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Basic Knowledge of Microscopy

Magi Hung

Classification of Microscopy

Light microscopy

Upright microscopy

Inverted microscopy

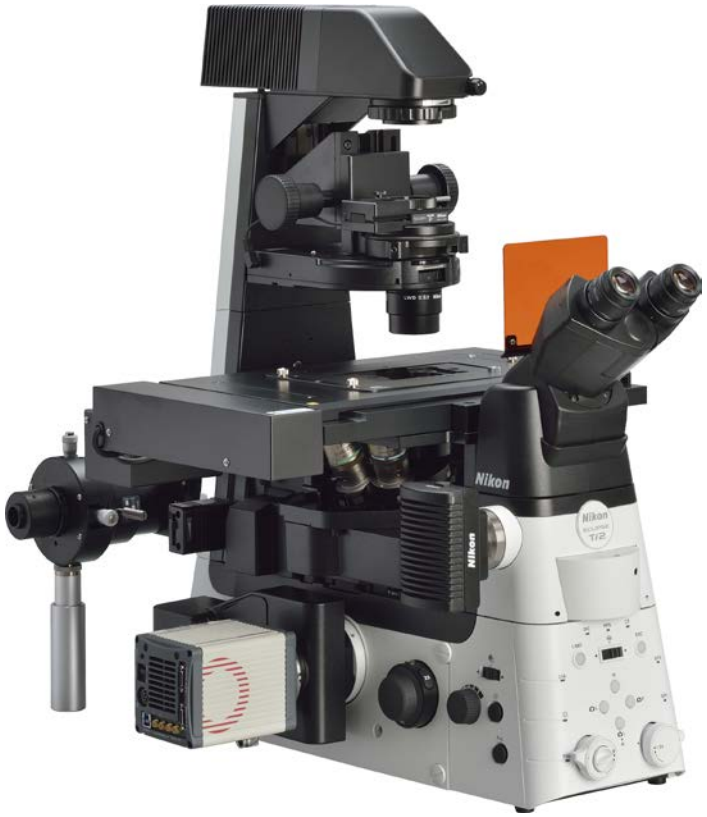
Stereomicroscopy

Laser scan microscopy (LSM)

Upright Microscopy

Generally for Slides or sample under shallow medium

Inverted Microscopy



Generally for sample attaching container bottom or slides.
(e.g. cell culture)

Optical train of compound microscope

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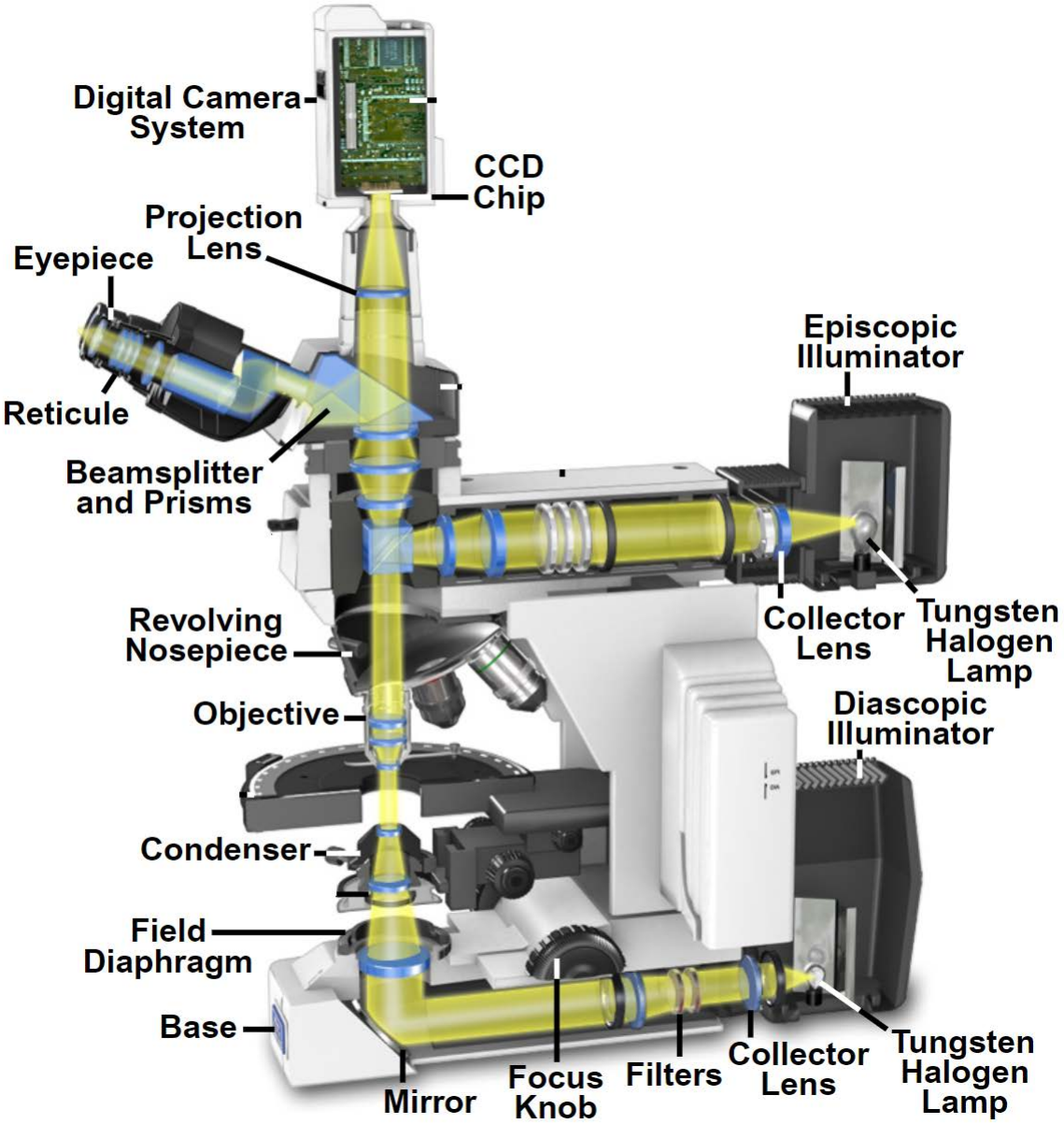
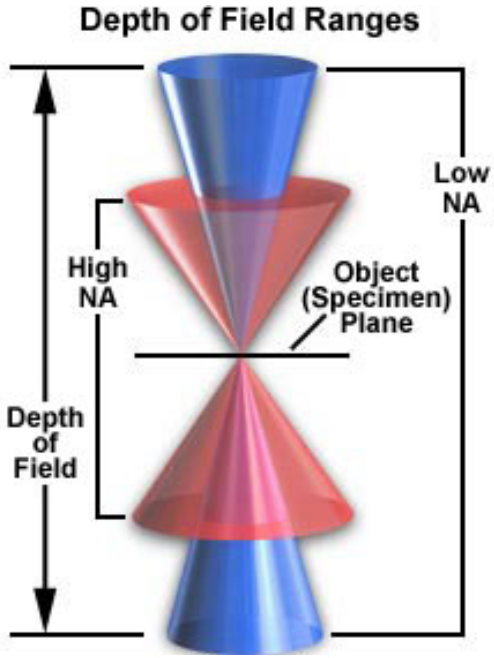


Nikon CFI60 200/60/25 Spec

200mm : tube lens focal length
60mm : objectives parfocal distance
25mm : diameter of thread

The characteristics of objectives

Numerical Aperture (N.A.)



