Introduction to light microscopy

A CAMDU training course

Claire Mitchell, Imaging specialist, L1.01, 08-10-2018

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Contents

1. Introduction to light microscopy
2. Different types of microscope
3. Fluorescence techniques
4. Acquiring quantitative microscopy data

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1. Introduction to light microscopy

1.1 Light and its properties

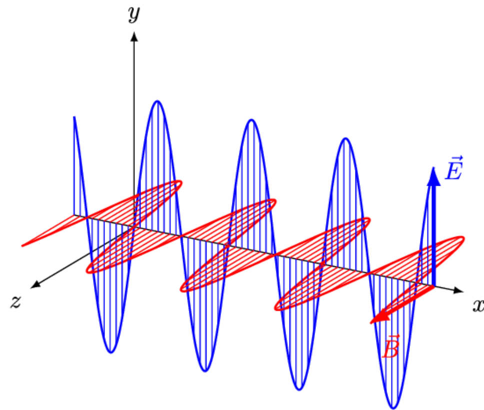
1.2 A simple microscope

1.3 The resolution limit

1.1 Light and its properties

1.1.1 What is light?

An electromagnetic wave



commons.wikimedia.org/wiki/File:EM-Wave.gif

AND

A massless particle

γ

www.particlezoo.net

1.1.2 Properties of waves

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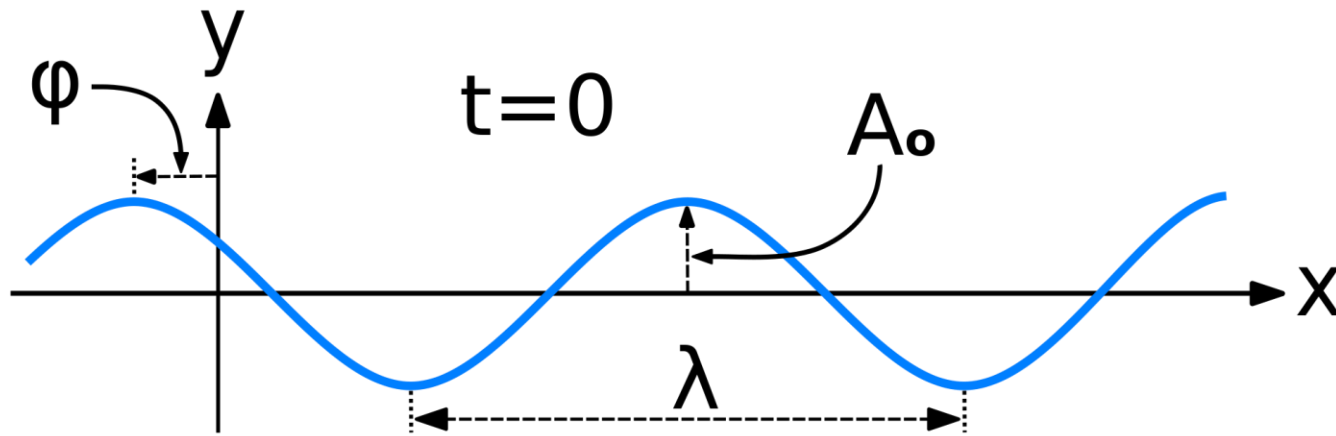
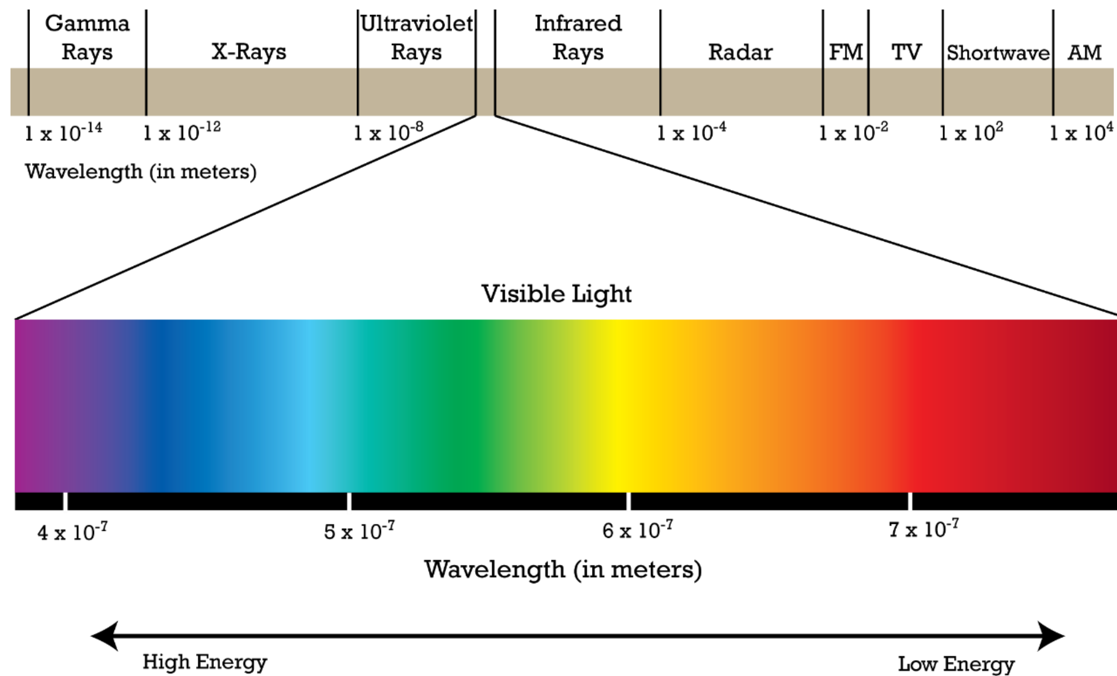


Light waves are transverse waves – they oscillate orthogonally to the direction of propagation

Important properties of light: wavelength, frequency, speed, amplitude, phase, polarisation

upload.wikimedia.org

1.1.3 The electromagnetic spectrum



pion.cz/en/article/electromagnetic-spectrum

$$E_{photon} = h\nu$$

$$c = \lambda\nu$$

E_{photon} = photon energy

h = Planck's constant

ν = frequency

c = speed of light

λ = wavelength

1.1.4 Refraction

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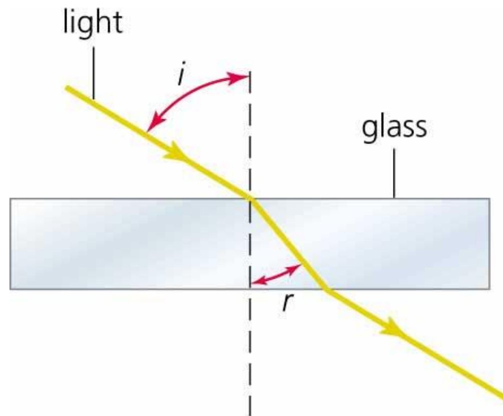
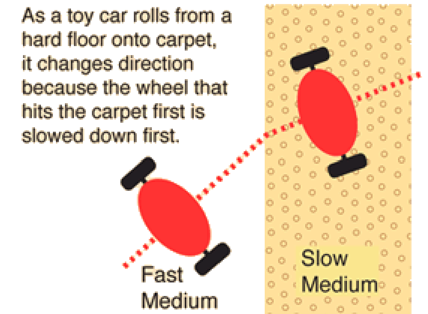
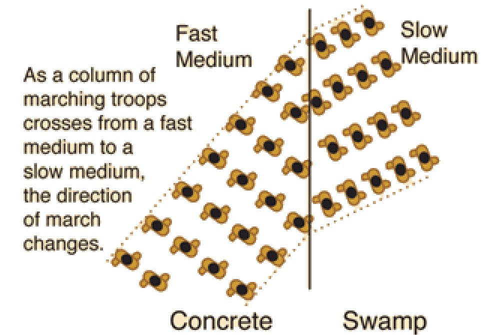
Light bends when it encounters a change in refractive index e.g. air to glass

www.thetastesf.com



files.askiitians.com





hyperphysics.phy-astr.gsu.edu/hbase/Sound/imgsou/refr.gif

1.1.5 Diffraction

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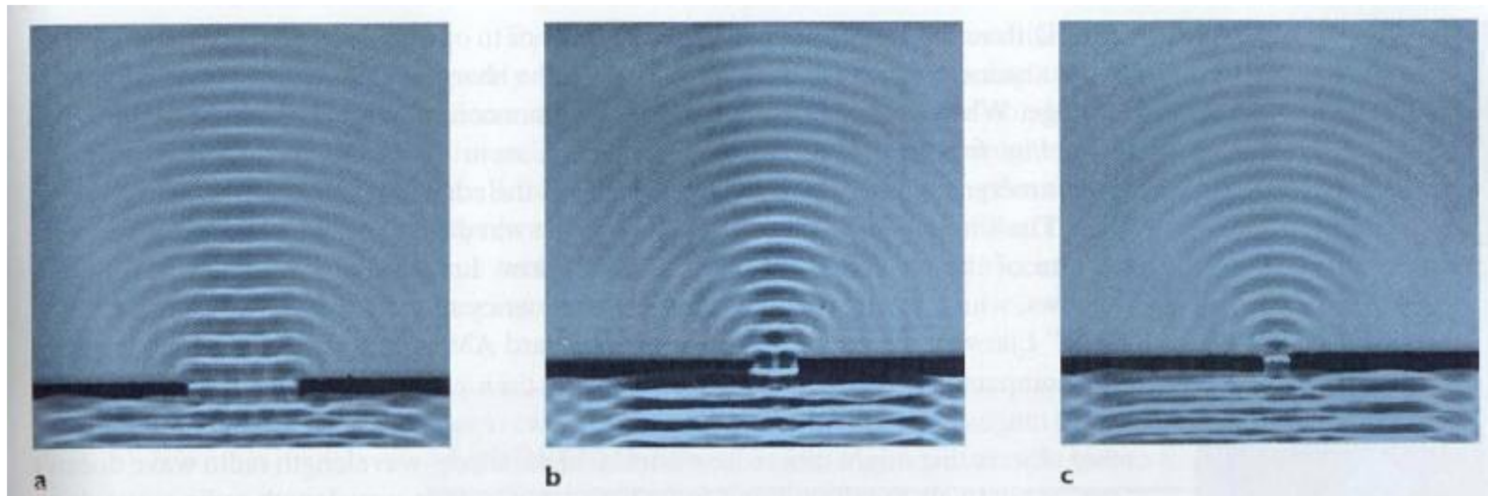
Light waves spread out when they encounter an aperture.



electron6.phys.utk.edu/light/1/Diffraction.htm

The smaller the aperture, the larger the spread of light.

1.1.6 Interference

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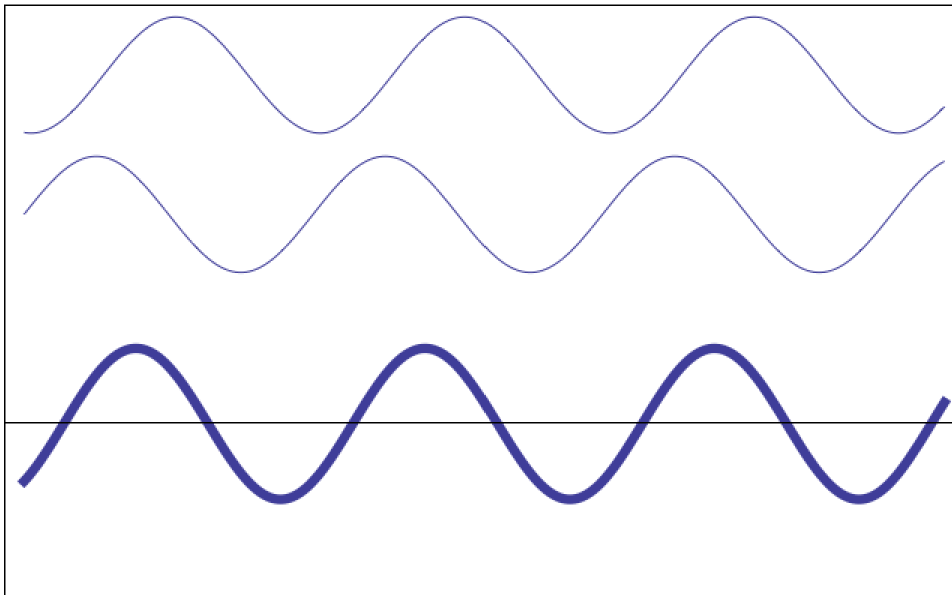

When waves overlap, they add together in a process called interference.

peak + peak = 2 x peak

trough + trough = 2 x trough

constructive

peak + trough = 0

destructive

www.acs.psu.edu/drussell/demos/superposition/superposition.html

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1.2 A simple microscope

1.2.1 Using lenses for refraction

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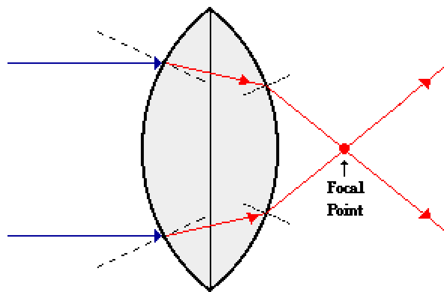
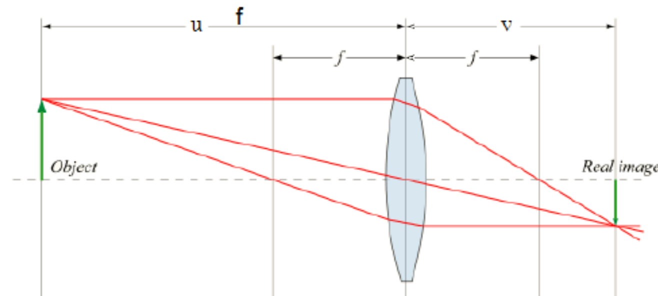


physicsclassroom.com



$$\frac{1}{f} = \frac{1}{u} + \frac{1}{v} \qquad m = \frac{v}{u}$$

cdn.education.com/files/

Light bends as it encounters each air/glass interface of a lens.

