GUIDE OF BASIC LIGHT MICROSCOPY

Lecture

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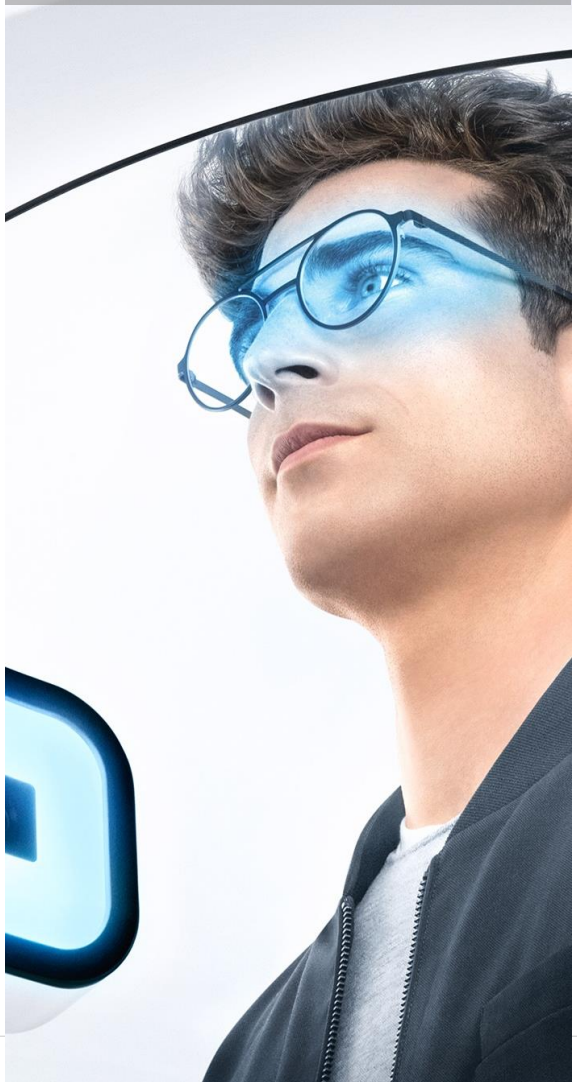
Application Specialist
Research Microscopy
Solutions

21 June 2024

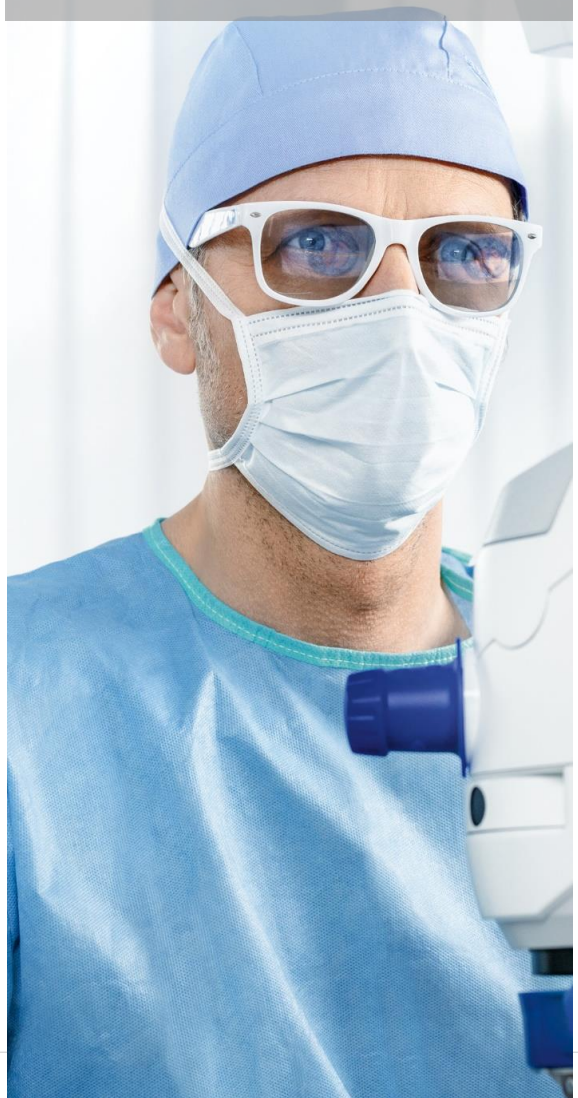
Agenda

ZEISS Optics

Consumer Markets



Medical Technology



Semiconductor Manufacturing

Industrial Quality & Research

SOFTWARE

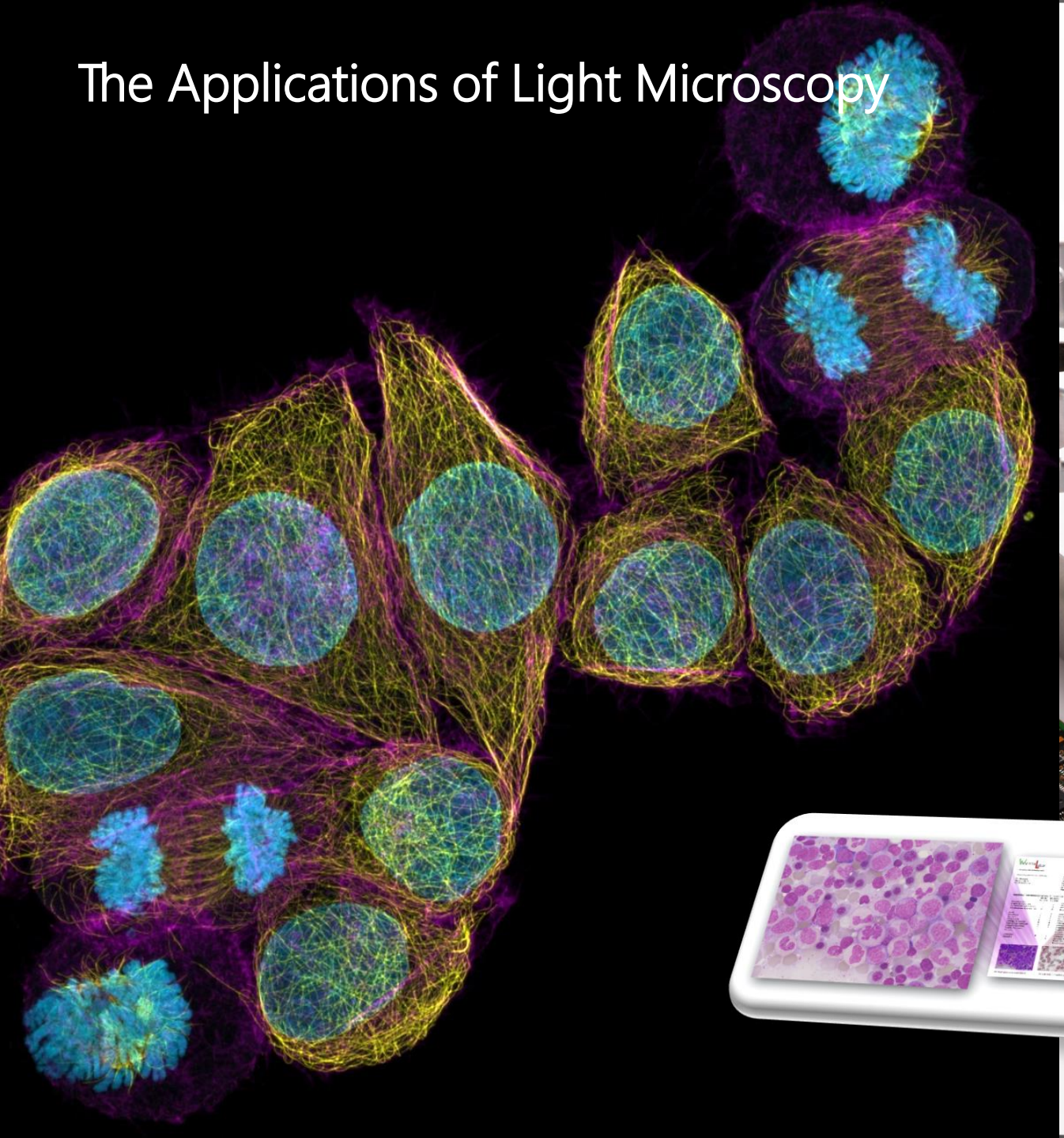
ZEISS ZEN lite

Your Free Version of ZEN for Basic Image Acquisition and Analysis

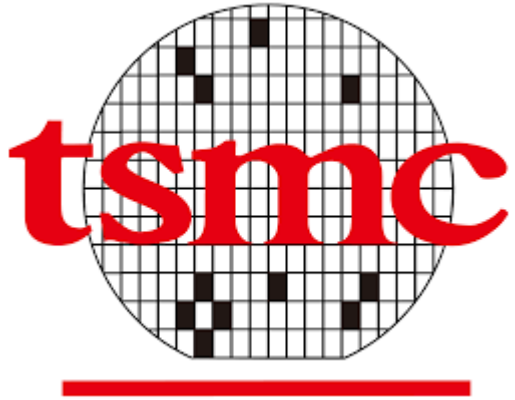
Download ZEN lite, your free copy of the powerful ZEN microscopy software. Use ZEN lite as a viewer for your CZI files or other standard file types. Perform image acquisition or fundamental image analysis and processing tasks.

- **Control of Axiocam microscope cameras**
- **Image transformation and measurements**
- **Essential image processing**

The Applications of Light Microscopy



The Applications of Light Microscopy



Insights For Science Discoveries

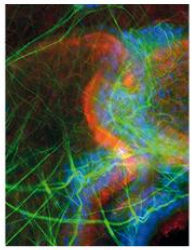
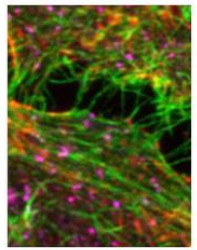
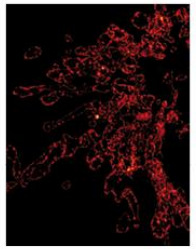
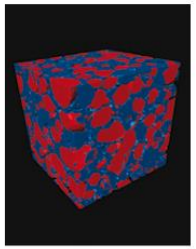


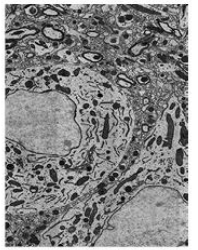
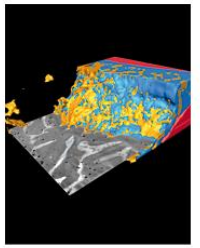
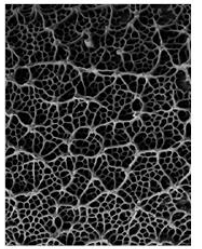
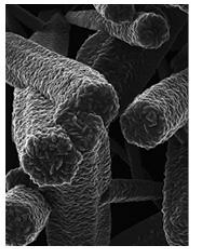








Stereo | Widefield | Confocal | Superresolution | X-ray | Scanning Electron | Multi-Beam Scanning Electron | Focused Ion Beam SEM | Field Emission Scanning Electron | Helium Ion

1 µm | 200 nm | 120 nm | < 50 nm | < 2 nm | < 1 nm | < 1 nm | 0.5 nm

LM

EM

Agenda

The Different Types Of Light Microscopes

Upright

Inverted

Stereo

The Different Types Of Light Microscopes

Upright

Inverted

Stereo

Sample size

The Different Types Of Light Microscopes

Upright

Inverted

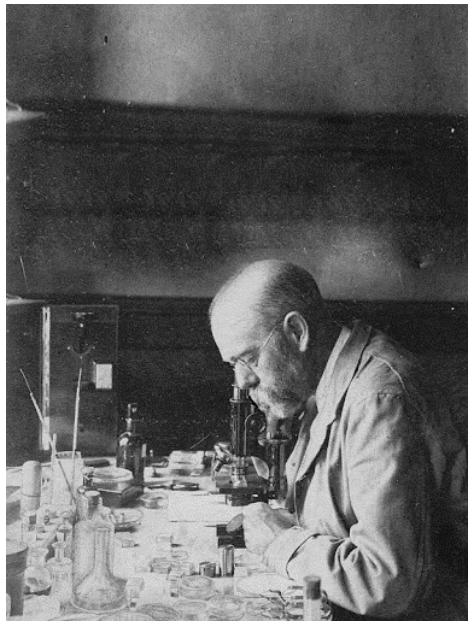
Stereo

Sample size

Upright Microscopes
Tuberculosis

Tuberculosis was one of the most dangerous diseases in the 19th century – causing millions of deaths worldwide.

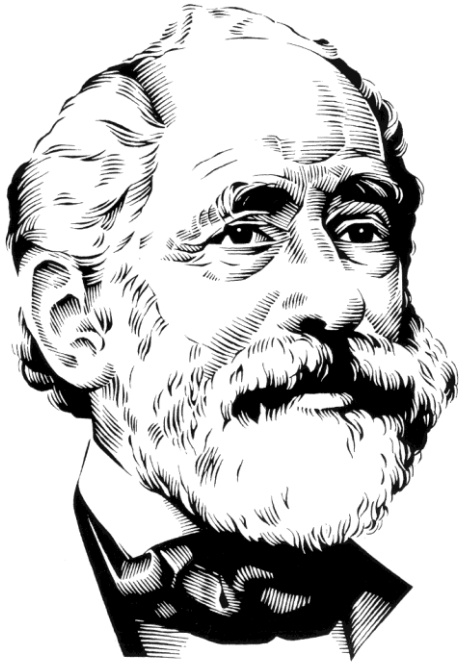
It was the ambition of leading scientists and doctors to find a treatment.



hospital balcony, Rochdale

Upright Microscopes

In 1857, Carl Zeiss developed his first microscope with an assembled optical system. Microscopy Solutions from ZEISS helped Robert Koch, identify Tuberculosis bacteria. And this was a key to fighting it.



Carl Zeiss
Founder

Upright Microscopes
Tuberculosis

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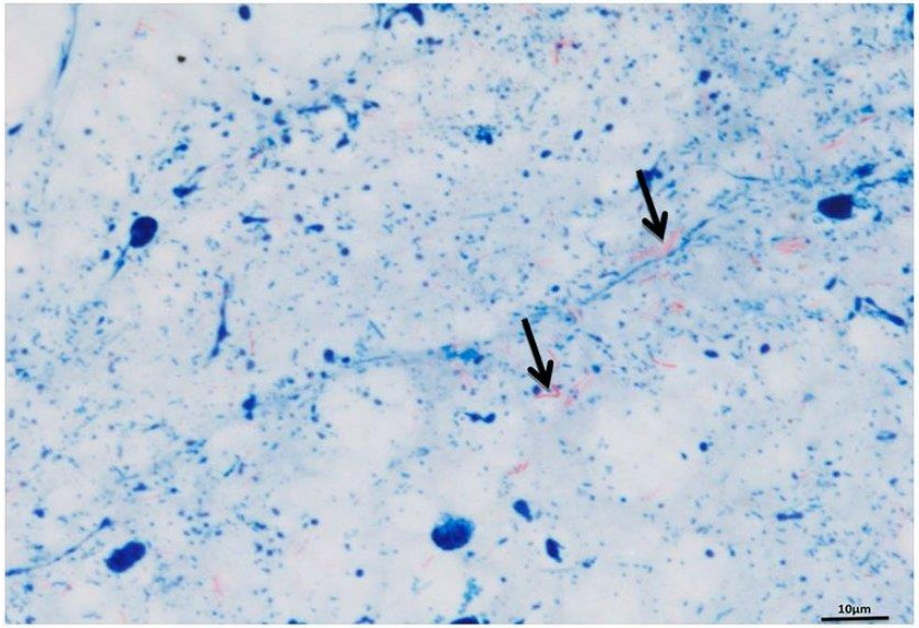
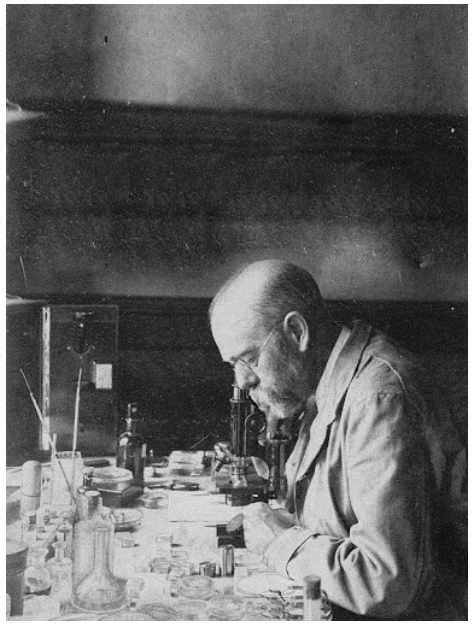
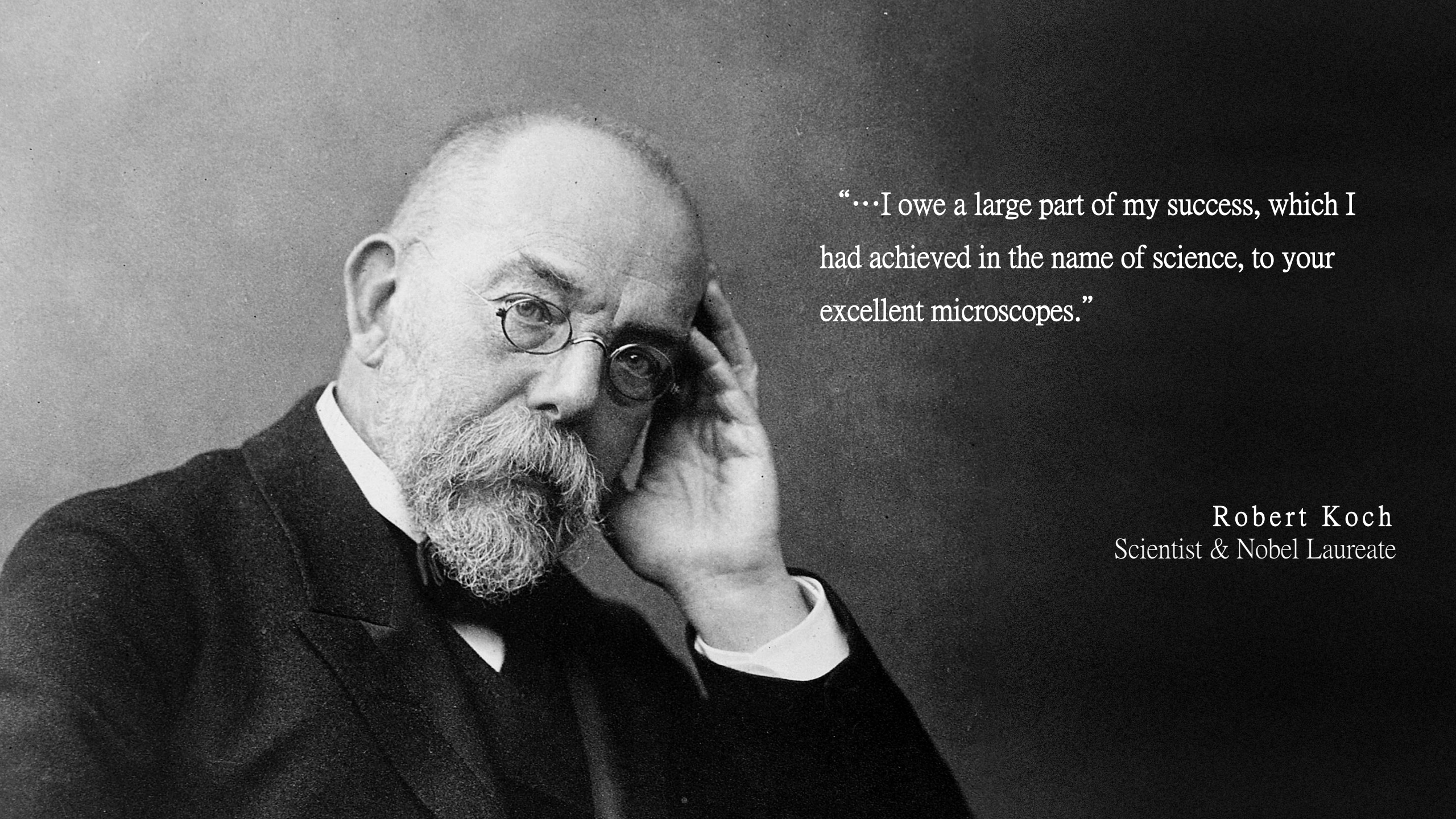


