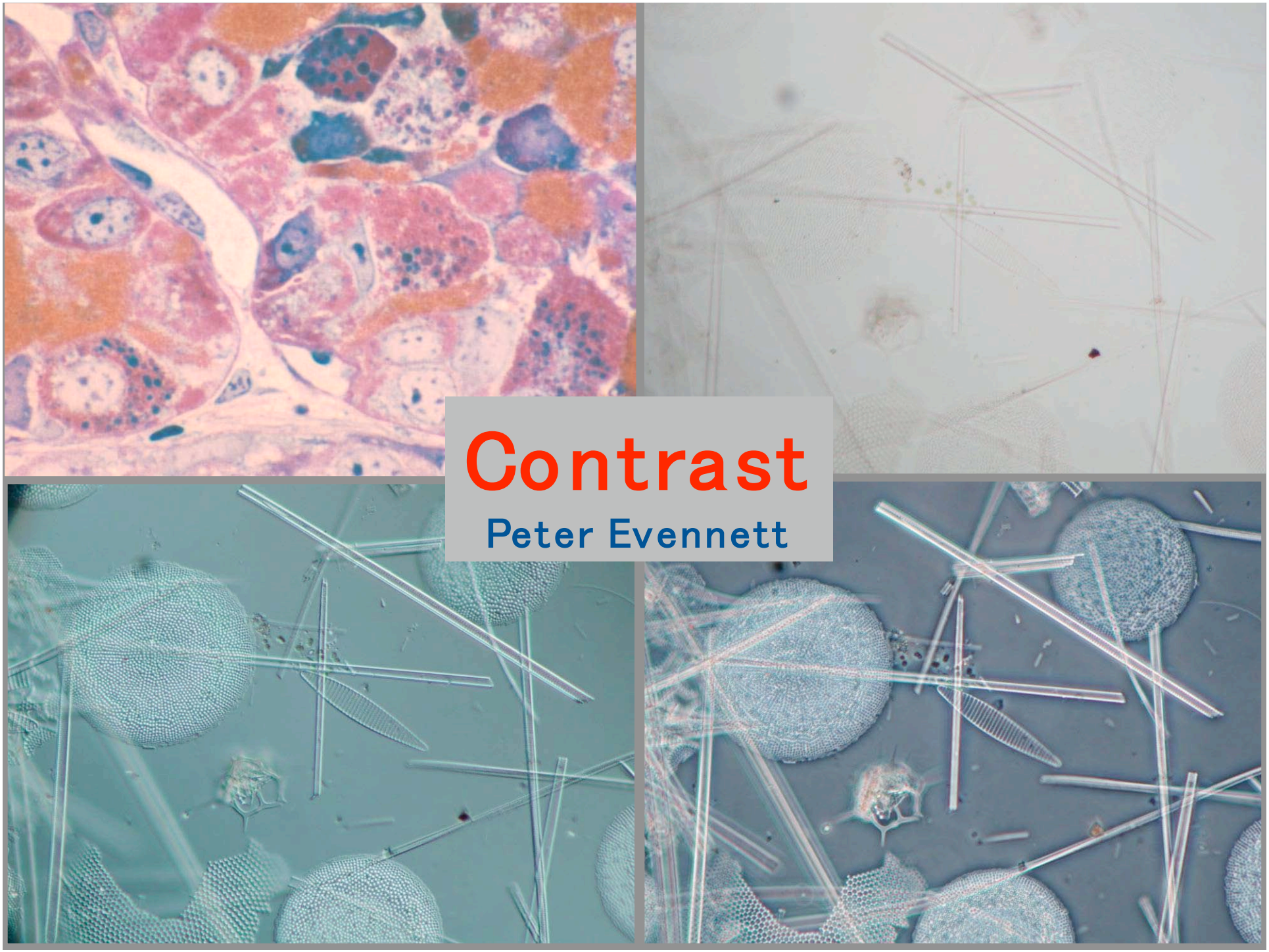
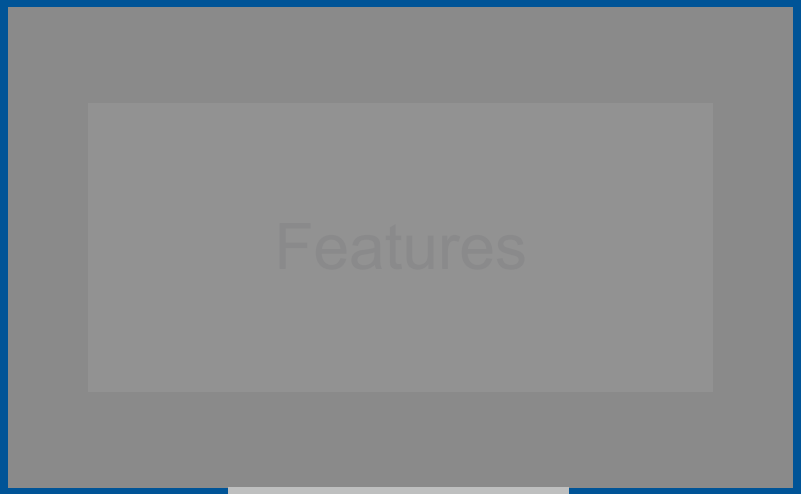


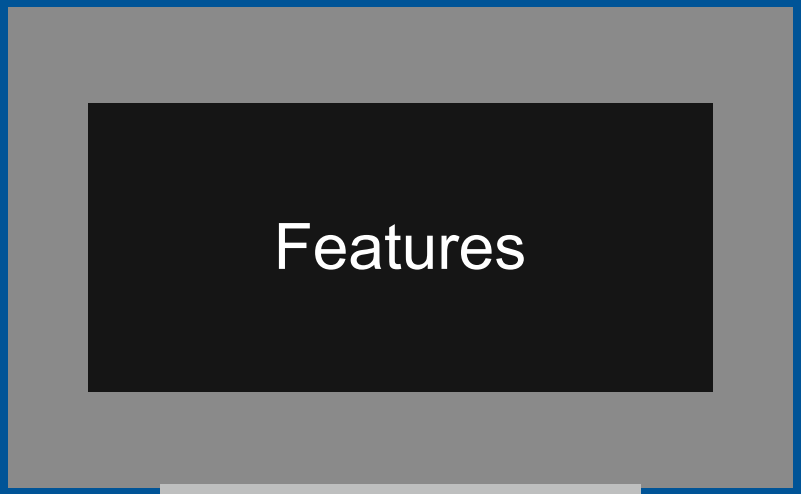
Contrast

Peter Evennett

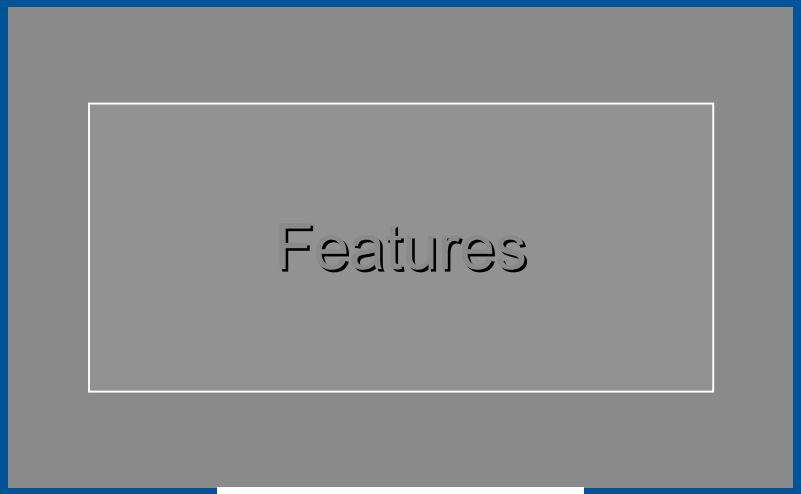
Increasing image contrast



Low contrast



Amplitude contrast



Edge contrast

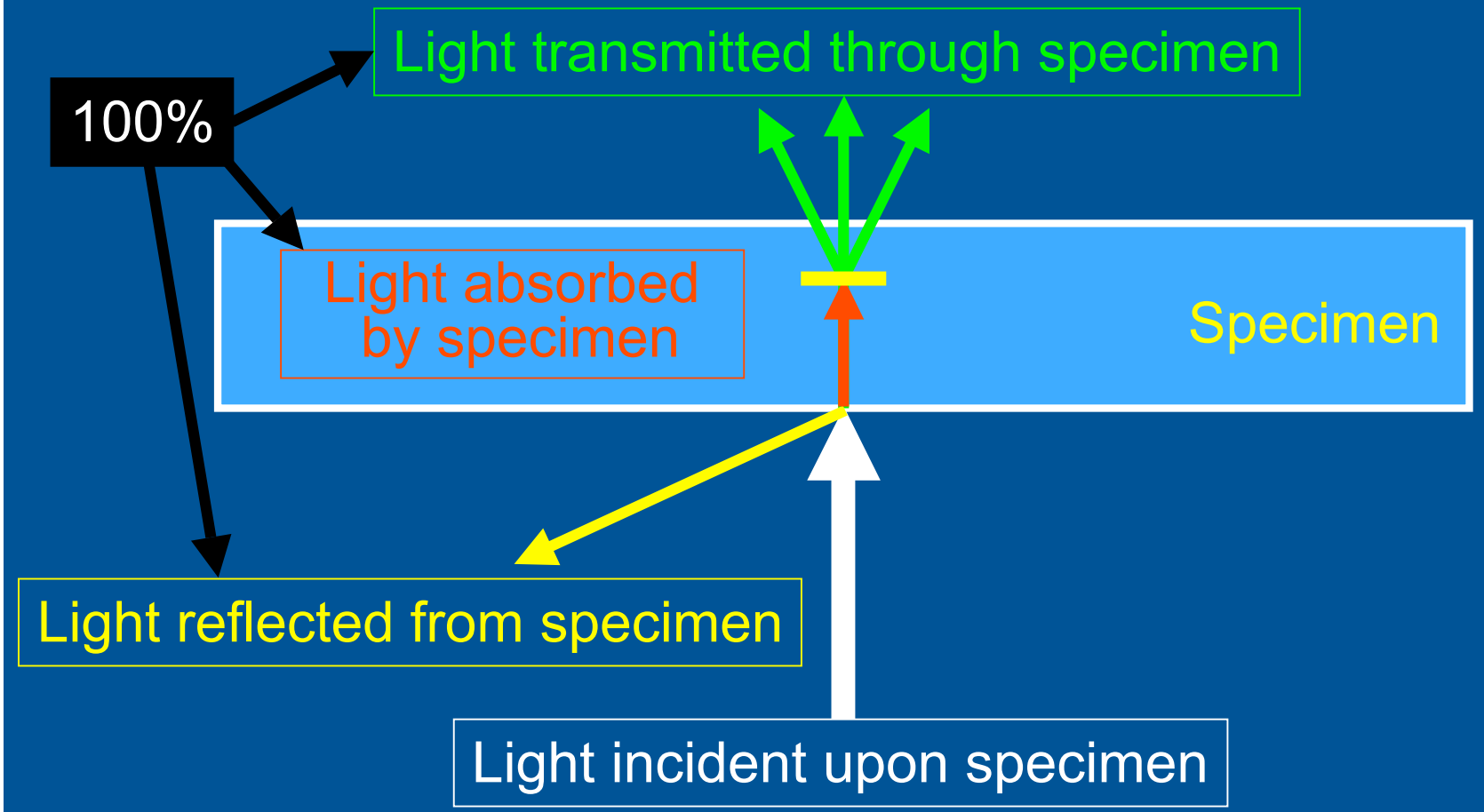
Colour contrast

Contrast may be altered:

- In the Specimen by
  - Staining
- In the Microscope by optical techniques
  - Colour Filters
  - Dark Field
  - Phase Contrast
  - Differential Interference Contrast
- In the Photographic Image by
  - Choice of film
  - Choice of developer
  - Choice of printing paper
- In the Video or Digital Image by
  - Electronic adjustments
  - Computer manipulation

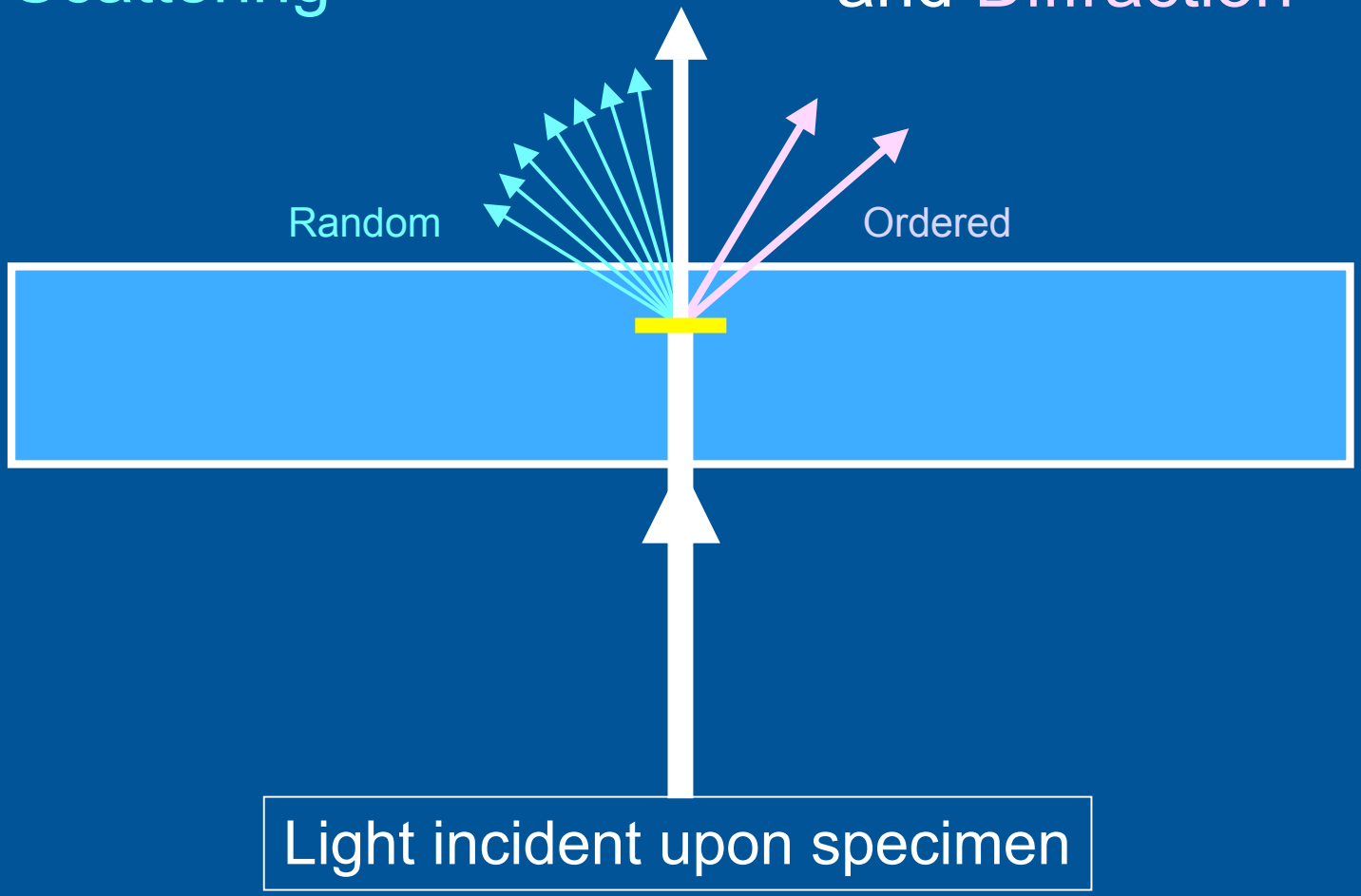
Effects of the specimen on light

- Absorption, Transmission and Reflection



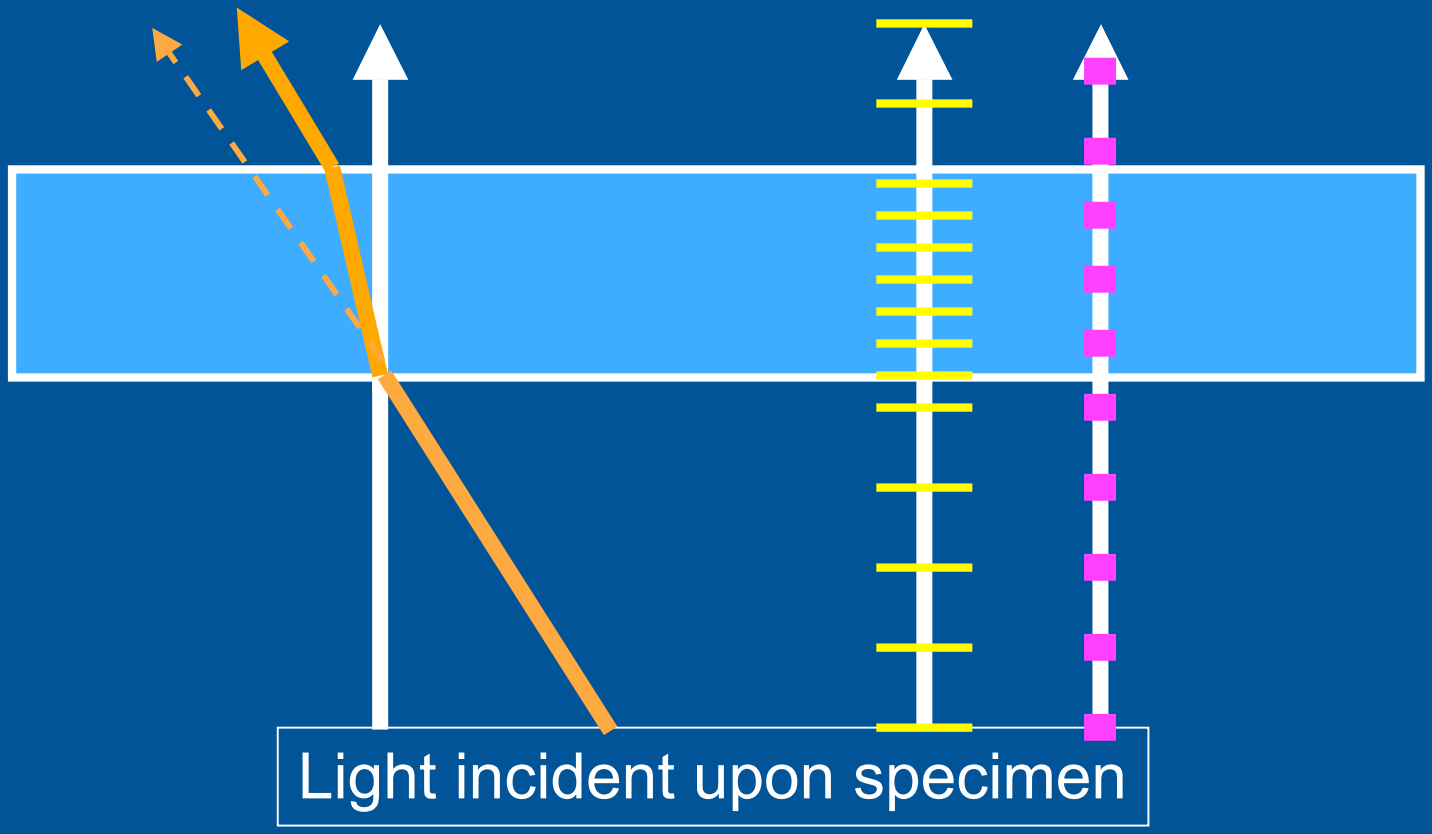
Effects of the specimen on light

- Absorption, Transmission and Reflection
- Scattering and Diffraction



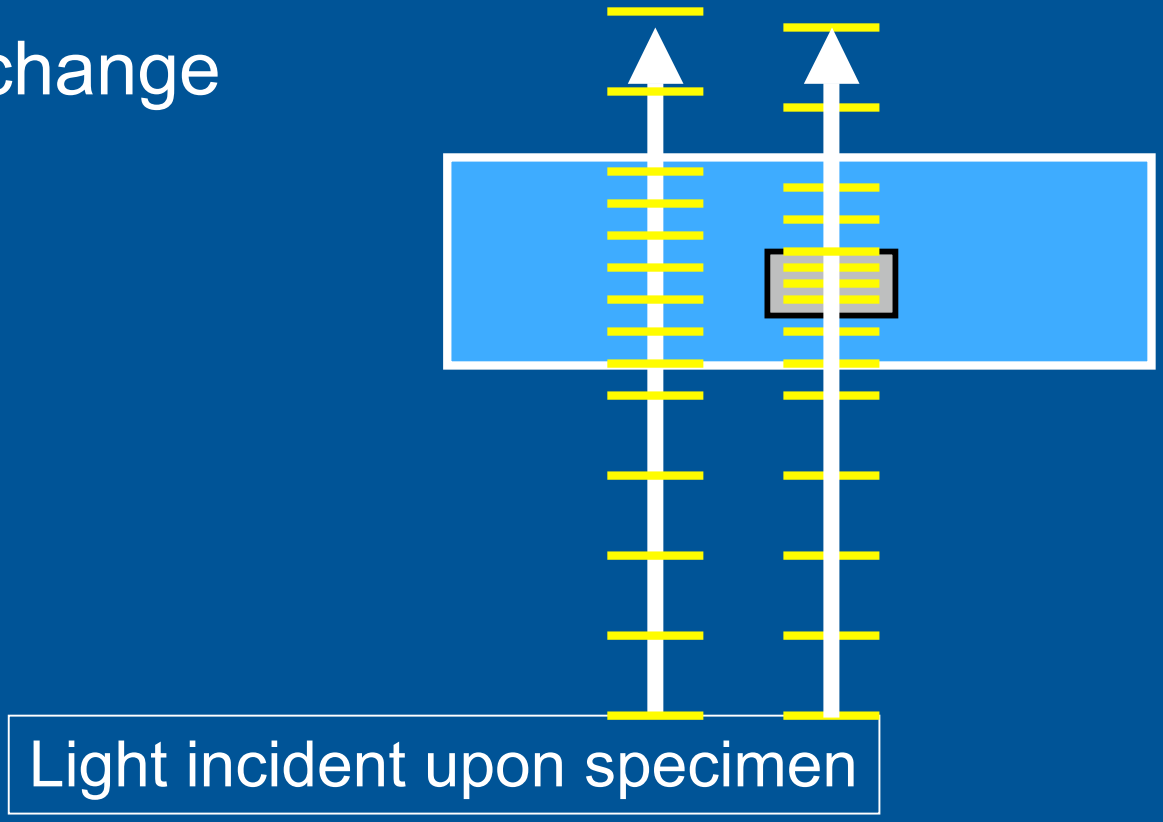
Effects of the specimen on light

- Absorption, Transmission and Reflection
- Scattering and Diffraction
- Refraction and Polarization



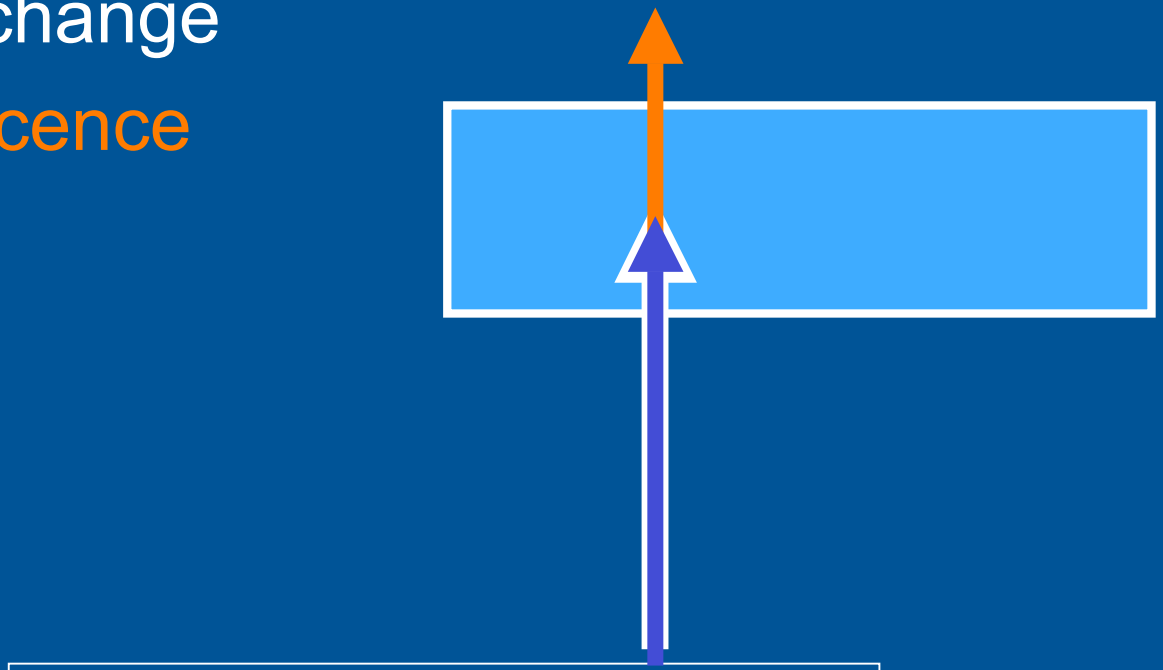
Effects of the specimen on light

- Absorption, Transmission and Reflection
- Scattering and Diffraction
- Refraction and Polarization
- Phase change