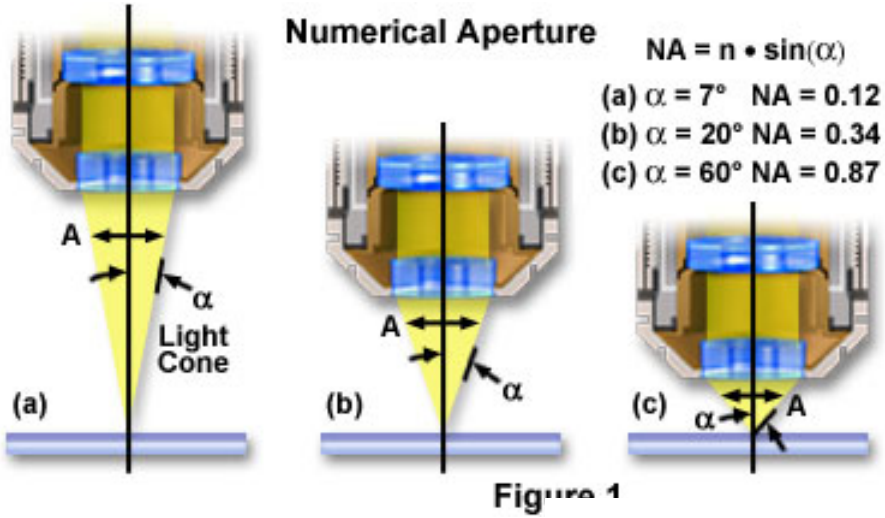


Figure 1

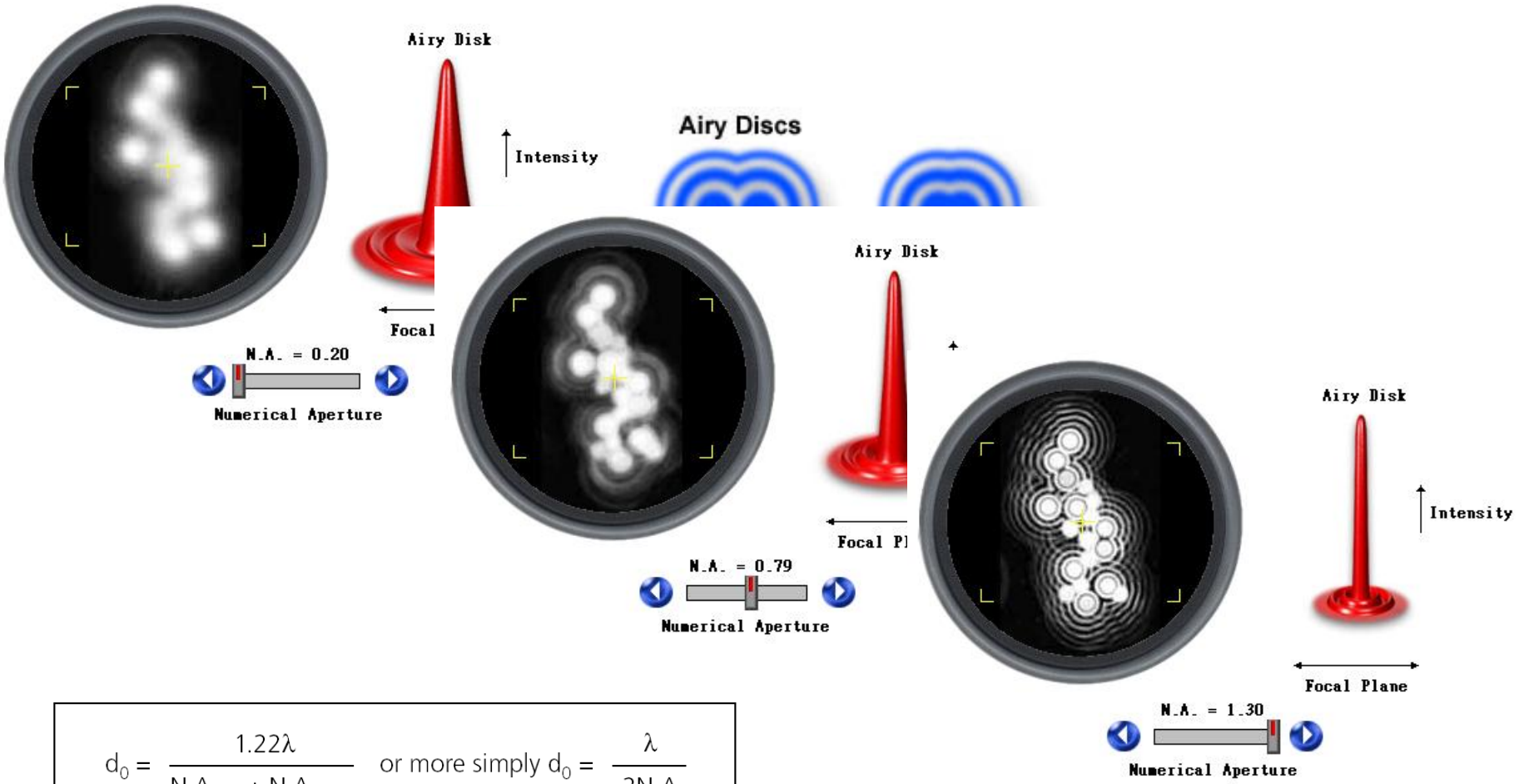
Numerical Aperture = N.A. = n · sin α

α is half the opening angle of the objective.

n is the refractive index of the immersion medium used between the objective and the object.

(n = 1 for air; n = 1.51 for oil or glass)

Resolution



$$d_0 = \frac{1.22\lambda}{N.A_{obj.} + N.A._{Cond}} \quad \text{or more simply } d_0 = \frac{\lambda}{2N.A}$$

λ = wavelength of light, e.g. 550 nm (green)

- **Resolving power, the limit up to which two small objects are still seen separately.**

