Light Microscopy

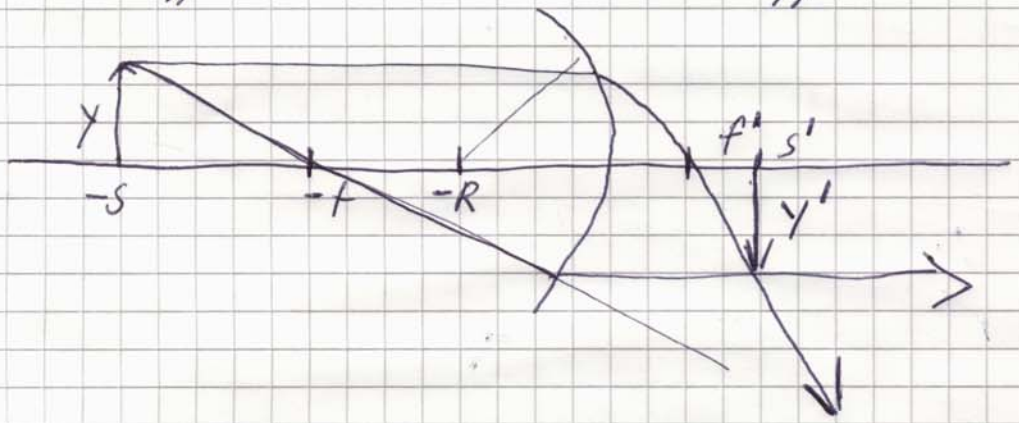
1. Basics Light Microscopy

Lit.: • Bergmann Schäfer Bd3 Optik p 148 ff
• Spector, Goldmann, Leinwand Cells, A Laboratory Manual Vol 2

cell size: eukaryotic cell 5–10 µm bacteria 1 µm
protein size: 5–10 nm

1.1 Ray Optics

refraction on a curved surface



refractive power: $\phi = \frac{n'-n}{R} = \frac{n'}{s'} - \frac{n}{s}$

focal length:

$$f' = \frac{n'}{\phi} = \frac{n'}{n'-n} R$$

$$f = -\frac{n}{\phi} = -\frac{n}{n'} f'$$

$$n\left(\frac{1}{R} - \frac{1}{s}\right) = n'\left(\frac{1}{R} - \frac{1}{s'}\right) \quad \text{Abbe}$$

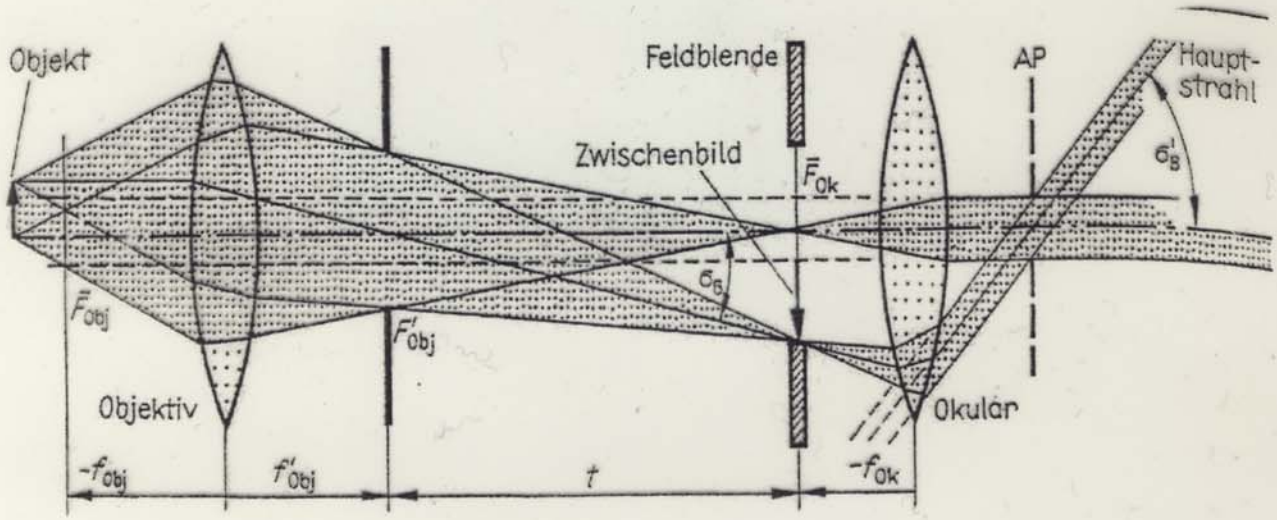
$$\frac{f'}{s'} + \frac{f}{s} = 1$$

magnification: $M = \frac{y'}{y} = \frac{n}{n'} \frac{s'}{s}$

History:
1538 Fracastoro principle
1590 Hans & Zacharias Janssen, Holland
17 century Leeuwenhoek
1869 Ernst Abbe

Vergrößerung Mikroskop

Abbildung mit zwei Sammellinsen.



