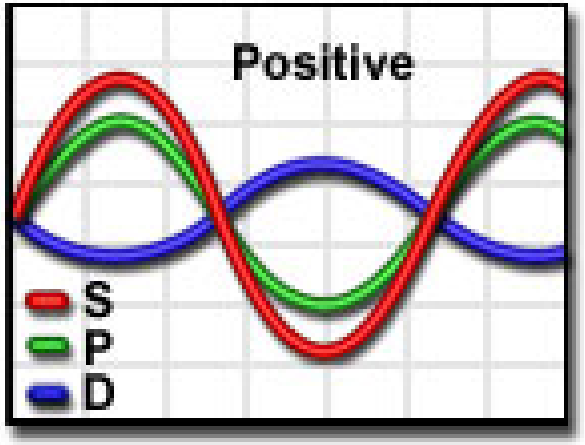


Phase contrast

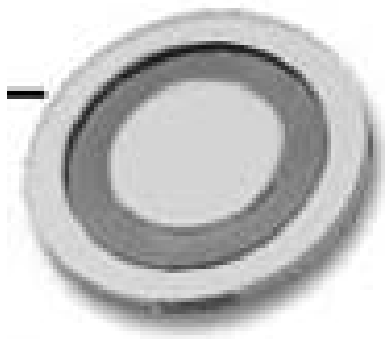


First separate S and D waves in the imaging path

Condenser annulus

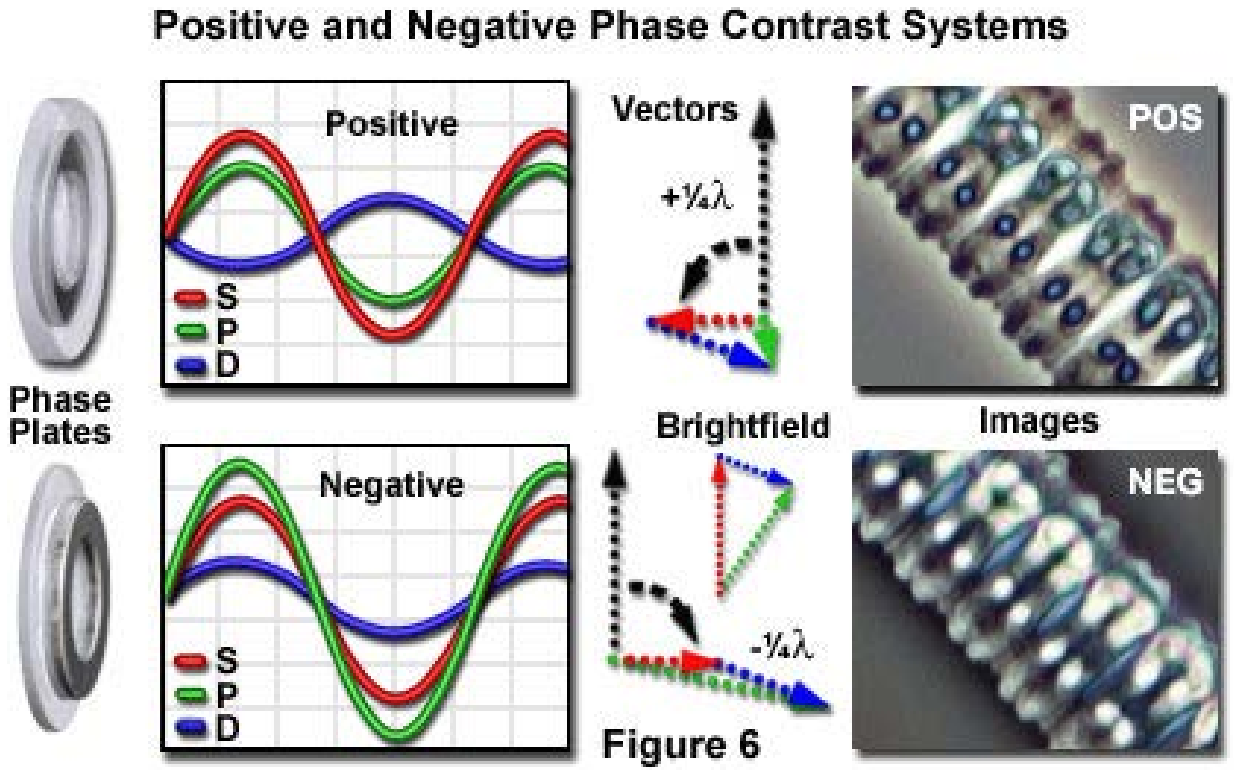


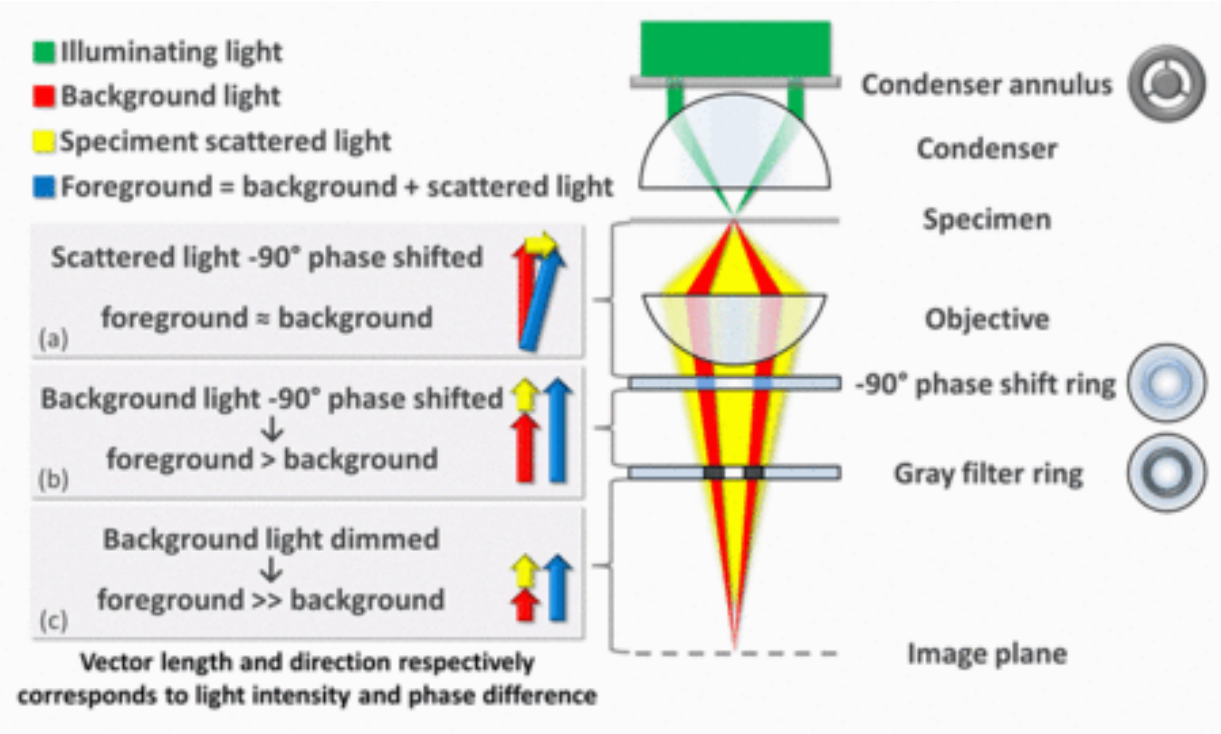
Phase ring



Introduce phase shift ONLY to S wave

Phasor description





$$C = \left[\frac{Ib - Is}{Ib}\right] * 100\%$$

Examining phase images

- Typically associated with density maps, which is a somewhat reasonable assumption