Contrast Methods

Bright Field

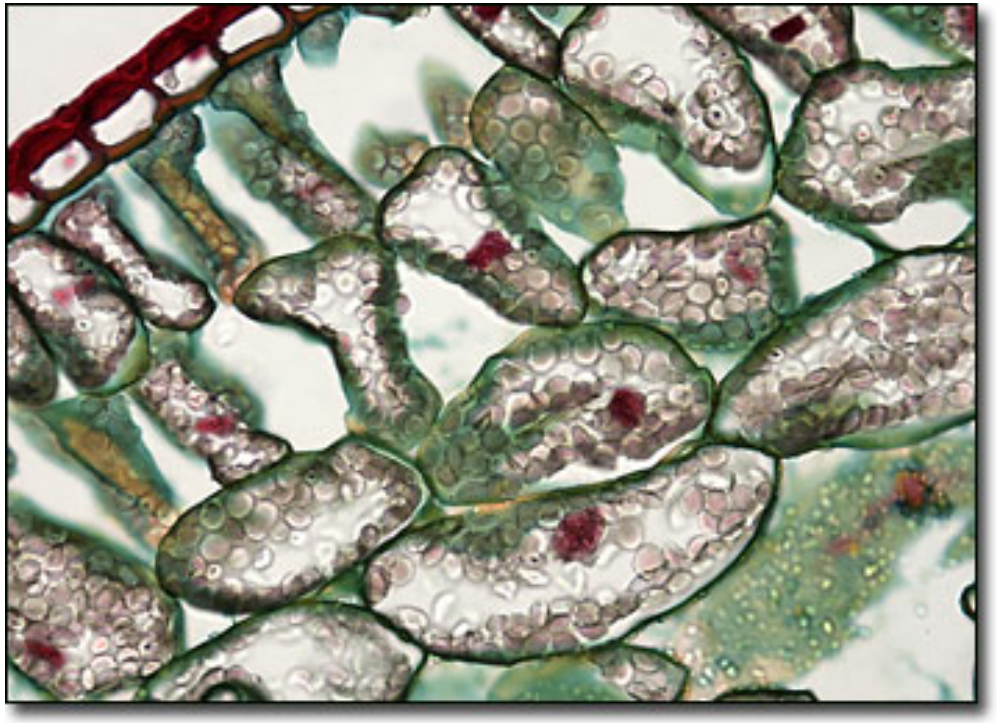
Dark Field

Phase Contrast

Differential Interference Contrast

Fluorescence

Bright Field



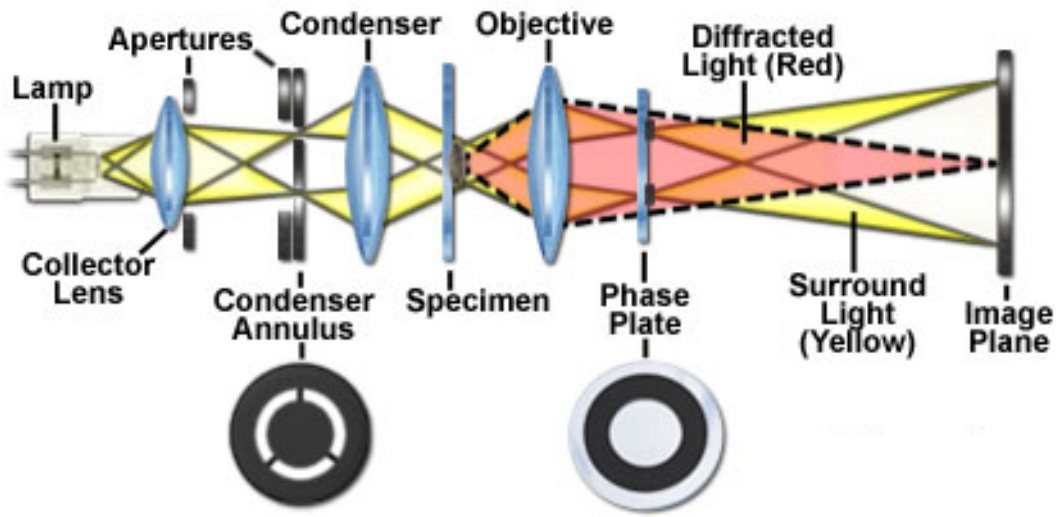
Hemlock Leaf

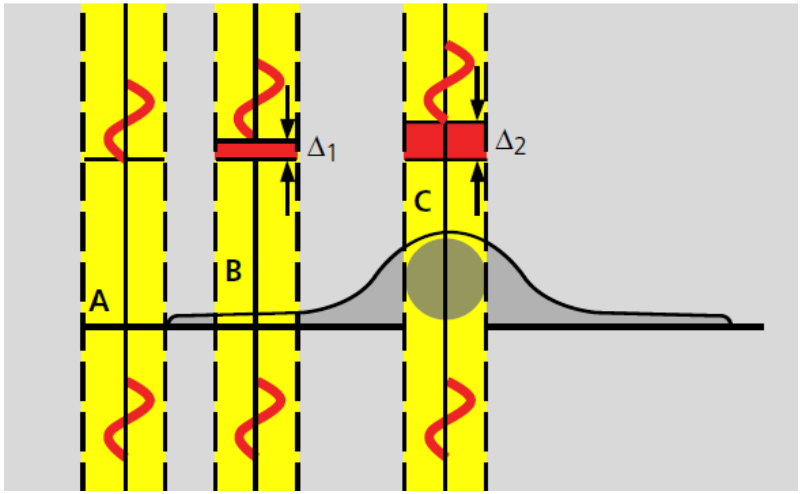
- Bright Field is the most universal technique used in light microscope.
- Usually used in samples with colorimetric staining or good contrast.

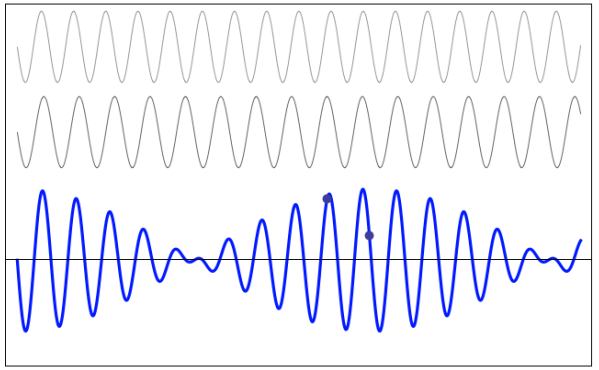
Dark Field

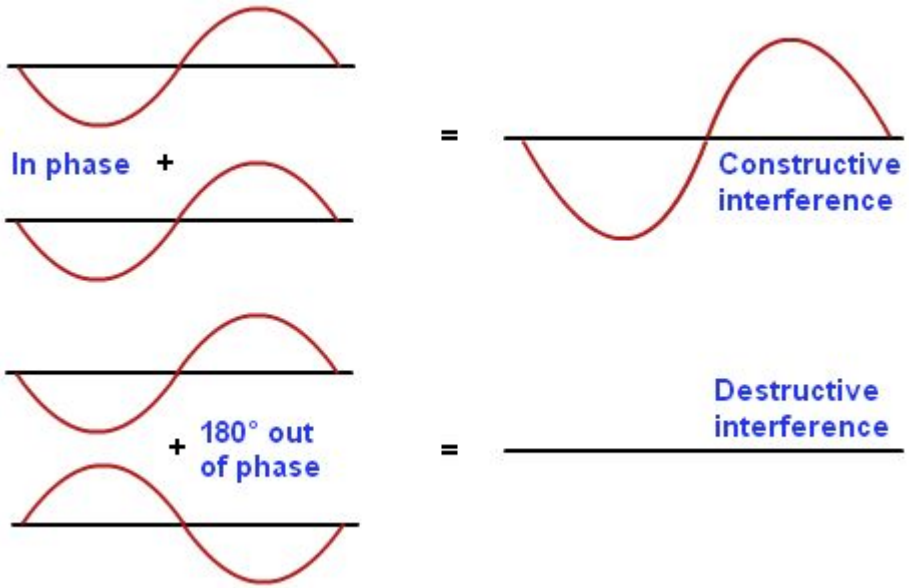
Fine structures can often not be seen in front of a bright background.