Strahlengang im Mikroskop

Mit dem Objektiv wird ein reelles Zwischenbild erzeugt, das mit dem Okular (oder einem Projektiv) nachvergrößert wird.
Okulare dienen der visuellen Beobachtung.
Das virtuelle Bild liegt im Unendlichen (Normalvergrößerung) oder in der deutlichen Sehweite ($s_d = 25$ cm). Häufig verwendet werden Okulare mit 10- bzw. 20-facher Vergrößerung.

Newton

$$f f' = x x'$$

with $s = f + x \qquad s' = f' + s'$

$$\Rightarrow M = \frac{n s'}{n' s} = -\frac{x'}{f'} = -\frac{f}{x}$$

$$s' = -\frac{f}{x} \cdot \frac{n'}{n} s$$

1.2. Magnification

show picture of principle elements of a microscope

$$f'_M = \frac{f'_{obj} \cdot f'_{ok}}{\Delta}$$

Δ : tube length distance between focal points of objective and eyepiece

eyepiece : 10×, 20×,
objective : 10×, 20×, 40×, 60×, 100×

Magnification

$$V = \frac{\beta}{\alpha} = \frac{250\,mm}{f'_M} = \frac{250\,mm}{f'_{ok}} \cdot \frac{\Delta}{f'_{obj}}$$