$$\delta = \frac{2\pi(n2 - n1)t}{\lambda}$$

- Accurate to think of optical path difference

$$\mathrm{OPD} = d_1 n_1 - d_2 n_2$$

Bright objects can have short d and high n, or long d and low n

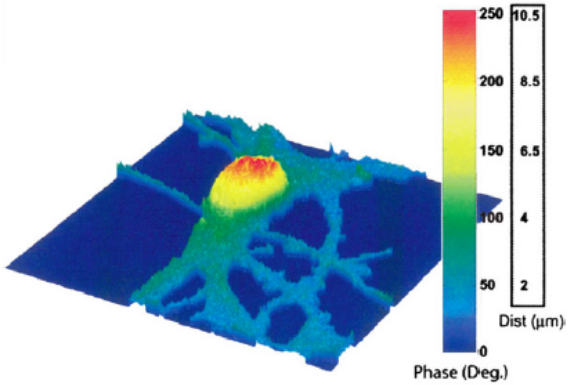


Figure 7 Quantitative phase image of a living mouse cortical neuron in culture obtained with DHM [from Fig. 2, Marquet et al. (2005)].

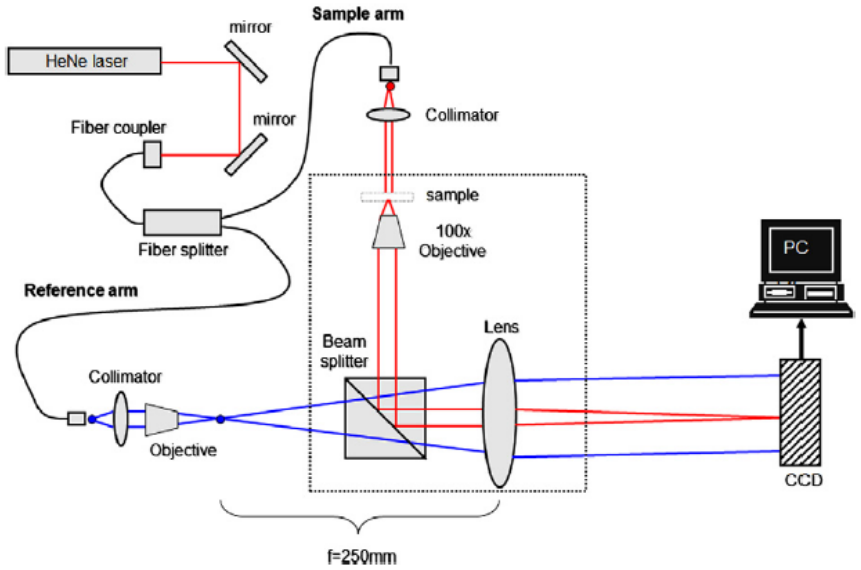


Figure 8 HPM experimental setup [from Fig. 1, Popescu et al. (2005)].

