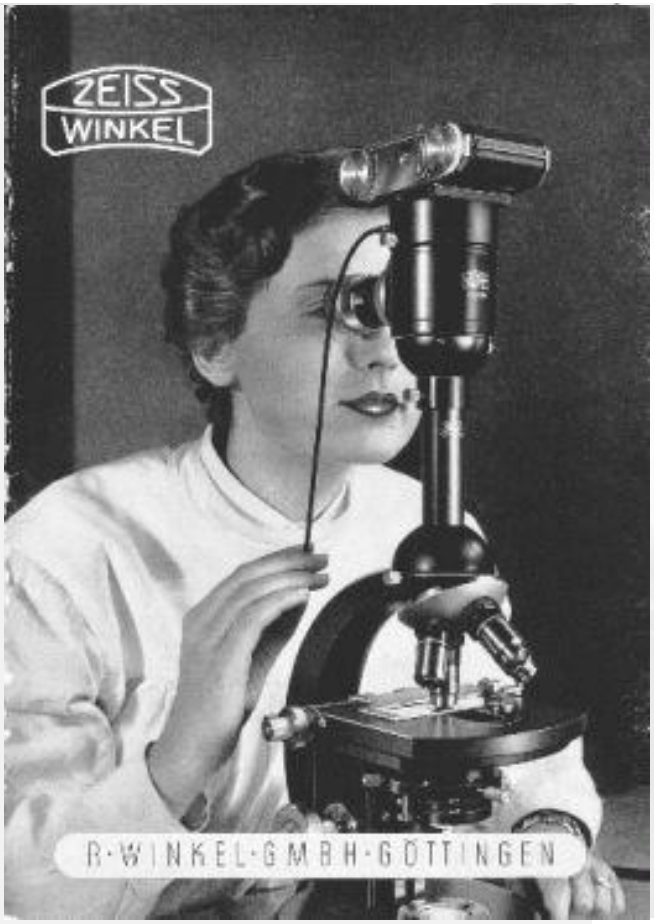
For participants of Microscopy Methods in Biomedicine
October 2025

PLEASE do not share with 3rd persons

Instrumentation for light microscopy

Components of microscope: light sources, objectives, fluorescent filters, cubes, principles of detection



Pavel Krist

pavel.krist@zeiss.com

Microscopes

How to orientate in the broad spectrum of products?

Stereomicroscope
Wide field microscope
Fluorescence microscope
TIRF system
Confocal microscope
Spinning disc
Multiphoton microscope
Superresolution microscope
Lightsheet microscope
Electron microscope
Other?

Microscopes

How to orientate in the broad spectrum of products?

Where to start?

Microscopes

How to orientate in the broad spectrum of products?

- Light microscopy instrumentation
- Contrast-enhancing techniques in optic
- Resolution and image formation in light microscopy
- Basics of fluorescence microscopy and immunolabeling
- Multi-dimensional laser confocal microscopy
- Introduction to live cell imaging
- Spinning disc confocal microscopy
- Light Sheet microscopy

- Introduction to image deconvolution
- Fluorophores
- FRAP + FCS
- FRET, FLIM
- Superresolution in light microscopy: STORM, SIM, STED
- Computative high resolution methods
- Quantitative phase microscopy

- Image acquisition by two- photon microscopy
- Introduction to image processing
- Image analysis and visualization in 3D
- Preparation of digital photographic documentation for publication

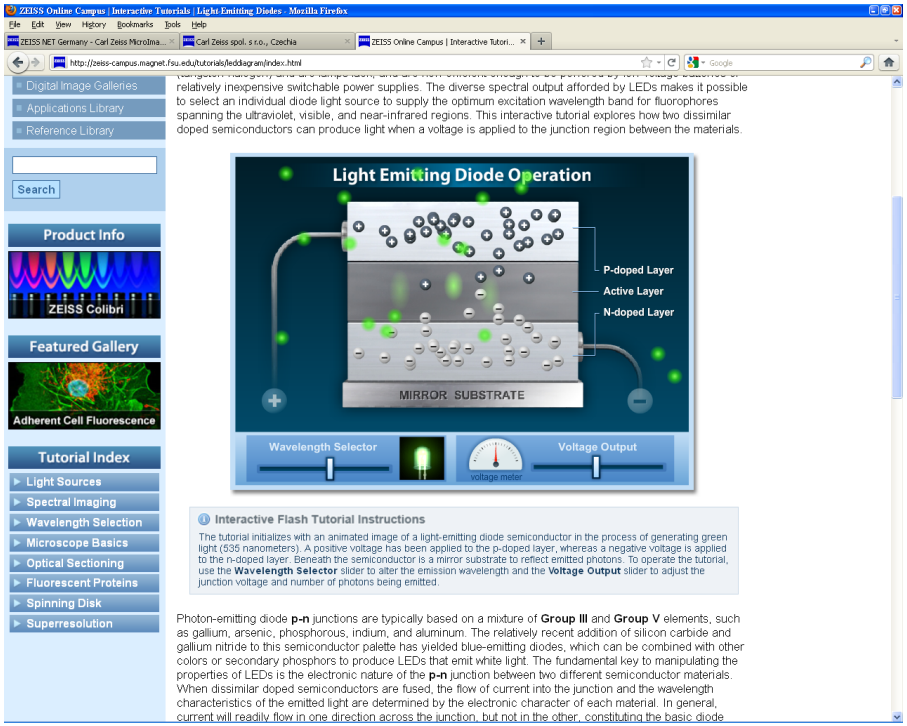
- Optical projection tomography
- Advanced electron microscopy techniques
- Preparing samples for TEM
- Image formation in transmission electron microscope
- Methodology for correct microscopy Scanning electron microscopy

Microscopes

How to orientate in the broad spectrum of products?

Where to start?

http://www.zeiss.com/campus = http://zeiss-campus.magnet.fsu.edu/
or www.google.com - „CARL ZEISS CAMPUS“



Microscopes

How to orientate in the broad spectrum of products?

ZEISS

Where to start?

https://www.youtube.com/watch?v=60_jgZtyR6U

Source:
Jan Peychl (MPI-CBG)

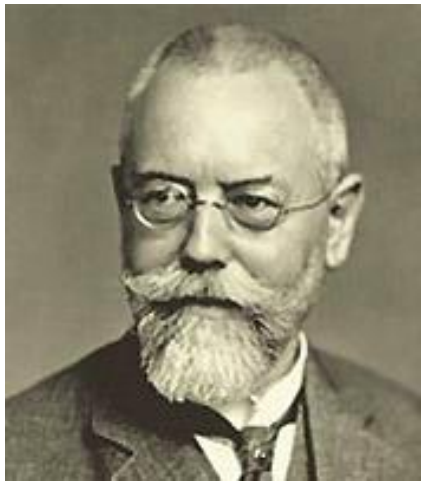
Microscopes

History of microscopy

Carl Friedrich Zeiss
(1816 - 1888)

Ernst Abbe
(1840 - 1905)

Otto Schott
(1851 - 1935)



August Köhler
(1866 - 1948)

Carl Zeiss – since 1846 - more than 176 years experience in production of microscopes

Microscopes

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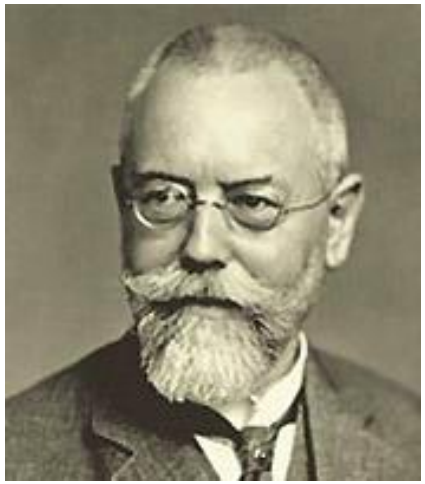


August Köhler
(1866 - 1948)

Carl Zeiss – since 1846

Leica – since 1849 Carl Kellner, 1865 Ernst Leitz, 1925 – Leica

Nikon – since 1917 (Nippon Kōgaku Tōkyō K.K.)

Olympus – since 1919 (Olympus Corporation)

+

many other companies

36 Nobel laureates worldwide use ZEISS instruments to achieve progress in science

1846

1905 **Robert Koch**
Physiology/Medicine

1906 **Santiago Ramón y Cajal Camillo Golgi**
Physiology/Medicine

1911 **Allvar Gullstrand**
Physiology/Medicine
In close collaboration with ZEISS staff

1925 **Richard Adolf Zsigmondy**
Chemistry
In close collaboration with ZEISS staff

1952 **Frits Zernike**
Physics
In close collaboration with ZEISS staff

1953 **Manfred Eigen**
Chemistry

1967 **Bert Sakmann**
Erwin Neher
Physiology/Medicine

1991 **Christiane Nüsslein-Volhard**
Physiology/Medicine

1995 **Günter Blobel**
Physiology/Medicine

1999 **Ahmed A. Zewail**
Chemistry

2001 **Sir Paul M. Nurse**
Leland H. Hartwell
Timothy Hunt
Physiology/Medicine

2001 **Eric A. Cornell**
Physics
In close collaboration with ZEISS staff

2002 **Sidney Brenner**
H. Robert Horvitz
John E. Sulston
Physiology/Medicine

2006 **Craig C. Mello**
Andrew Z. Fire
Physiology/Medicine

2008 **Harald zur Hausen**
Physiology/Medicine

2008 **Osamu Shimomura**
Martin Chalfie
Roger Tsien
Chemistry
In close collaboration with ZEISS staff

2010 **Andre Geim**
Konstantin Novoselov
Physics

2011 **Dan Shechtman**
Chemistry

2012 **Sir John B. Gurdon**
Shinya Yamanaka
Physiology/Medicine

2013 **Eric Betzig**
Stefan W. Hell
William E. Moerner
Chemistry
In close collaboration with ZEISS staff

2014 **John O'Keefe**
May-Britt Moser
Edvard I. Moser
Physiology/Medicine

2018 **Arthur Ashkin**
Gérard Mourou
Donna Strickland
Physics

Seeing beyond

Microscopes

How to orientate in the broad spectrum of products?

Where to start?

What defines which microscope technique you need?

- Stereomicroscope
- Wide field microscope
- Fluorescence microscope
- TIRF system
- Confocal microscope
- Spinning disc
- Multiphoton microscope
- Superresolution microscope
- Lightsheet microscope
- Electron microscope
- Other?

Microscopes

How to orientate in the broad spectrum of products?

Where to start?

What defines which microscope technique you need?

Microscopes

How to orientate in the broad spectrum of products?

Where to start?

What defines which microscope technique you need?

THE SAMPLE

The impressive capabilities of the modern microscope can tempt workers to think it can accomplish miracles. However, no amount of optical sectioning or post-processing can reconstruct a perfect picture from bad input.

(Scott Fraser, 1990)

Garbage In, Garbage Out.

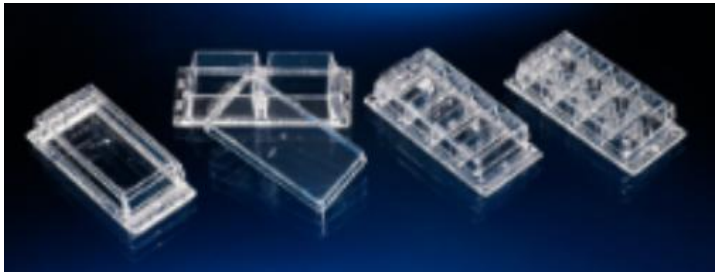
Instrumentation for light microscopy

Microscope and its parts

- 0,17 mm cover glass
- No cover glass
- Petri dish, multiwell plates, LabTek, Nunc™
- POC, POC-R
- Other specimen carriers
- Glass / Plastic???

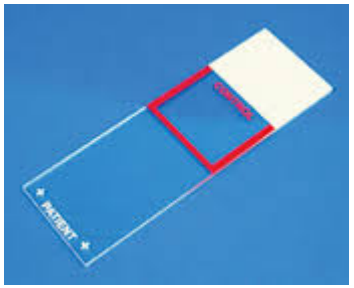
VERY IMPORTANT

Cover glasses, high performance, D=0.17mm, box with 1000 pc. Size 18x18 mm² type 1 1/2 H as per ISO 8255-1 with restricted thickness-related tolerance D=0.17 mm +/- 0.005 mm refractive index = 1.5255 +/- 0.0015, Abbe number = 56 +/-2 recommended for applications with high numerical aperture objectives

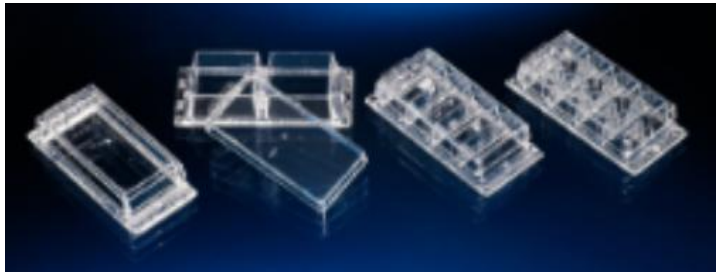


Instrumentation for light microscopy

Microscope and its parts



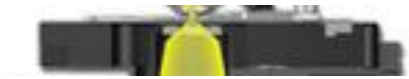
Sample



Instrumentation for light microscopy

Microscope and its parts

Sample
Microscope stage



Stages

- Manual
  - routine work

- Motorized
  - multiple positions
  - tile scans
  - more comfort at higher magnification

Instrumentation for light microscopy

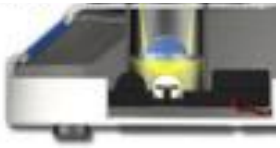
Microscope and its parts

TL

Light sources
- transmitted light
  - halogen
  - LED

Sample
Microscope stage

Light source



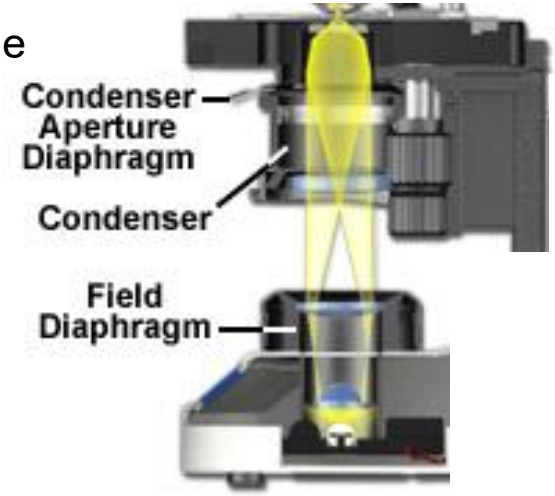
Instrumentation for light microscopy

Microscope and its parts

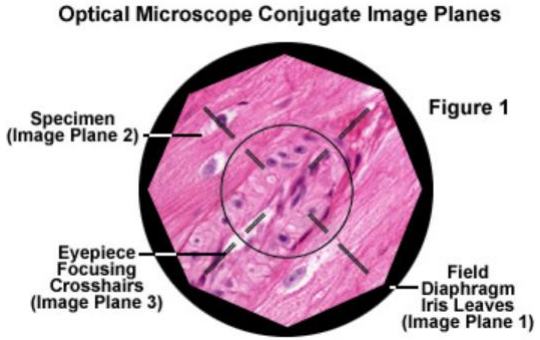
Proper configuration of the microscope in regards to illumination is possibly one of the most misunderstood concepts in optical microscopy, and is a **critical parameter that must be fulfilled in order to achieve optimum performance**.

TL

Sample
Microscope stage



Light source



Microscopes

History of microscopy

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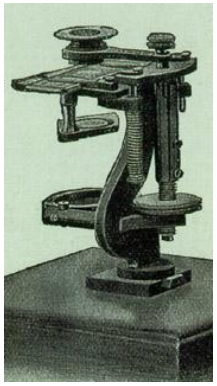
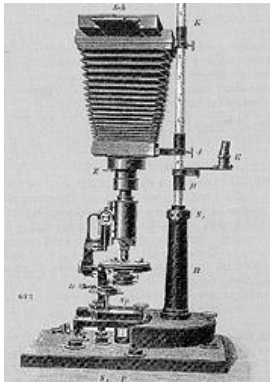


