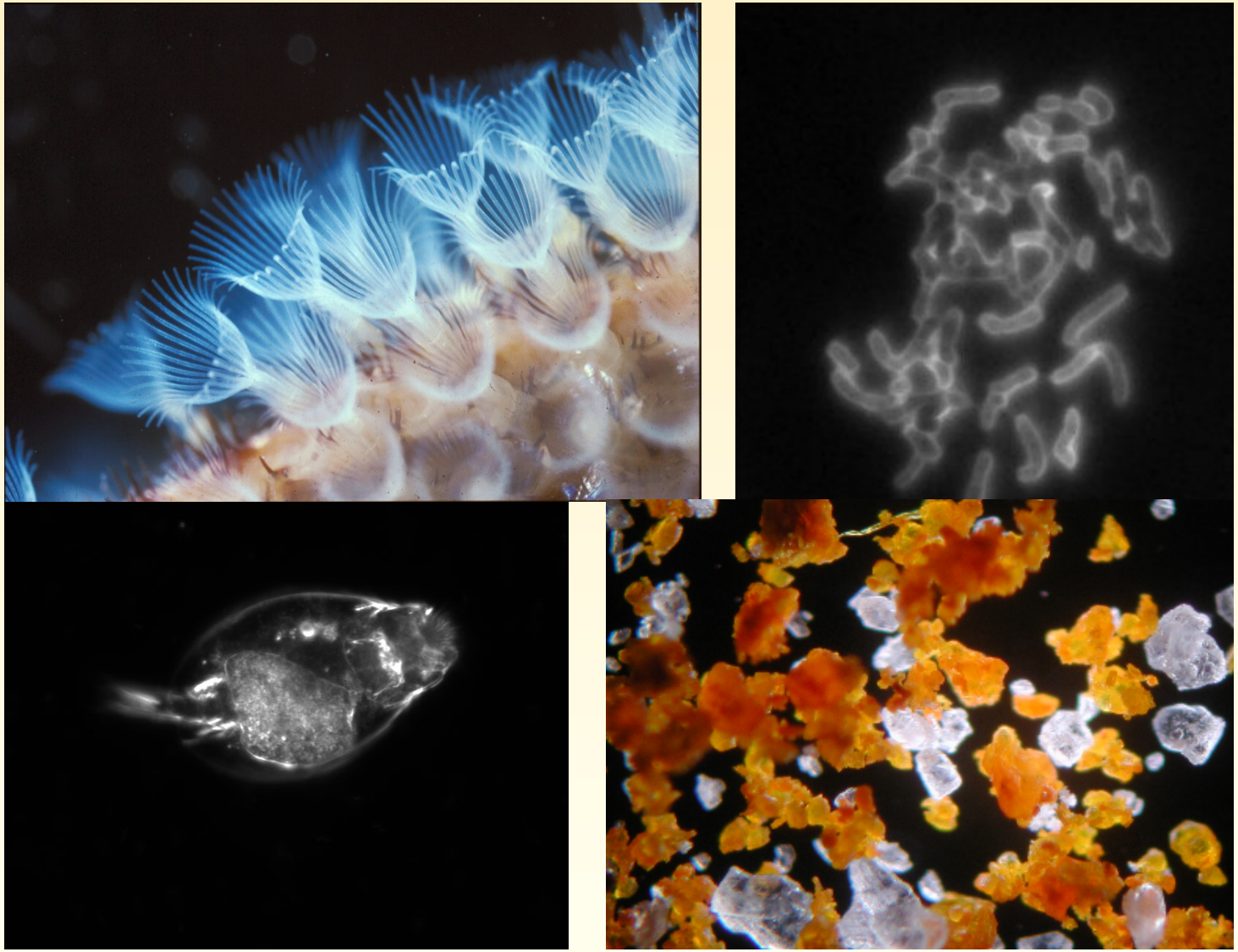
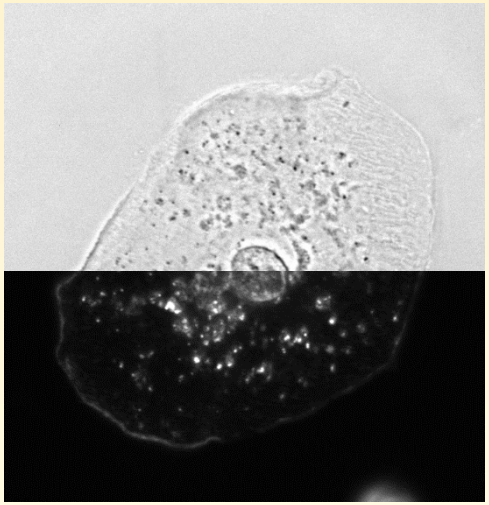
2. Dark field microscopy



