

Intelligent Analysis of Biomedical Images

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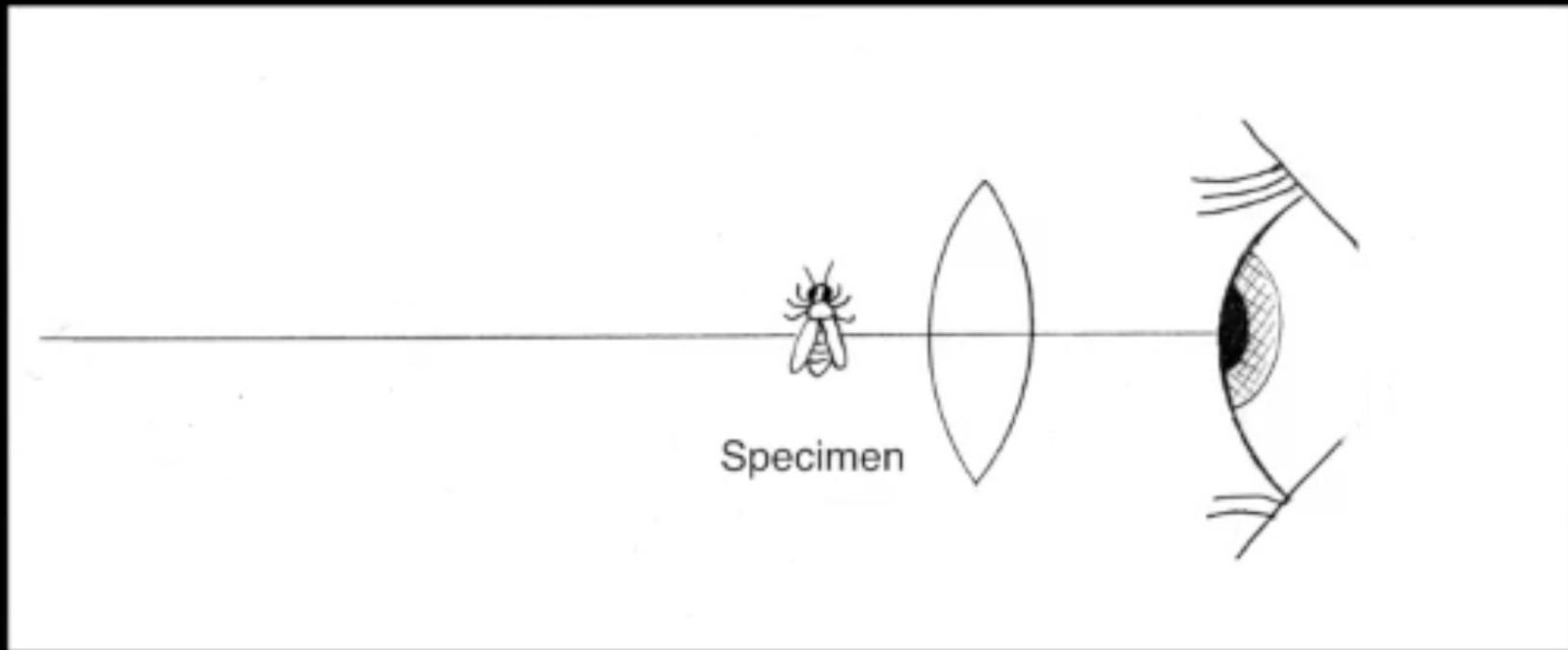
Fall 2023