A curved surface can be used to change the angle of the rays.

A single lens is enough to induce magnification – a magnifying glass

1.2.2 How do we achieve magnification?

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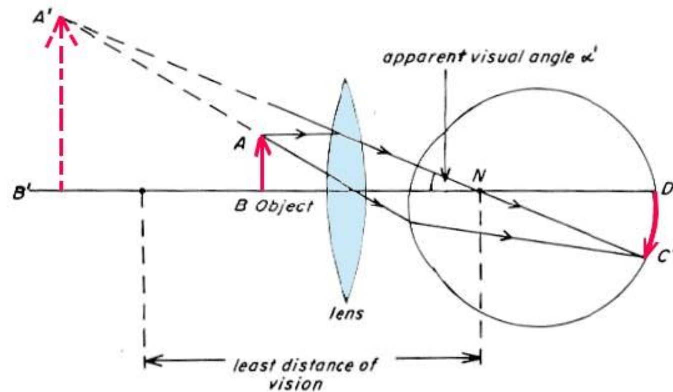
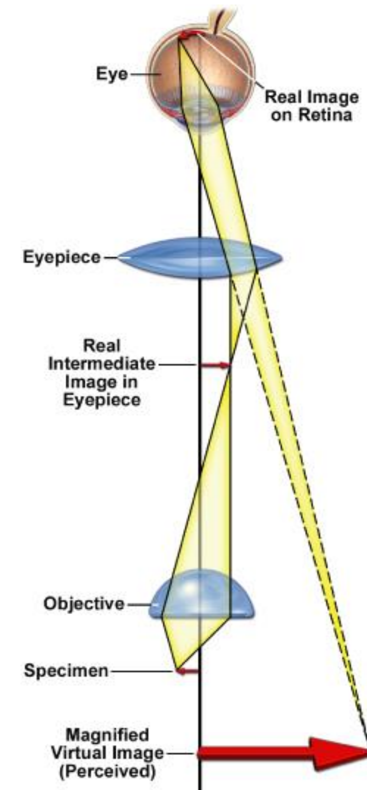


quekett.org

Magnifying glass – single lens to magnify an image

Compound microscope – a series of lenses creates a magnified image on the detector (eye/camera). Greater magnification possible with higher fidelity



Fundamentals of light microscopy and electronic imaging, Murphy and Davidson

1.2.3 Set-up of a simple microscope

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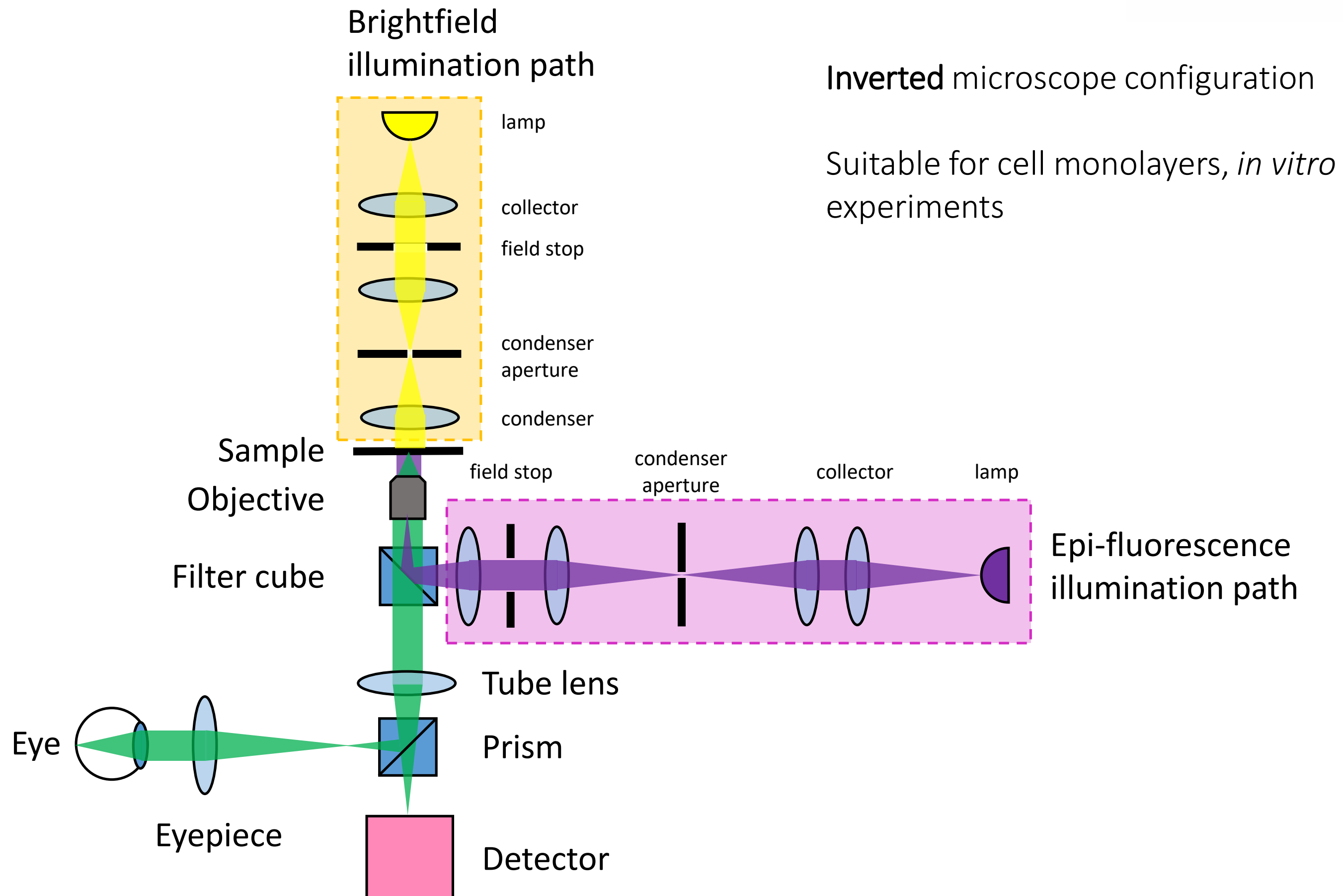

Inverted microscope configuration

Suitable for cell monolayers, *in vitro* experiments

1.2.4 Set-up of a simple microscope

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Eye

Eyepiece

Detector

Prism

Tube lens

Epi-fluorescence illumination path

Filter cube

Objective

Sample

lamp

collector

condenser aperture

field stop

condenser

condenser aperture

field stop

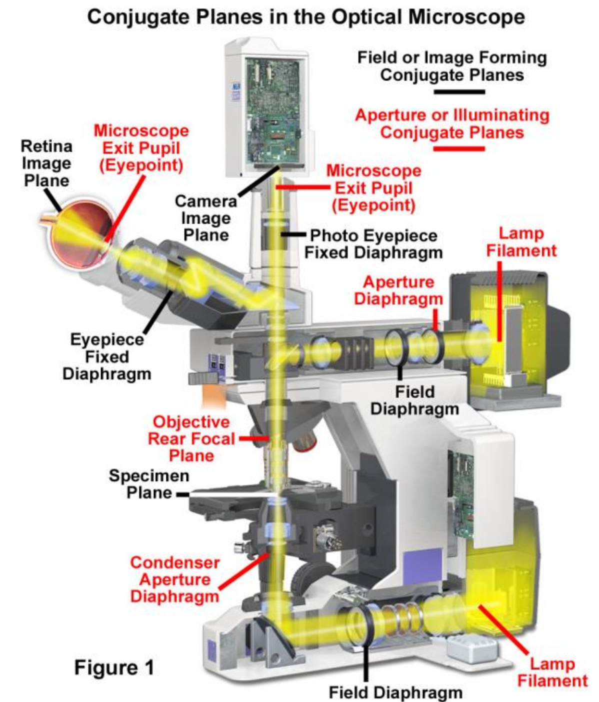
collector

lamp

Brightfield illumination path

Upright microscope configuration

Suitable for *in vivo* microscopy and thick tissue imaging



Understanding Conjugate Planes And Köhler Illumination
Michael W. Davidson And Thomas J. Fellers

1.2.3 Set-up of a simple microscope

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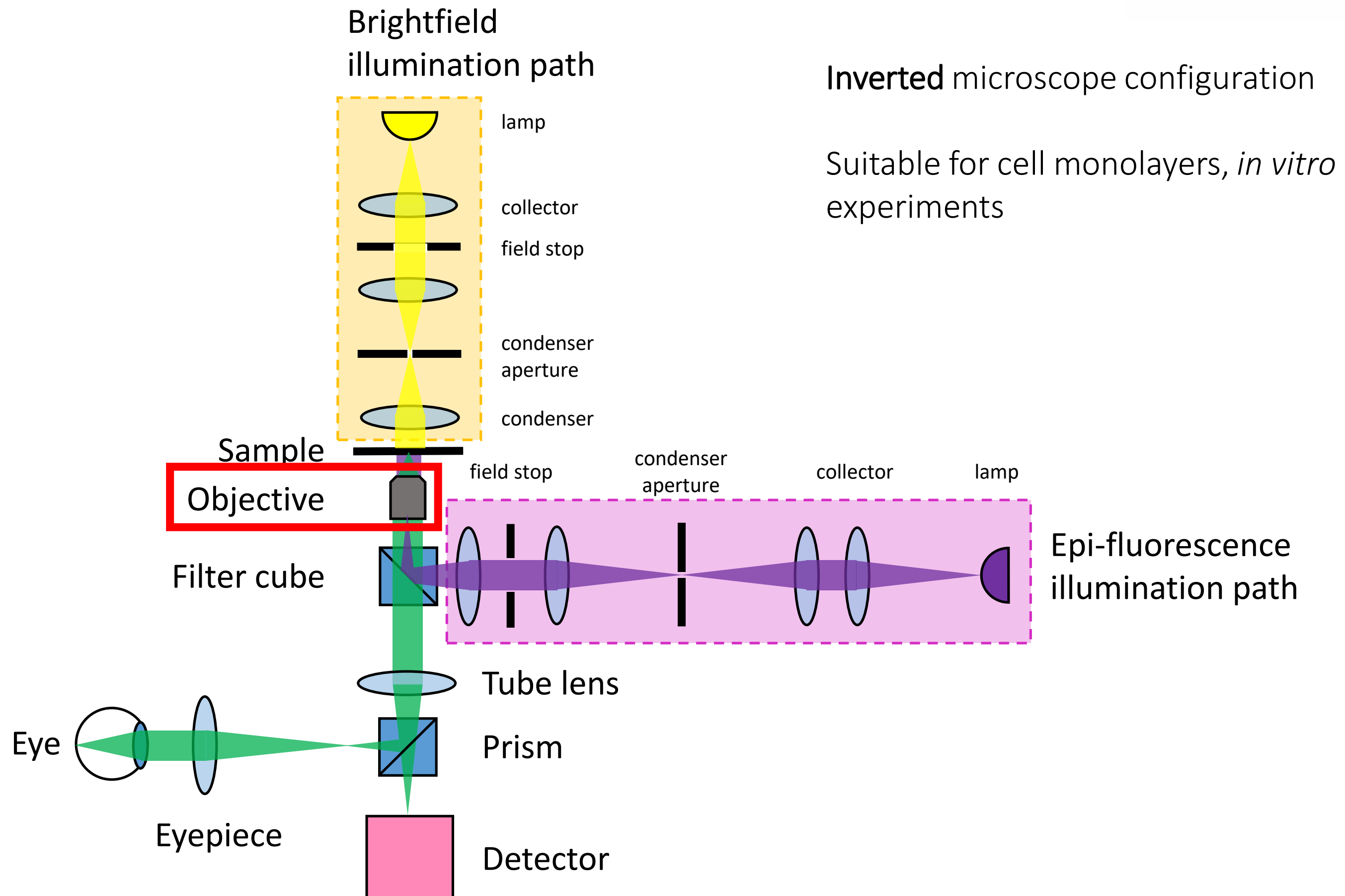

Inverted microscope configuration

Suitable for cell monolayers, *in vitro* experiments

1.2.5 The microscope objective

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A series of lenses acting a single magnifying lens with superior optical characteristics

Microscopes typically have >1 objective to change total magnification

@fabio_pazzano

olympus.magnet.fsu.edu/primer/anatomy/specifications.html

1.2.6 Choice of objective

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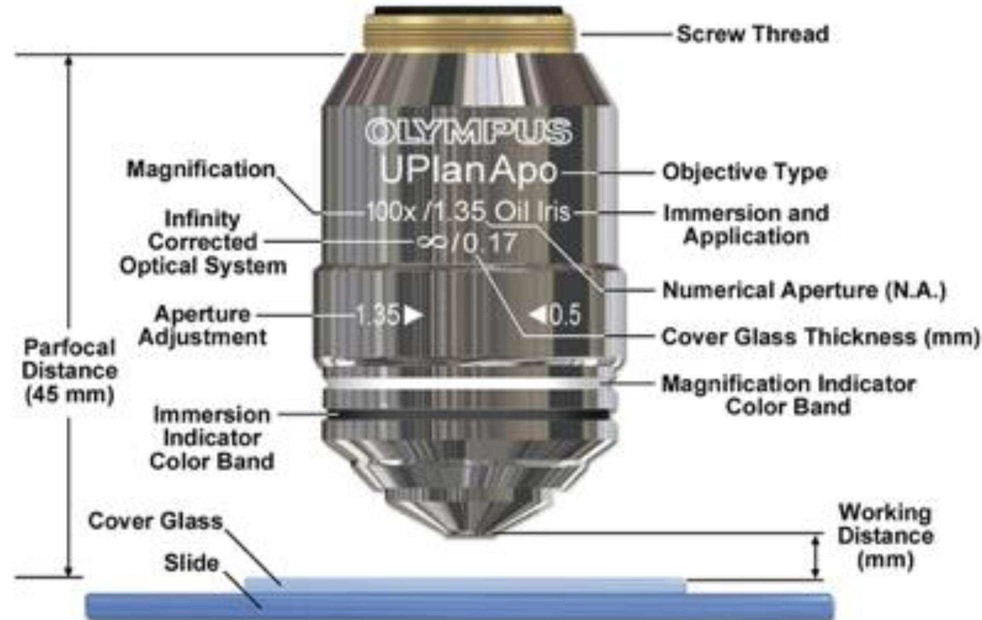

Mag.	1×	2×	4×	10×	20×	40×	50×	60×	100×
Code	Black	Gray	Red	Yellow	Green	Light Blue		Dark Blue	White
Imm. Med.	Oil		Water		Glycerin		Oil/Water/Glycerin		
Code	Black		White		Orange		Red		

1.2.7 Objective designs

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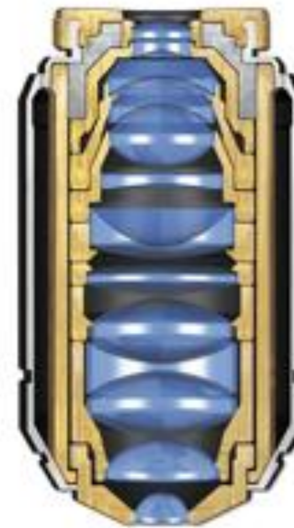
Achromat

Fluorite



Plan Apochromat

Figure 4.11

Objective designs. Apochromatic objectives may contain 12 or more lens elements to give bright, flat images with excellent color correction across the visual spectrum. Fluorite objectives have fewer lens components and produce sharp, bright images. These objectives exhibit excellent color correction and transmit UV light. Achromatic objectives are not as well corrected but are excellent for single color imaging.