2. Dark field microscopy

Brightfield:

- Contrast mainly due to the absorption of light through the specimen
- For *transparent* and *very thin* specimen often not very useful → objects hardly absorb the light



Cheek cells

- For such objects methods like **Darkfield** (or phase contrast) can be used
- In transparent specimen refraction occurs at the borders of structures with different densities
- With dark-field these boundaries can be made visible

2. Dark field - Principle

Bright field
(Transmitted-light)

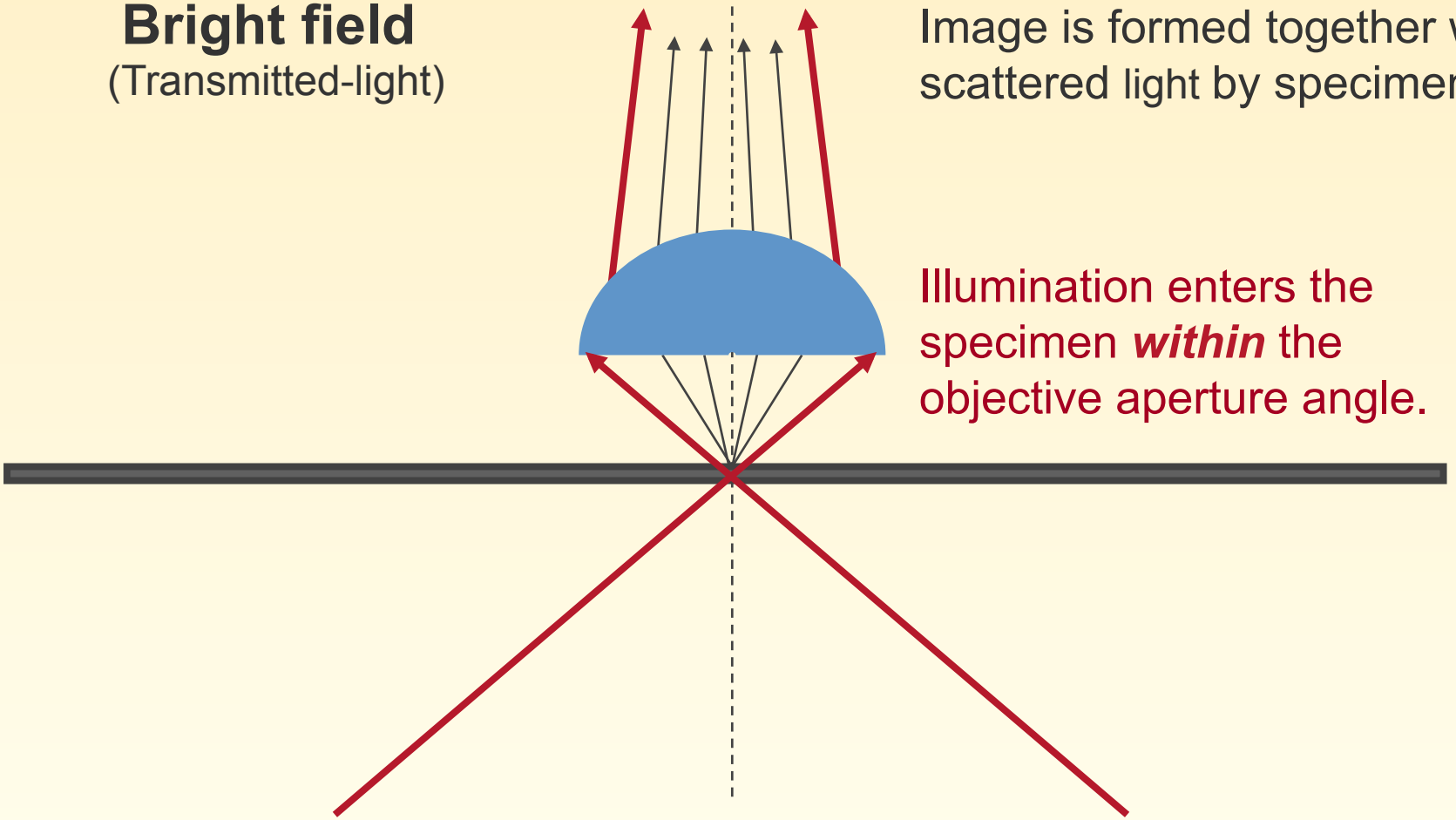


Image is formed together with scattered light by specimen.