Courtesy: Some slides are adopted from the videos in iBiology

Introduction to the Microscopy

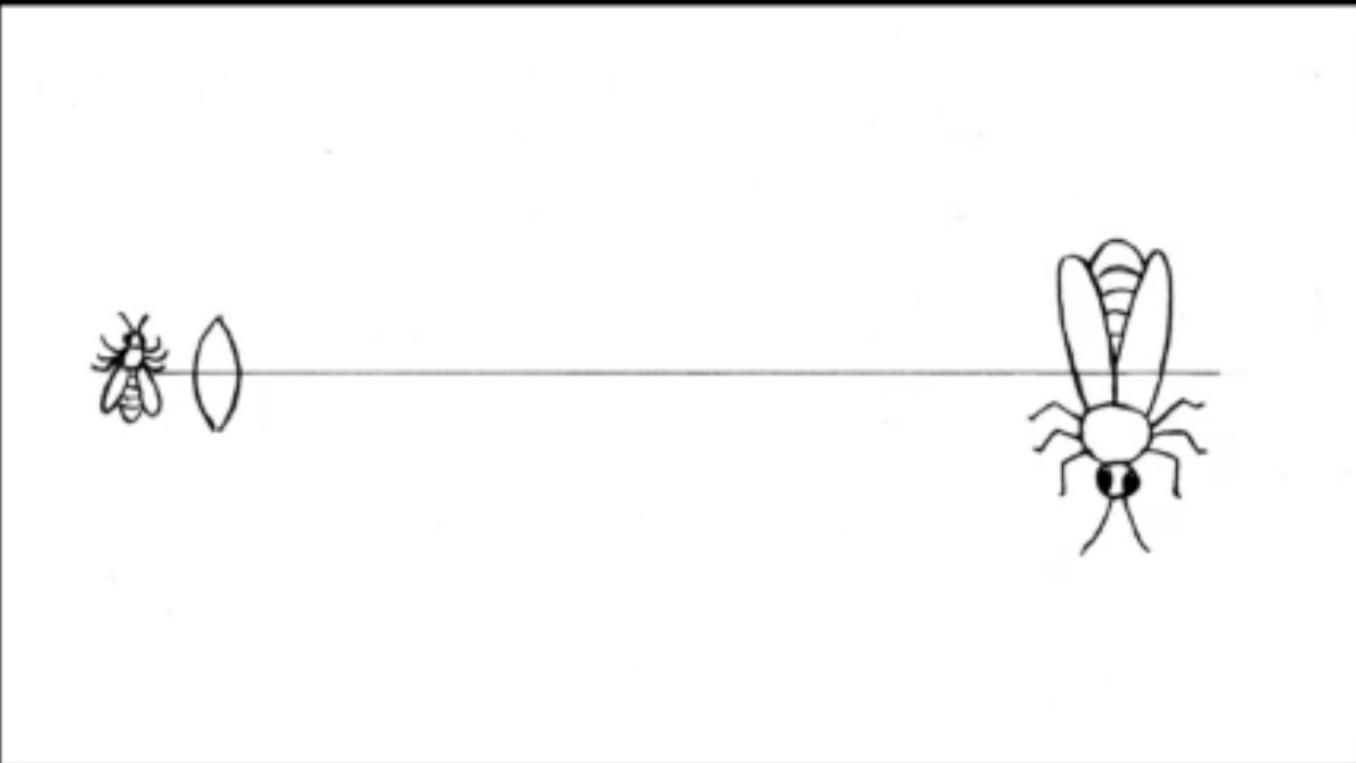
History of early microscopes

“Simple microscope” or magnifying glass

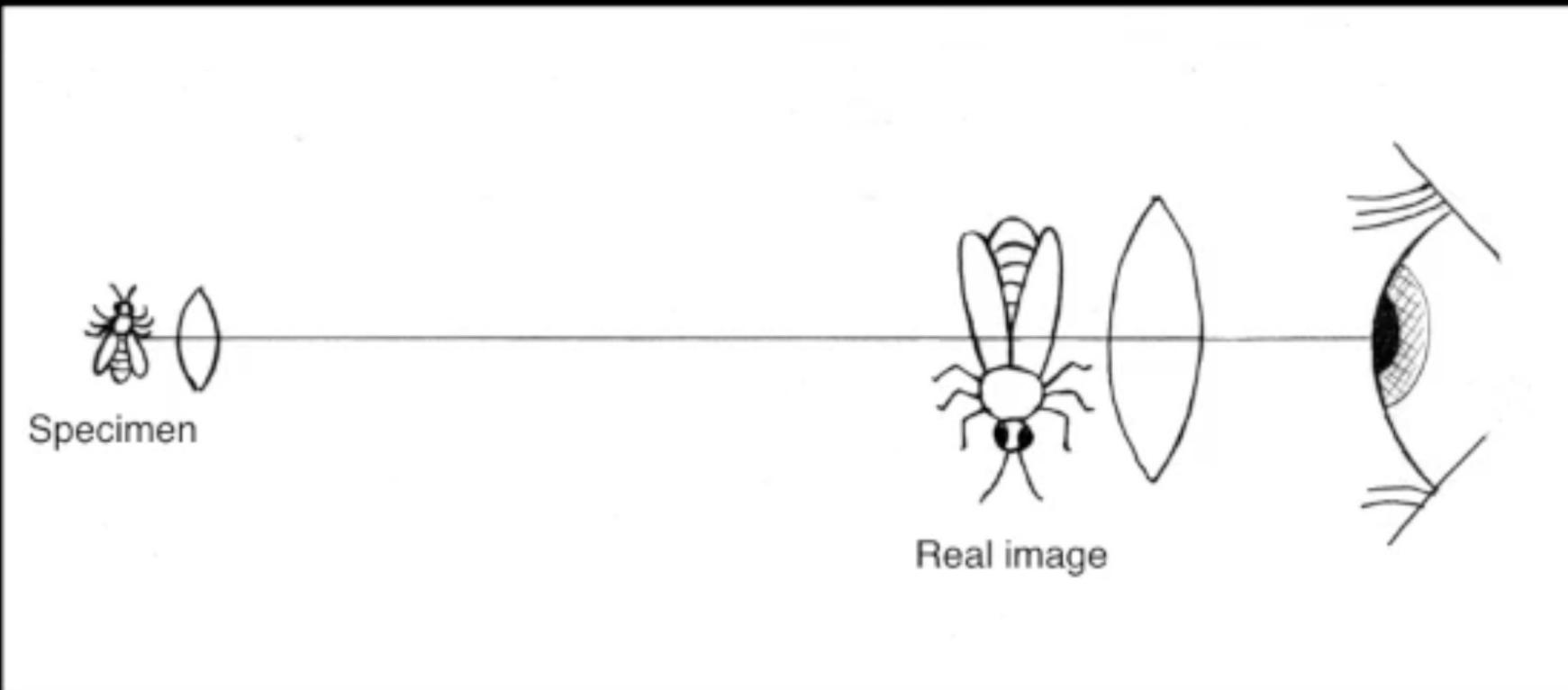


A single lens forms a “virtual” image of the specimen on the retina of the eye

A single lens used to produce an enlarged “real” image in space

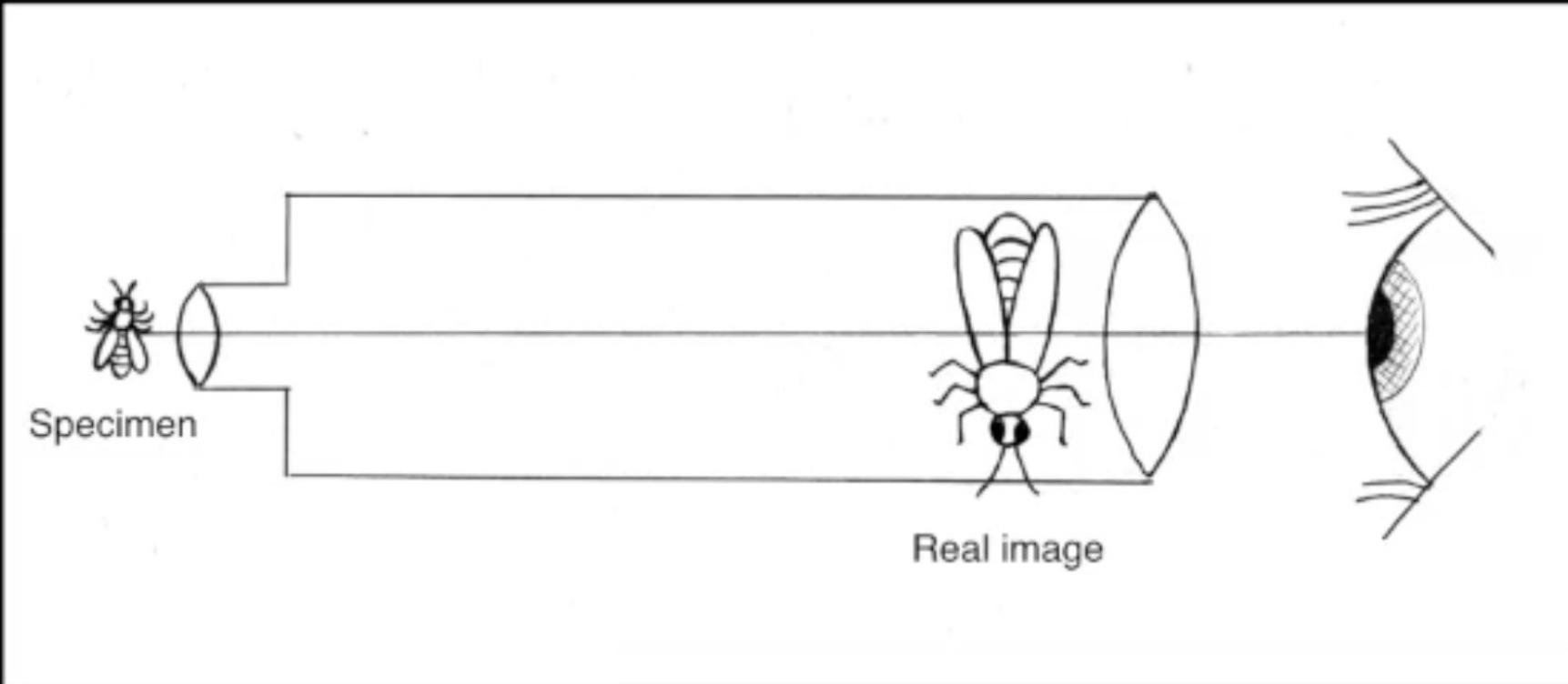


Compound Microscope



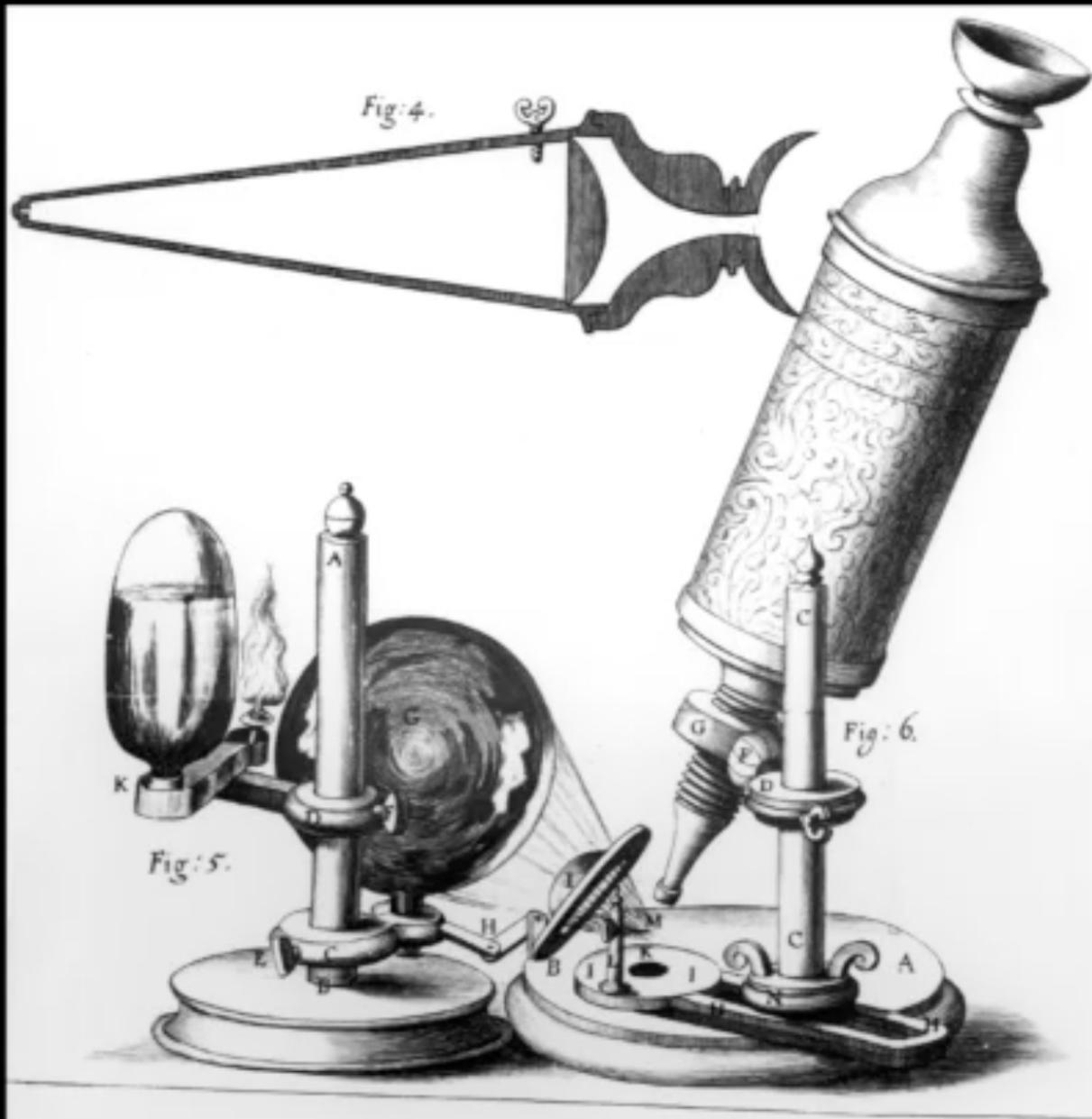
A second lens is used as a magnifying glass to view the “real” image