$$d = \frac{\lambda}{2n \sin\alpha}$$

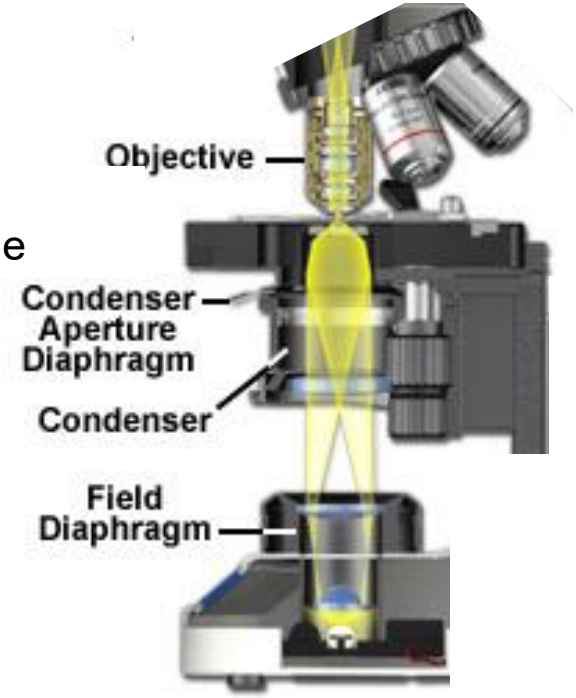


Instrumentation for light microscopy

Microscope and its parts

TL

Sample
Microscope stage



Light source

Instrumentation for light microscopy

Microscope and its parts

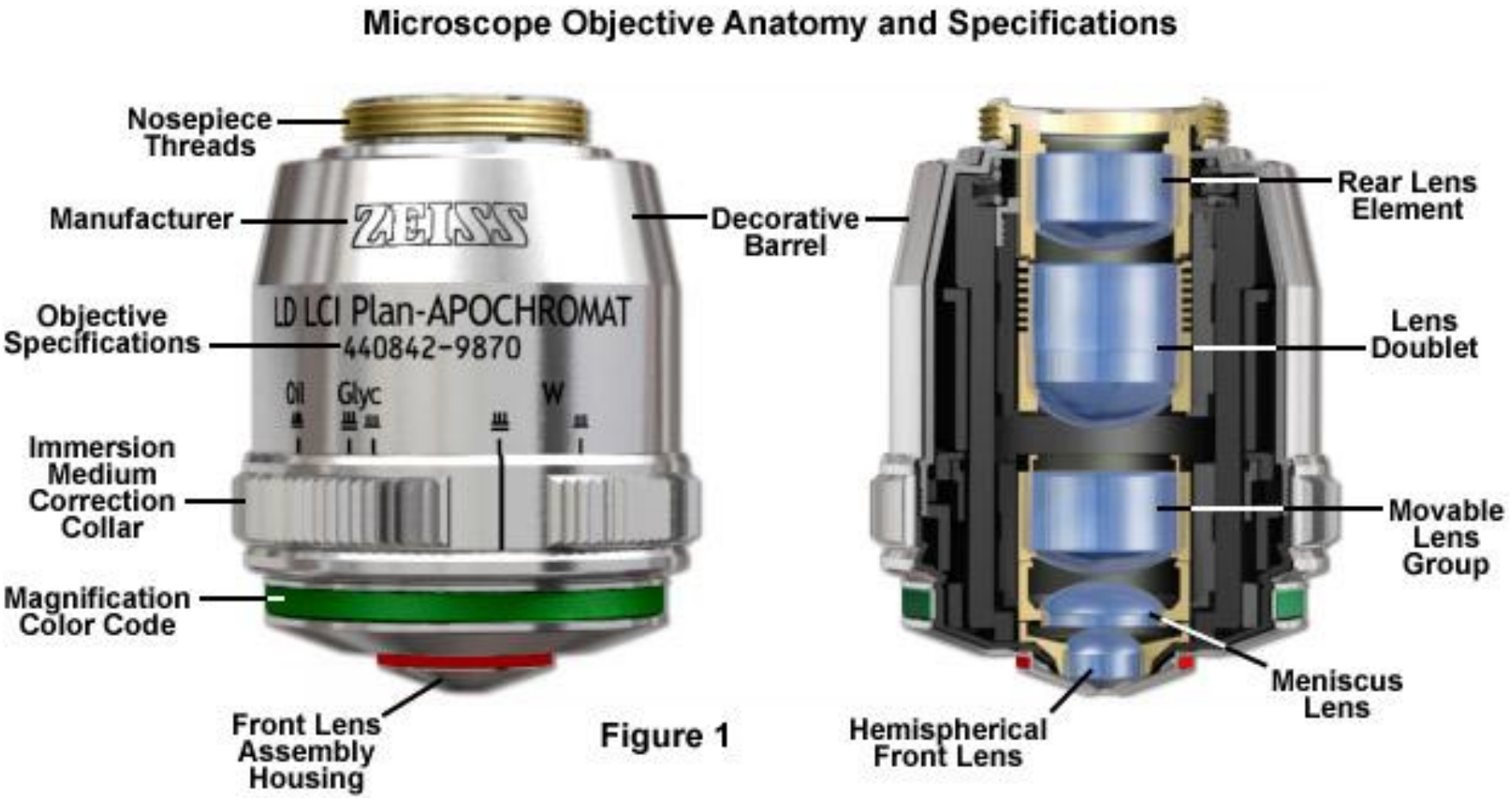


Figure 1

Instrumentation for light microscopy

Microscope and its parts

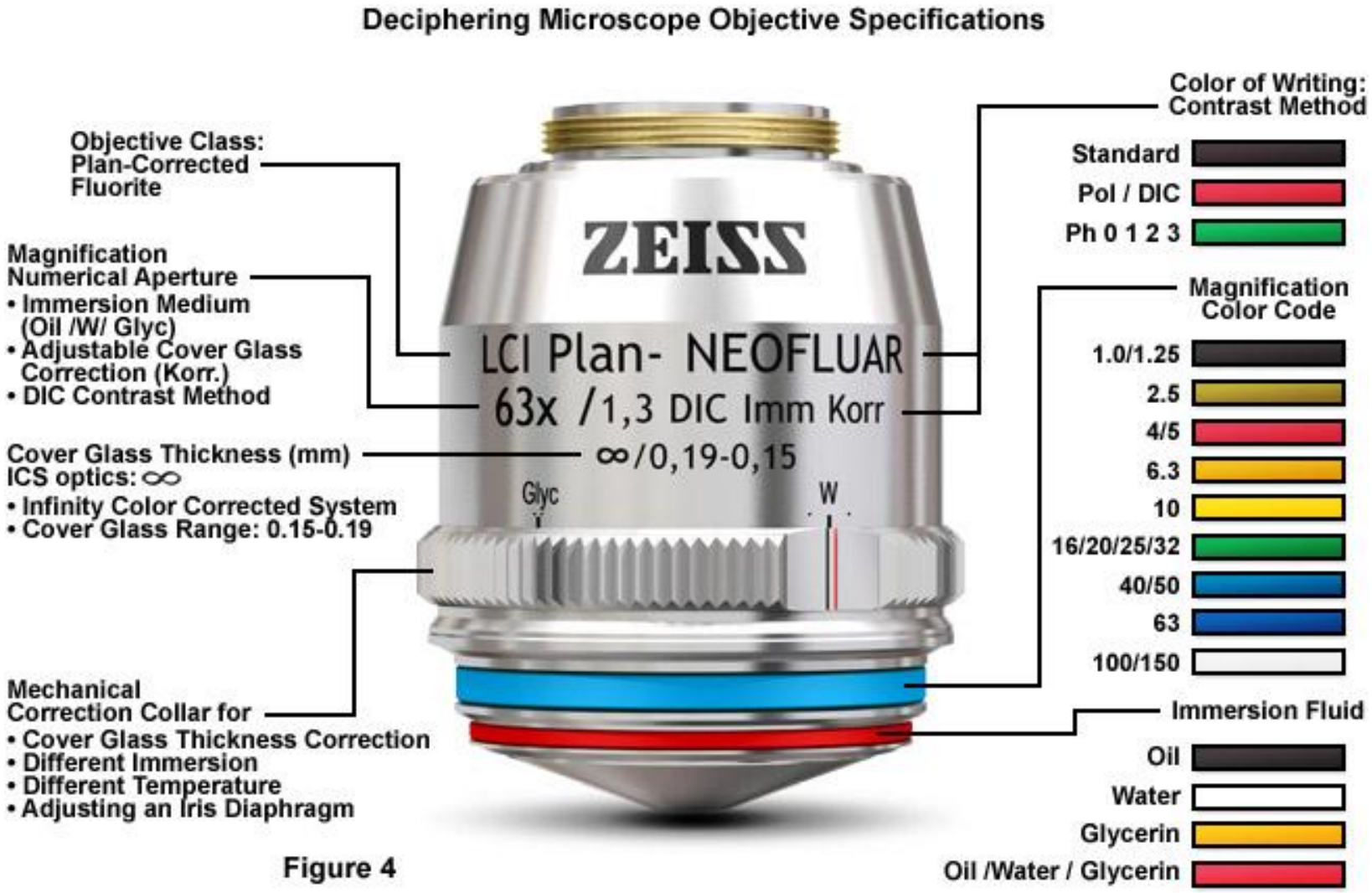


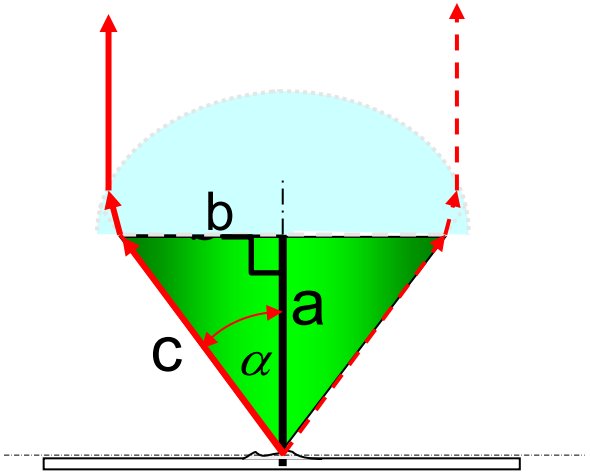
Figure 4

What matters more when choosing an objective?

Magnification

- 1x / 1.5x
- 2.5x
- 4x / 5x
- 6.3x
- 10x
- 16x/20x/25x/32x
- 40x / 50x
- 63x
- 100x / 150x

Numerical Aperture



$$\frac{b}{c} = \sin \alpha$$

$$NA = n \times \sin \alpha$$

n → Refractive Index

Effect of immersion oil on NA

$NA = n \times \sin \alpha$

Instrumentation for light microscopy

Microscope and its parts

Effect of immersion oil on NA

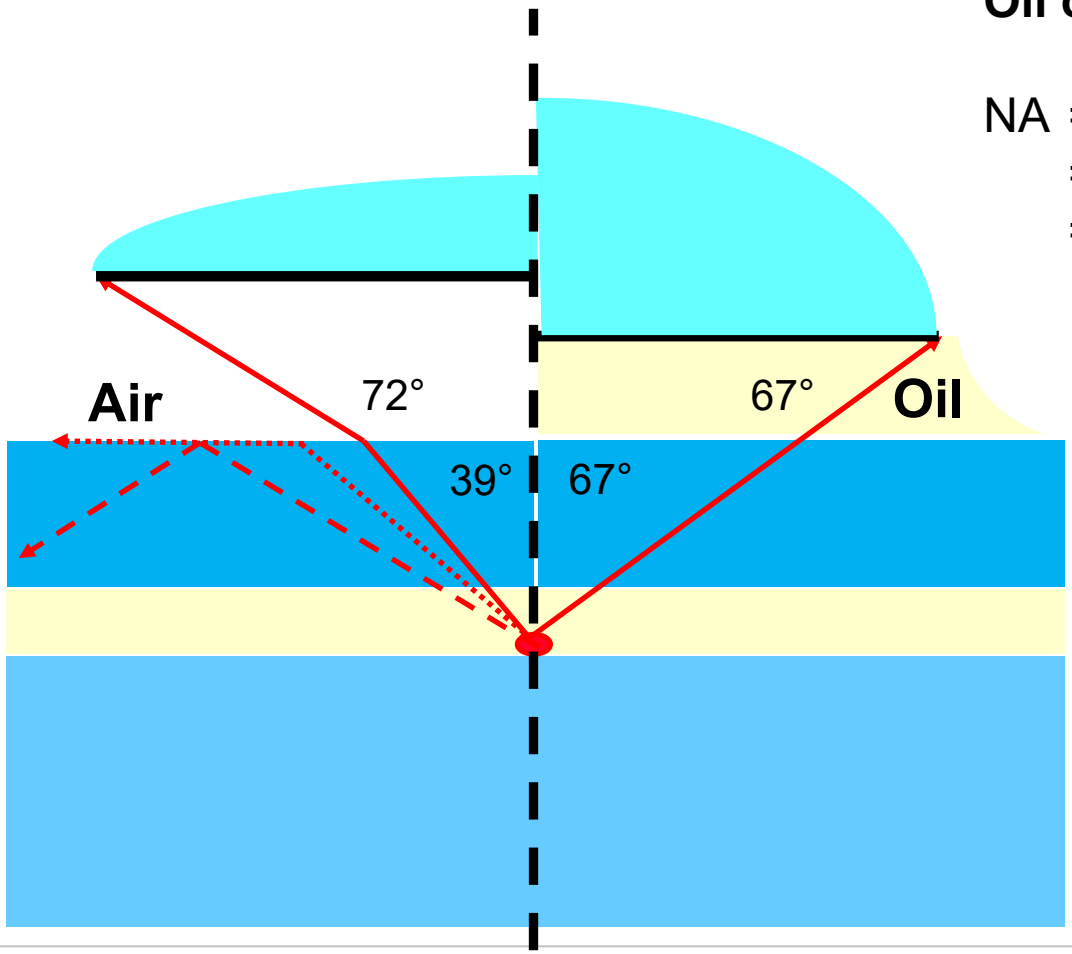
$NA = n \times \sin \alpha$

Dry objective

NA = 1 x sin 72°
= 1 x 0,95
= 0,95

Oil objective

NA = 1,515 x sin 67°
= 1,515 x 0,92
= 1,4



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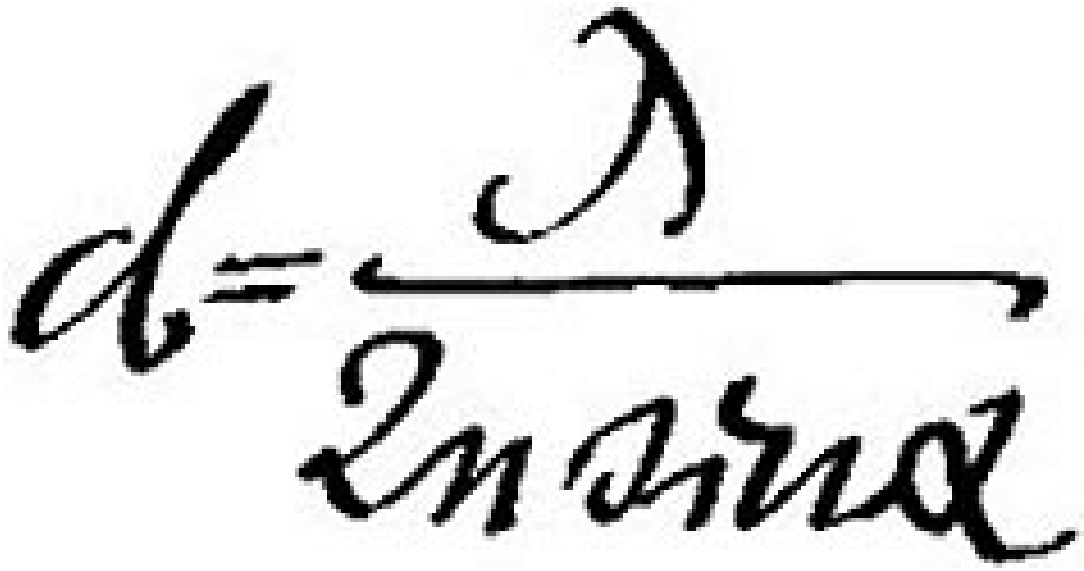
Instrumentation for light microscopy

Microscope and its parts



Ernst Abbe
(1840 - 1905)

Resolution is the minimum separation distance between two point objects that are clearly resolved.

$$d = \frac{\lambda}{2 \times NA}$$

$$NA = n \times \sin \alpha$$

n $\rightarrow$ Refractive Index

What matters more when choosing an objective?

Numerical Aperture

Resolution depends on NA

Light transmission of an objective depends on NA^2

Depth of field of an objective is (approximately) inversely proportional to NA^2

$$d = \frac{\lambda}{2 \times NA}$$

$$NA = n \times \sin \alpha$$

n $\rightarrow$ Refractive Index

Effect of immersion oil on NA

Possible combinations

Sample: fixed
in water (live cell)

Cover glass: no (1.000) or water dipping lens (1.330)
yes (1.515)

Immersion: no = air (1.000)
water (1.330)
silicon (1.406)
glycerol (1.456)
oil (1.515)

The relative refractive index of an optical medium 2 with respect to another reference medium 1 (n21) is given by the ratio of speed of light in medium 1 to that in medium 2. (wikipedia)

$$n_{21} = \frac{v_1}{v_2}$$

Effect of immersion oil on NA

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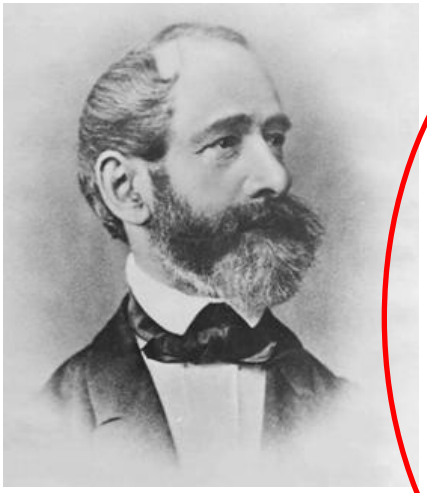
Microscopes

History of microscopy



Carl Friedrich Zeiss
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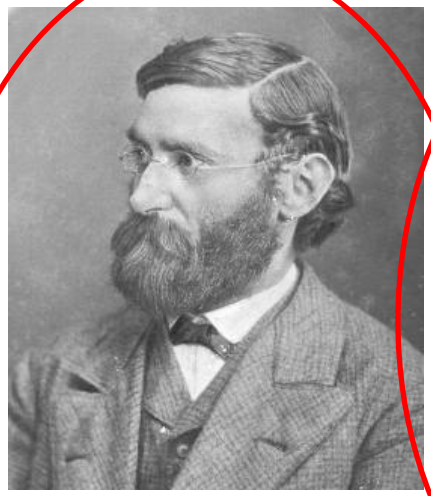

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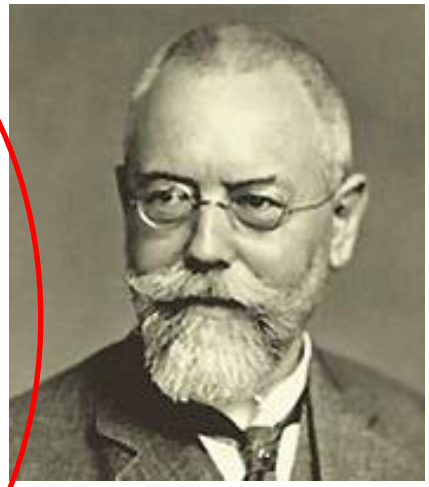

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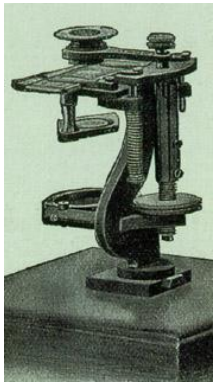

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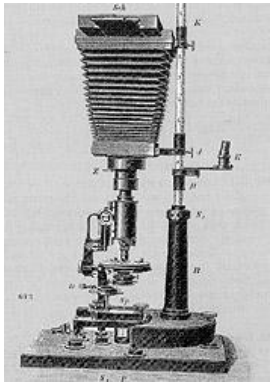


Instrumentation for light microscopy

Microscope and its parts

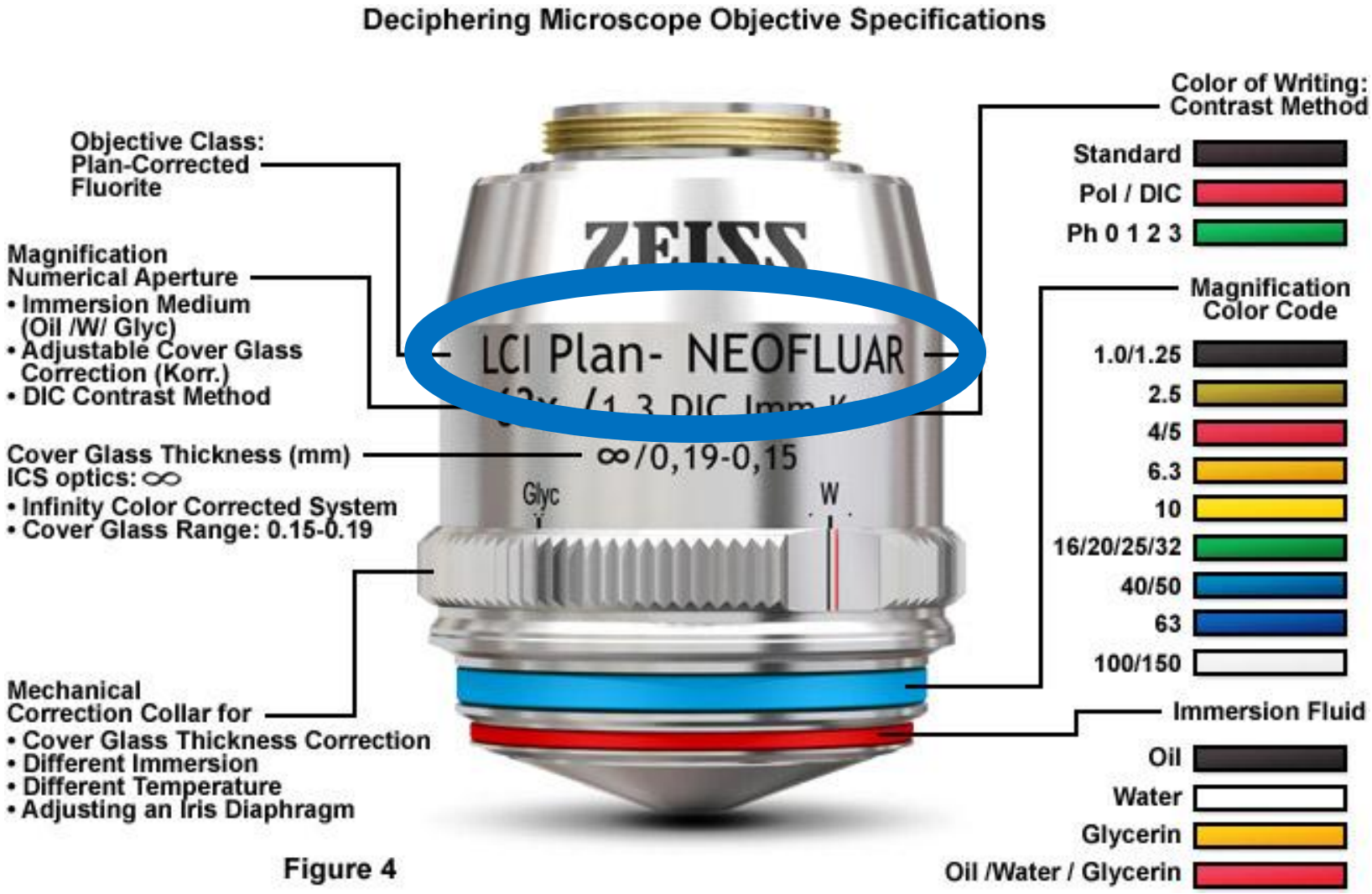


Figure 4

Instrumentation for light microscopy

Microscope and its parts

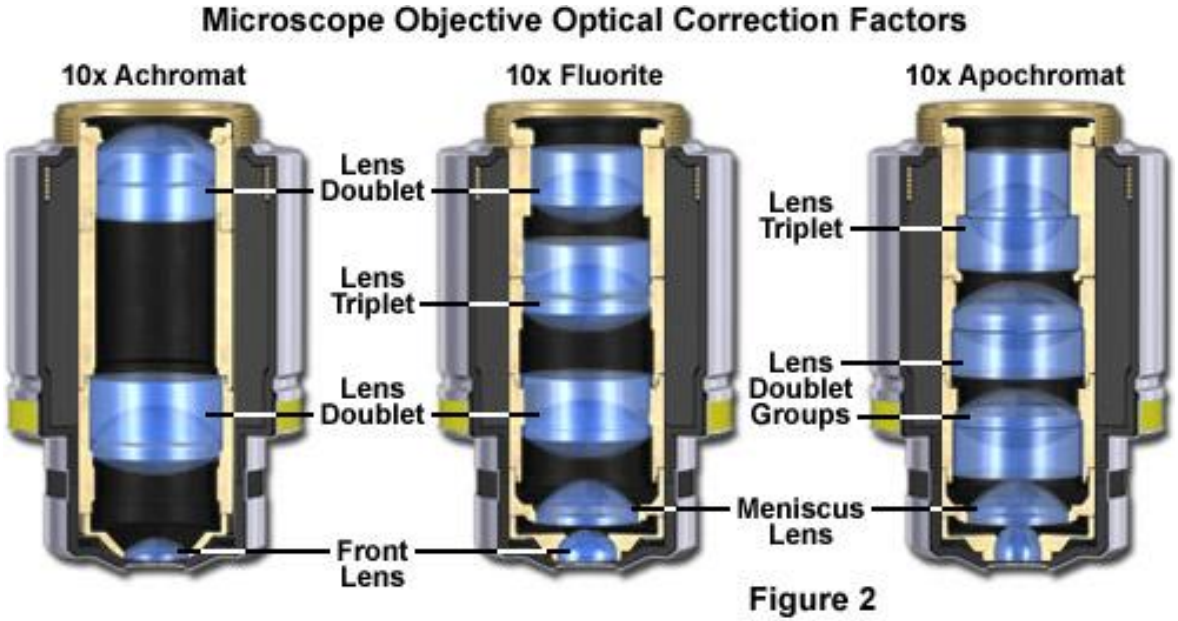


Figure 2

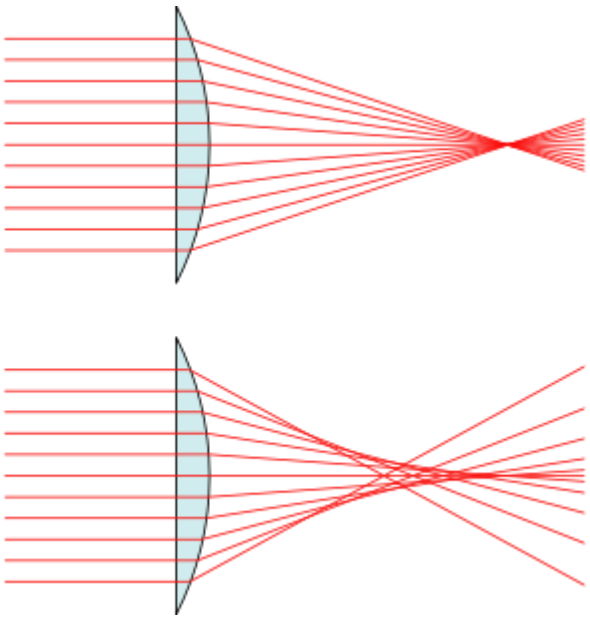
Microscope Objective Correction for Optical Aberration

Objective Specification	Spherical Aberration	Chromatic Aberration	Field Curvature
Achromat	1 Color	2 Colors	No
Plan Achromat	1 Color	2 Colors	Yes
Fluorite	2-3 Colors	2-3 Colors	No
Plan Fluorite	3-4 Colors	2-4 Colors	Yes
Plan Apochromat	3-4 Colors	4-5 Colors	Yes