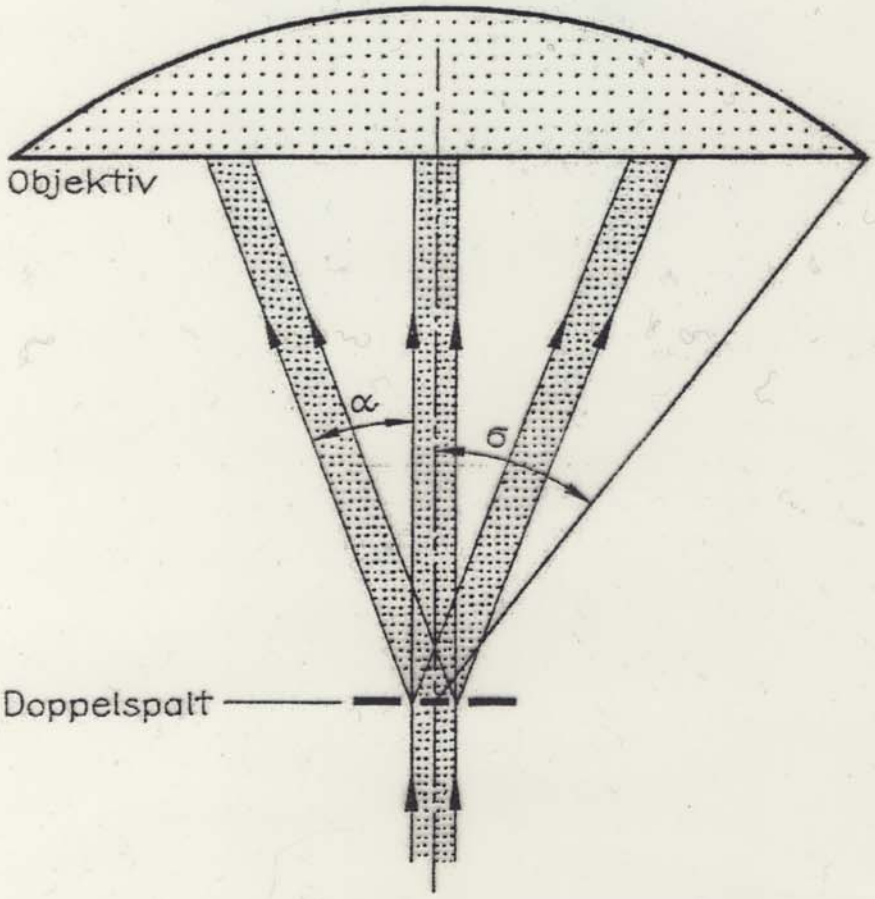
~~α : angle object appears in eye~~

$$V = 50 - 2000$$

α : opening angle of the object

β : opening angle of picture

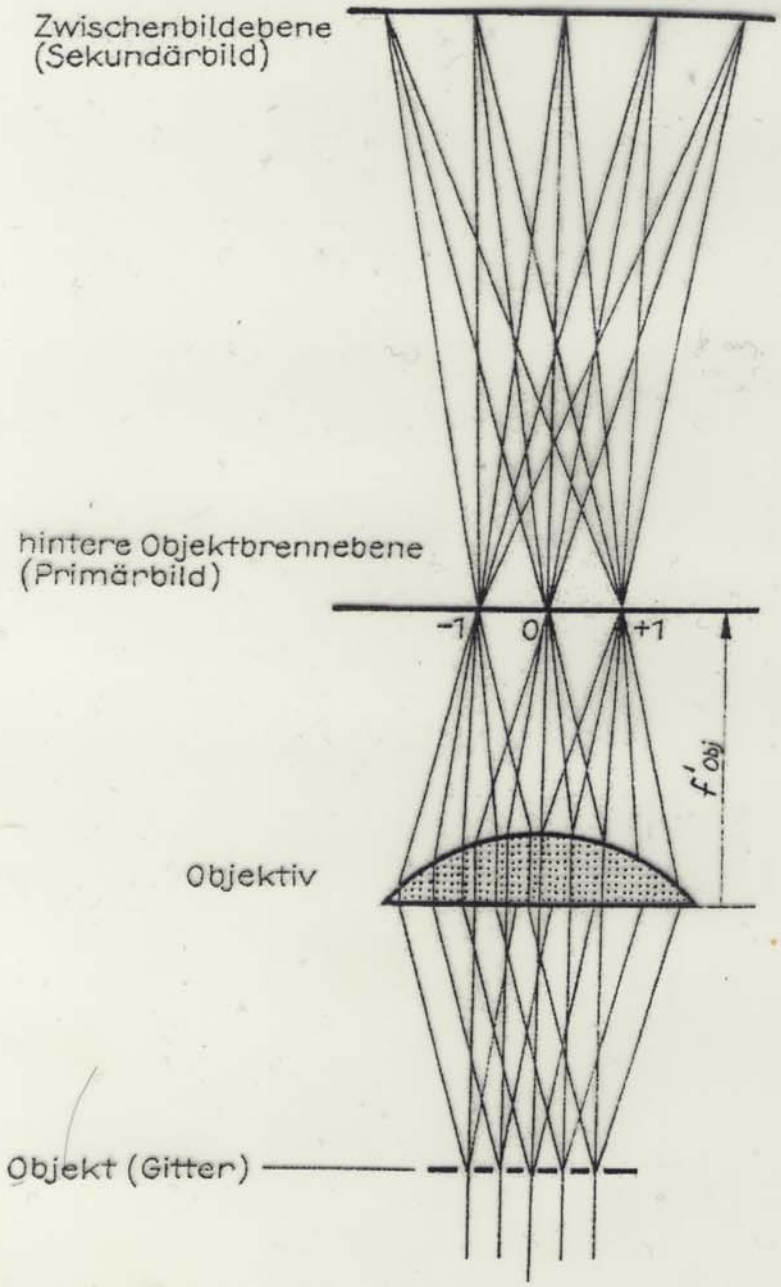
Ein Objekt, das aus zwei dicht benachbarten Gitterspalten im Abstand d_{min} besteht, wird dann noch aufgelöst, wenn mindestens die ersten Beugungsordnungen (m = +1, 0, -1) in das Objektiv gelangen:



Begrenzung des Auflösungsvermögens durch die numerische Apertur

Auflösungsvermögen

Nach Abbe (1873) müssen zum genaueren Verständnis der Bildentstehung im Mikroskop die Beugungs- und Interferenzeffekte des Lichts betrachtet werden.



1.3. Resolution

Abbé 1873:

picture in microscope is result of diffraction and interference!

show picture!