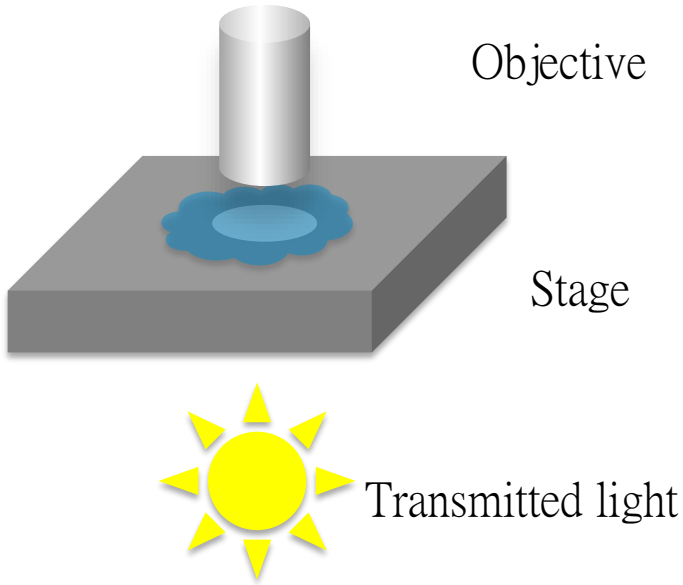
Dezemon, Zingue & Weber, P. & Soltani, Fatma & Raoult, D. & Drancourt, M.. (2018).



hospital balcony, Rochdale


"…I owe a large part of my success, which I had achieved in the name of science, to your excellent microscopes."
Robert Koch
Scientist & Nobel Laureate

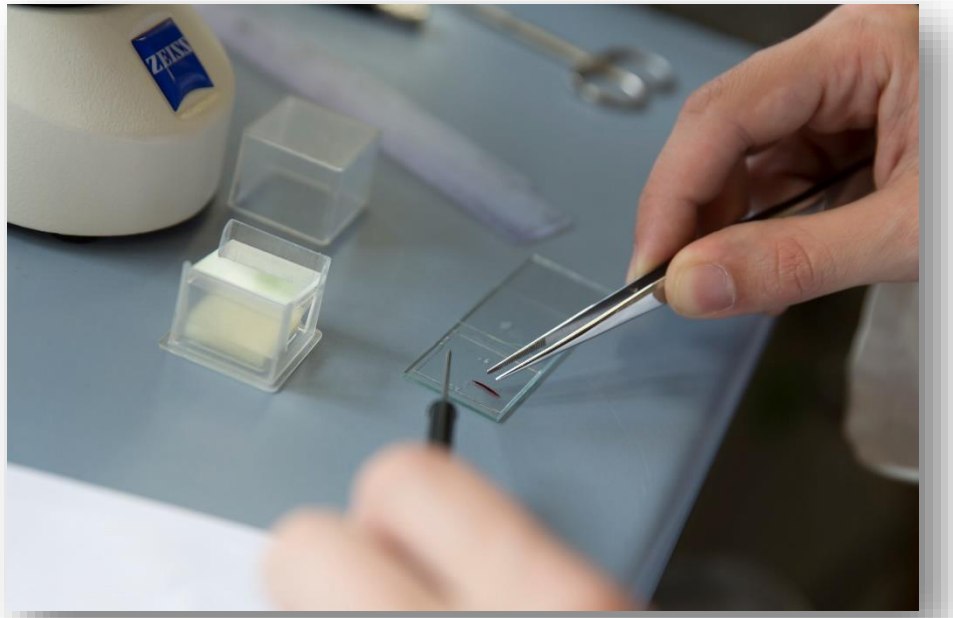
Upright Microscopes



Upright Microscopes

- Highest BF resolution
- Shortest working distance

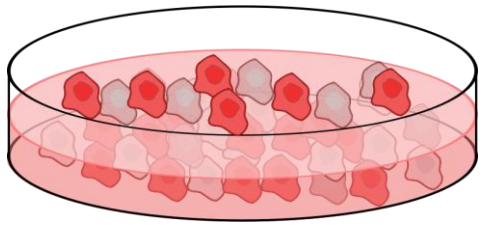
Suitable **for slide observation**



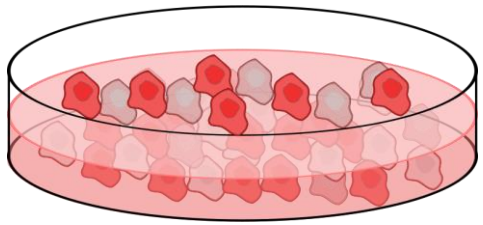
Upright Microscopes

- Highest BF resolution
- Shortest working distance

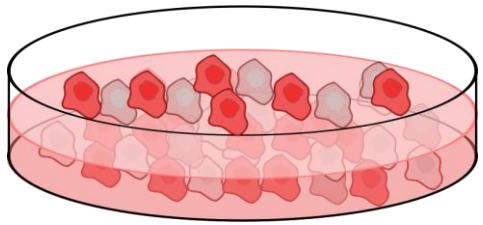
Suitable for slide observation
How about culture dishes?



Upright Microscopes

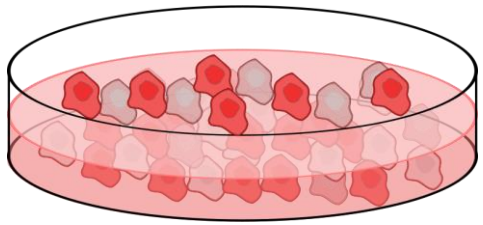


Upright Microscopes



Inverted Microscopes

1818-1883 A.D John Lawrence Smith



Inverted Microscopes

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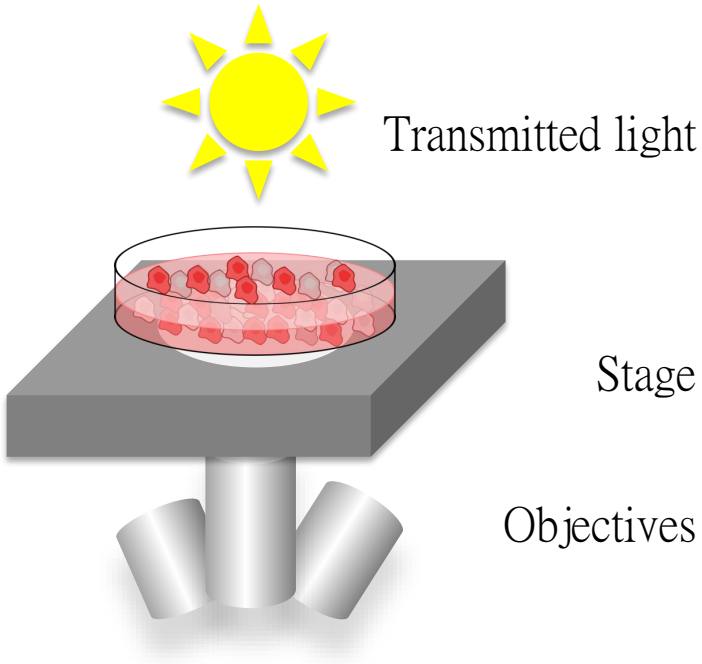
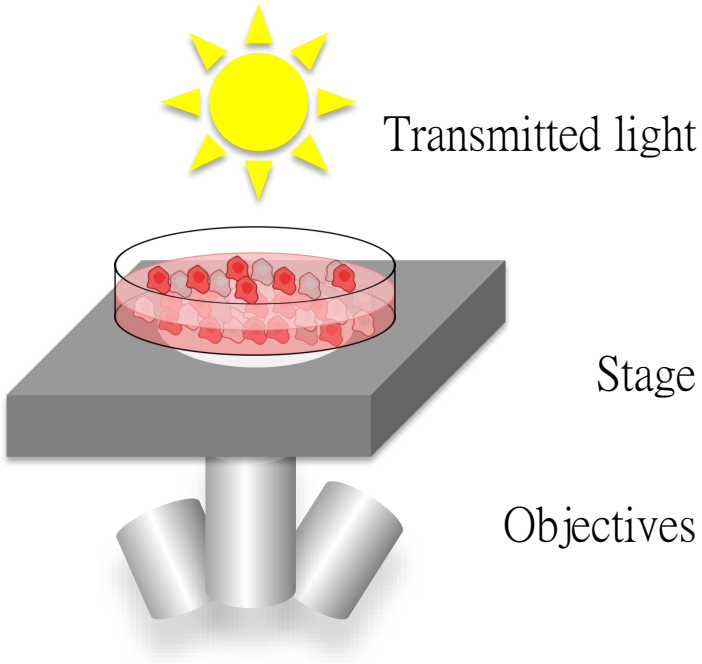


Inverted Microscopes
The Best Choice for Multi-tasking

- Live cell imaging
- Long working distance
- BF resolution < Upright Microscope

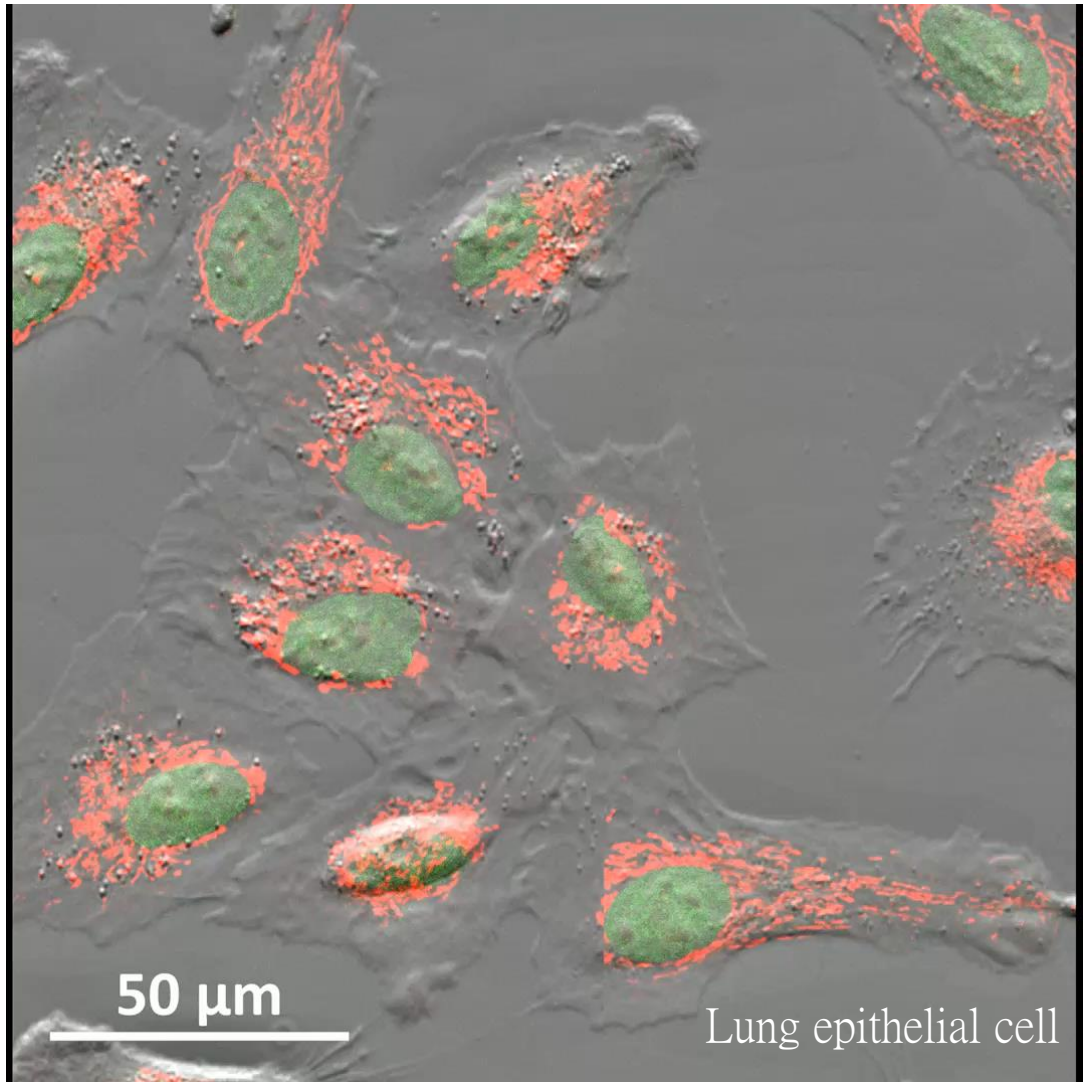
  (Max. Condenser N.A.=0.55)

- Slides
- Petri dish
- Multiwell plate
- Flask



Inverted Microscopes
Live Cell Imaging

37℃, 5% CO_2, Living cell Incubation System



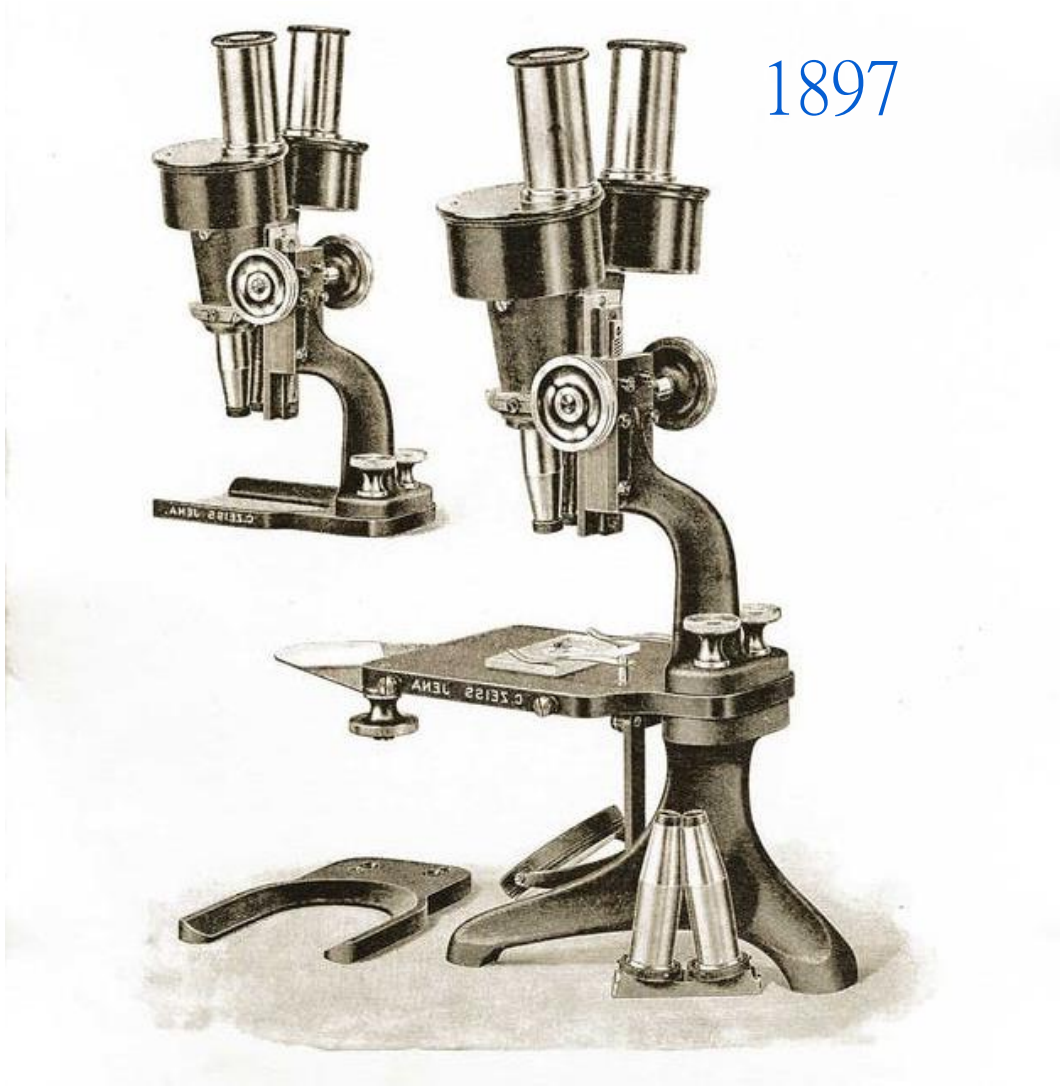
Inverted Microscopes
Micro Manipulation

In Vitro Fertilization (IVF) centers

Intracytoplasmic sperm injection (ICSI)

Intracytoplasmic morphologically selected sperm injection (IMSI)

Stereo Microscopes
3D Vision



Stereo Microscopes
3D Vision

Stereo Microscopes
Versitile 3D Vision

- 3D vision
- Longest working distance
- Flexible illumination
- Low magnification

- Large object observation
  - Plant
  - Zebrafish
  - Drosophila
  - Mouse dissection…..