More internal lenses
-> reduced aberrations
-> increased cost!

1.2.8 Numerical aperture

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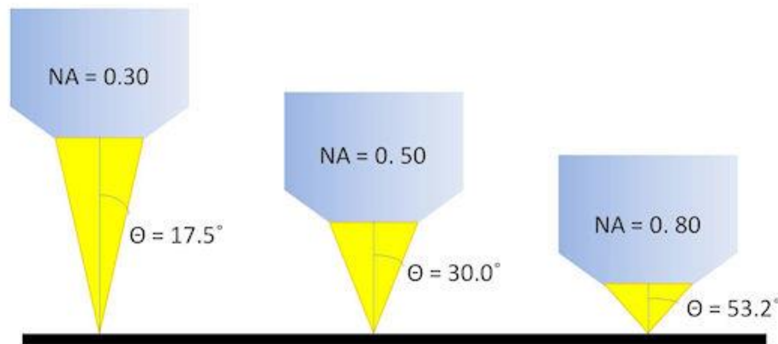
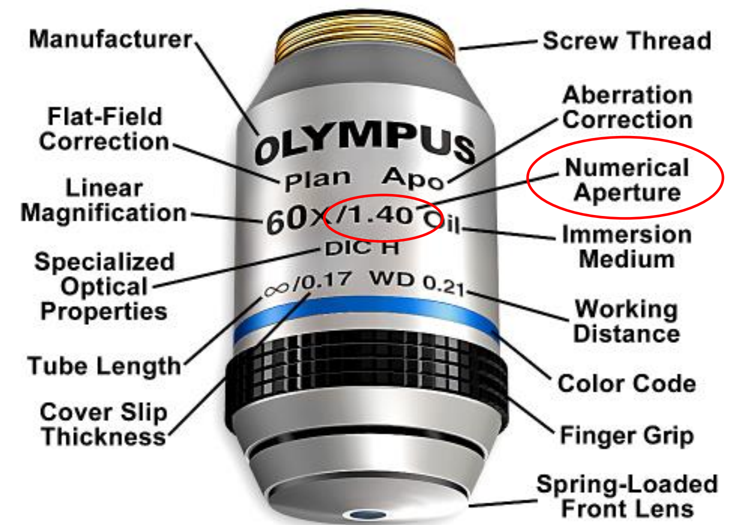
- Number defining the opening angle of an objective
- $NA = n sin\theta$ n = refractive index of immersion medium
- Typically between 0.3 – 1.46 for research microscopes



www.sheffield.ac.uk/kroto



stevegallik.org

1.2.9 Numerical aperture

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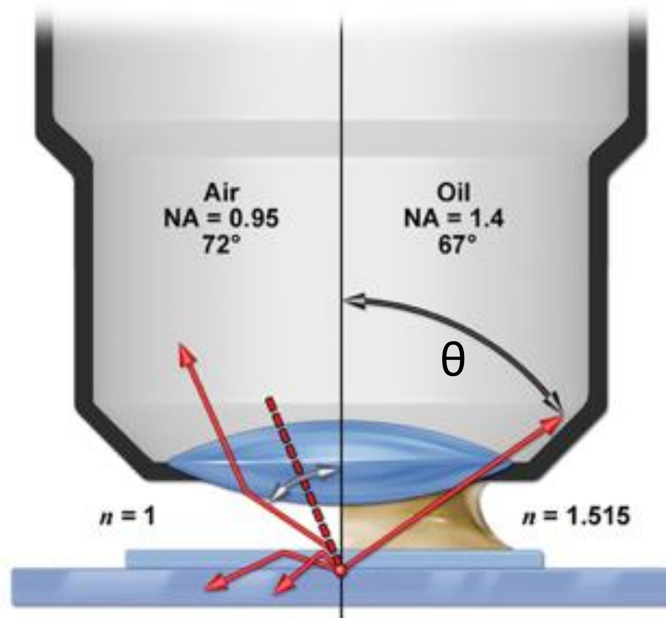

Figure 6.2

Effect of immersion oil on increasing the angular extent over which diffracted rays can be accepted by an objective. For dry objectives, NA is limited, because rays subtending angles greater than about 39° (angle formed at dotted line with the normal) are lost by total internal reflection and never enter the lens (downward deflected red arrows). This compares with an acceptance angle of 67° in the case of an oil immersion lens and accounts for the ability of an oil lens to collect much larger angles of diffracted light. Note, however, that the estimated maximum NA of 0.95 for the dry lens is actually calculated from the acceptance angle of 72° (double-headed arrow). The larger acceptance angle is due to the refraction of light at the air: coverslip interface. Snell's law, the critical angle for total internal reflection, and the refractive indices of the glass, oil, and air are all that are required to calculate these relationships. The newest TIRF objectives designed for use with special immersion oil have NA values of up to 1.49.

$$NA = n \bullet \sin(\theta)$$

1.2.3 Set-up of a simple microscope

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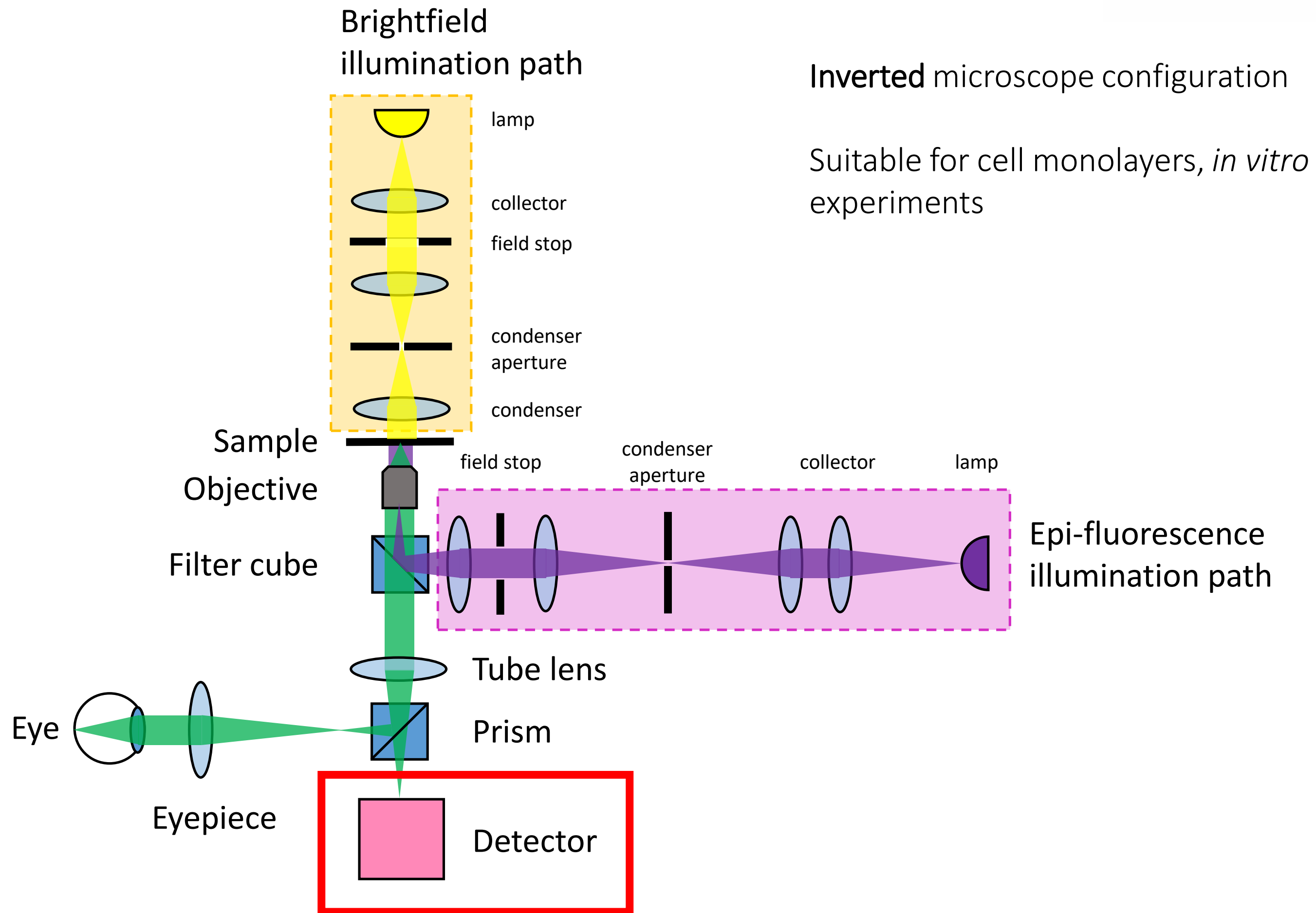

Inverted microscope configuration

Suitable for cell monolayers, *in vitro* experiments

1.2.10 Detectors

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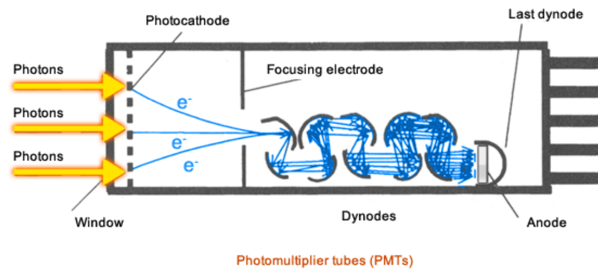
Turns photons into electrons!

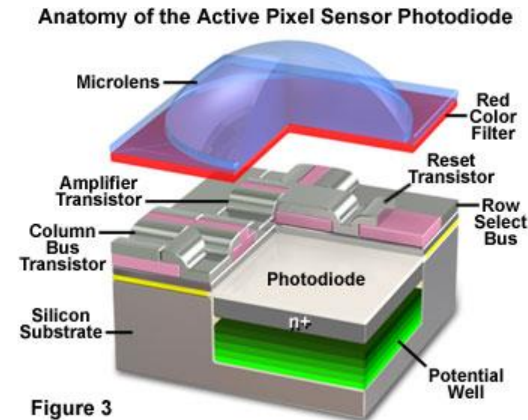
Point detectors i.e. PMTs



ammrf.org.au/myscope/confocal/confocal/lasers.php

Detector arrays i.e. cameras

- Arrays of individual sensors 5-20 μm in size
- Each pixel is a semi-conductor (doped silicon)
- Electrons are produced in proportion to the light intensity according to the photoelectric effect.