Effects of the specimen on light

- Absorption, Transmission and Reflection
- Scattering and Diffraction
- Refraction and Polarization
- Phase change
- Fluorescence



Light incident upon specimen

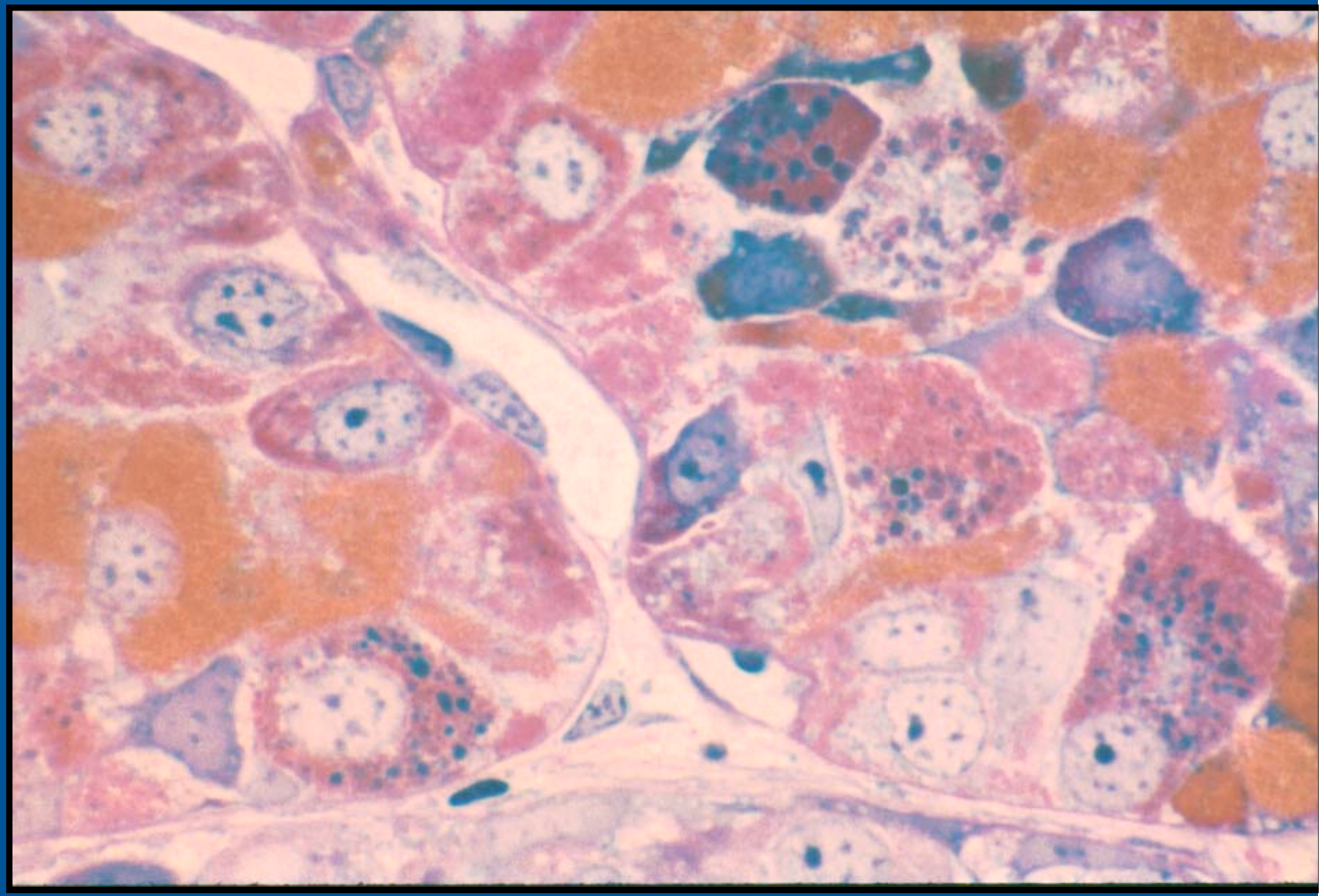
Effects of the specimen on light

- Absorption, Transmission and Reflection
- Scattering and Diffraction
- Refraction and Polarization
- Phase change
- Fluorescence

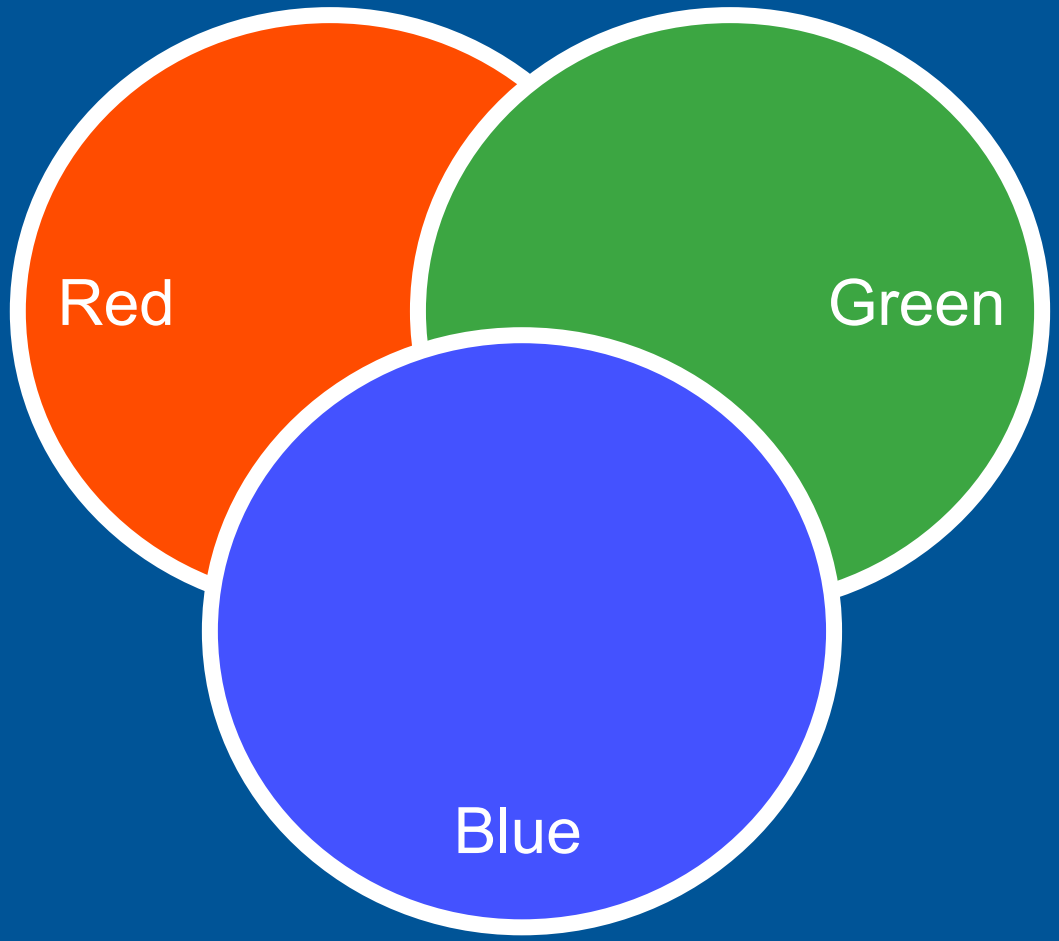
The contrast mechanism converts these effects into variations of *brightness* or colour

- the only variations that the human eye, photographic film or electronic sensors are able to detect

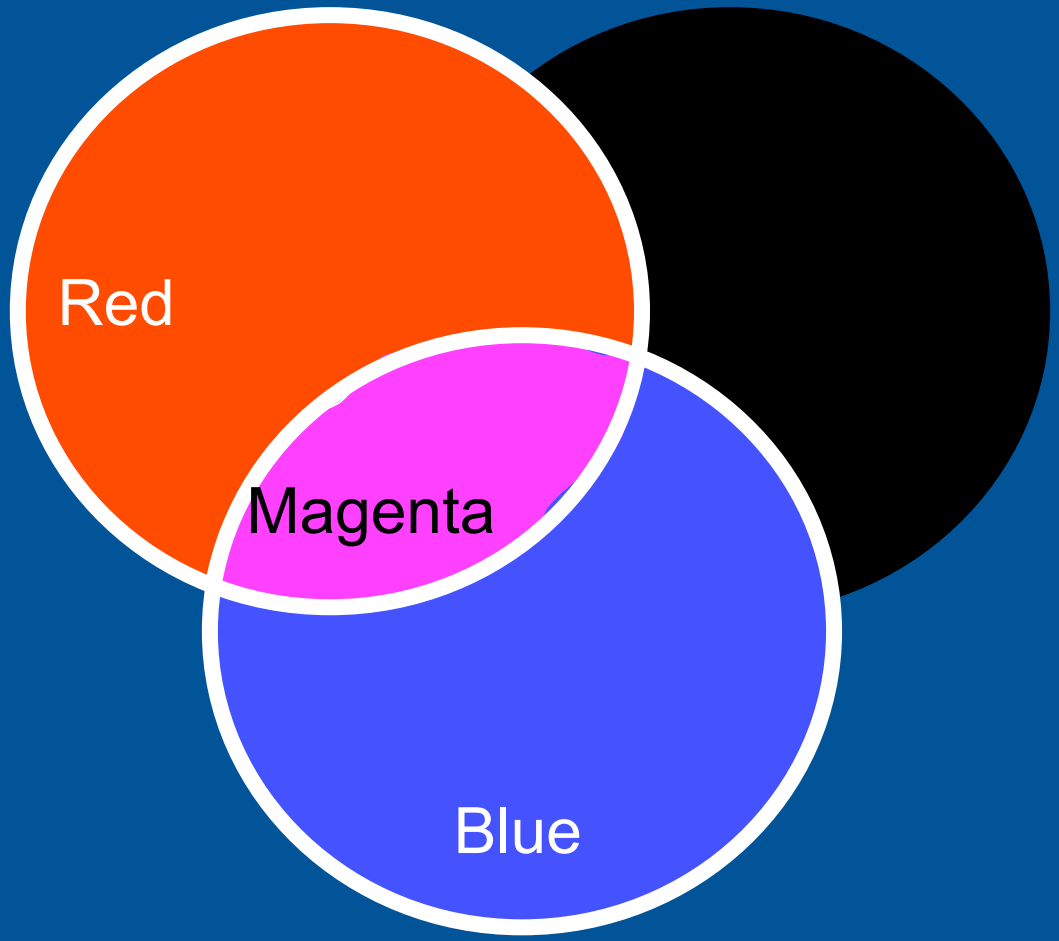
Stained section of *Xenopus* pituitary gland



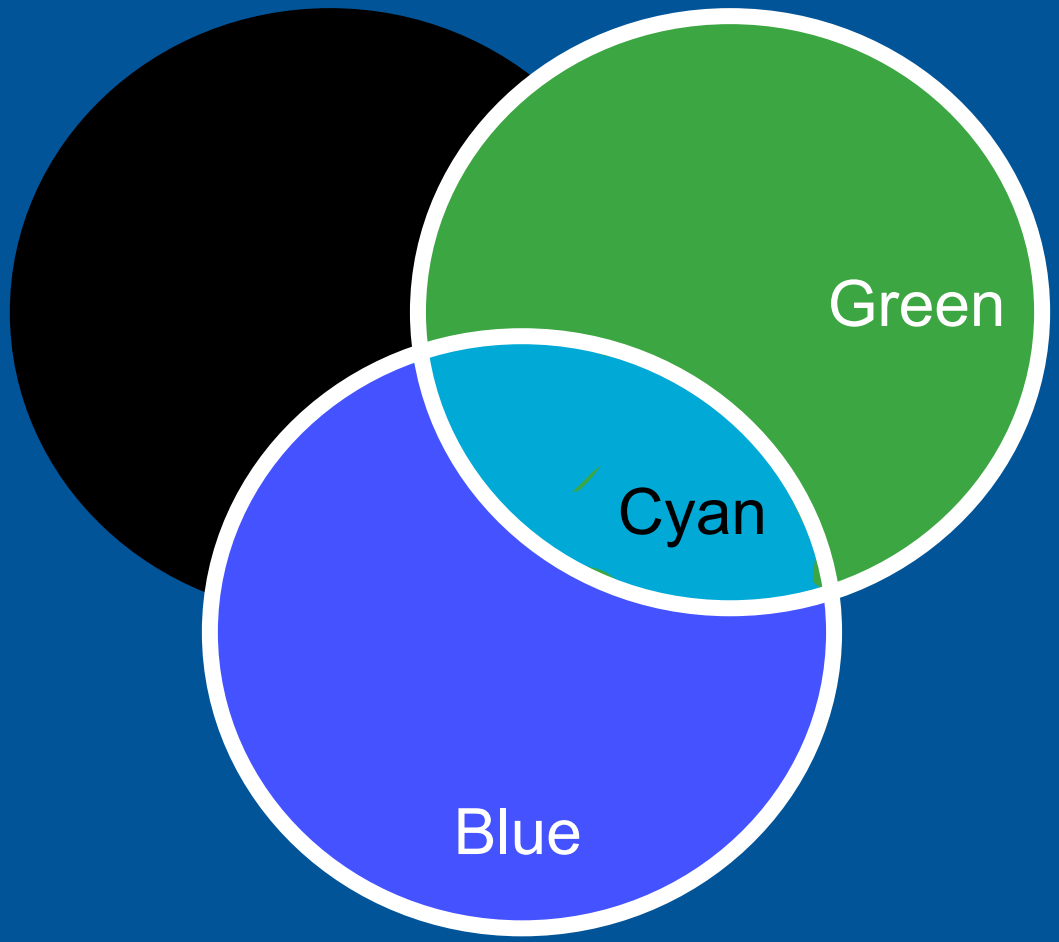
Three Primary Colours



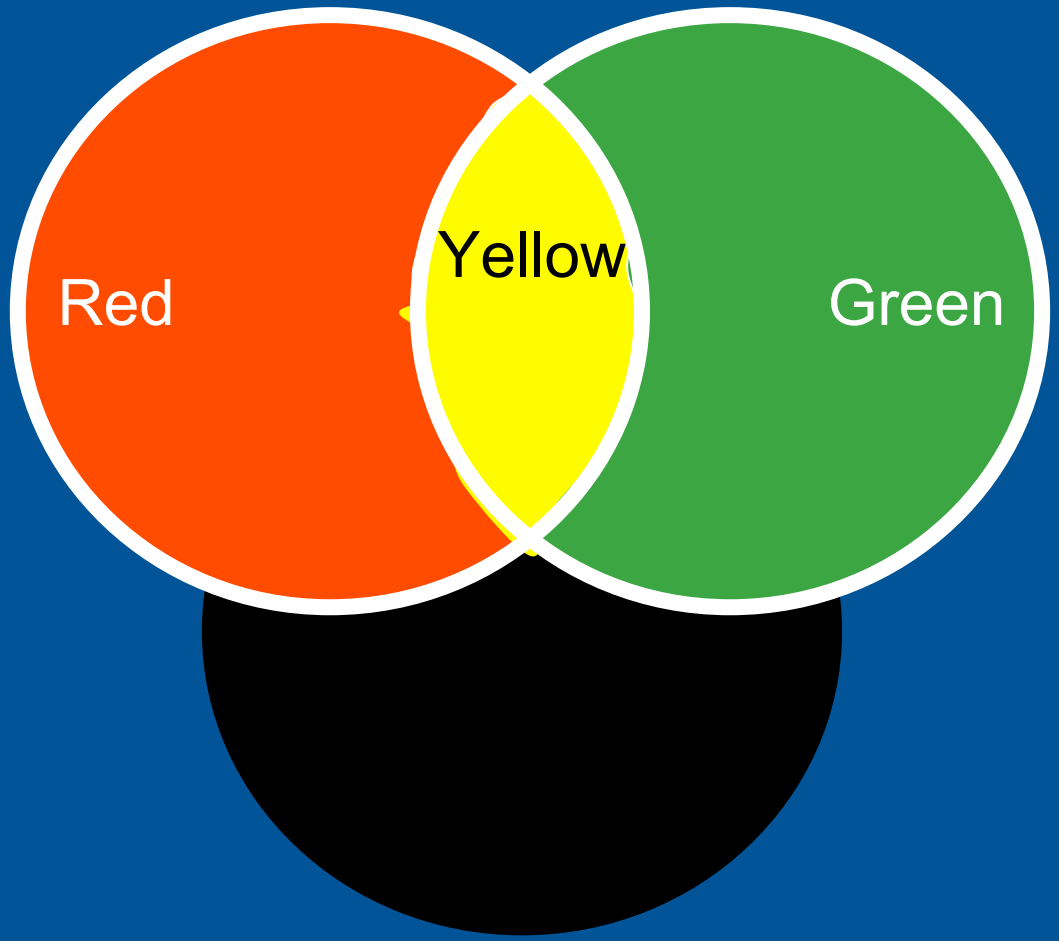
Mixing coloured lights - Secondary Colours



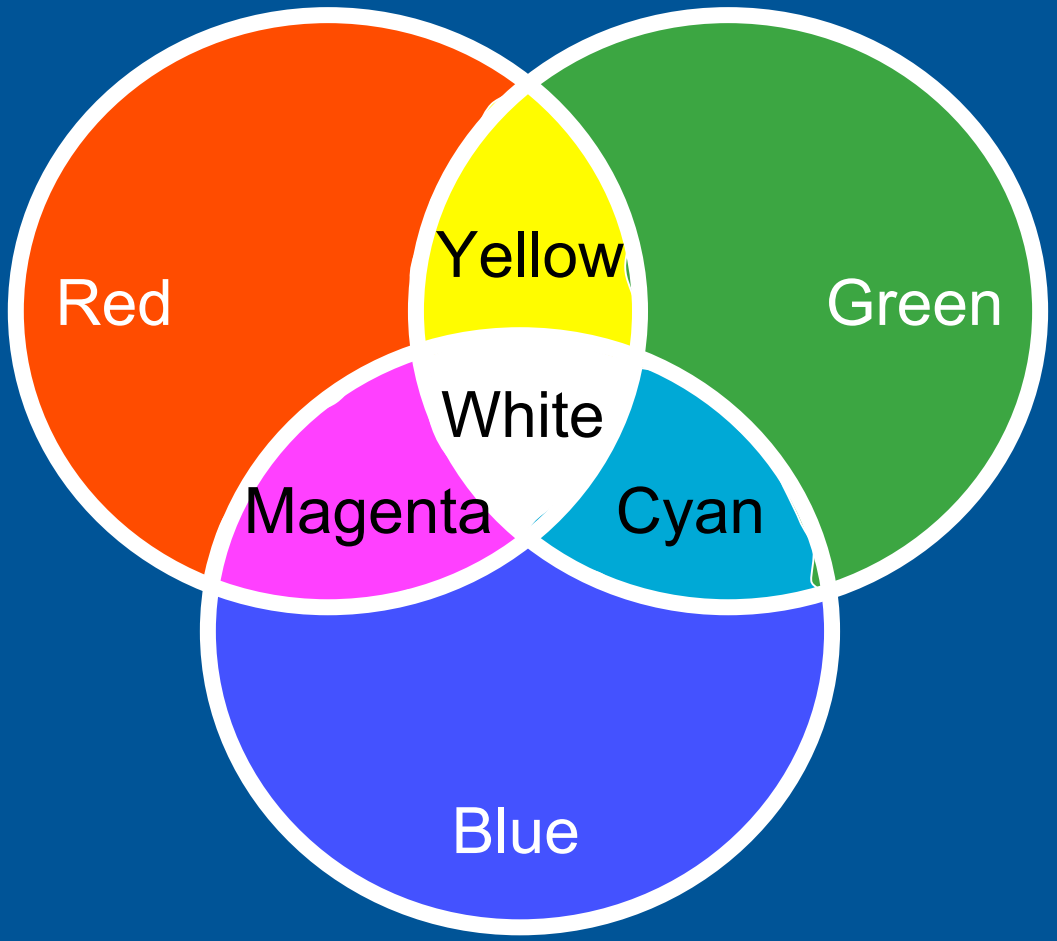
Mixing coloured lights - Secondary Colours



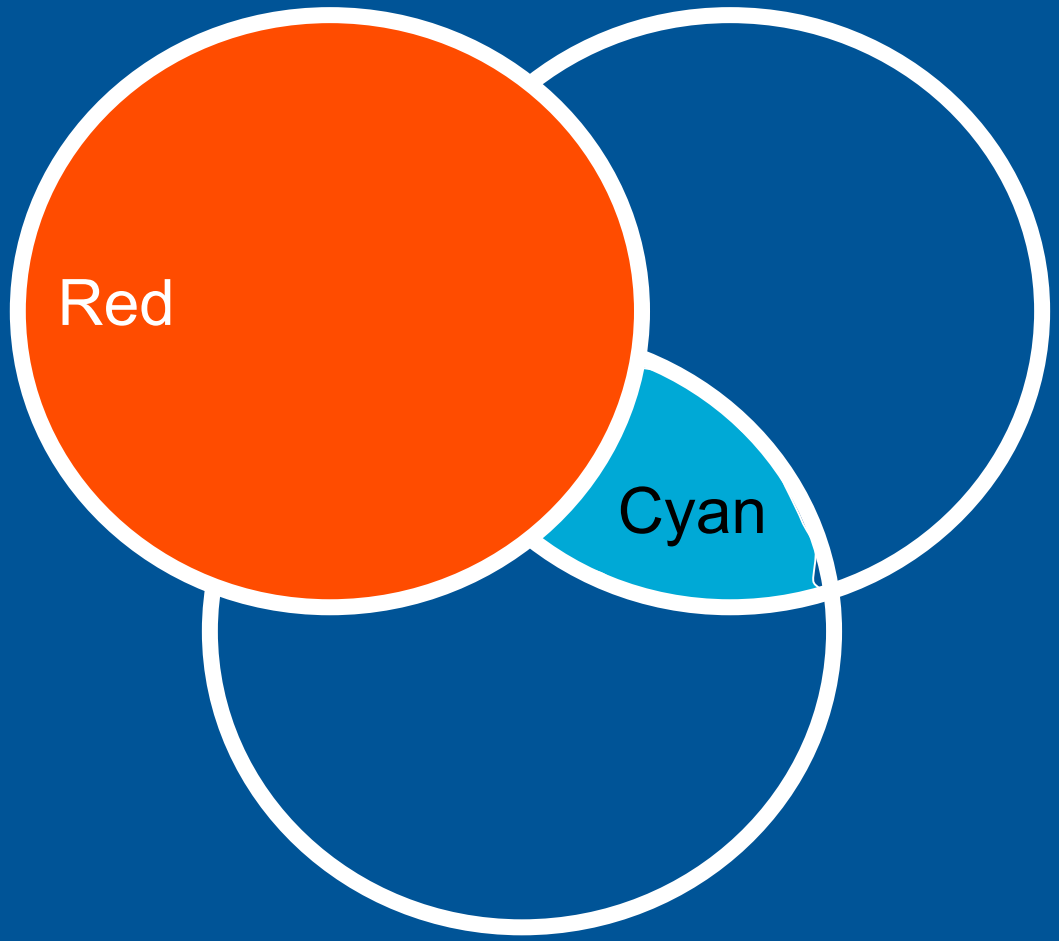
Mixing coloured lights - Secondary Colours