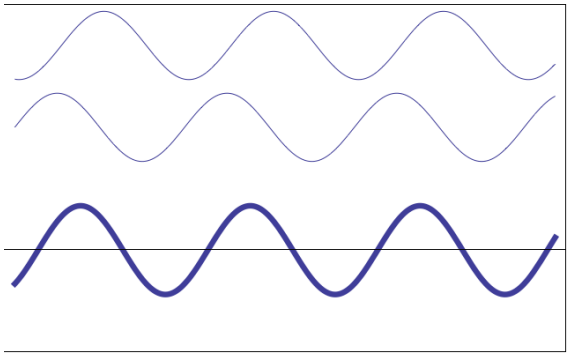
Phase Contrast



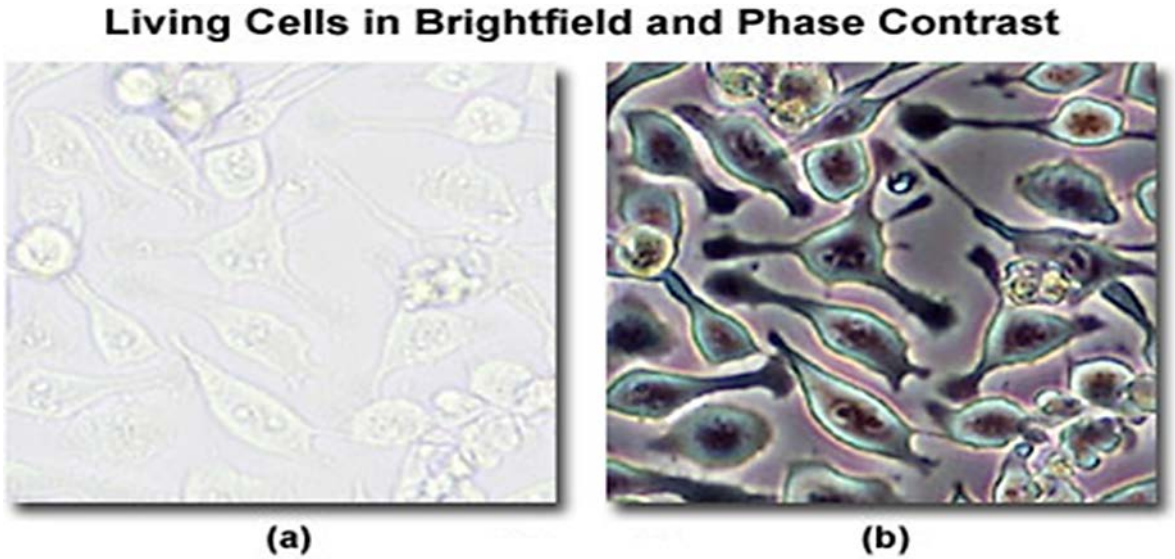


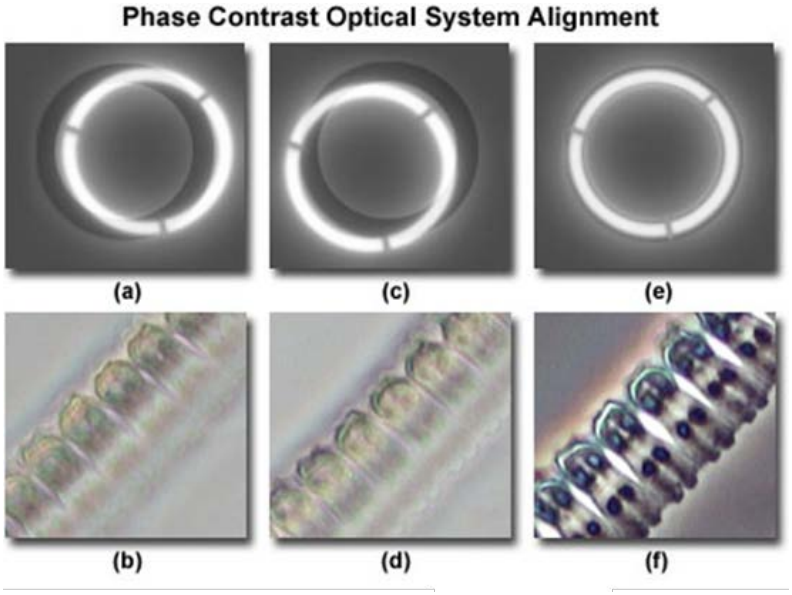






Phase Contrast





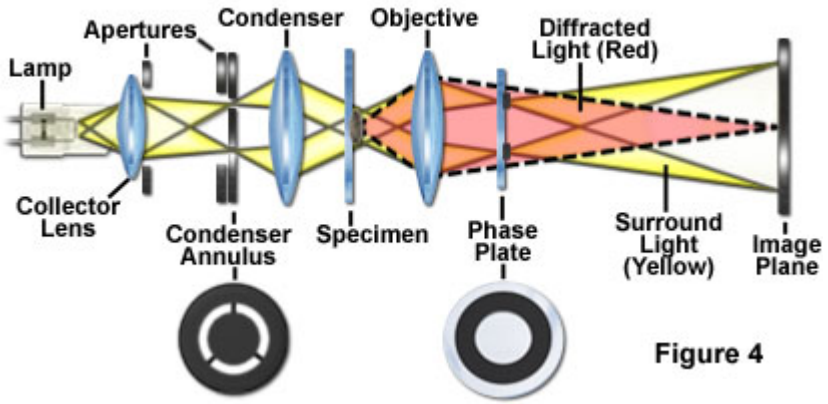


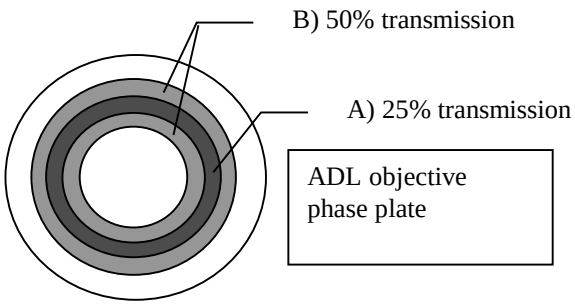
Figure 4

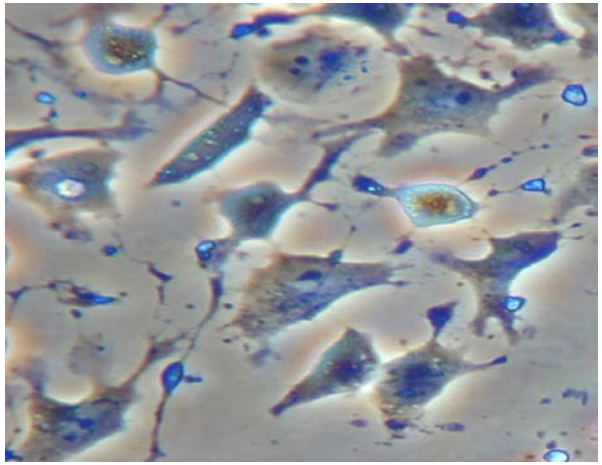
Apodized Phase Contrast

Image with much wilder tonal contrast range

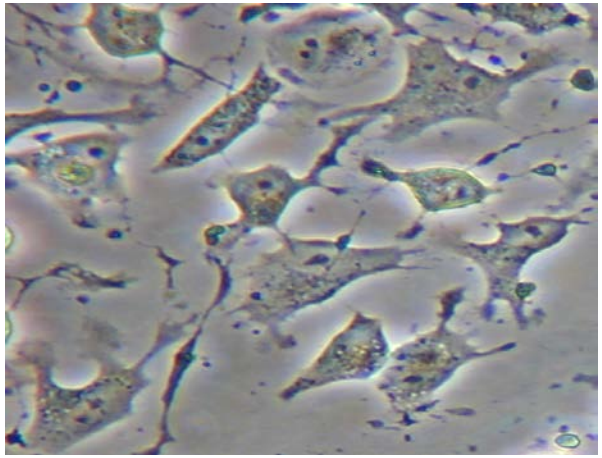
See detail like never before

Almost no halo





Previous Phase Image



New Phase (APC) Image

Differential Interference Contrast

