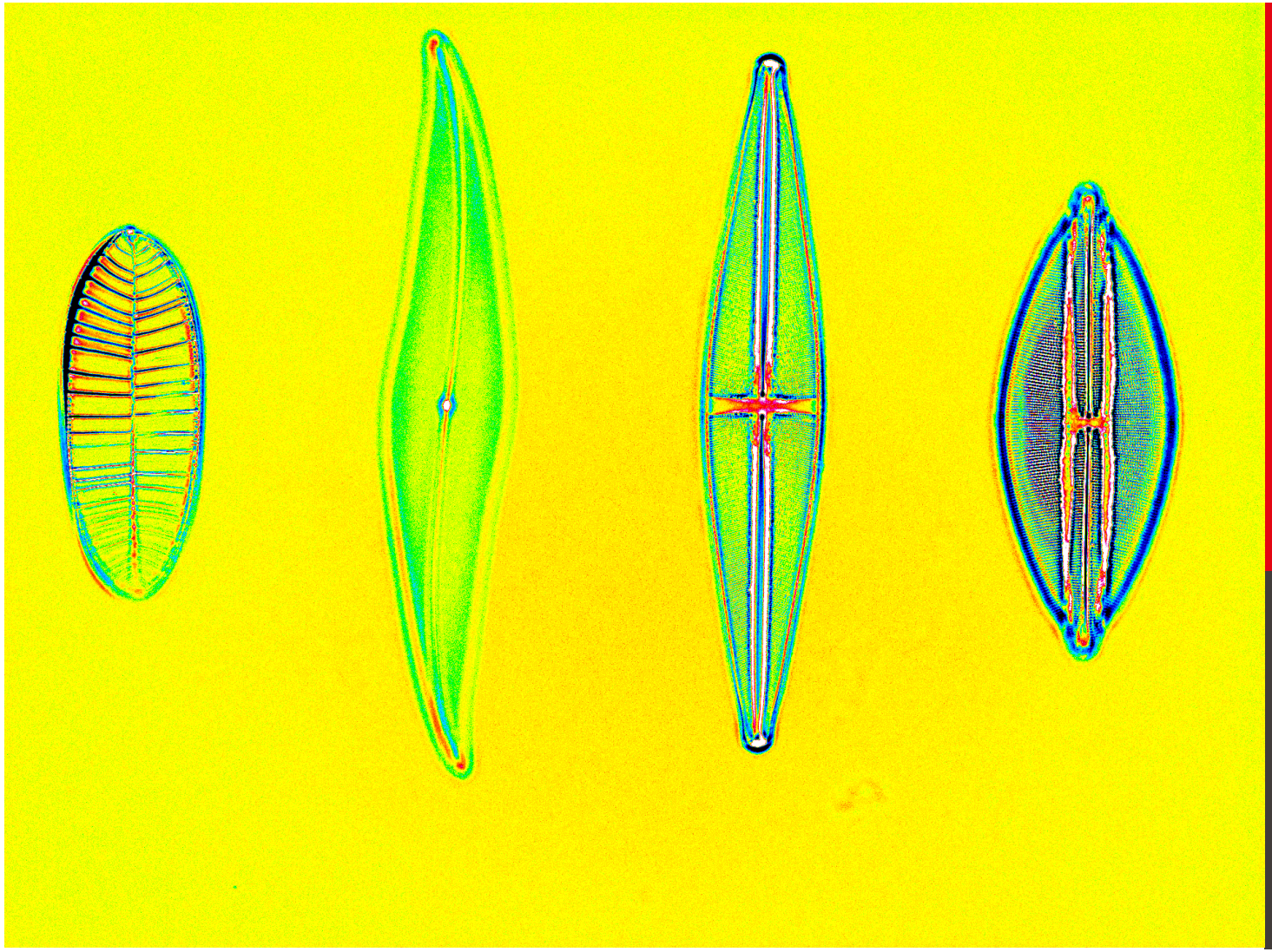


Biomicroscopy II

Microscopy Basic & Terminology