Instrumentation for light microscopy

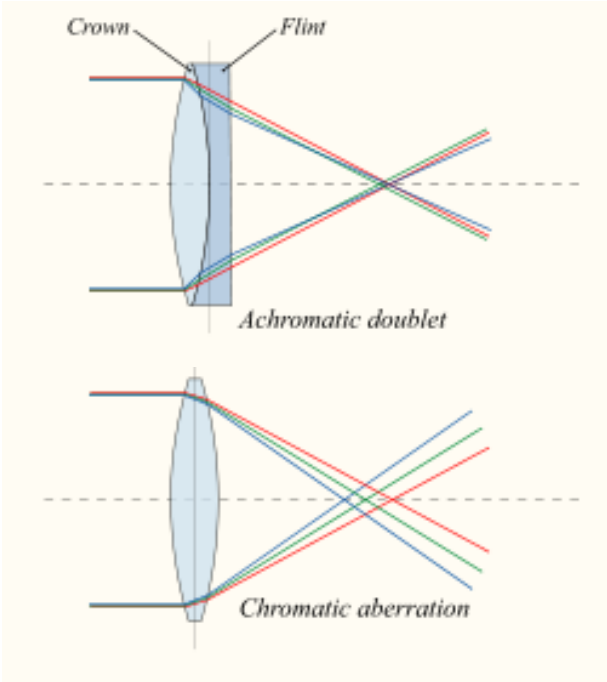
Microscope and its parts

Spherical abberation



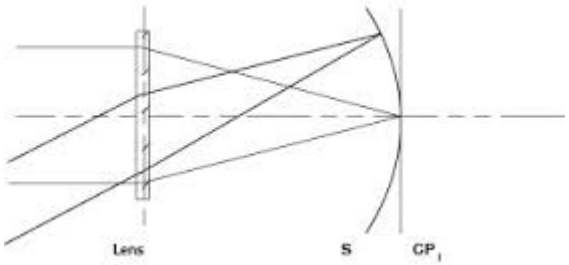
failure of a lens to focus all rays passing the glass in different places to the same point

Chromatic abberation



failure of a lens to focus all colors to the same convergence point

Field curvature



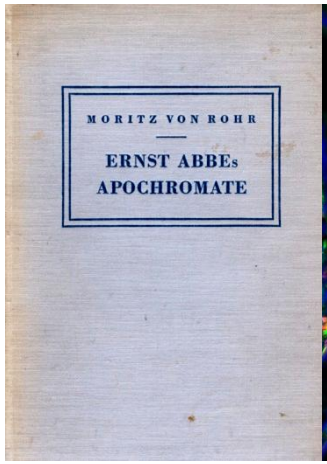
focusing a flat surface as a spherical surface

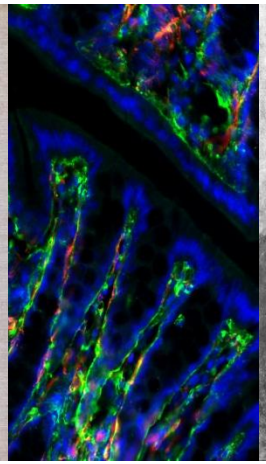
Instrumentation for light microscopy

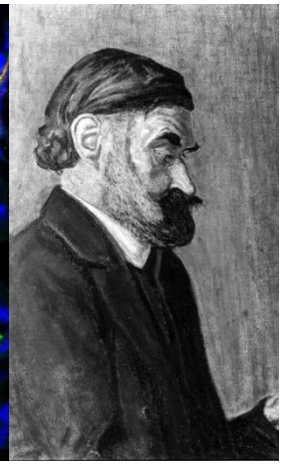
Microscope and its parts

CARL ZEISS APOCHROMATs are fully color corrected for
no longer only 3 – 4 spectral lines,
but **for a full SPECTRAL range!**

A bit of history….
The apochromatic correction was invented by Prof. Ernst Abbe in 1886 at CARL ZEISS







Instrumentation for light microscopy

Microscope and its parts

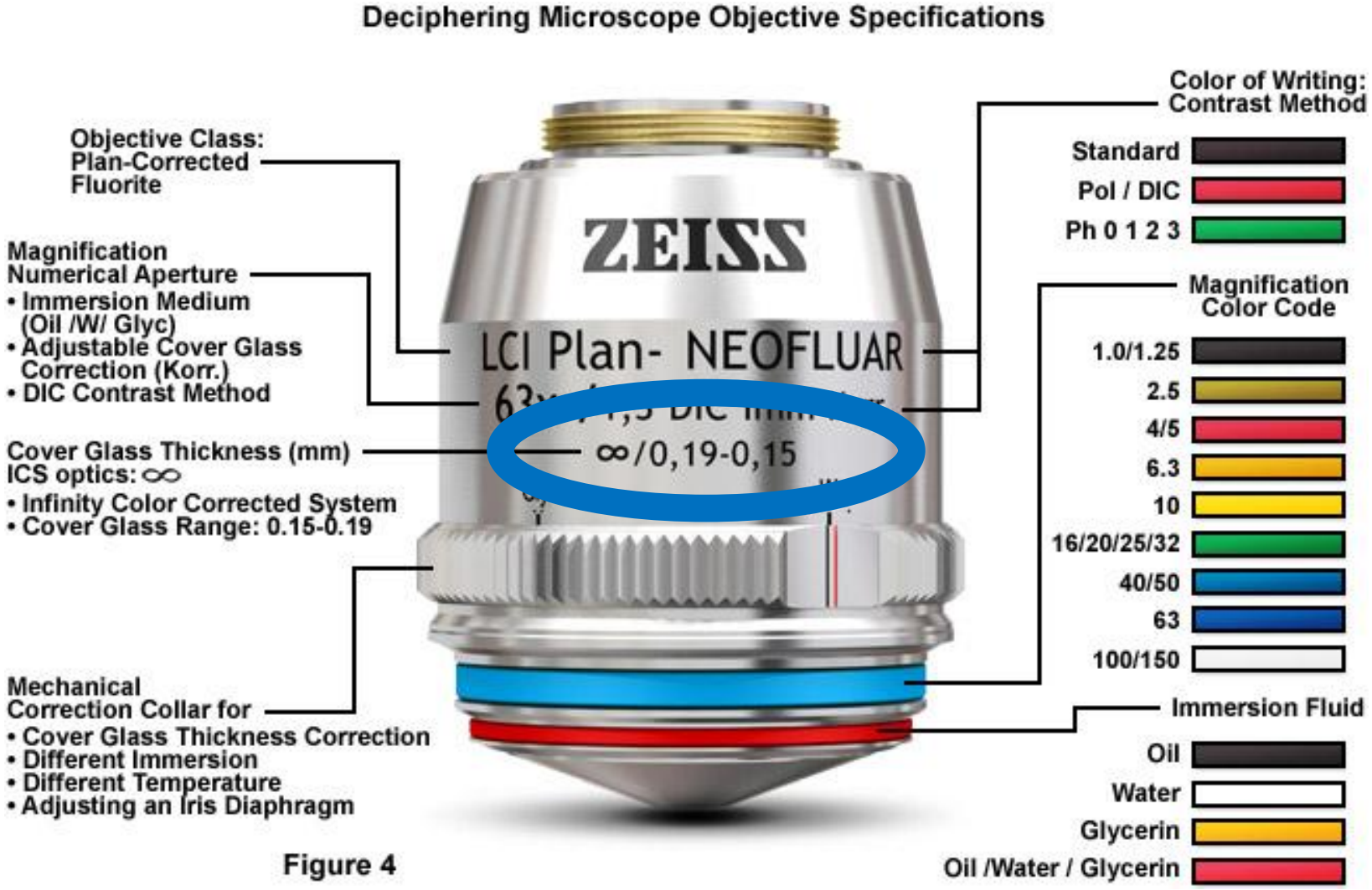


Figure 4

Instrumentation for light microscopy

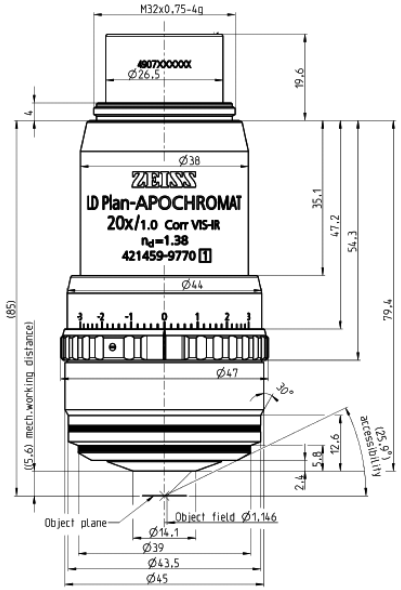
Microscope and its parts

OBJECTIVES

- Dry objectives
- Objectives corrected for 0,17 mm cover glass
- Objectives with correction collar
- Immersion objectives: oil, water, glycerol, silicon oil
- Dipping objectives
  (electrophysiology applications)

- Life cell imaging objectives
  (correction for different temperatures)

- Special objectives
  (clearing solutions etc.)



Instrumentation for light microscopy

Microscope and its parts

OBJECTIVES

http://www.zeiss.com/microscopy/en_de/products/microscope-components/objectives.html

Or search at
www.google.com
For
„ZEISS objectives“

Instrumentation for light microscopy

Microscope and its parts

OBJECTIVES

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For
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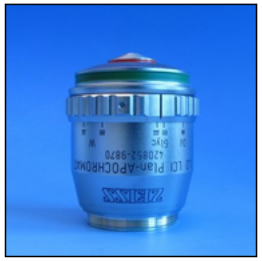
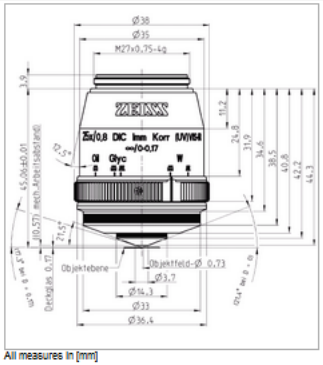
→ Transmittance curve	Objective LD LCI Plan-Apochromat 25x/0.8 Imm Corr DIC M27
	420852-9870-000
	Basket
Price	→ Price
Magnification	25x
Numerical Aperture	0.8
Working Distance [mm]	0.57 at cover glass 0.17
Coverglass Thickness [mm]	0-0.17
Thread Type	M27x0.75
Immersion	Oil, Glycerine and Water
Field of View [mm]	18
Parfocal Length [mm]	45.06
Long Distance (LD)	■
Correction Ring (Korr)	■
Iris (Iris)	
Optical System	Infinity Color Corrected System (ICS)
Flatness	★★★★★
Color Correction	★★★★★
Biomedical Applications	
Fluorescence	■
- Multichannel	★★★★★
- Ultraviolet Transmission	★★★
- Infra Red Transmission	★★★★
BrightField (H)	■
Differential Interference Contrast (DIC)	★★★★★
High Contrast DIC (HC DIC)	
PlasDIC Contrast	
Phase Contrast (PH)	
VAREL Contrast	
Hoffman Modulation Contrast (HMC)	
Polarization Contrast (POL)	
Materials (Reflected Light) Applications	
BrightField (H)	
BrightField/DarkField (HD)	
Reflected Light DIC (RL DIC)	
High Contrast DIC (HC DIC)	
DIC with circular polarized light (C-DIC)	
Total Interference Contrast (TIC)	
Polarization Contrast (POL)	
Options	
Confocal Microscopy	■
- Ultra Violet	★★★★
- VIS (visible light)	★★★★★
NLO-IR / 2 Photon	★★
Total Internal Reflection Fluorescence (TIRF)	
ApoTome	■
Microdissection	

Objective LD LCI Plan-Apochromat 25x/0.8 Imm Corr DIC M27



Objective LD LCI Plan-Apochromat 25x/0.8 Imm Corr DIC M27 for oil, water or glycerine immersion (D=0-0.17mm) (WD=0.57mm at D=0.17mm) incl. immersol 518 F, oiler 20 ml

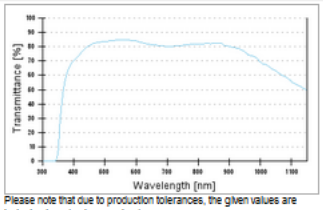
Mechanical Dimensions



All measures in [mm]

mech. Arbeitsabstand = mechanical working distance
Deckglas = cover glass
Objektebene = object plane
Objektfeld = object field
Ausleuchtung = illumination
Probenzugänglichkeit = specimen accessibility

Transmittance curve



Please note that due to production tolerances, the given values are typical only and not guaranteed.

DIC sliders

DIC slider LCI PN 25x/0.8 II
Item Number: 426947-0000-000
DIC slider LCI PN 25x/0.8 II
→ Price | Compatibility | Basket

Other Accessories

Adapter 30mm with optics and DIC slider mount
Item Number: 424516-9040-000
Adapter 30mm with optics and DIC slider mount for adaptation of objectives M27 with parfocal length of 45 mm
Note: This item is no longer available.
→ Price | Compatibility

Adapter 30mm with optics and DIC slider mount
Item Number: 424516-9041-000
Adapter 30mm with optics and DIC slider mount for adaptation of objectives M27 with parfocal length of 45mm
→ Price | Compatibility | Basket

Objective heater 33/38 S1 (D)
Item Number: 411860-9067-000
Objective heater 33/38 S1 (D) - with oil discharge channel - clamping via front dia. 33 mm - max. objective dia. 38 mm - temperatu ...
→ Price | Compatibility | Basket

Transmission grid L1 ApoTome for Axio Imager
Item Number: 423664-0000-000
Transmission grid L1 ApoTome for Axio Imager
Note: This item is no longer available.
→ Price | Compatibility

Transmission grid PL ApoTome
Item Number: 000000-1151-003
Transmission grid PL ApoTome for Axioplan 2
Note: This item is no longer available.
→ Price | Compatibility

Transmission grid VL ApoTome
Item Number: 000000-1151-004
Transmission grid VL ApoTome for Axio Observer and Axiovert 200
Note: This item is no longer available.
→ Price | Compatibility

Immersion

Box of lens cleaning paper (300 sheets)
Item Number: 462975-0000-000
Box of lens cleaning paper (300 sheets)
→ Price | Compatibility | Basket

Immersion glycerine ne=1.455, bottle 10 ml
Item Number: 462959-0000-000

Instrumentation for light microscopy

Microscope and its parts

Which objective will you choose?

63x/1,4 oil immersion

100x/1,4 oil immersion

Instrumentation for light microscopy

Microscope and its parts

Which objective will you choose?

63x/1,4 oil immersion

100x/1,4 oil immersion

Instrumentation for light microscopy

Microscope and its parts

Which objective will you choose?

40x/1,4 oil immersion

63x/1,4 oil immersion

100x/1,4 oil immersion

Instrumentation for light microscopy

Microscope and its parts

Which objective will you choose?

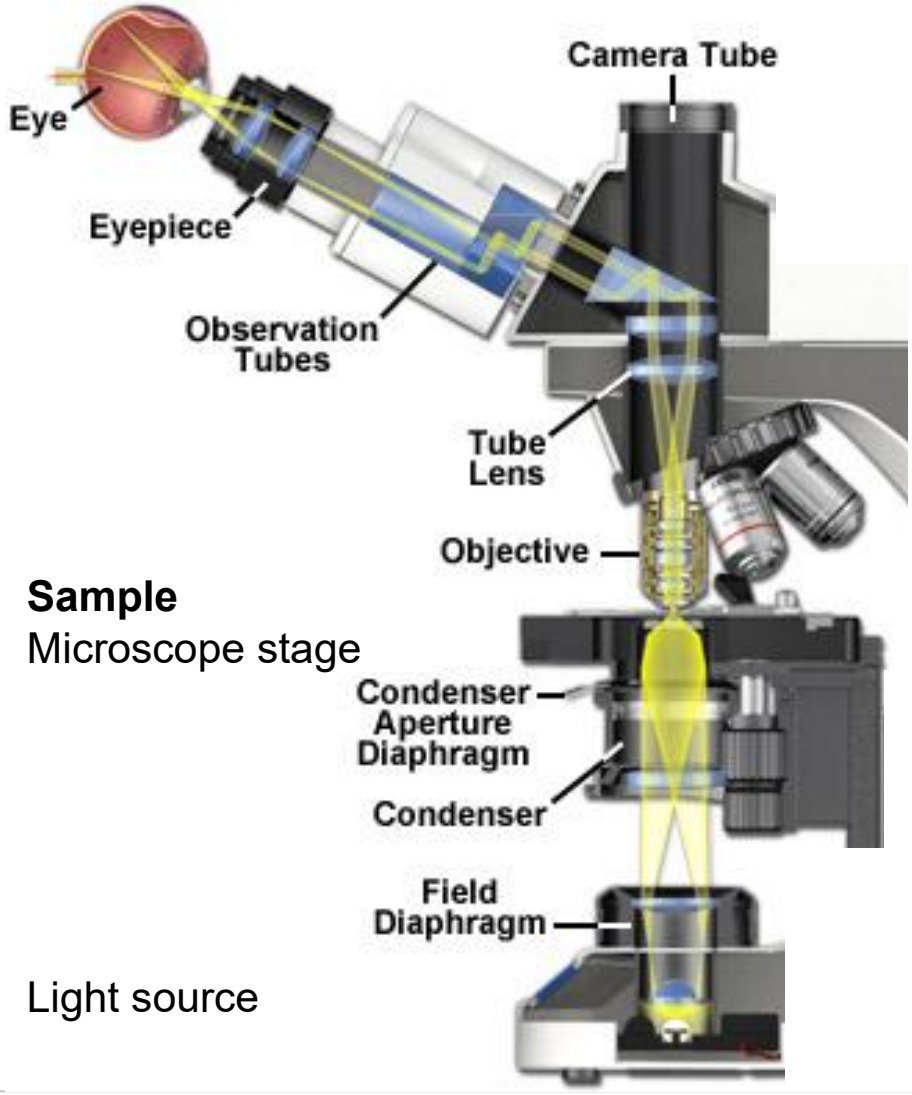
40x/1,4 oil immersion
(WD=0.13mm)

63x/1,4 oil immersion
(WD=0.19mm)

100x/1,4 oil immersion
(WD=0.17mm)

Instrumentation for light microscopy

Microscope and its parts



Sample
Microscope stage

Light source

TL

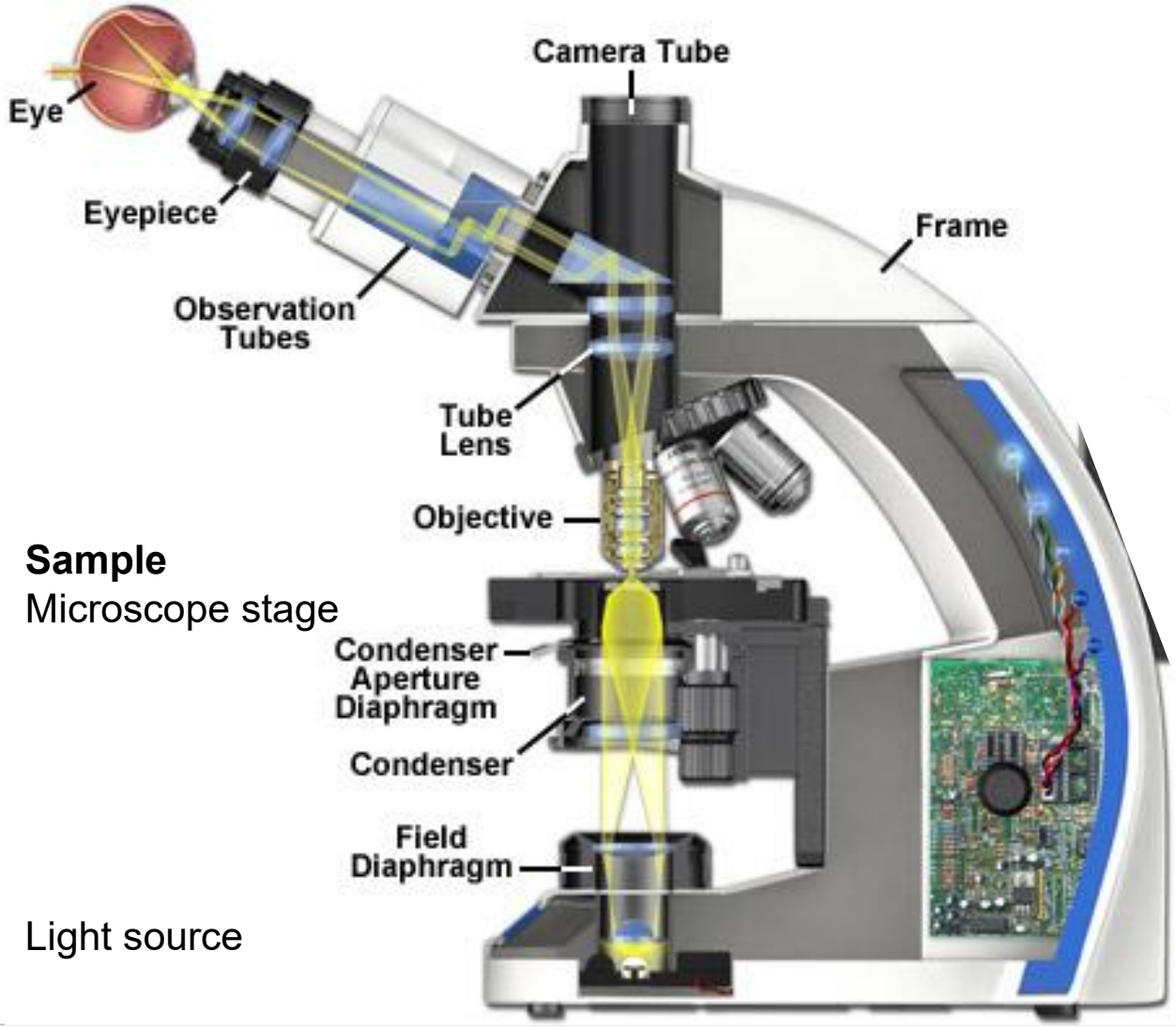
Tube
- Standard
- Ergonomic tiltable
- Photo tube

Eyepieces
- 10x, 16, 25x, other?
- Field of View
- Correction for diopters

Camera port
- 50/50%, 0/100%
- 20/80%
- Camera adapter (1x, 0,63x, ???)