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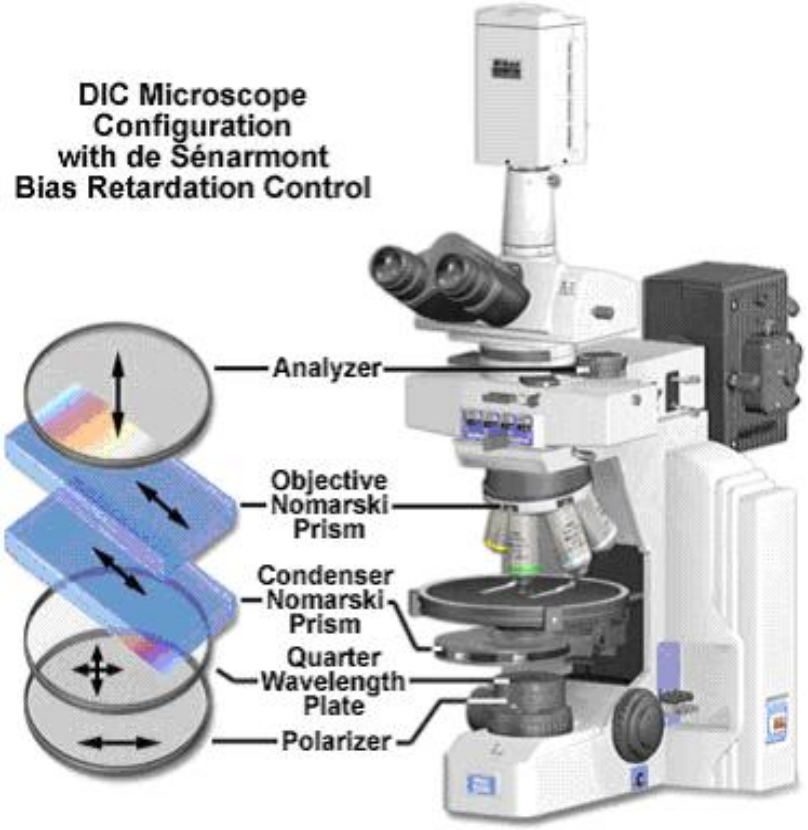
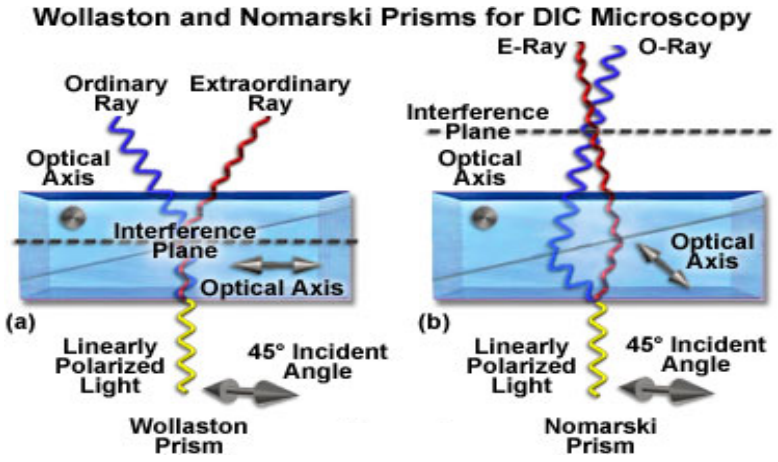
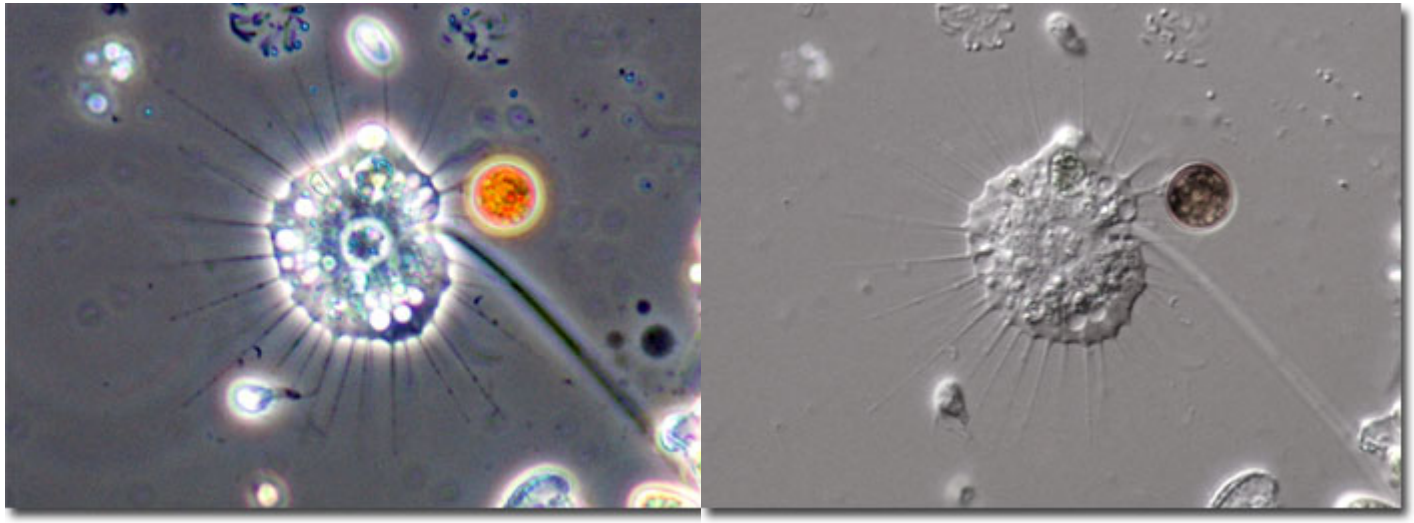




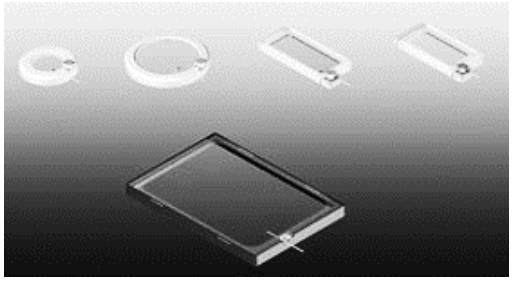
HeLa Cell Culture



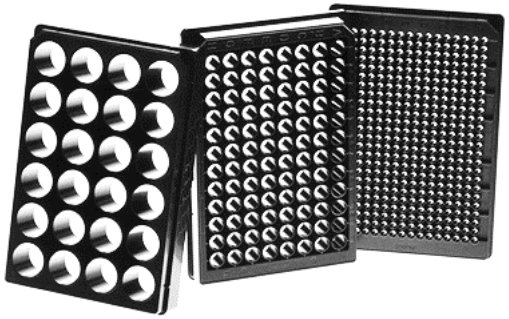
Heliozoans (*Actinophrys sol*)

Drawback of DIC

Glass vessel only; from cover to bottom.

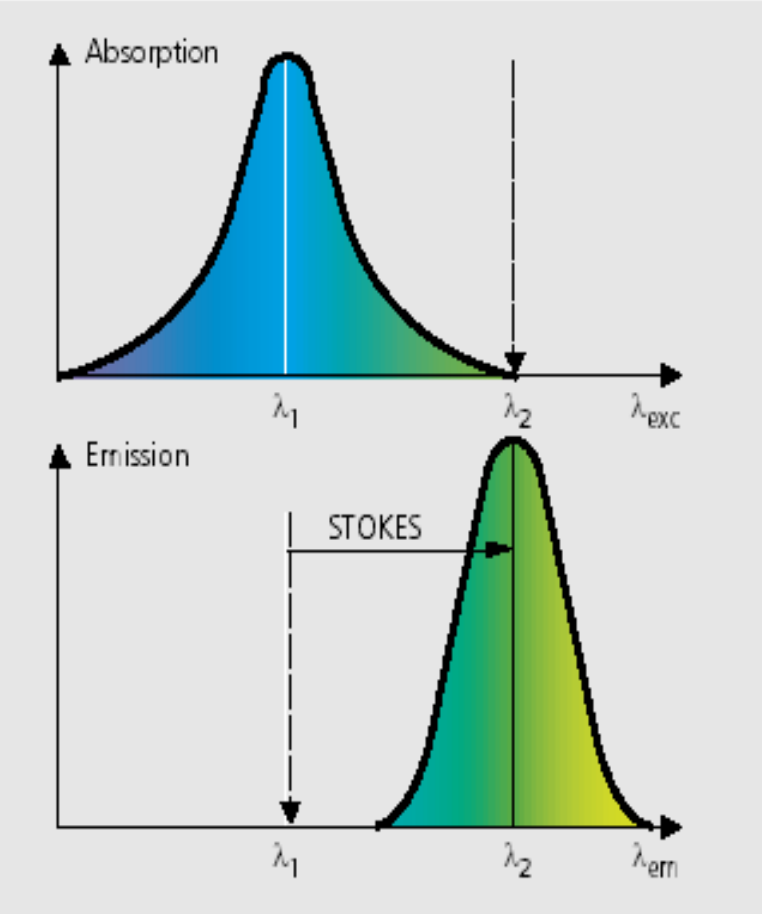


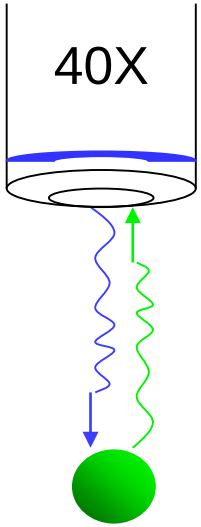
Vessel cover



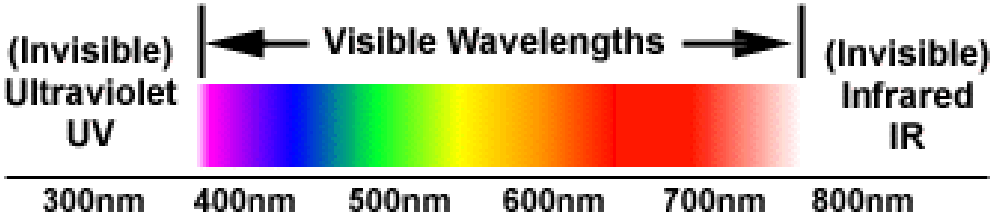
When Plastic meets polarized light!