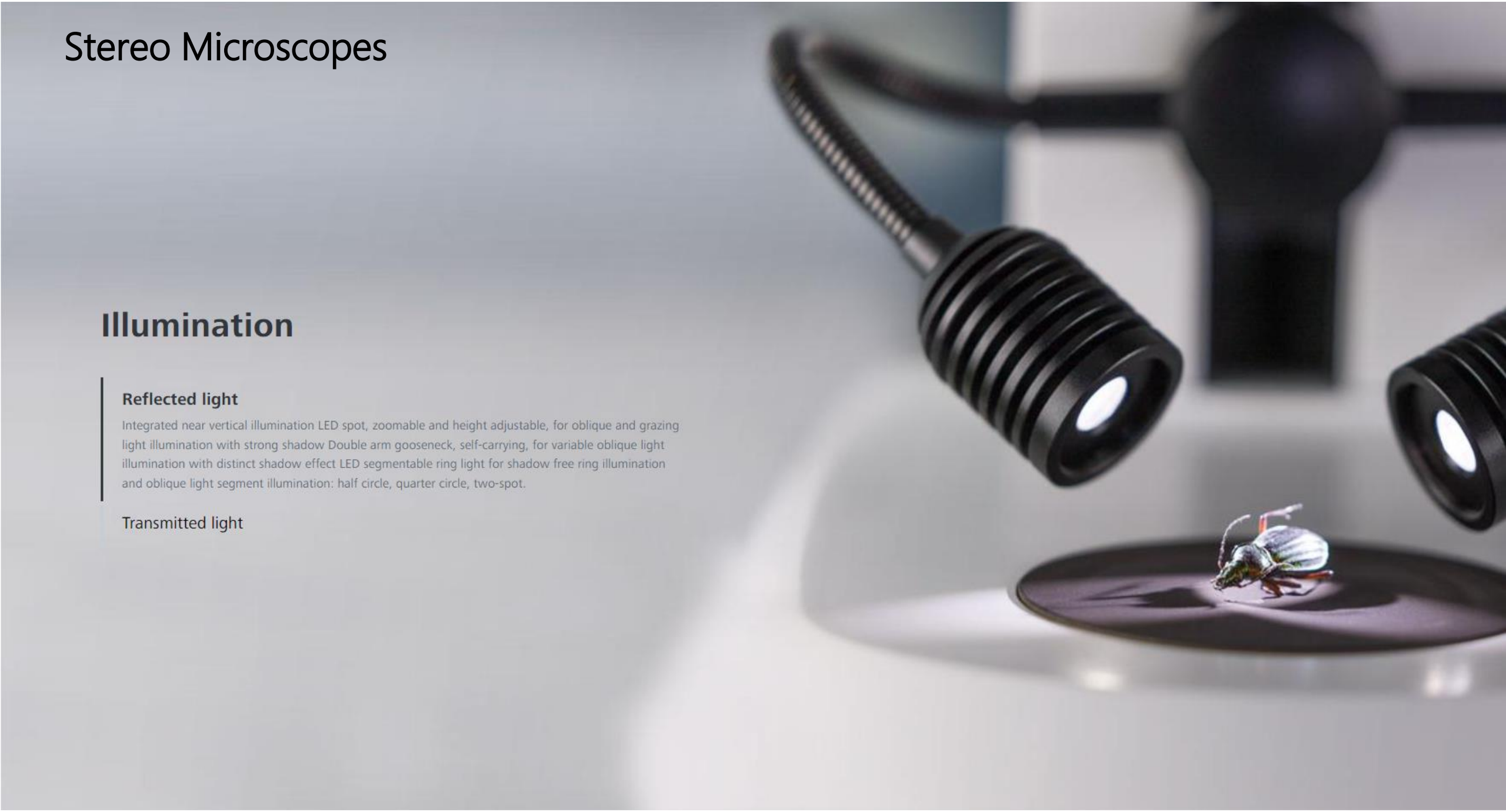
Stereo Microscopes

Illumination

Reflected light

Integrated near vertical illumination LED spot, zoomable and height adjustable, for oblique and grazing light illumination with strong shadow Double arm gooseneck, self-carrying, for variable oblique light illumination with distinct shadow effect LED segmentable ring light for shadow free ring illumination and oblique light segment illumination: half circle, quarter circle, two-spot.

Transmitted light



Stereo Microscopes

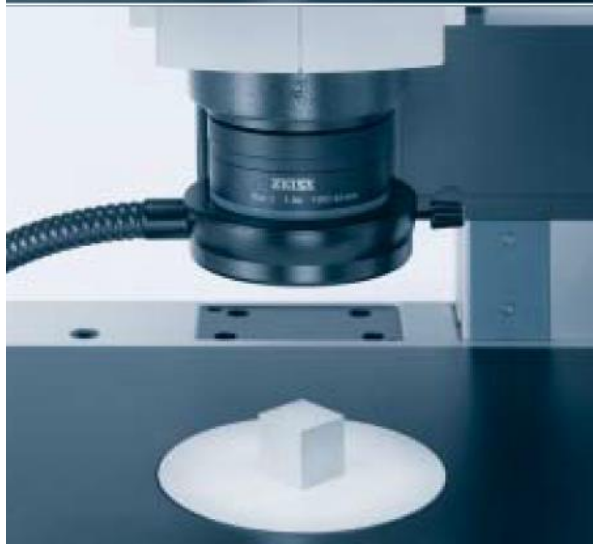
Illumination

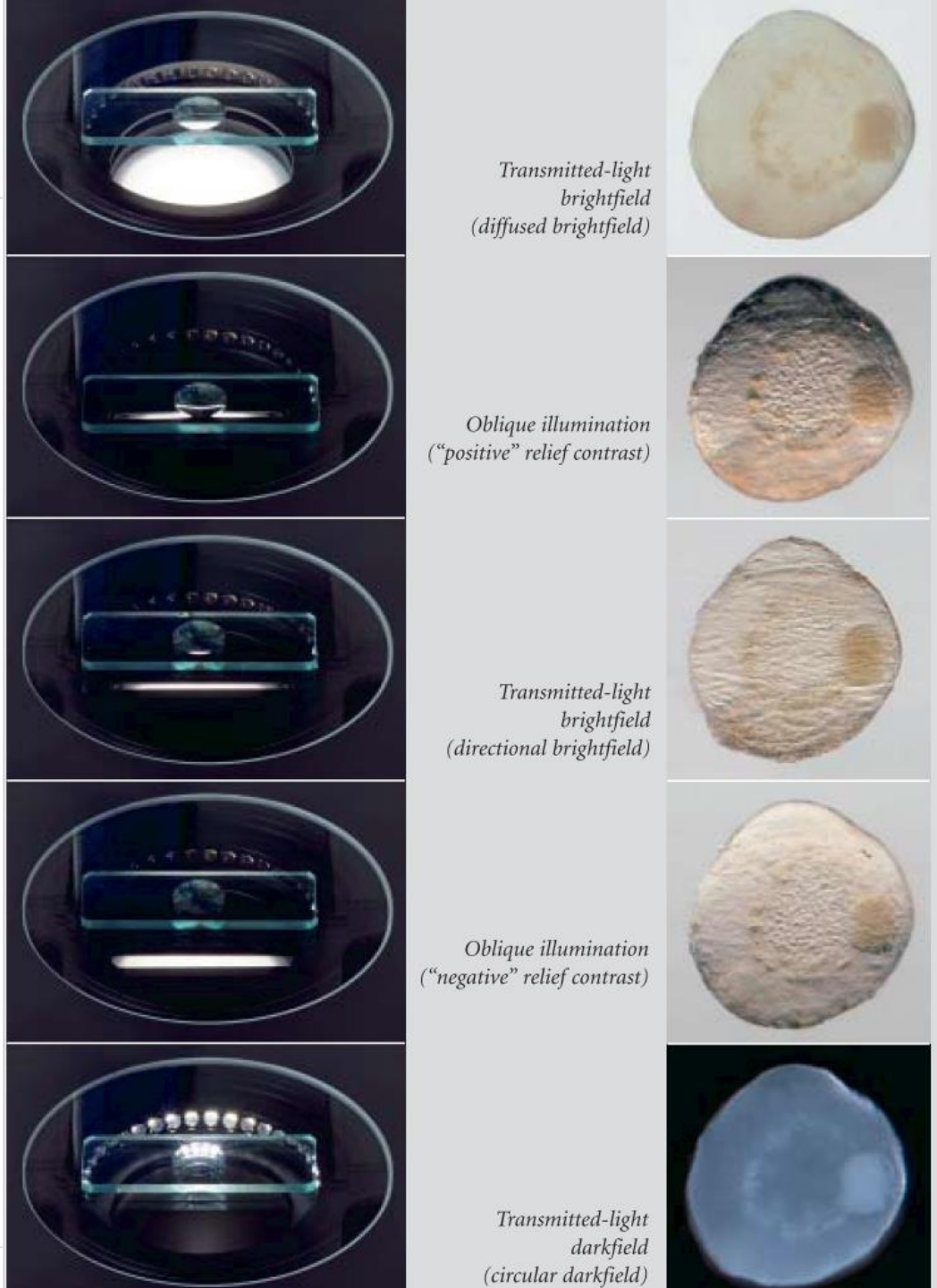
Reflected light

Transmitted light

Flat transmitted light base for brightfield and darkfield illumination Tiltable mirror base for brightfield, darkfield and oblique light illumination.

Stereo Microscopes
Illumination





The Different Types Of Light Microscopes

Upright

Inverted

Stereo

Sample size

Agenda

Magnification And Resolution

- Magnification? 100x? 1000x? 999999999x?

Magnification And Resolution

- Magnification? 100x? 1000x? 999999999x?
- ~1500x is the limit of Light Microscopes, magnification above 1500x is meaningless
- Why?.

Magnification And Resolution

Magnification And Resolution

Magnification alone is not enough:

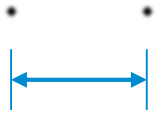
Resolution determines what we see.

Resolution Of Your Eyes

Definition:

The resolution limit is reached, when two point-like objects can not be imaged as two distinct structures anymore.

The **distance** between the objects is called the resolution limit.



d = 10 cm

d = 0.1 cm

Resolution Of The Microscopes

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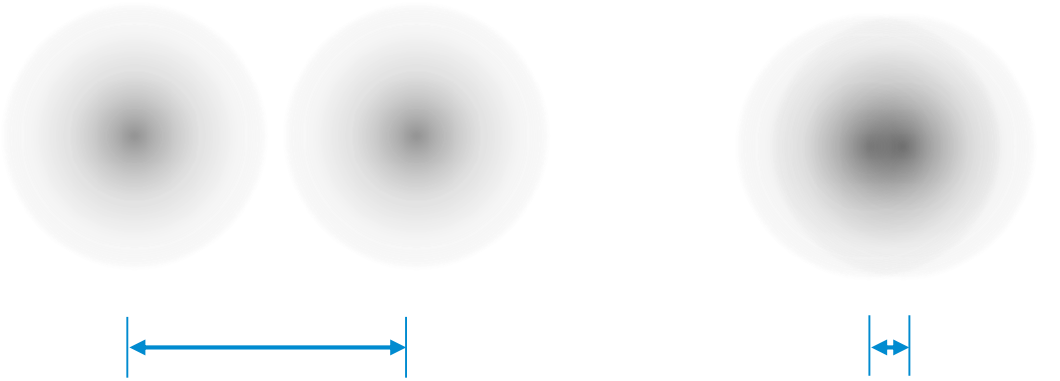
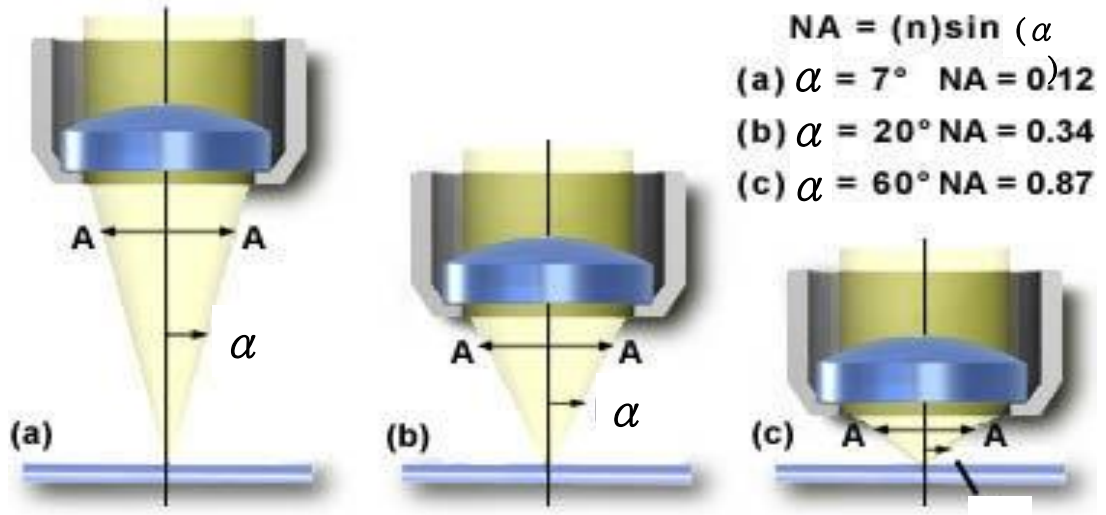


Resolution - N.A.
Numerical Aperture

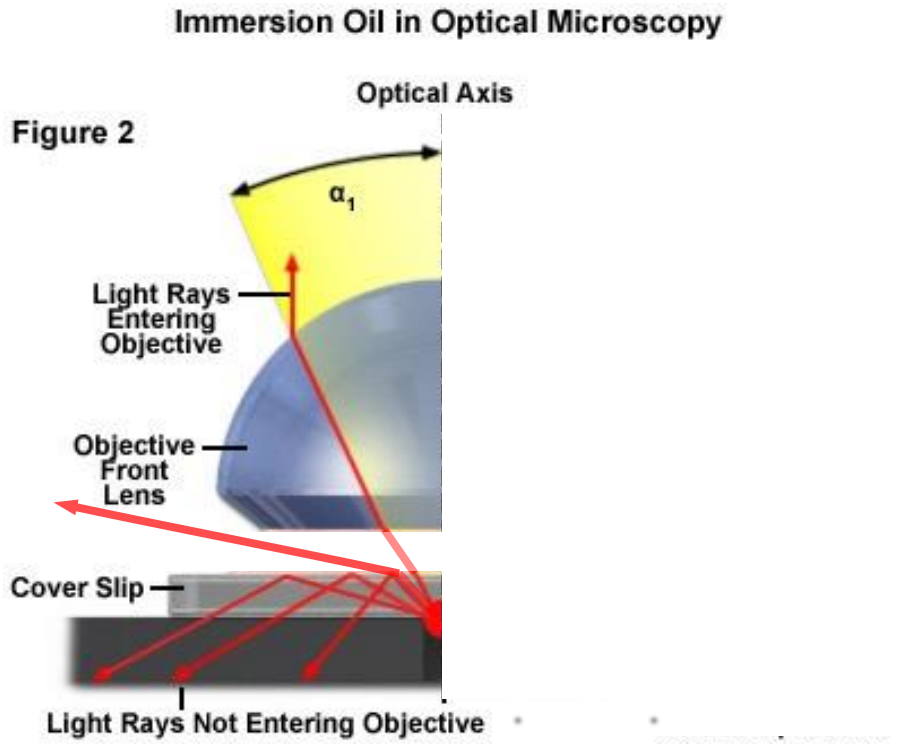
N.A. value: The angle of light (α) into objective

Resolution - N.A.
Numerical Aperture



		α
Magnification:	Low	High
Working distance:	Long	Short
N.A. value:	Low	High

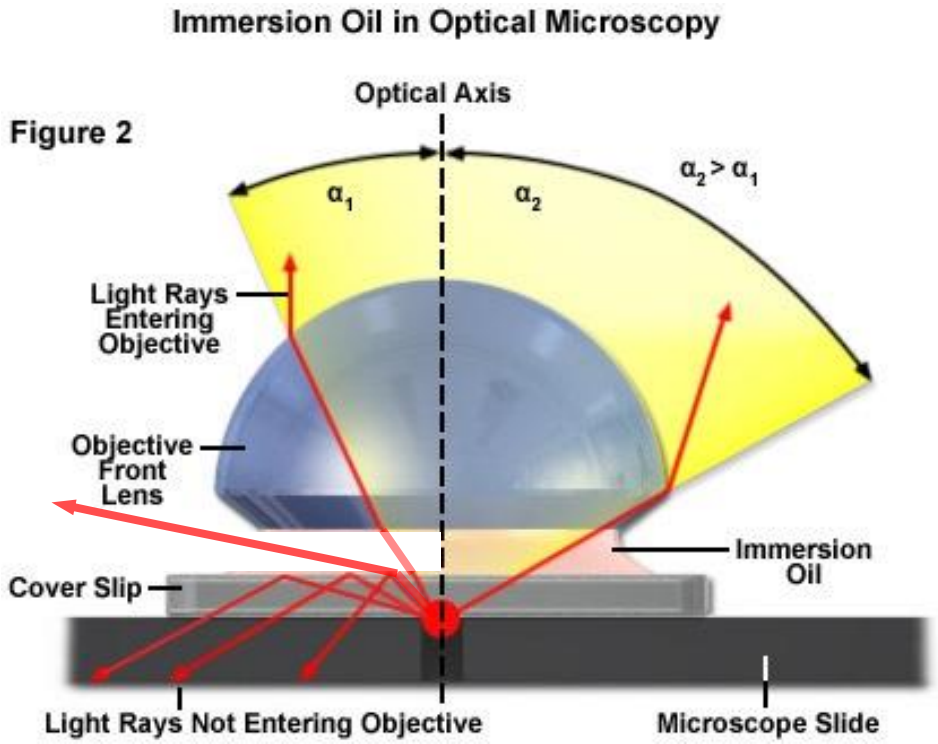
Resolution - N.A.