Instrumentation for light microscopy

Microscope and its parts



Sample
Microscope stage

Light source

TL

Frame
- Robust metal frame
- Ergonomic

Electronics
- Programmable buttons
- TFT display
- Remote control
- Motorized parts
- Connection the PC
- Synchronization
- Trigger IN / OUT
- **SAFETY**
- other

Instrumentation for light microscopy

Microscope and its parts

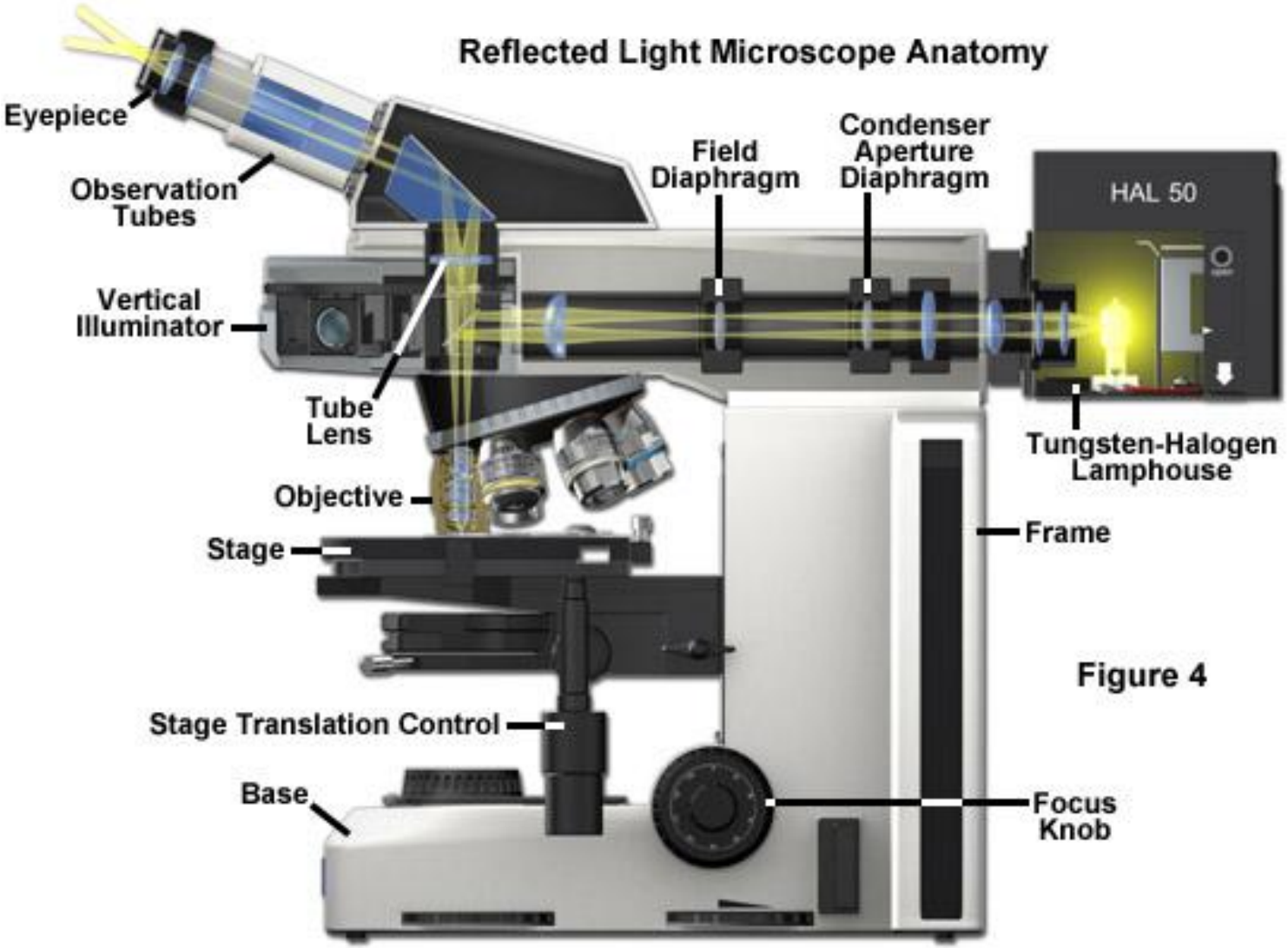


Figure 4

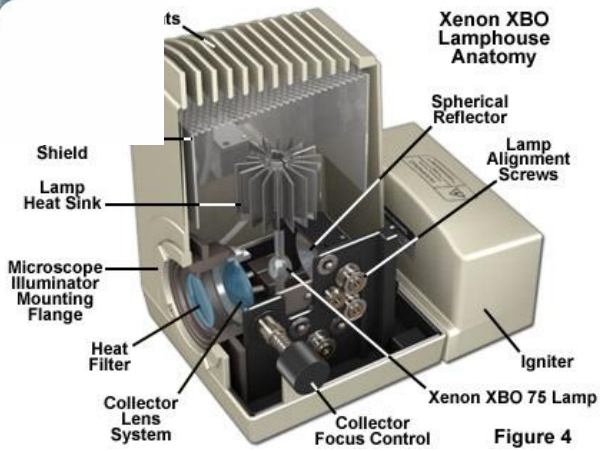
RL

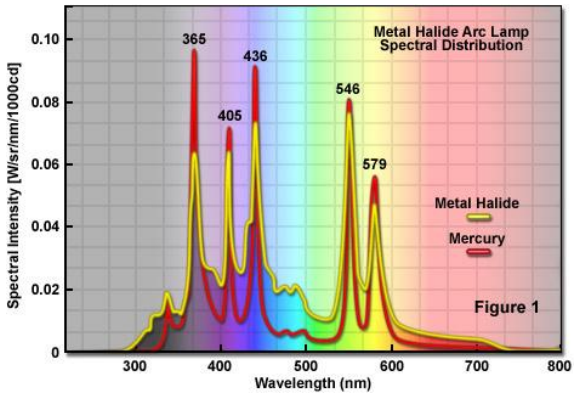
Instrumentation for light microscopy

Microscope and its parts

Parameters to consider:

- Needed power
- Color / Spectrum
- Life time
- Speed





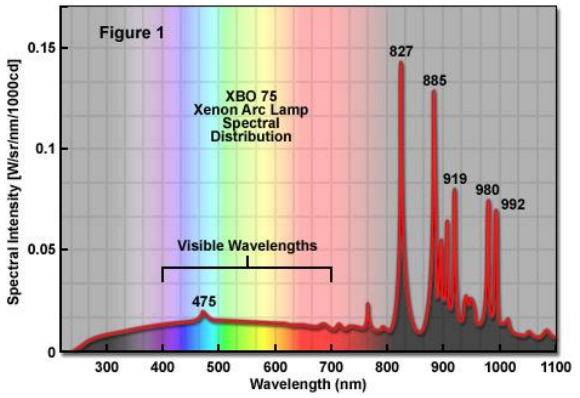
Light sources

- TL: transmitted light

- halogen
- LED

- RL: reflected light

- halogen / LED
- mercury burner - fluorescence
- metal halide - fluorescence
- Xenon - fluorescence
- laser - fluorescence LSM



Instrumentation for light microscopy

Microscope and its parts

Parameters to consider:

- Needed power
- Color / Spectrum
- Life time
- Speed

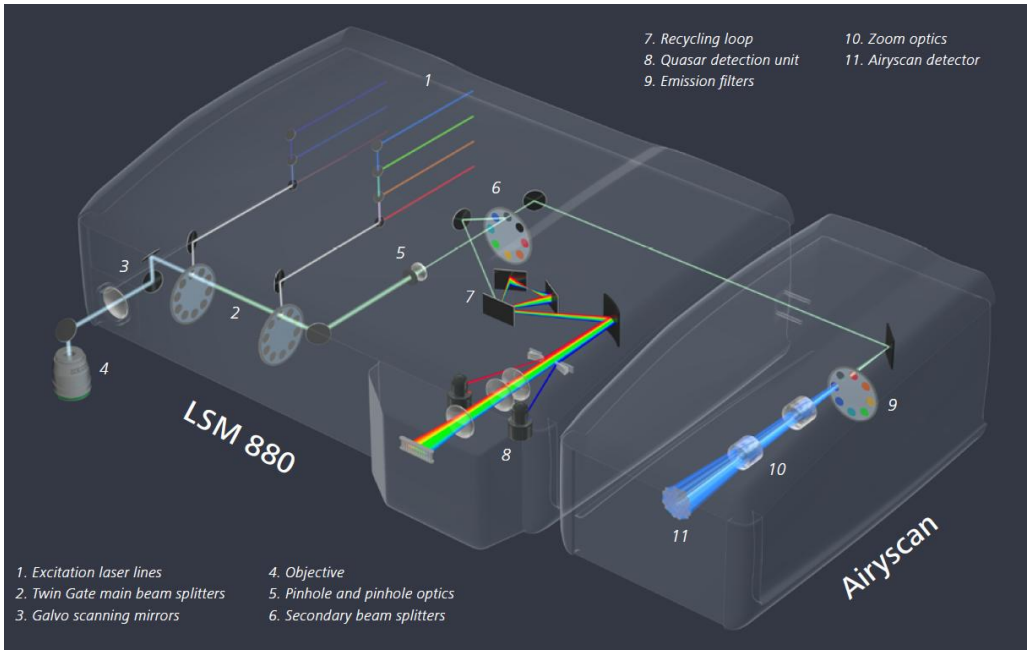
Light sources

- TL: transmitted light

- halogen
- LED

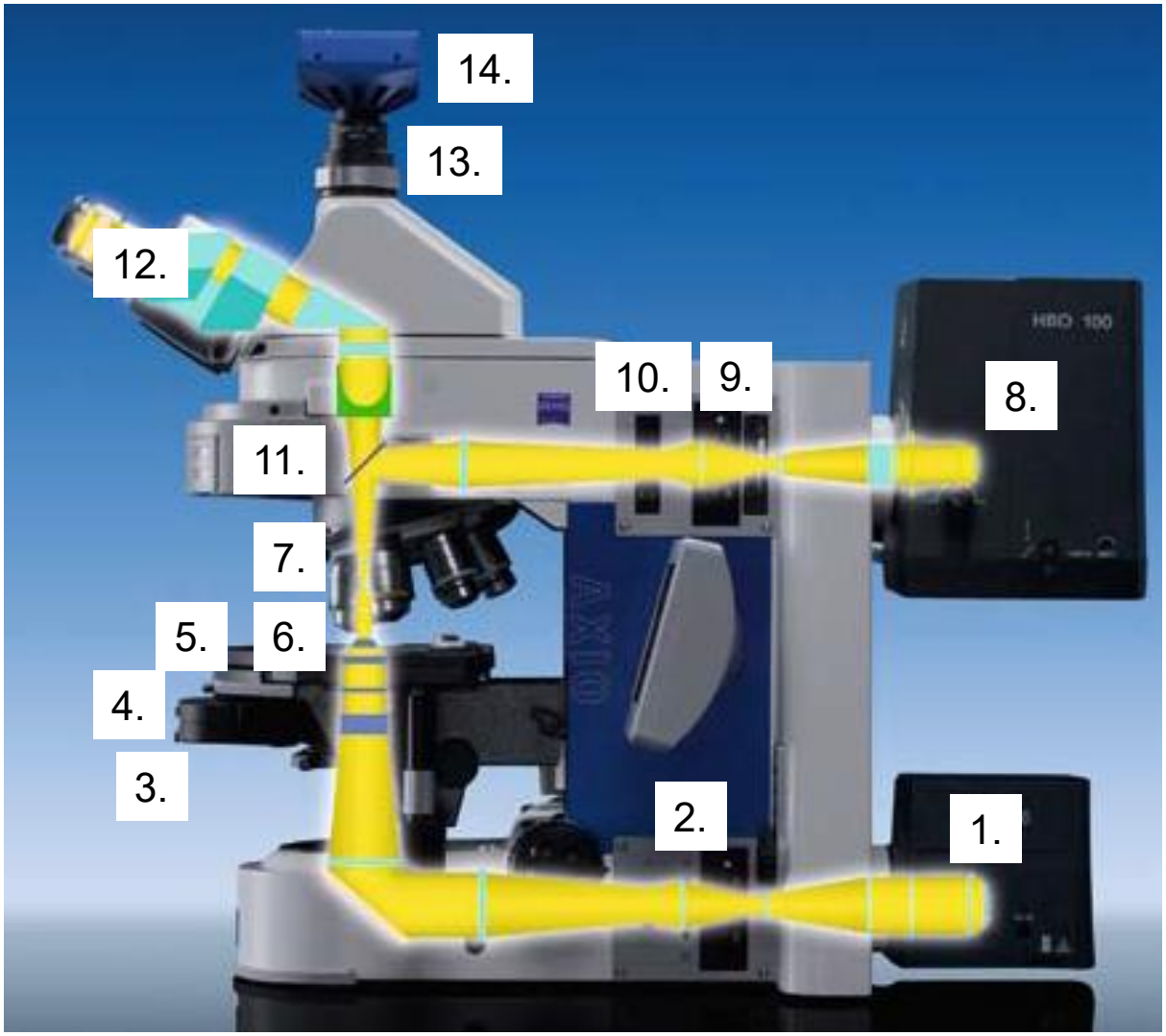
- RL: reflected light

- halogen / LED
- mercury burner - fluorescence
- metal halide - fluorescence
- Xenon - fluorescence
- laser - fluorescence LSM



Instrumentation for light microscopy

Microscope and its parts



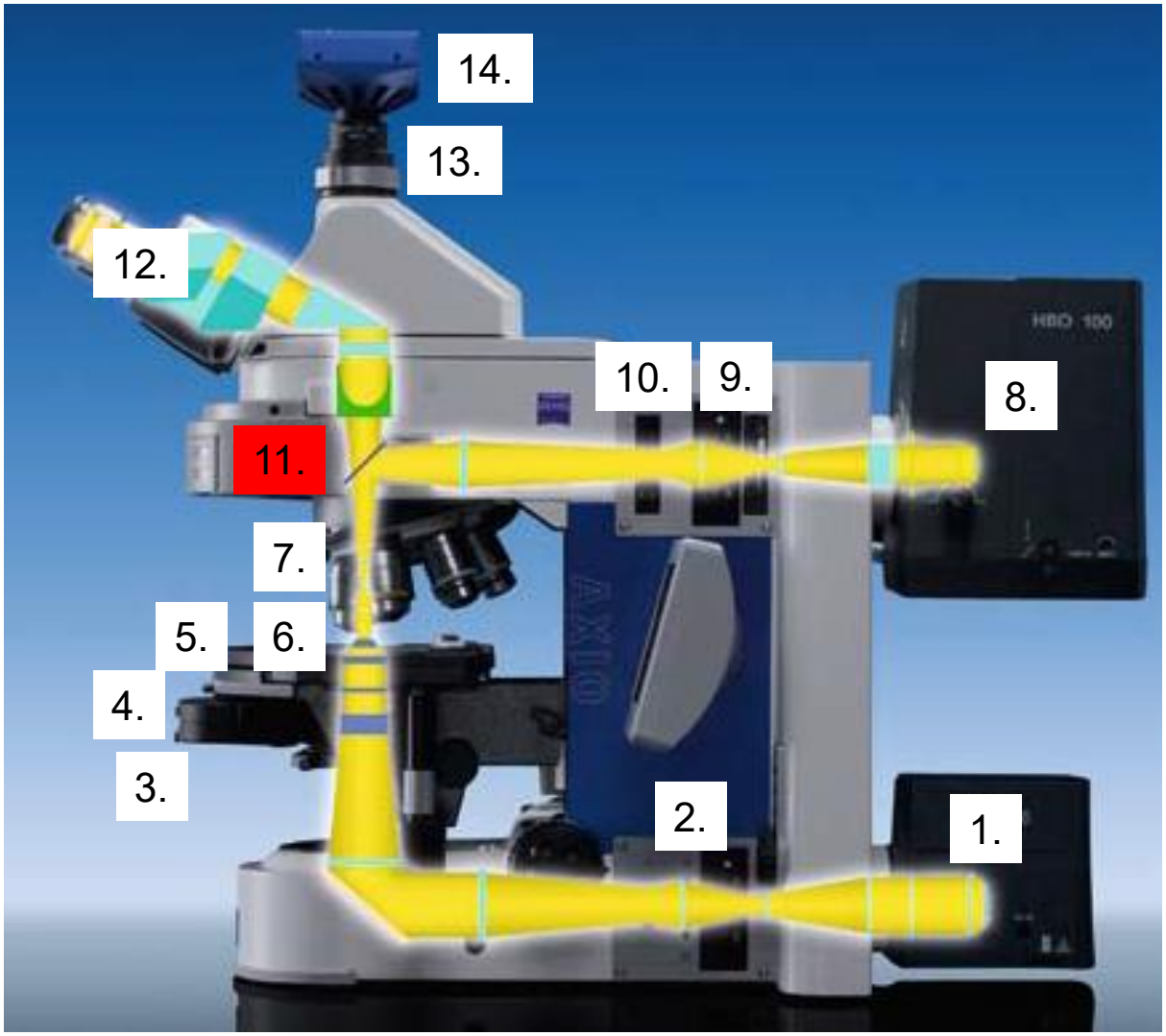
1. Transmitted light source
2. TL field diaphragm
3. Condenser
4. TL aperture diaphragm
5. Stage
6. Specimen
7. Objective

8. Reflected light source
9. RL aperture diaphragm
10. RL field diaphragm
11. Reflector

12. Ocular
13. Camera adapter
14. Camera

Instrumentation for light microscopy

Microscope and its parts



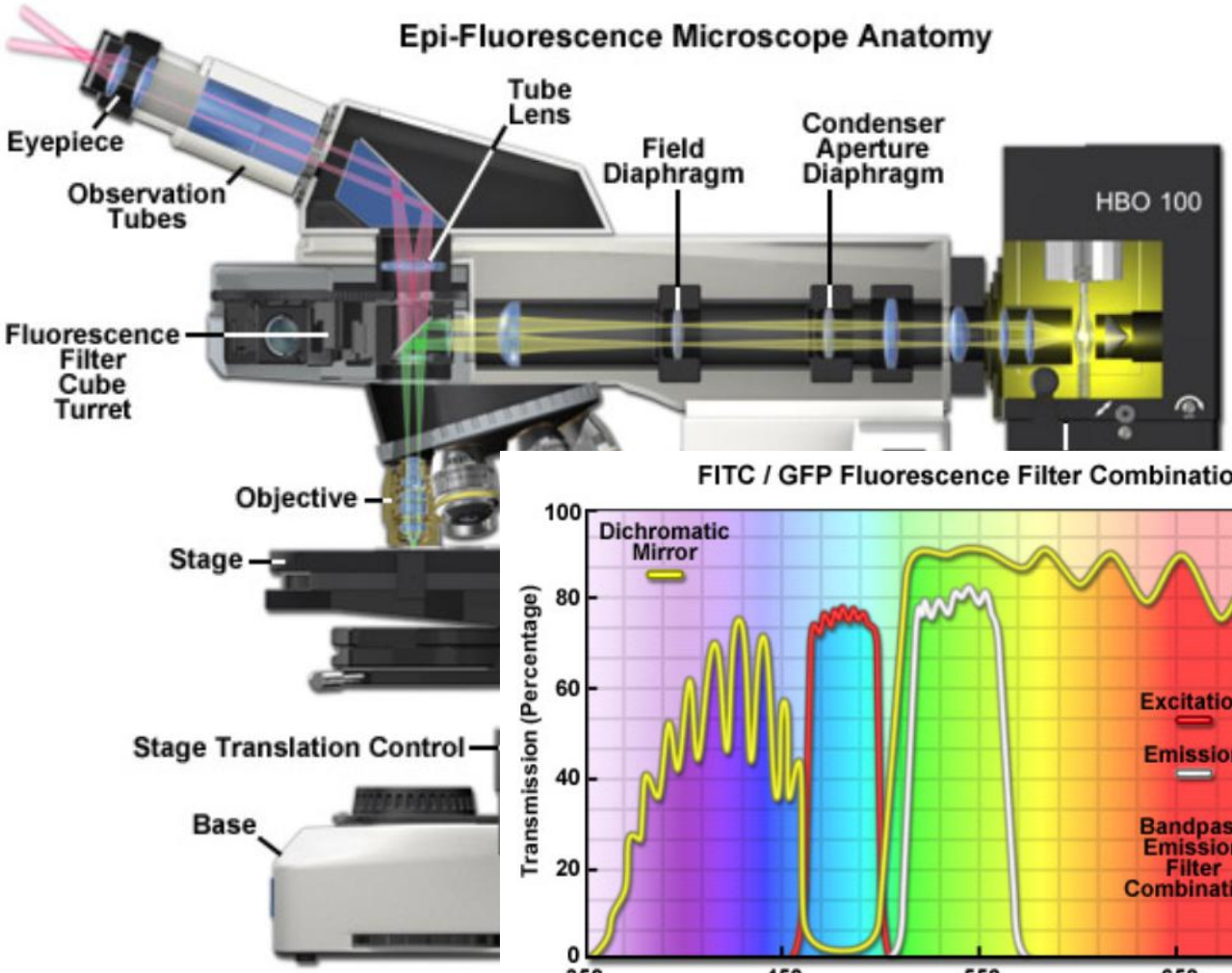
1. Transmitted light source
2. TL field diaphragm
3. Condenser
4. TL aperture diaphragm
5. Stage
6. Specimen
7. Objective

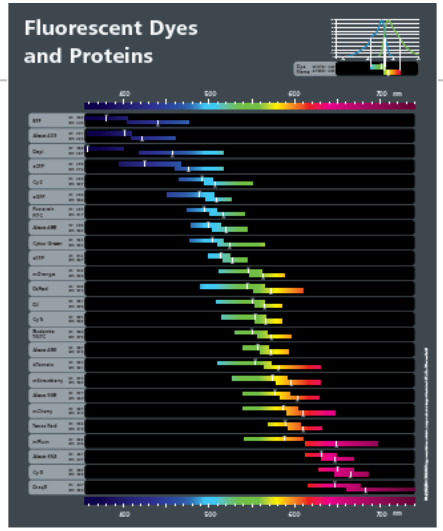
8. Reflected light source
9. RL aperture diaphragm
10. RL field diaphragm
11. **Reflector**

12. Ocular
13. Camera adapter
14. Camera

Instrumentation for light microscopy

Microscope and its parts





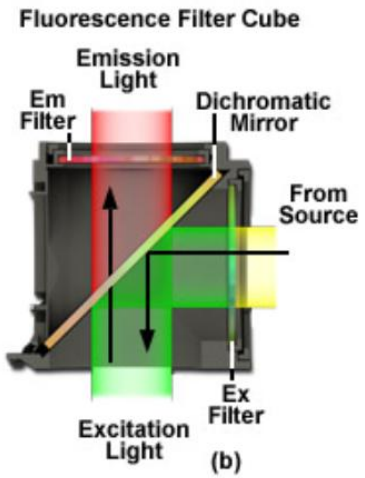
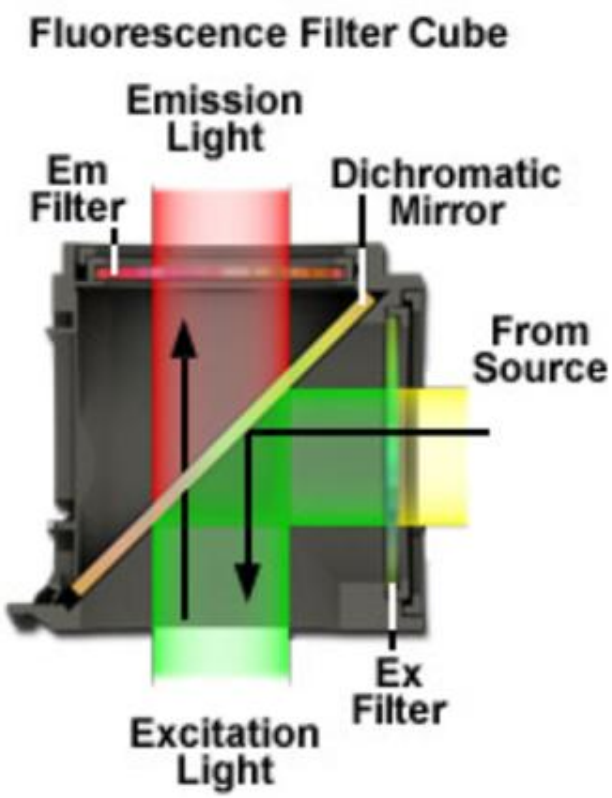