Illumination enters the specimen ***within*** the objective aperture angle.

2. Dark field - Principle

Dark field
(Transmitted-light)

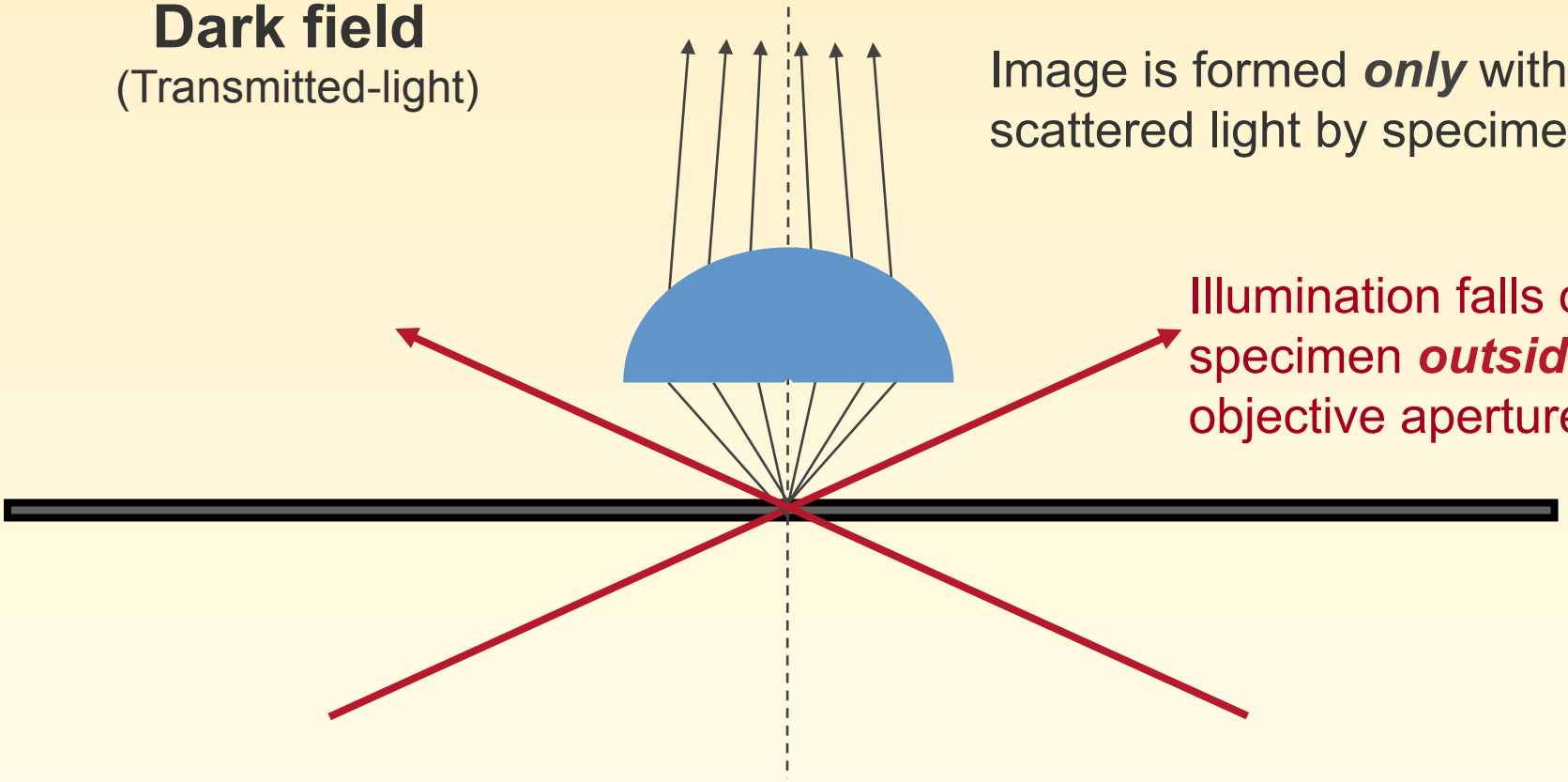


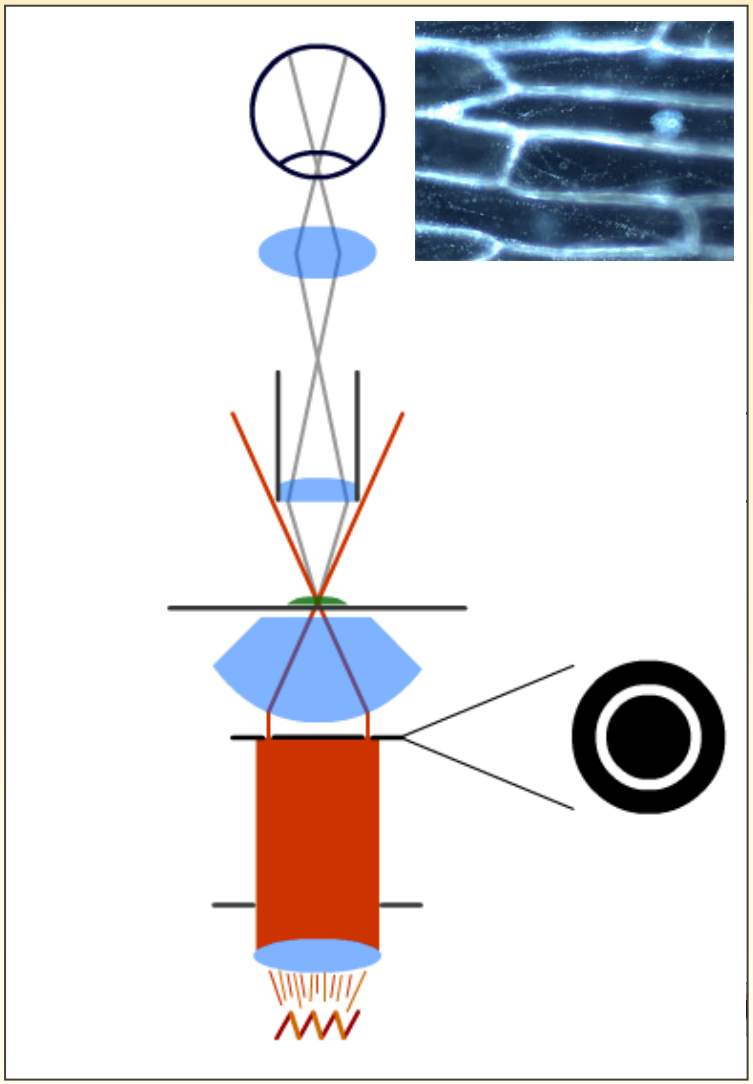
Image is formed ***only*** with scattered light by specimen.

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

Illumination occurs that no direct light from the condenser enters the lens.
If there is no specimen in the light path it gives a uniform black image.

Drawing by © Peter Evennett

2. Dark field – The Microscope



- Central annulus reduces the light to an outer ring *(in the front focal plane of the condenser)*