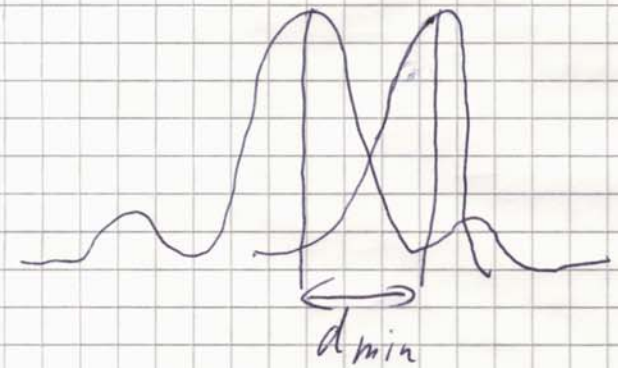
two close by objects with distance d_{min} can be imaged when at least the first diffraction peaks reach the objective!

show picture!

⇒ numerical aperture $A_{Obj} = n \sin\alpha$

$$\Rightarrow \quad A_{Obj} = n \sin\alpha \geq \sin\alpha = \frac{\lambda}{d_{min}}$$

$$\Rightarrow \boxed{d_{min} \geq \frac{\lambda}{A_{Obj}}}$$



$$\boxed{d_{min} = \frac{0,61\,\lambda}{n \sin\alpha}}$$

air objectives

$n = 1 \qquad A_{obj} = 0.95 \Rightarrow \theta \approx 72^\circ$

immersion objectives

$n = 1.33$ (water)

$n = 1.45 - 1.52$ (immersion oil)

$\Rightarrow A_{obj} \approx 1.4 - 1.6$

$$\Rightarrow d_{min} = \frac{480\,nm}{1.6} = 300\,nm$$

1.4. Intensity and Information

Intensity $\quad J = \dfrac{d^2\phi}{d\Omega\, dA}$ — transmitted light power

in reality: $\quad J = \dfrac{1}{\Lambda} \dfrac{d^2\phi}{d\Omega\, dA}$

Λ | light transmission value!