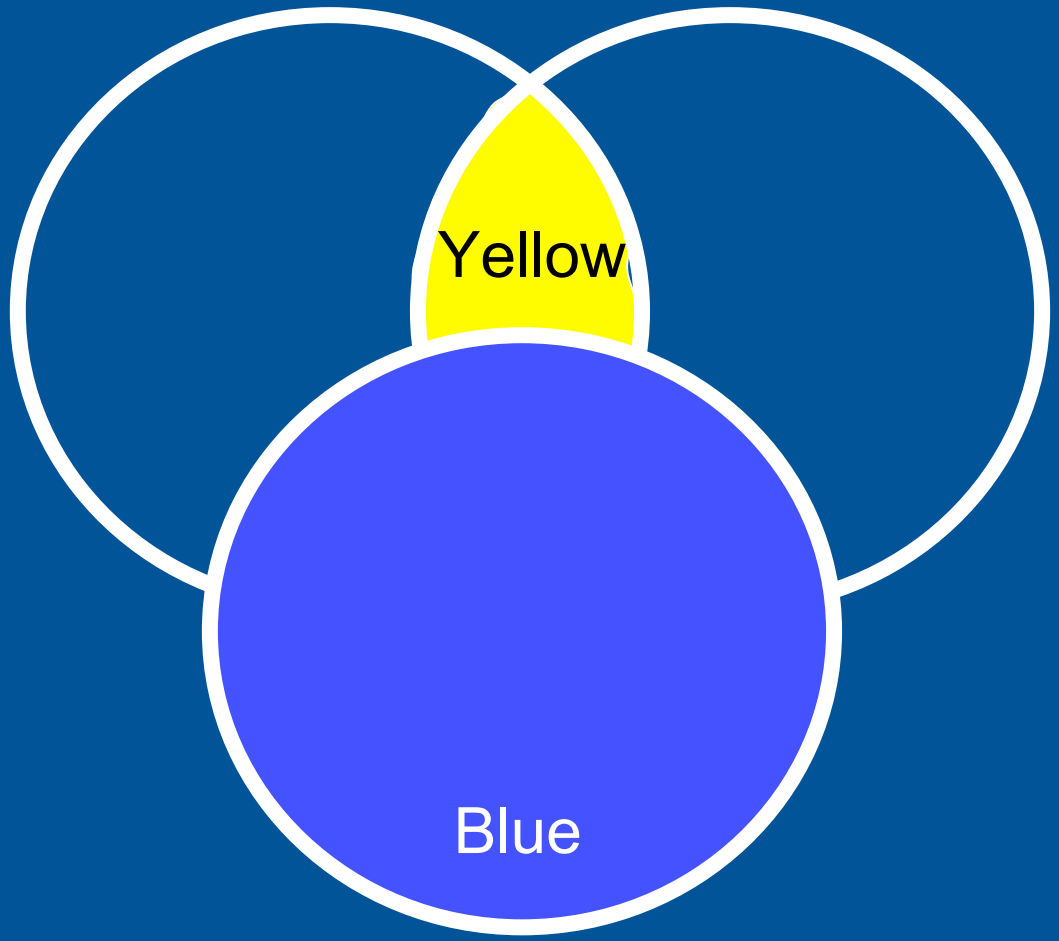
Mixing coloured lights - Secondary Colours and white



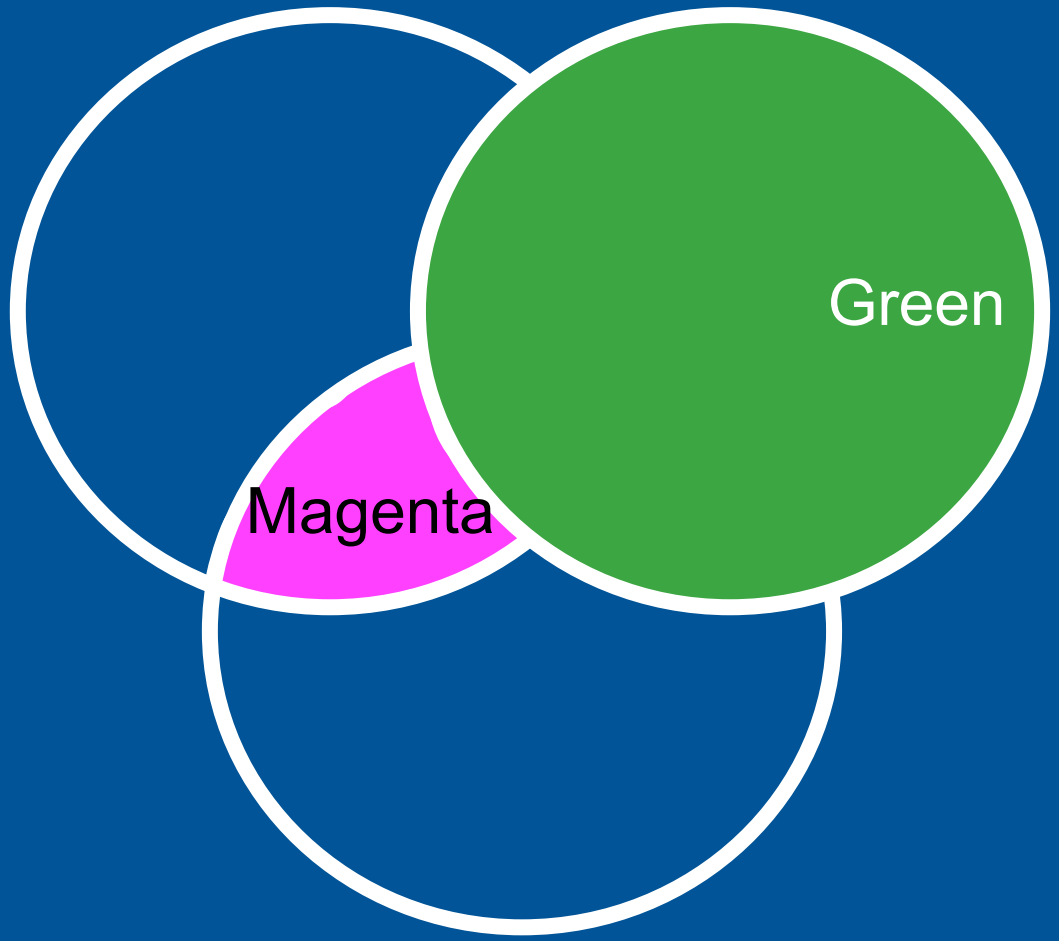
Complementary colours

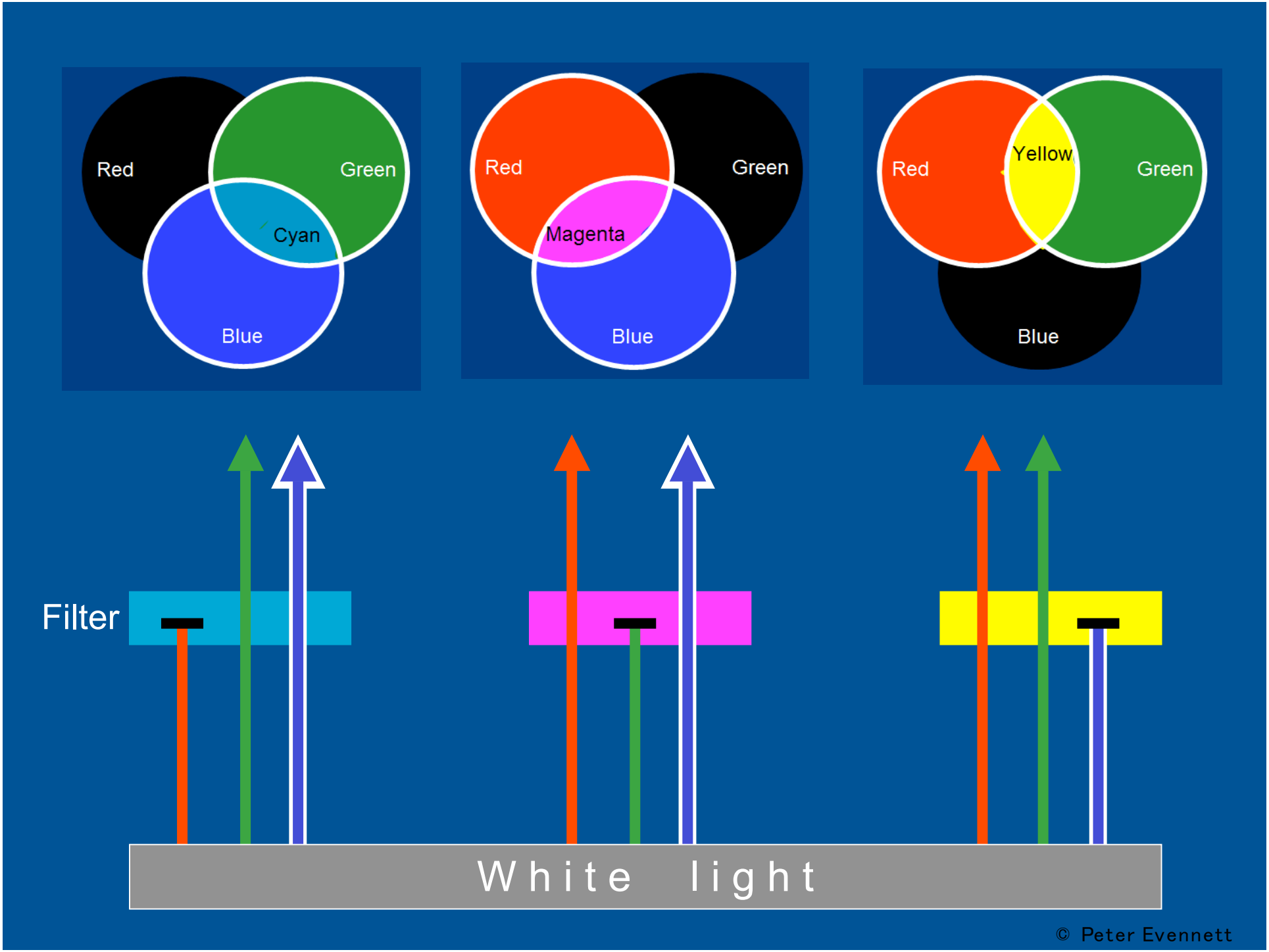


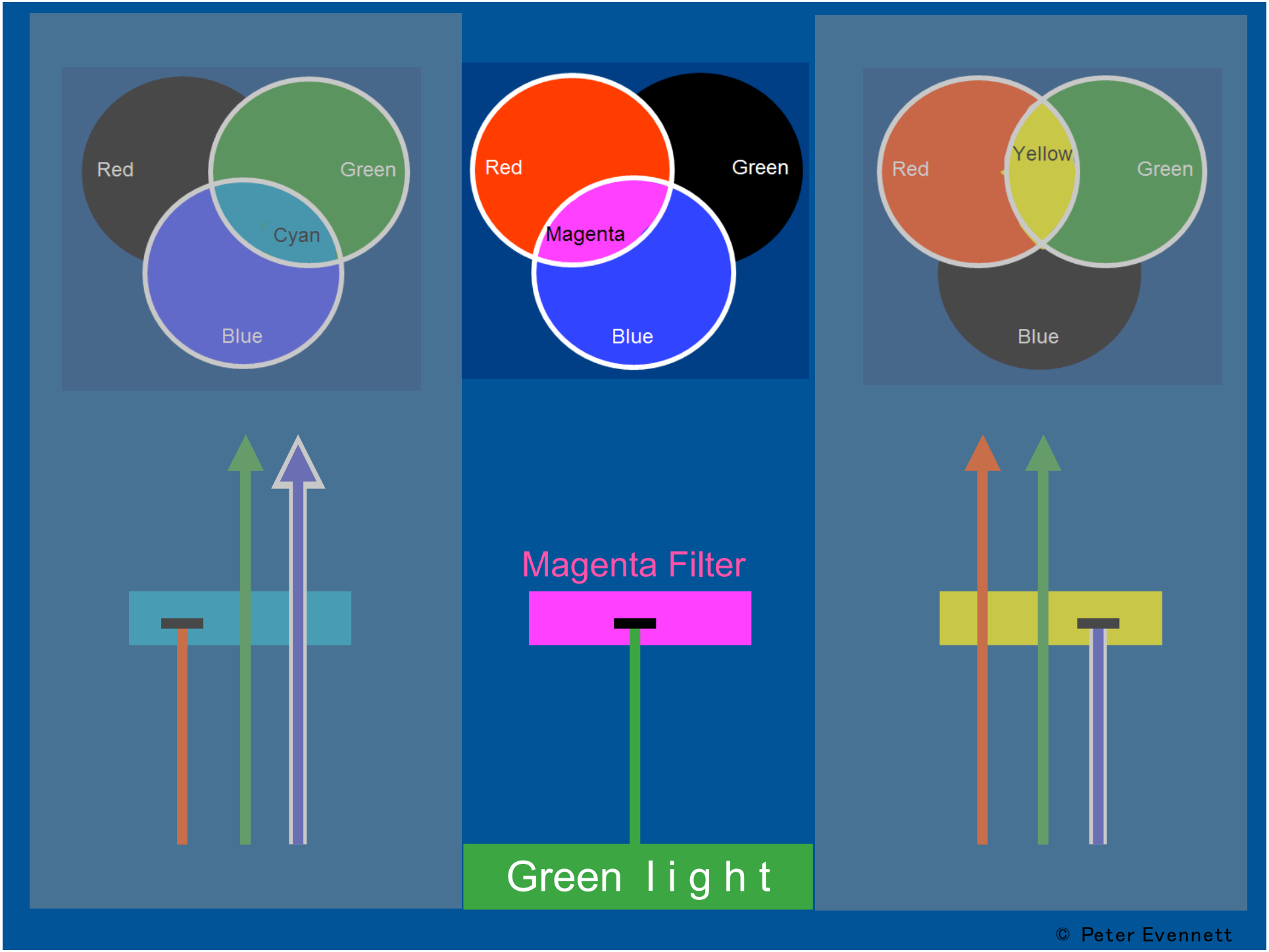
Complementary colours



Complementary colours




Red
Green
Cyan
Blue
Red
Green
Magenta
Blue
Red
Yellow
Green
Blue
Filter
White light
© Peter Evennett


Red
Green
Cyan
Blue
Red
Green
Magenta
Blue
Red
Yellow
Green
Blue
Magenta Filter
Green light
© Peter Evennett

Transmitted-light
Bright field

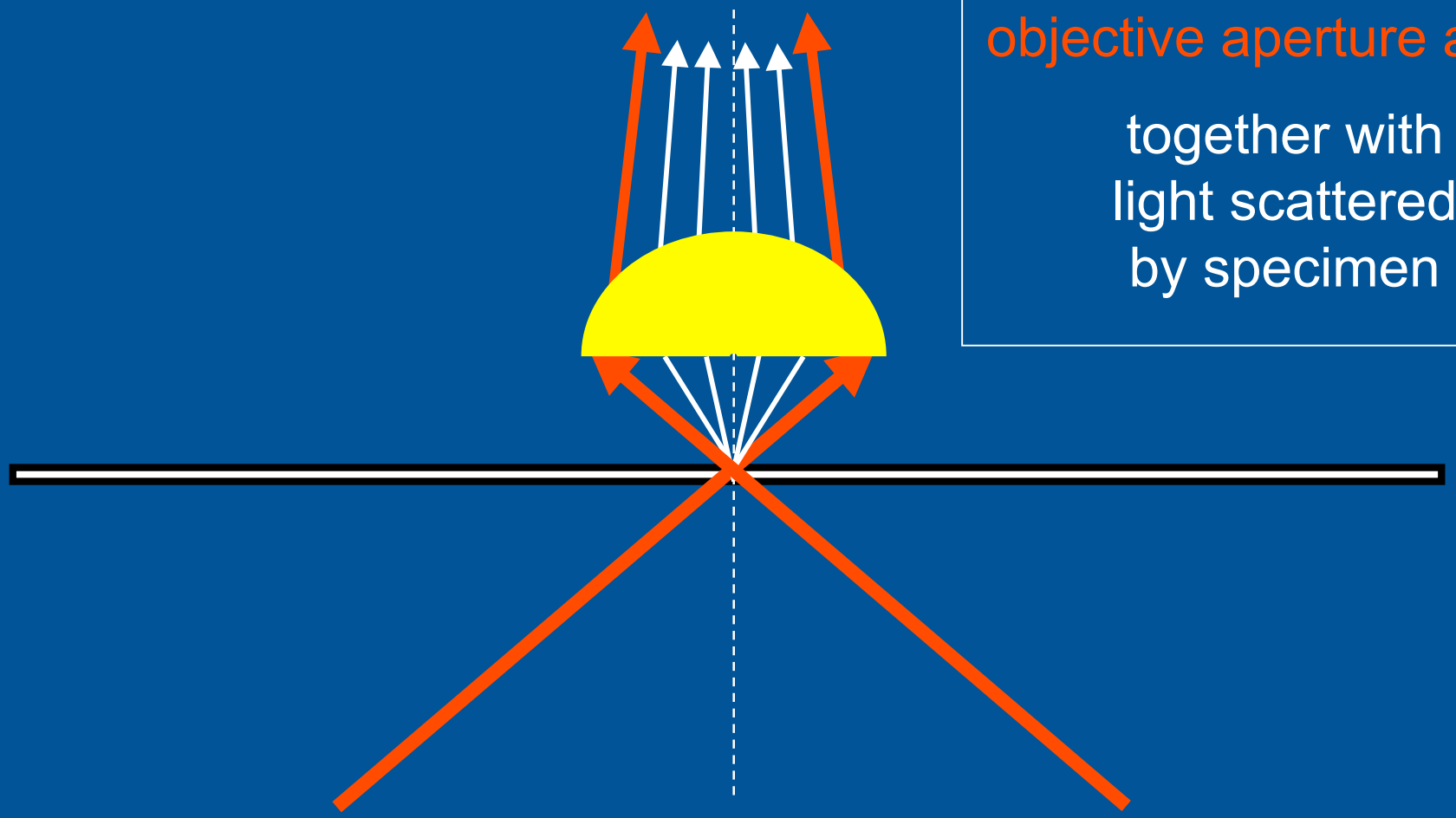
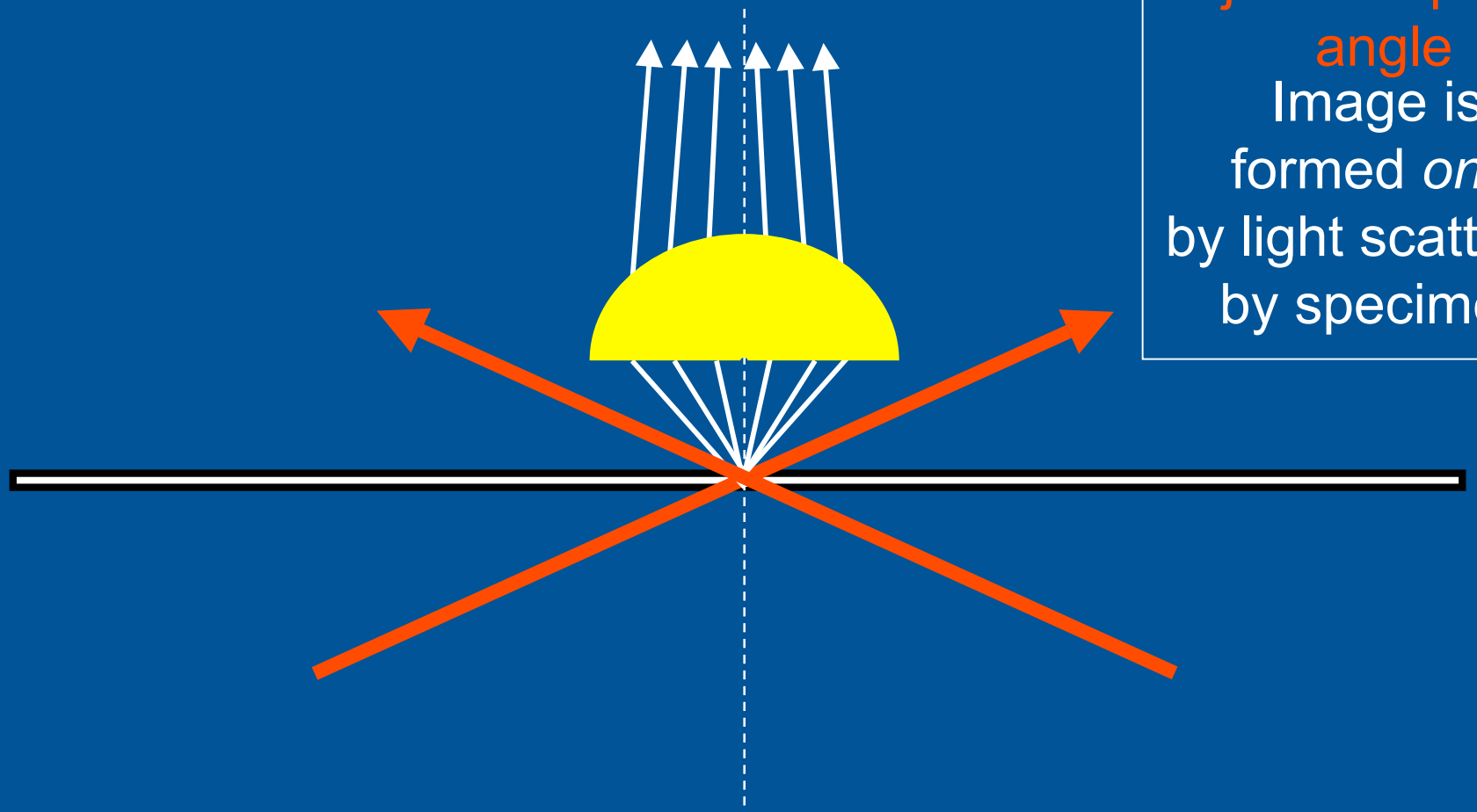