Figure 4

Instrumentation for light microscopy

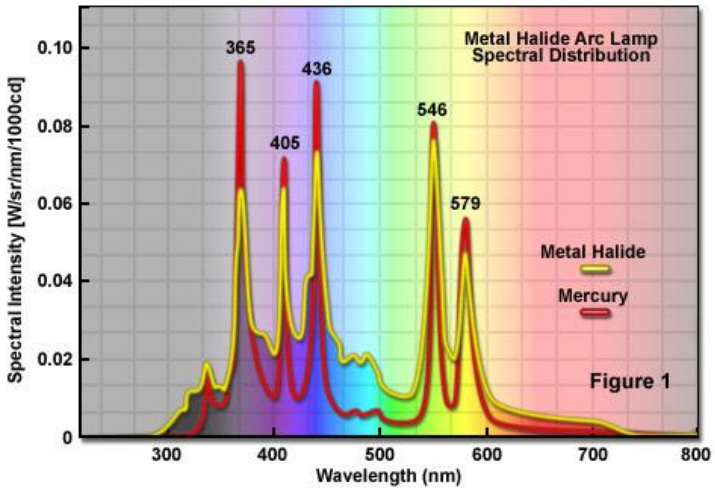
Microscope and its parts



Example

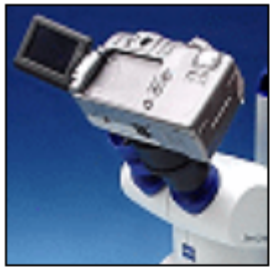
	Filter set 38 HE	Filter set 09
Excitation filter	BP 470/40	450-490
Dichroic beam splitter	FT 495	510
Emission filter	BP 525/50	LP 515

BP = Band Pass 470/40 = light between 450 – 490 nm
LP = Long Pass LP 515 = light above 515 nm
SP = Short pass SP 515 = light bellow 515

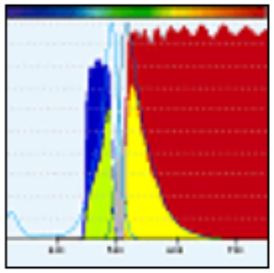


Instrumentation for light microscopy

Microscope and its parts



Camera Assistant
Here you find appropriate adaptors for Micro Photography



Filter Assistant
Here you find fluorochromes and proper filter sets for your fluorencence microscope

Objective Assistant
Here you find objectives for your favourite Contrast Methods

http://www.zeiss.com/microscopy/en_de/products/microscope-components/filtersets-fluorescent-dyes.html

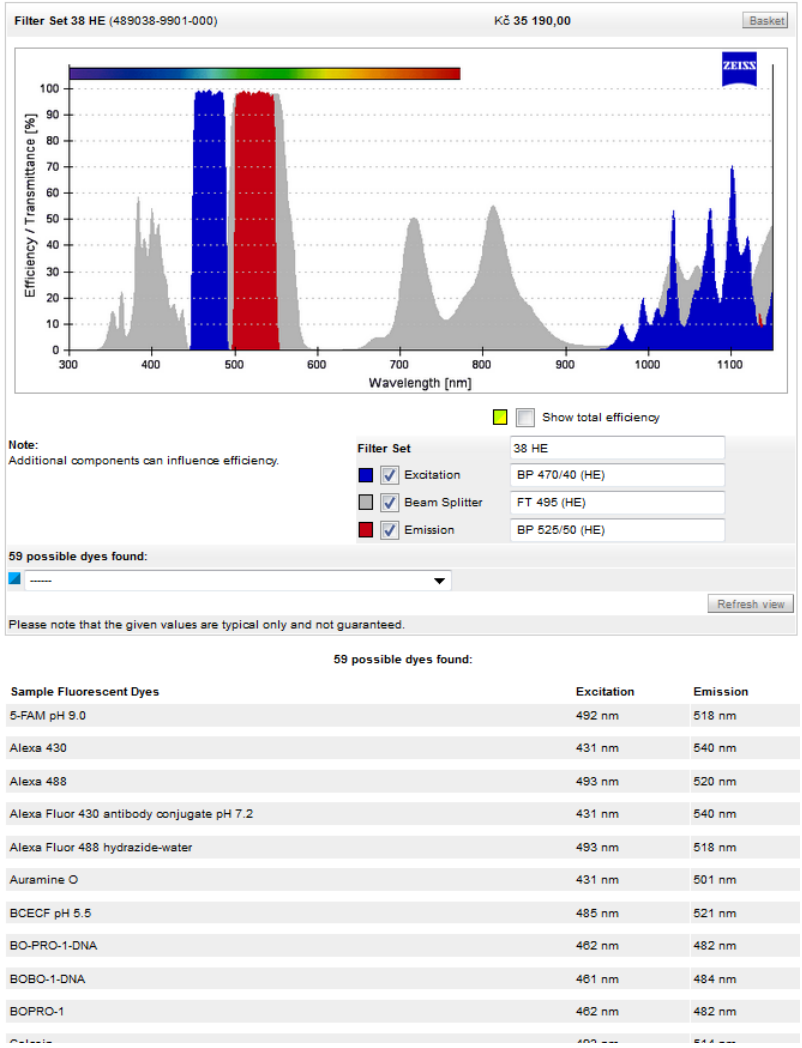
Or search at
www.google.com
for
„ZEISS filtersets“

Fluorescence Dye and Filter Database

- Overview Dyes
- Overview Filter Sets
- Interactive Fluorescence Dye and Filter Database
- Frequently Asked Questions (FAQ)

Instrumentation for light microscopy

Microscope and its parts

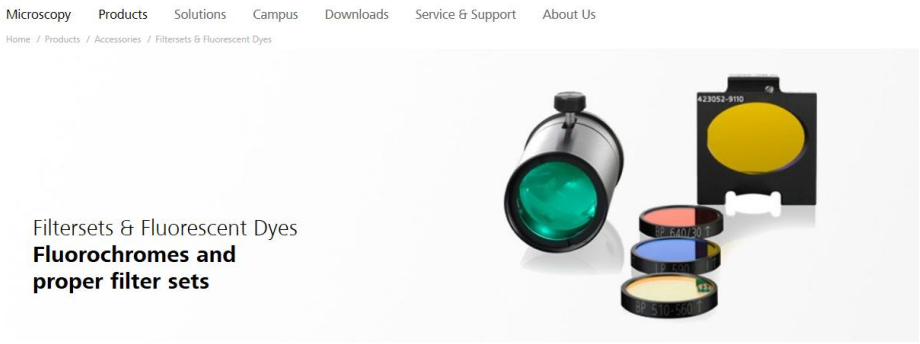


59 possible dyes found:

Sample Fluorescent Dyes	Excitation	Emission
5-FAM pH 9.0	492 nm	518 nm
Alexa 430	431 nm	540 nm
Alexa 488	493 nm	520 nm
Alexa Fluor 430 antibody conjugate pH 7.2	431 nm	540 nm
Alexa Fluor 488 hydrazide-water	493 nm	518 nm
Auramine O	431 nm	501 nm
BCECF pH 5.5	485 nm	521 nm
BO-PRO-1-DNA	462 nm	482 nm
BOBO-1-DNA	461 nm	484 nm
BOPRO-1	462 nm	482 nm

http://www.zeiss.com/microscopy/en_de/products/microscope-components/filtersets-fluorescent-dyes.html

Or search at
www.google.com
for
„ZEISS filtersets“



Fluorochromes and Proper Filter Sets for Your Fluorescence Microscope

Visit our online shop to learn more about fluorescent dyes and filter sets for applications in microscopy.

› More

Ask your ZEISS › contact about Filtersets & Fluorescent Dyes now!

Instrumentation for light microscopy

Microscope and its parts

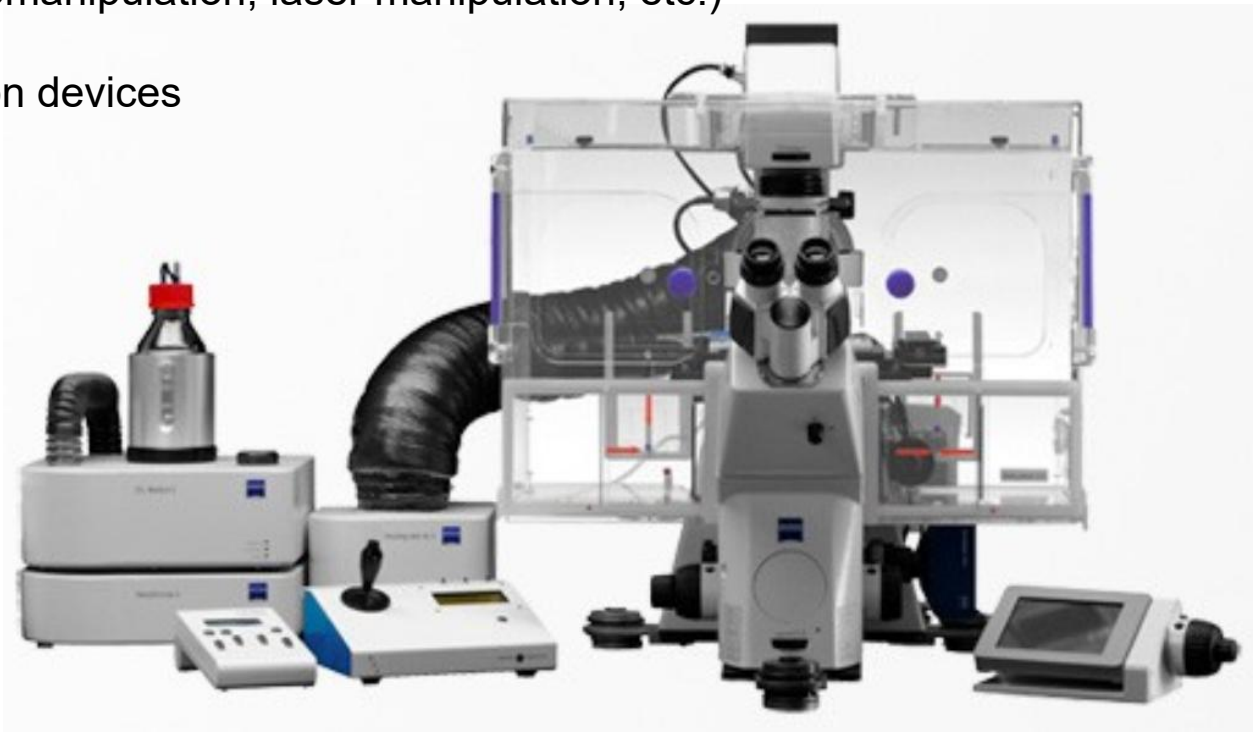
Other hardware parts

- Polarization: polarizer / analyzer
- Contrasting methods:
  (phase contrast, modulation contrast, Differencial interference contrast, etc)
- Color filters
- Structure illumination
- Manipulation devices (micromanipulation, laser manipulation, etc.)

- Life cell imaging = incubation devices
  - temperature control
  - pH control (*via* CO_2)
  - O2 control (*via* N_2)
  - humidifier

- Antivibration table

- Other



Instrumentation for light microscopy

Microscope and its parts

Common parts
- microscope body
- condenser
- stage
- objectives
- oculars
- other optics
- inputs / outputs
- light sources

Microscope design
- Stereo microscope
  - Greenough design
  - telescopic design
- zoom microscopes
- upright microscopes
- inverted microscopes
- Lightsheet microscopes
- other type of microscopes

Light sources
- transmitted light
  - halogen
  - LED
- reflected light
  - halogen / LED
  - mercury burner
  - metal halide
  - Xenon
  - laser

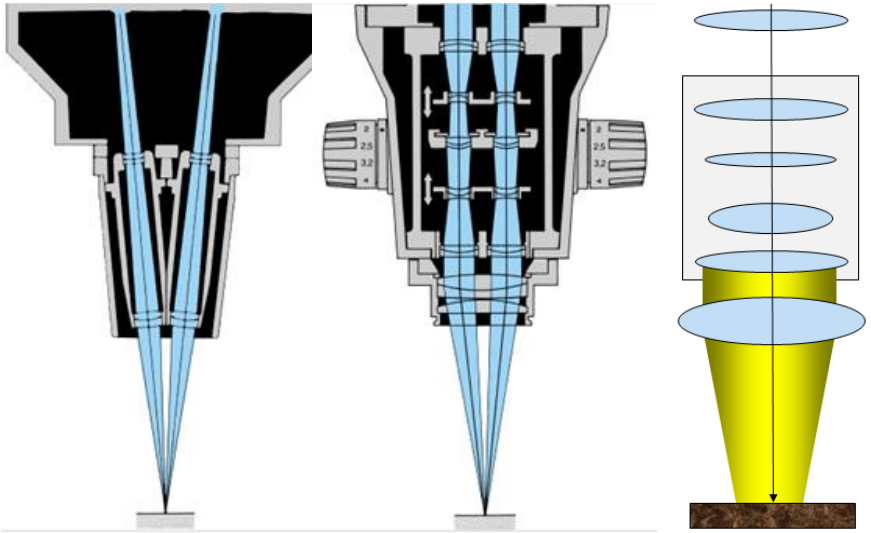
Detectors
- types of detectors
  - eyes
  - camera
  - PMT etc.
- parameters
  - resolution
  - speed
  - sensitivity

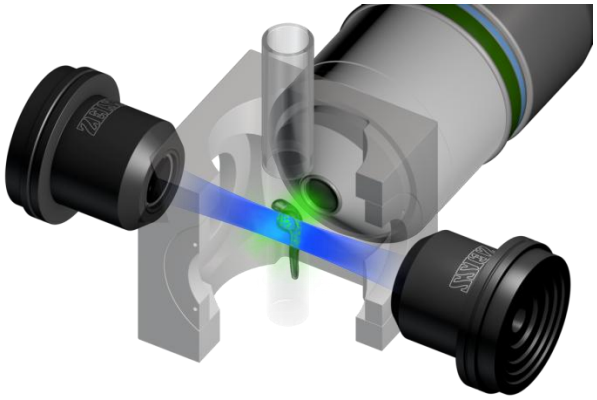
Instrumentation for light microscopy

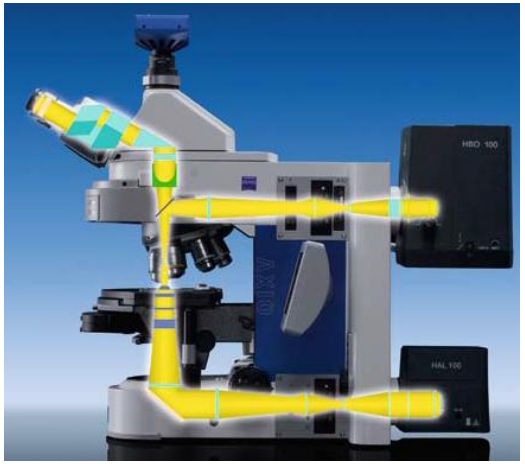
How to orient in the broad spectrum of products?

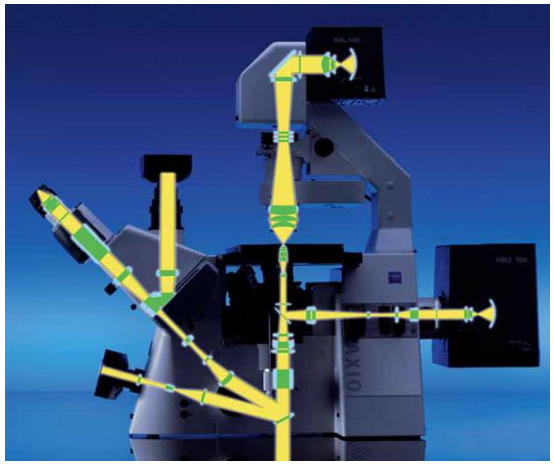
Microscope design

- stereomicroscope
  - Greenough design
  - telescopic design
- zoom microscopes
- upright microscopes
- inverted microscopes
- Lightsheet microscopes
- other type of microscopes







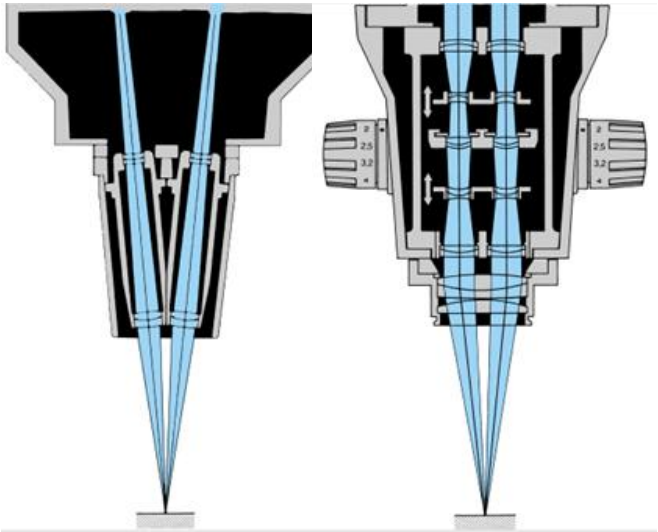


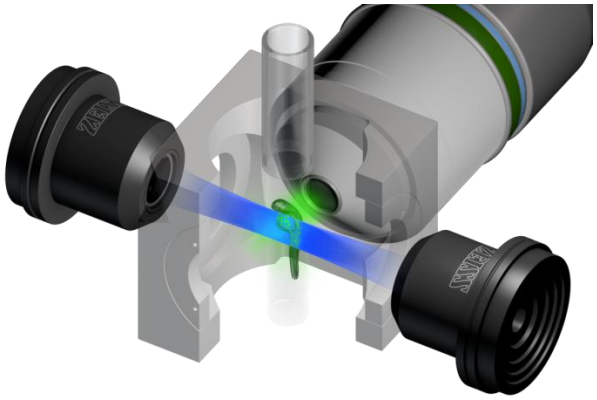
Instrumentation for light microscopy

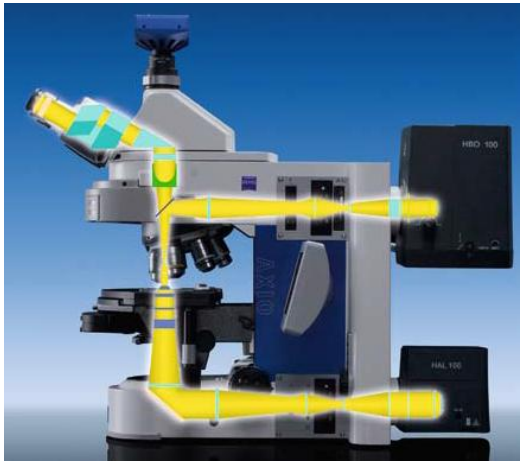
How to orient in the broad spectrum of products?

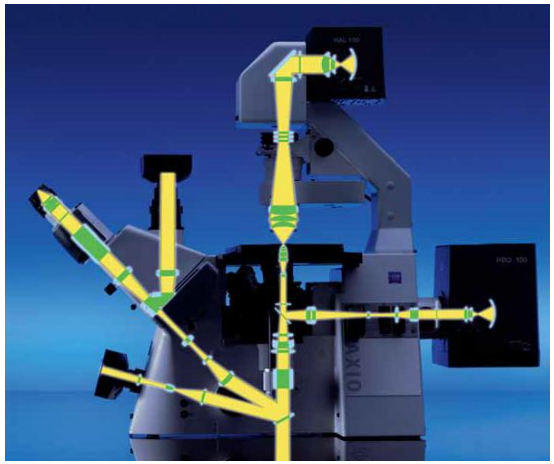
Microscope design

- stereomicroscope
  - Greenough design
  - telescopic design
- zoom microscopes
- upright microscopes
- inverted microscopes
- Lightsheet microscopes
- other type of microscopes



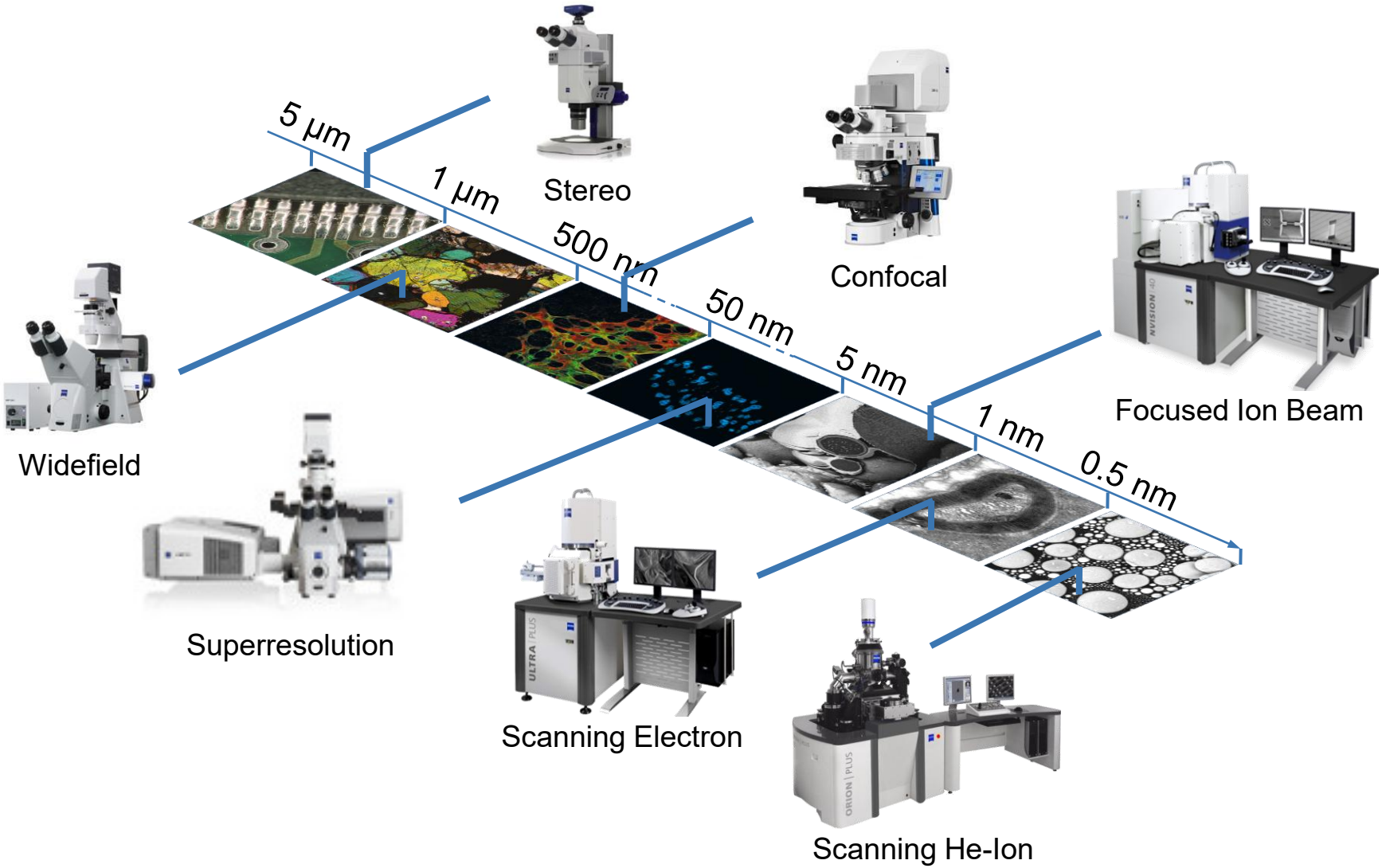






Instrumentation for light microscopy

How to orient in the broad spectrum of products?