The Principle of Fluorescence

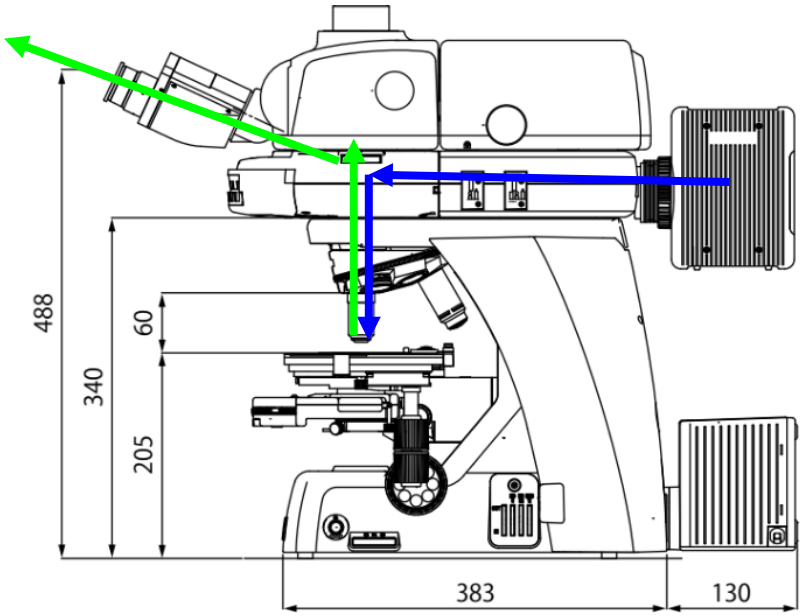


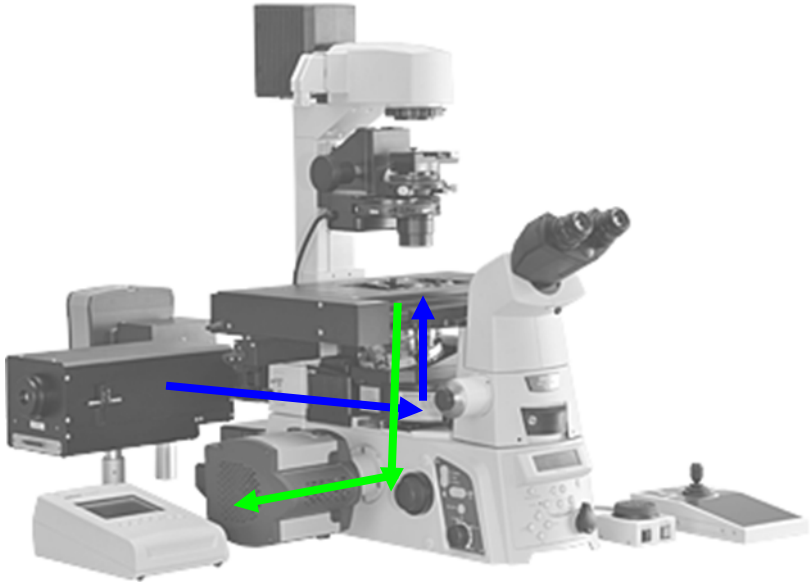


Stoke shift

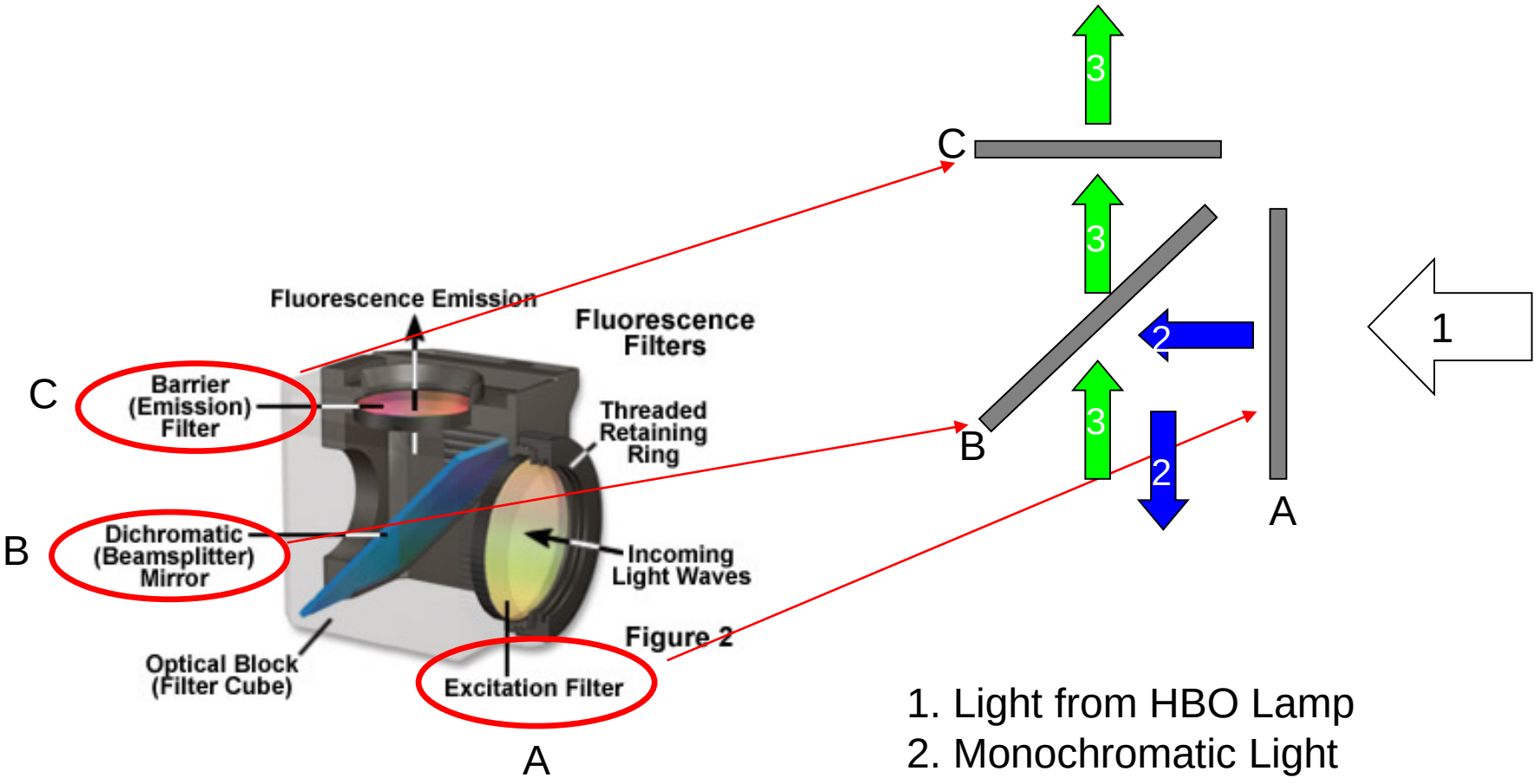


Light path of Fluorescence



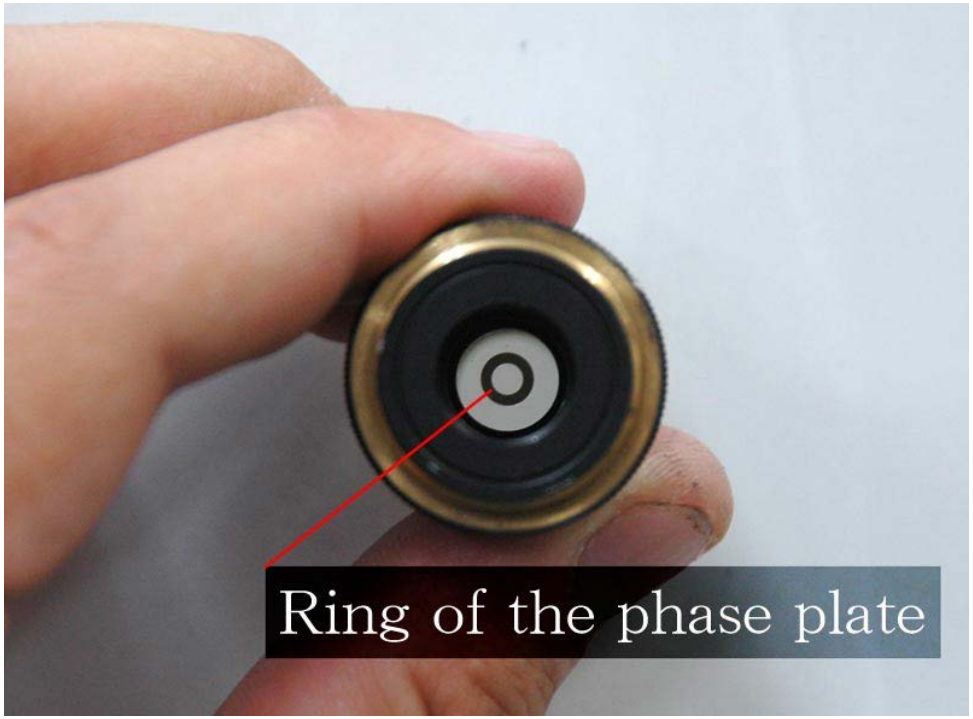


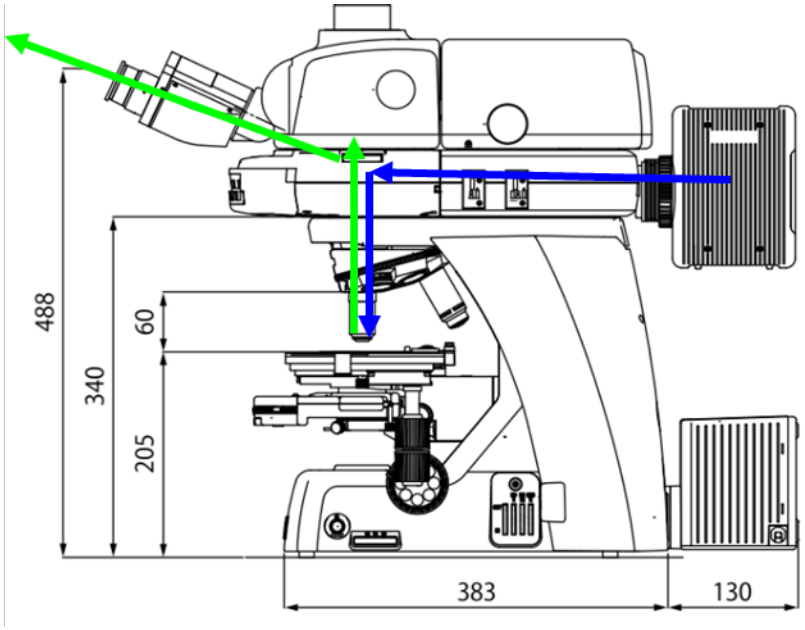
Filter Cube



1. Light from HBO Lamp