Compound Microscope

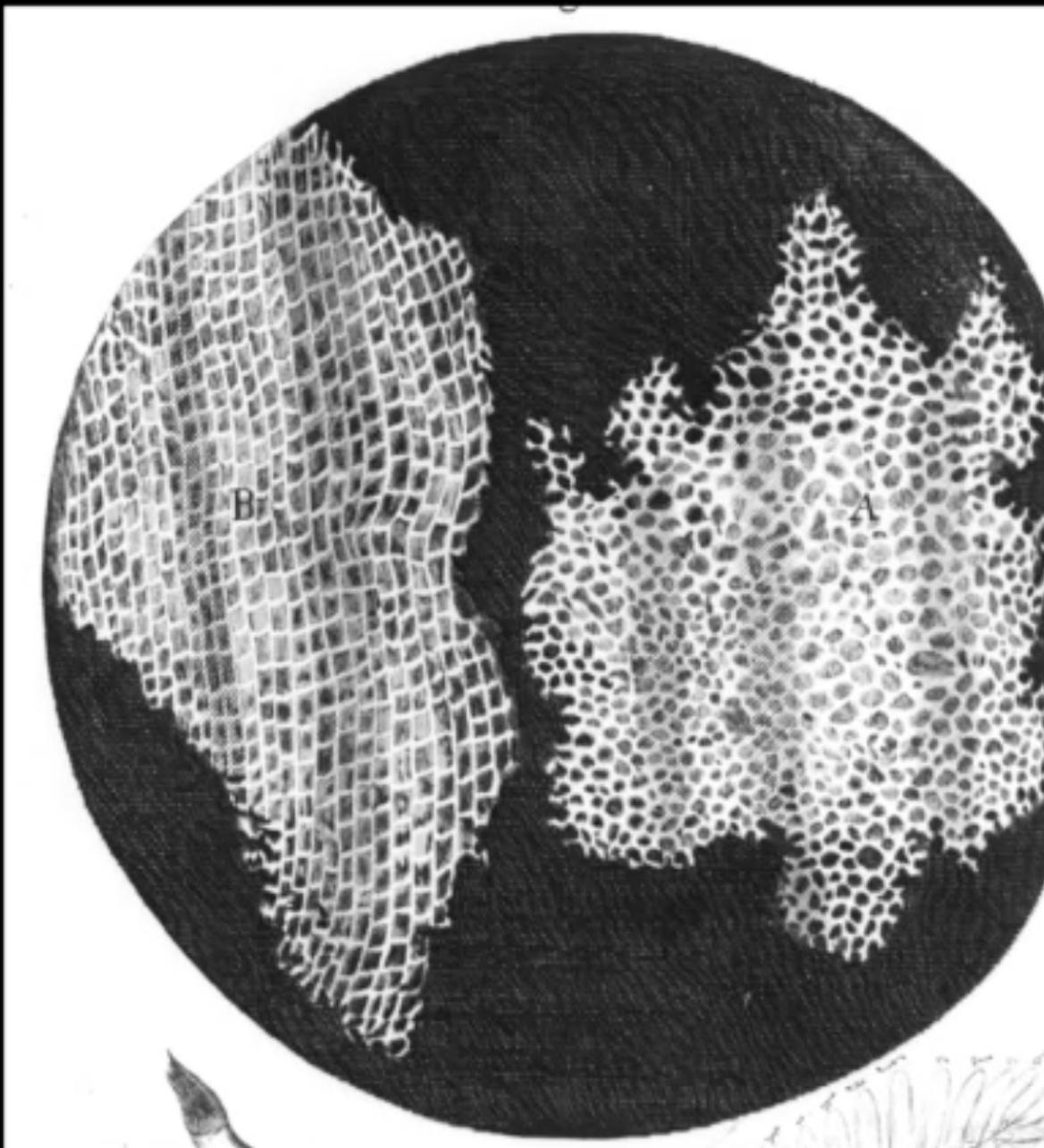


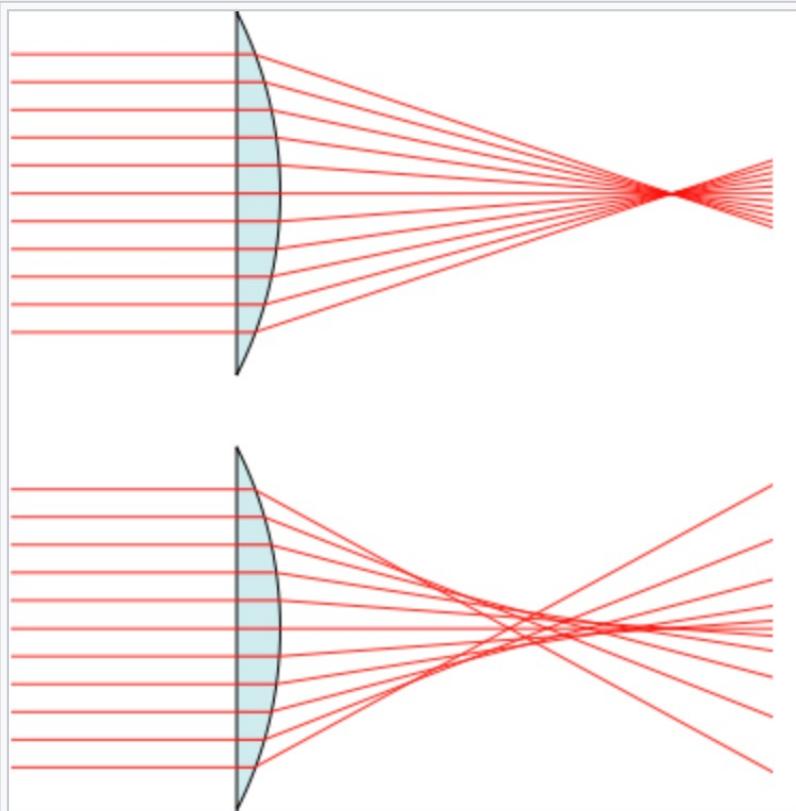
A second lens is used as a magnifying glass to view the “real” image

Robert Hooke's Microscope 1665



Cork cells, Robert Hooke 1665

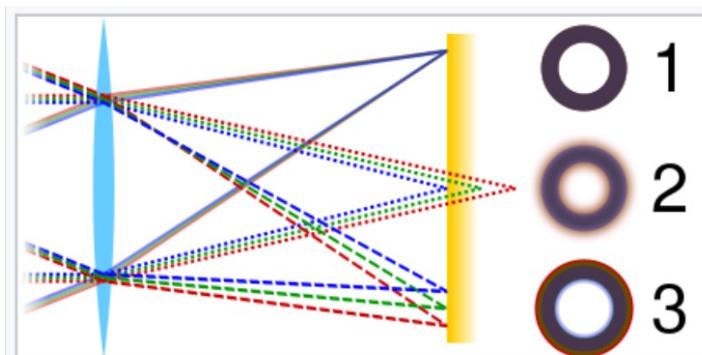
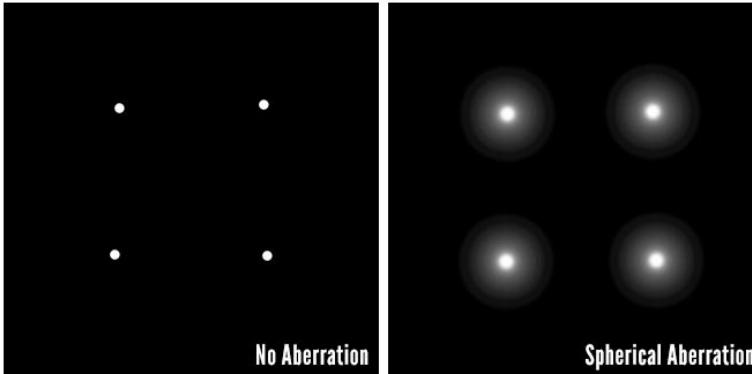




On top is a depiction of a perfect lens without spherical aberration: all incoming rays are focused in the [focal point](#).