Image formed from illumination which enters ***within*** objective aperture angle

together with light scattered by specimen

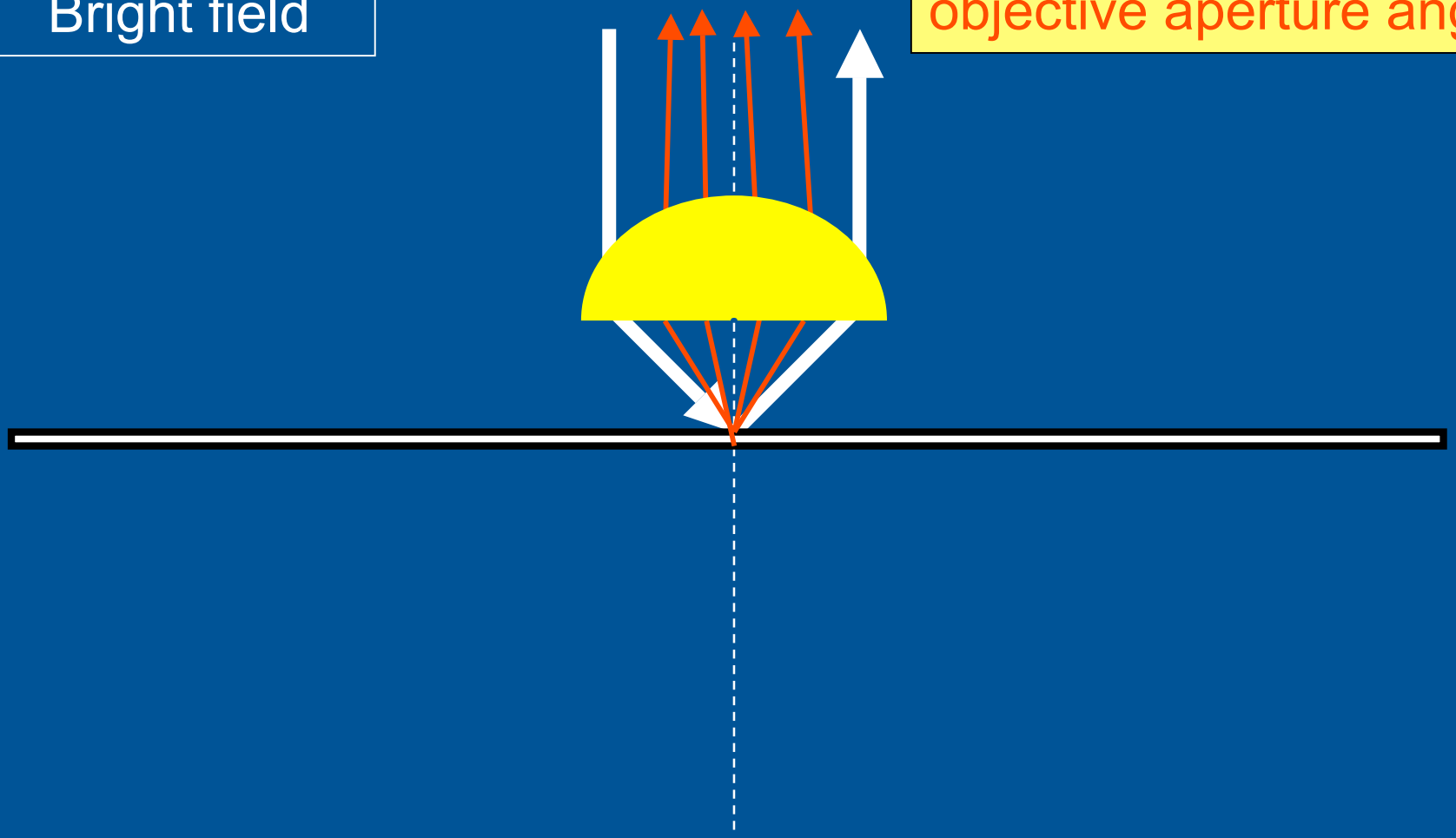
Transmitted-light
Dark field



Illumination falls on specimen ***outside*** objective aperture angle

Image is formed *only* by light scattered by specimen

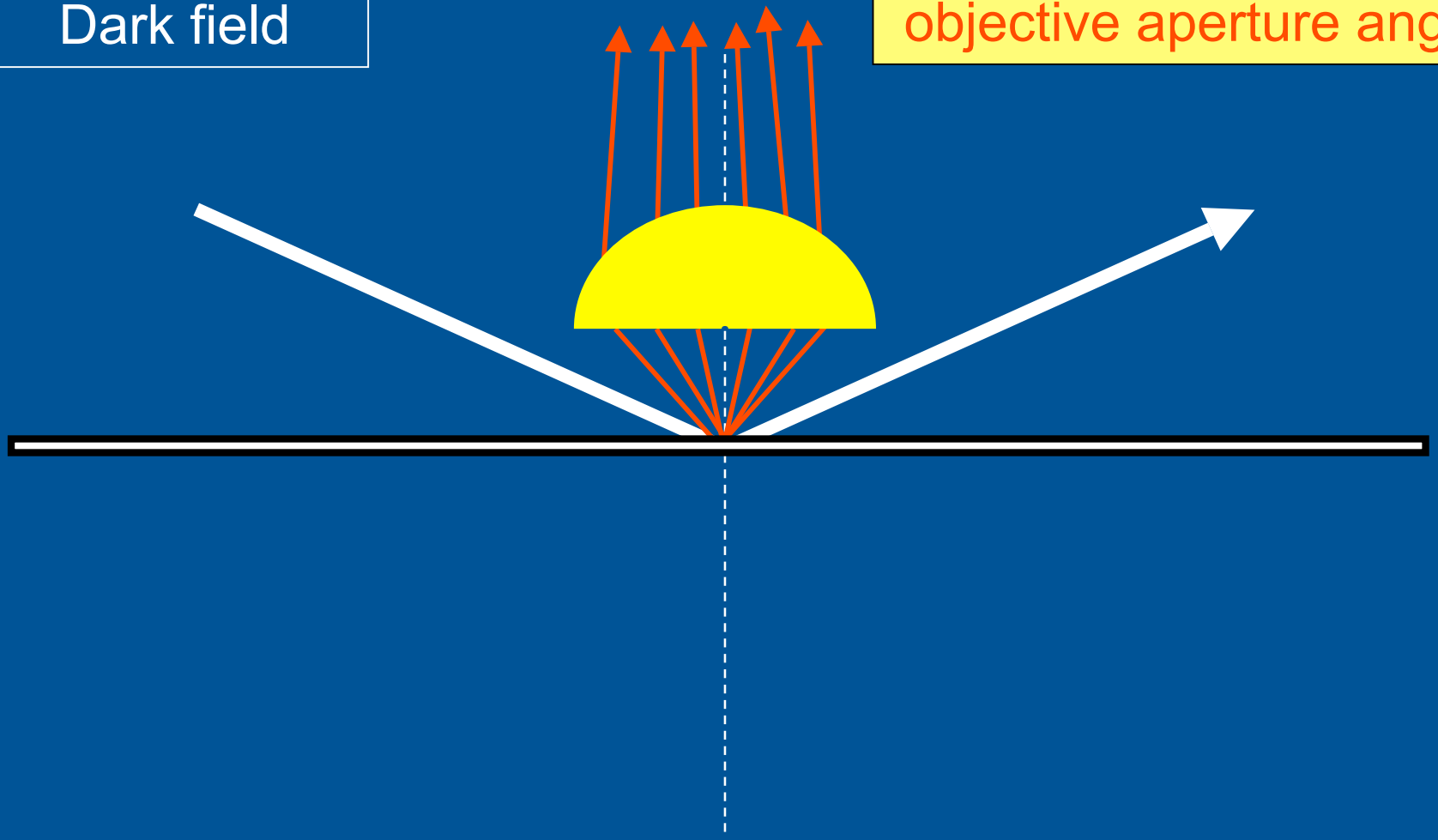
Reflected-light
Bright field

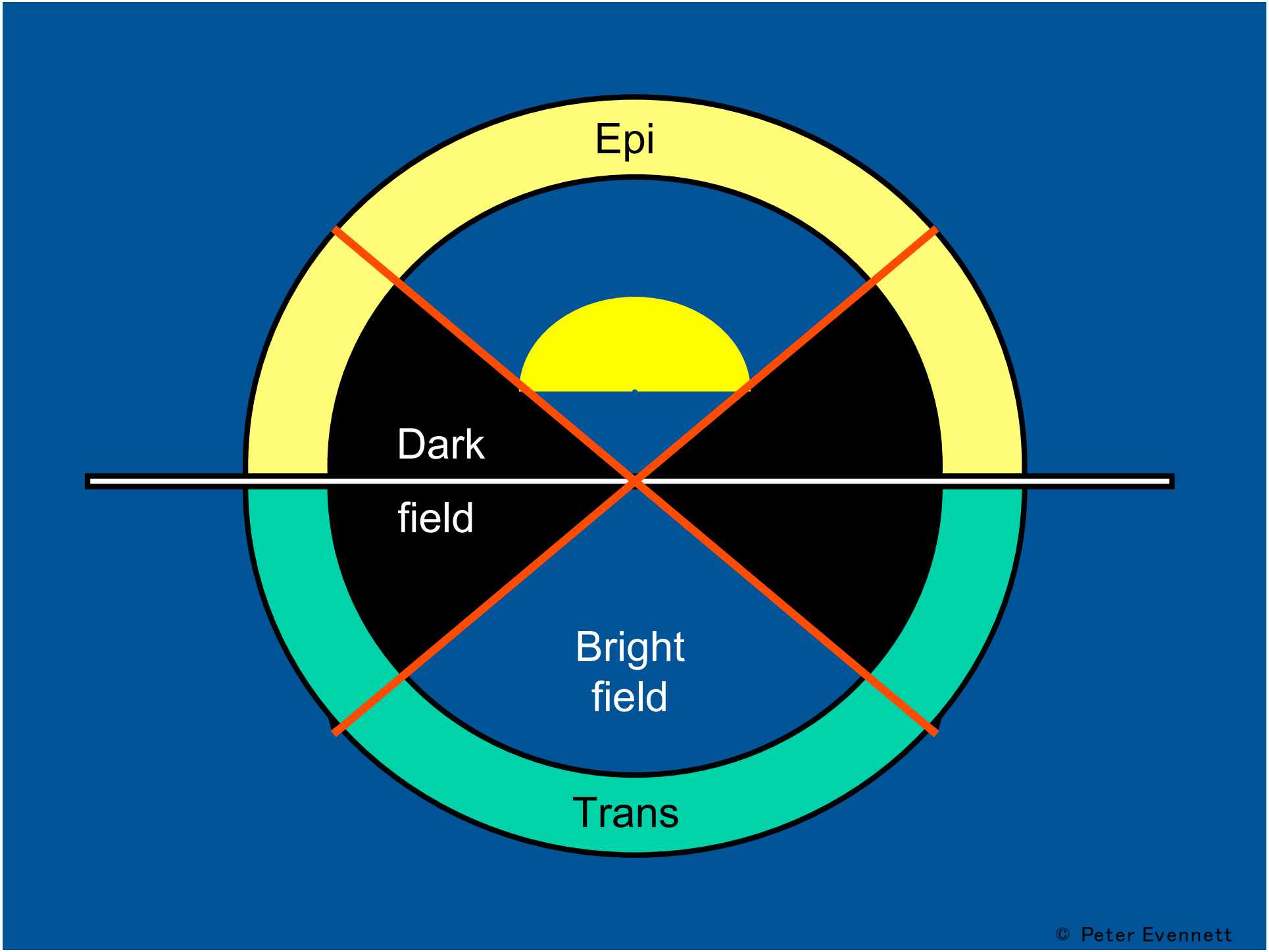


Bright field:
Illumination from ***within*** objective aperture angle

Reflected-light
Dark field

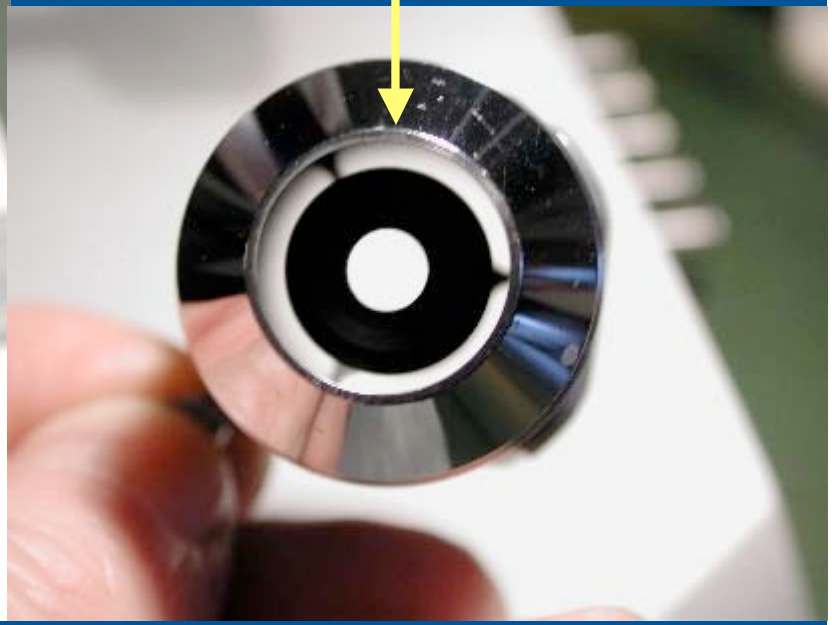
Dark field:
Illumination from ***outside*** objective aperture angle

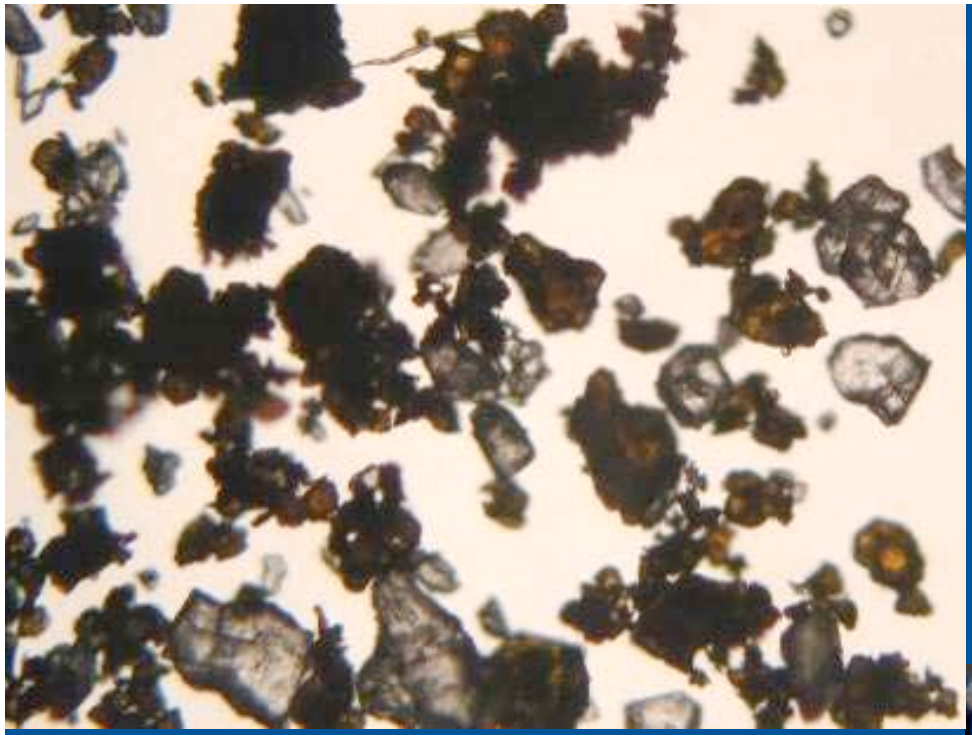




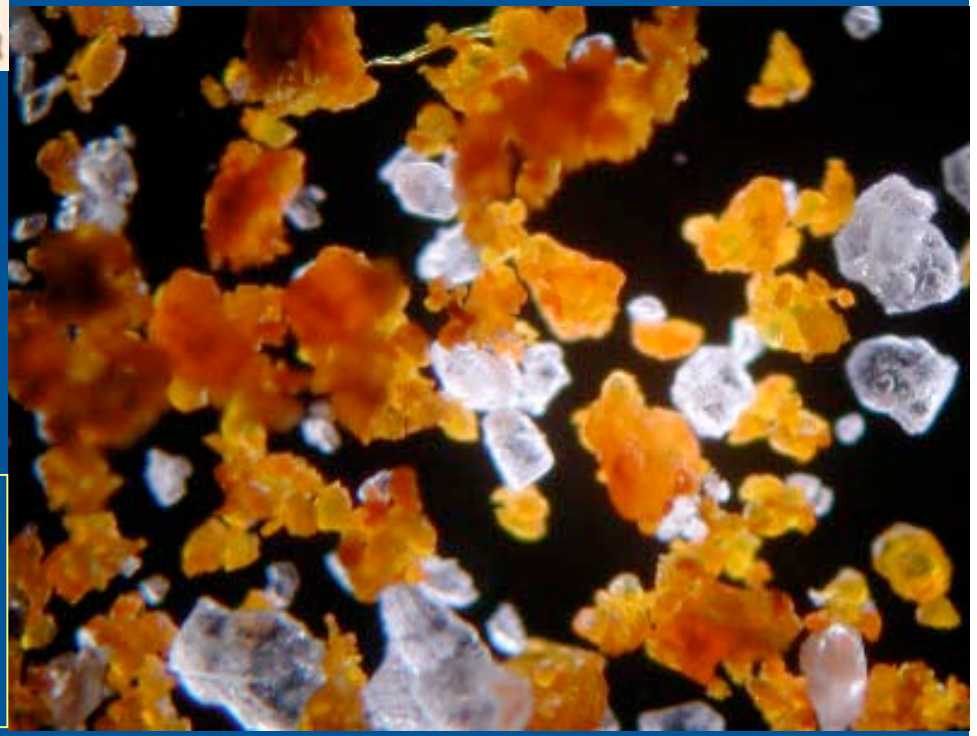
Normally-functioning objective lens

Surround with reflecting end delivering light at oblique angle on to surface of specimen





Transmitted-light
Bright field



Reflected-light
Dark field

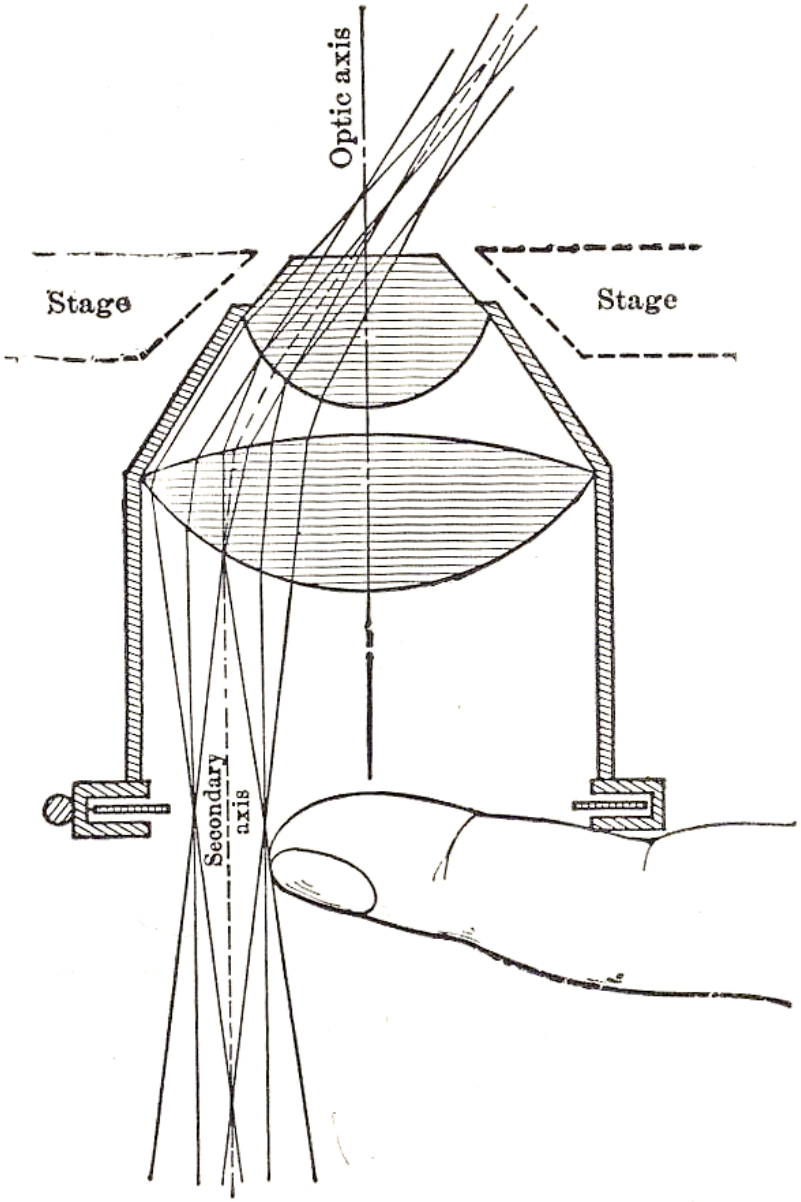
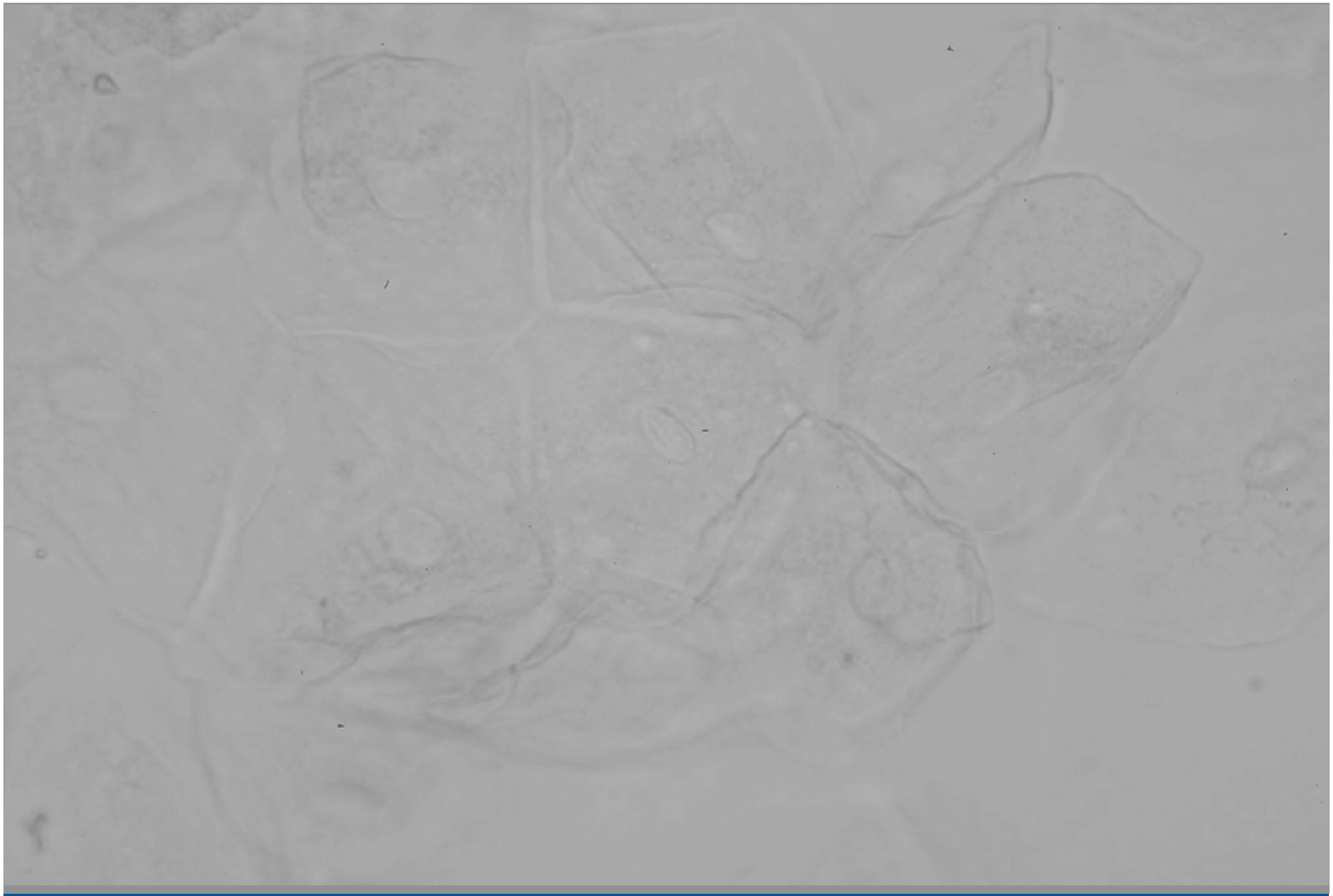


FIG. 62. OBLIQUE LIGHT WITH A CONDENSER.
(From Chamot).

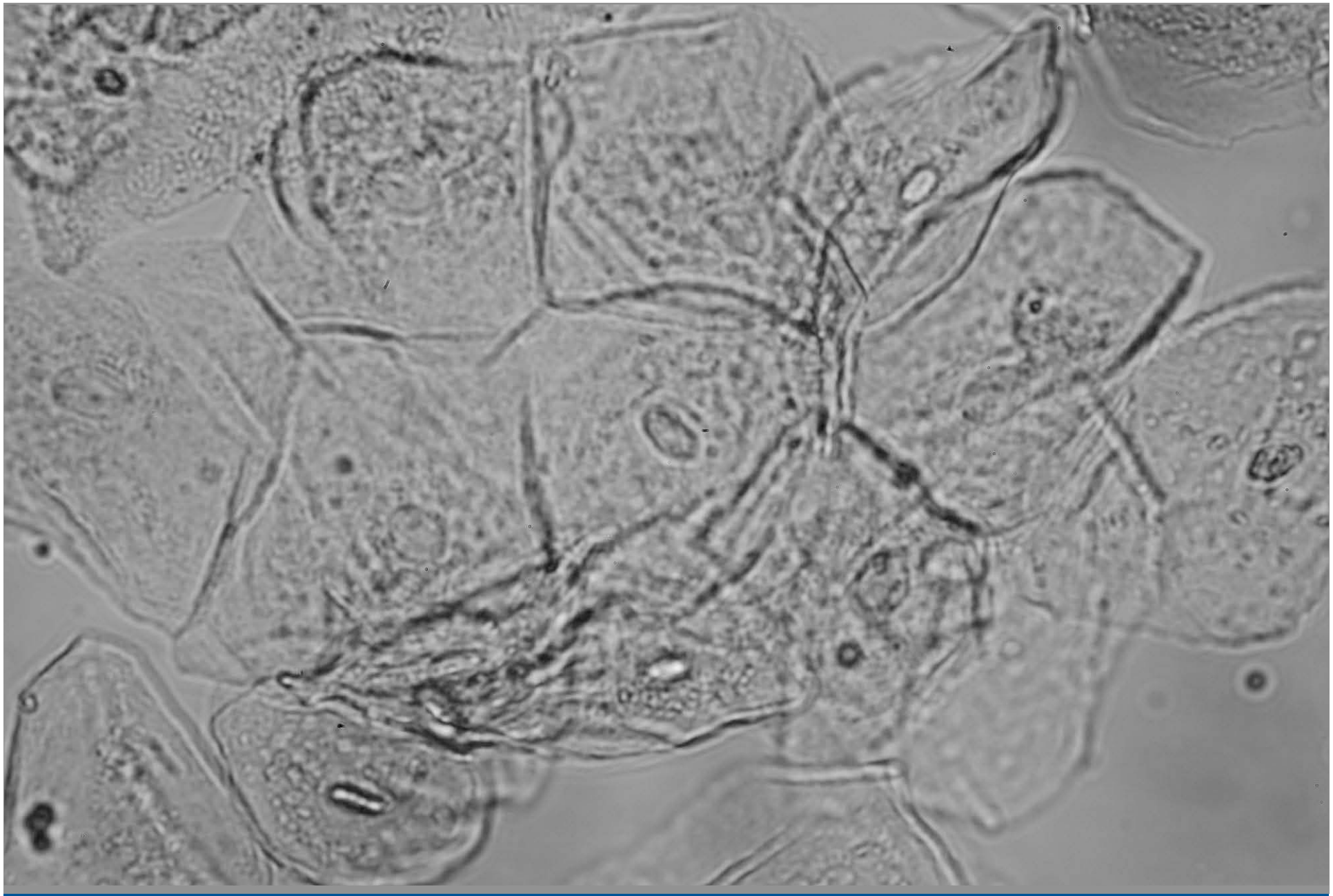
‘Digital’ oblique illumination

- ie using your finger!