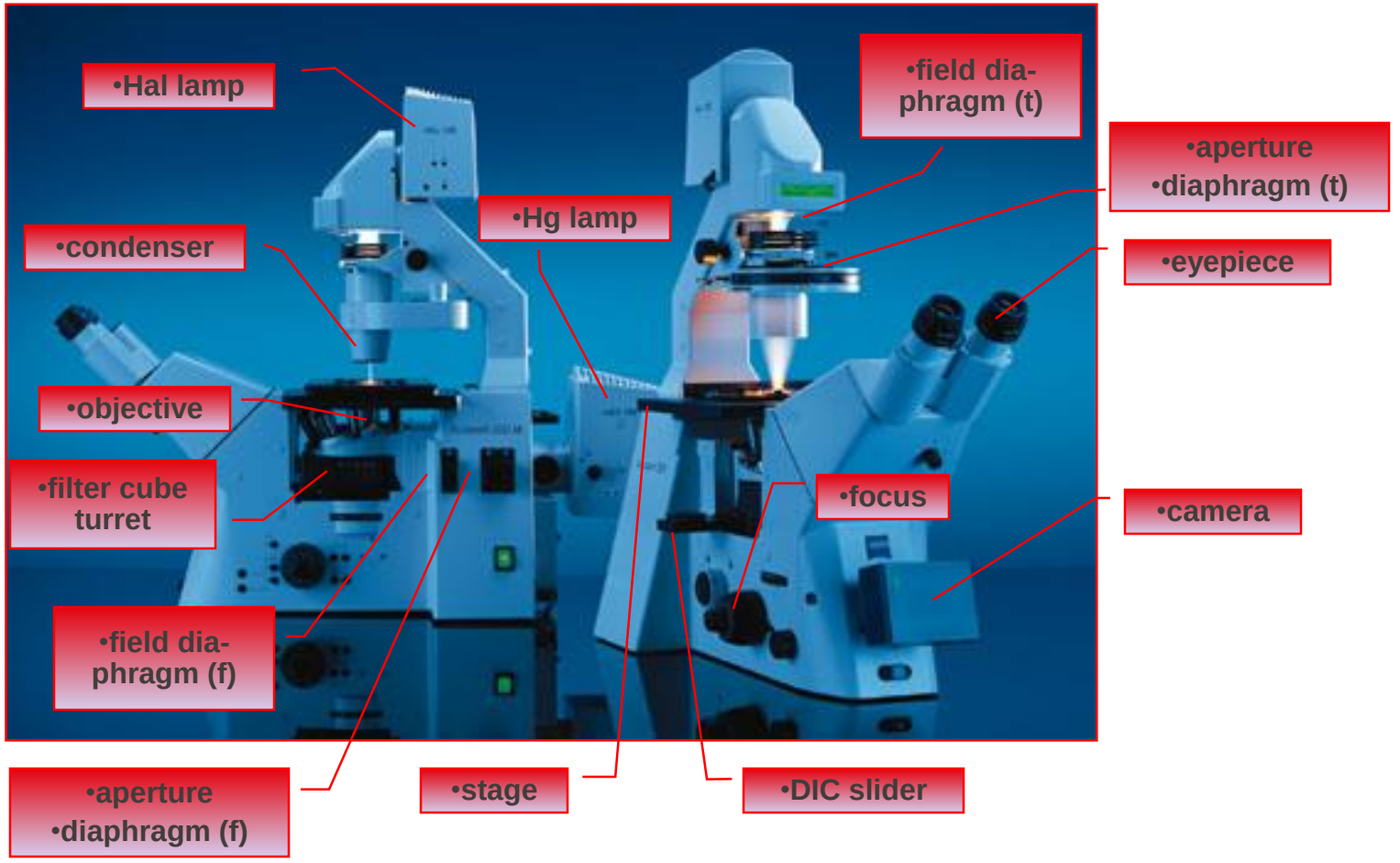
École polytechnique fédérale de Lausanne