The bottom example depicts a real lens with spherical surfaces, which produces spherical aberration: The different rays do not meet after the lens in one focal point. The further the rays are from the [optical axis](#), the closer to the lens they intersect the optical axis (positive spherical aberration).

(Drawing is exaggerated.)



Comparison of an ideal image of a ring (1) and ones with only axial (2) and only transverse (3) chromatic aberration



Photographic example showing high quality lens (top) compared to lower quality model exhibiting transverse chromatic aberration (seen as a blur and a rainbow edge in areas of contrast.)

Leeuwenhoek's Microscope, ca 1700



Nuclei in Onion cells: Brown's microscope

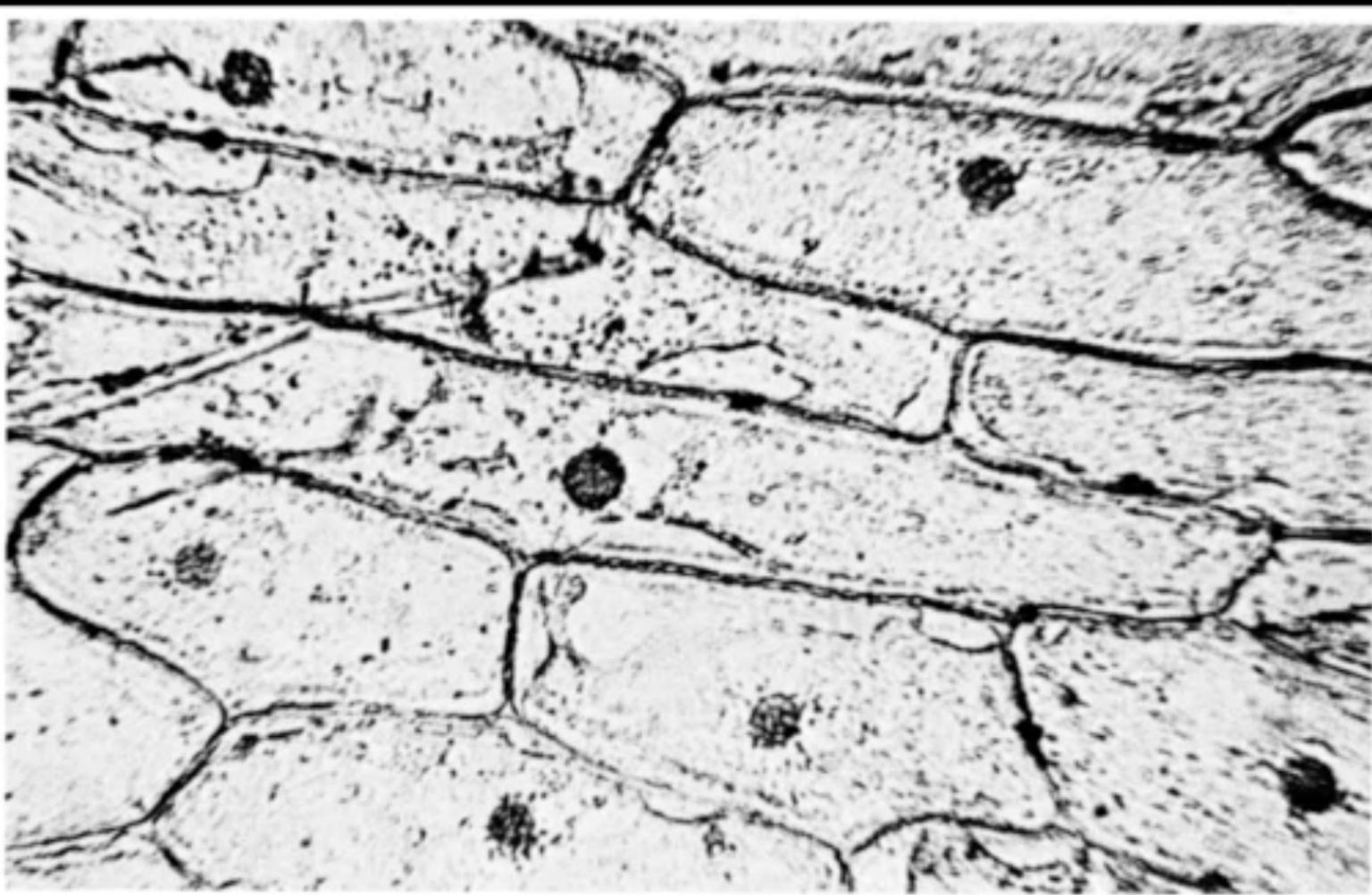


FIG. 25 Twelve cells of *Allium* epidermis, viewed through a medium-power lens of Brown's microscope (Fig. 24), show cell-walls and nuclei with great clarity. Contrary to popular belief, aberrations are slight, and the image quality compares favourably with modern microscopes fitted with fully corrected lenses. (From: Brian Ford "Single Lens")