- With a suitable N.A. of the lens no direct light gets into the objective lens
- with no sample in the beam path → no light comes into the objective and the image gets dark
- At the phase boundaries of the sample light is refracted and scattered.
- Light then is captured by the objective lens
- See bright flash of the structures on a dark background

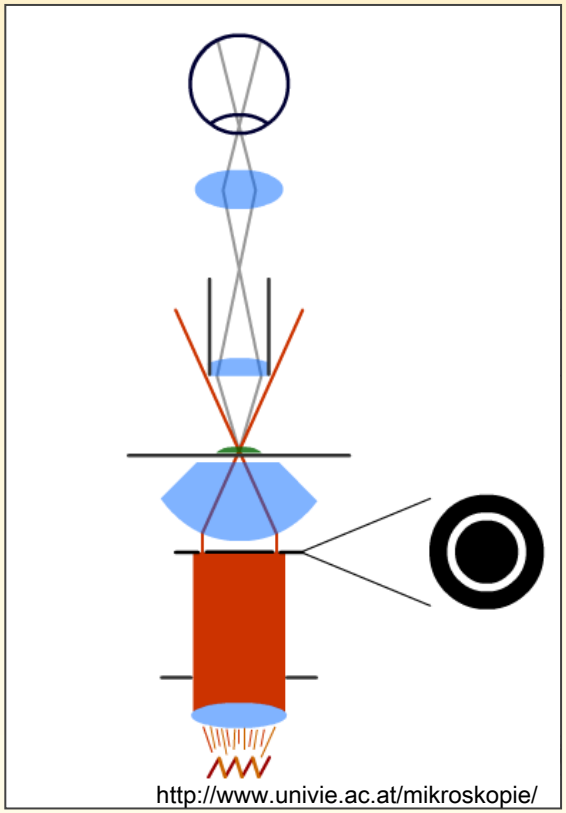
Cave

The N.A. of the objective has to be smaller than the N.A. of the condenser!

Picture by © http://www.univie.ac.at/mikroskopie/

2. Dark field – The Microscope

The size of the annulus in the condenser must fit to the N.A. of the lens.