olympus-lifescience.com

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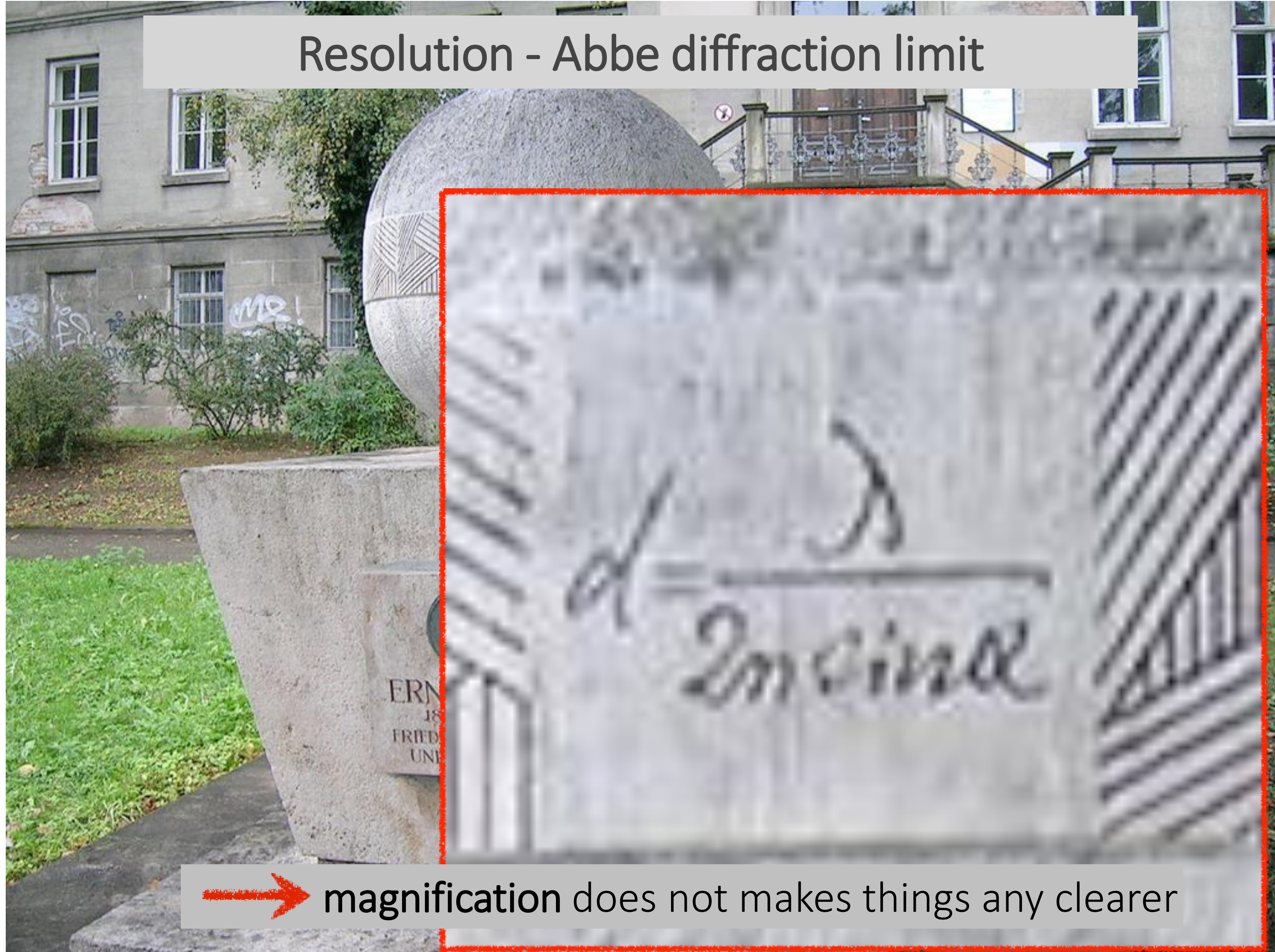
1.3 The resolution limit


Resolution - Abbe diffraction limit
$d = \frac{\lambda}{2n \sin \alpha}$
magnification does not makes things any clearer

1.3.1 The point spread function

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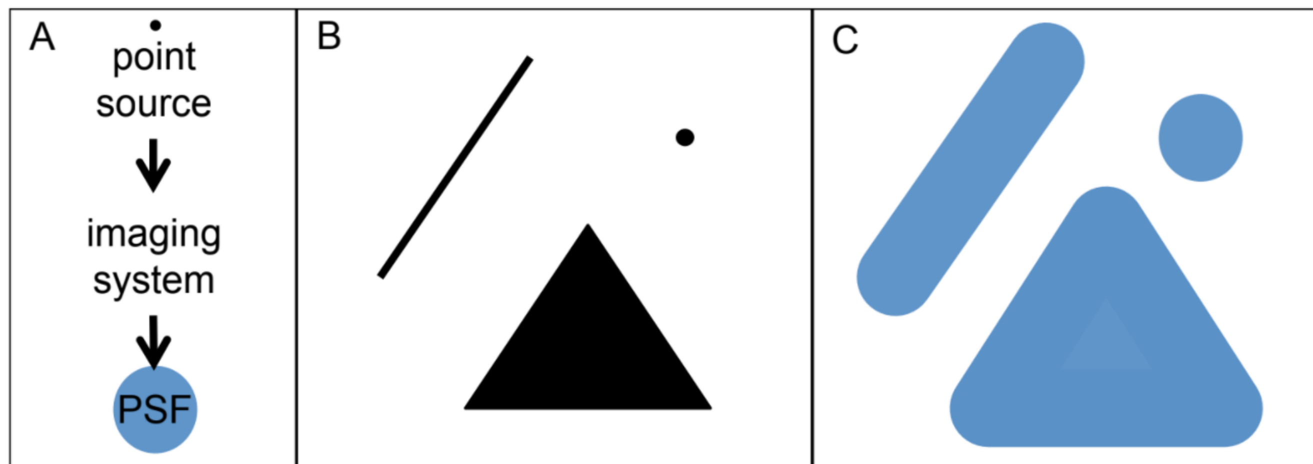
- We cannot focus light to an infinitely small point
- What we detect instead is a point spread function (PSF)
- The PSF acts to blur our image *at every point in the image*



1.3.2 Resolution in light microscopy

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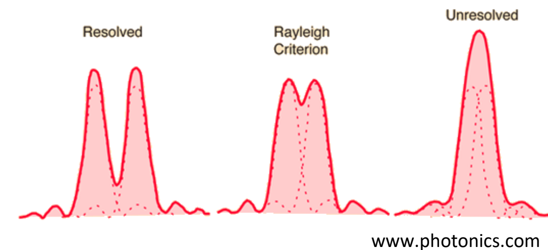
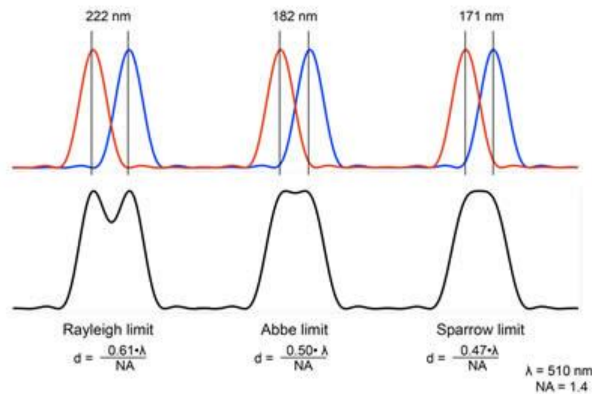
Resolution (r) is the minimum distance at which two points can be separated.



www.photonics.com



advanced-microscopy.utah.edu

3 resolution criteria:

- astronomers use Sparrow
- microscopists typically use Rayleigh

1.3.4 Rayleigh criterion for spatial resolution

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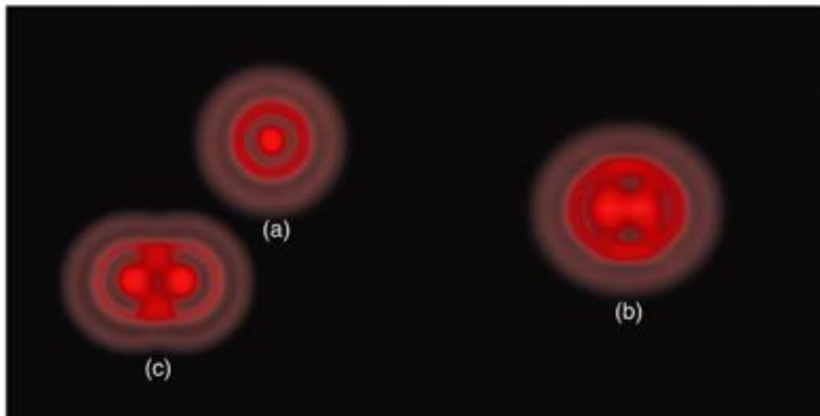

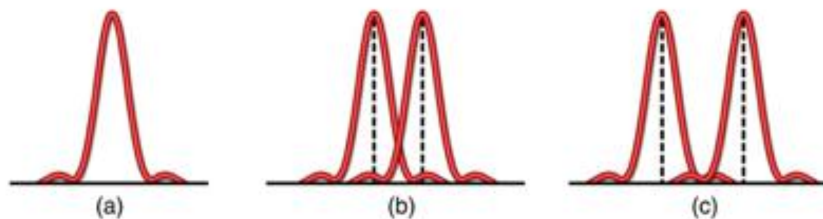

Figure 6.3

Rayleigh criterion for spatial resolution. (a) Profile of a single diffraction pattern: The bright Airy disk and 1st and 2nd order diffraction rings are visible. (b) Profile of two disks separated at the Rayleigh limit such that the maximum of a disk overlaps the first minimum of the other disk: The points are now just barely resolved. (c) Profile of two disks at a separation distance such that the maximum of each disk overlaps the second minimum of the other disk: The points are clearly resolved.

$$r = 0.61 \bullet \lambda \bullet NA^{-1}$$

r is the minimal distance of two closely spaced diffraction spots that can be resolved

1.3.4 PSF and resolution

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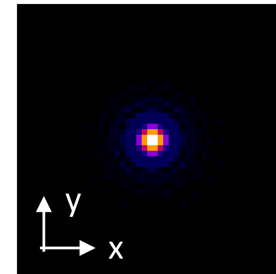
- Broader PSFs lead to worse resolution
- The Rayleigh criterion:

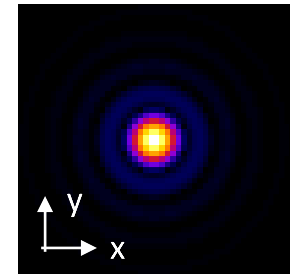
$$r = \frac{0.61\lambda}{NA}$$

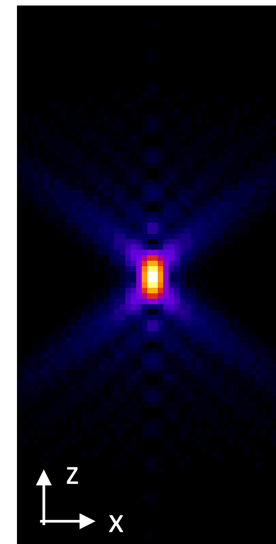
λ = wavelength of light

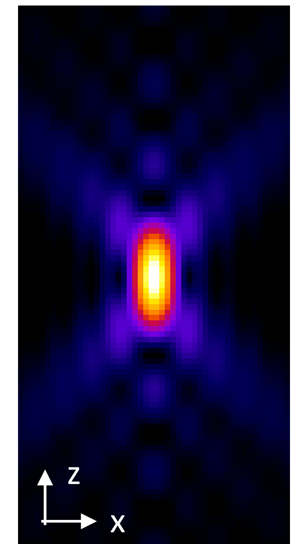
NA = numerical aperture of the objective

γ = 0.7







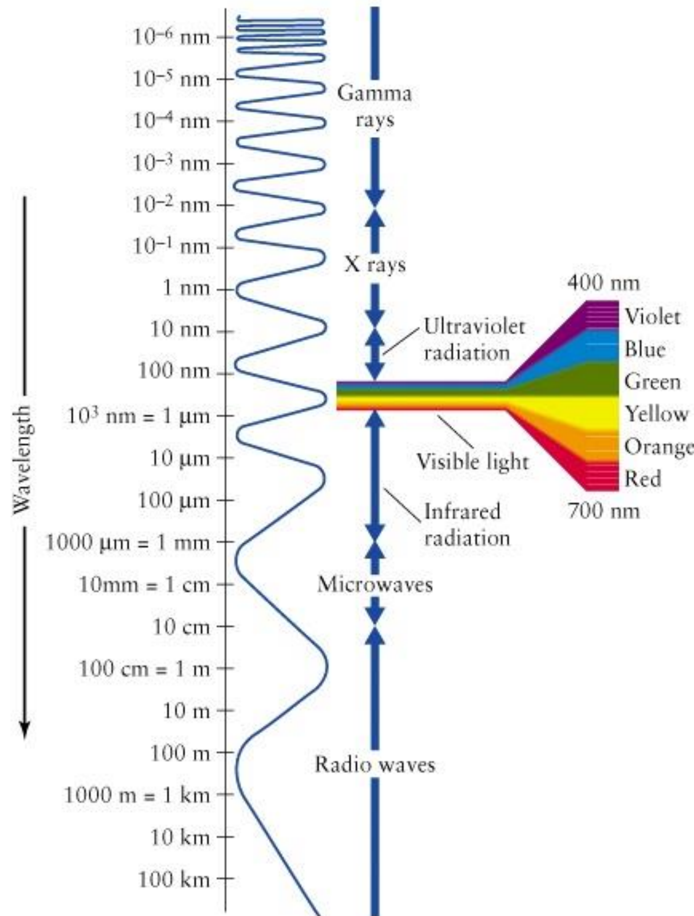


1.4 NA oil objective

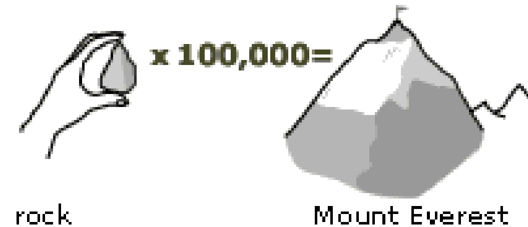
0.75 NA air objective

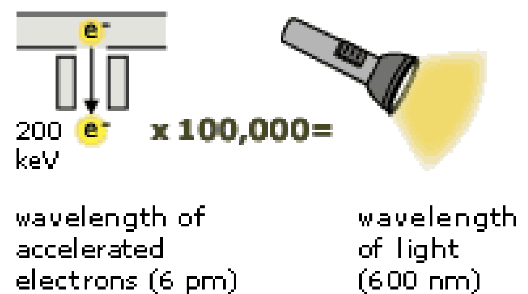
PSFs calculated using PSF Generator in Fiji

1.3.5 Resolution – correlates with wavelength



$$d = \frac{0.61\lambda}{NA}$$





1.3.5 NA and resolution

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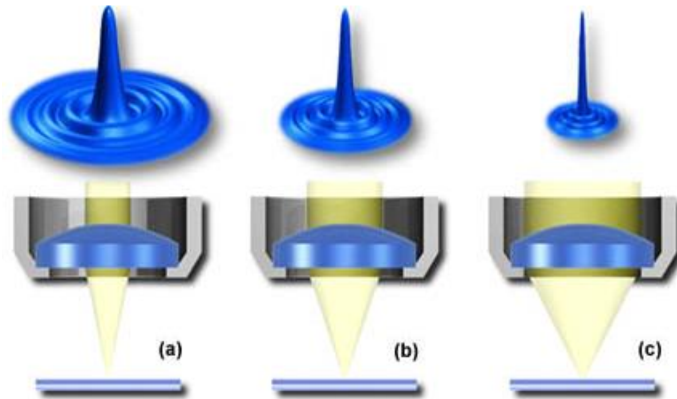
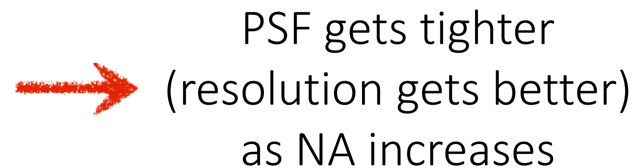


The Figure illustrates the effect of numerical aperture on the size of Airy disks imaged with a series of hypothetical objectives of the same focal length, but differing numerical apertures. With small numerical apertures, the Airy disk size is large, as shown in (a). As the numerical aperture and light cone angle of an objective increases however, the size of the Airy disk decreases as illustrated in(b) and (c).