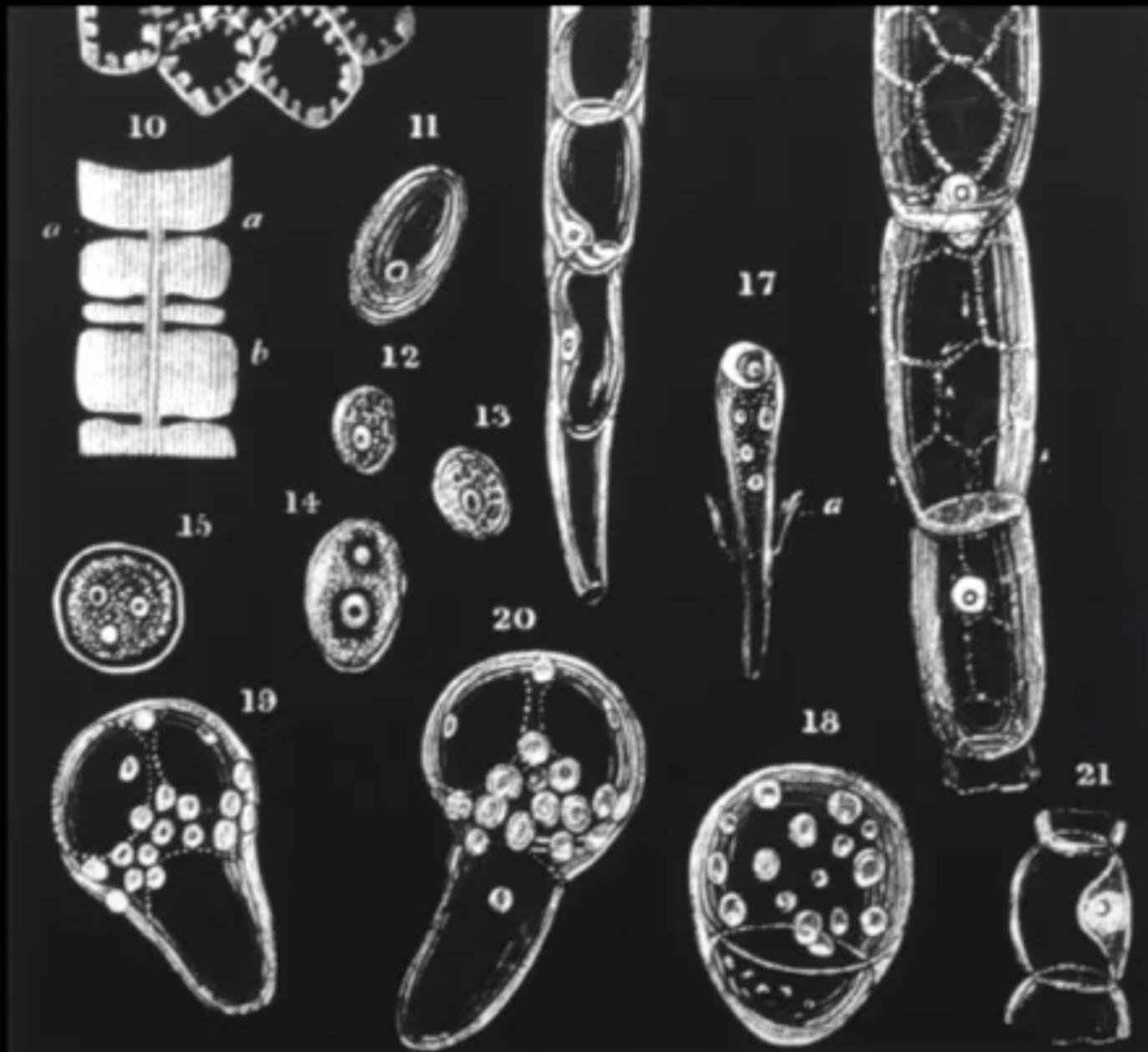
Robert Brown's Simple Microscope 1820



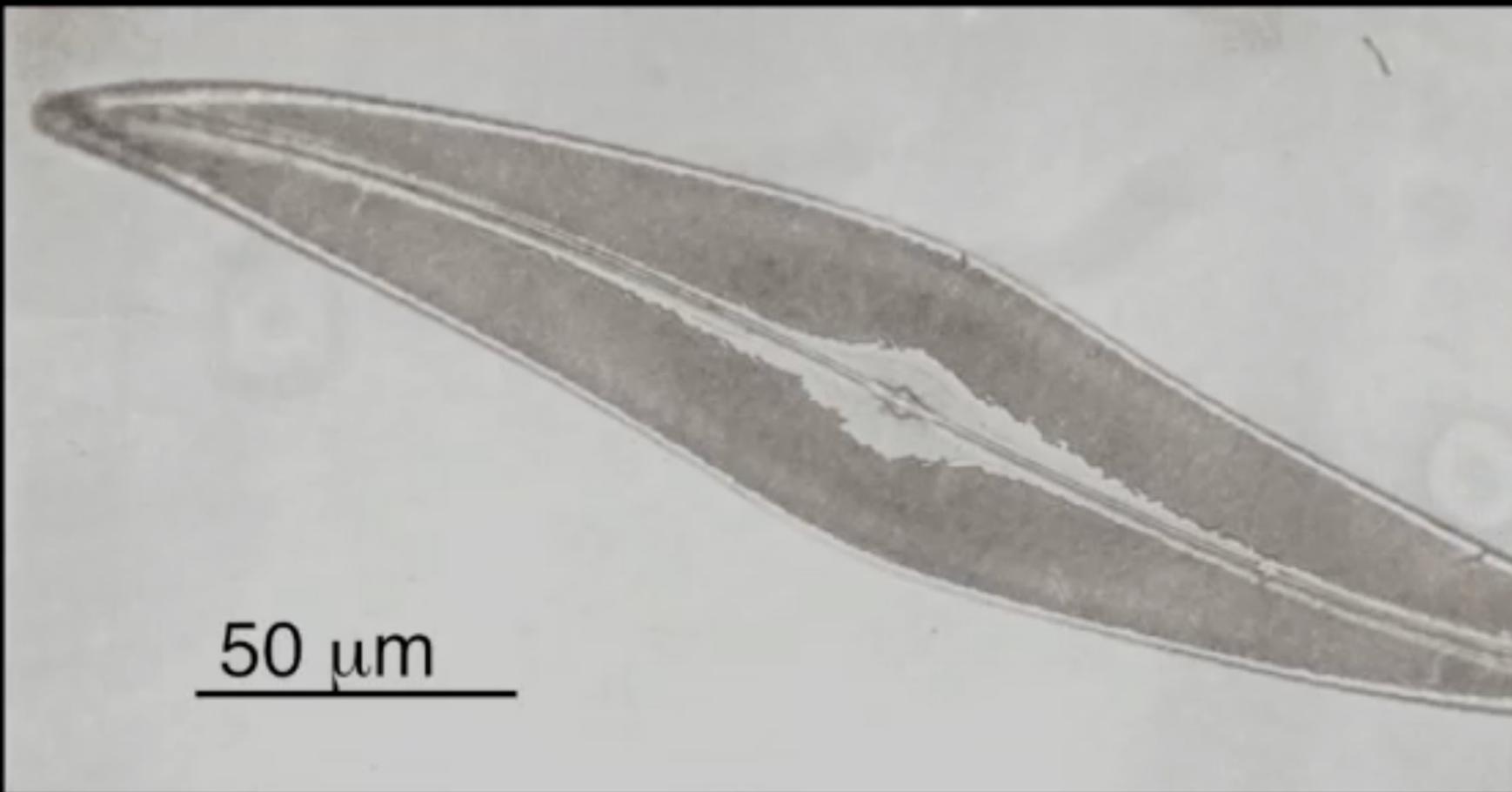
FIG. 28 The simple microscope in its final years, exemplified by this fine example in the collections at the Herbarium, Kew. It was Robert Brown's property, and features a rotatable double-sided mirror and a fine-focusing control mounted below the coarse-focusing knob. As a research instrument for student or field use, it would be well fitted for present-day applications (p. 156).

From:
Brian Ford
"Single Lens"

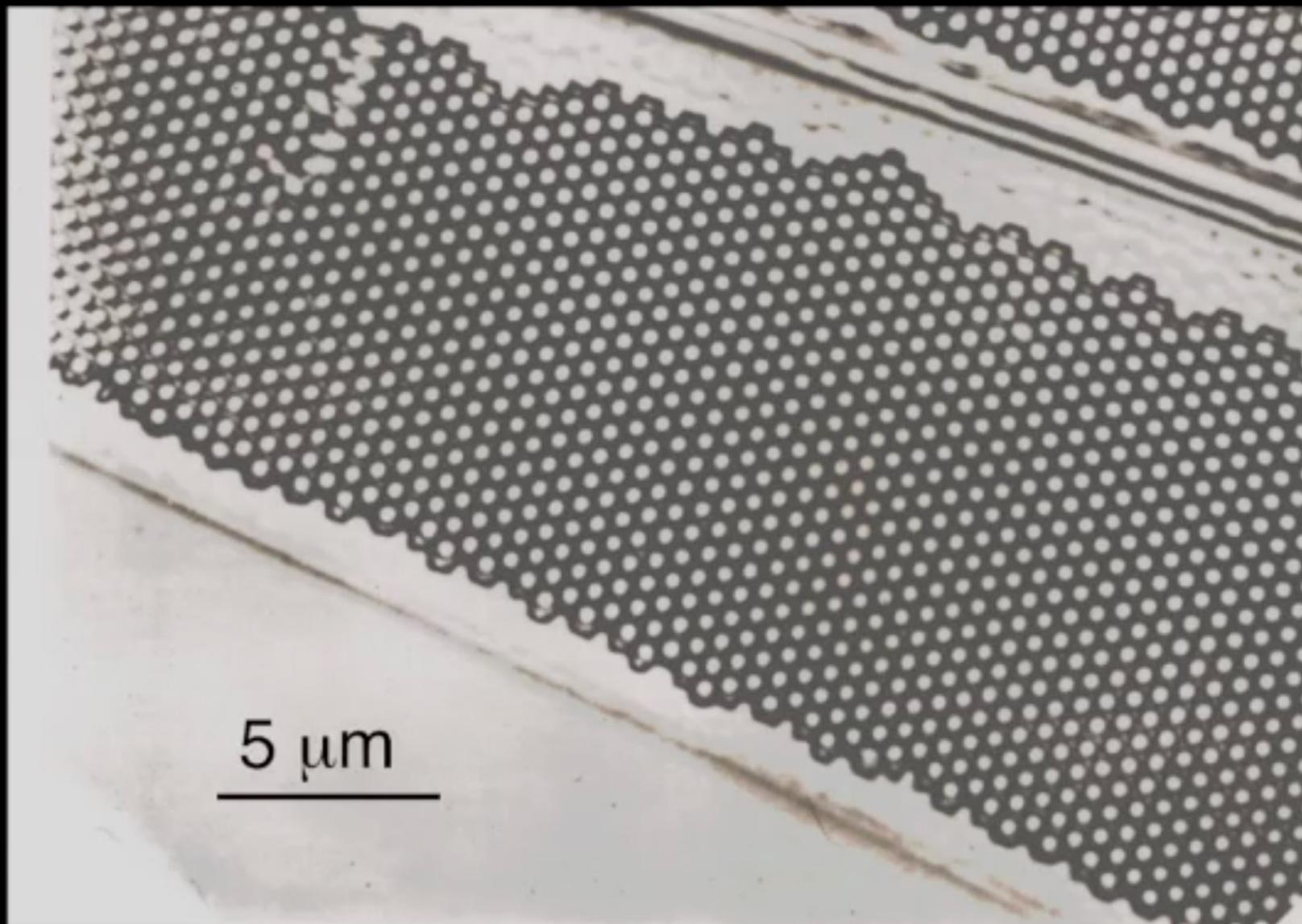
Cell theory: Schleiden and Schwann 1838



**Diatom (*Pleurosigma*)
Abbe 1888**



Diatom (*Pleurosigma*) Abbe 1888



Bright and Dark Field and Phase-Contrast Microscopy

Microscopes Are Designed with 3 Essential Functions

- Magnification (to make structural detail visible to the detector (eye, camera))
- Resolution (limited by wavelength, not lens aberrations)
- Contrast (most biological specimens are transparent at visible wavelengths)