DF-link

- Too small:
  direct light gets into the objective
- Too big:
  sample is illuminated insufficiently, and the structures don't shine bright enough.
- Special objectives

2. Dark field – Pros and Cons

Advantages

- Small, unstained, objects can be observed with high contrast, especially thin specimens
- Objects near and even below the resolution limit cause signals when the light is strong enough.
- Dark field illumination, especially at low magnification, is very easy to implement and cheap
- In contrast to bright field there are no optic phenomena like streaks or shadows

Disadvantages

- Not for thicker specimens
  many signals from different focal planes around the dark field effect
- Requirements on the cleanliness of equipment and preparation is very high
  interfering signals occur
- Due to the opening angle of the condenser the NA of the objectice needs to be reduced
  the resolution is reduced compared to bright field and other contrast methods like phase contrast or DIC

2. Dark field – The Microscope

- Setup:
  - Köhler the microscope
  - Choose dark field annulus or phase ring 3 (Ph 3) in front focal plane of condenser
  - Choose the right objective: NA smaller than NA of the condenser

- Specimen:
  - dark field is good for small single scatterers
  - ciliated cells
  - nanoparticles
  - blood cells, sperm cells
  - diatomes
  - cheek cells

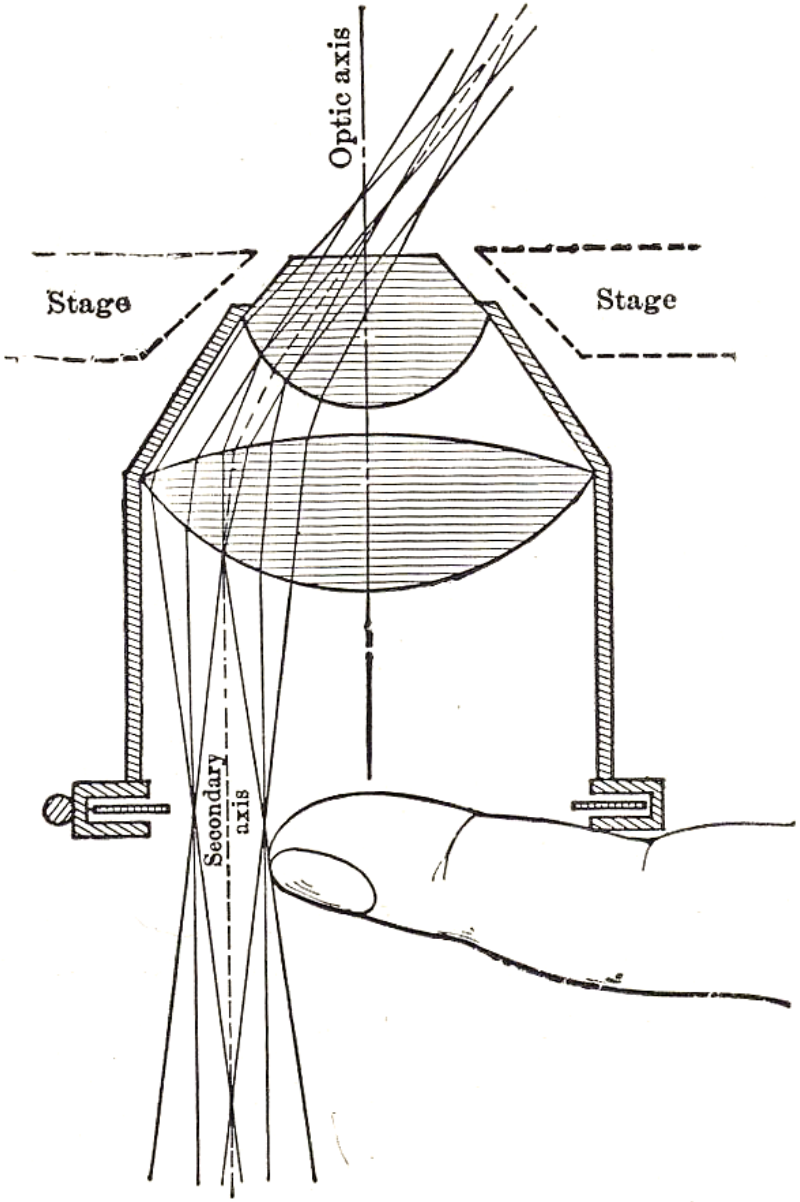


FIG. 62. OBLIQUE LIGHT WITH A CONDENSER.
(From Chamot).

‘Digital’
oblique illumination

using your finger!

A ‘no-cost’ option
for most microscopes