Microscope Designs

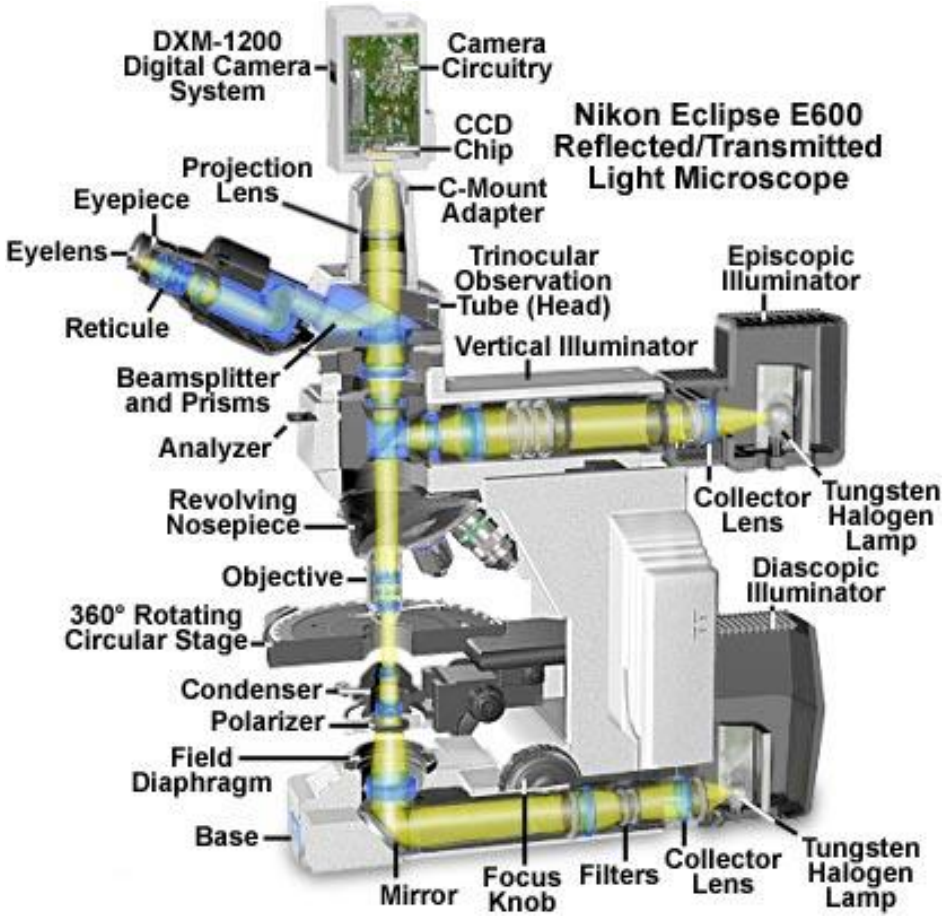
- Upright
- Used in biology mostly for fixed specimens