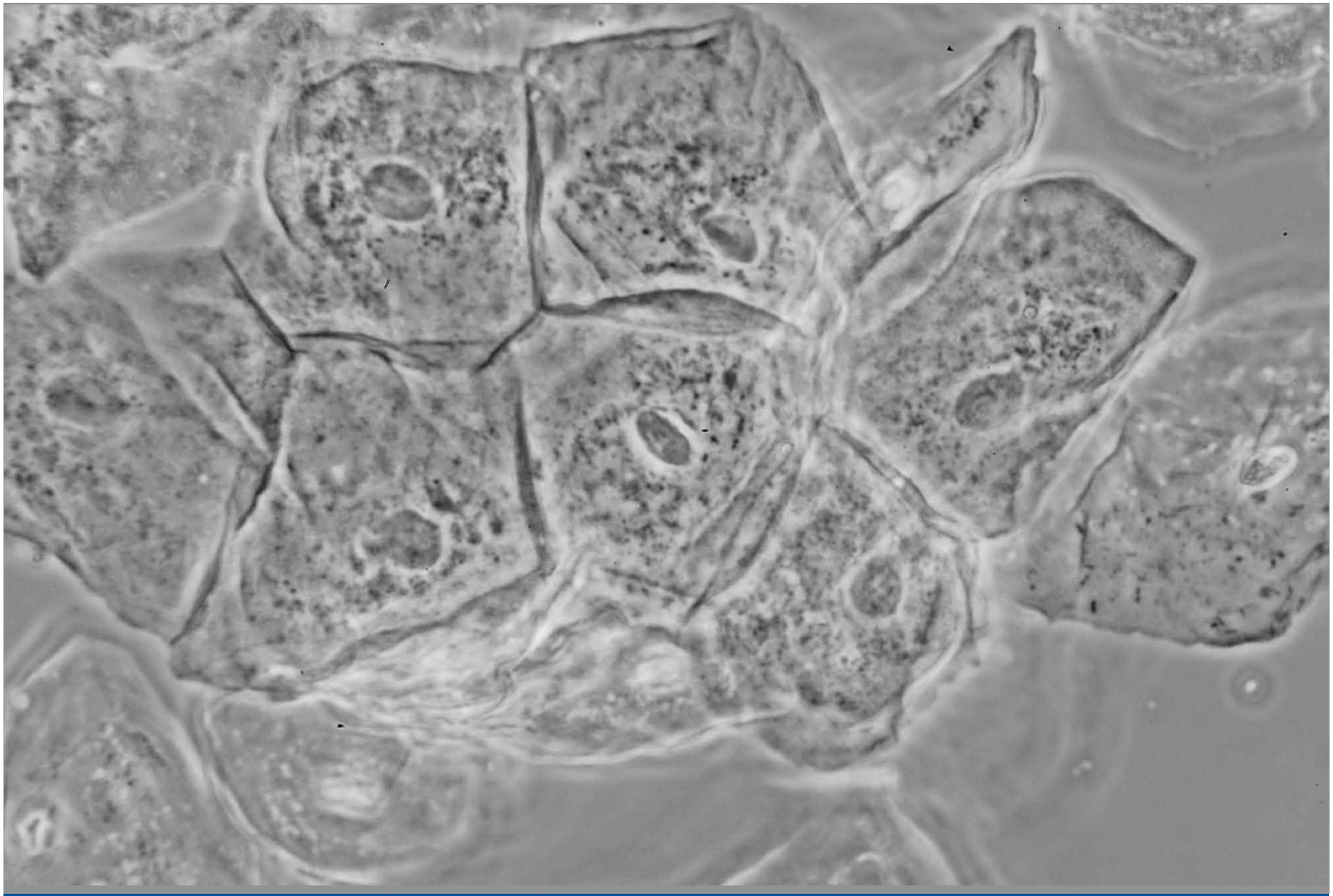
A ‘no-cost’ option for most microscopes



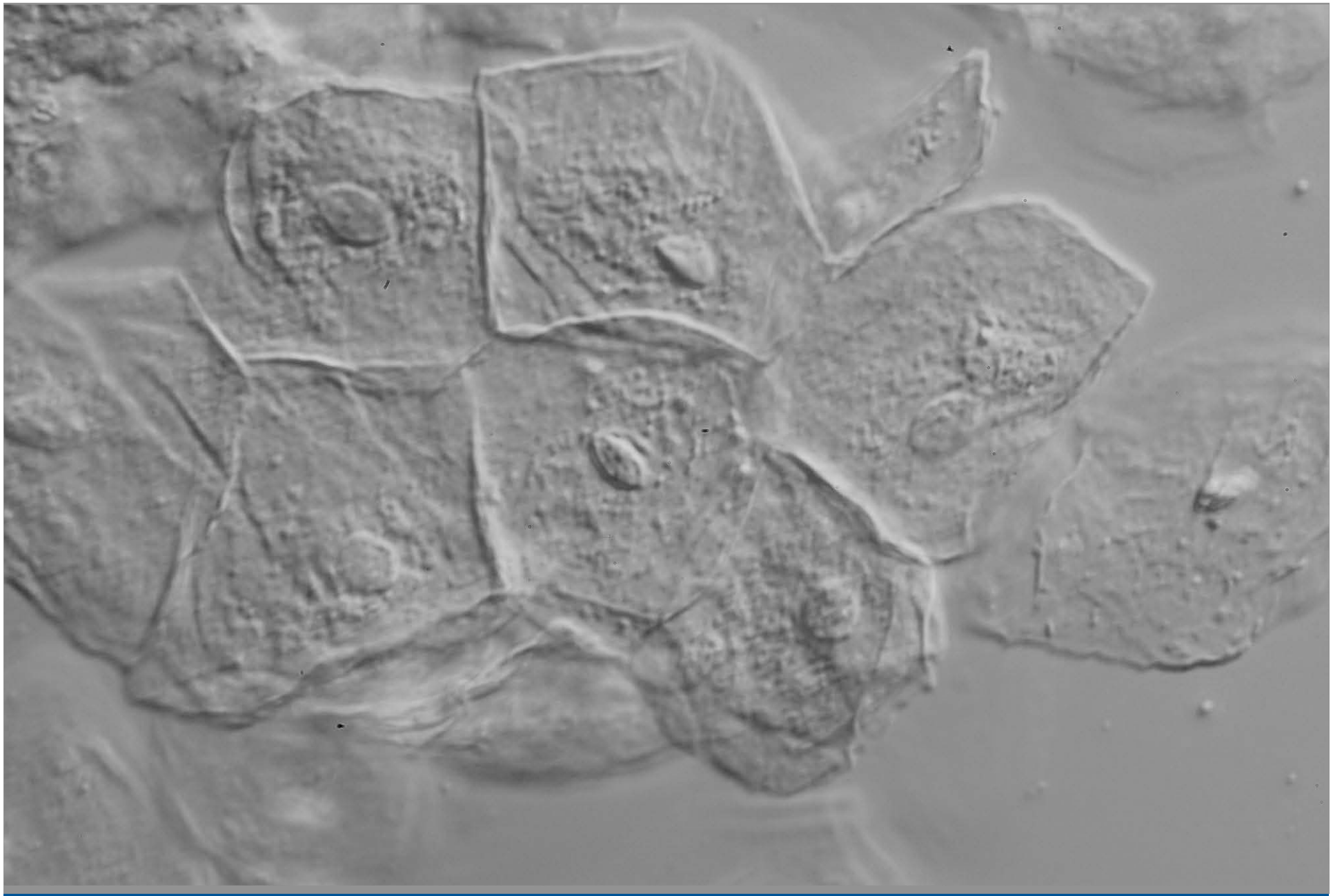
Bright field, full illuminating aperture