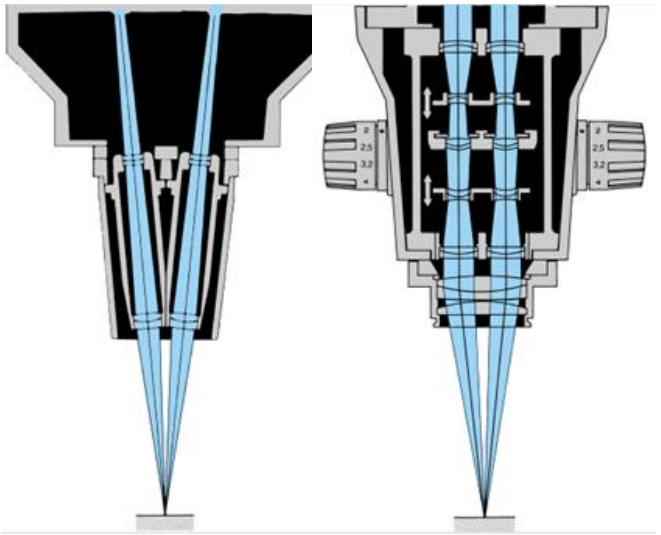


Instrumentation for light microscopy

How to orientate in the broad spectrum of products?

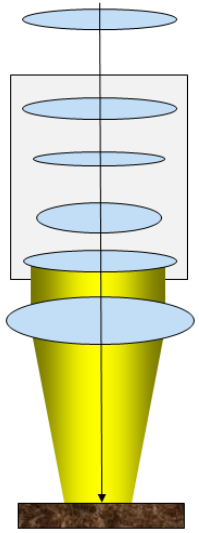
Stereomicroscope
Zoom microscope
Lightsheet microscope

Big field of view - large samples
Manipulation possible
Long working distance
Lower resolution

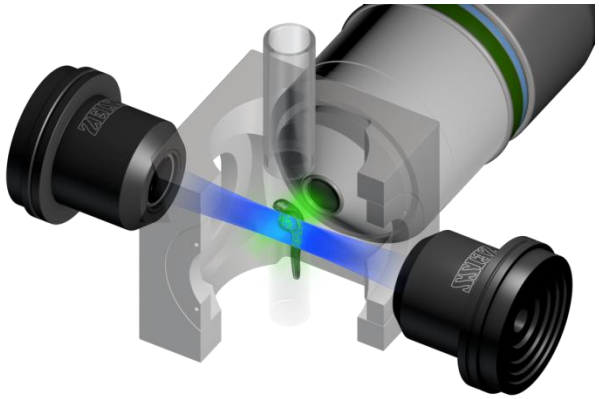


Greenough design

Telescopic design



Zoom microscope



Lightsheet fluorescence microscope

Instrumentation for light microscopy

How to orientate in the broad spectrum of products?

Upright microscope
Inverted microscope
Confocal microscope
etc.

⇨

Small field of view
High resolution

Instrumentation for light microscopy

Microscope and its parts

QUESTION:

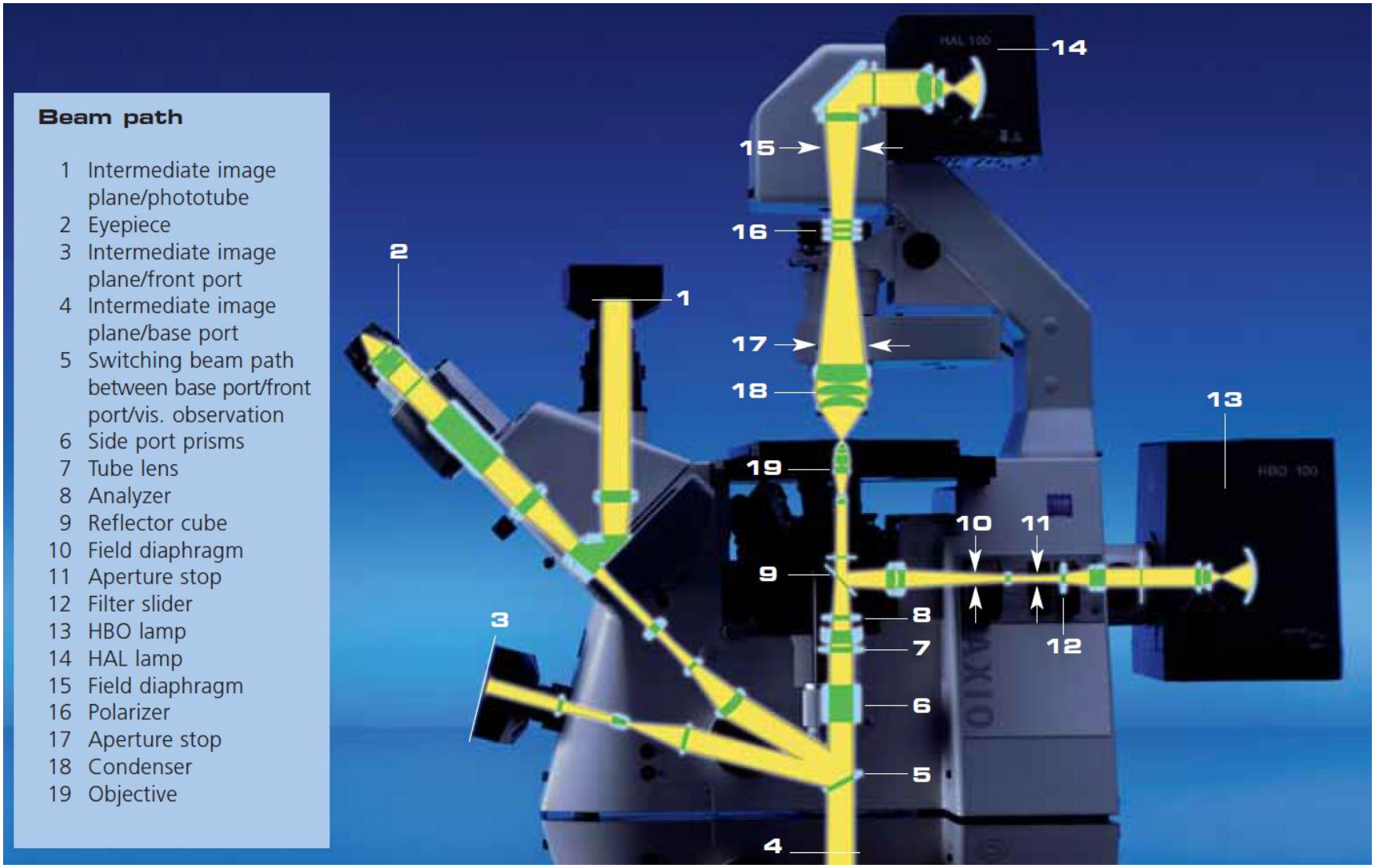
Upright microscope

or

Inverted microscope

Instrumentation for light microscopy

Microscope and its parts



Instrumentation for light microscopy

Microscope and its parts

QUESTION:

Upright microscope

or

Inverted microscope

Instrumentation for light microscopy

Microscope and its parts

QUESTION:

Upright microscope

or

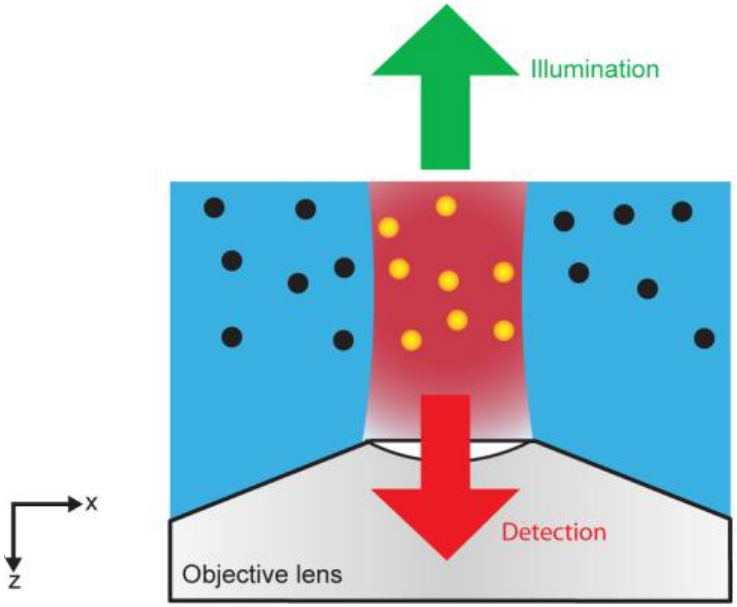
Inverted microscope

Fixed stage upright microscope

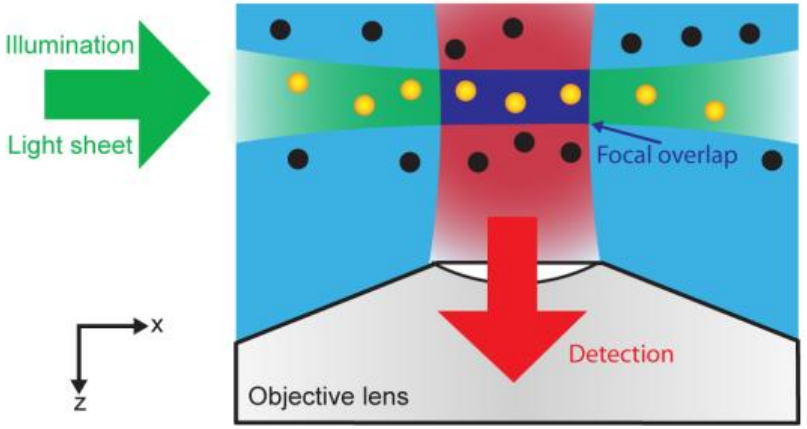
Instrumentation for light microscopy

Microscope and its parts

Epi-illumination



Lightsheet illumination



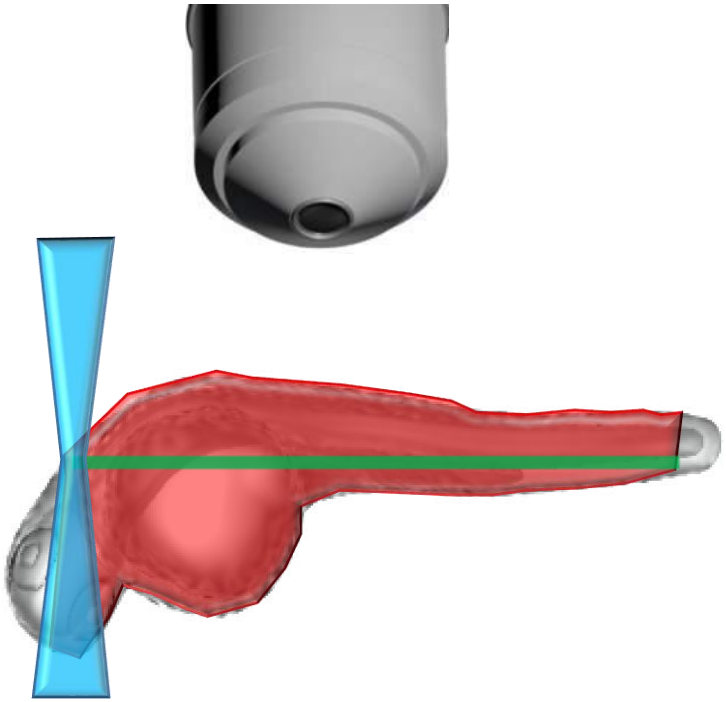
- Inherent optical sectioning capability of the illumination method
- No excitation of out-of-focus fluorescence

Figure from the PhD thesis of Jörg Ritter (2011), University of Bonn, Germany

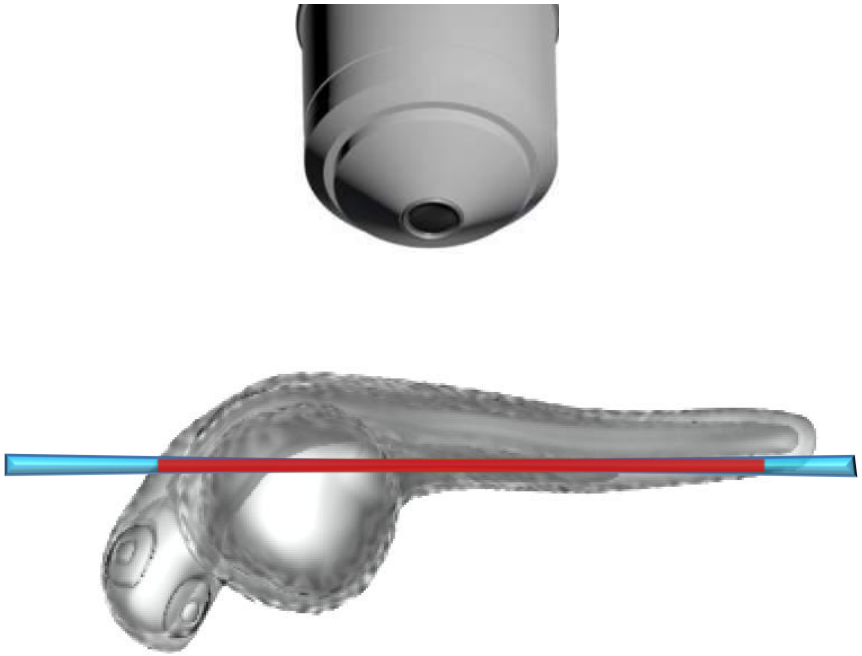
Instrumentation for light microscopy

Microscope and its parts

Epi-illumination (e.g. LSM)



Lightsheet illumination



Less photo-damage

- Long periods of observation
- Fast imaging
- Multiview imaging

Instrumentation for light microscopy

Microscope and its parts

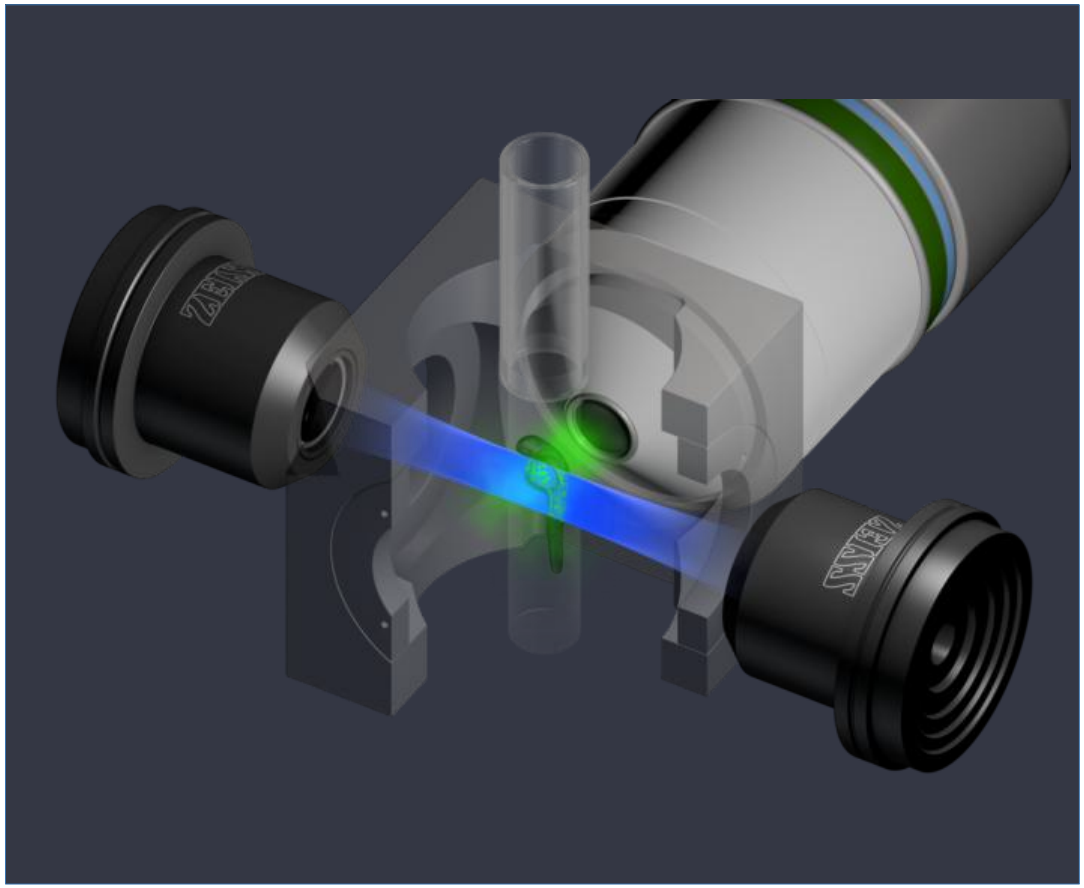
LSFM
SPIM

Illumination beam path

- Horizontal Microscope needed
- Laser beam is shaped into a Lightsheet using a cylindrical lens
- Scanning mirrors move the sheet along the focal plane (y-direction)

Detection beam path

- Horizontal Microscope needed
- Decoupled from illumination beam path
- Oriented 90º to illumination beam path



Instrumentation for light microscopy

Microscope and its parts

Common parts
- microscope body
- condenser
- stage
- objectives
- oculars
- other optics
- inputs / outputs
- light sources

Microscope design
- Stereo microscope
  - Greenough design
  - telescopic design
- zoom microscopes
- upright microscopes
- inverted microscopes
- Lightsheet microscopes
- other type of microscopes

Light sources
- transmitted light
  - halogen
  - LED
- reflected light
  - halogen
  - mercury burner
  - metal halide
  - Xenon
  - LED
  - laser

Detectors
- types of detectors
  - eyes
  - camera
  - PMT, GaAsP, etc.
- parameters
  - resolution
  - speed
  - sensitivity

Instrumentation for light microscopy

Microscope and its parts

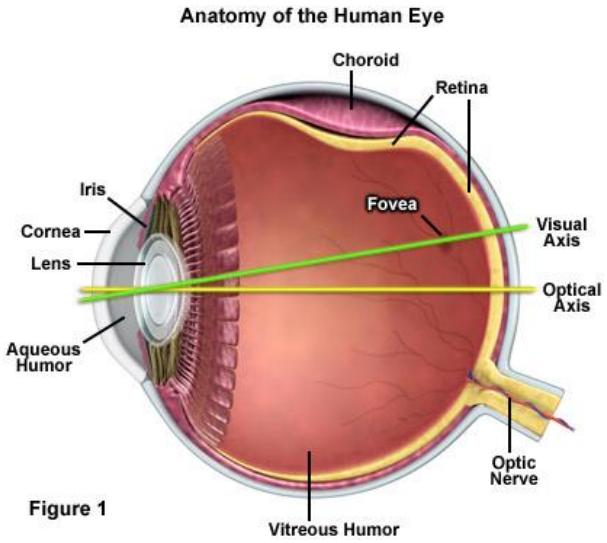


Figure 1

Detectors

- types of detectors
  - eyes
  - camera
  - PMT etc.

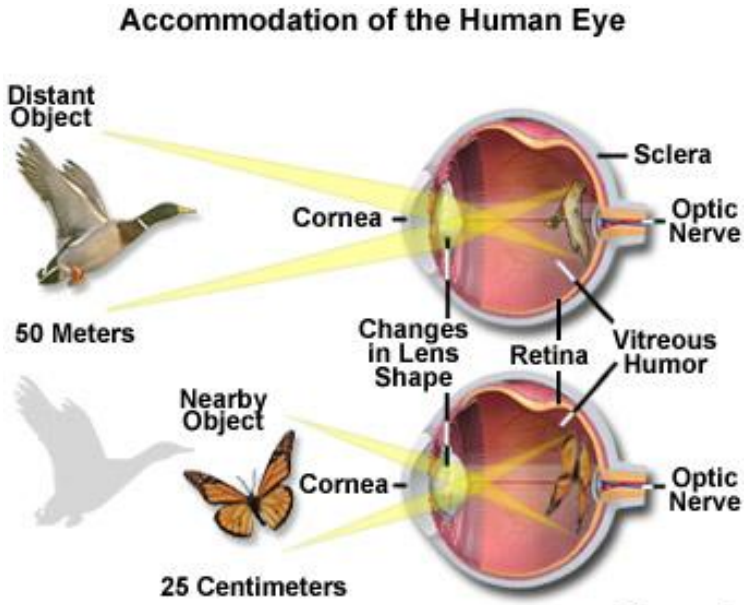


Figure 2

Instrumentation for light microscopy

Microscope and its parts

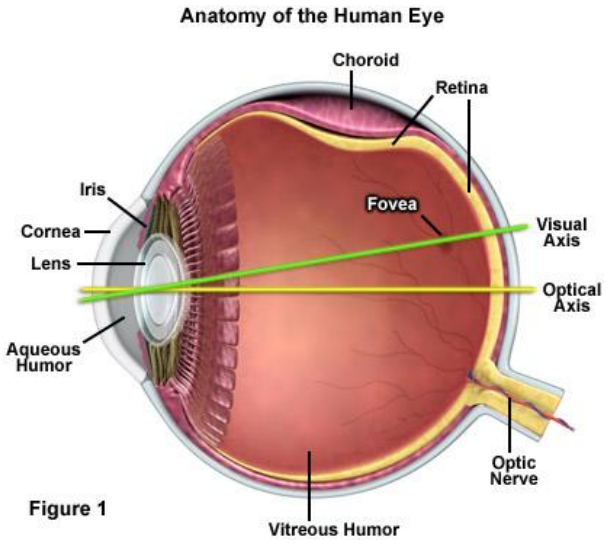


Figure 1

Detectors

- types of detectors
  - eyes
  - camera
  - PMT etc.