Phase artifacts

Shade off and halos are associated with the fact that all the higher order rays of the s wave get blocked by the phase ring

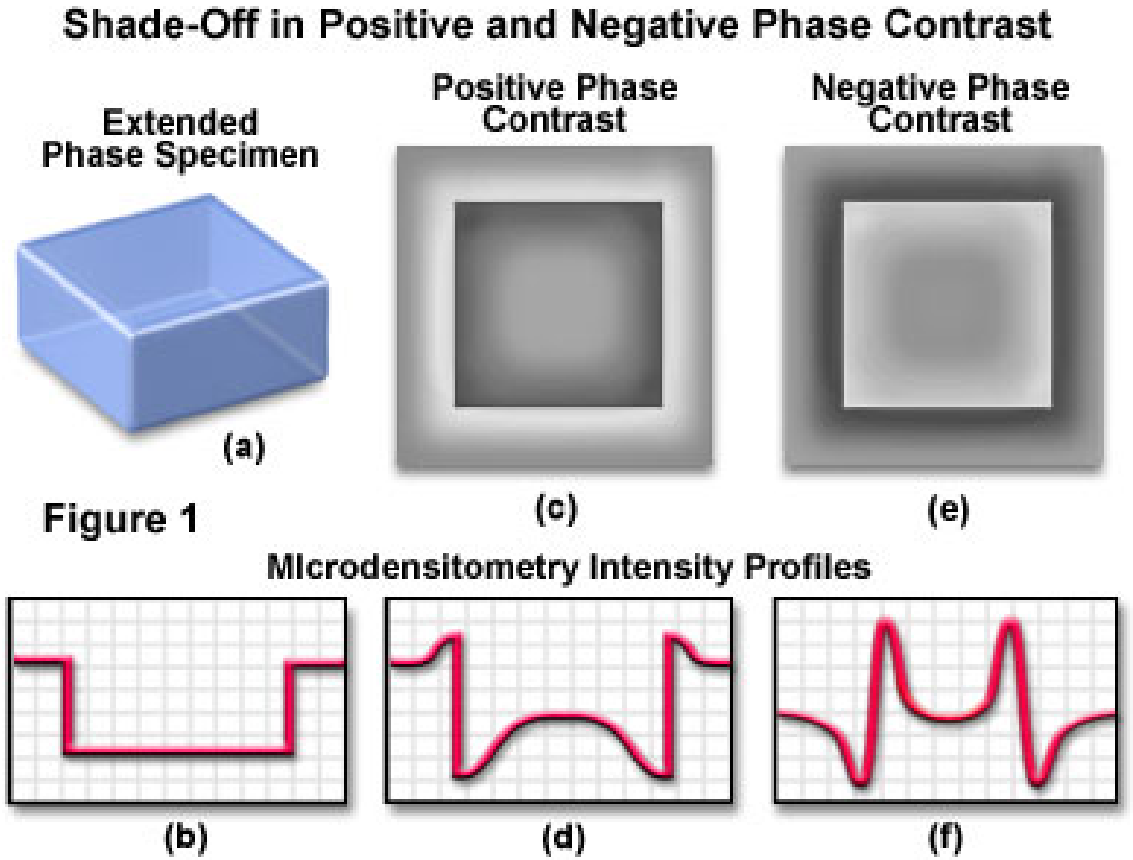