Light emitted by radiated object

$$P(x,y) = A(x,y)\, e^{ik\,W(x,y)}$$

picture

$$F(l_x, l_y) = c \iint P(x,y)\, e^{-ik(x l_x + y l_y)/z}\, dx\, dy$$

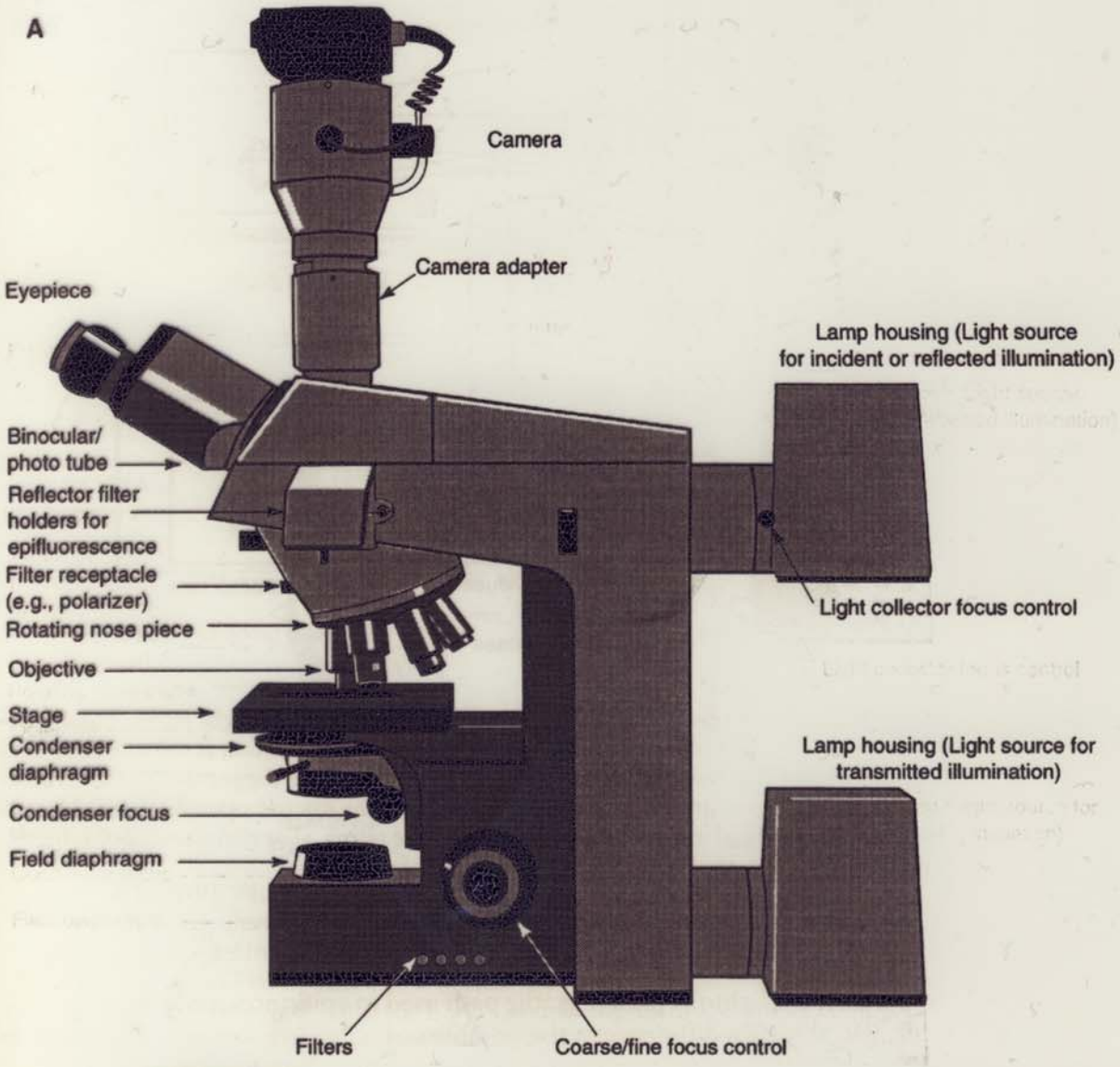
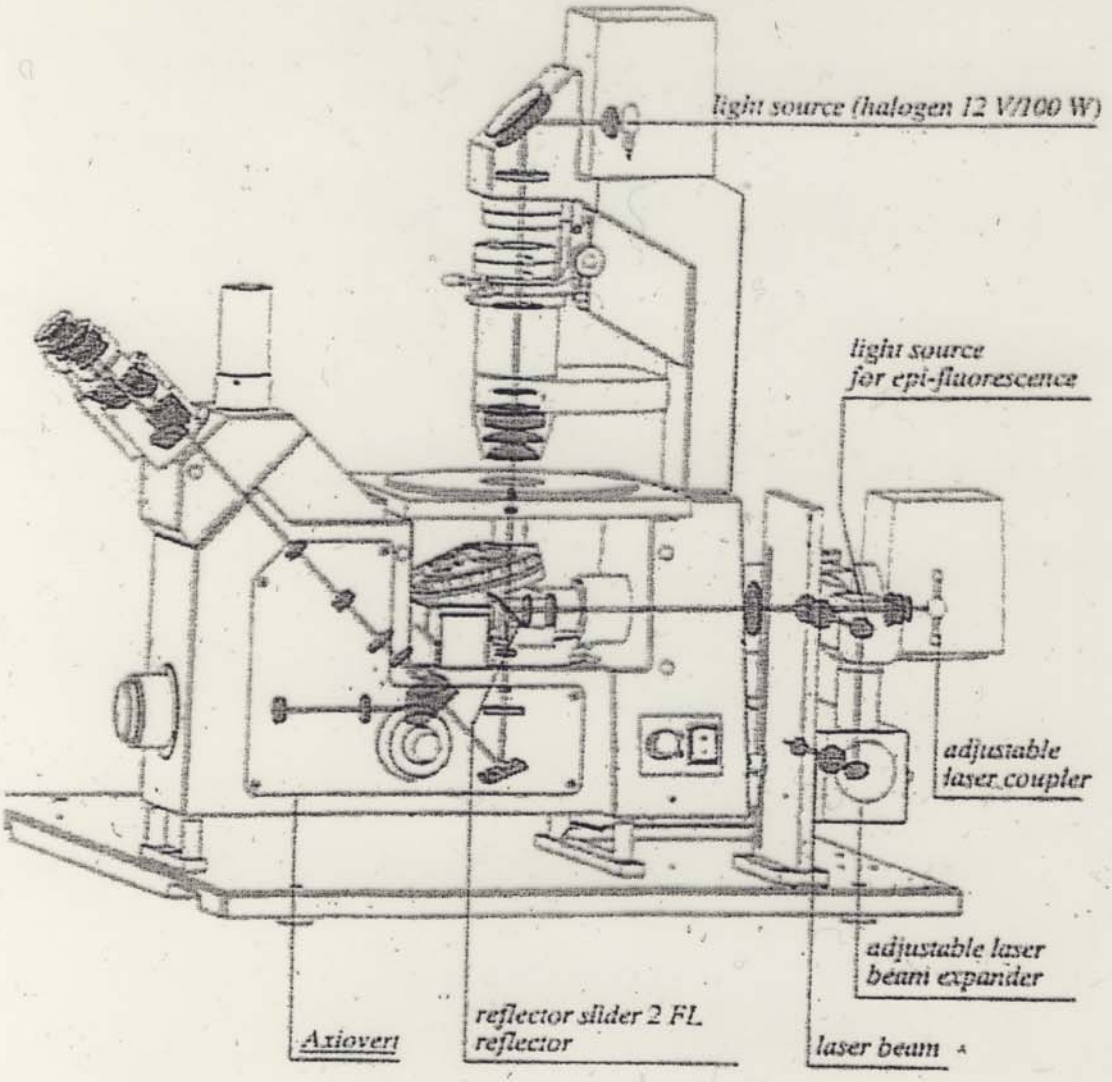