Numerical Aperture (NA) = η • sin α

Air η = 1 Oil / Glass η = 1.51

Resolution - N.A.



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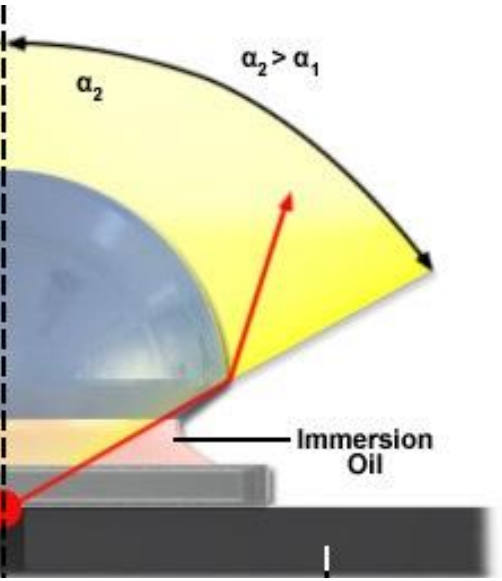
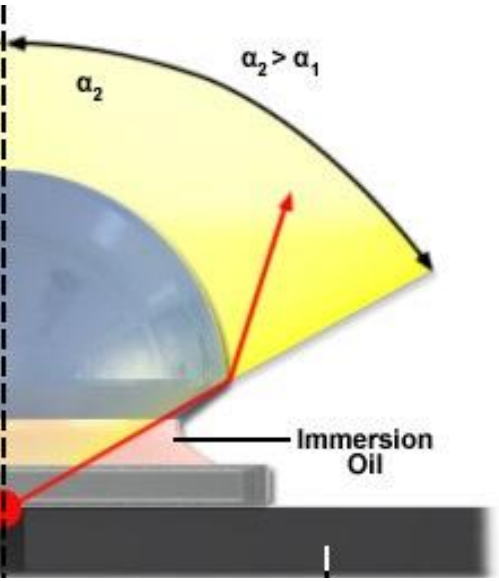


$$d_0 = \frac{1{\cdot}22\lambda}{N.A_{obj.} + N.A._{Cond}} \quad \text{or more simply } d_0 = \frac{\lambda}{2N.A}$$

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

Ernst Abbe
Partner

Resolution - N.A.



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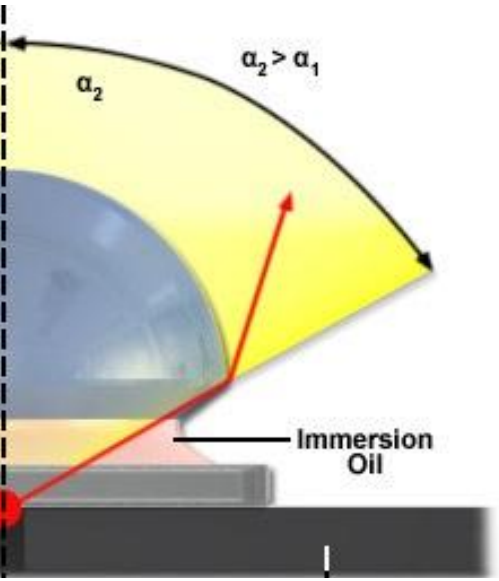
The resolution of light microscope **d_0= 200~300 nm**

Ernst Abbe
Partner

Resolution - N.A.



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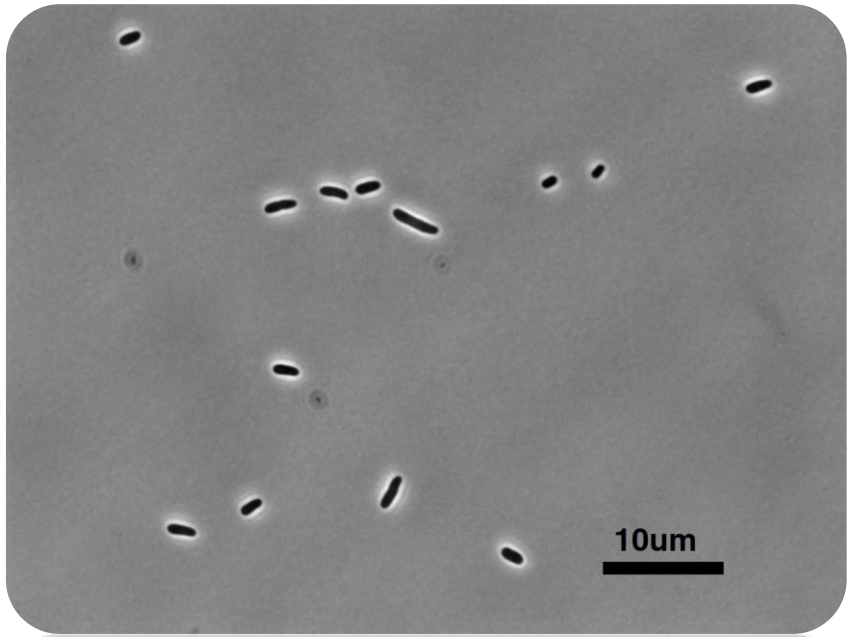


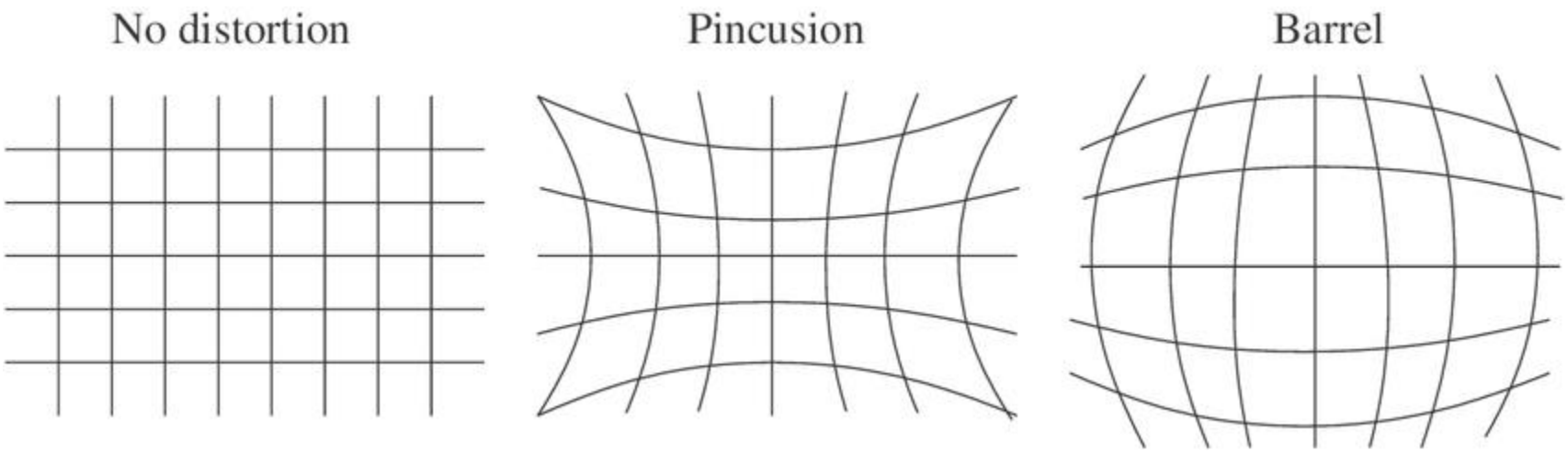
E. Coli 0.5 x 2µm

Ernst Abbe
Partner

Agenda

Aberrations Of Light
Distortion

- Distortion 畸變



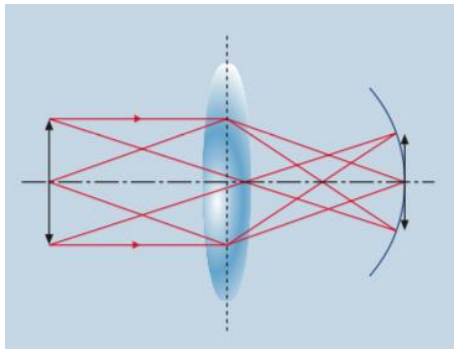
Aberrations Of Light
Distortion

Distortion

Corrected

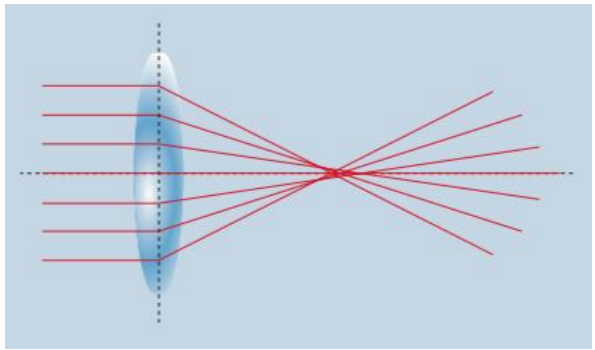
Aberrations Of Light

場曲
Field curvature



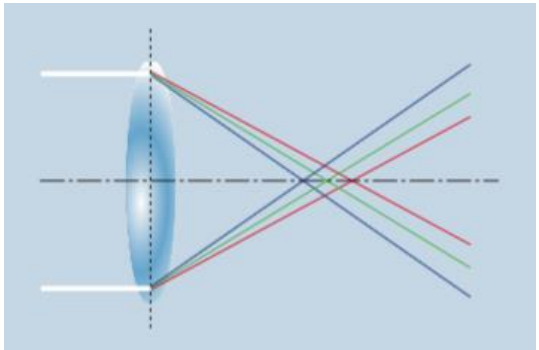
Cover glass thickness
Specimen thickness

球差
Spherical aberrations



Refractive index
Specimen thickness

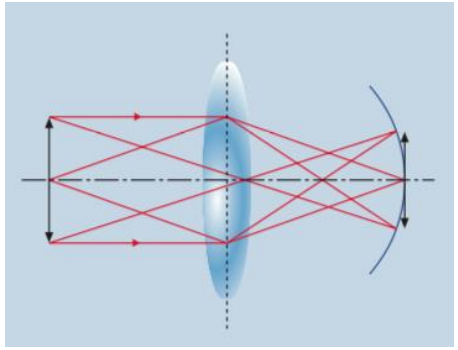
色差
Color aberrations



High N.A.
High Magnification

Aberrations Of Light
Field Curvature

Field curvature



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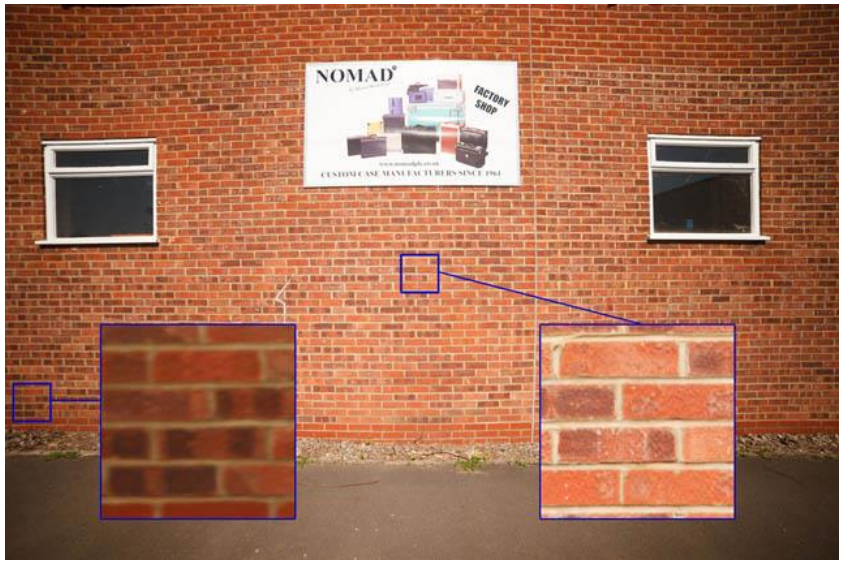
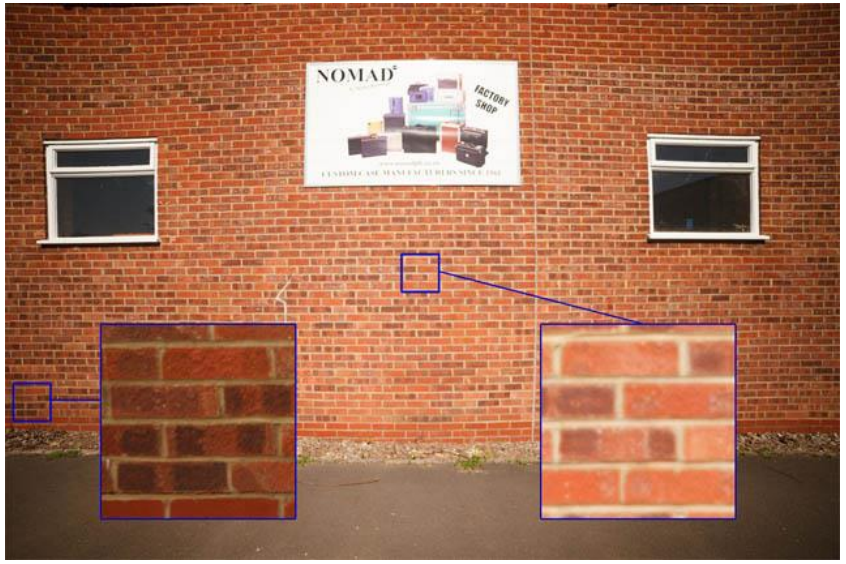
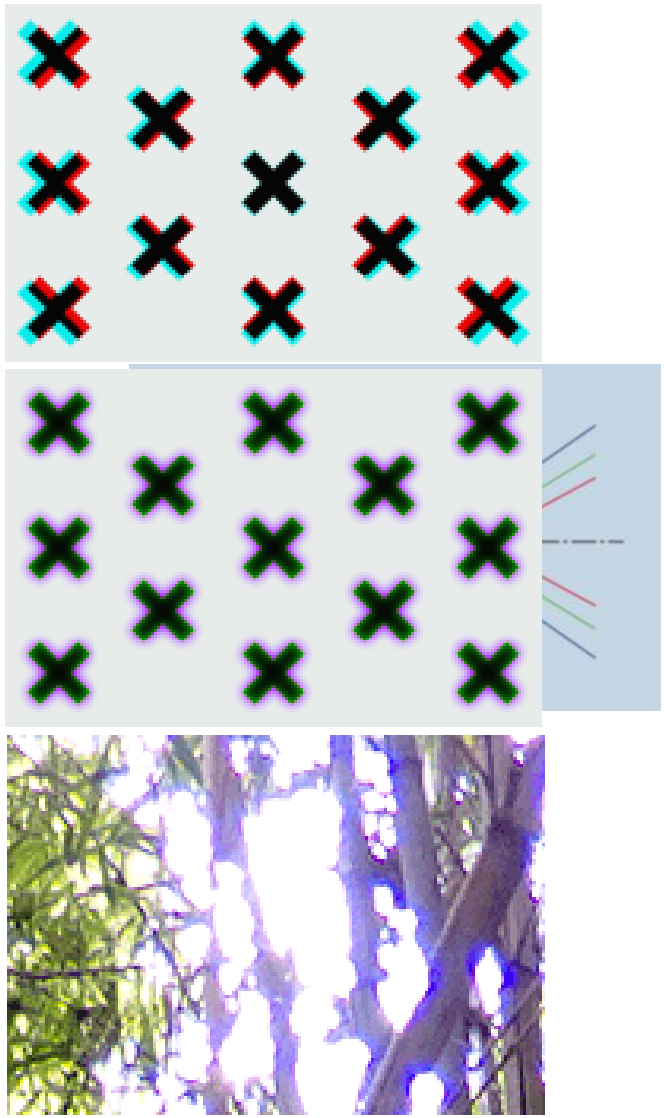
Specimen thickness