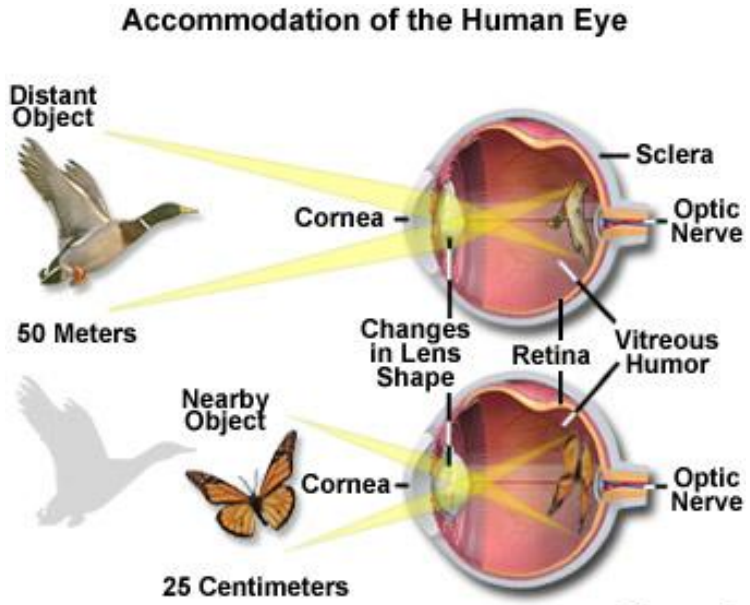
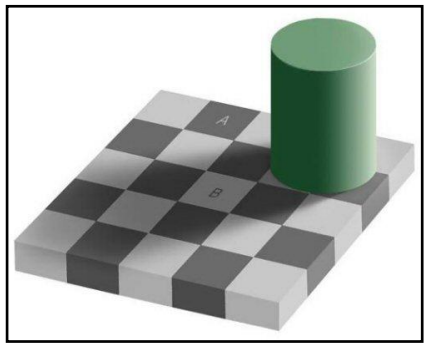
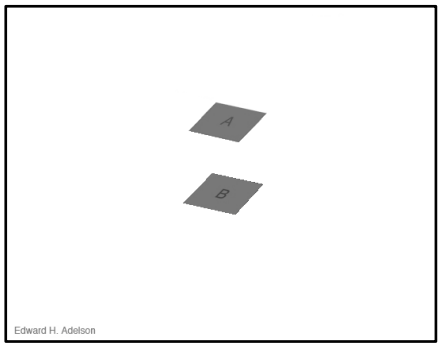
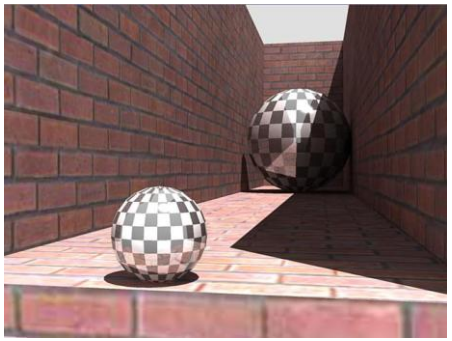
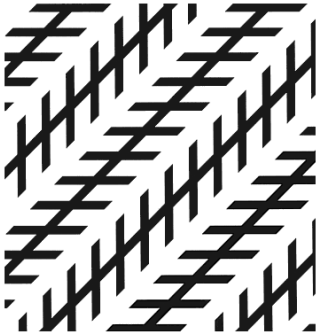


Figure 2









Instrumentation for light microscopy

Microscope and its parts

3 / 5 / 6 / 12 Mpix
mono / color
CCD / CMOS

USB 3.0 connection
- universal
- fast

Special cameras

SOFTWARE

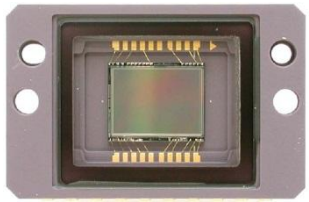
ZEN

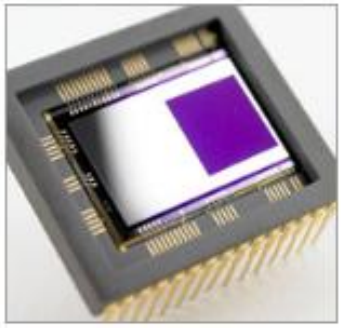
arivis

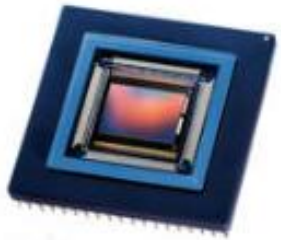
Instrumentation for light microscopy

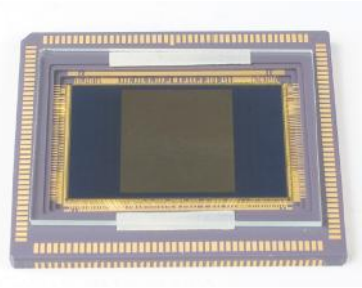
Microscope and its parts - cameras

- **CCD** Charge Coupled Device
- **BT CCD** Back Thinned CCD
- **EM CCD** Electron Multiplication CCD
- **BT EM CCD** Back Thinned EMCCD
- **CMOS** Complementary Metal Oxide Semiconductor
- **sCMOS** „scientific CMOS“
- **BSI CMOS** Back Side Illuminated CMOS









Digital EM - CCD Cameras

EM - CCD Sensor Technology

EMCCD technology:

- Frame Transfer Architecture
- Charge amplification designed to eliminate the effect of read noise on imaging

- At short exposure times the unavoidable read noise is the MOST significant noise source for biological imaging

- Produced in low volumes
- QE from 50% up to 95% back tinned
- Affected by smear effects

- Without active EMGAIN higher read noise then CCD → less sensitive

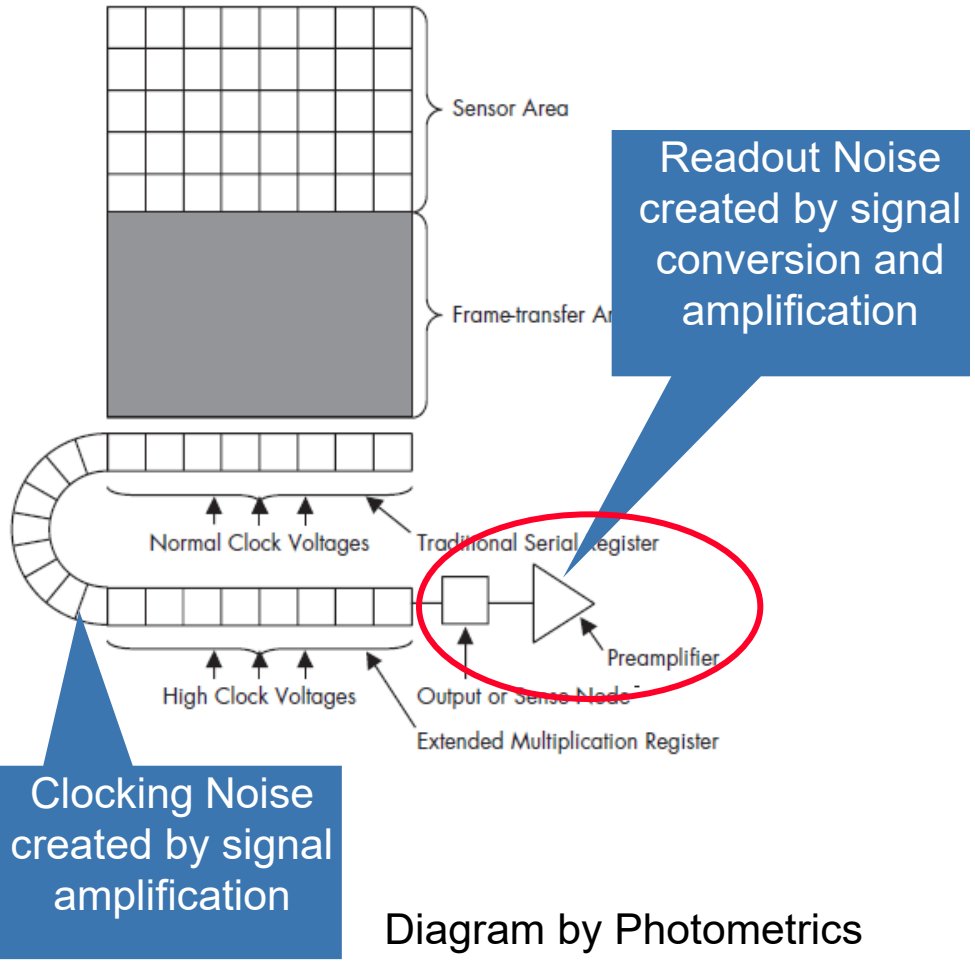
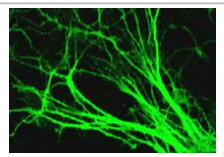


Diagram by Photometrics

Instrumentation for light microscopy

Microscope and its parts - PMT



Photon

p

Photoelectric effect:
Photocathode

Dynodes

Anode

Electron

e-

Output
Meter

-

+

Power Supply
(Gain = Volts)

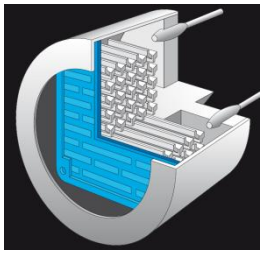
+ - bright Pixel

Instrumentation for light microscopy

Microscope and its parts - PMT

PMT (Photo Multiplier Tube)

- Standard

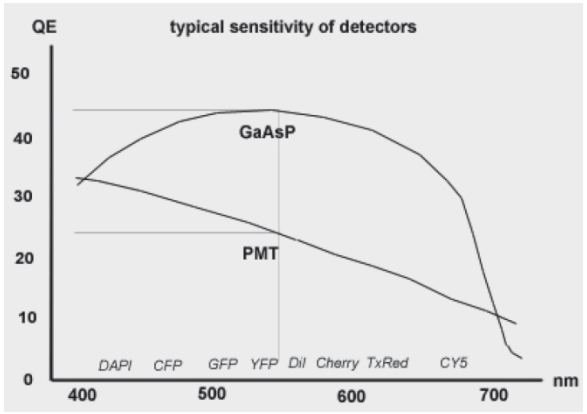


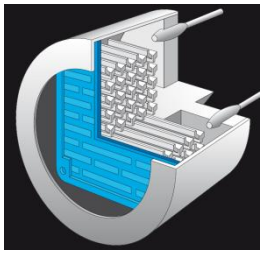
Instrumentation for light microscopy

Microscope and its parts - PMT

PMT (Photo Multiplier Tube)

- Standard
- GaAsP



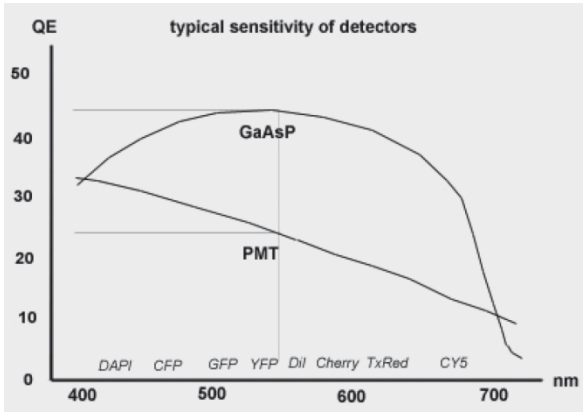


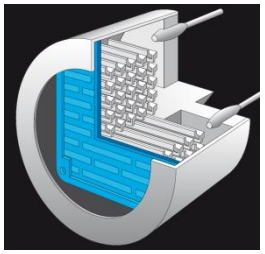
Instrumentation for light microscopy

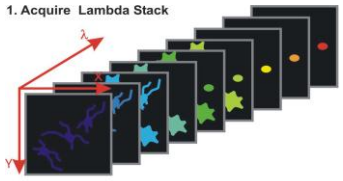
Microscope and its parts - PMT

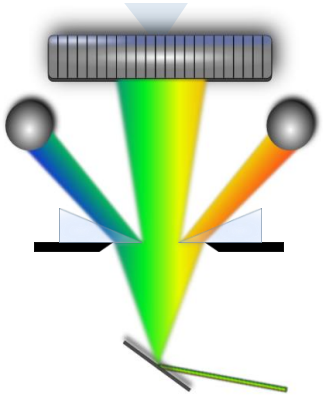
PMT (Photo Multiplier Tube)

- Standard
- GaAsP
- Clever PMT:
  - Spectral
  - Spectral GaAsP







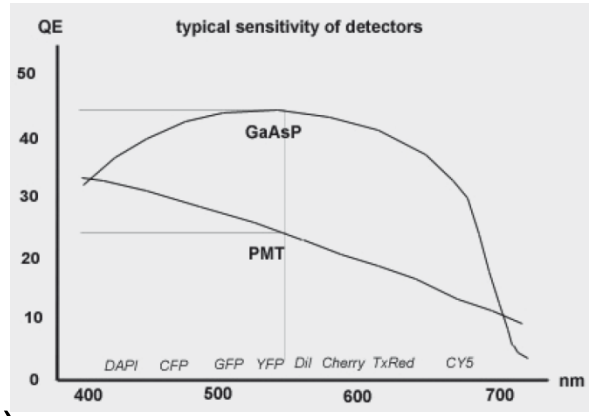


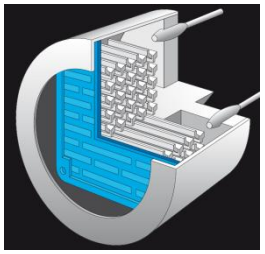
Instrumentation for light microscopy

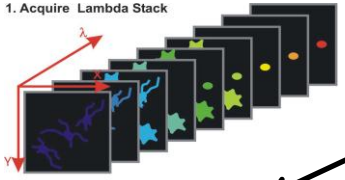
Microscope and its parts - PMT

PMT (Photo Multiplier Tube)

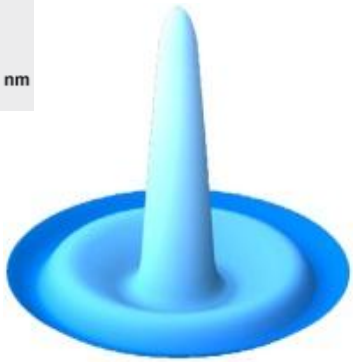
- Standard
- GaAsP
- Clever PMT:
  - Spectral
  - Spectral GaAsP
  - Spacial GaAsP (Airyscan)

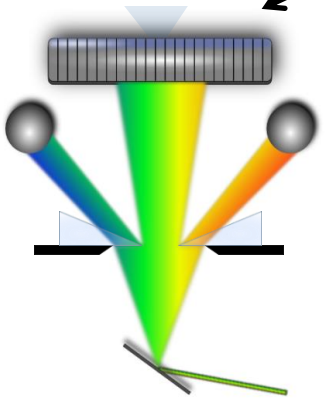






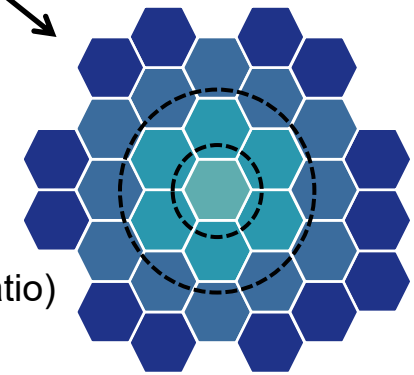
Array detector





Spectral information
SIMULTANEOUSLY
(Lambda scan)

Spacial information
SIMULTANEOUSLY
(high resolution,
increased signal to noise ratio)



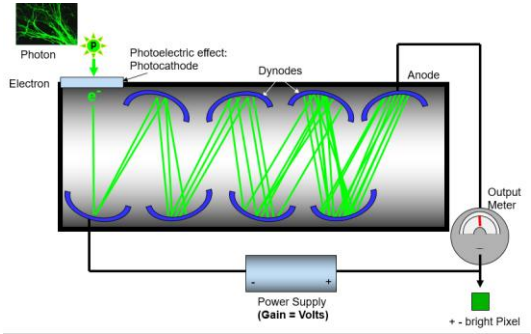
Instrumentation for light microscopy

Microscope and its parts

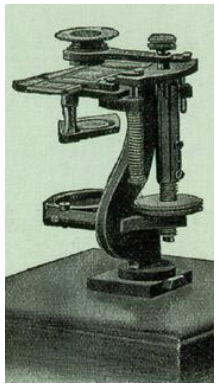
Detectors

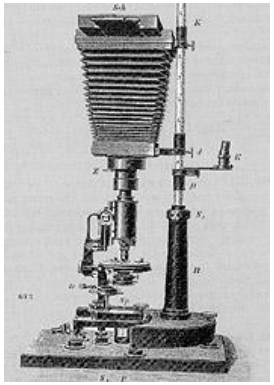
- types of detectors
  - eyes
  - camera
  - PMT etc.

- parameters	**CAMERA (wide-field)**	**PMT (LSM)**
- resolution	low to high	versatile
- speed	all pixels in one shot	point scanner
- sensitivity	very high	lower