FIGURE 94.2
The light microscope. (*A*) Basic components of the light microscope arranged for transmitted and incident illumination. *(figure continues)*


light source (halogen 12 V/100 W)
light source
for epi-fluorescence
adjustable
laser coupler
adjustable laser
beam expander
Axiovert
reflector slider 2 FL
reflector
laser beam

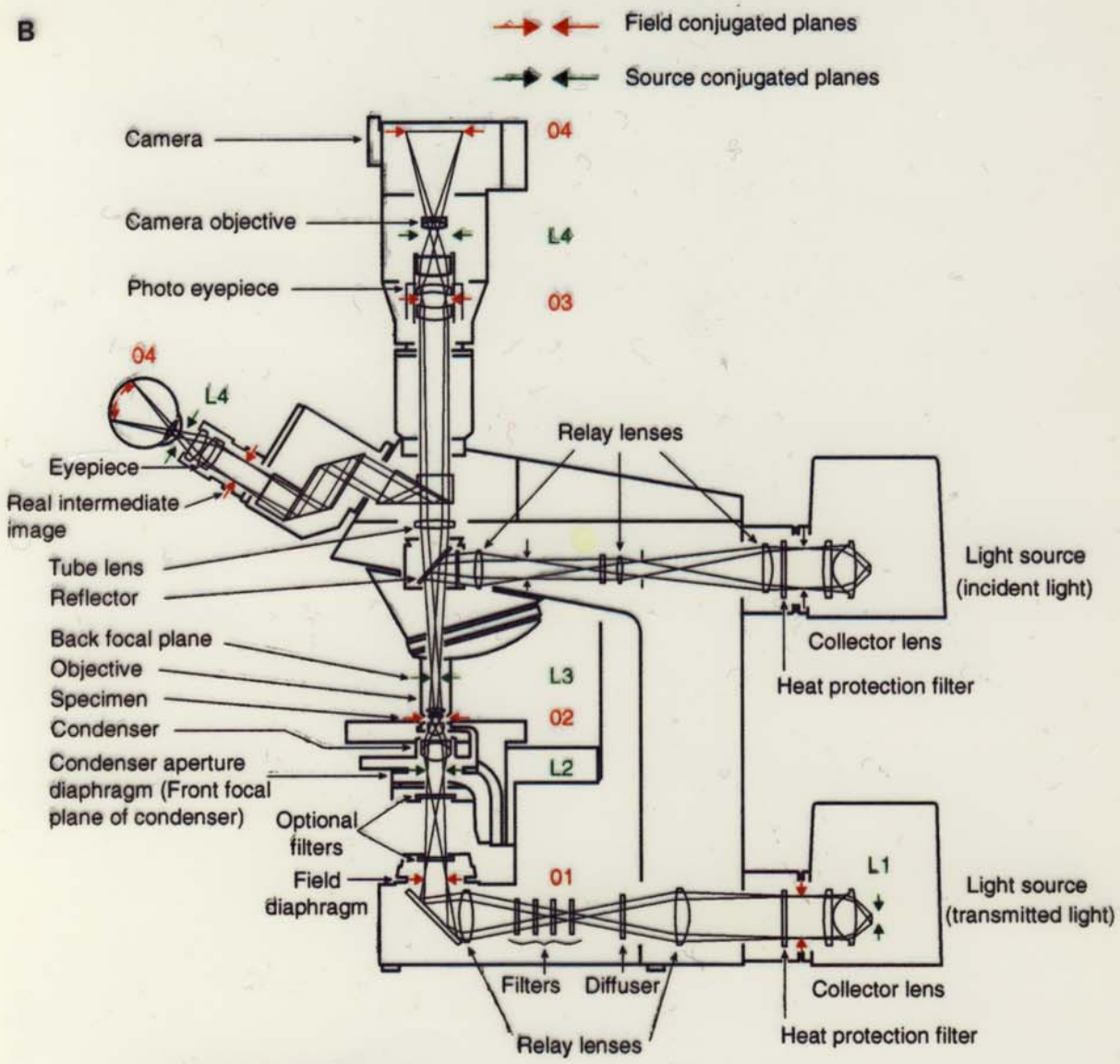


FIGURE 94.2 (continued)
(*B*) Diagrammatic representation of the transmitted and incident light paths. Light from