Resolution

- Recall that the resolution of an imaging device is defined as the **minimum distance, d, that two bright points that are d apart are detectable.**

Limit of resolution is given by,

$$\text{Limit of resolution} = d = \frac{0.61\lambda}{NA} = \frac{0.61\lambda}{\mu \sin \alpha}$$

where NA =Numerical Aperture of the microscope,

μ = Refractive index of the medium,

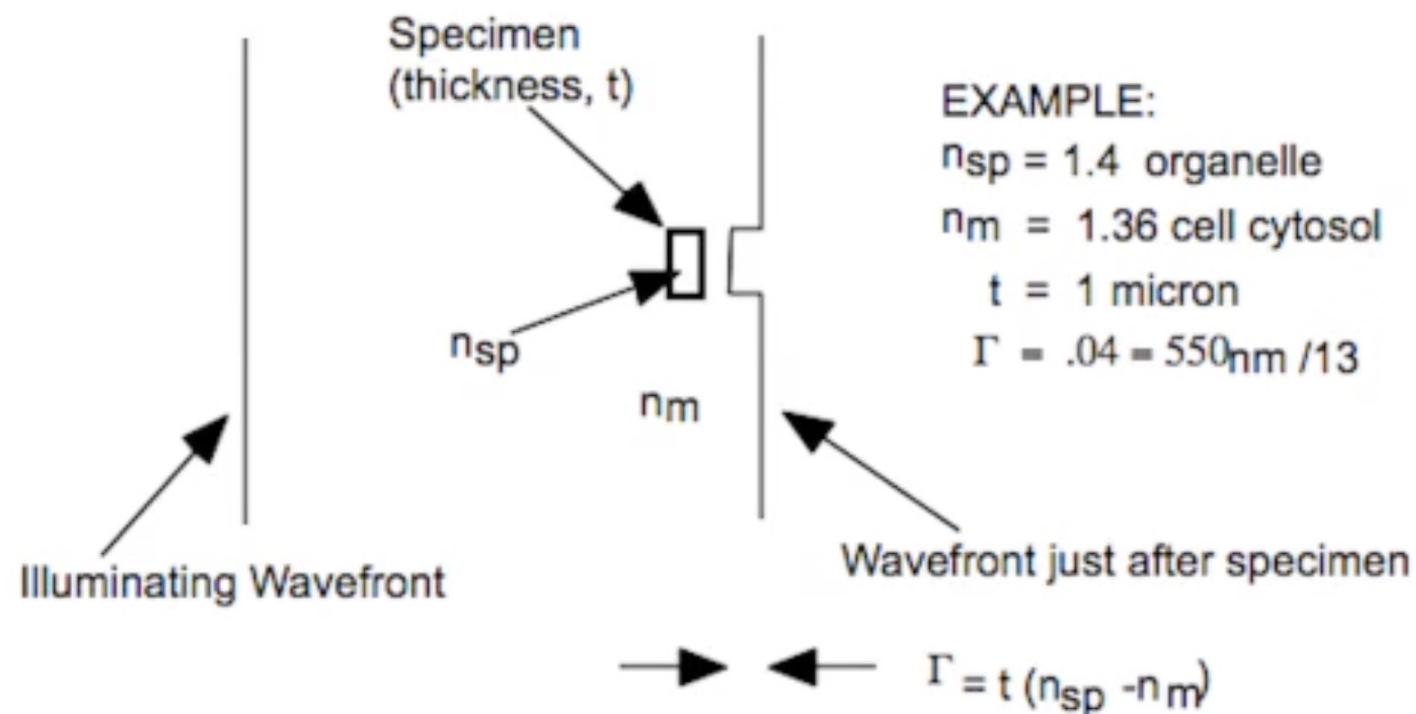
α = Half angle with the optical axis,

λ = Wavelength of light used.

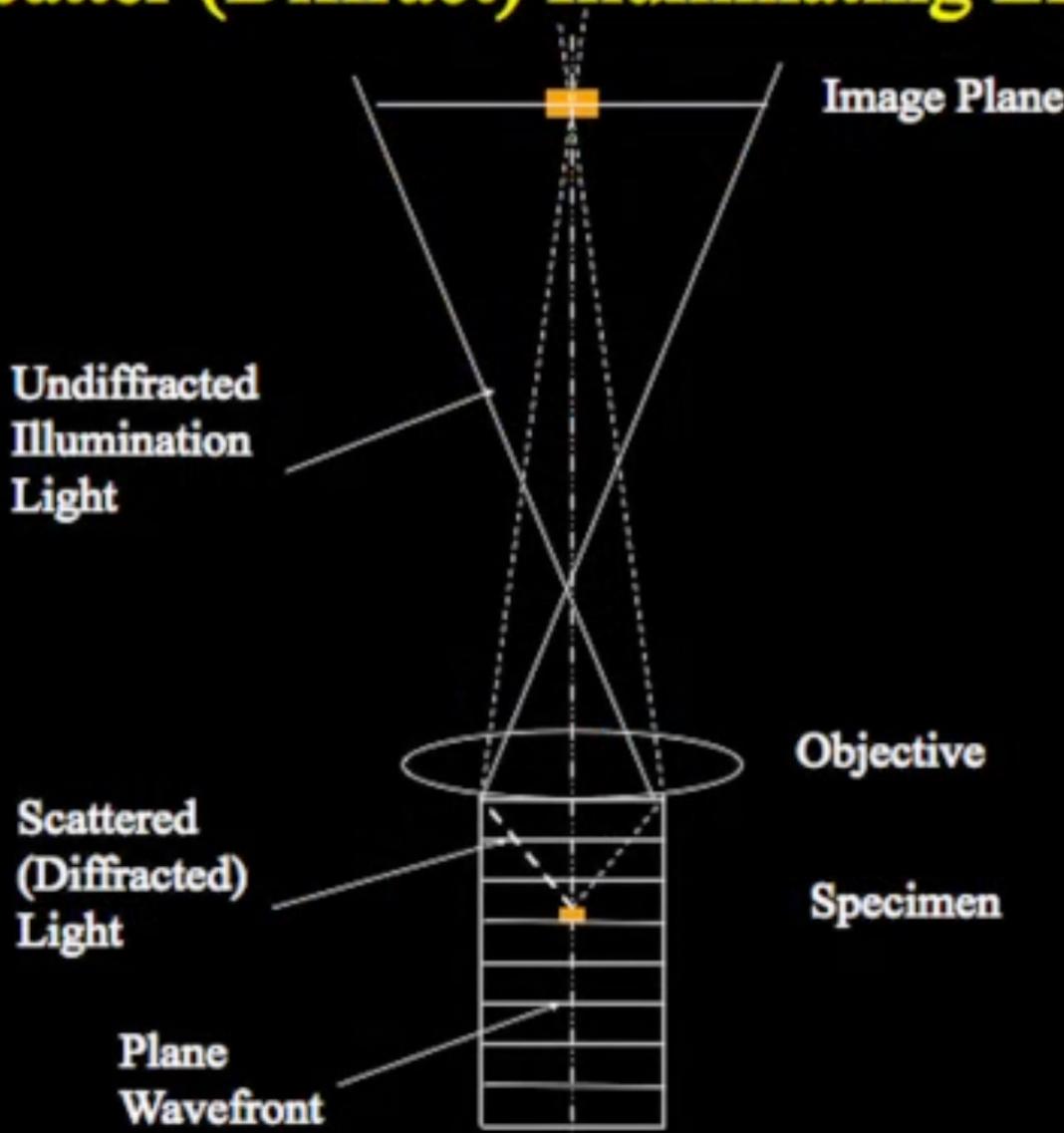
Contrast in Light Microscopy

- Absorption:
 - -Color Dyes: Histology
 - -Epi-Fluorescence
- Refractive Index Differences
 - in Specimen Structural Detail from Background (Media, Cytosol)