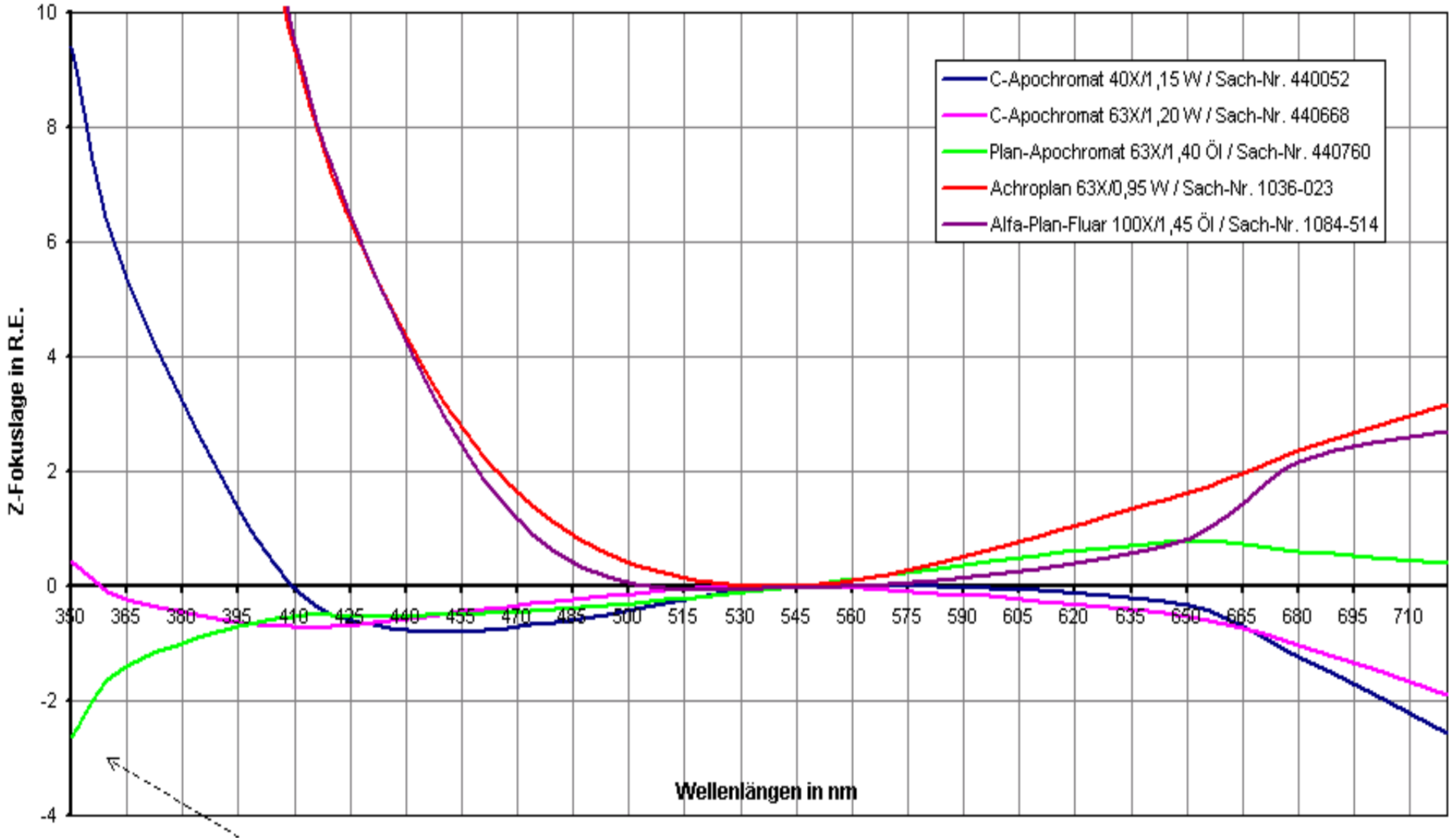


Aberrations Of Light

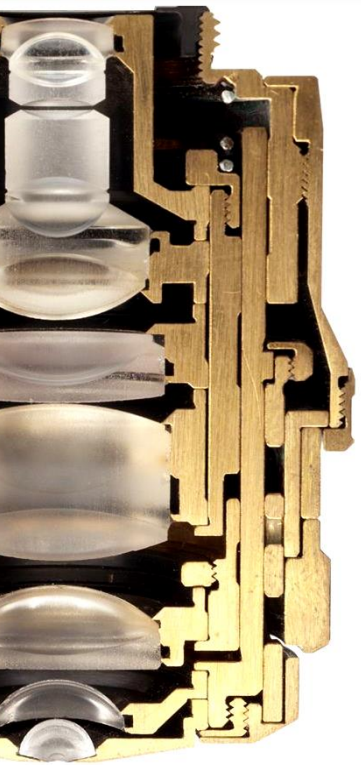
Color / Chromatic aberration



Aberrations Of Light



The Objective



Labeling of the Objective
Objective class, special designations are used for this, e.g.
LD for Long Working Distance

Magnification / Numerical Aperture
plus additional details on
- immersion medium (Oil /W/ Glyc)
- adjustable cover glass correction (Korr.)
- contrast method

Tube Length / Cover Glass Thickness (mm)
ICS optics: ∞
Infinity Color Corrected System
standard cover glass: 0.17
without cover glass: 0
insensitive: -

Mechanical Correction Collar
- cover glass thickness correction
- different immersion
- different temperature
- adjusting an iris diaphragm

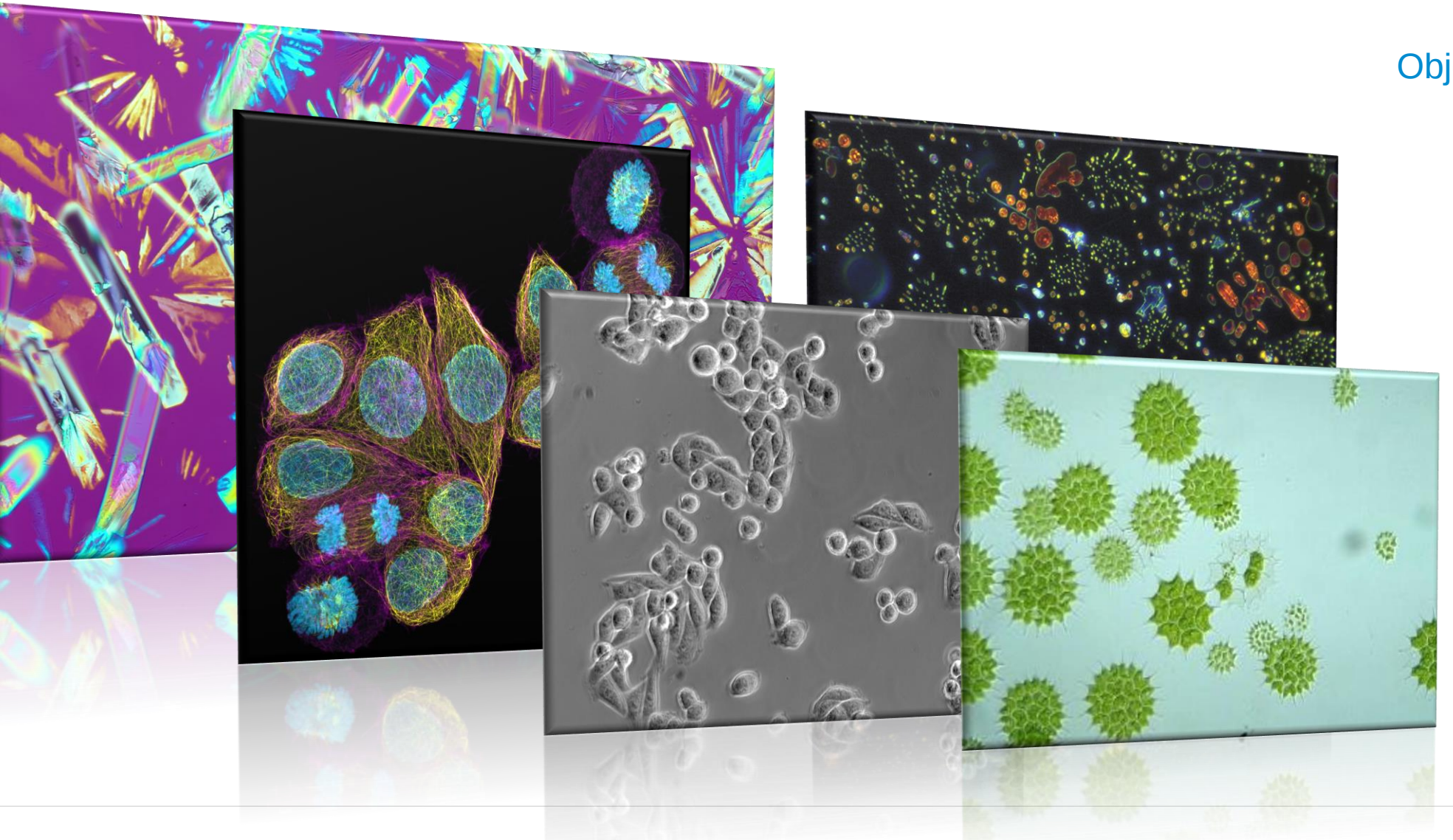
Color of writing
Contrast method
- Standard
- Pol / DIC
- Ph 0 1 2 3

Color Coding of Magnification
- 1.0/1.25
- 2.5
- 4/5
- 6.3
- 10
- 16/20/25/32
- 40/50
- 63
- 100/150

Immersion Fluid
- Oil
- Water
- Glycerin
- Oil /Water / Glycerin

Agenda

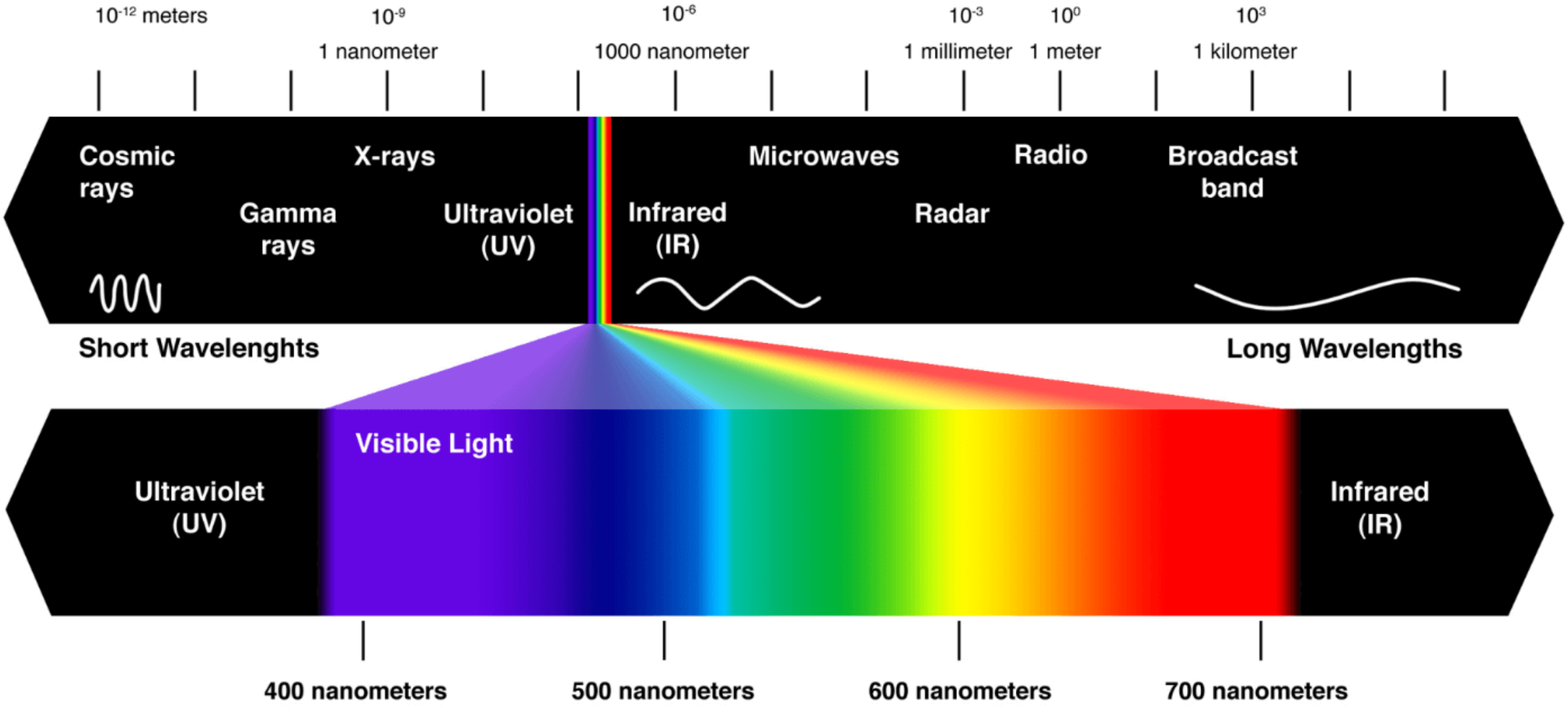
Contrast Methods
Vivid and Brilliant Colors



Objects v.s Background

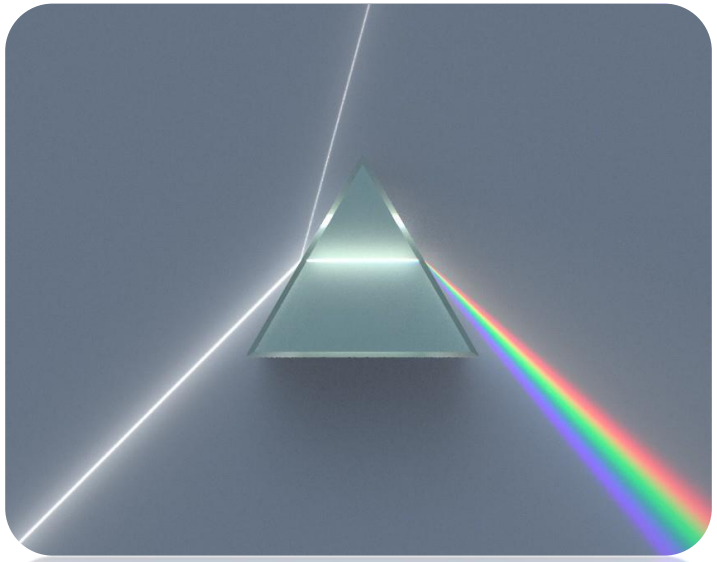
Contrast Methods

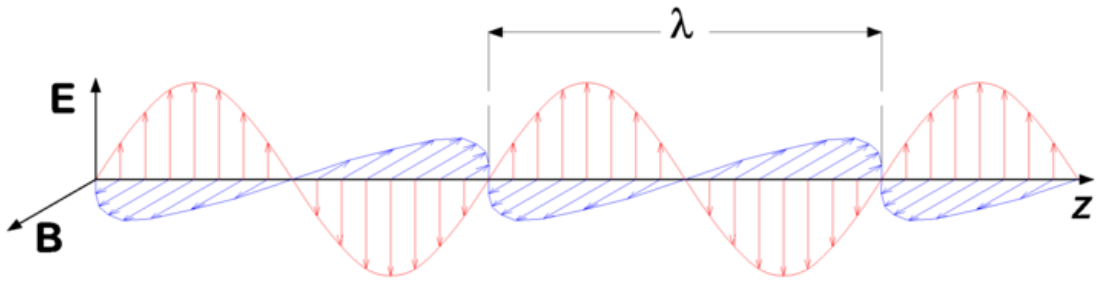
Light Is The Messenger of Information



Contrast Methods
Light Is The Messenger of Information

- Visible light (400 nm – 750nm)
- Light is particle. - *Issacs Newton*
- Light is wave. - *Robert Hooke, Christiaan Huygens, Leonhard Paul Euler*
- Light could be polarized. - *Thomas Young*

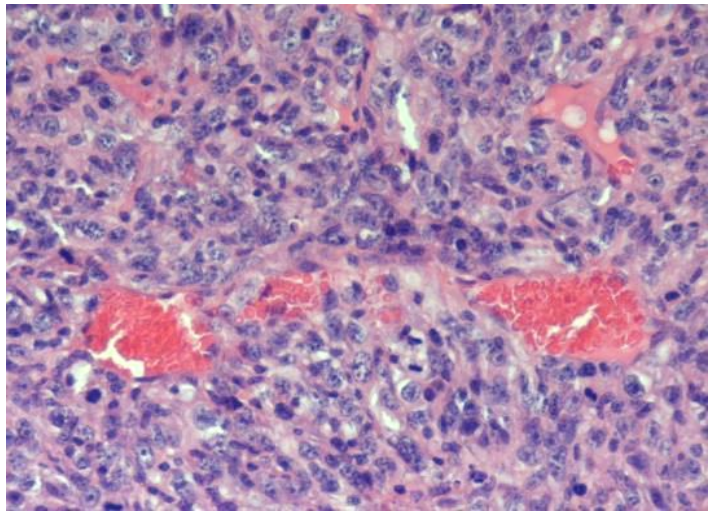




Contrast Methods
Bright Field (H)

- Light from the bulb or LED
- Sample should be colorful
- Dyes were applied on sample