The Airy disk is an expression for the point-spread function amplitude and intensity of a perfect instrument, free of aberration.



$$NA = n \cdot sin\theta$$

MICROSCOPY RESOURCE CENTER
OLYMPUS

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Recap of Section 1

- Properties of light
  - What is light? Waves and particles
  - Refraction, diffraction and interference
- Light microscopy theory
  - From refraction to magnification
  - Objectives
  - Detectors
- The resolution limit
  - The point spread function
  - Resolution and wavelength
  - Resolution and NA

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2. Different types of light microscope

2.1 Brightfield

2.2 Fluorescence microscopy

2.3 Volumetric microscopy

2.4 Super-resolution

2.1 Brightfield

1.2.3 Set-up of a simple microscope

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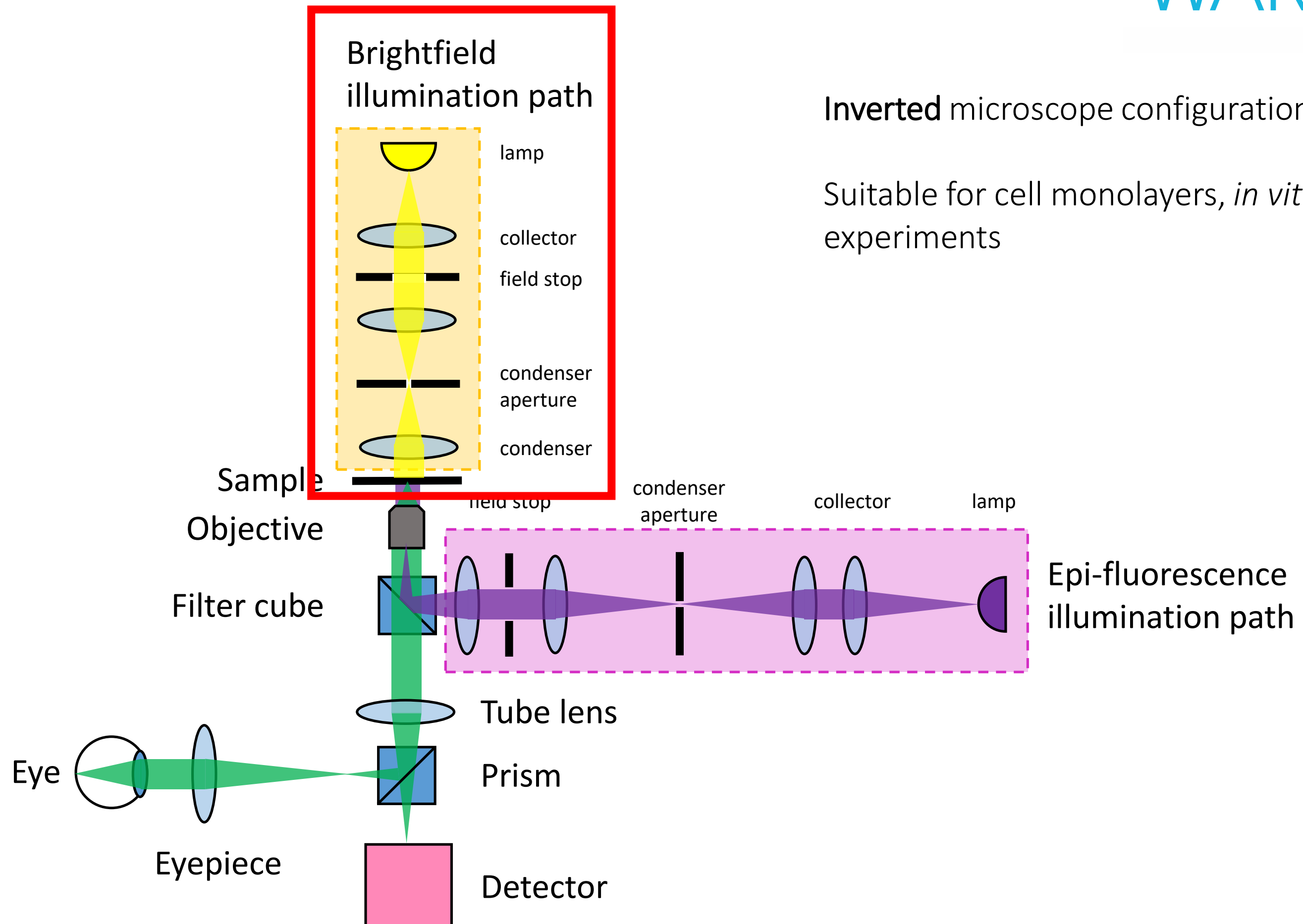

Inverted microscope configuration

Suitable for cell monolayers, *in vitro* experiments

2.1.1 Label-free imaging

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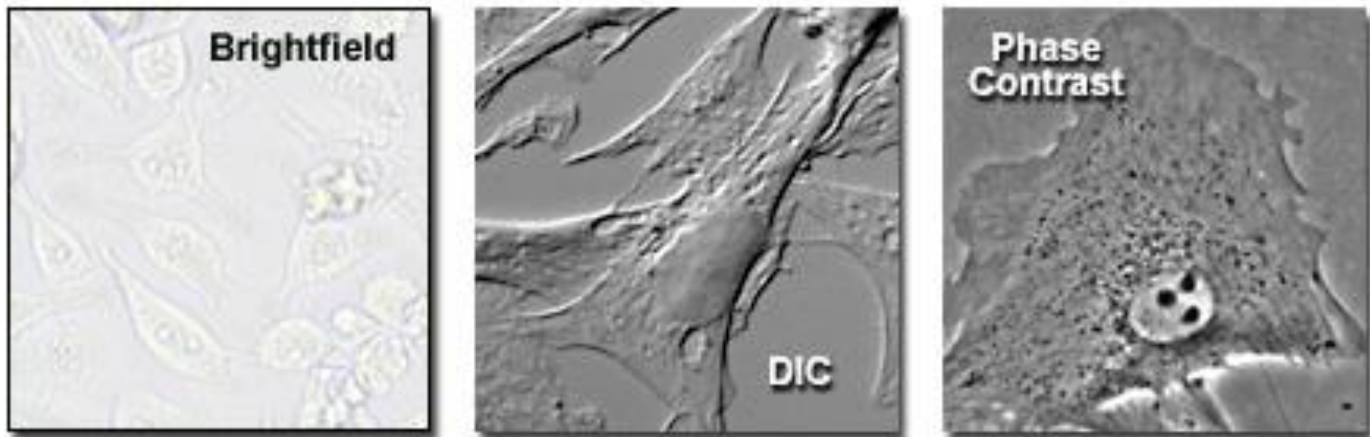
- Cells and other biological samples are quite transparent and difficult to see.
- Turning phase shifts into variations in intensity/amplitude
- Introduction to **phase contrast** and **differential interference contrast**.



zeiss-campus.magnet.fsu.edu/articles/livecellimaging/techniques.html

2.1.2 Phase contrast

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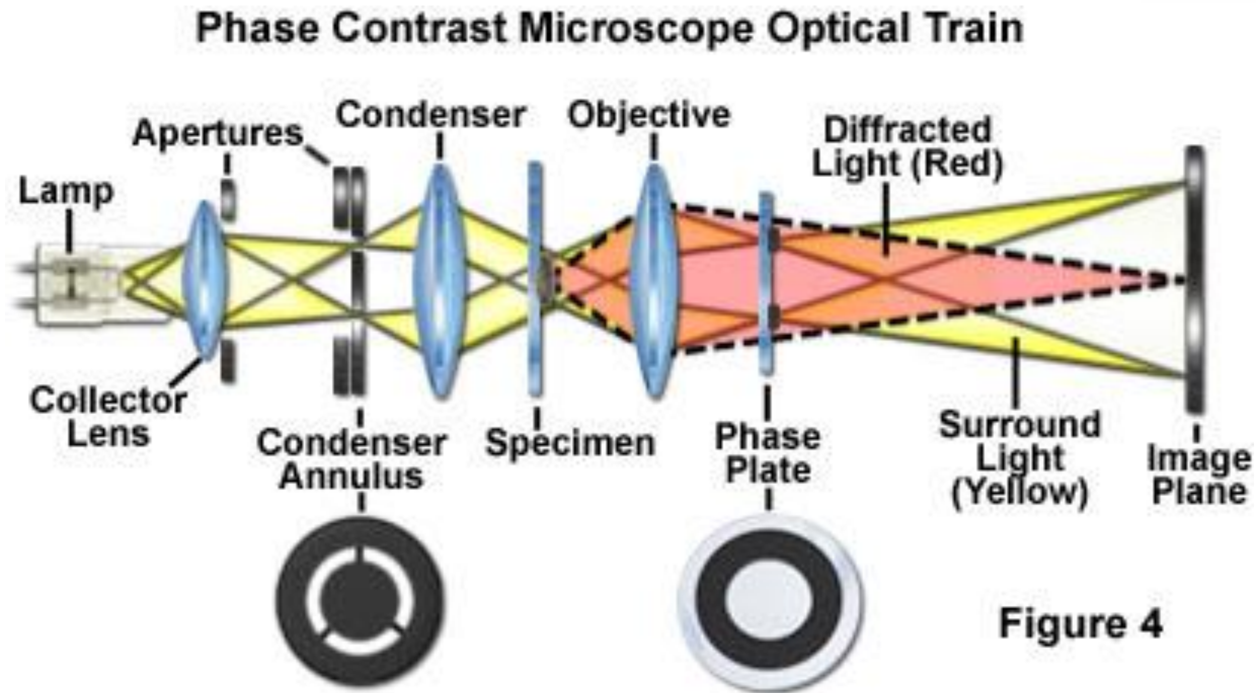
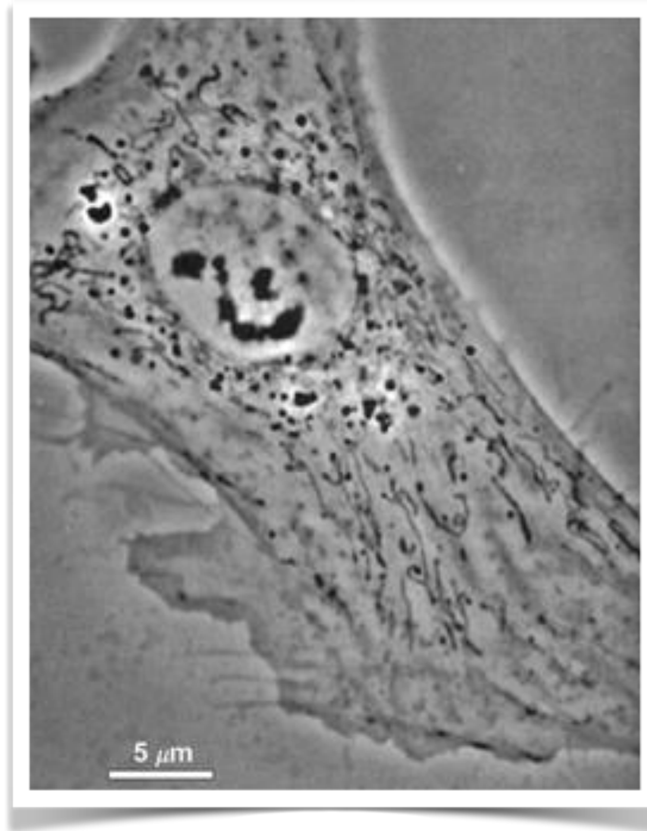


www.microscopyu.com

Pros:

- Low cost
- Less susceptible to poor alignment

2.1.3 Phase contrast



2.1.4 Differential interference contrast (DIC)

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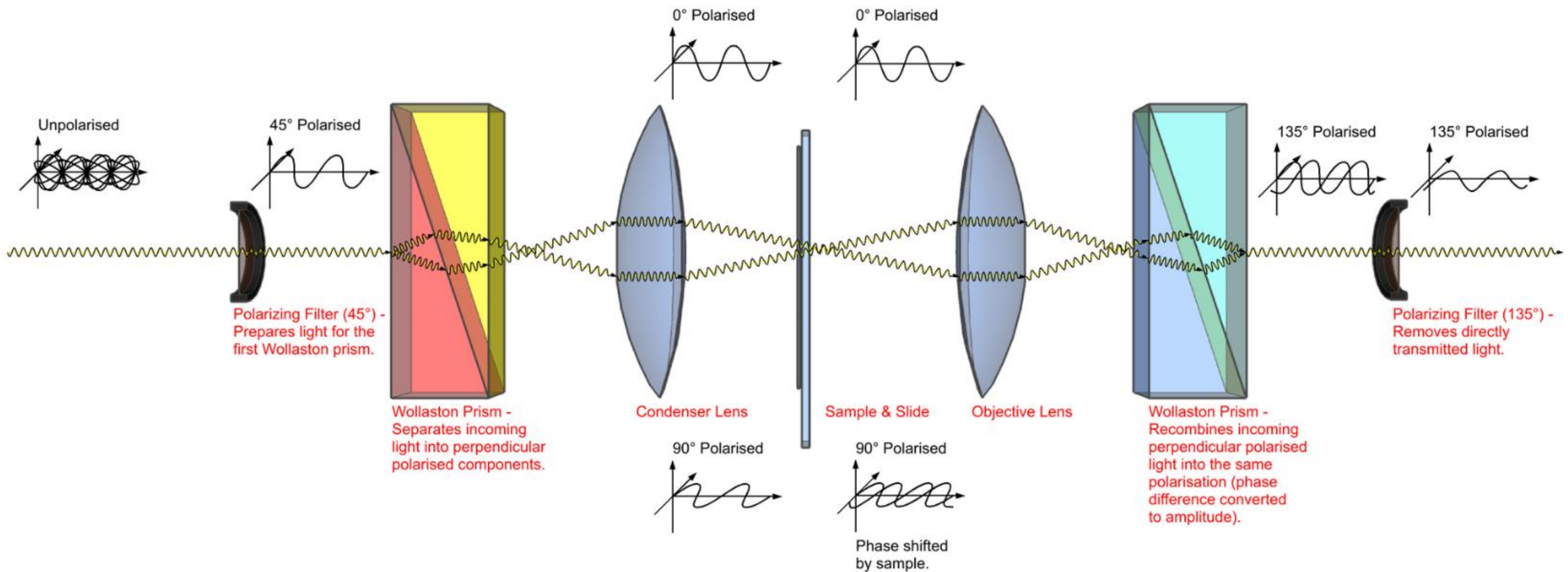

Pros:

- No halos
- 3D effect

bio.libretexts.org

2.1.5 Differential Interference Contrast

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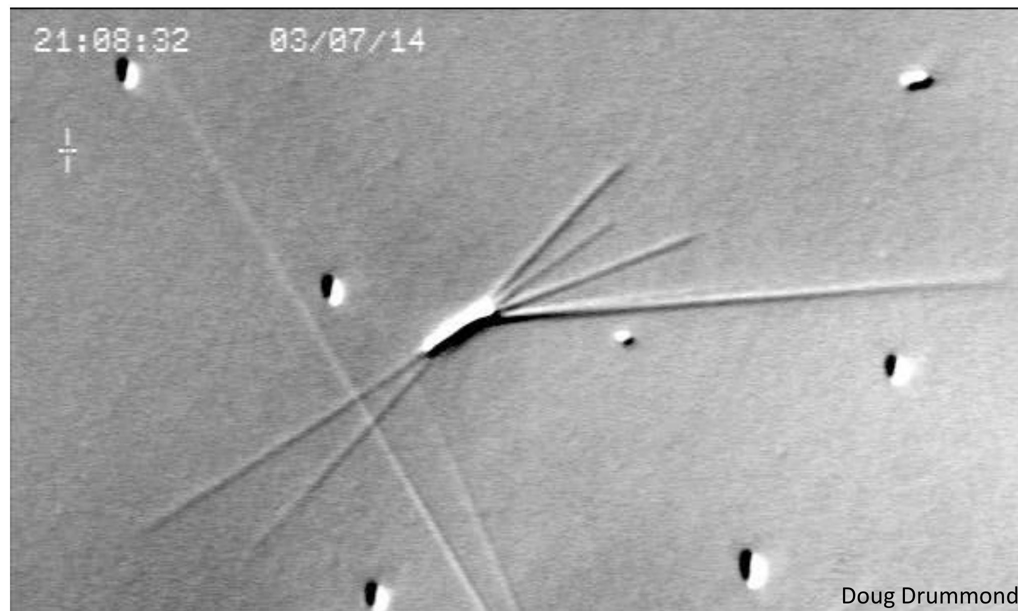
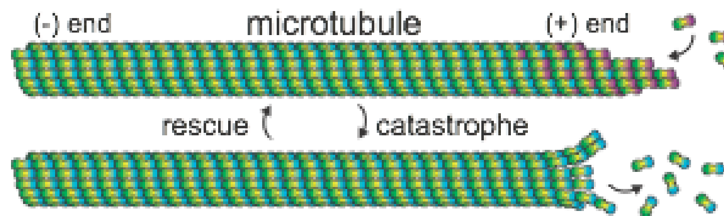


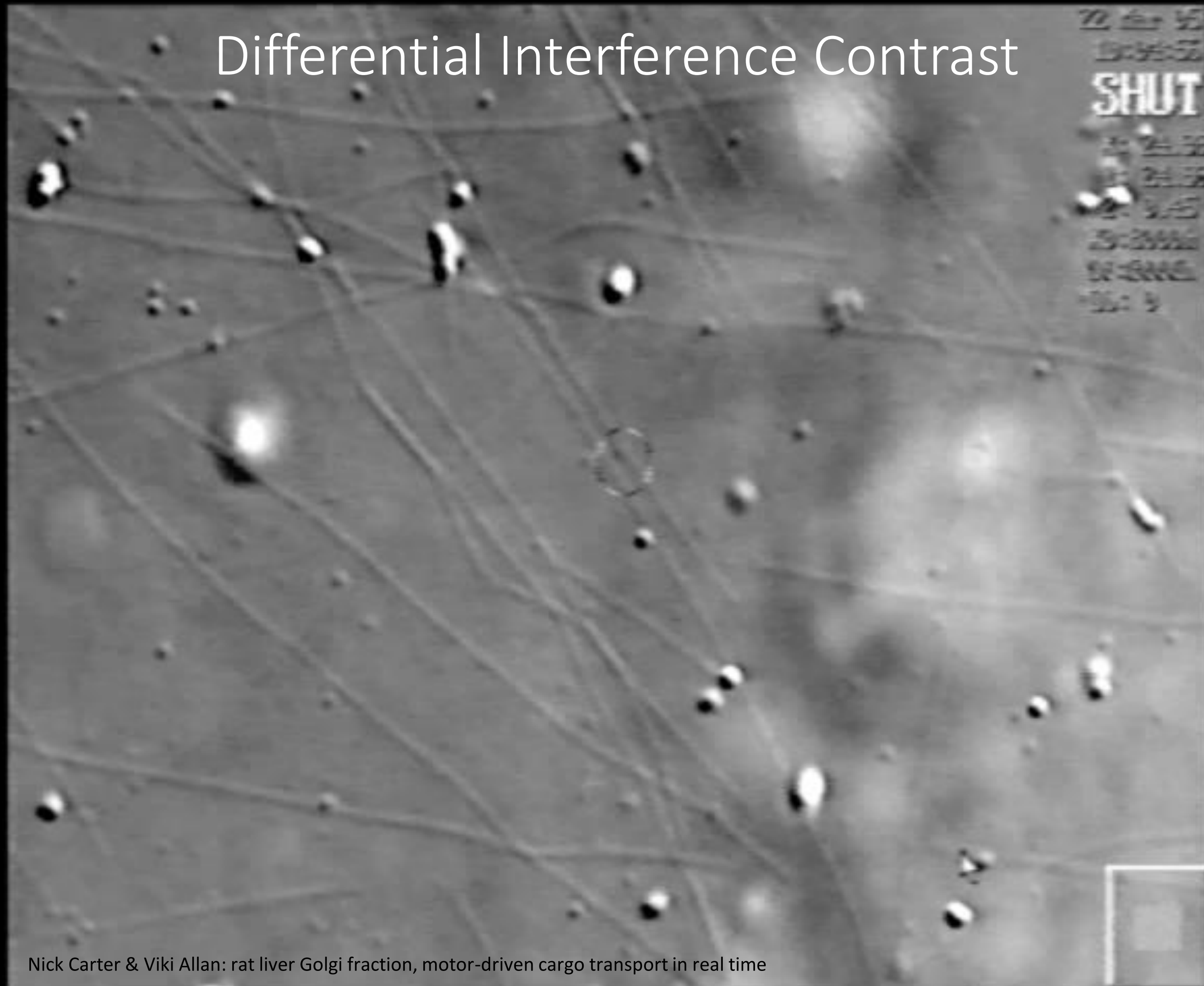
Doug Drummond



Anne Straube

Differential interference contrast visualises objects based on a difference in their refractive index. These microtubules are only 25nm wide, but appear to be about 300nm wide. When two move close together, they appear as one.

Differential Interference Contrast



Nick Carter & Viki Allan: rat liver Golgi fraction, motor-driven cargo transport in real time

2.1.6 Transmission techniques

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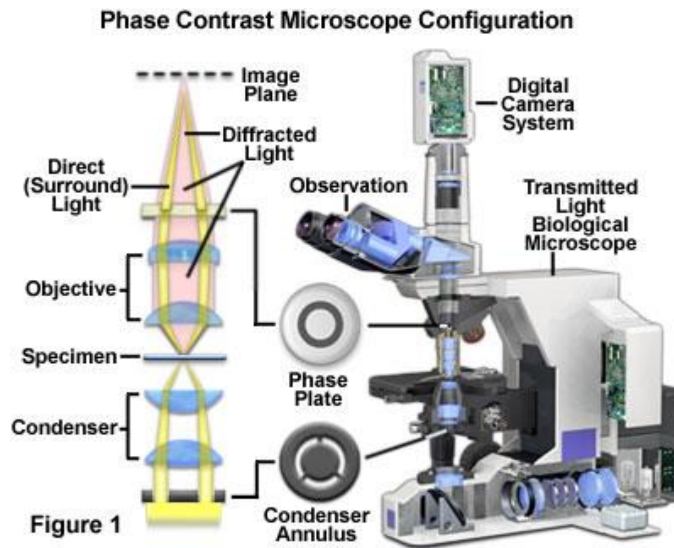

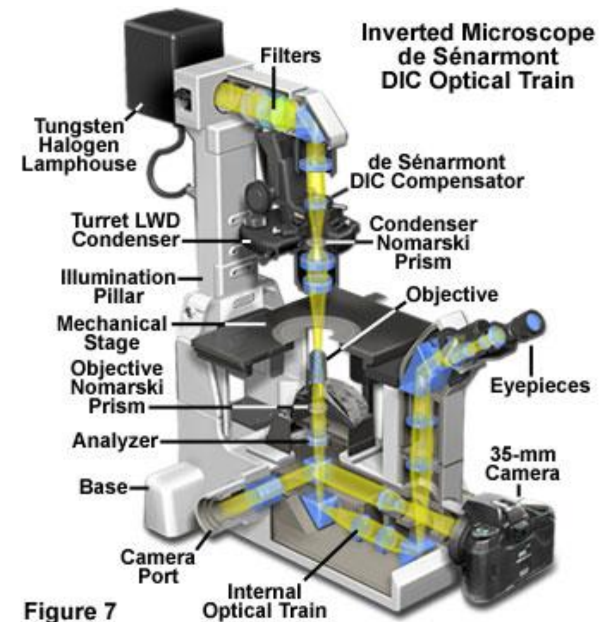

Pros:

- Low cost
- Less susceptible to poor alignment

Pros:

- No halos
- 3D effect

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Nikon
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2.2 Fluorescence microscopy

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1.2.3 Set-up of a simple microscope

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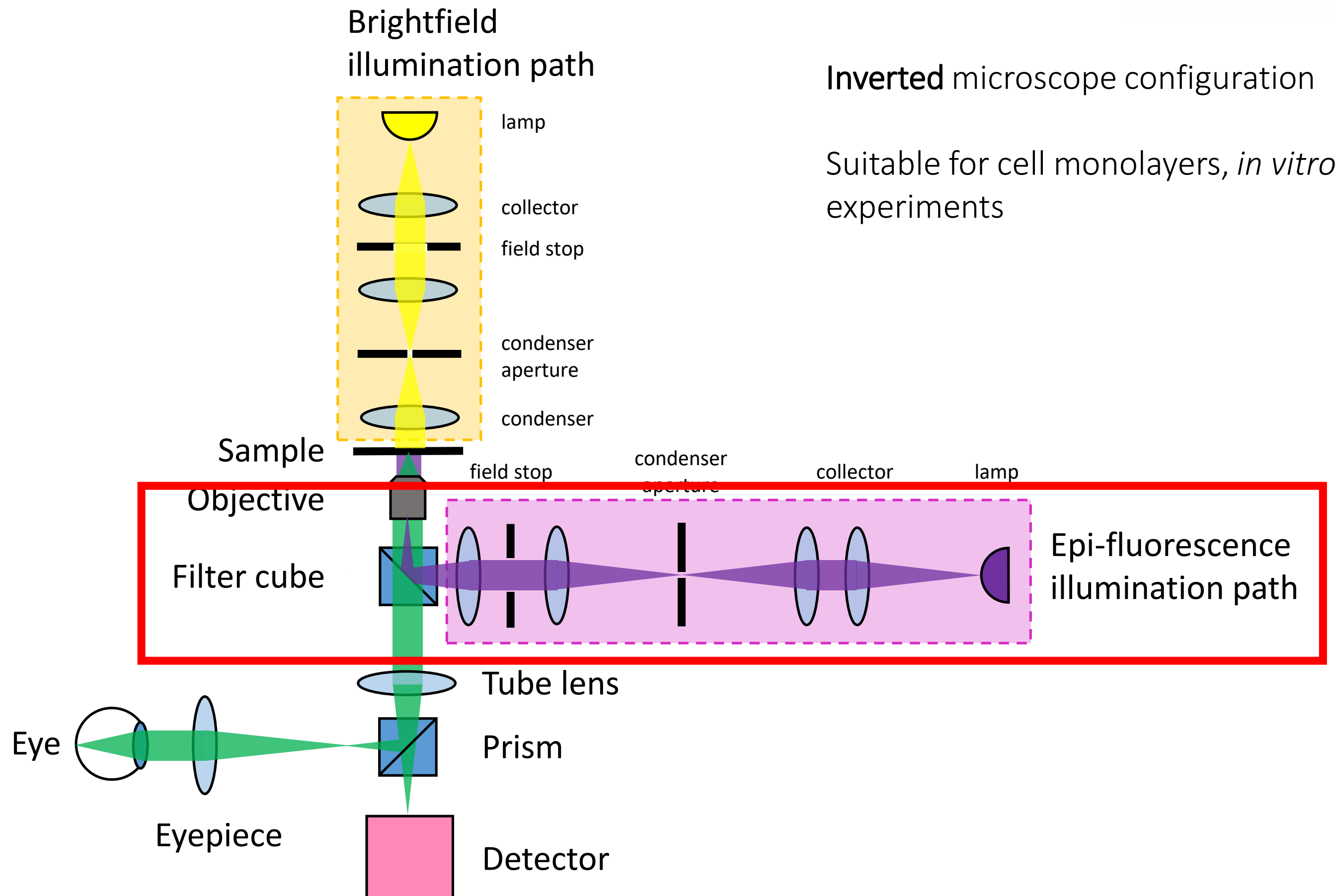
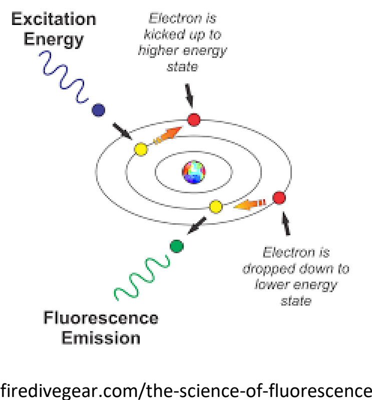