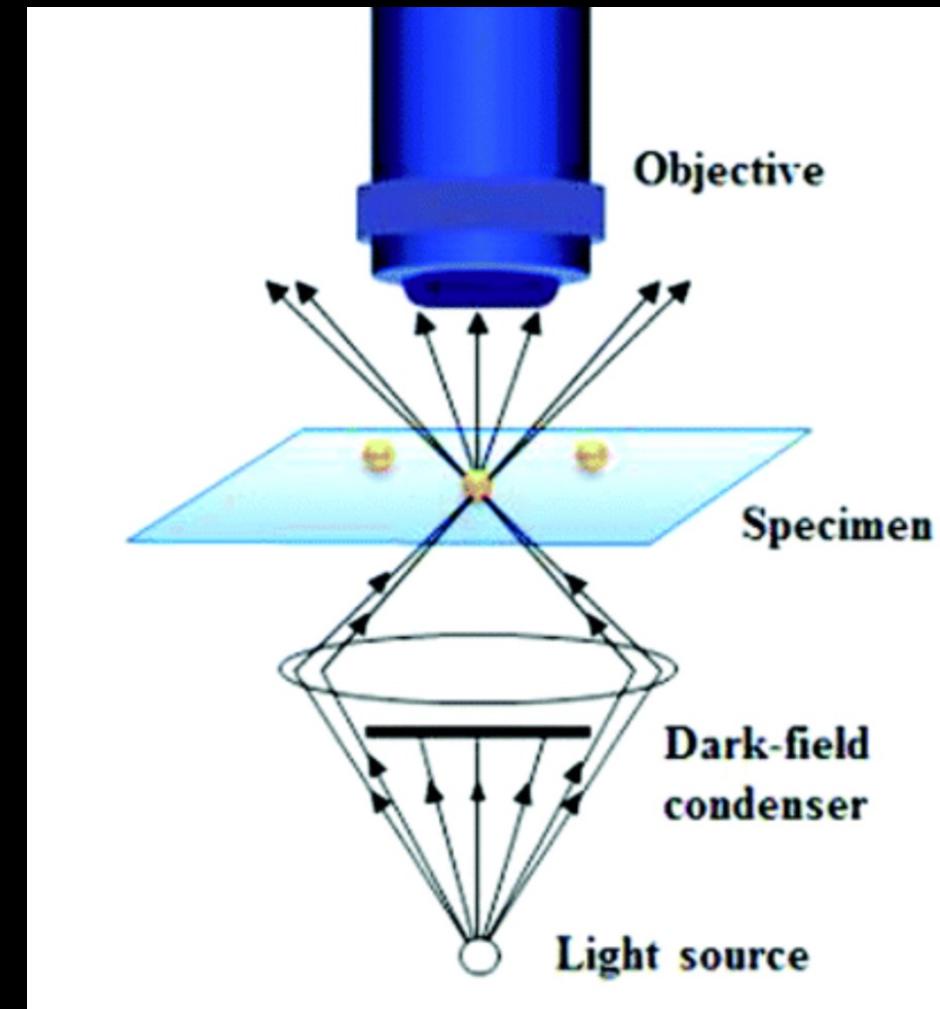
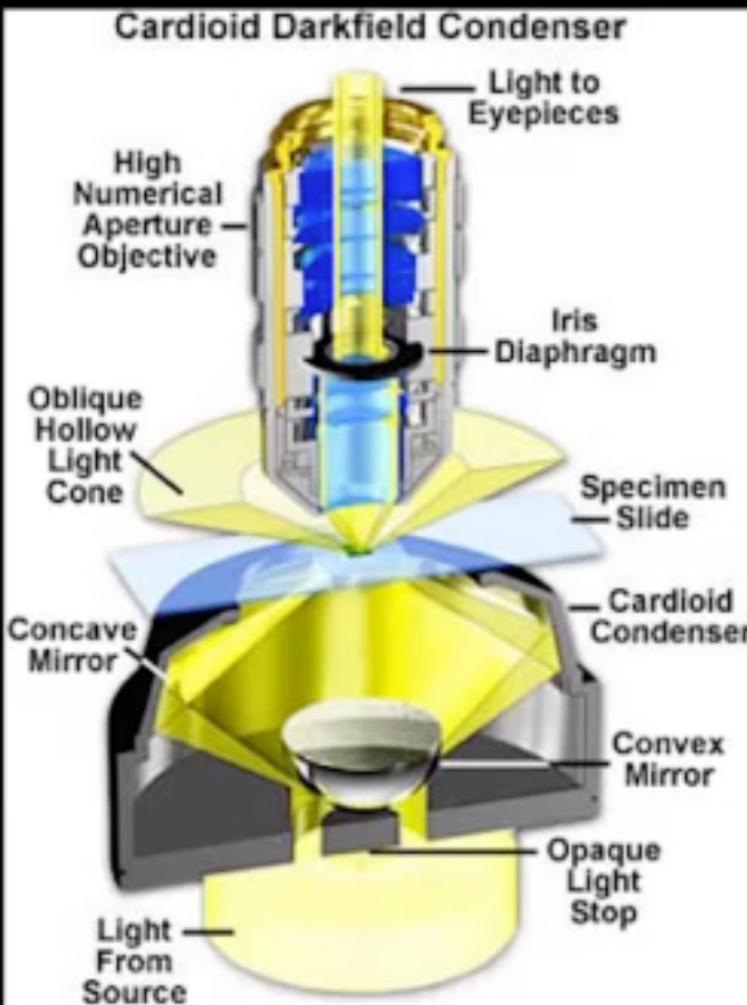
Specimens with a Higher Refractive Index Than the Background Produce a Retardation of Light



Retardations in Transparent Specimens Scatter (Diffract) Illuminating Light



Darkfield Microscopy: Objective Collects Only Scattered (Diffracted) Light; Rejects Illumination Light

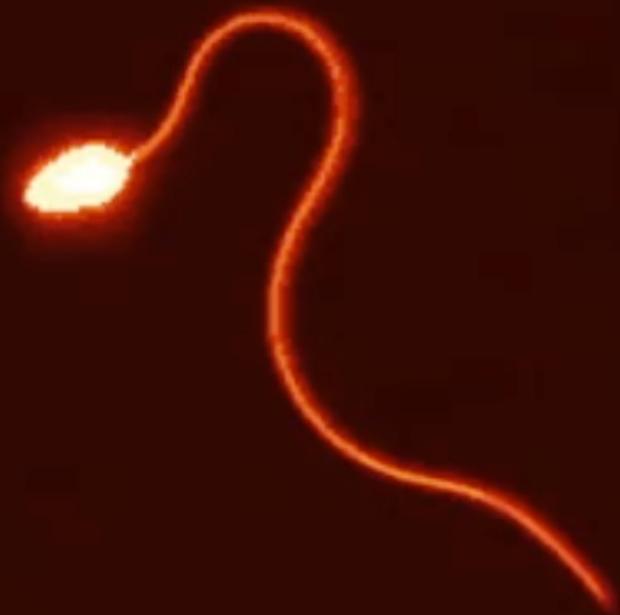


Source: [Link](#)

Advantages of Darkfield

- High sensitivity- Black background provides high contrast to see scattered light from small (~25 nm) objects.
- Excellent for low magnification outlines of individual cells such as sperm and flagella.