2. Monochromatic Light
3. Fluorescence Light returning from the Specimen

Reduced intensity due to phase plate

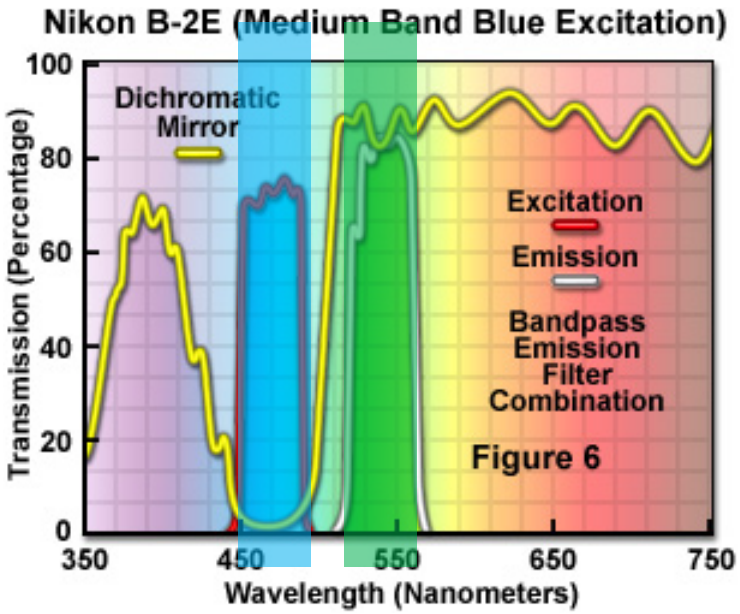




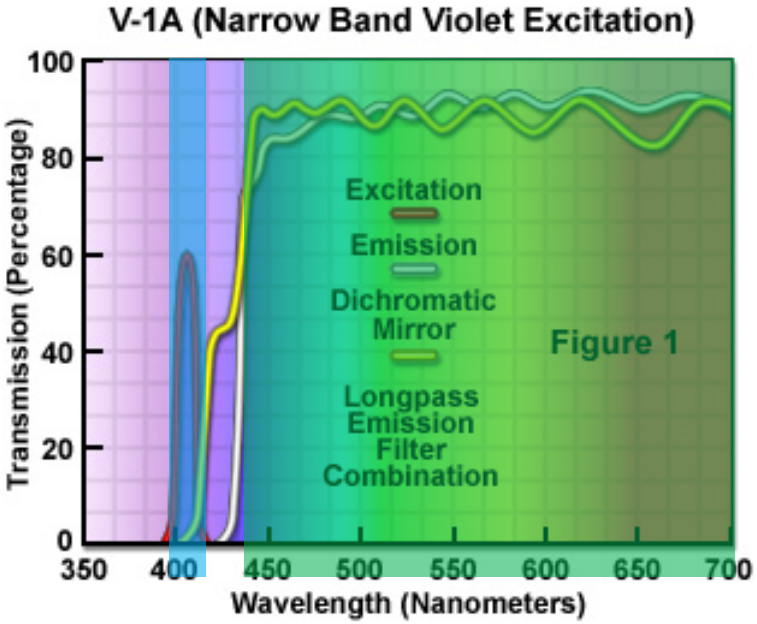
- Objective serve as condenser in FL imaging, excitation light output is reduced by phase plate.

- Emission intensity also reduced by phase plate.