Bright field, small illuminating aperture

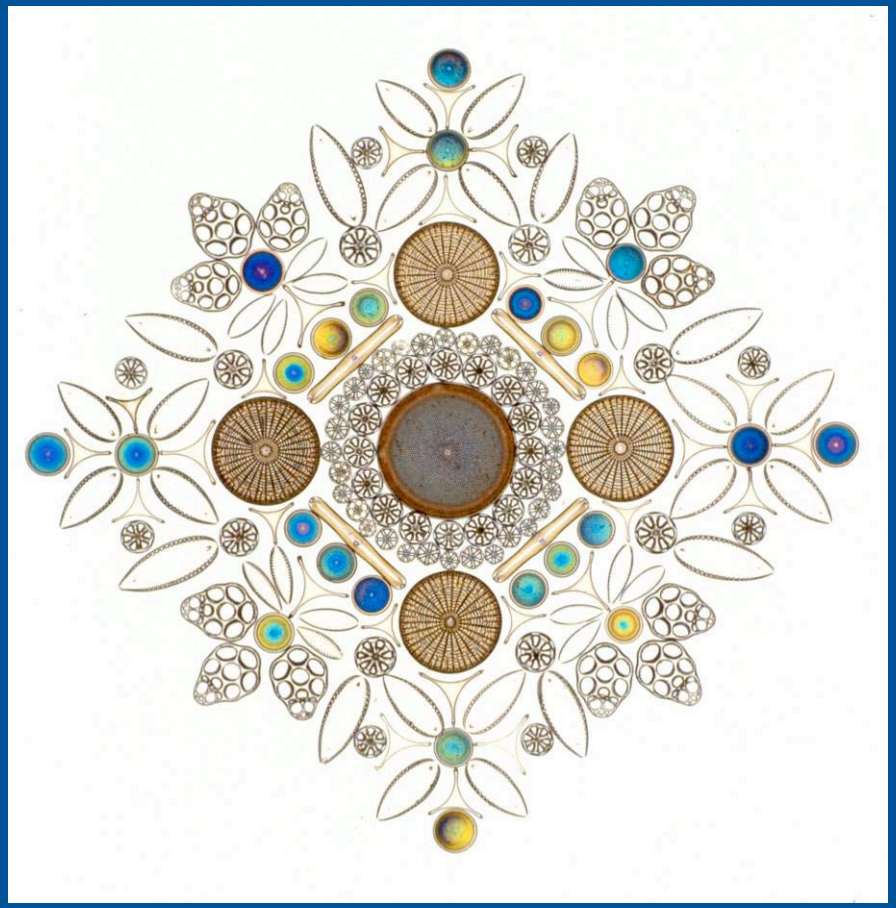


Phase contrast

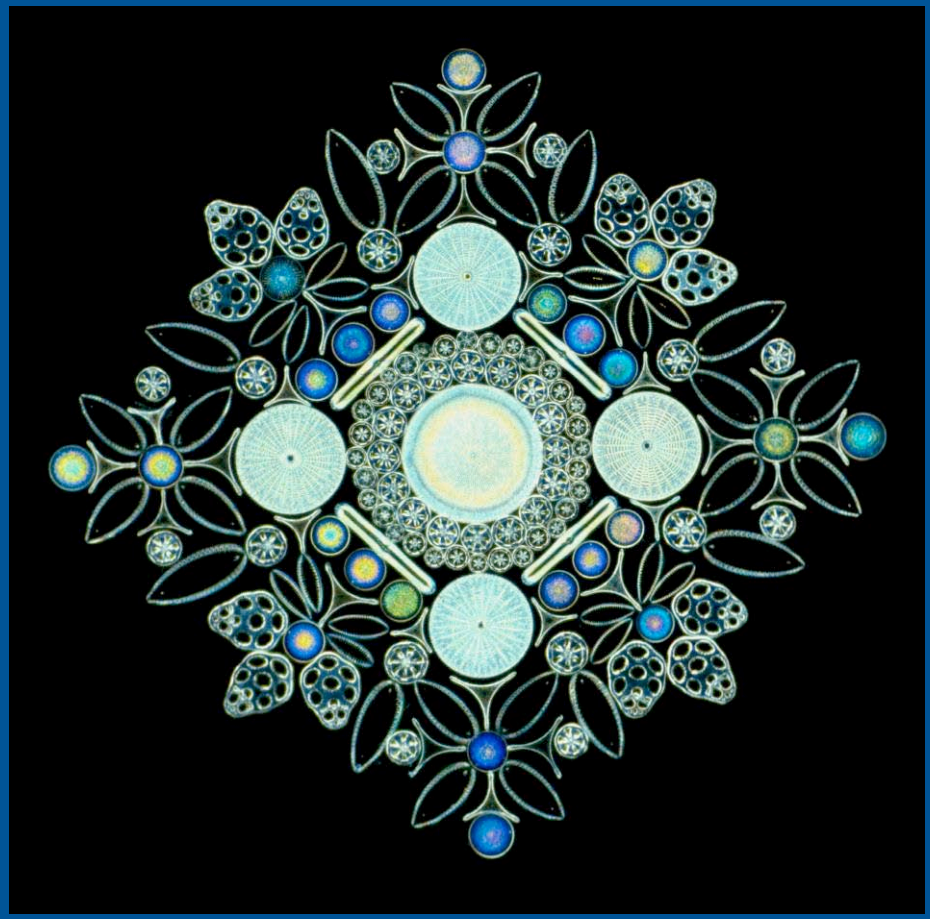


Differential interference contrast

Diatom arrangement: transmitted-light



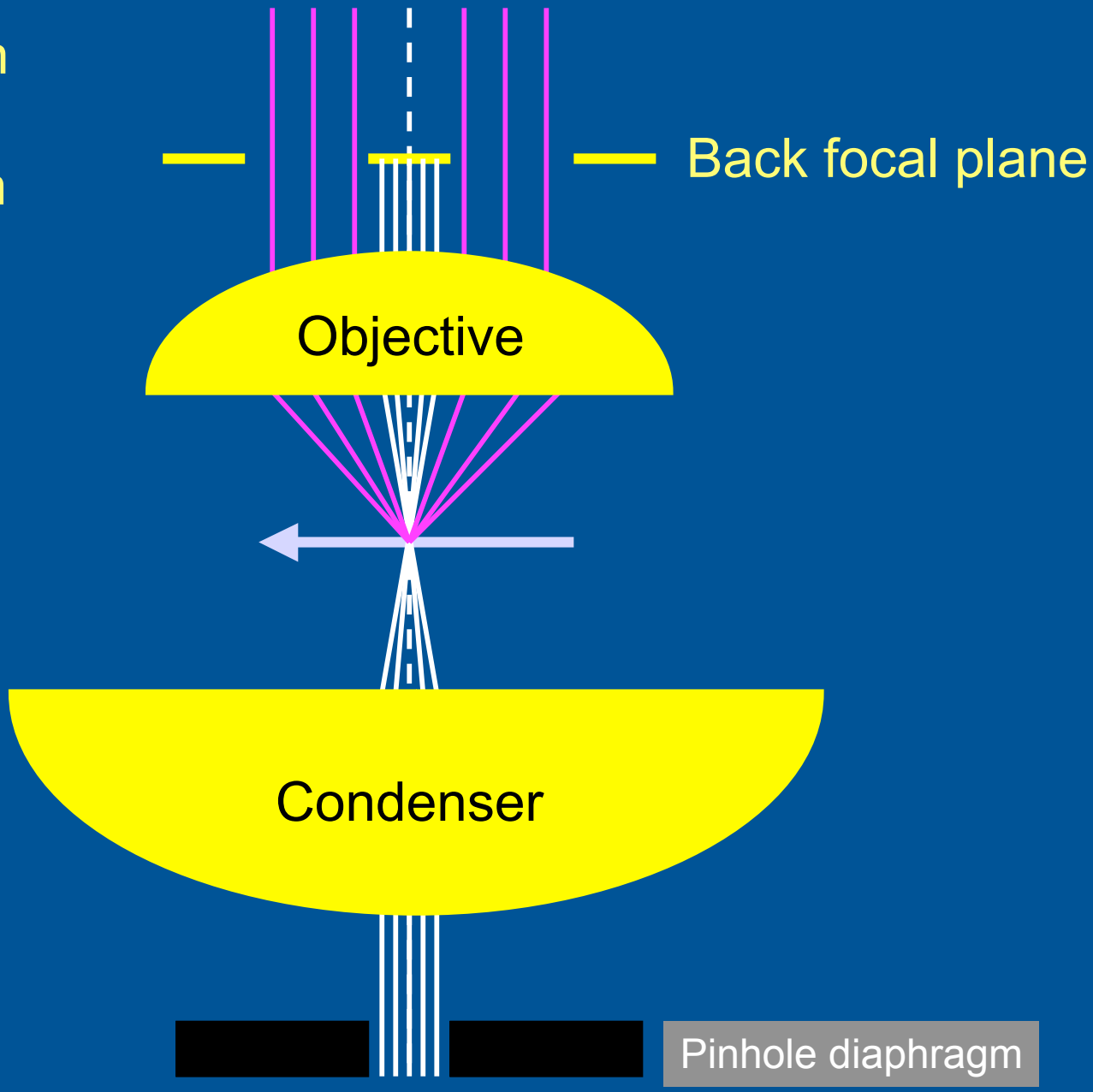
Bright field

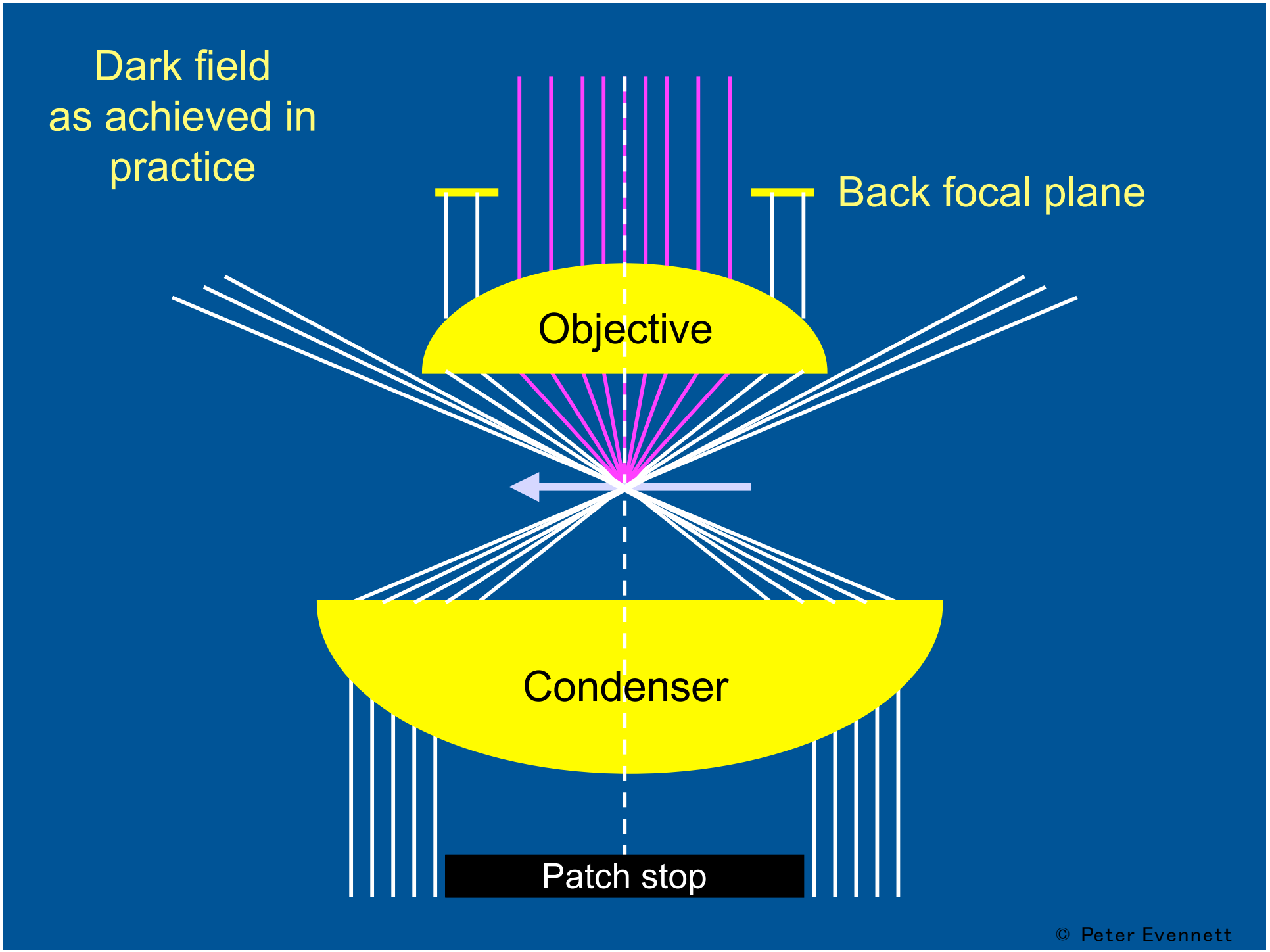


Dark field

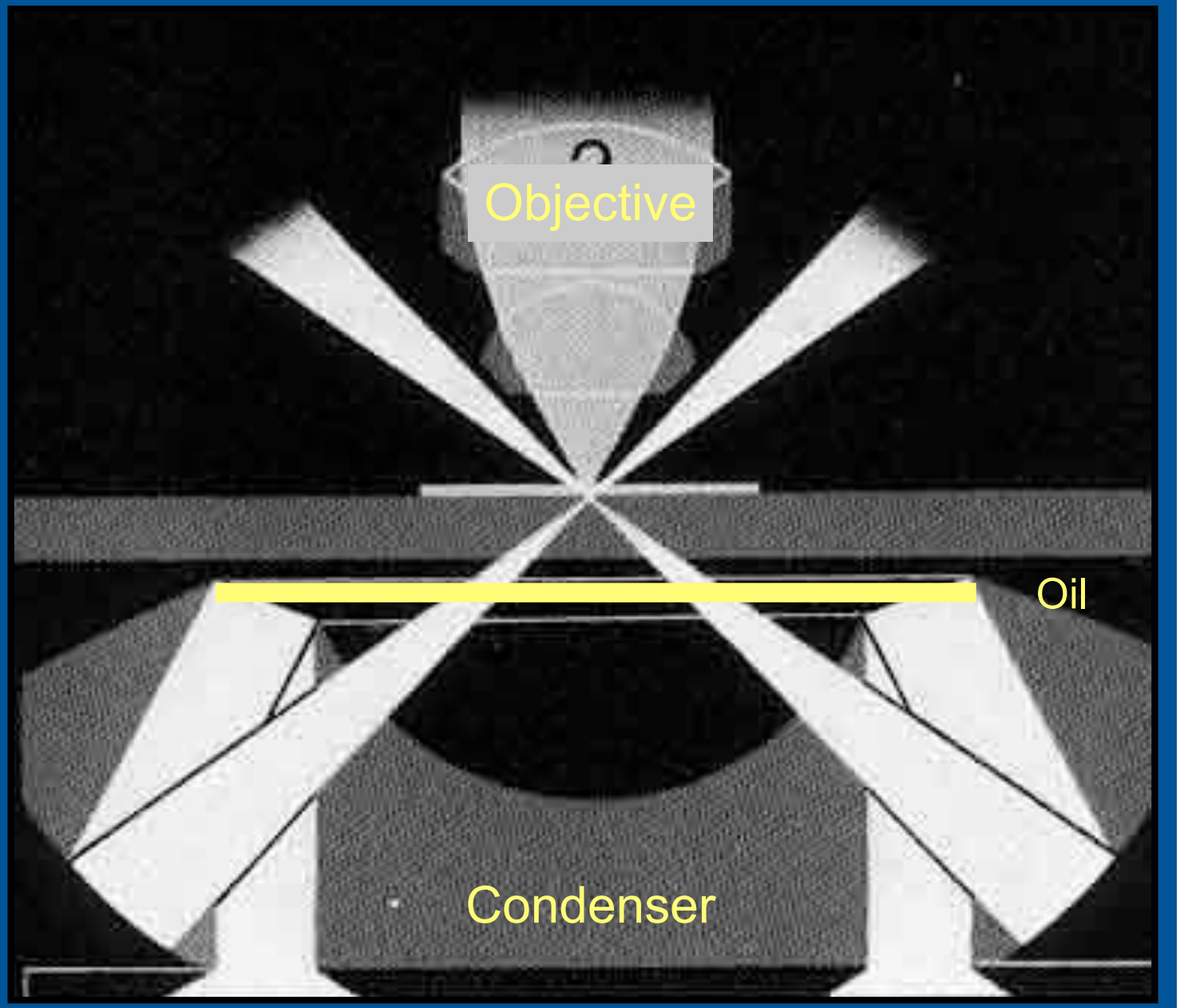
Bryozoa: dark field

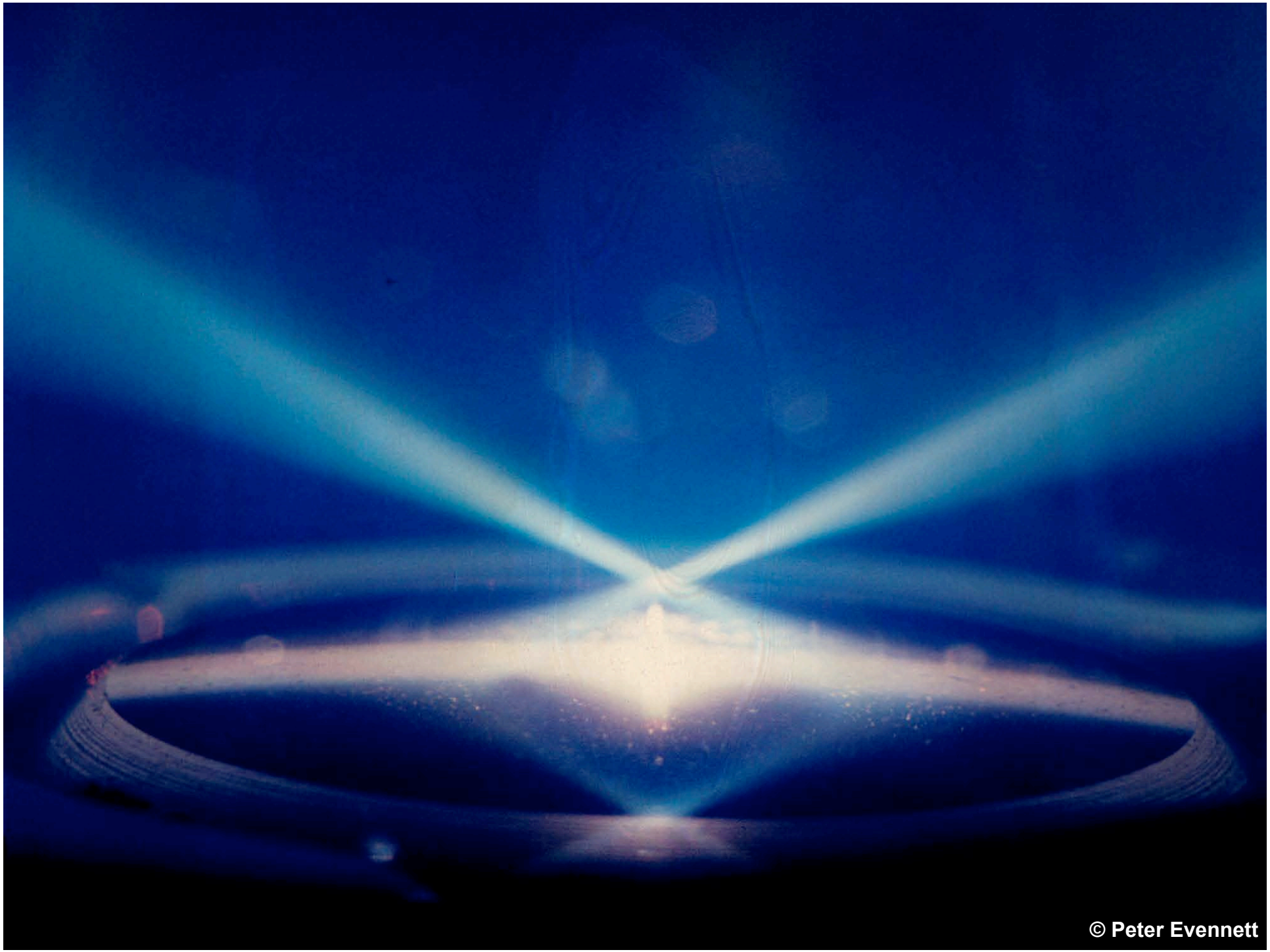
Dark field
as achieved in
diffraction
demonstration



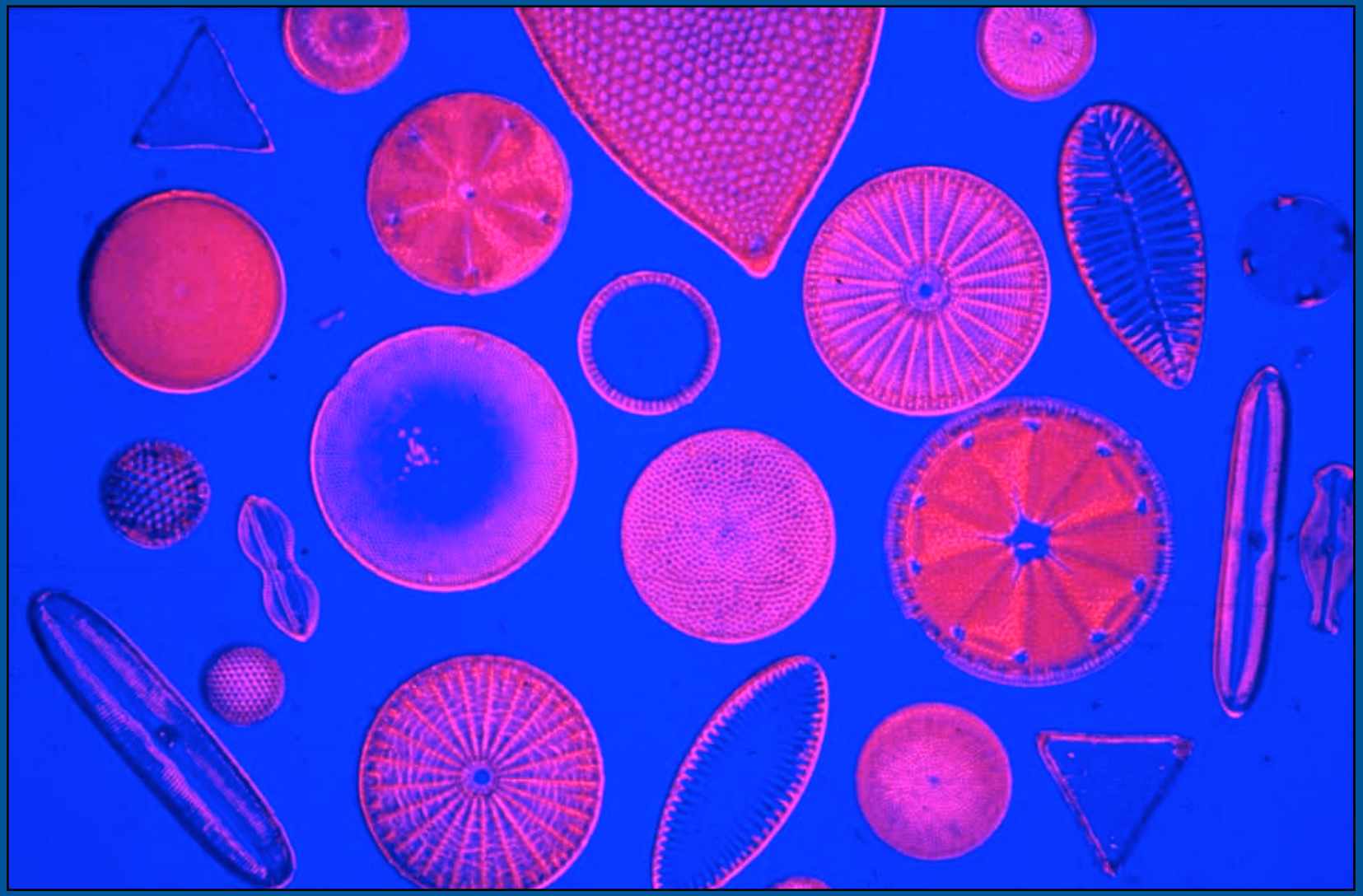

Dark field
as achieved in
practice
Back focal plane
Objective
Condenser
Patch stop
© Peter Evennett