Darkfield of Beating Sea Urchin Sperm Axoneme

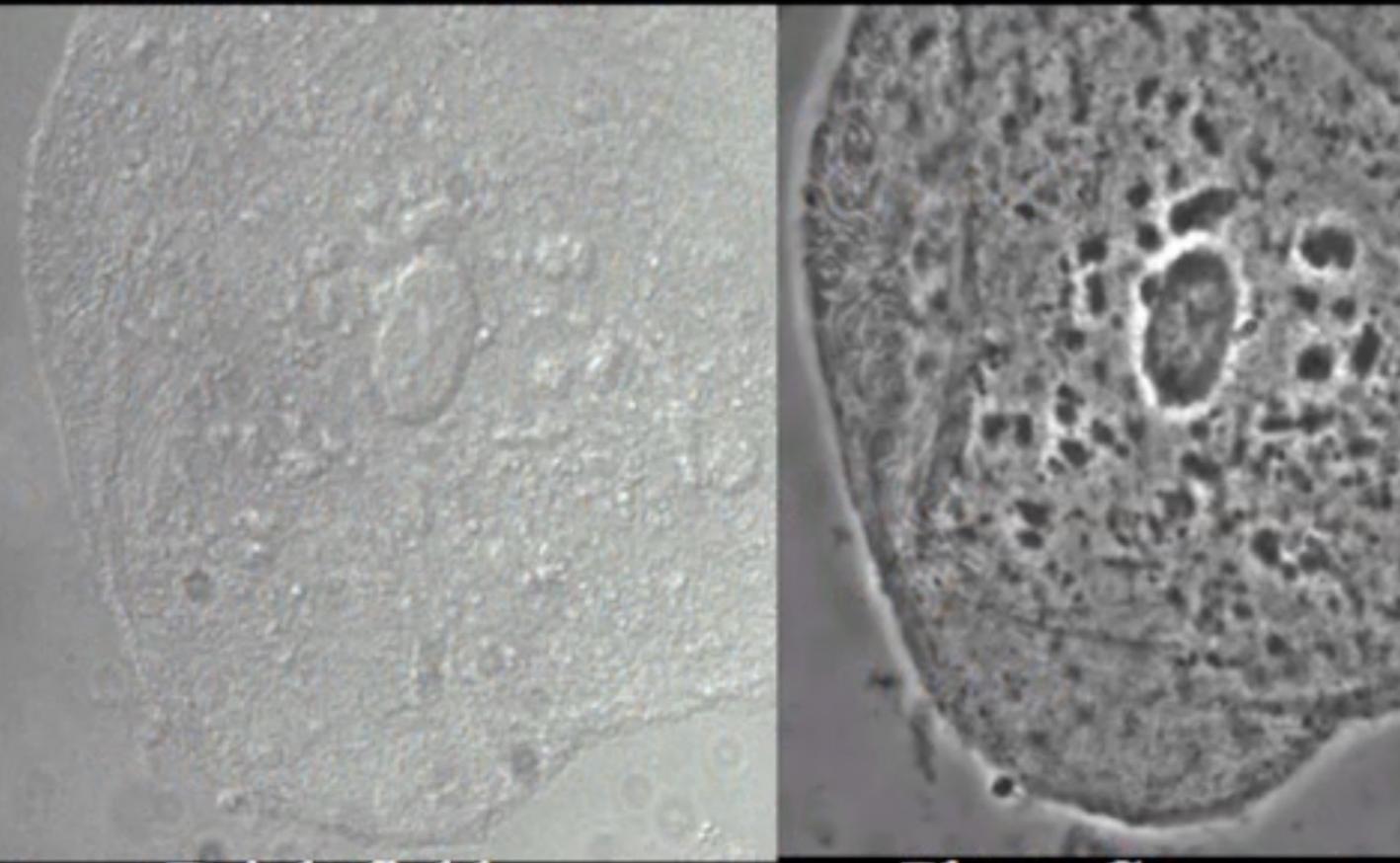


Shalin Mehta, Oldenbourg Lab,
MBL
Woods Hole, MA

Disadvantages of Darkfield

- Resolution limited by need for $NA_{cond} > NA_{obj}$
- Scattered light in thick specimens lowers contrast of fine structural detail
- Poor depth of field
- Images of internal cellular structure often inaccurate because missing interference with undiffracted light
- Often needs special, bright light sources

Phase Contrast Gives Contrast to Structural Detail in Transparent Specimens at Highest Objective NA

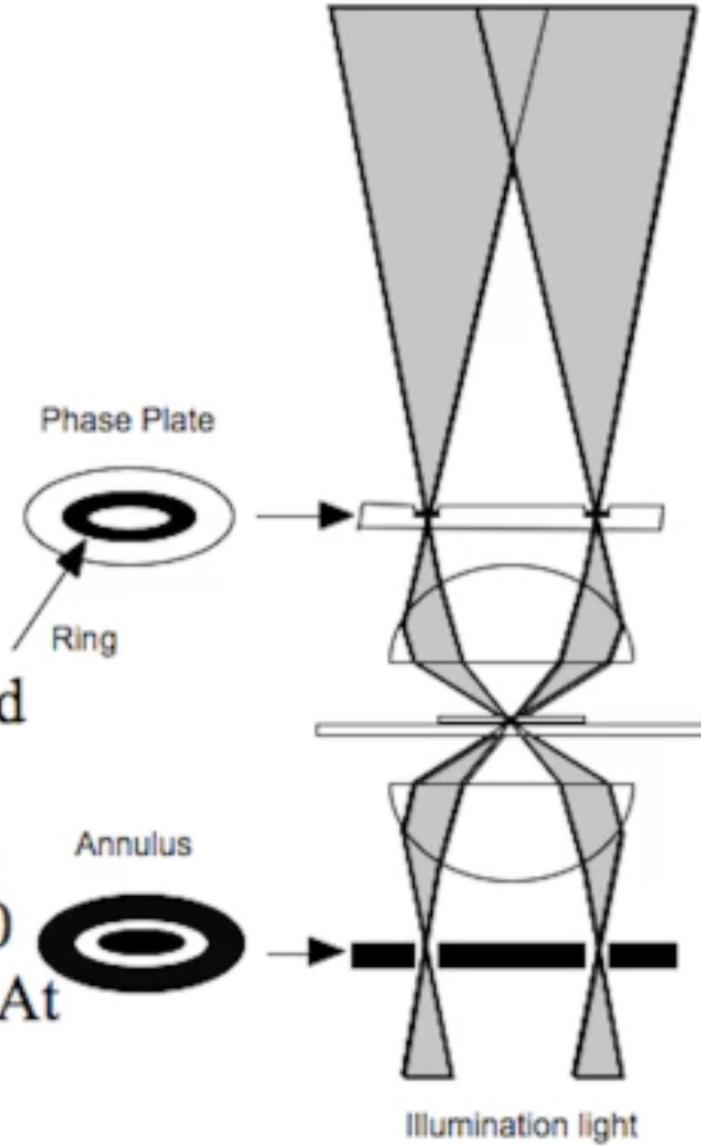


Brightfield

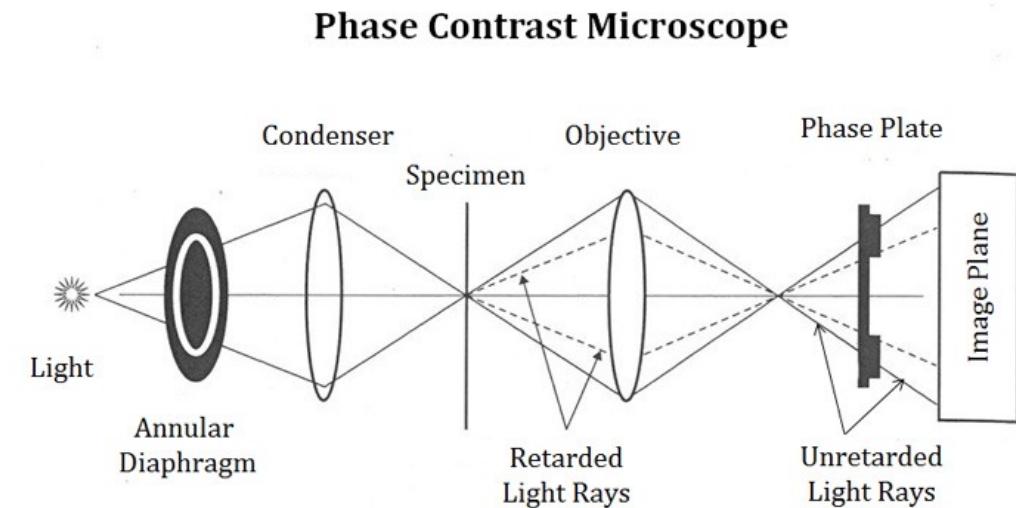
Phase Contrast

(Cheek Cell, $NA_{obj} = 1.4$)

Phase Contrast:
Uses
Annular Ring as
Condenser Stop
and Phase Ring
in Objective
Back Focal Plane



Phase Advance $\lambda/4$ and
Attenuate 75% The
Undiffracted Light So
Diffracted Light is 180
Degrees Out of Phase At
Image Plane to Give
Dark Contrast



Source: [Link](#)