the source to final image either in the camera or on the human retina is shown. Four field conjugated planes (represented by red arrows) and four source conjugated planes (represented by green arrows) are within the optical system of the microscope. The last field-conjugated plane is the final image in the camera or on the retina. (For definitions of 01, 02, 03, 04 and L1, L2, L3, L4, see Fig. 94.3A).

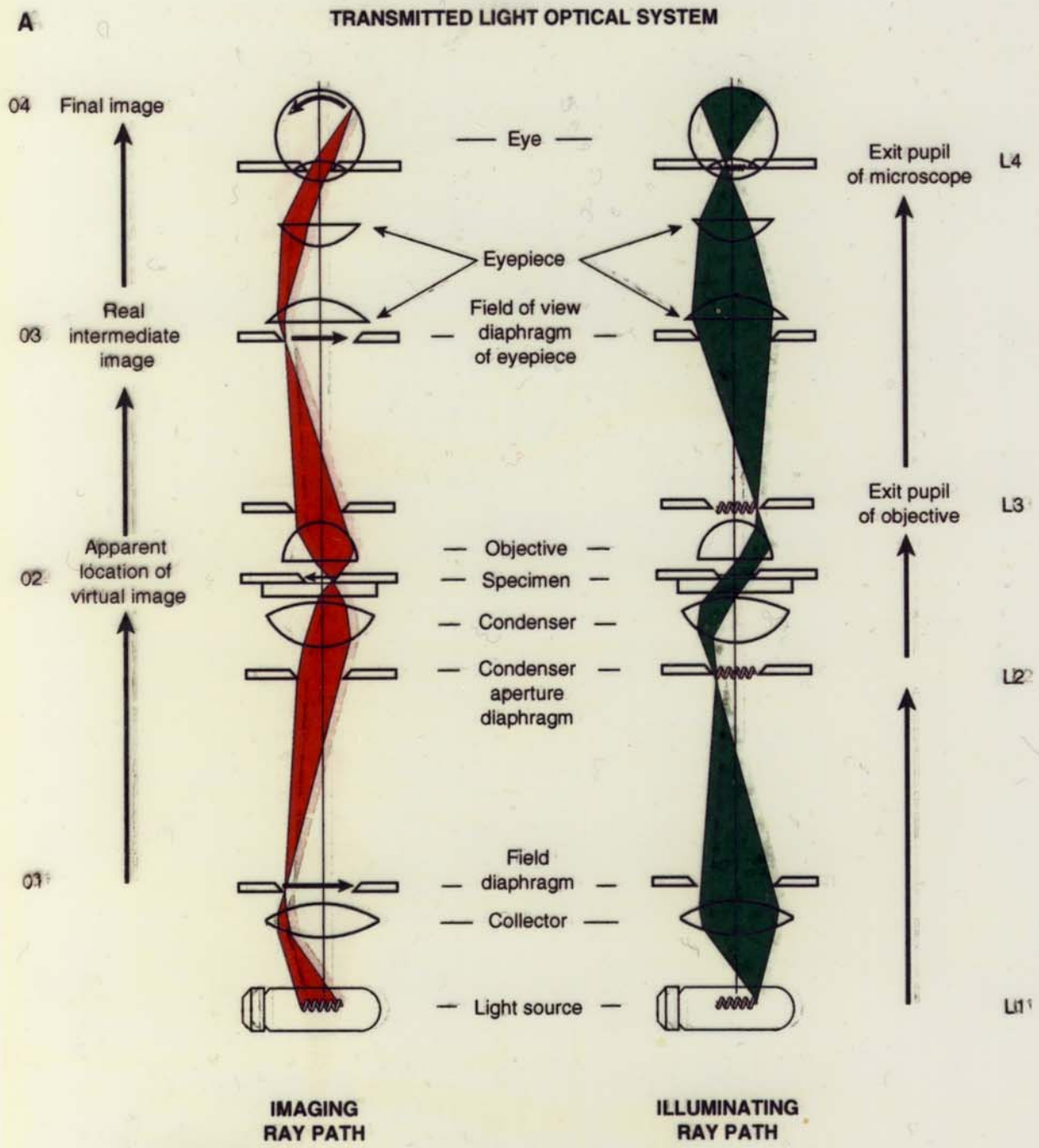
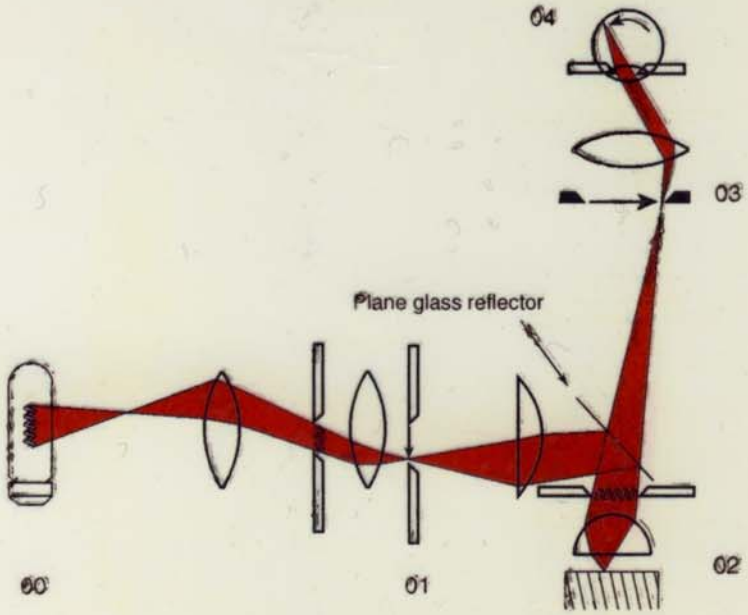