Figure 1

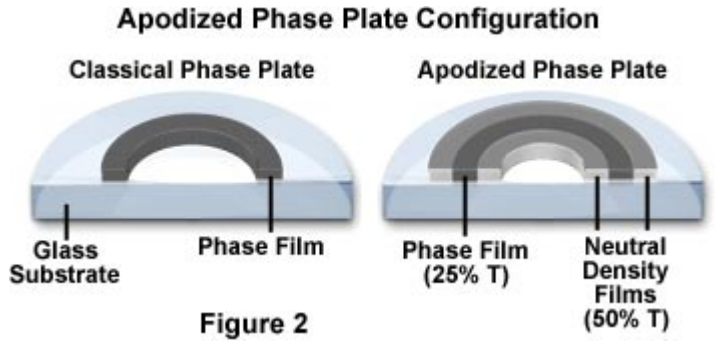


Figure 2

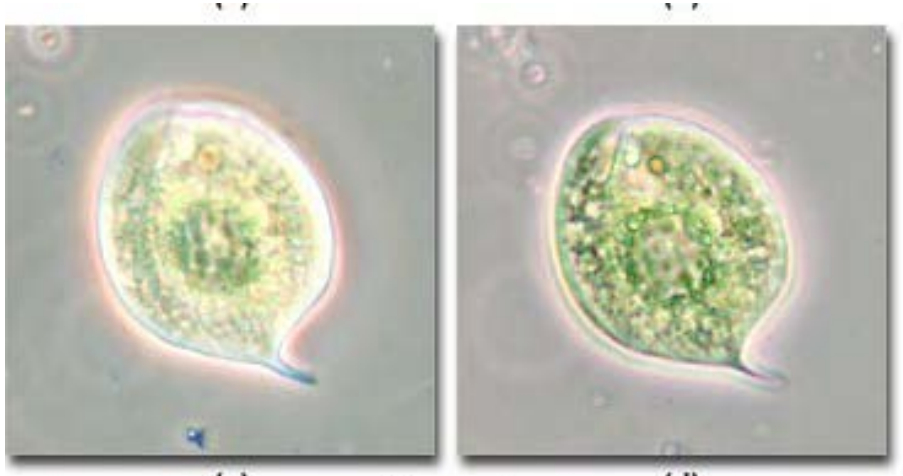
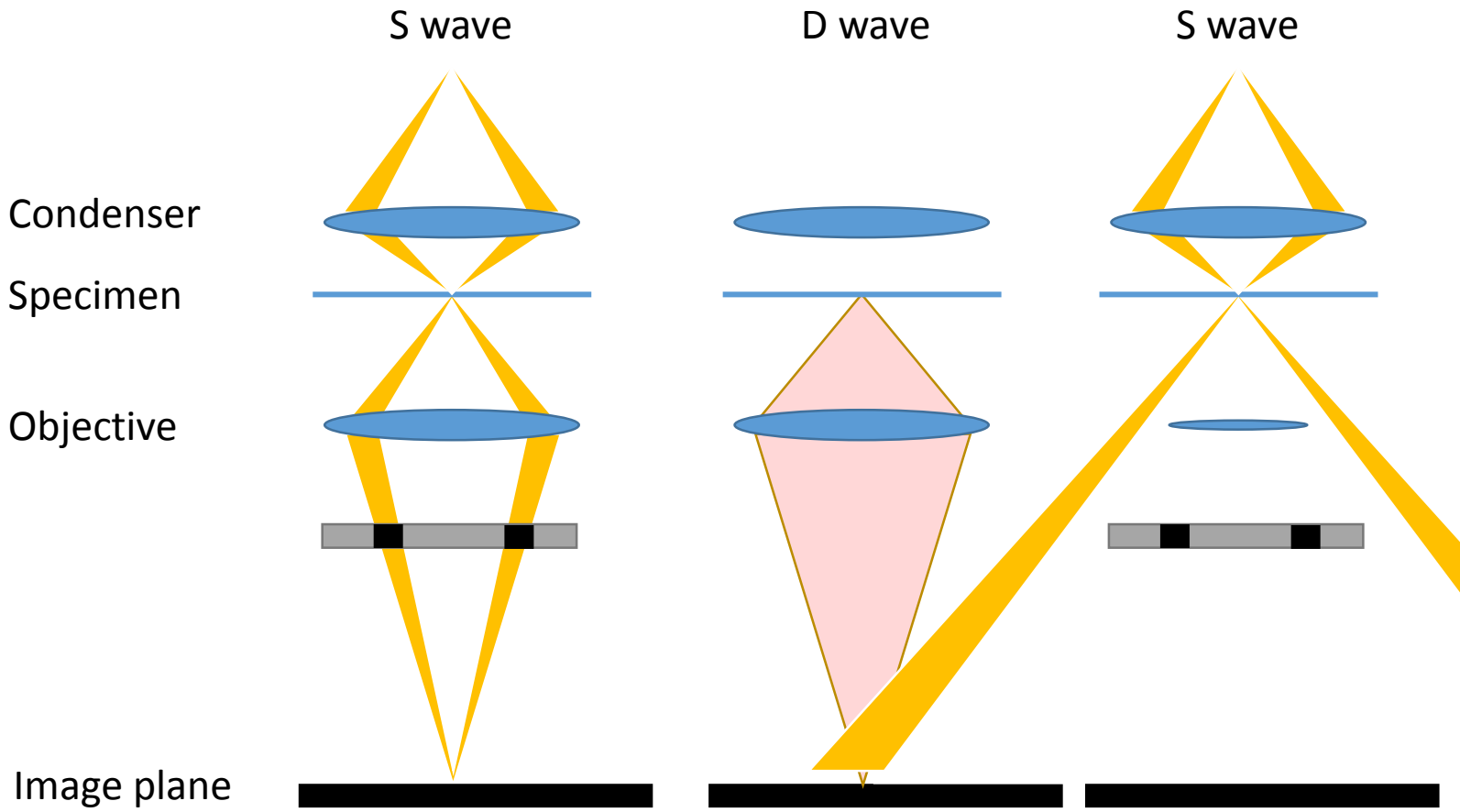