- Inverted
- Widely used in biology for living cell imaging

Main microscope components



Anatomy of microscope



- Two independent
- illumination paths:
- Transmission
- Fluorescence

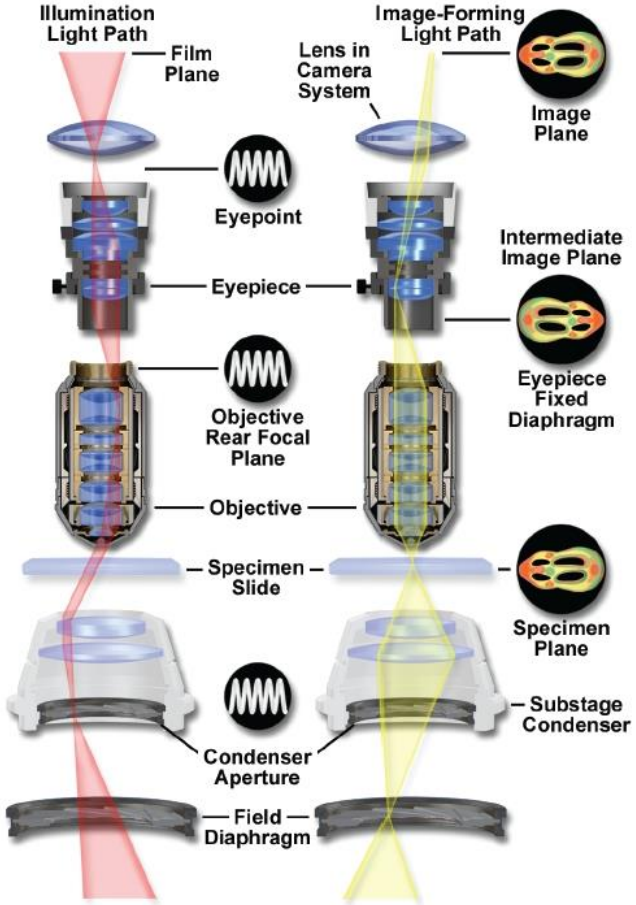
- Components for
- contrasting methods:
- DIC
- Dark field
- Phase contrast

Requirements for illumination

- Uniform over whole field of view
- Has all angles accepted by objective
- Allows optimize image brightness/contrast
- Allows continuous change of intensity
- Allows continuous change of field of view
- Change in illumination and imaging parts do not effect each other