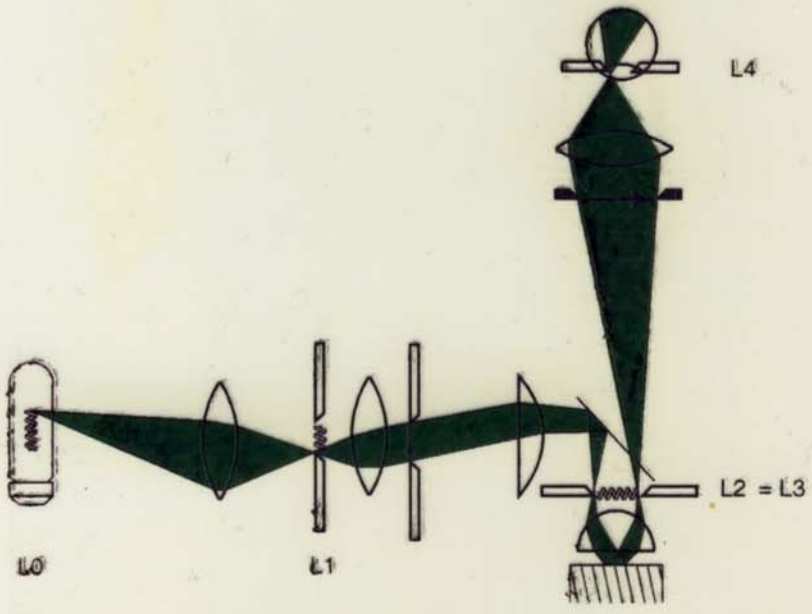
FIGURE 94.3
Köhler illumination. (*A*) Ray paths in Köhler transmitted light for a finitely corrected microscope. In the imaging ray path, 01, 02, 03, and 04 represent the image conjugated planes (see also Fig. 94.2B). The arrows in the imaging ray path indicate image orientation. In the illuminating ray path, L1, L2, L3, and L4 represent the source conjugated planes (see Fig. 94.2B). (*B*) Ray path in incident light Köhler illumination (epifluorescence). In the imaging ray path, 00, 01, 02, 03, and 04 represent the image-conjugated planes. In the illuminating ray path, L0, L1, L2, L3, and L4, represent the source-conjugated planes. Because the objective serves also as condenser, L2 and L3 are coincident.

B

INCIDENT LIGHT OPTICAL SYSTEM



IMAGING RAY PATH



ILLUMINATING RAY PATH

FIGURE 94.3 *(continued)*