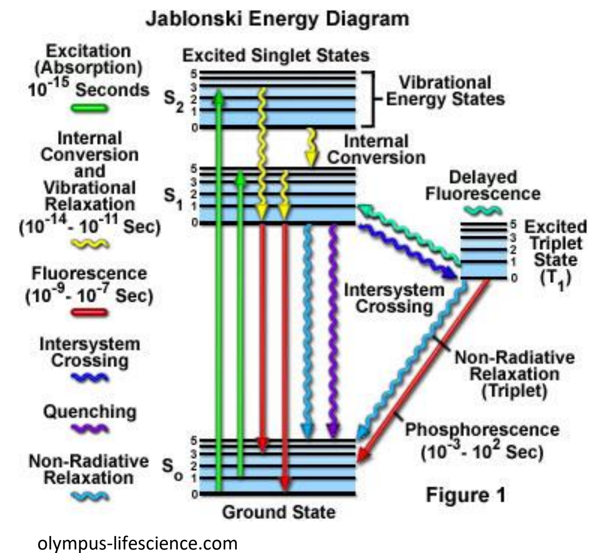
2.2.1 What is fluorescence?

WARWICK

An atom/molecule absorbs a photon and an electron is promoted to a higher energy level



firedivegear.com/the-science-of-fluorescence



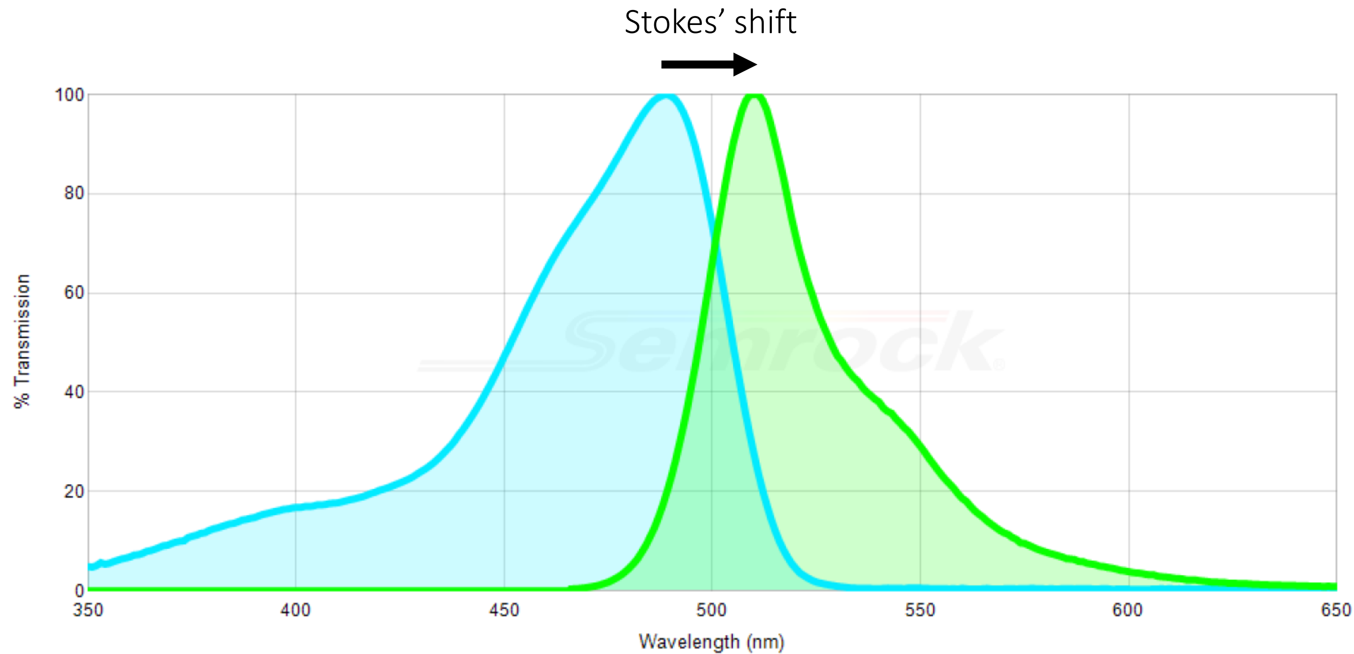
olympus-lifescience.com

Some energy is lost through molecular collisions (internal conversion)

The electron moves back to the ground state, a lower energy (longer wavelength) photon is emitted

2.2.2 Fluorescence excitation/emission spectrum

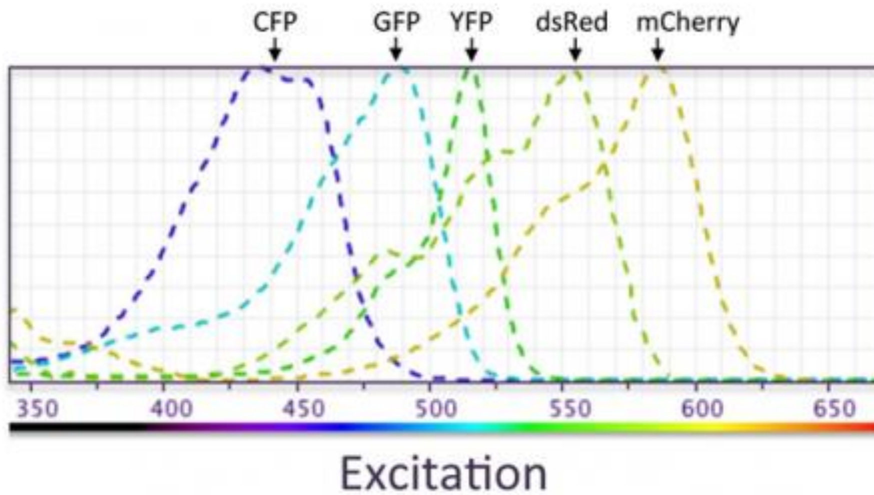
WARWICK

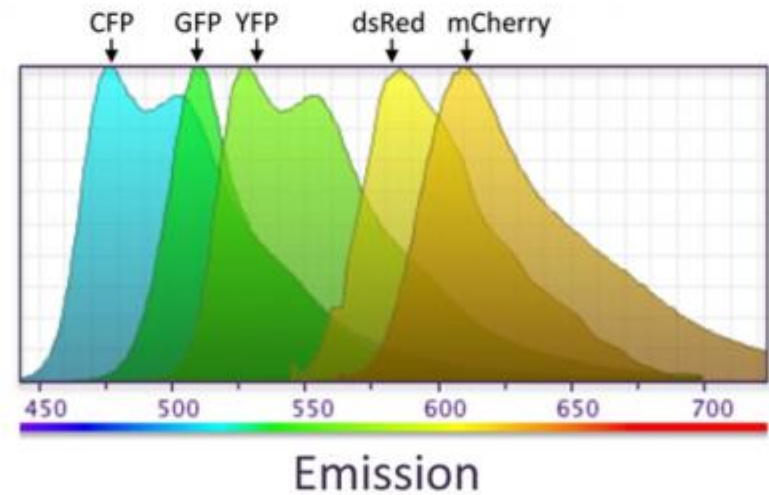


Generated using searchlight.semrock.com

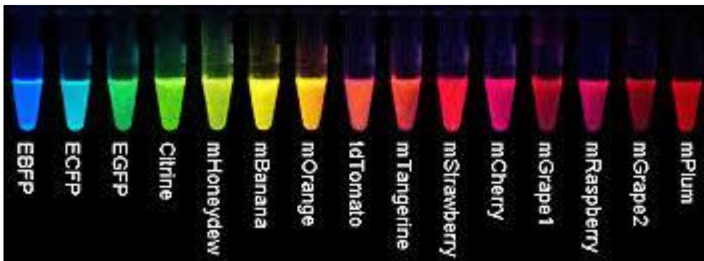
Excitation/emission spectrum of green fluorescent protein (GFP)

2.2.3 Multicolour fluorescence

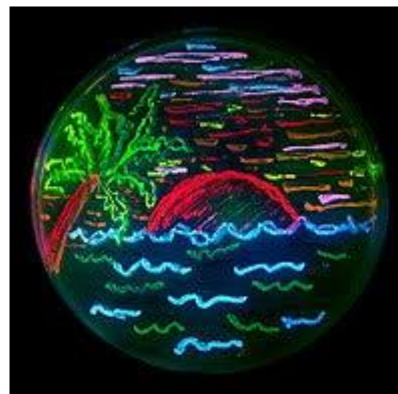




www.lifesci.dundee.ac.uk



www.conncoll.edu



Wikipedia

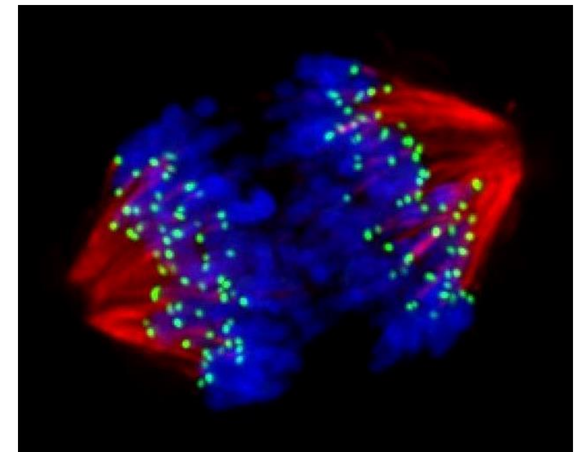
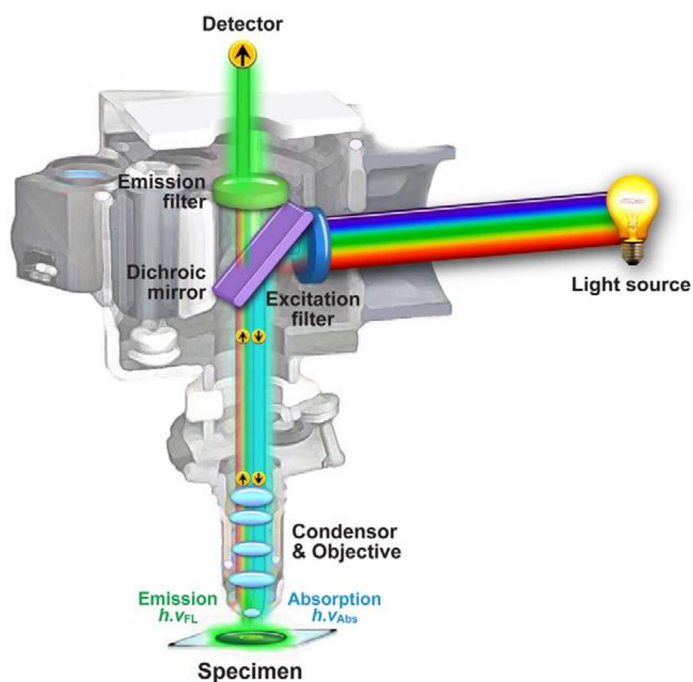


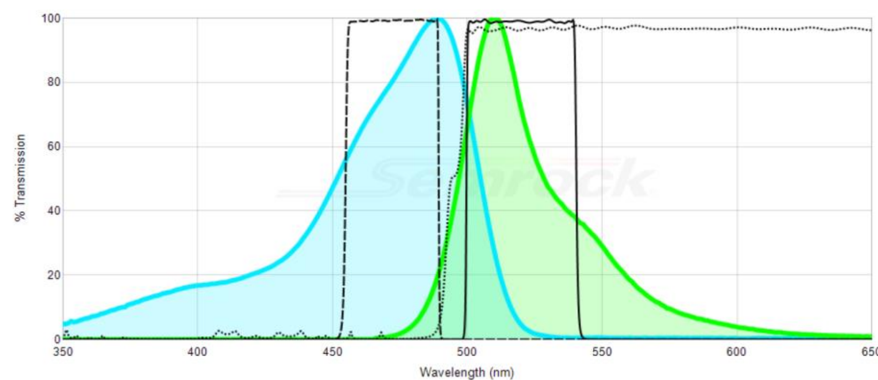
Image taken by Phil Auckland of CMCB, red is microtubules, blue is DNA and green is kinetochores