Figure 3

Dark field microscopy

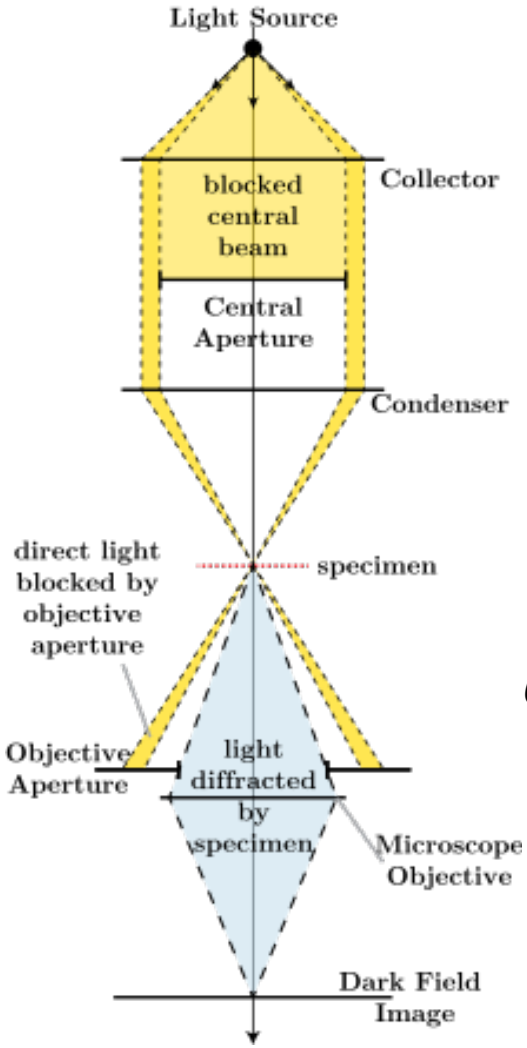
- Get rid of the S wave all together
- Now the diffracted light will appear on a dark background

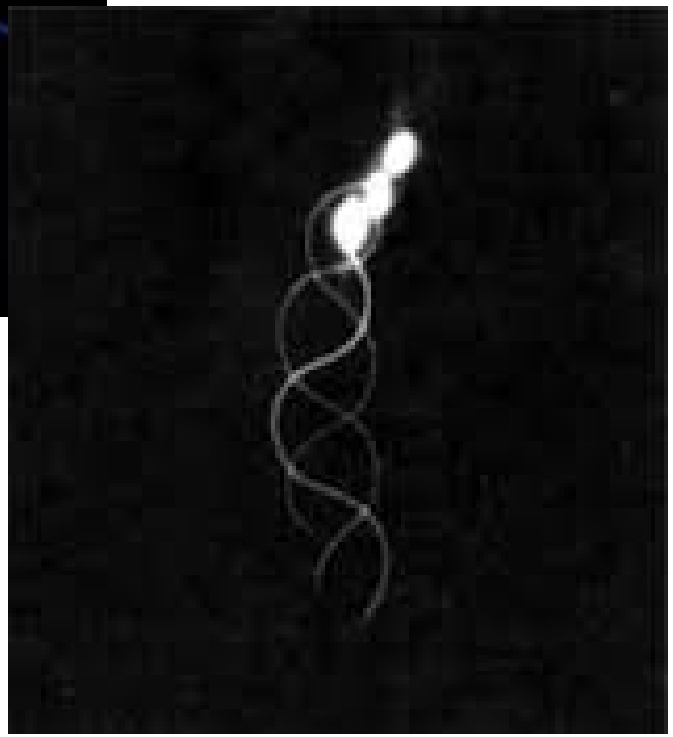


First separate S and D waves in the imaging path

Darkfield considerations

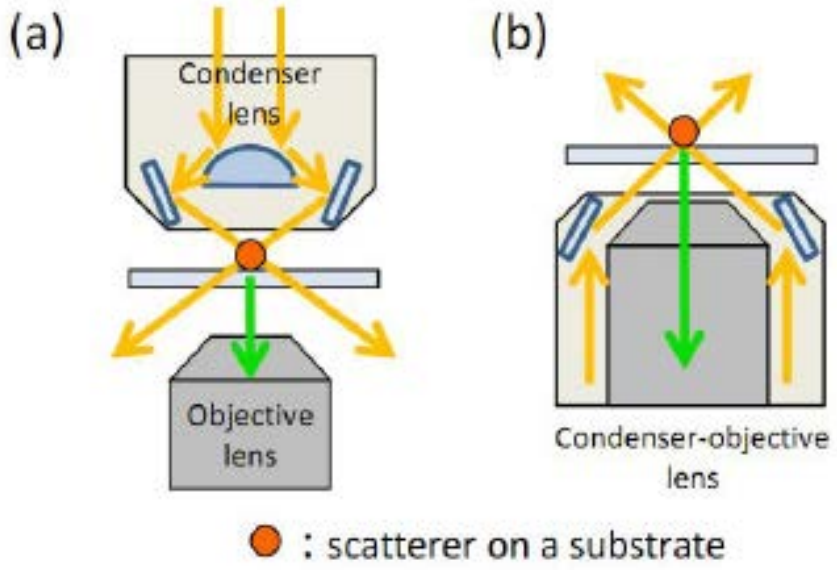
- Numerical aperture of condenser MUST be higher than objective
- Brightness corresponds to highly refractive objects
- Can see objects that would otherwise be smaller than diffraction limit
- Dust and dirt are highly visible