Instrumentation for light microscopy

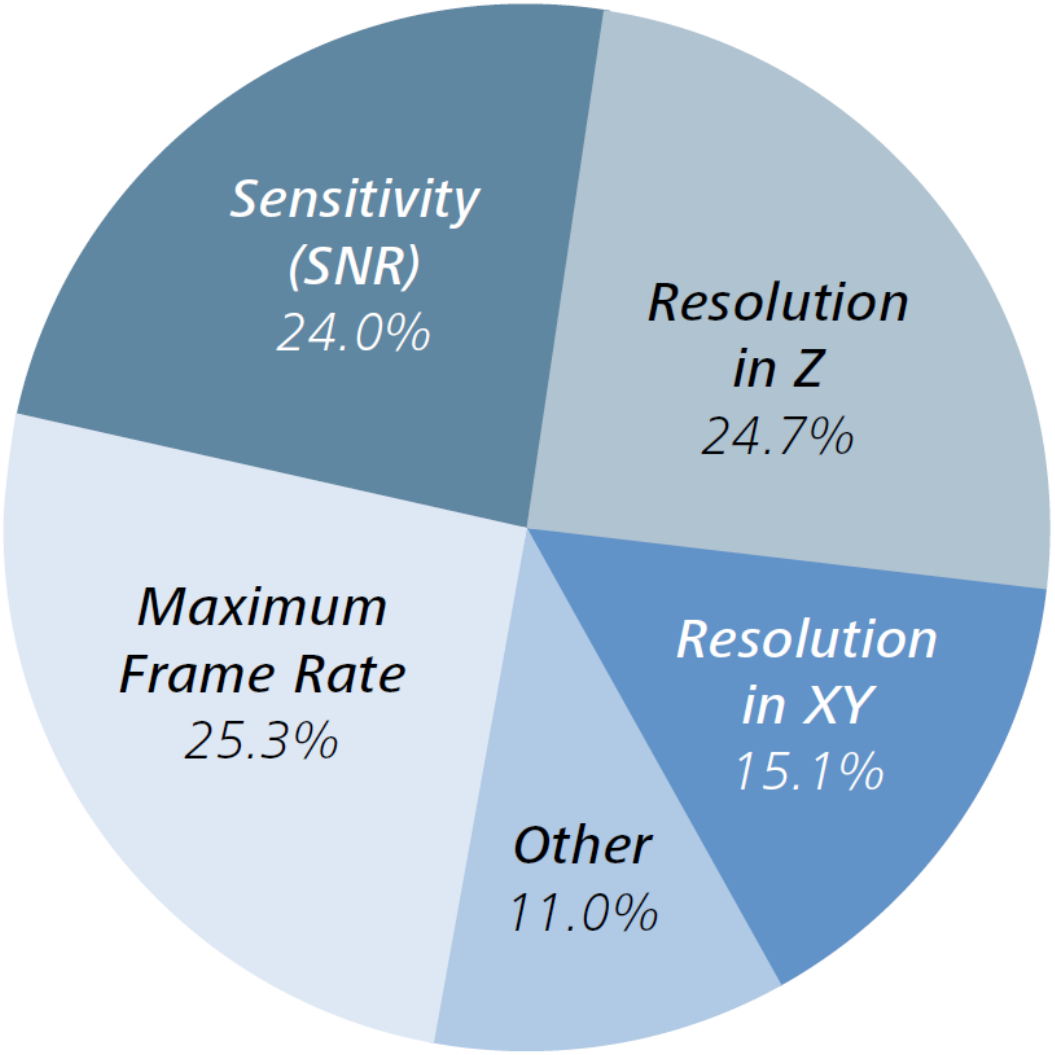




History **Presence**

Instrumentation for light microscopy

Survey among researchers

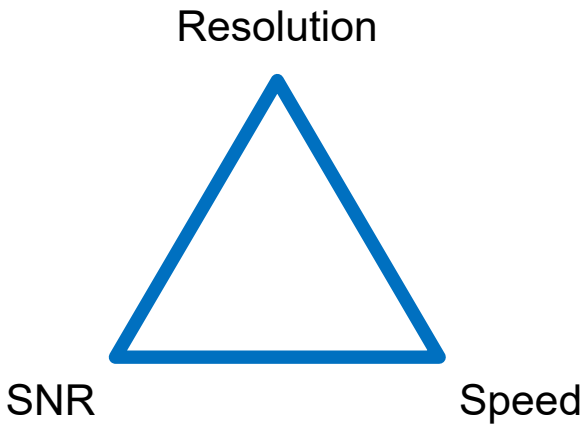


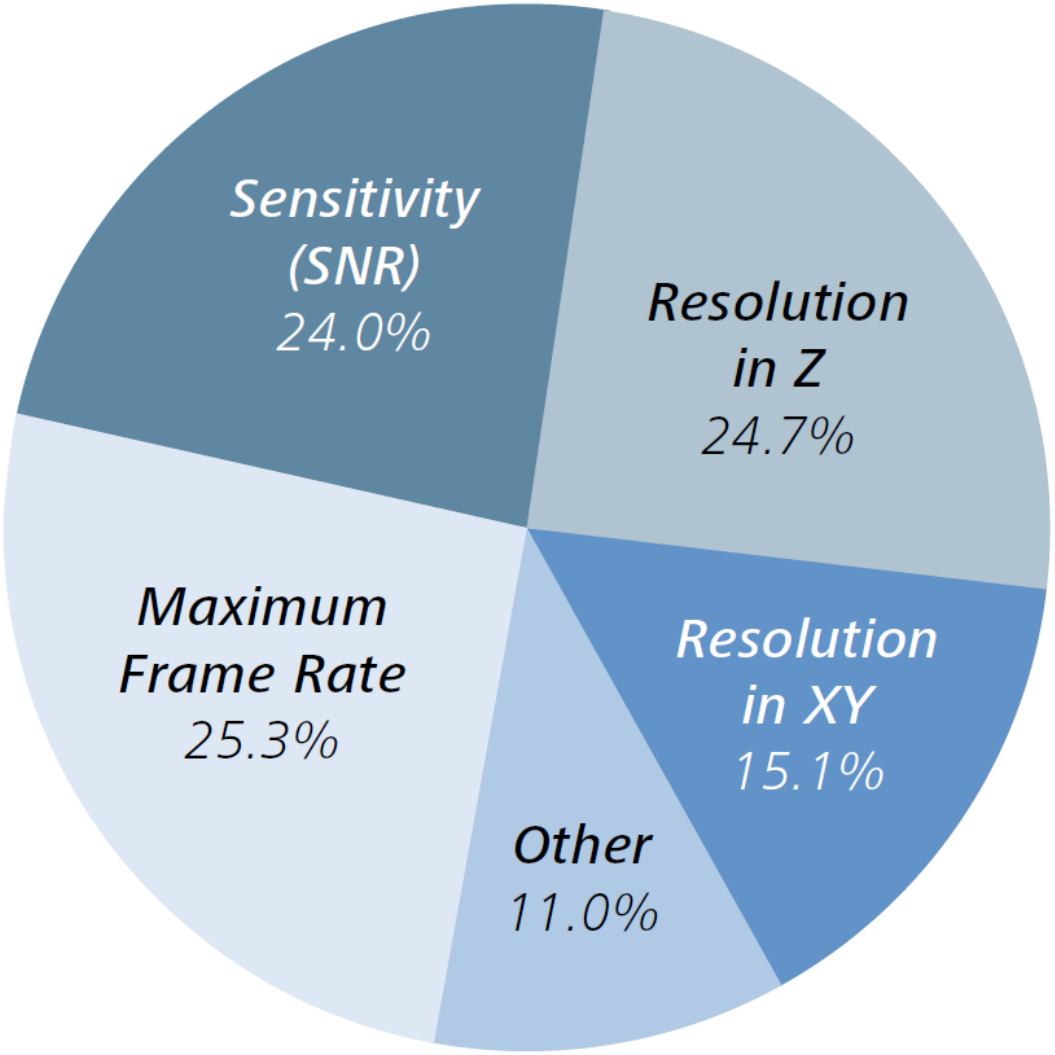
THE SAMPLE

Instrumentation for light microscopy

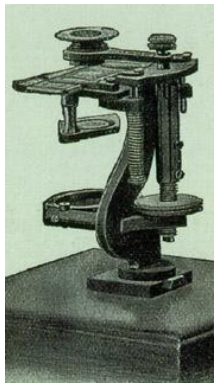
Survey among researchers

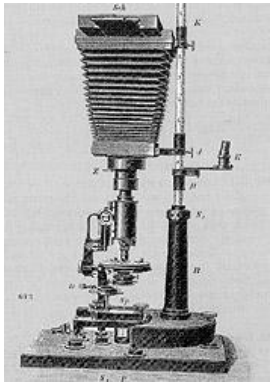
BUT





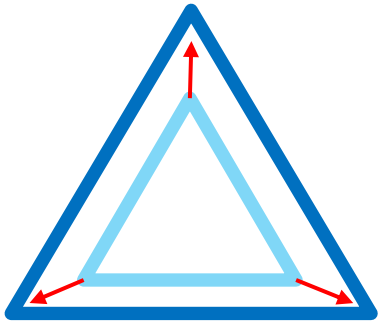
Instrumentation for light microscopy



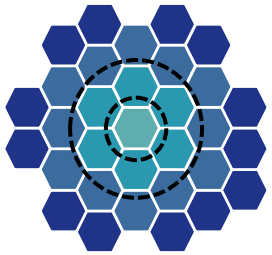


History **Presence** **Future**

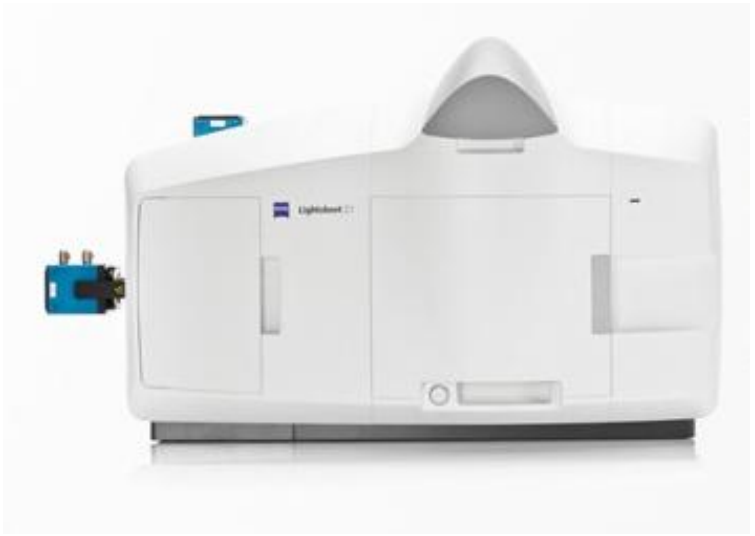
Instrumentation for light microscopy



Already NOW!!!

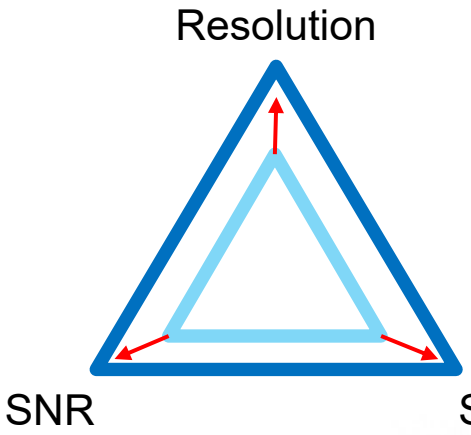


ZEISS LSM 990 with Airyscan 2



ZEISS Lightsheet.7

Instrumentation for light microscopy

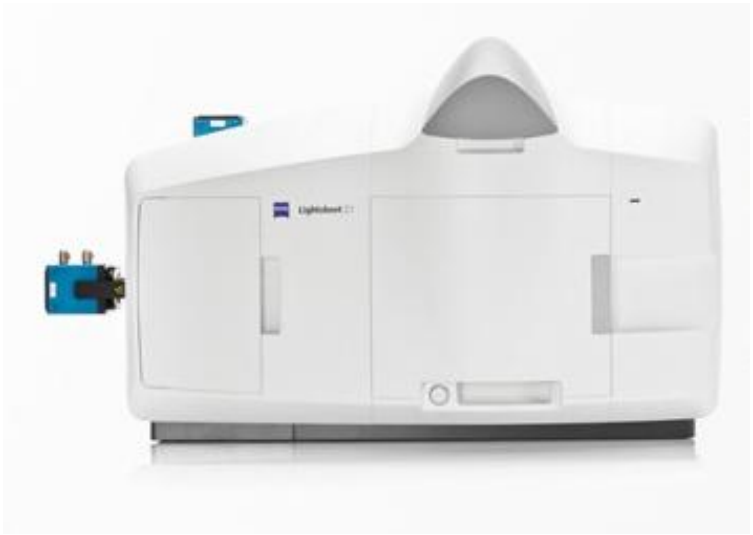


Already NOW!!!

and

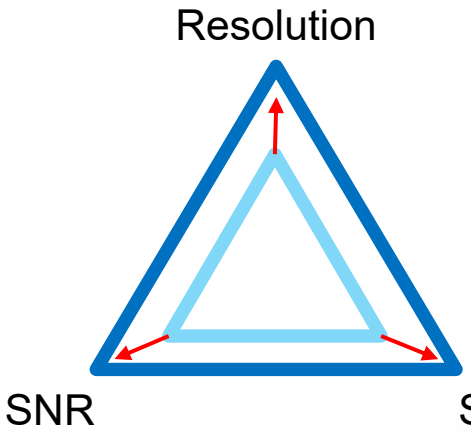
New great ideas / techniques are coming!

ZEISS LSM 990 with Airyscan 2



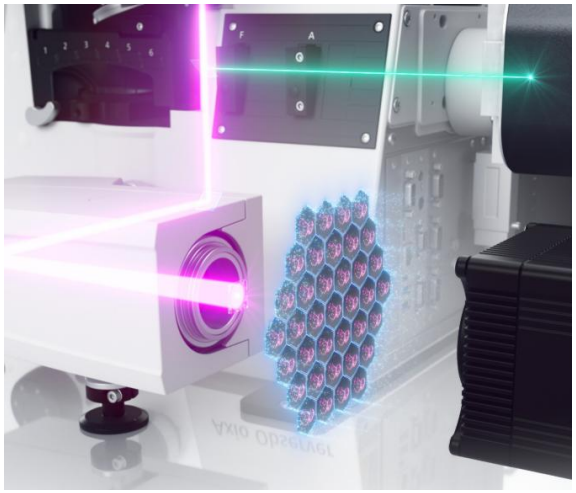
ZEISS Lightsheet.7

Instrumentation for light microscopy



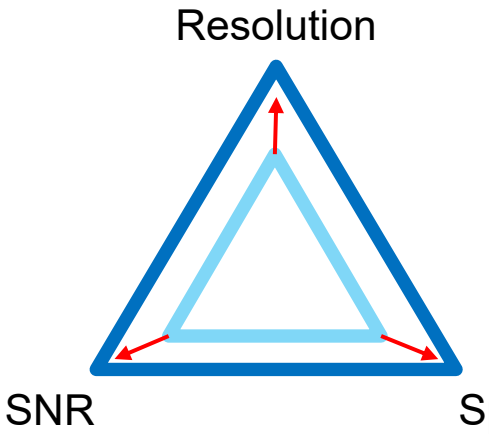
Already NOW!!!
and
New great ideas / techniques are coming!

ZEISS LSM 990 with Airyscan 2



ZEISS Lightfield4D

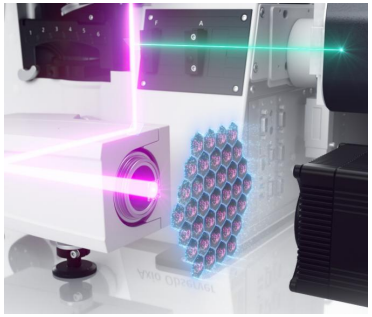
Instrumentation for light microscopy



BUT BE AWARE!!!

The <u>SAMPLE</u> is the most important part

ZEISS LSM 990 with Airyscan 2



ZEISS Lightfield4D

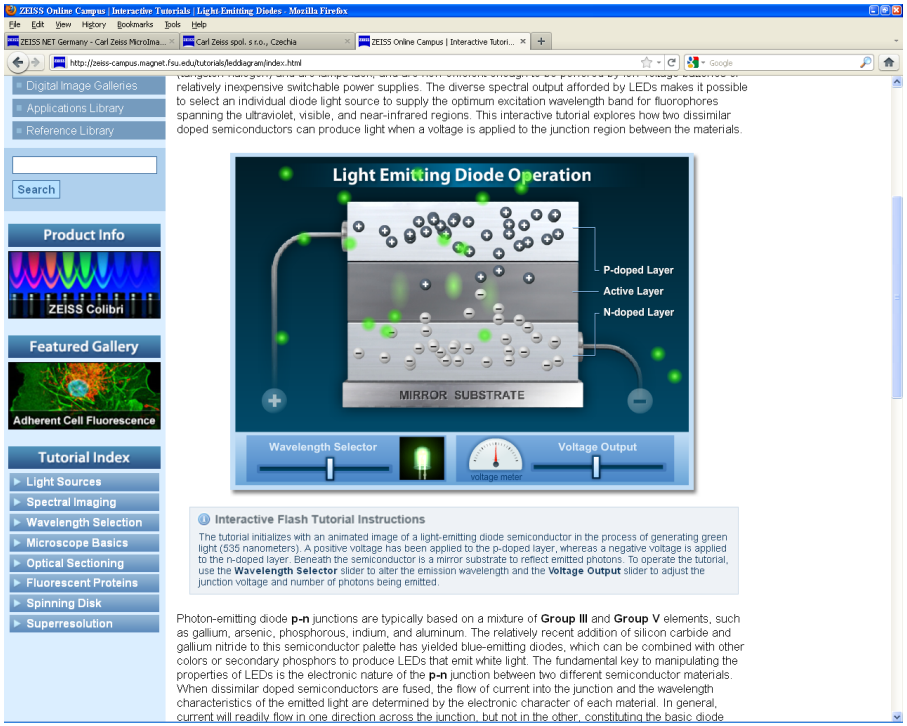
ZEISS Lightsheet.7

Microscopes

How to orientate in the broad spectrum of products?

LOST???

http://www.zeiss.com/campus = http://zeiss-campus.magnet.fsu.edu/
or www.google.com - „CARL ZEISS CAMPUS“



Microscopes

How to orient in the broad spectrum of products?

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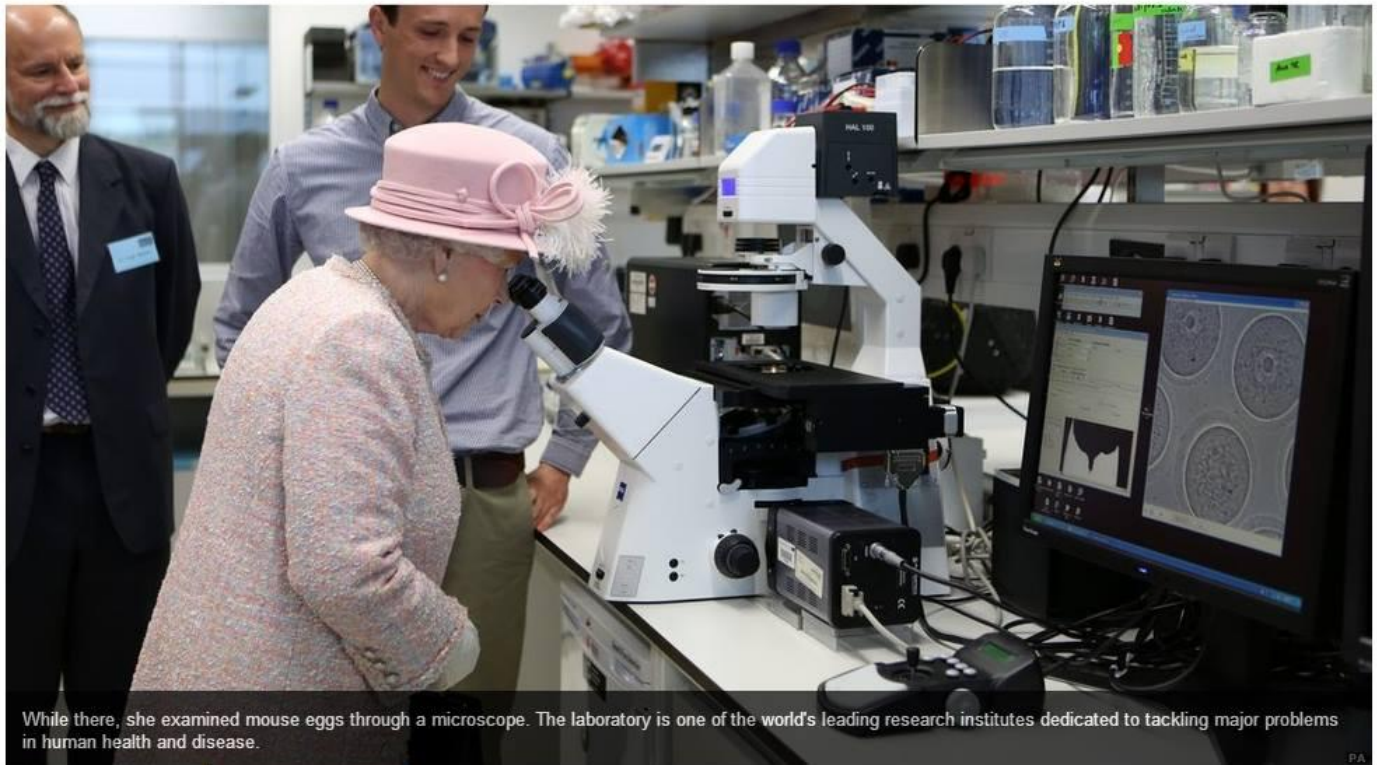
23 May 2013 Last updated at 15:11 GMT

In pictures: The Queen and Duke of Edinburgh in Cambridge



While there, she examined mouse eggs through a microscope. The laboratory is one of the world's leading research institutes dedicated to tackling major problems in human health and disease.

Microscopes

BUT before you need to know

Stereo microscope
Wide field microscope
Fluorescence microscope
TIRF system
Confocal microscope
Spinning disc
Multiphoton microscope
Superresolution microscope
Lightsheet microscope
Electron microscope
Other?

Microscopy overview

- Upright microscopes
- Electrophysiology microscopes
- Inverted microscopes
- Stereomicroscopes
- Zoom microscopes
- Confocal microscopes
- Superresolution
- Imaging systems
- Laser microdissection
- Electron microscopy

Microscopy overview

YOU HAVE THE CHOICE = USE IT!!!

- Upright microscopes – PrimoStar, AxioScope, AxioImager
- Electrophysiology microscopes – AxioExaminer
- Inverted microscopes – PrimoVert, AxioVert, AxioObserver
- Stereomicroscopes – Stemi, SteREO Discovery
- Zoom microscopes – AxioZoom.V16
- Confocal microscopes – Airyscan, LSM 980, 900, 980 NLO
- Superresolution – ELYRA.7 – SMLM and LS
- Imaging systems – Lightsheet.71, Spinning Disc, Apotome.2
- Laser microdissection – PALM Microbeam, Microtweezers, Combi system
- Electron microscopy – GeminiSEM, Sigma, MultiSEM, Merlin, EVO, Crossbeam, Orion

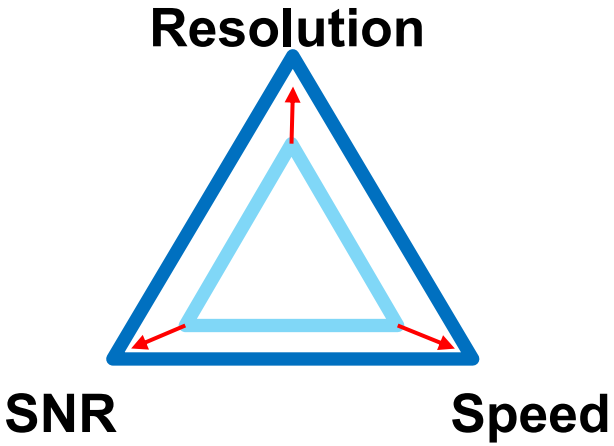
ONLY WITH

ZEISS

We make it visible.

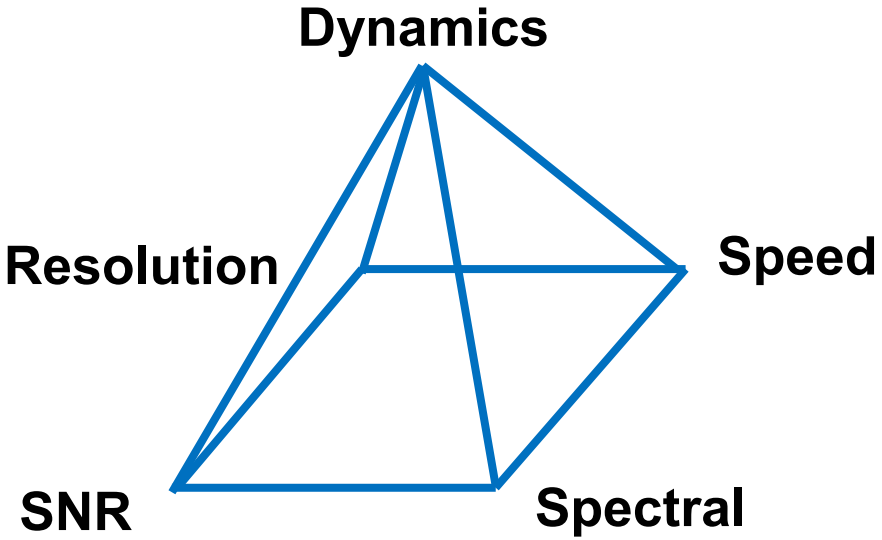
Theory vs. Reality

THE SAMPLE



Theory vs. Reality

THE SAMPLE

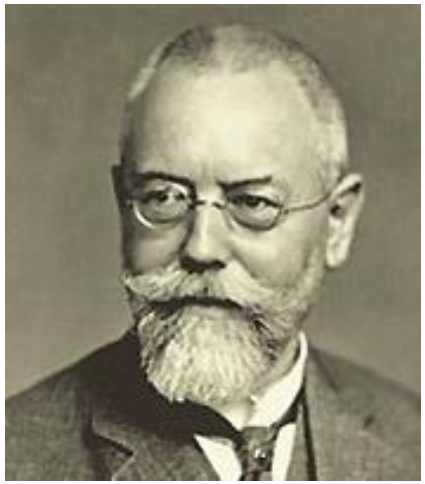


Light microscopy

Carl Friedrich Zeiss
(1816 - 1888)

Ernst Abbe
(1840 - 1905)

Otto Schott
(1851 - 1935)



August Köhler
(1866 - 1948)

http://www.zeiss.com/campus

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We make it visible.