Algae with green color



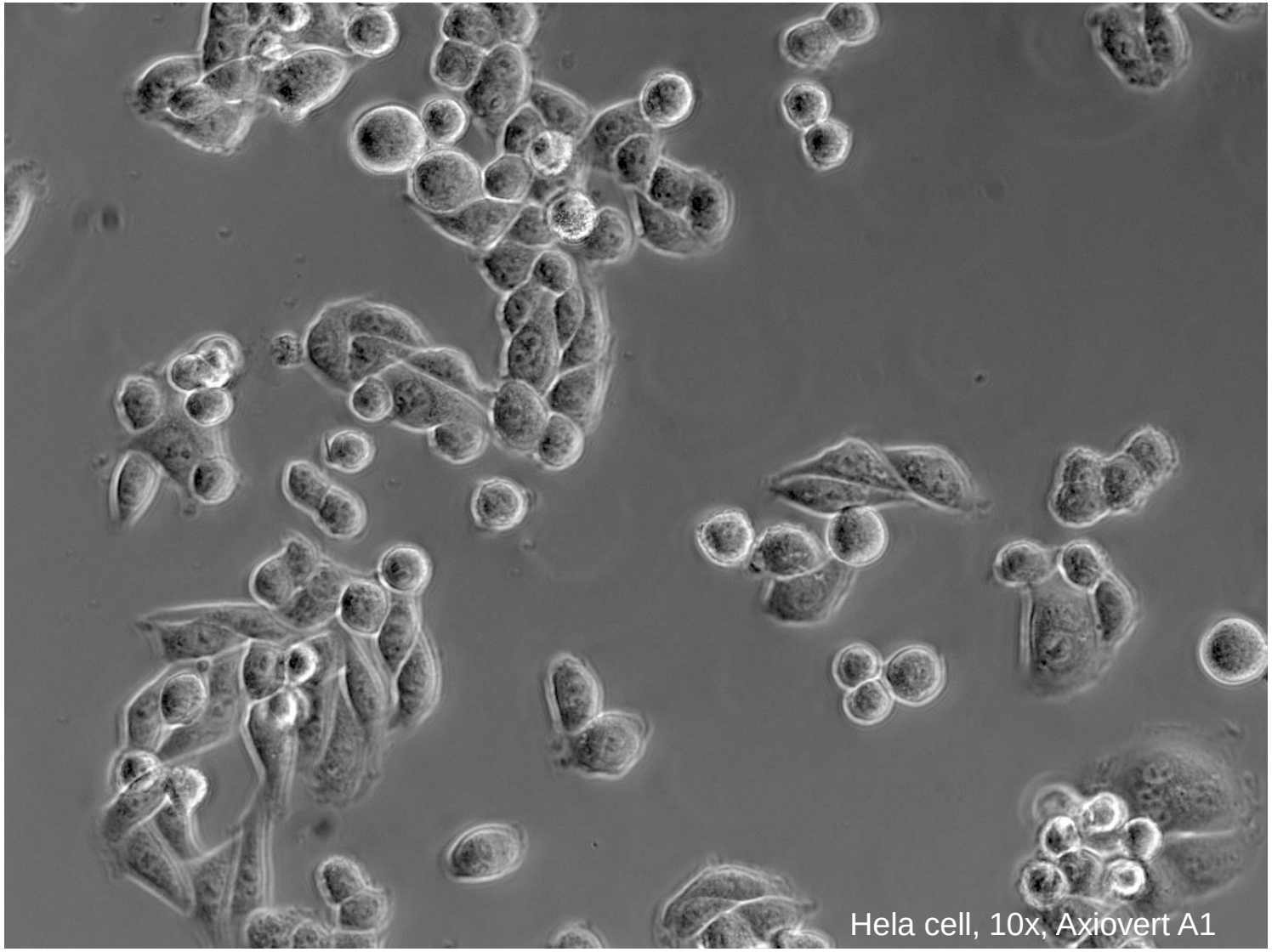
Tissue slide with immune staines

Contrast Methods
Dark Field (D)

- Block the light from the bulb or LED
- Dark background
- Only light diffracted by the sample was observed
- Darkfield objectives, annlar stop
- Tiny thin seams, filaments, edges

Contrast Methods

Phase Contrast (Ph)



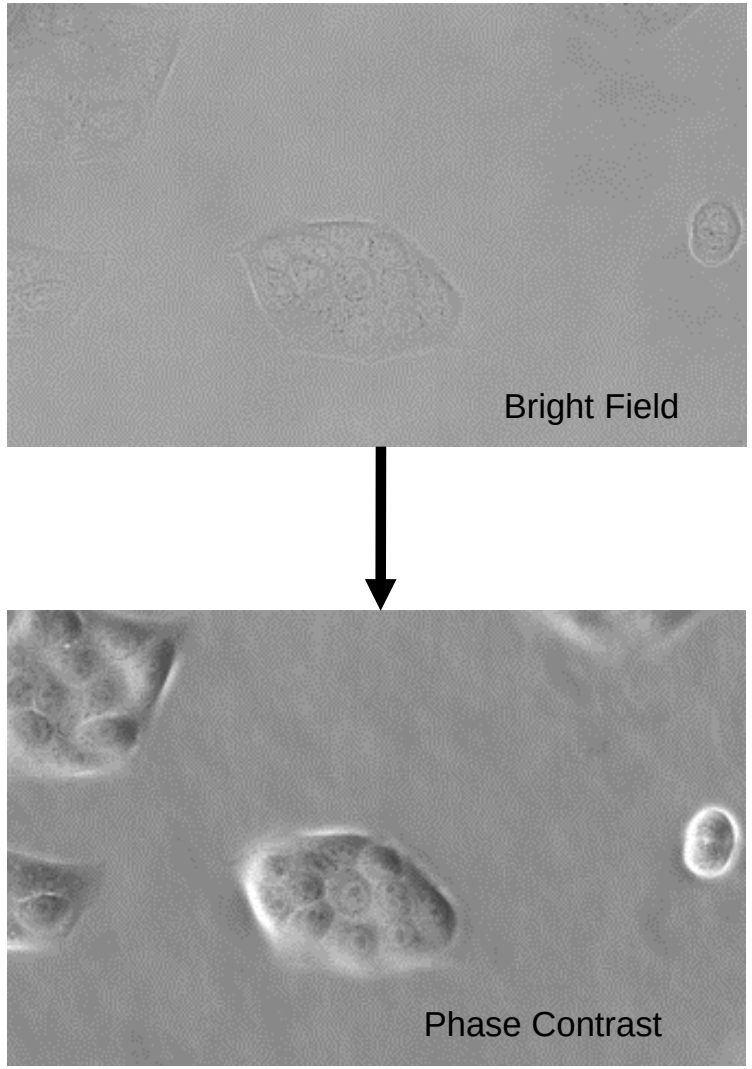
Contrast Methods
Phase Contrast (Ph)

- Nobel Prize for Physics, Dutch, 1953
- The most popular contrast method among cell biology labs
- Lights were “retarded” due to the thickness of the sample

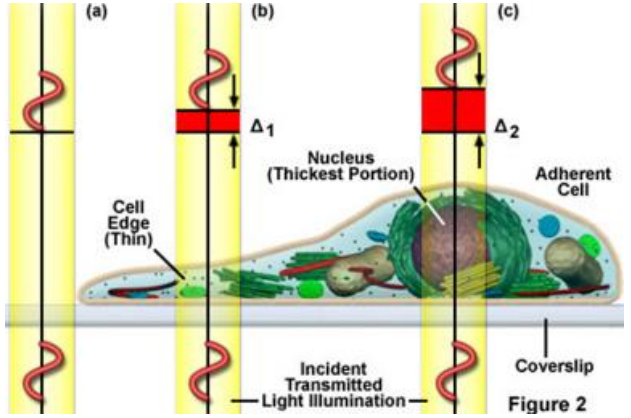
- Need a **Phase Stop**
- Phase contrast objectives
- Colorless samples

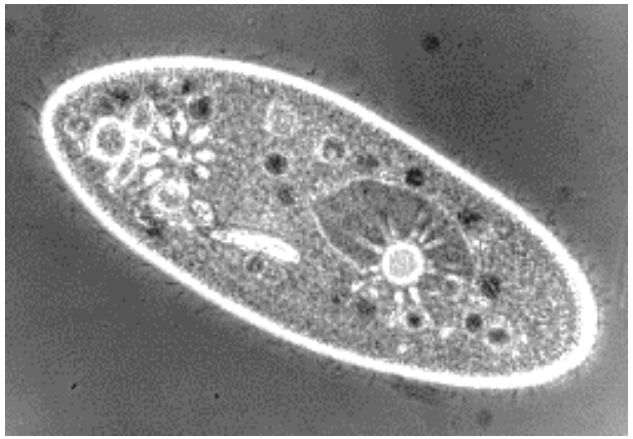
Photo from the Nobel Foundation archive.

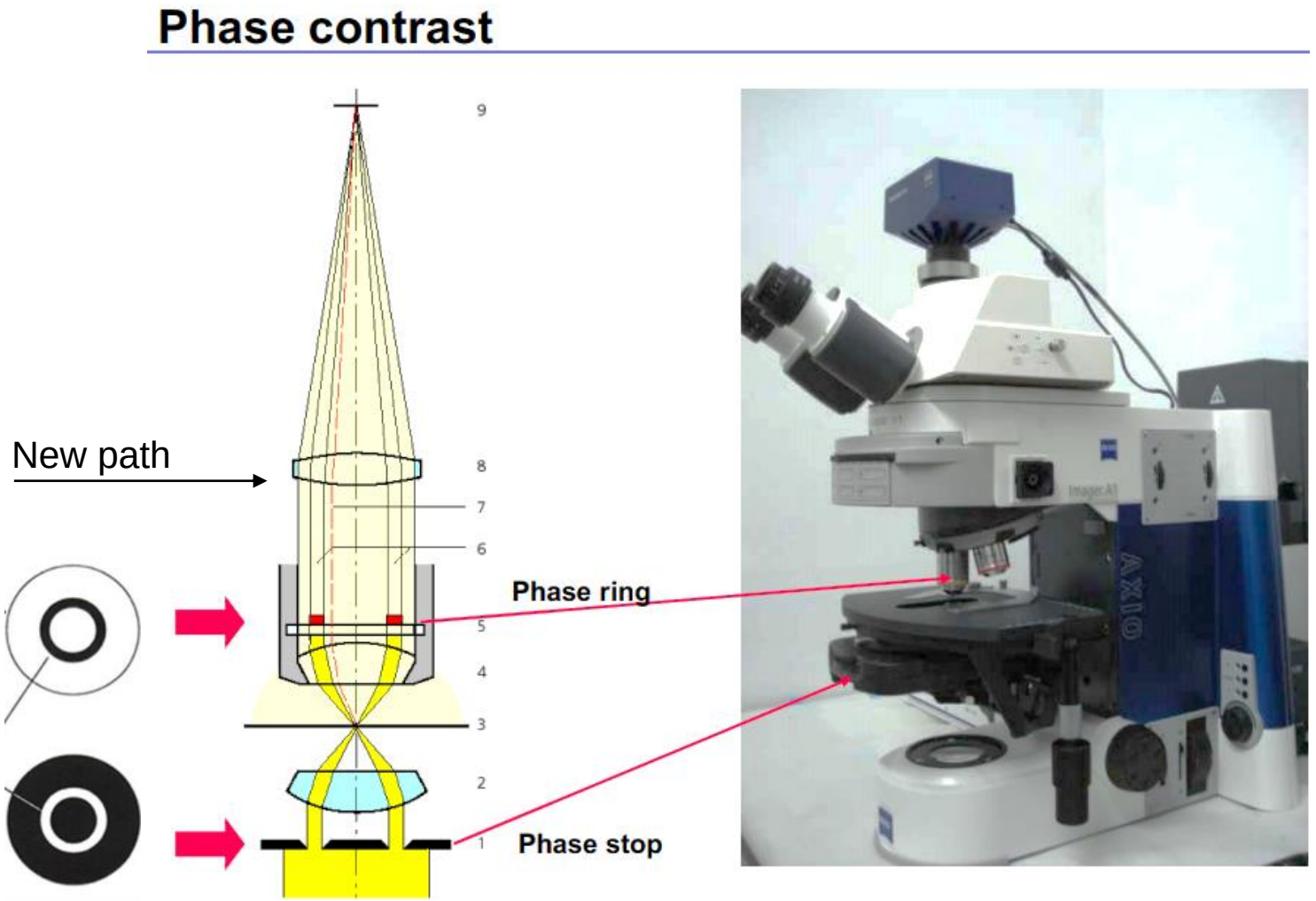
Frits Zernike



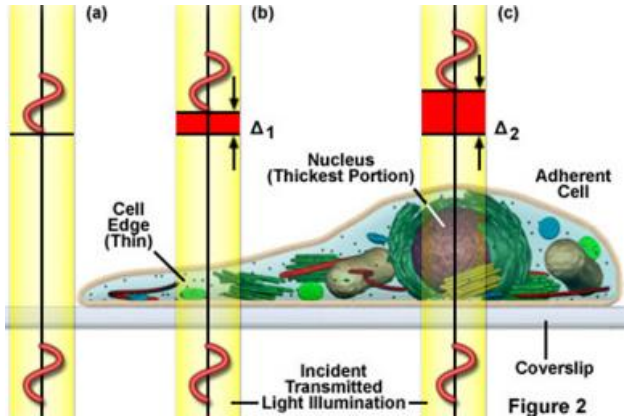
Contrast Methods
Phase Contrast (Ph)

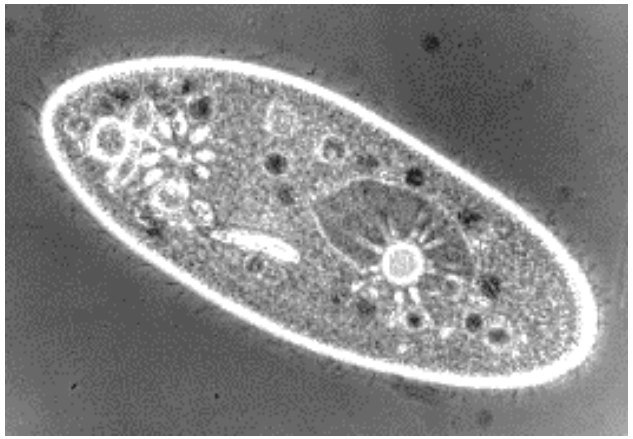


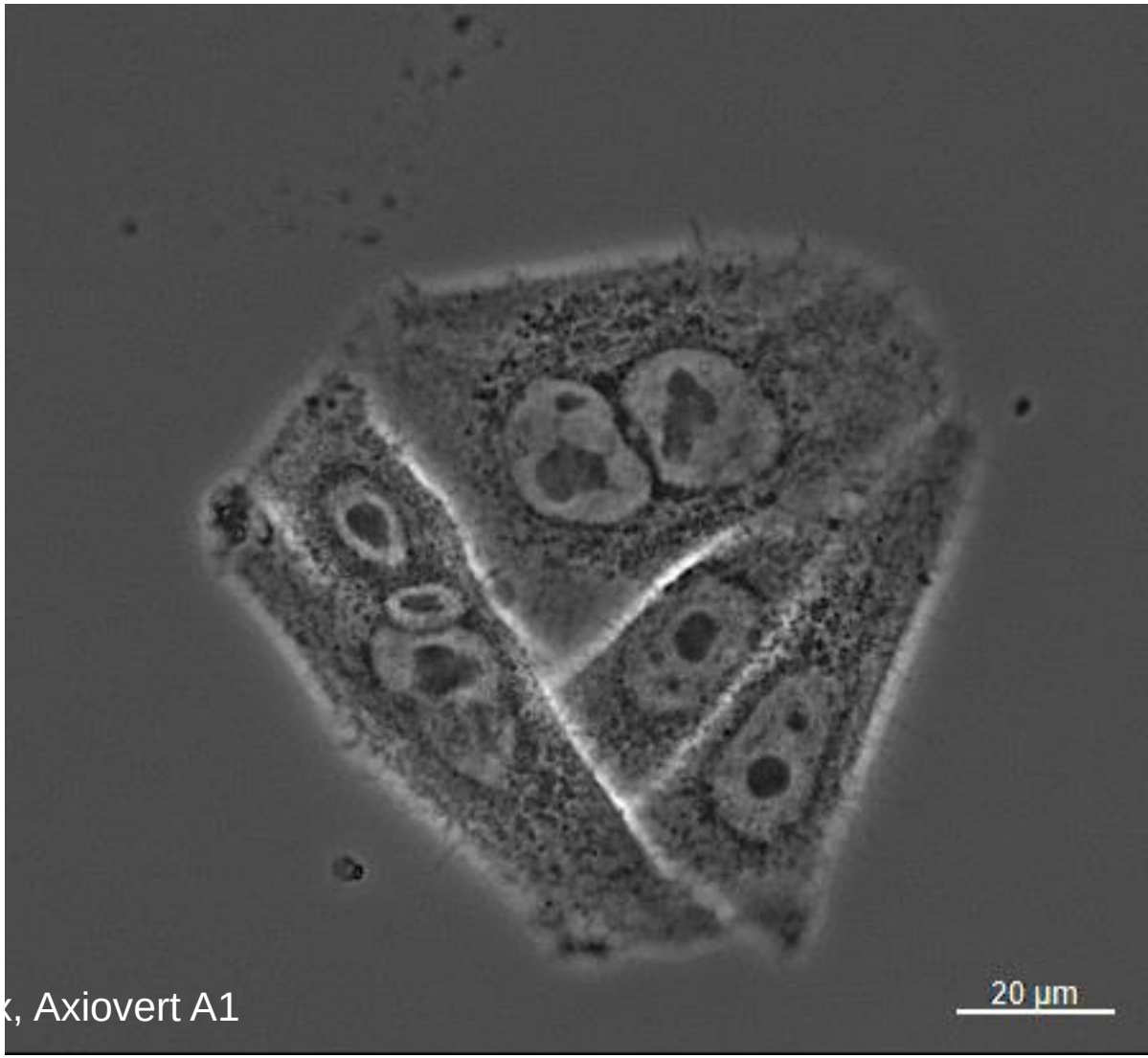




Contrast Methods
Phase Contrast (Ph)