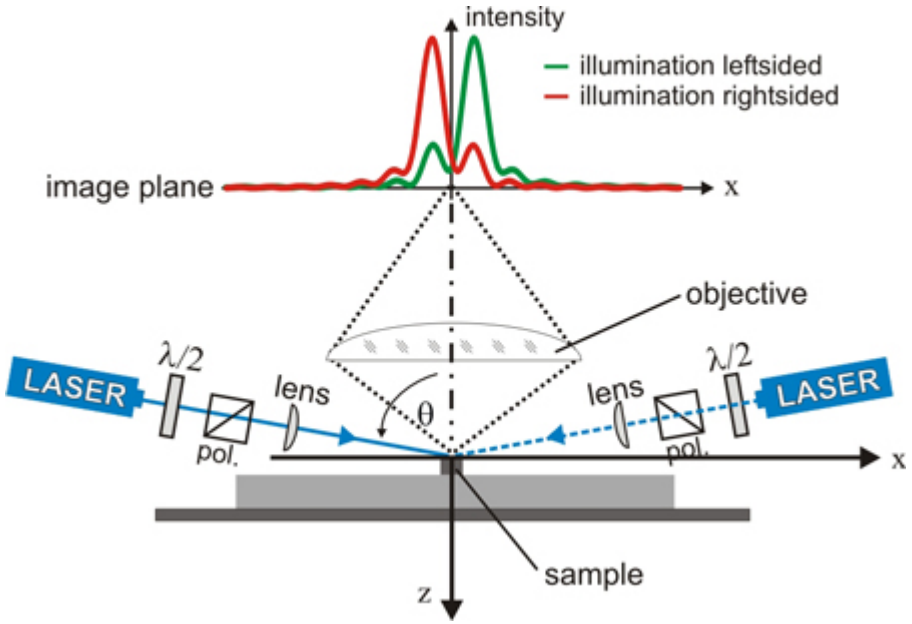




$$C = \left[\frac{Ib - Is}{Ib}\right] * 100\%$$

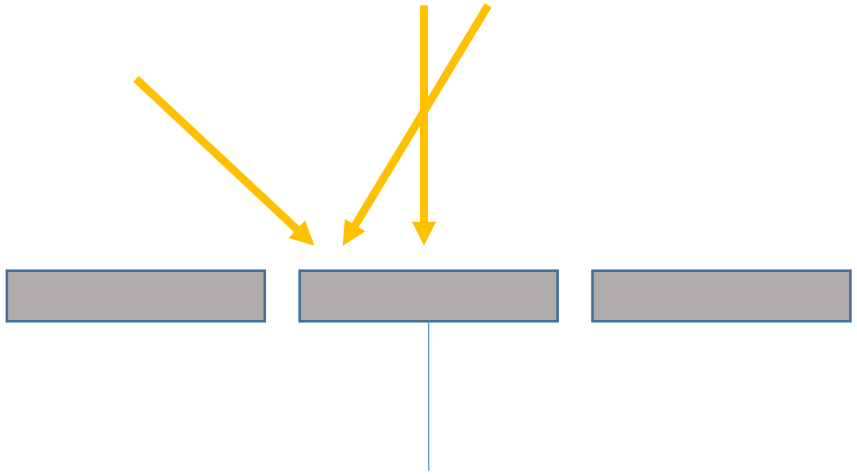
Dark field optical setups



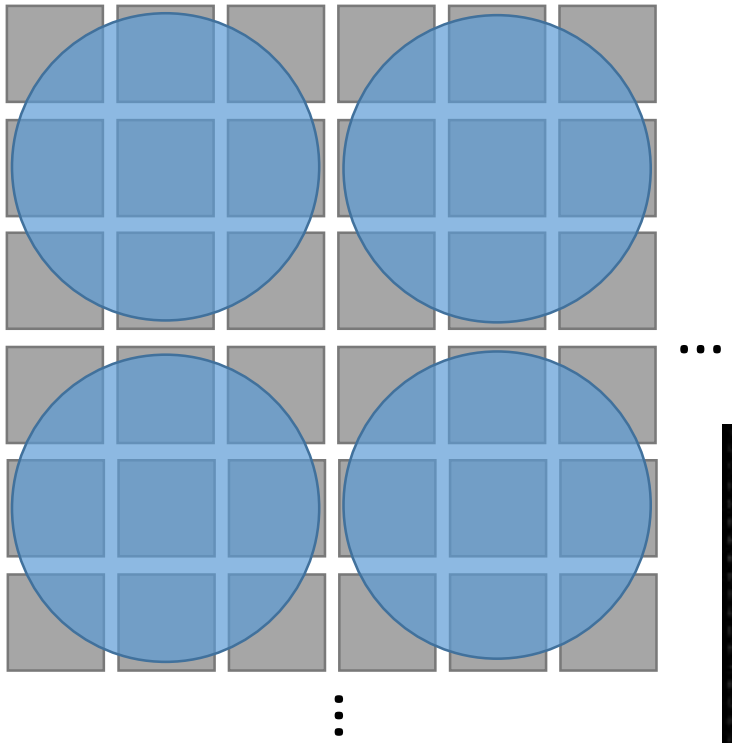


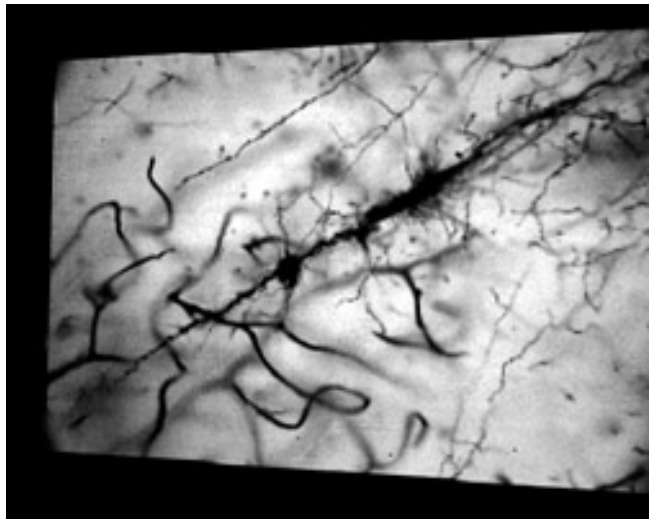