Dark field condenser operating by reflection

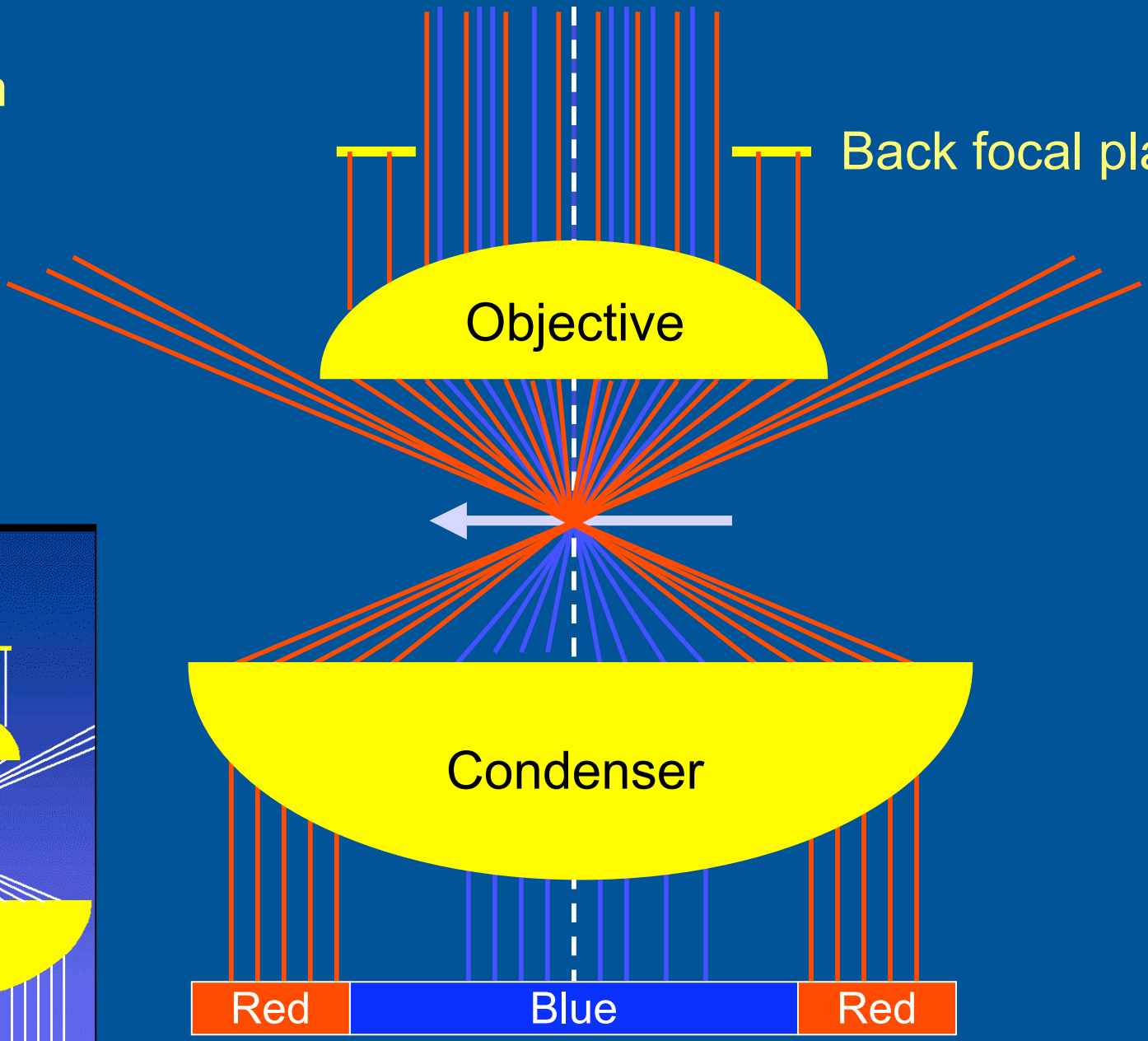


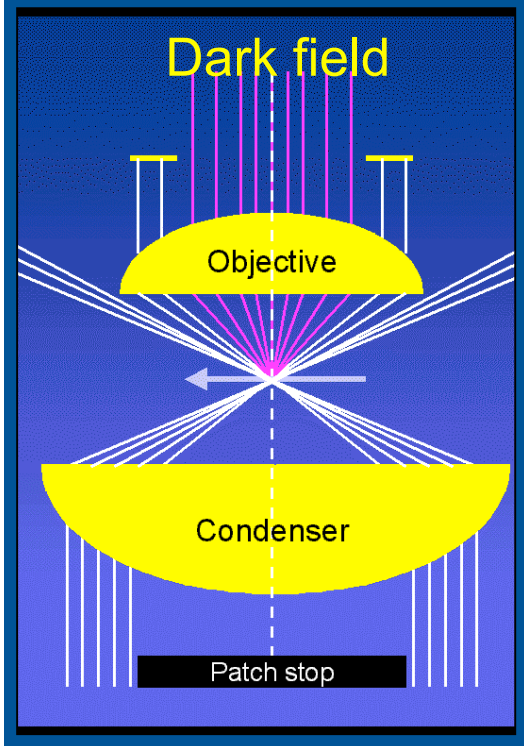

© Peter Evennett

Diatoms: Rheinberg illumination



Rheinberg illumination

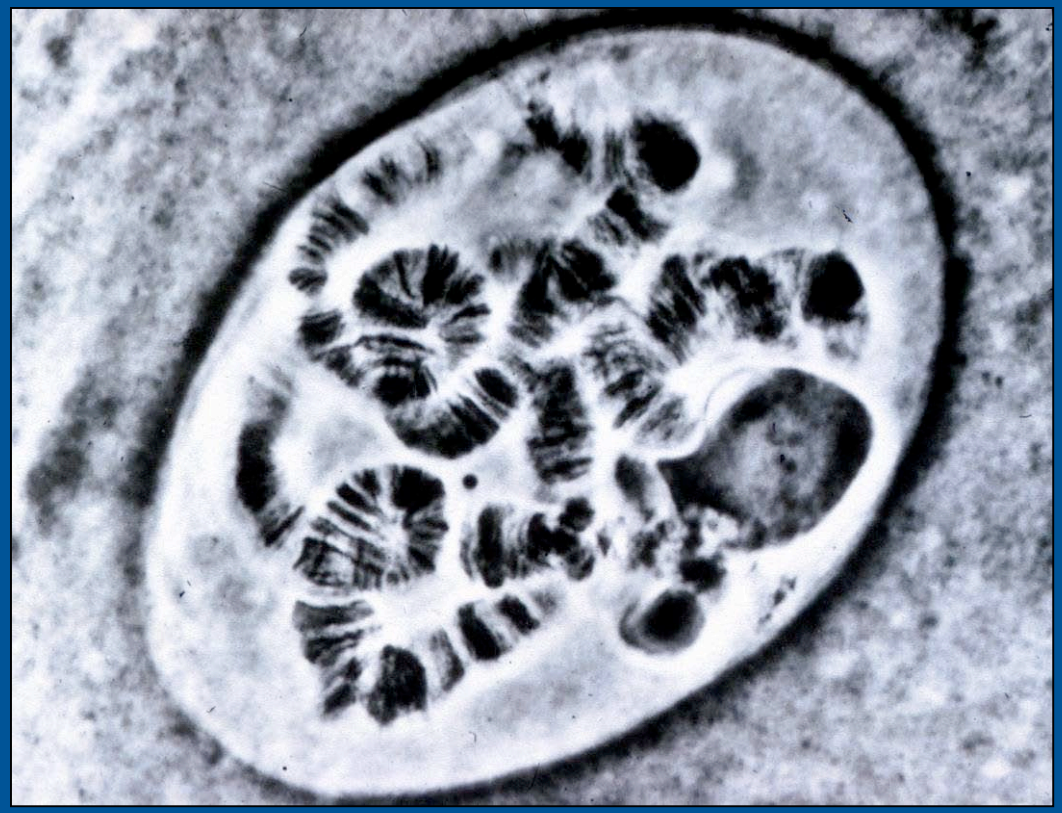




Phase contrast 1933

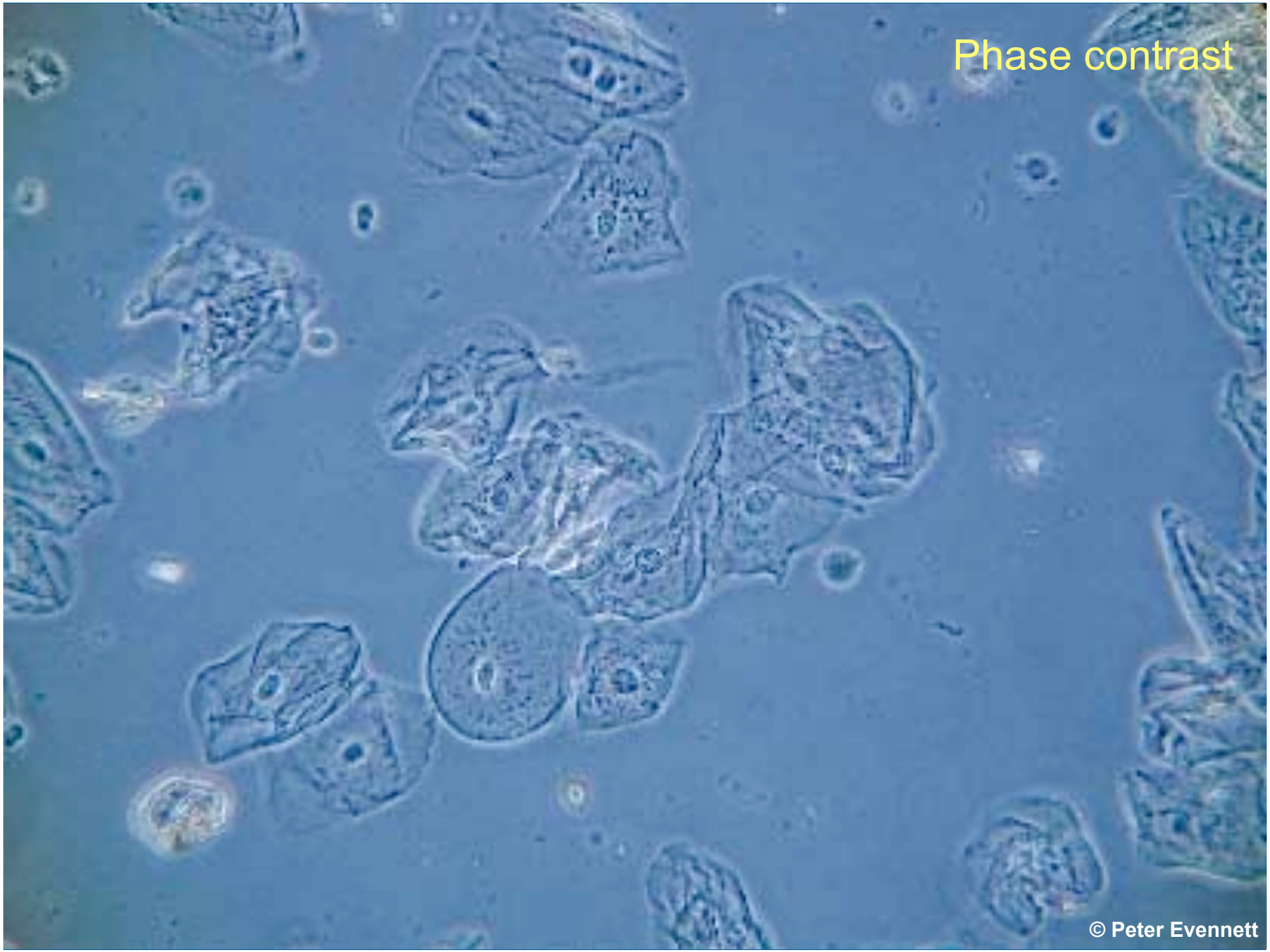
Frits Zernike

1886 - 1966

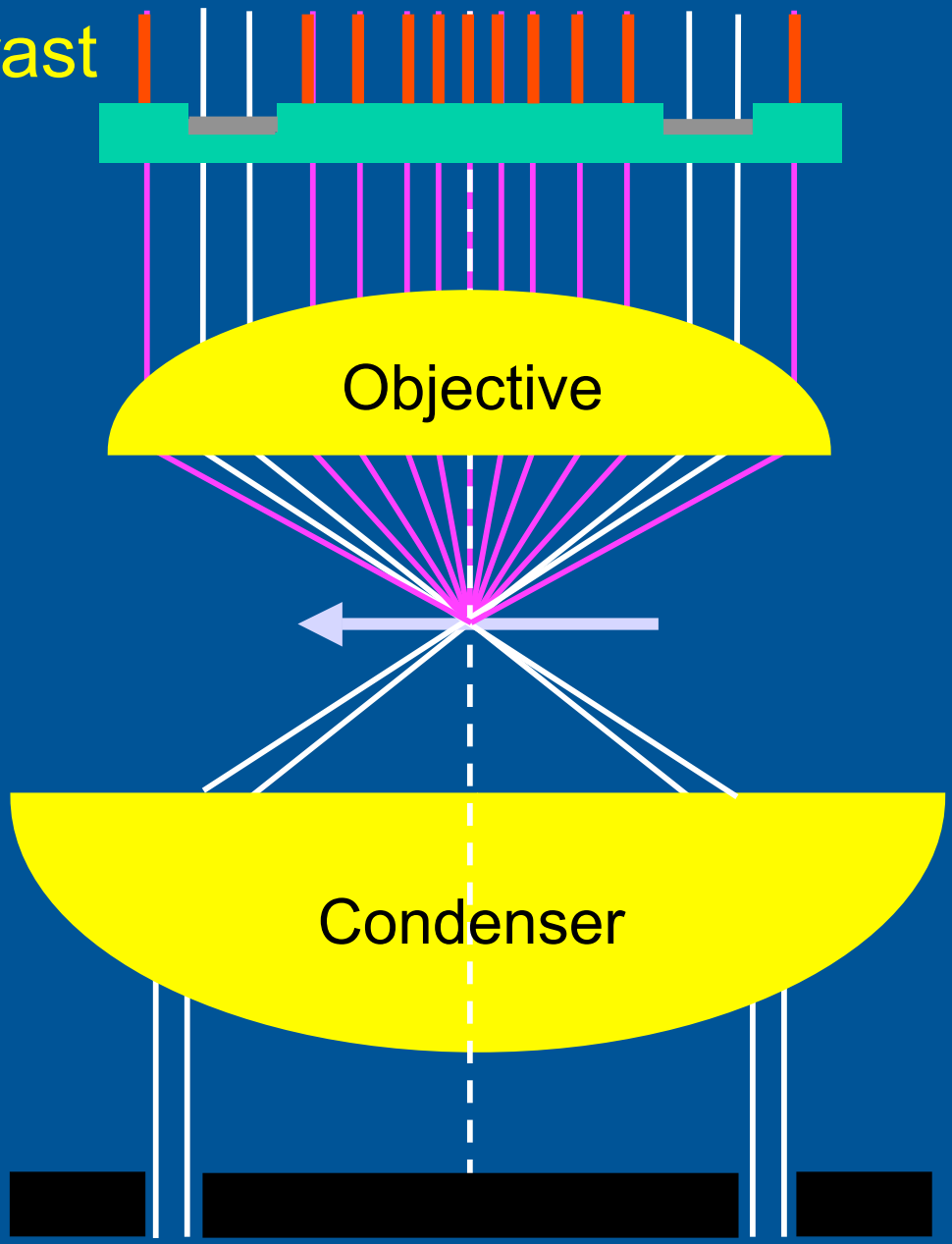


Chromosomes of *Chironomus*

Phase contrast Kurt Michel 1942


Phase contrast
© Peter Evennett

Phase contrast



Phase plate retards scattered light another ¼λ, providing ½λ phase difference

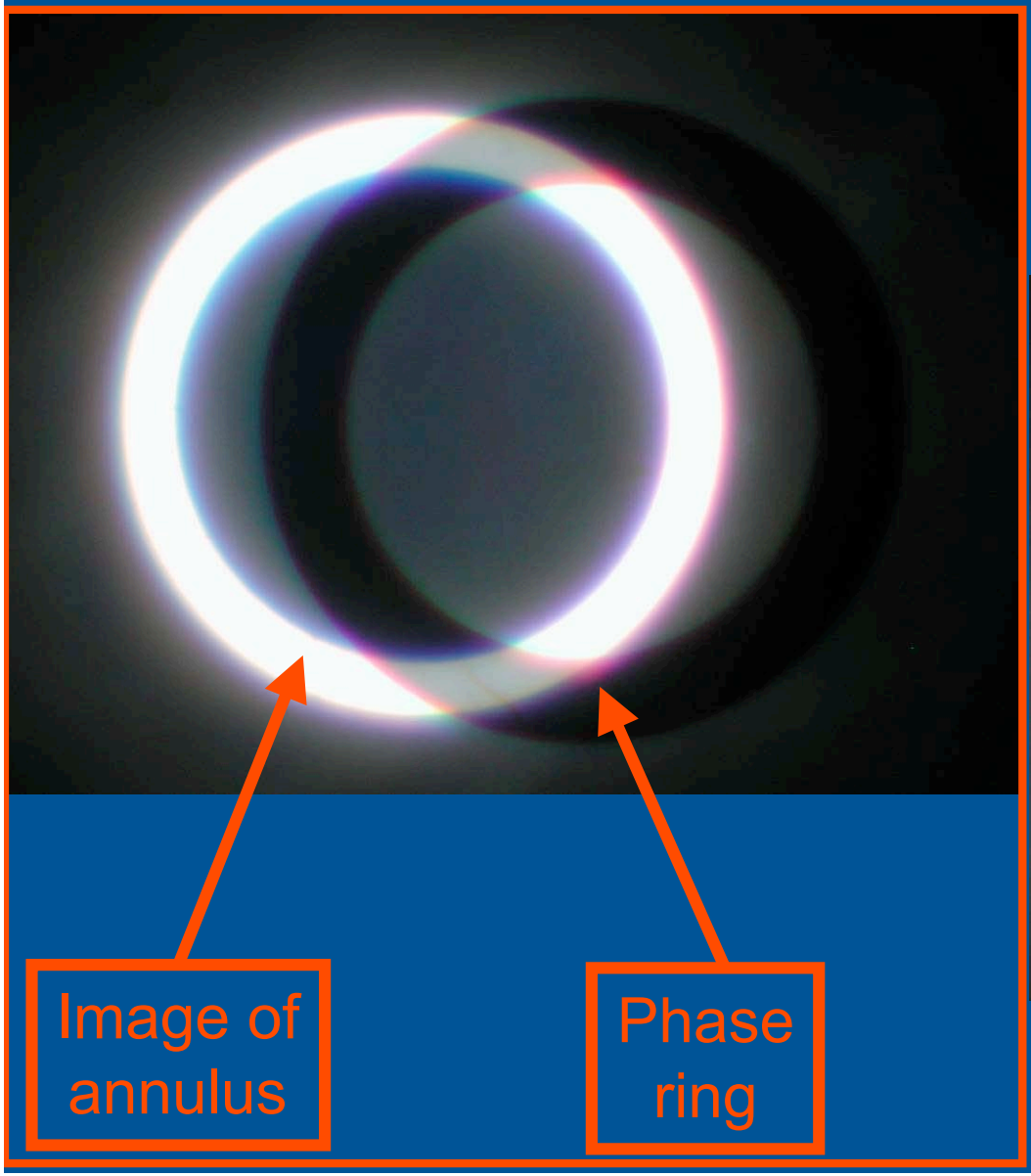
Specimen scatters light into objective

and retards it a little - about ¼λ

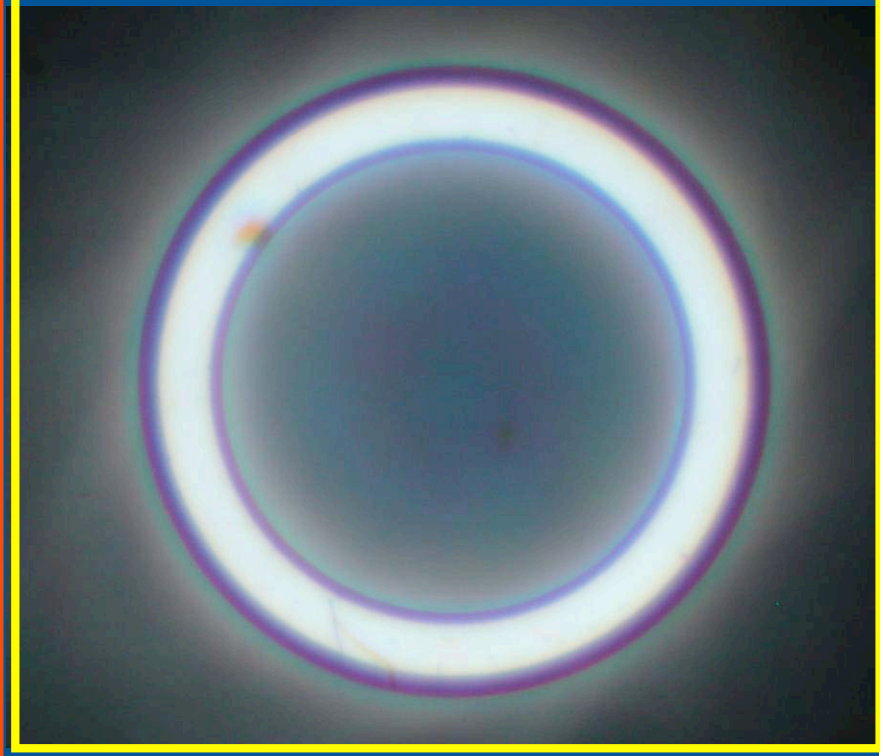
Illuminating annulus in front focal plane of condenser

Adjustment of illuminating annulus

as seen in the back focal plane of the objective



Illuminating annulus adjusted so that its image coincides with phase ring



Phase contrast set

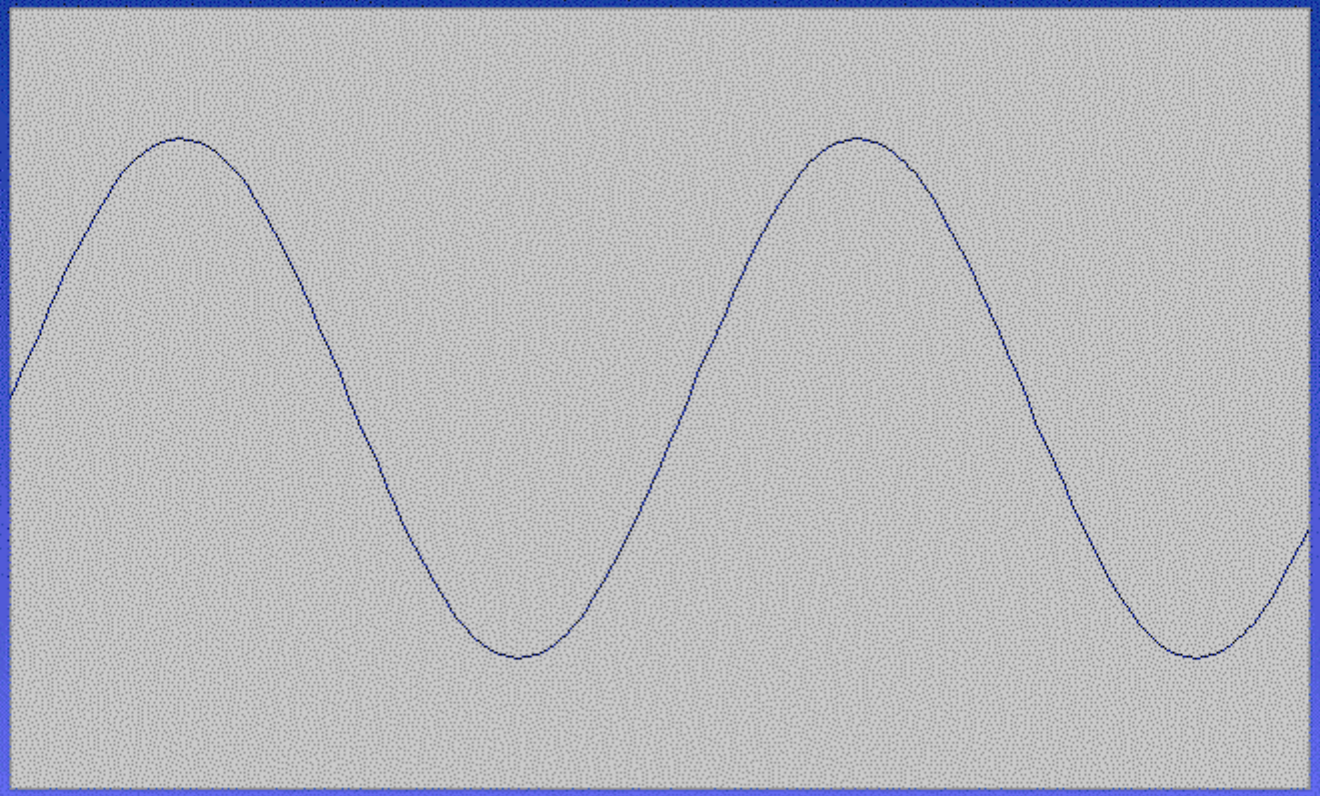
Phase contrast

- Because they consist of areas of different refractive index from their surroundings, transparent, non-absorbing objects produce small differences in the *phase* of light which encounters them, but only small differences in its *amplitude*.

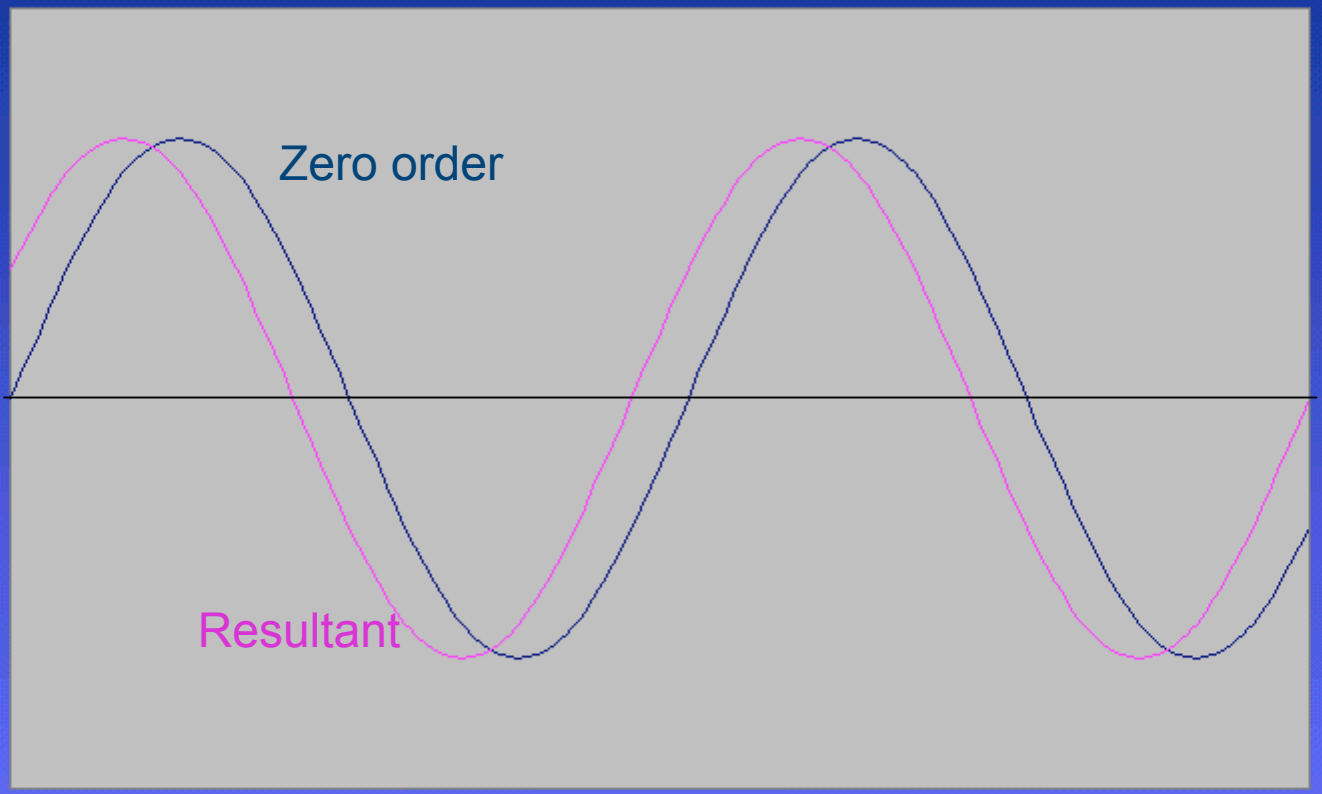
- Thus they are invisible in the microscope image.

- Phase contrast is a technique for converting an *invisible image* into a visible image.

Unmodified ‘zero-order’ beam

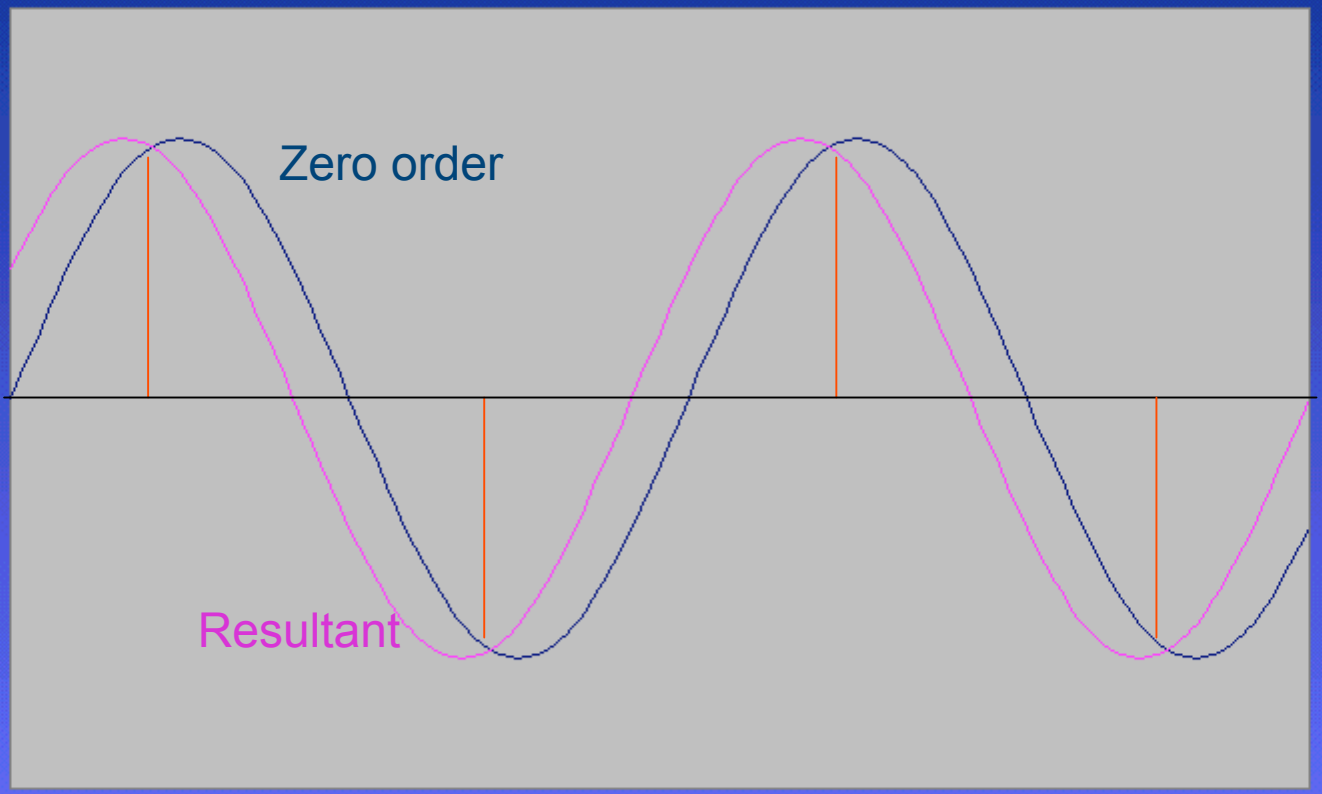


Resultant beam in image of non-absorbing object

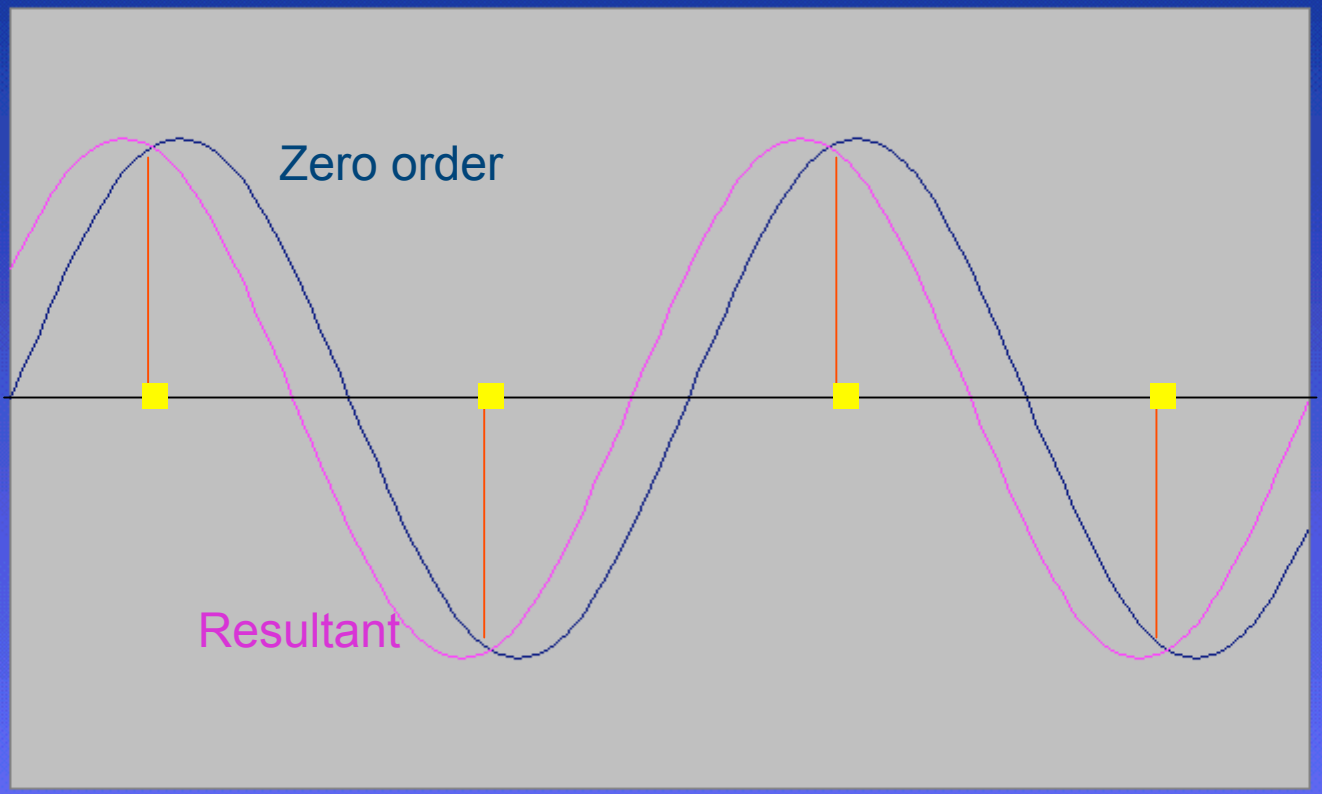


Resultant beam is slightly retarded from zero-order beam

Positions where amplitudes are equal

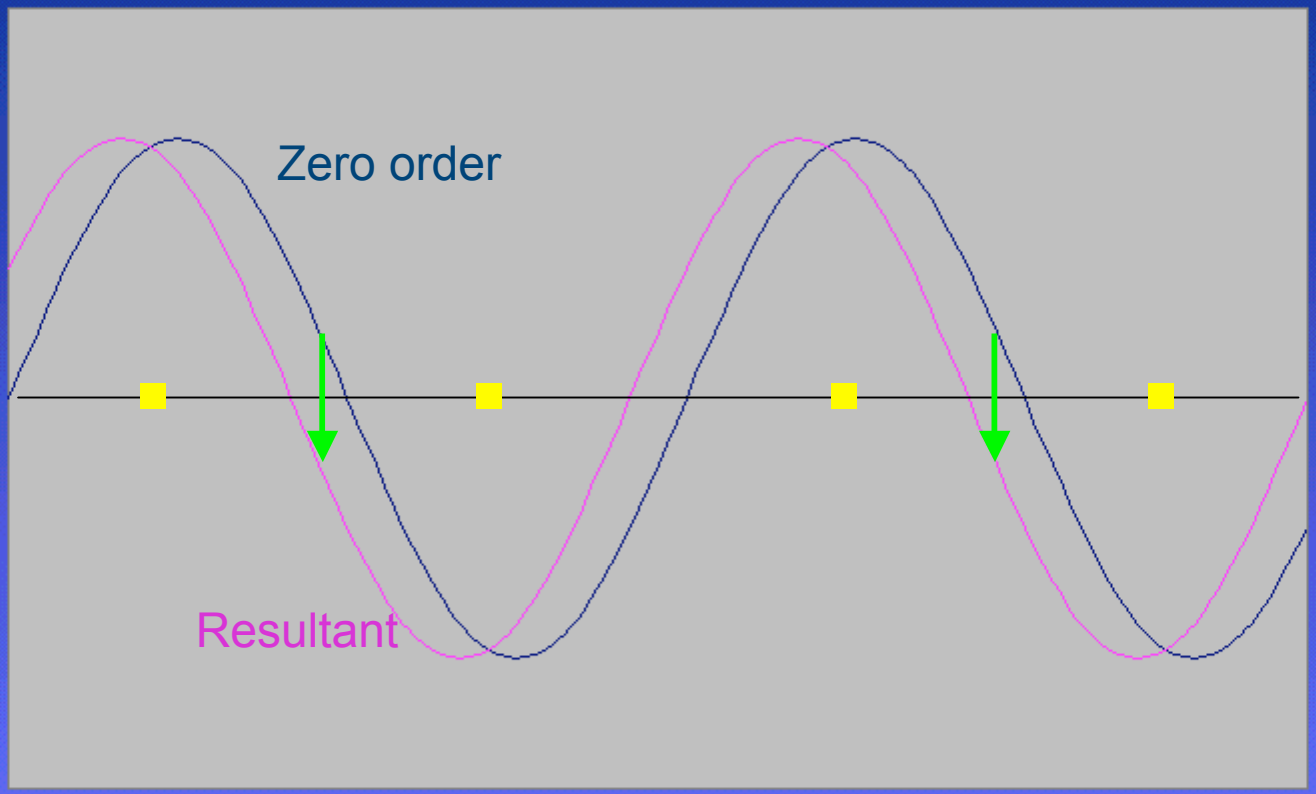


Positions where amplitudes are equal



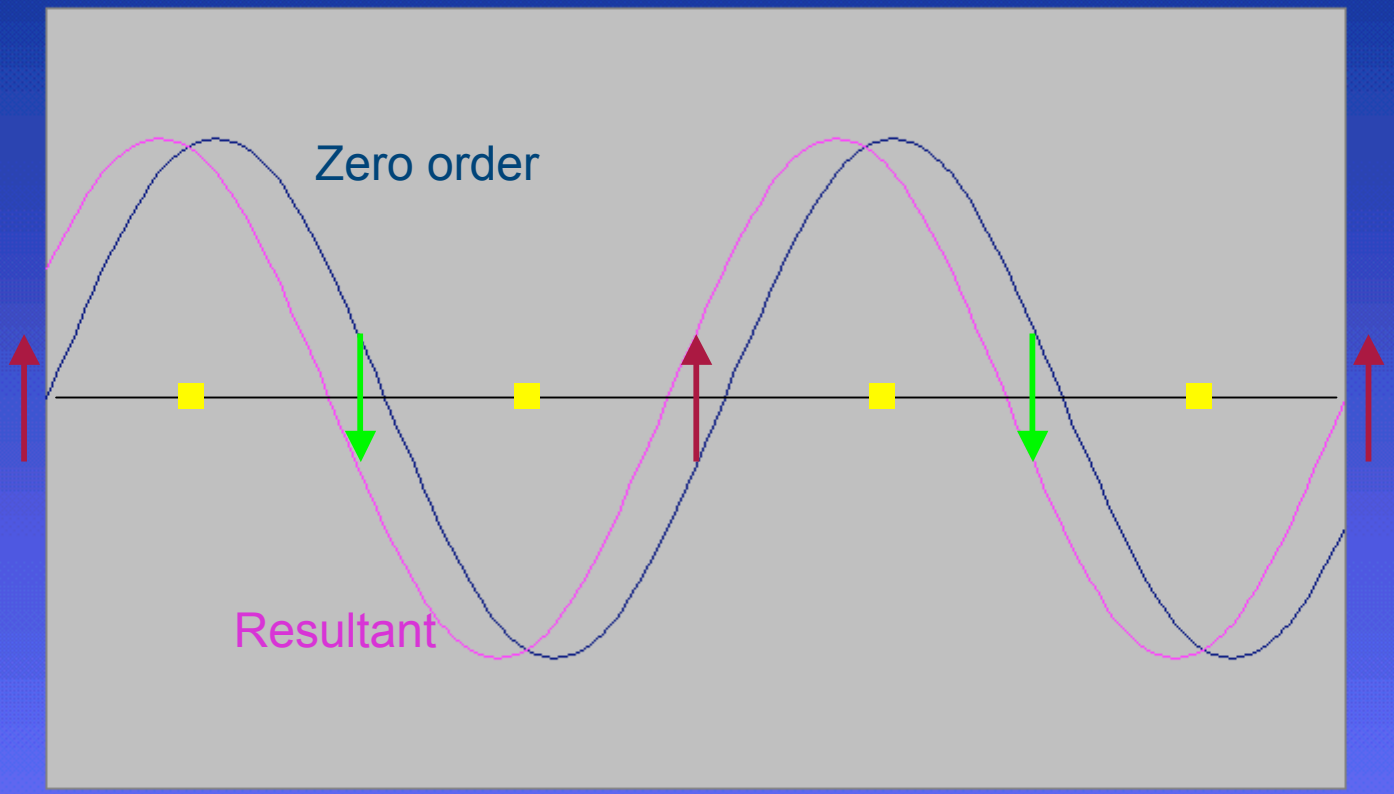
In these positions the diffracted ray must have a value of zero