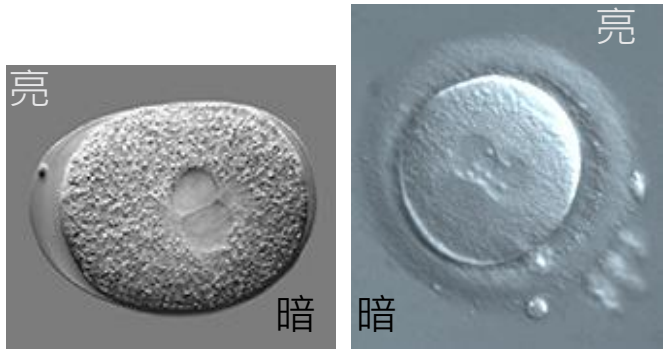


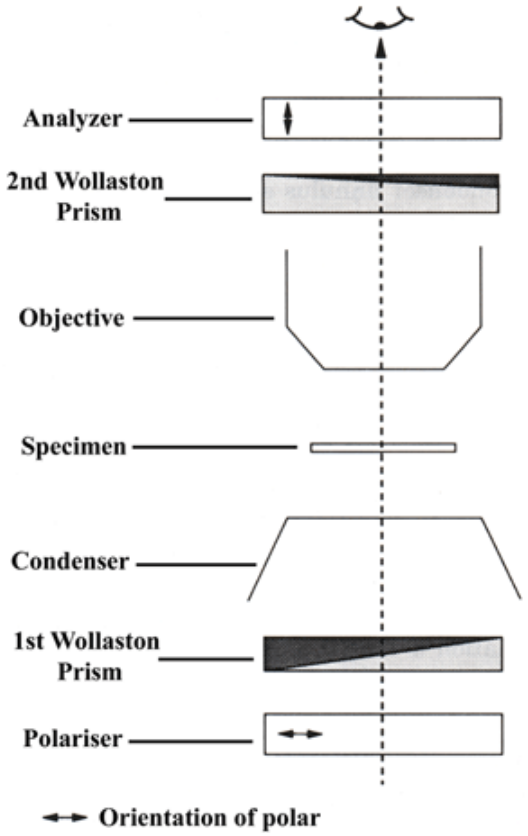


Contrast Methods
Differential Interference Contrast (DIC)

- Installed on high-end microscopes
- Looks “ relief ”
- High resolution

- Polarizer, analyzer, Wollaston prism x2
- POL objectives





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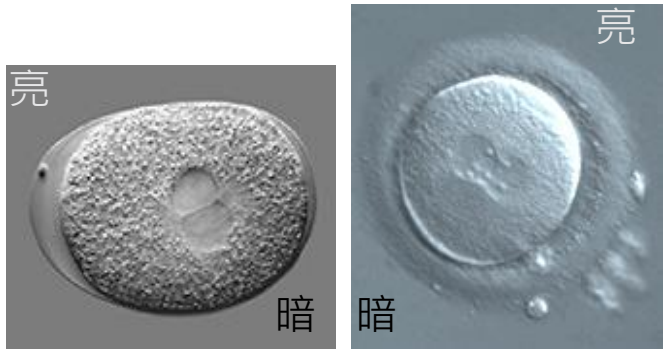
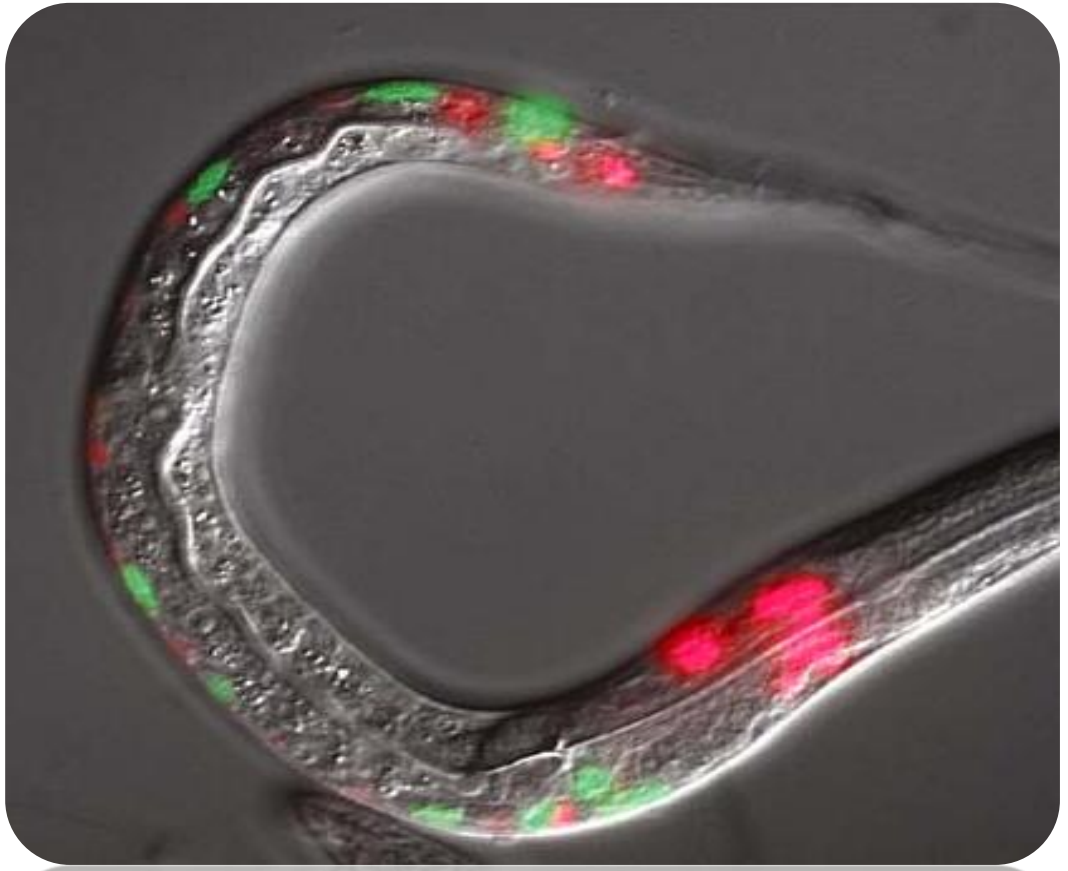
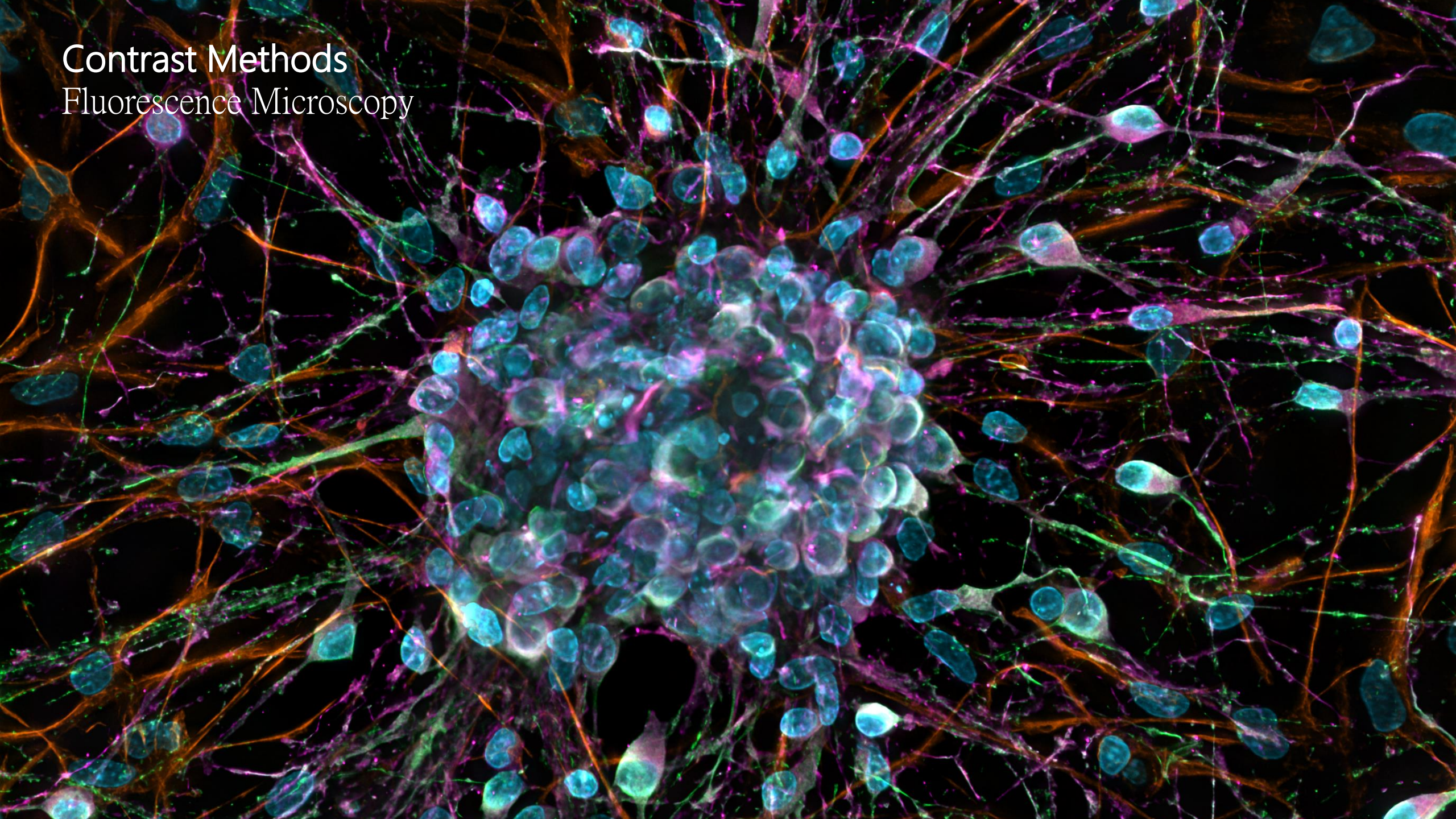




Contrast Methods

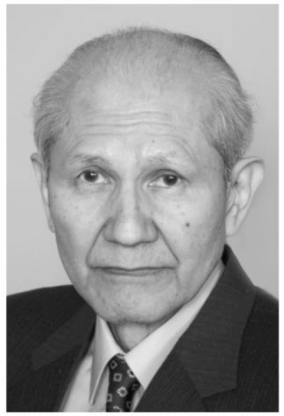
Fluorescence Microscopy



Contrast Methods
Fluorescence Contrast (FL)

- Specific, precision to molecule level
- Multiple staining
- High resolution
- 4D imaging
- Fluorescence bleaching ☹
- Gene transfection, fluorescent dyes
- Fluorescence filters
- Fluorescent light sources

The Nobel Prize in Chemistry 2008



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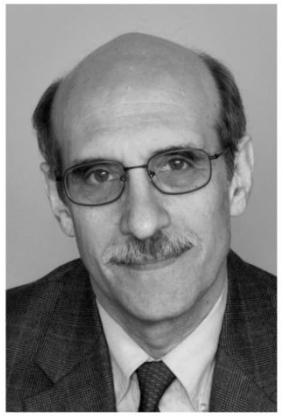
Osamu Shimomura



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Martin Chalfie

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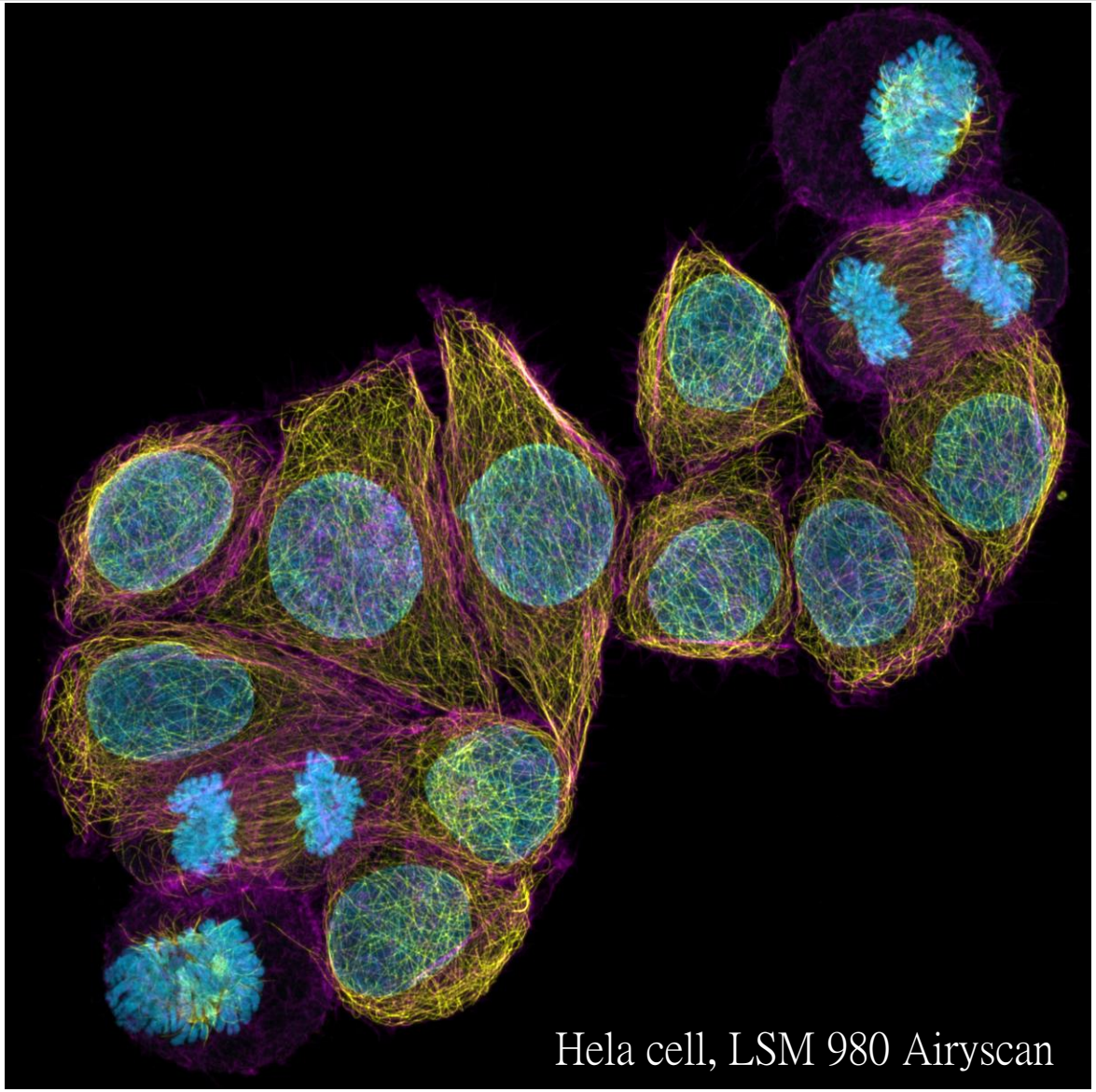
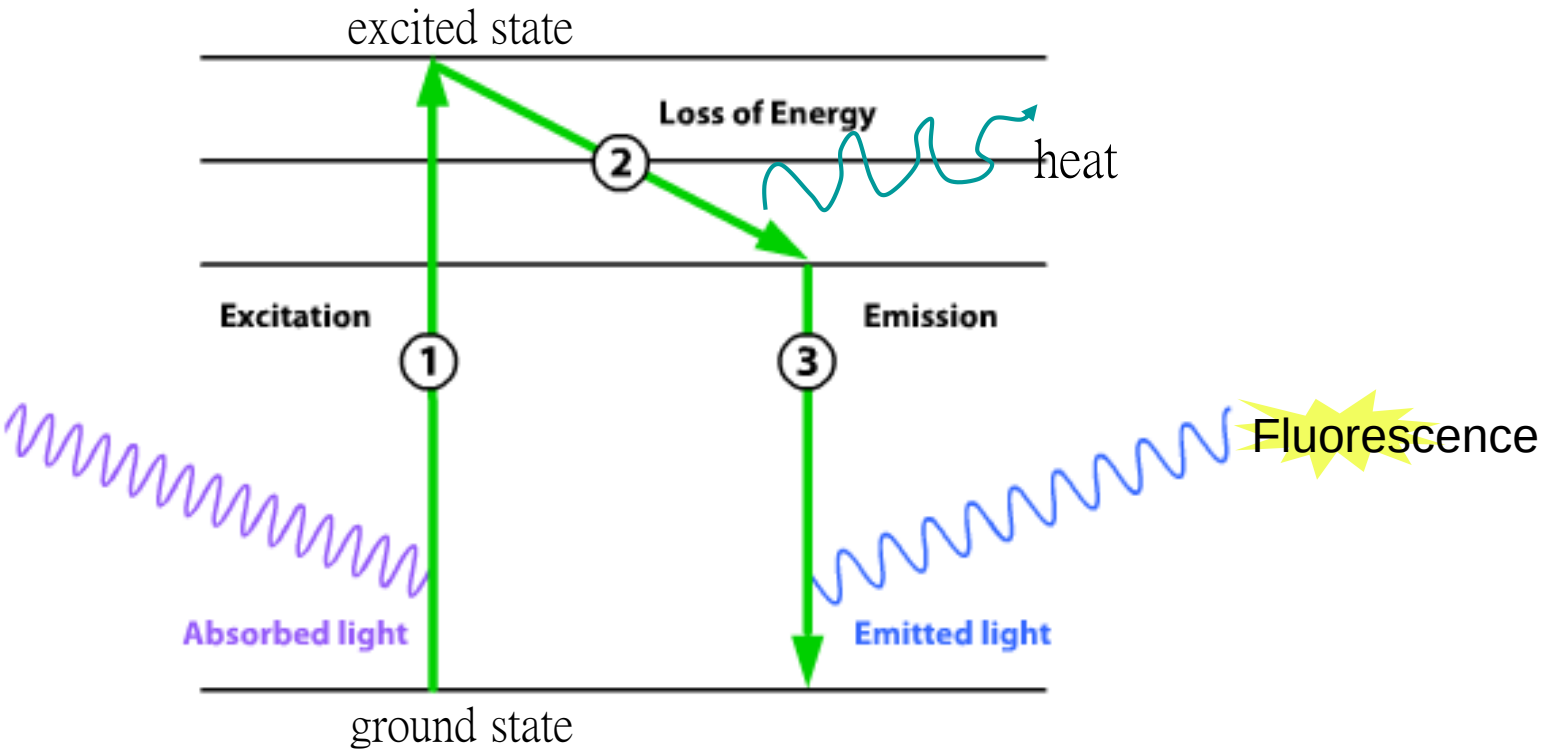
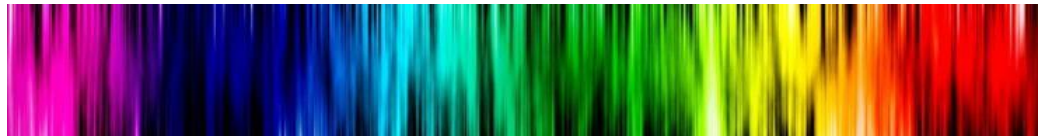
Roger Y. Tsien