Light field microscopy

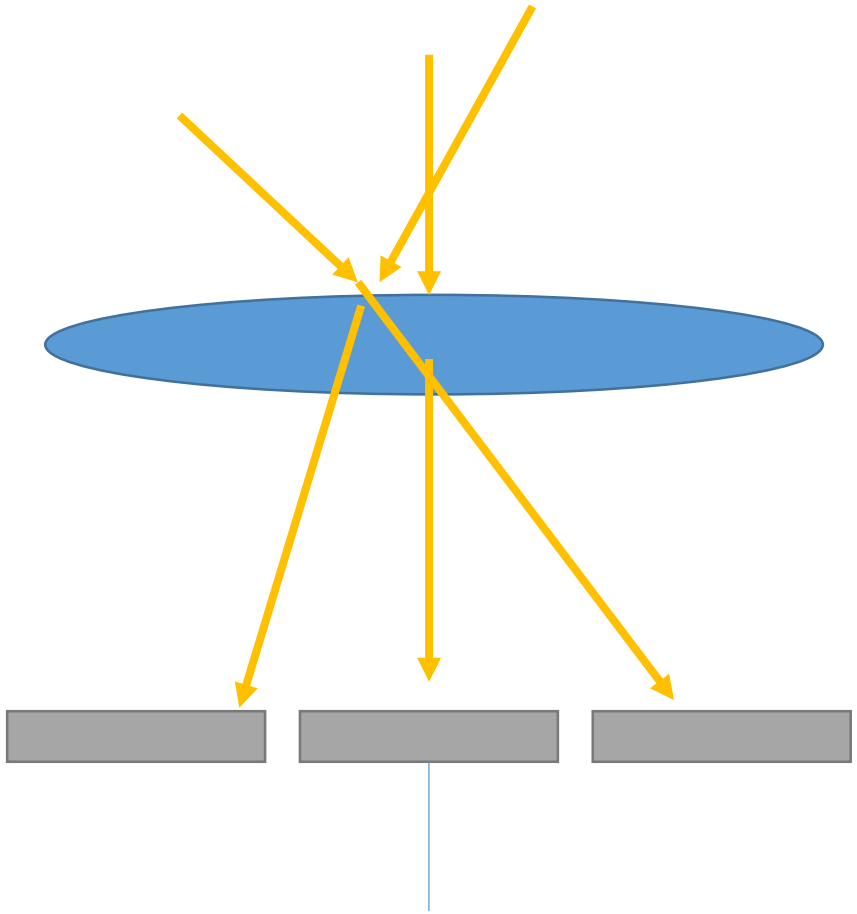
- Light has both a position and an angle
- At the camera pixel, we only know the current position, so it’s impossible to tell where it came from in a 3D image