Realized in Kohler illumination

Conjugated planes in optical microscopy

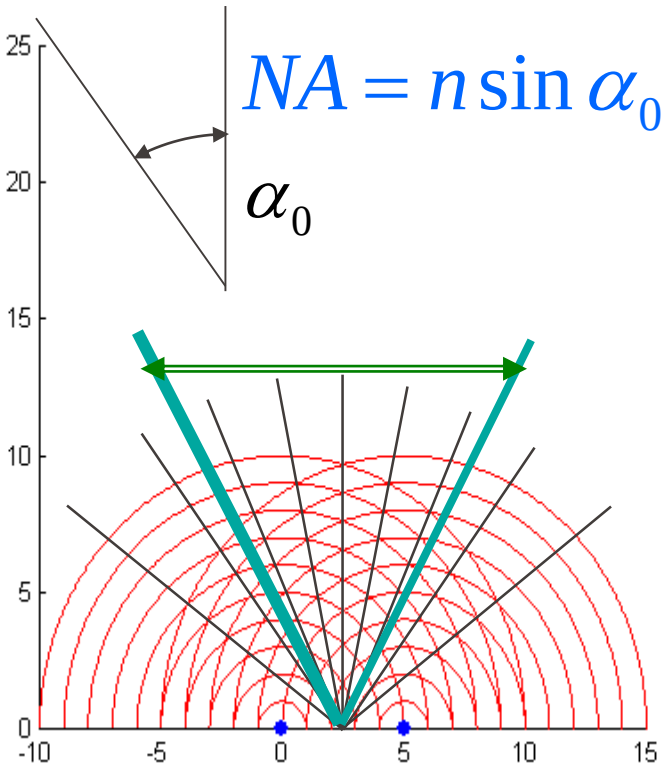


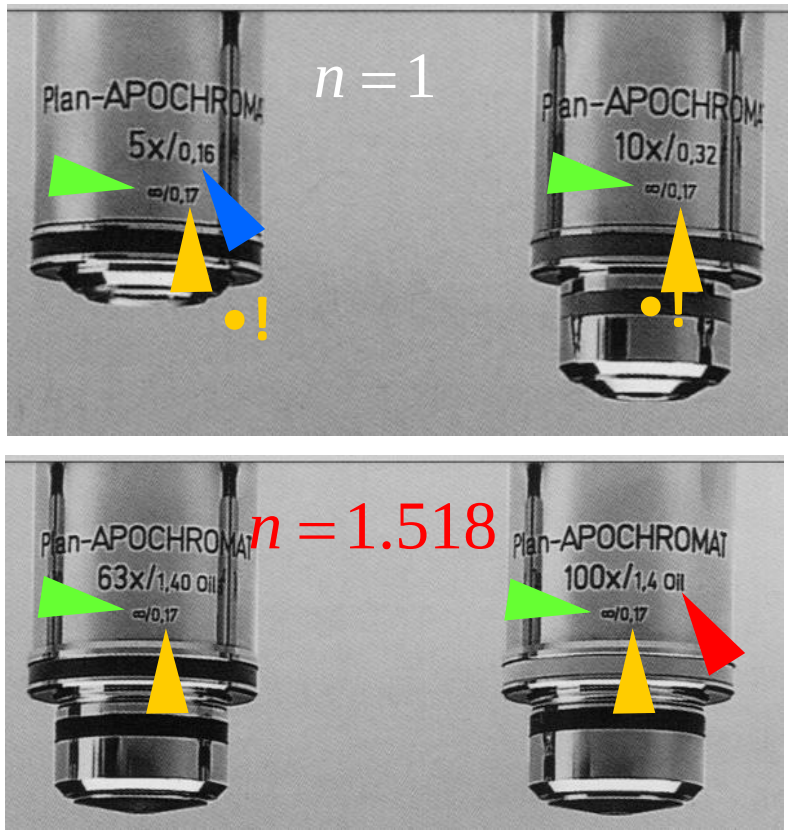
- Image forming light path
- (Observed with eyepiece)
- 1. Variable field diaphragm
- 2. Specimen plane
- 3. Intermediate image plane
- 4. Image plane (camera, retina)

- Illumination light path
- (Observed with Bertrand lens)
- 1. Lamp (filament, arc)
- 2. Condenser aperture diaphragm
- 3. Objective rear (back) focal plane
- 4. Eyepoint (exit pupil of microscope)

- Conjugated = imaged onto each other
- Has one diaphragm in every path
- If light at given plane is focused in one
- path, it is parallel in other path