Type of Filter Cube



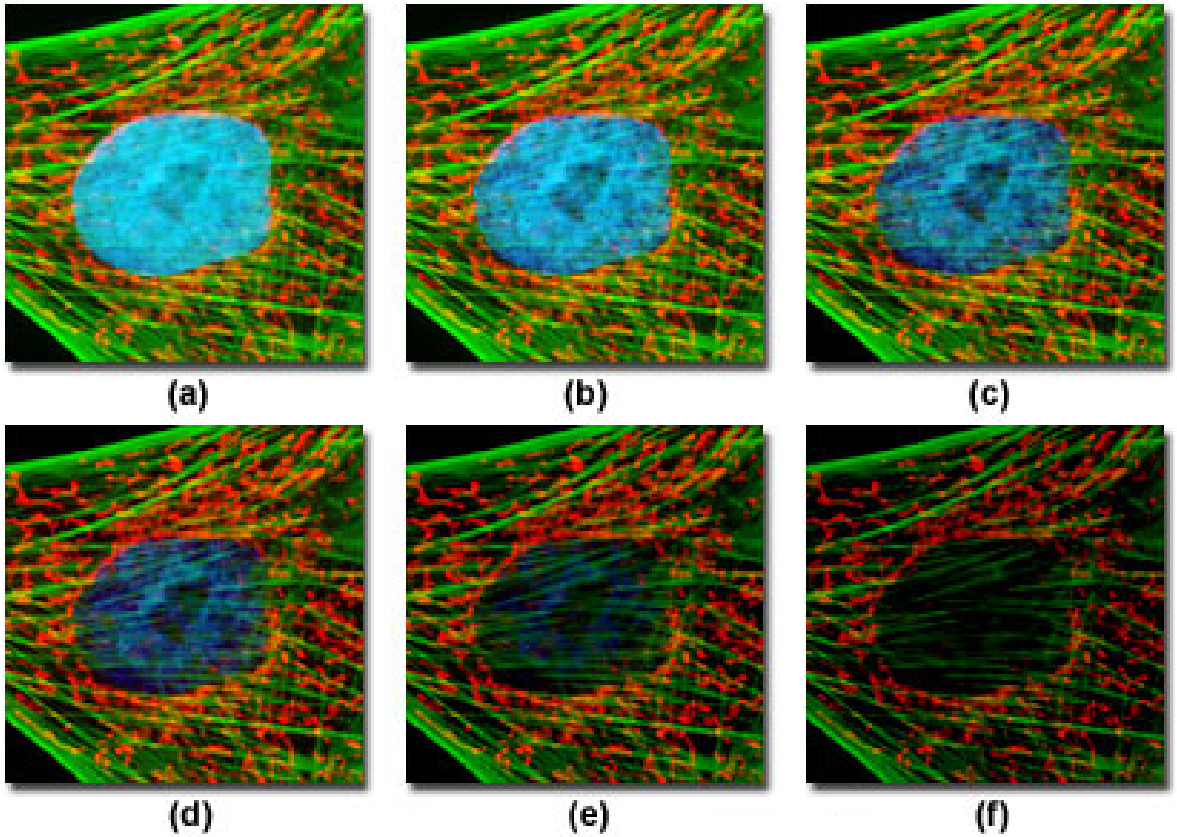
Bandpass emission filter



Longpass emission filter

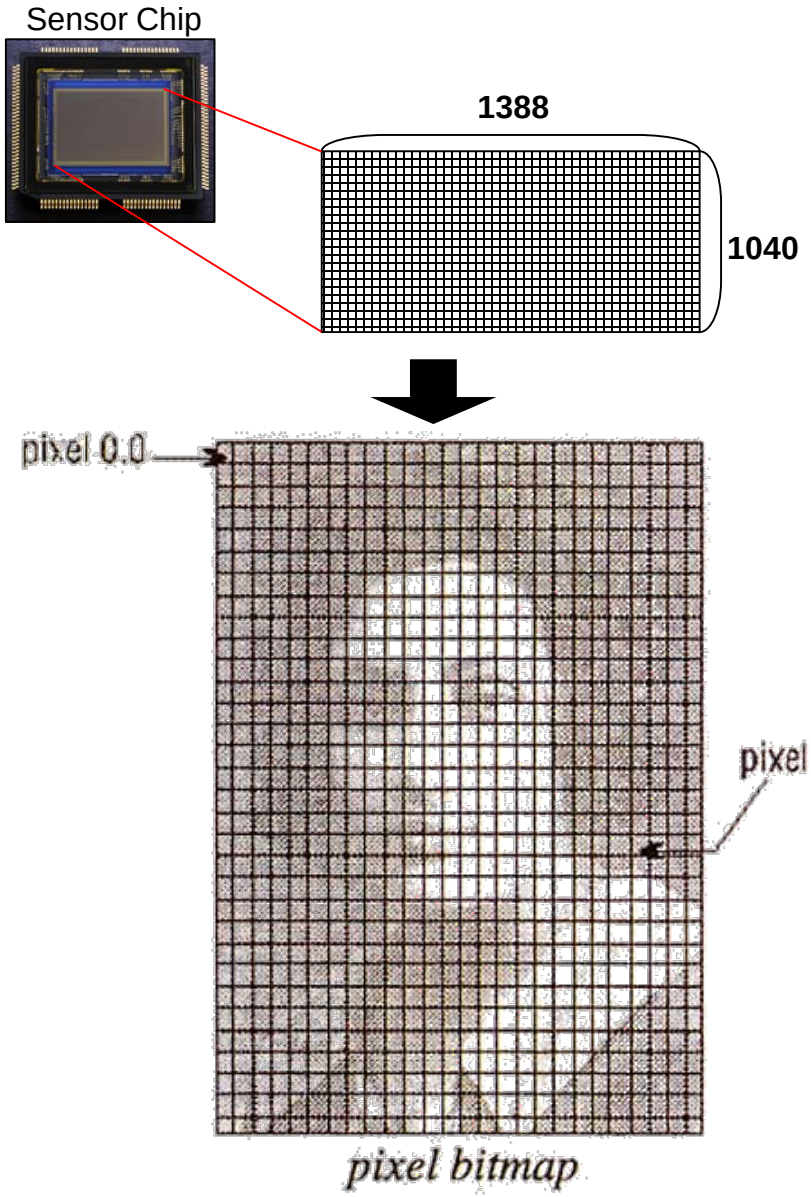
The Photo-bleaching of Fluorescence

Indian Muntjac deer epidermis fibroblast cells



Nuclei :Hoechst 33258(blue fluorescence)
Mitochondria : MitoTracker Red CMXRos (red fluorescence)
Cytoskeleton :Alexa Fluor 488 (green fluorescence)

Image Digitalization Principle



Basic Component of digital image:

- **Pixel (X,Y)**
- **Channels (RGB or CYMK)**
- **Intensity (12 bit = 0~4096)**

Bits does matter!

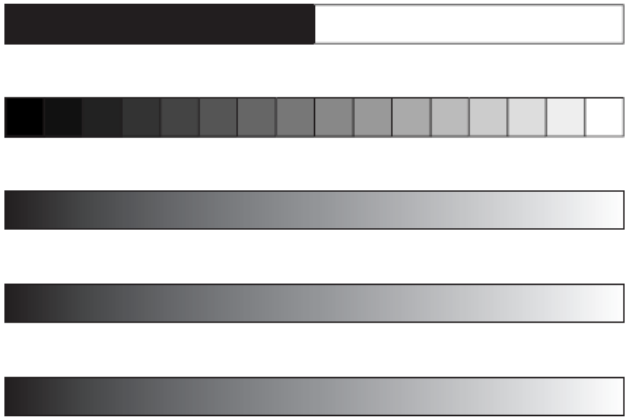
1 Bit = 2^1 = 2 steps

4 Bit = 2^4 = 16 steps

8 Bit = 2^8 = 256 steps

12 Bit = 2^{12} = 4096 steps

16 Bit = 2^{16} = 65536 steps



Note: The human eye can discriminate roughly 200 shades of gray!

Image Format (Pixel)	Uncompressed File Size (8 Bit)
256 x 256	64 K
512 x 512	256 K
1024 x 1024	1 MB
2048 x 2048	4 MB

Pixels x bits /8= Bytes/Frame

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turning *vision* into information