Positions where amplitude of resultant is *less* than that of zero order



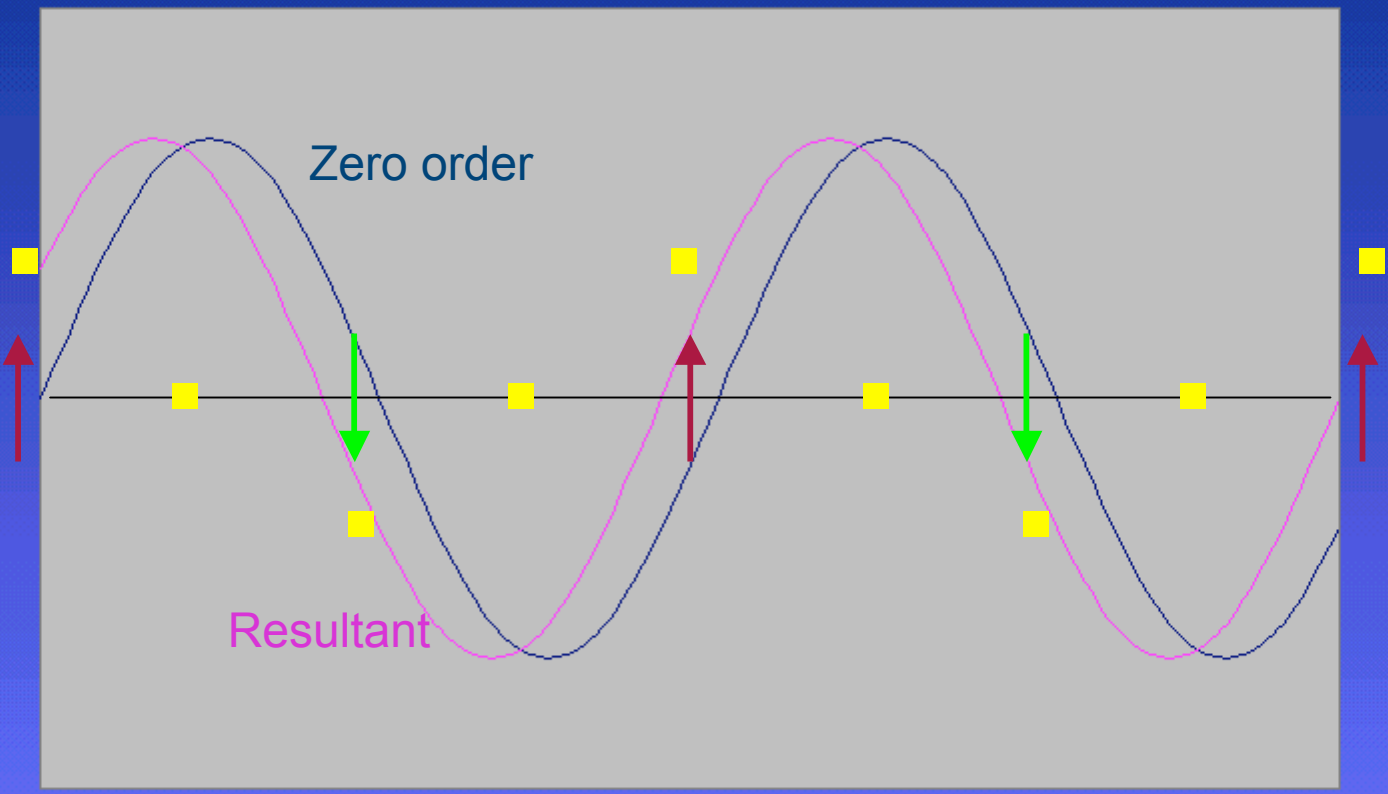
In these positions the diffracted ray must have a negative value

Positions where amplitude of resultant is *greater* than that of zero order

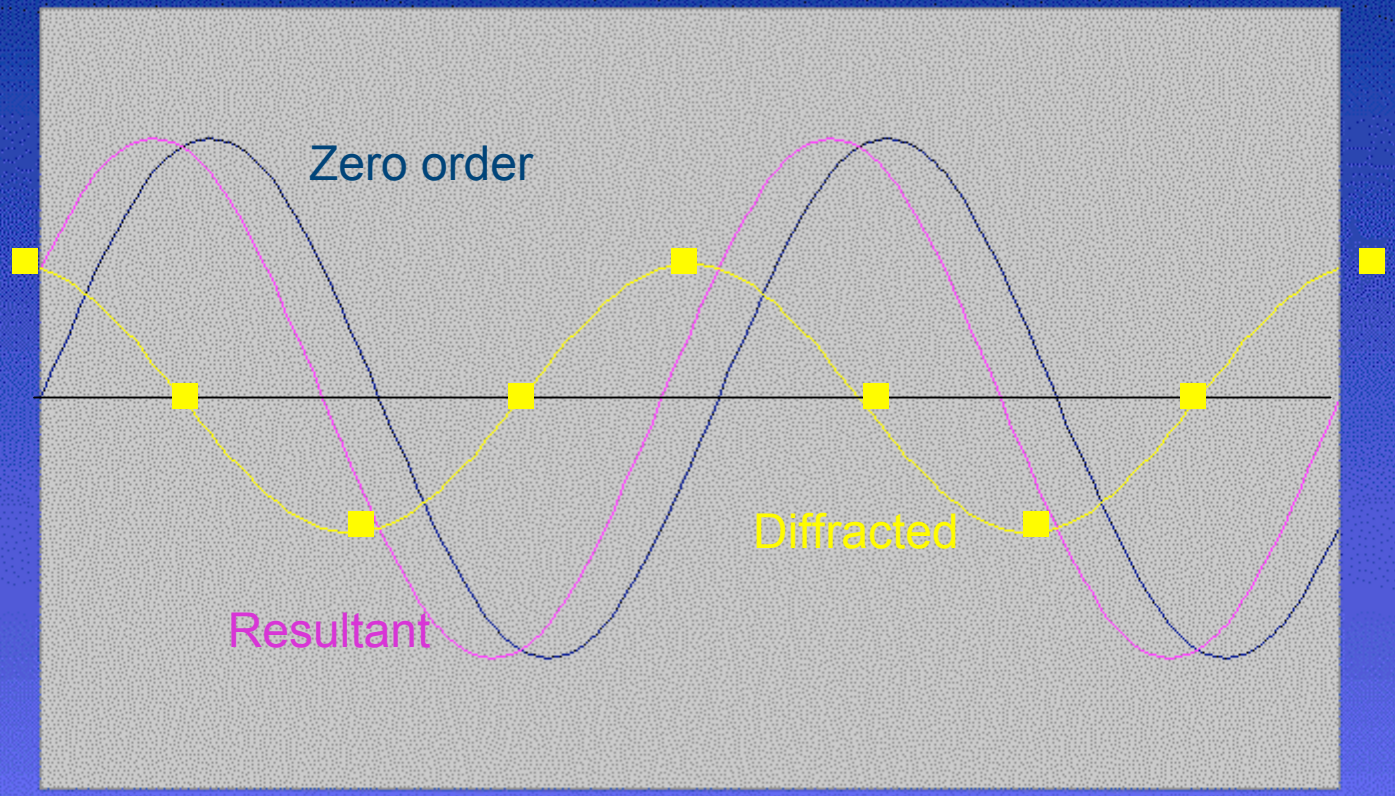


In these positions the diffracted ray must have a positive value

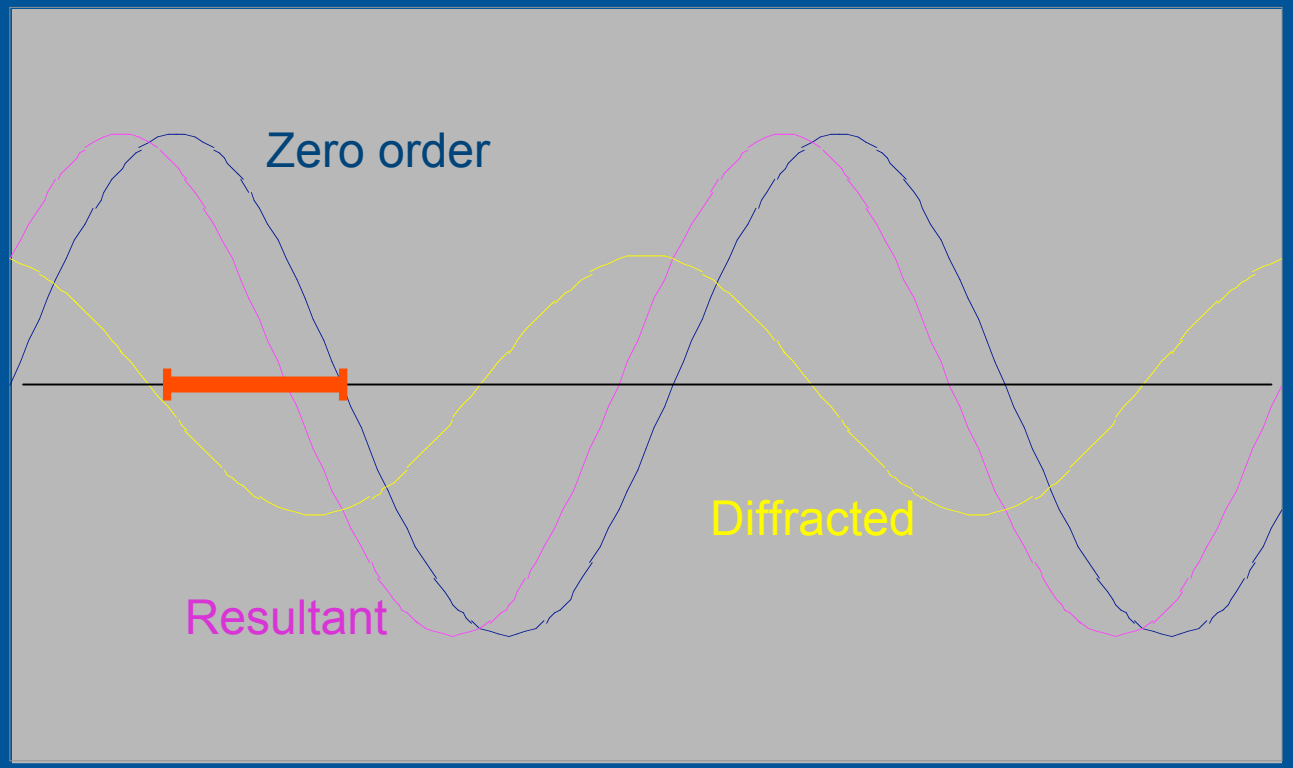
Points for plotting the diffracted ray



Diffracted ray required to convert zero order into resultant



Diffracted ray is one quarter wavelength behind zero order



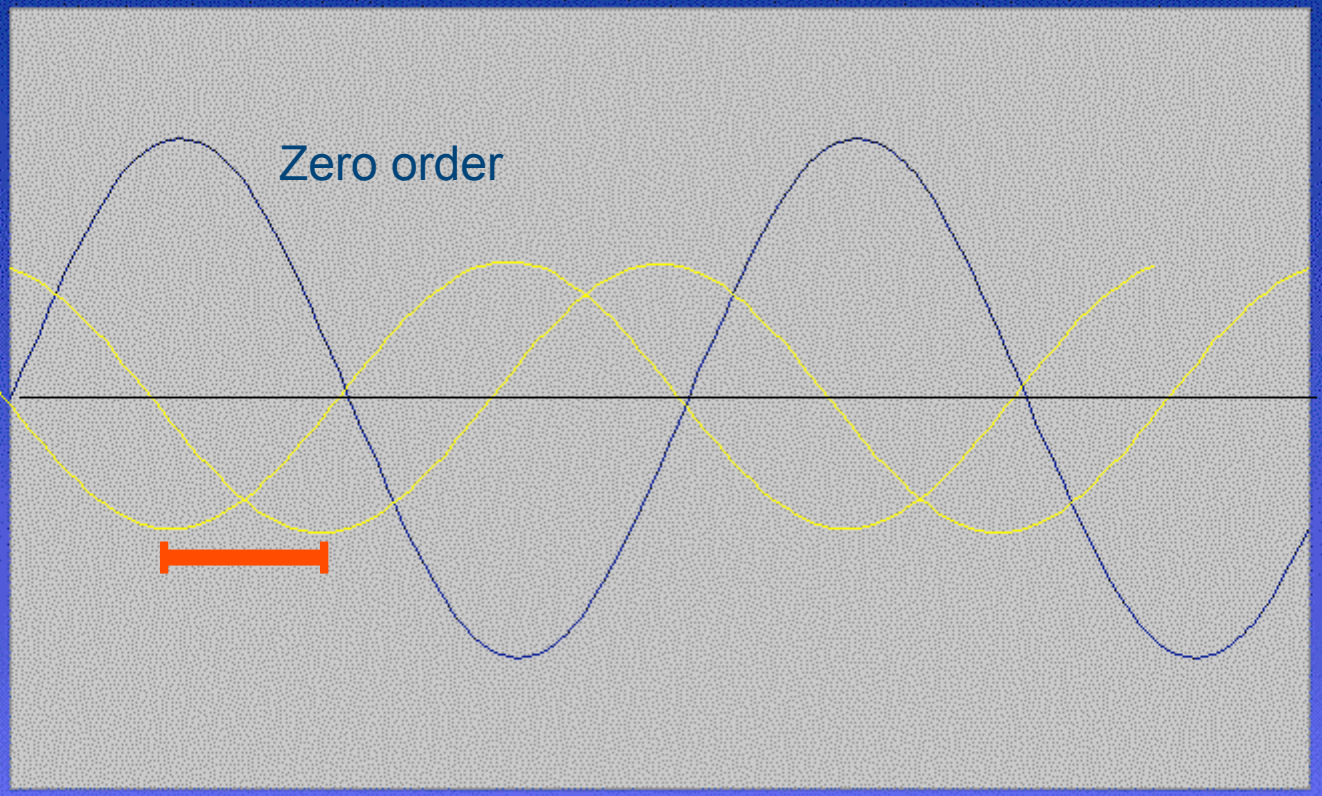
Quarter of a wavelength

The diffracted ray differs from the zero-order by one quarter of a wavelength

…and we know that this leads to an invisible image because of lack of contrast.

- We know too that with an *absorbing* object, there is one-half a wavelength difference, and this leads to good contrast.
- If we were able to convert the quarter-wavelength difference into a half-wavelength difference, the non-absorbing object would appear in the image as if it were an absorbing object.

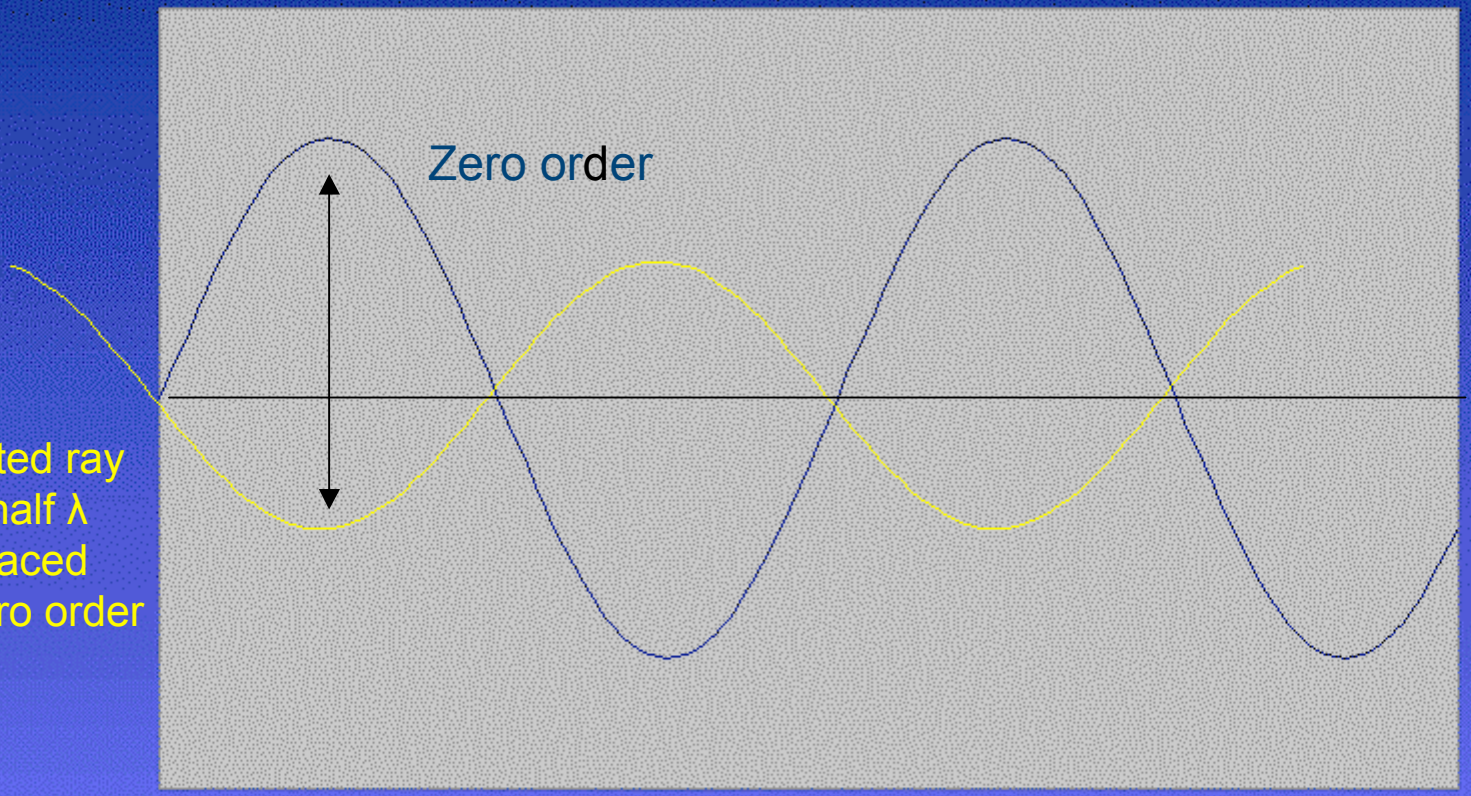
Diffracted ray retarded by another one quarter wavelength



Diffracted ray shifted to left by a further λ/4 by phase plate

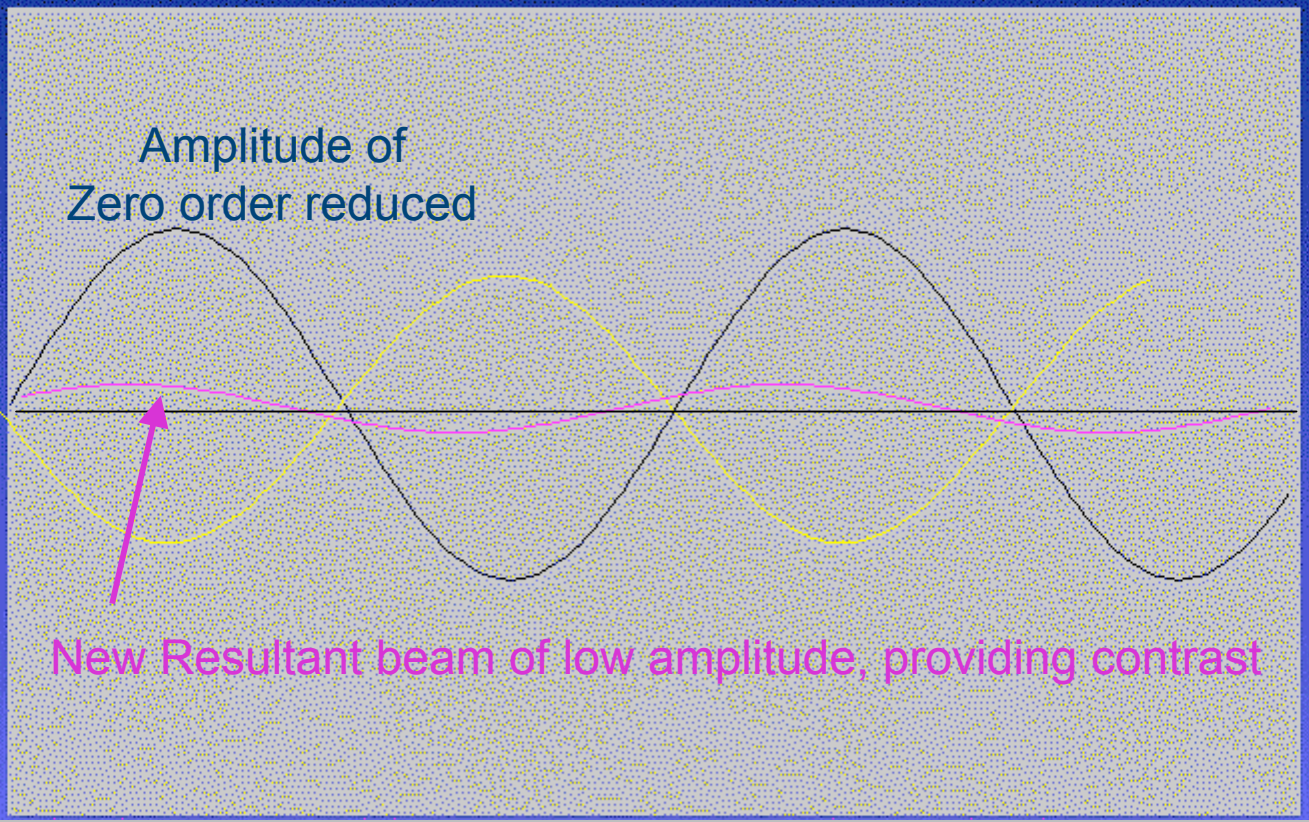
Diffracted beam now approximately half a wavelength behind zero order

Diffracted ray now one half wavelength behind zero order



The diffracted ray is now in a position to interfere destructively with the zero order, but it is of lower amplitude

Diffracted ray now one half wavelength behind zero order *and* amplitude of zero order reduced



The diffraction pattern of our non-absorbing object has been converted into a diffraction pattern similar to that of an absorbing object - so the image looks like an image of an absorbing object.