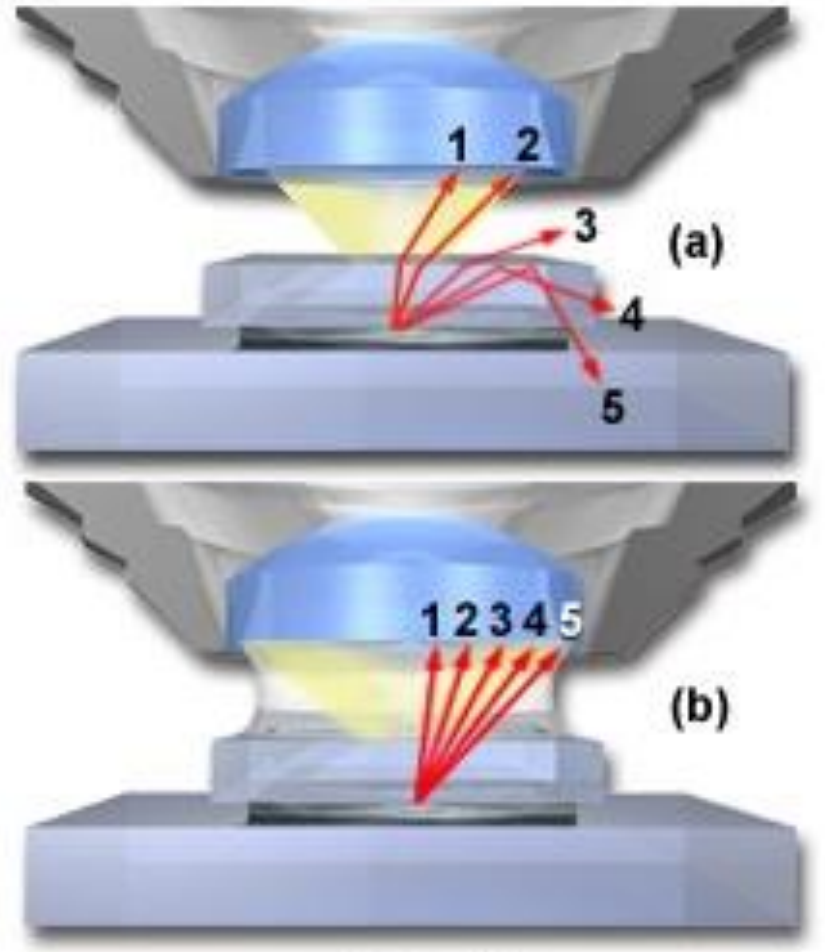
Numerical aperture of objective





The NA defines how much light (signal) and how many diffraction orders (resolution) are captured by the objective.

Role of immersion

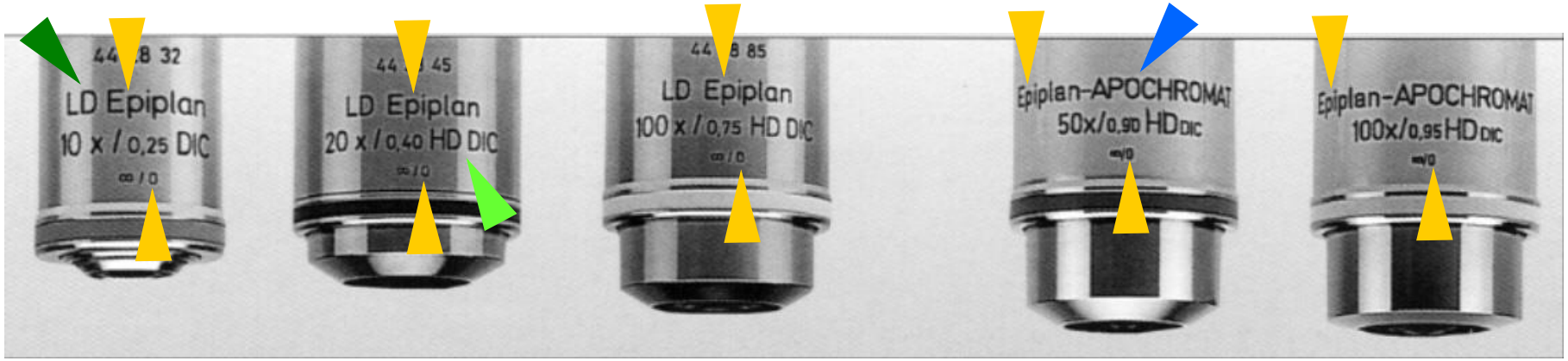


- NA =n sin α
- Refractive indices:
  - Air - 1.003
  - Water - 1.33
  - Glycerol - 1.47
  - Oil - 1.52
- Immersion media increase the NA of an objective or a condenser by brining the beams with higher incidence angle into the light path

Working distance and parfocal length

- Parfocal distance
  - Distance from objective shoulder untill specimen plane
  - 45 mm for most manufactures, 60 mm for Nikon CFI 60
- Working distance
  - Distance from front edge of objective untill cover slip
  - Varies from several mm untill several hundreds micrometers. Special long working distance objective are available.