Contrast Methods
Fluorescence Contrast (FL)

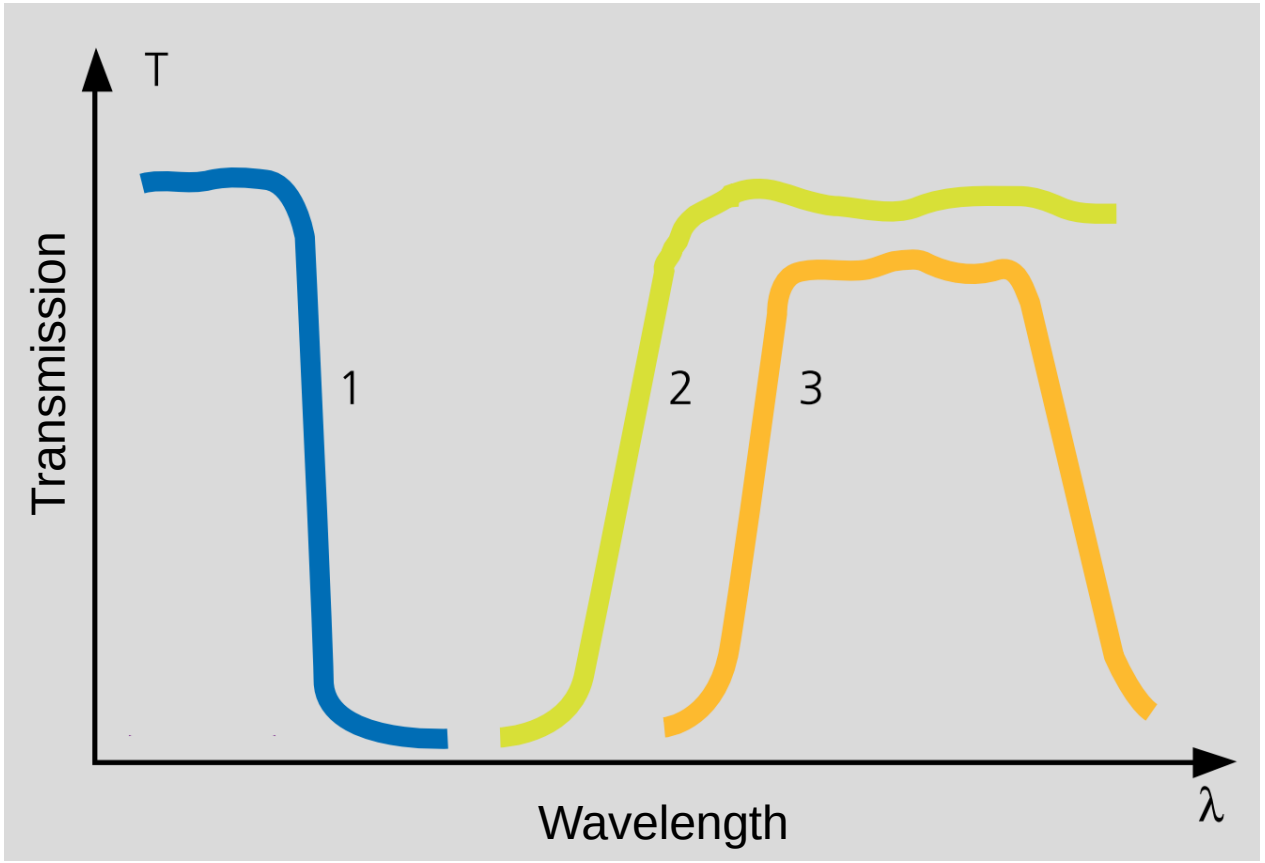




Fluorescence Hardwares
The Filter Sets

Fluorescence Hardwares
The Filter Sets

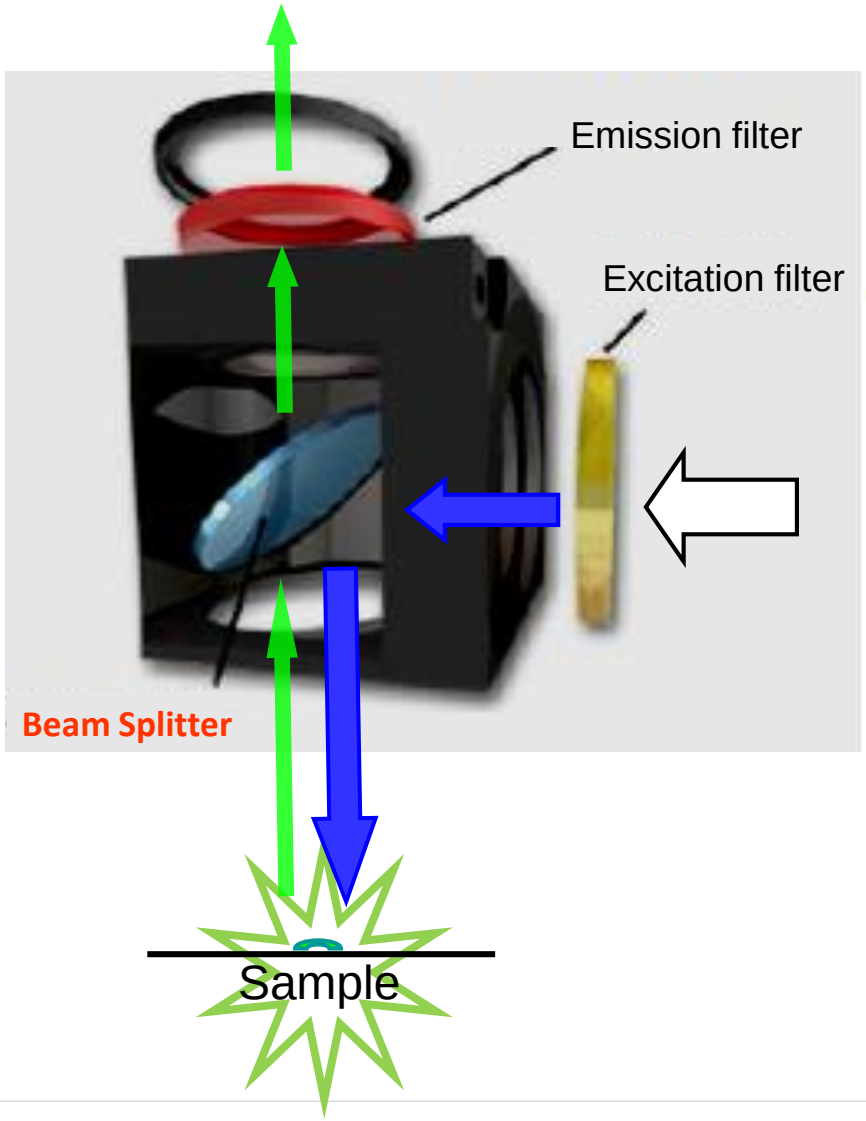
Fluorescence Filters

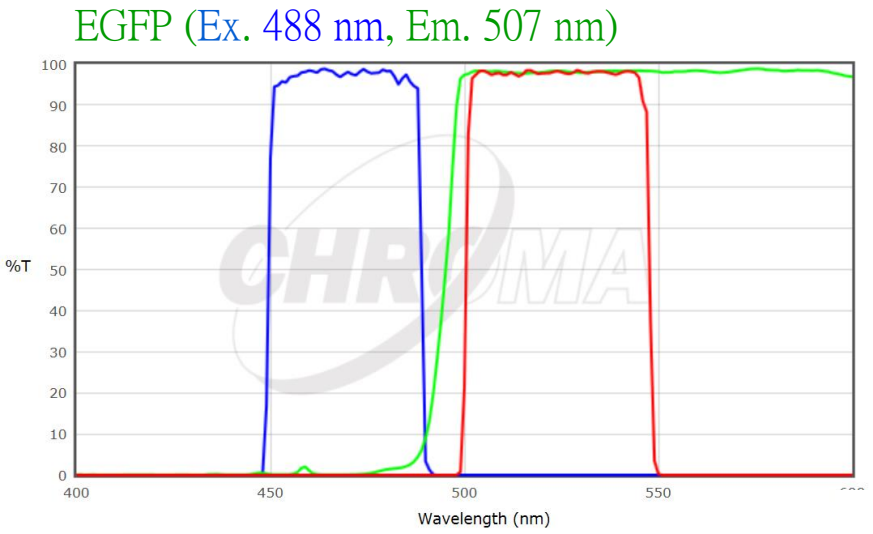


1. Shortpass filter
2. Longpass filter
3. Bandpass filter

Fluorescence Filters

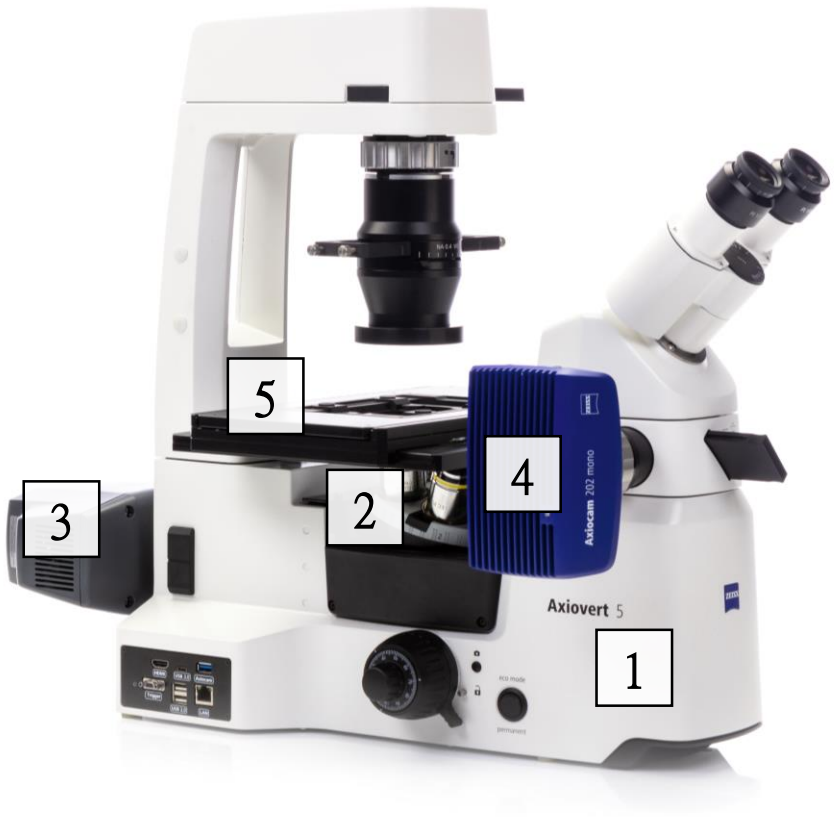
- Fluorescence filter cube



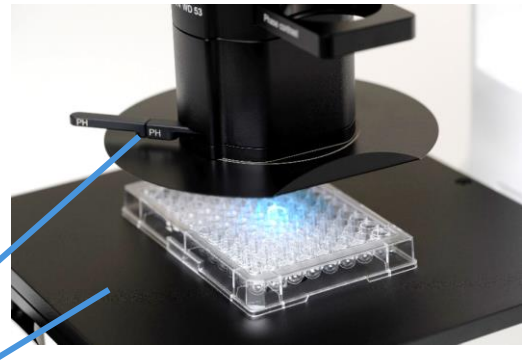


FILTERS	TYPE
ET470/40x	EX
T495lpxr	BS
ET525/50m	EM

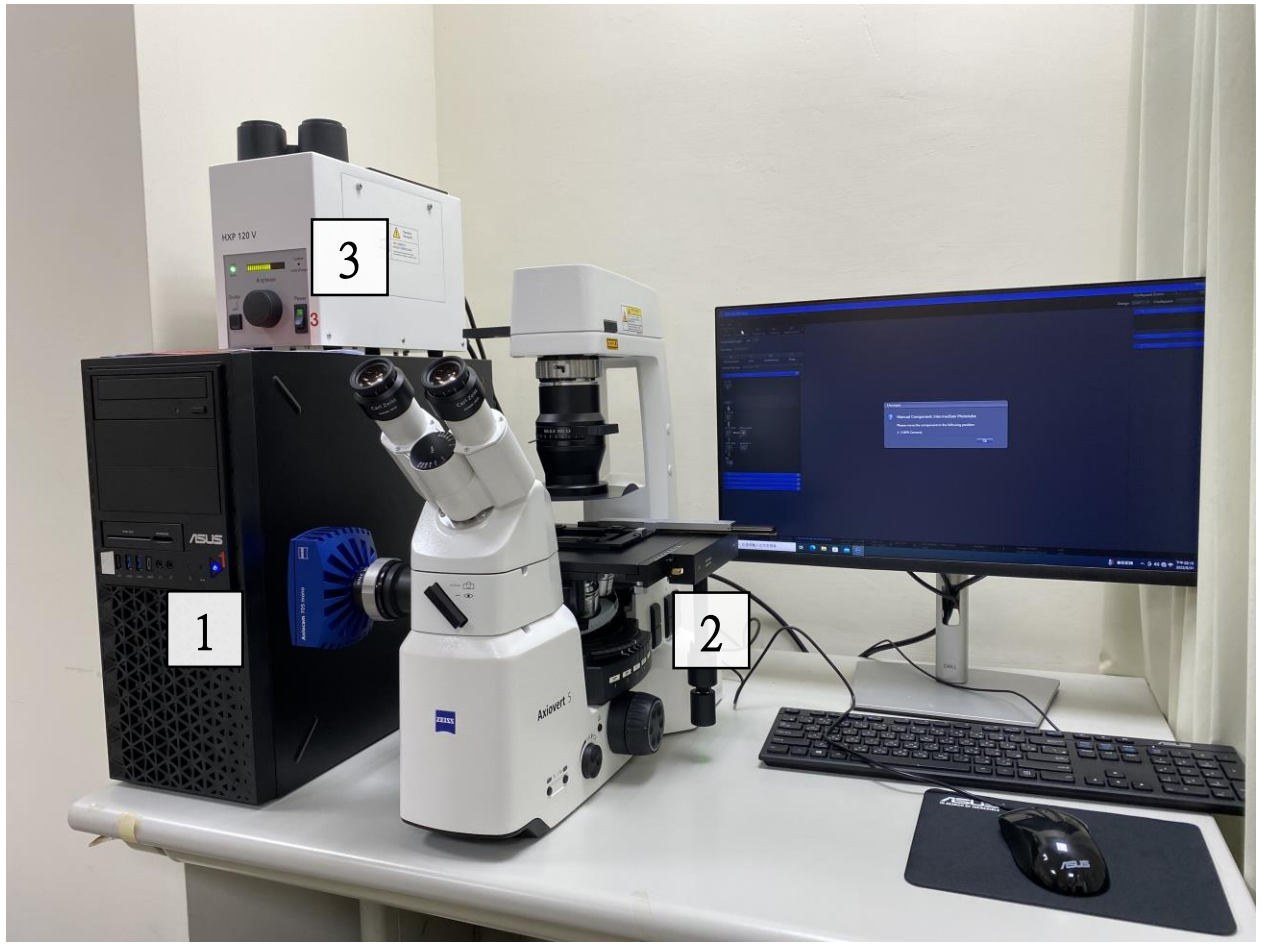
Components of Axiovert 5



1. Microscope
2. Objectives
3. Illumination
4. Camera
5. Accessories
   - Light shield
   - Stage
   - Mounting frame
   - Filter set



Starting Axiovert 5 in 3 steps



1. Computer
2. Microscope
3. Illumination

Click the ZEN icon on the desktop to enter the software

Seeing beyond

Seeing beyond