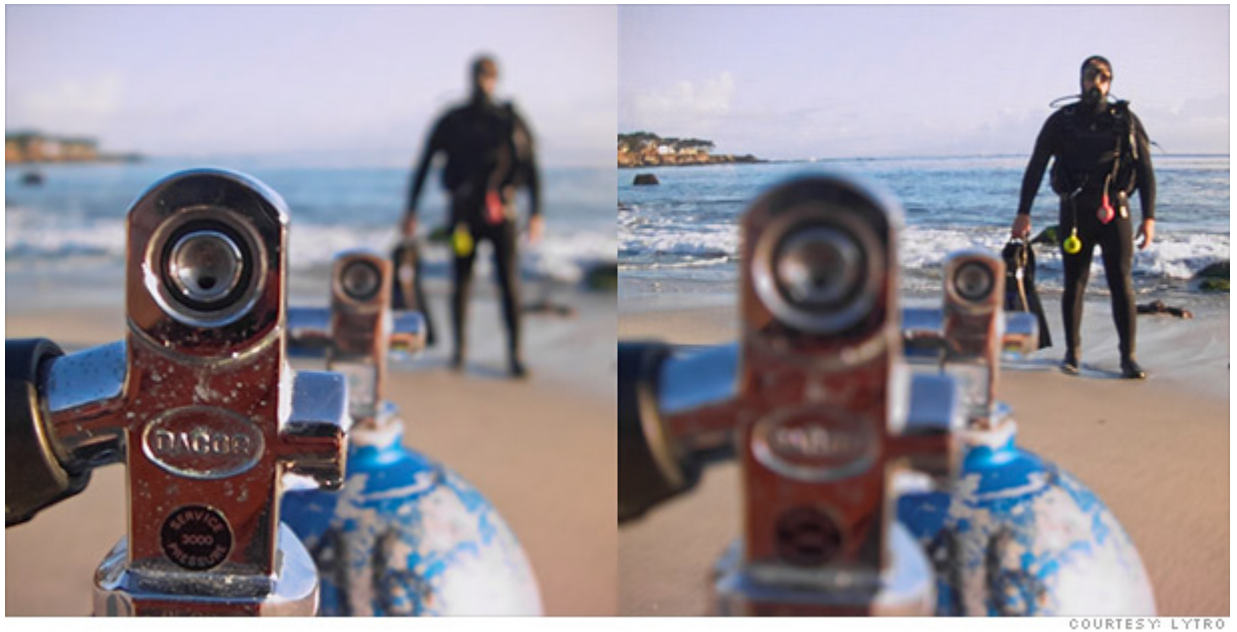
Light field microscope





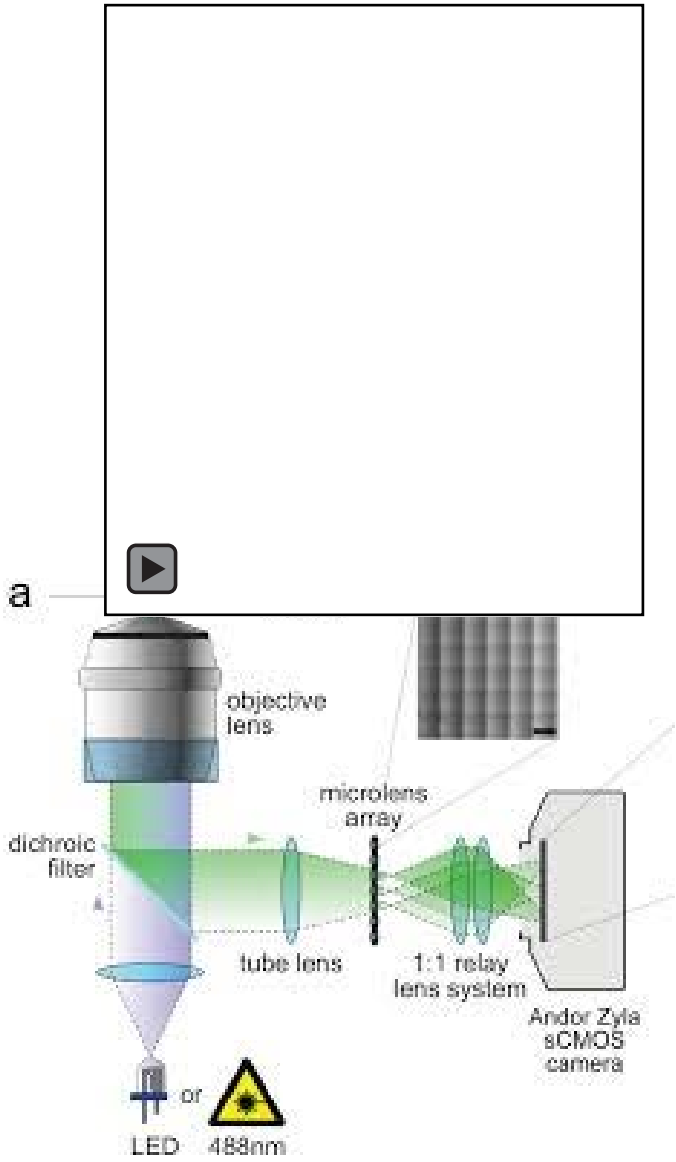


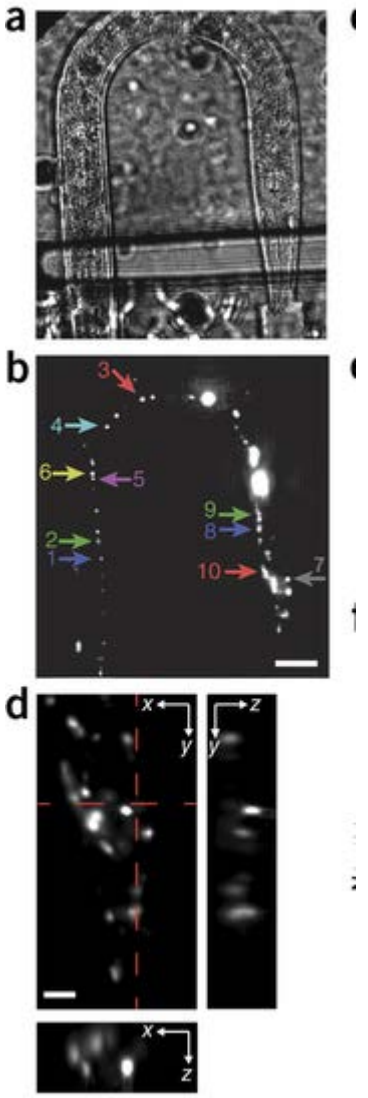
Lightfield examples



Information about both position and angle

- 1 pixel -> 25 pixels, loss of resolution and brightness
- Depth information acquired in a single image
- Easy to implement





And on to matlab?