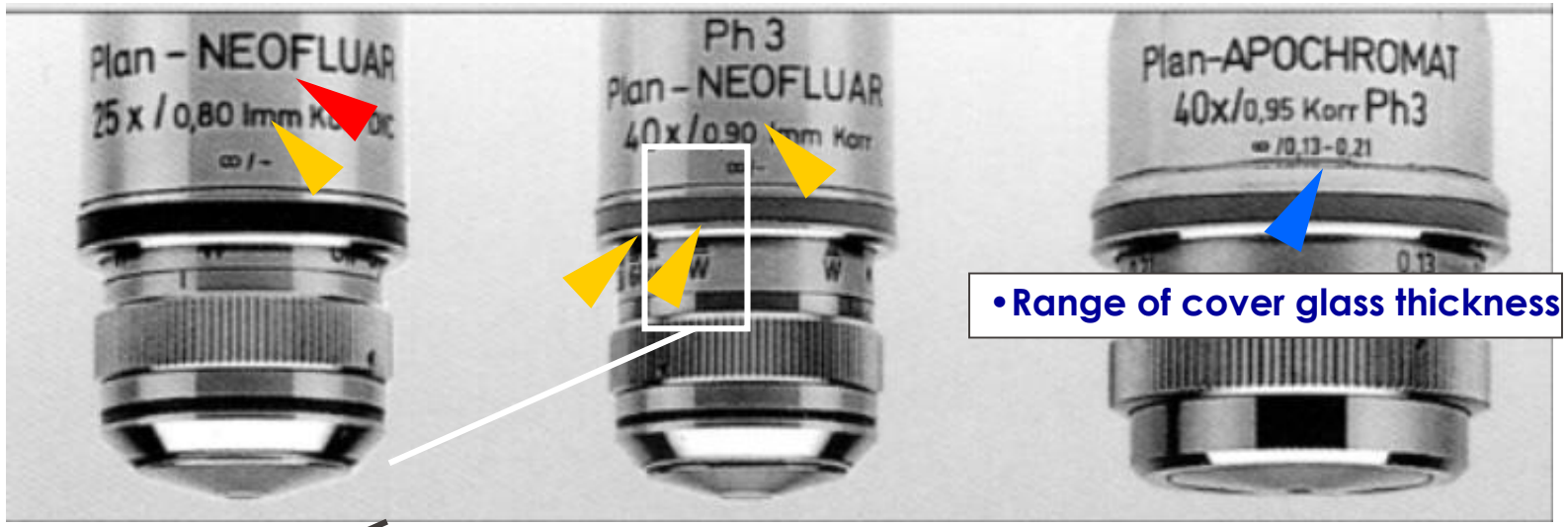
Engravings on objectives



- Epi = observation from above (**0 = no cover glass**)
- LD = **long** (working) **distance**
- **plan** = minimal curvature in the image plane
- **APOCHROMAT** = especially color corrected
- HD = **hell/dunkel** = bright/dark field
- DIC = **differential interference contrast** (low strain optics for polarized light)

Objectives with correction collars

NEOFLUAR optics is less color corrected than APOCHROMAT



- Ph = phase contrast (3 specifies matching condenser)

- Different immersion media under various cover glass conditions

Total microscope magnification

- Defined by magnification of objective, eyepiece and intermediate magnification
  - $M_{tot}=M_{obj} \times M_{int} \times M_{eyepiece}$
- Objective magnification defined by focal lengths of tube lens and objectives
  - $M_{obj}=f_{tl}/f_{obj}$
- Tube lens has a standardized value for specific manufacture
- Zeiss, Leica, Olympus 165 mm, Nikon 200 mm
- Typical magnification ranges:
  - M_{obj}: 2x÷100x
  - M_{int}: 1.5x÷2.5x
  - M_{obj}: 10x÷25x

Useful magnification range

- Microscope resolution is limited by NA and wavelength.
- Enlargement of image does not necessarily resolve new features.
- Excessively large magnification is called empty magnification.
- The Airy disk on retina/camera should not exceed two cell/pixel sizes.
- Useful magnification = 500-1000 x NA of objective

M_{obj}	$M_{eyepiece}$	NA_{obj}	M_{tot}	M_{useful}	Magnification
10x	10x	0.35	100	175-350	low
40x	10x	0.70	400	350-700	ok
100x	10x	1.40	1000	700-1400	ok
100x	15x	1.40	1500	700-1400	empty