2.2.4 How do we filter light?

WARWICK



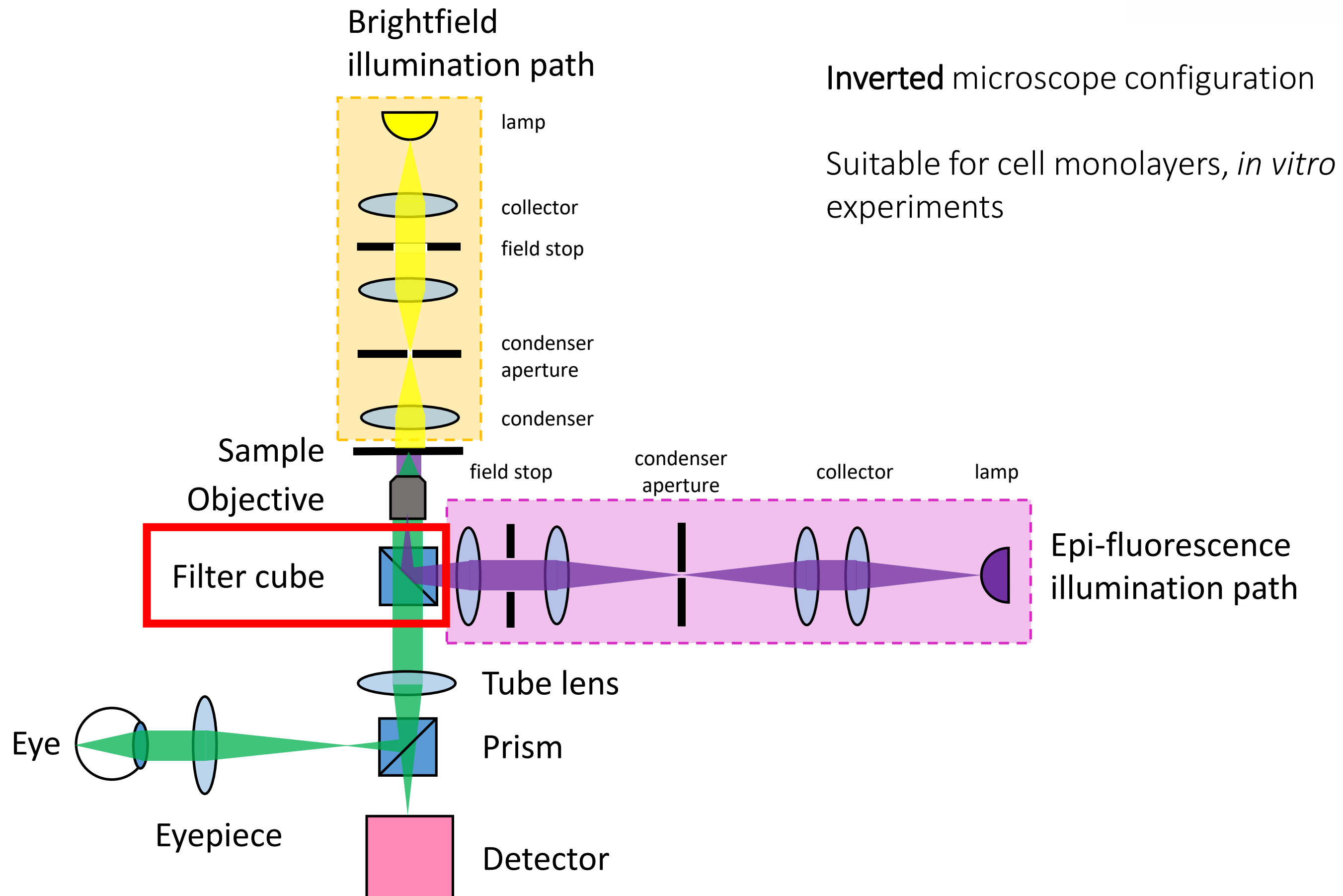
Ishikawa-Ankerhold et al, Molecules 17(4):4047-132. (2012)



Generated using searchlight.semrock.com

1.2.3 Set-up of a simple microscope

WARWICK



Inverted microscope configuration

Suitable for cell monolayers, *in vitro* experiments

2.2.5 Fluorophore Excitation

WARWICK

%T

100
90
80
70
60
50
40
30
20
10
0

350 400 450 500 550 600 650 700 750 800

Wavelength (nm)

ZET405/488/561/647x
ZT405/488/561/647rpc
ZET405/488/561/647m-TRF

EGFP Excitation Spectra
EGFP Emission Spectra

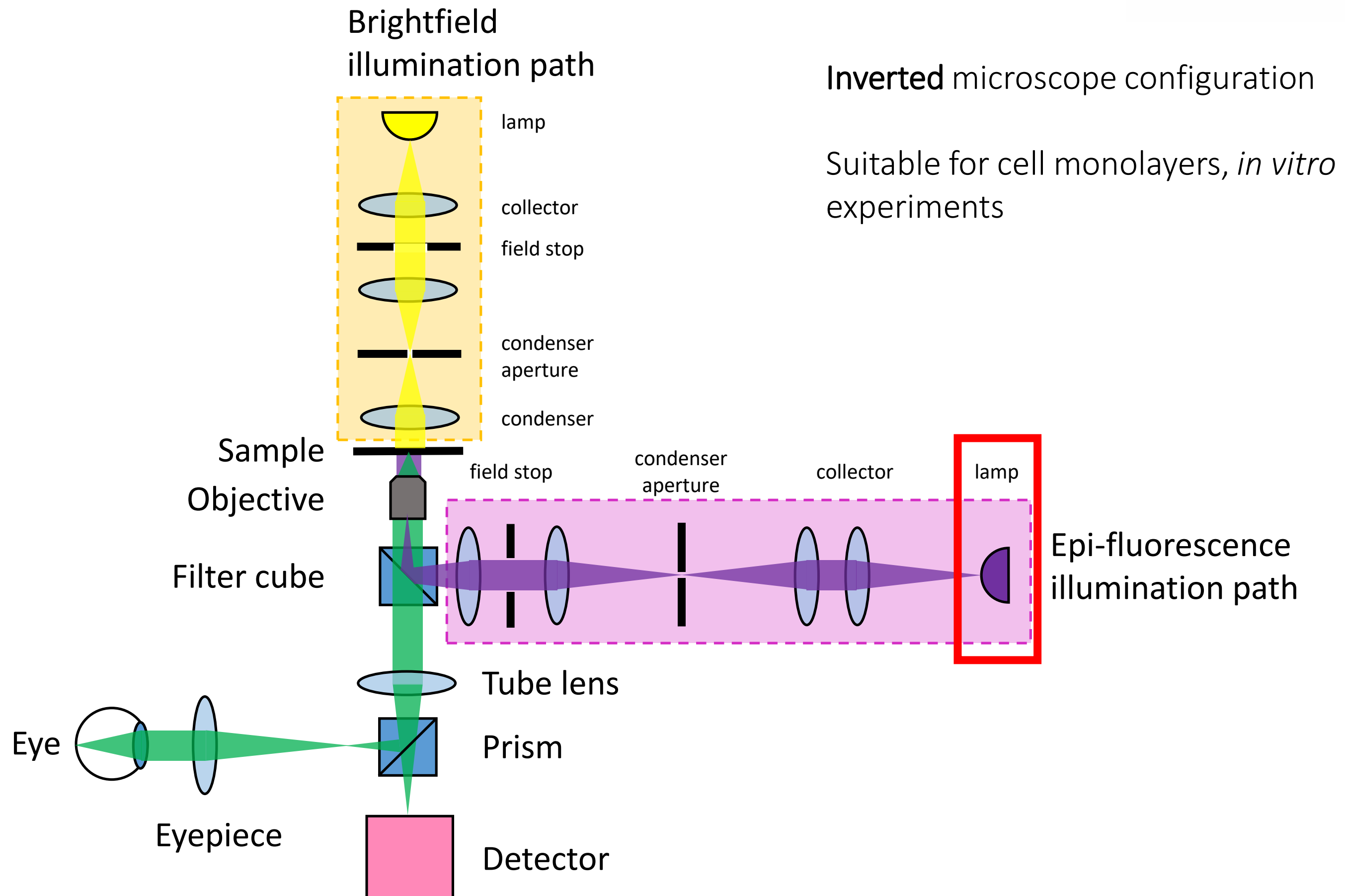
Alexa Fluor 568™ Excitation Spectra
Alexa Fluor 568™ Emission Spectra

Alexa Fluor 647™ Excitation Spectra
Alexa Fluor 647™ Emission Spectra

TRF89902 - ET - 405/488/561/647nm Laser Quad Band Set for TIRF applications

1.2.3 Set-up of a simple microscope

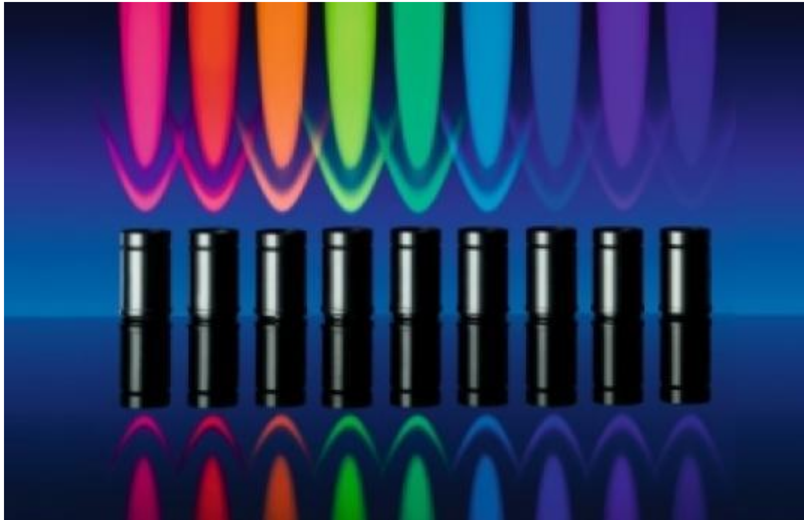
WARWICK



Inverted microscope configuration

Suitable for cell monolayers, *in vitro* experiments

2.2.6 Fluorescence microscopy excitation sources



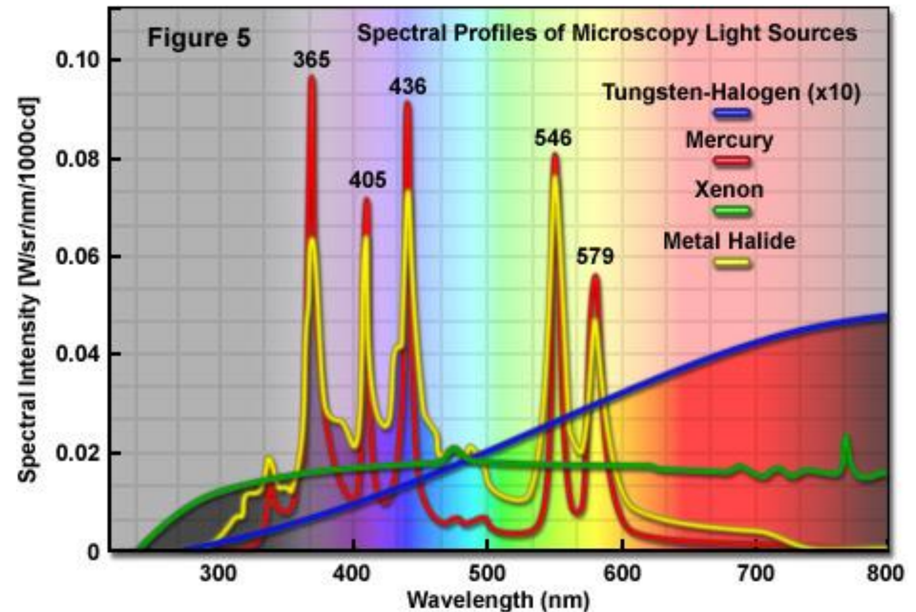
microscopy-analysis.com

- Lamps
- LEDs
- Lasers

2.2.7 Lamps

WARWICK

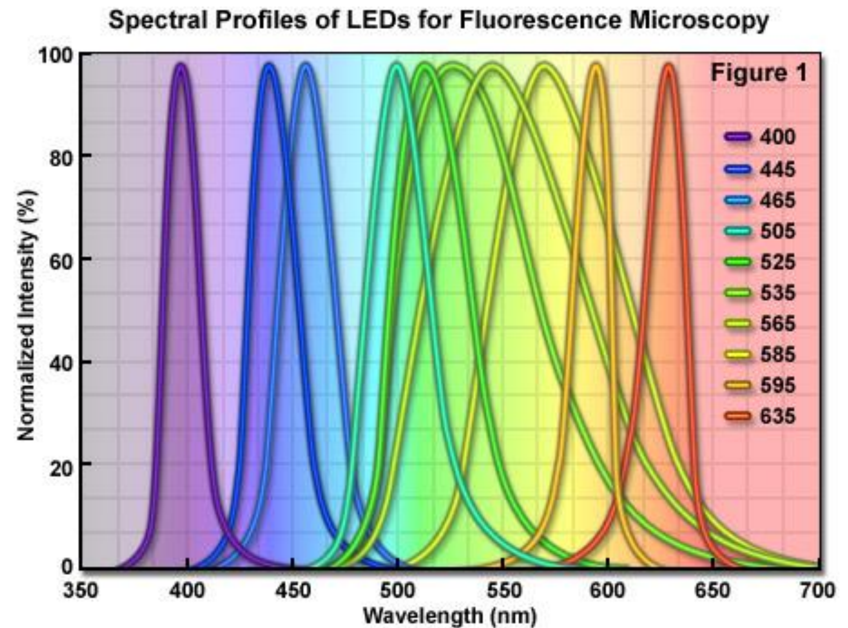
- Broad spectrum lamps which are filtered down using excitation filters
- Limited lifetime
- Need time to warm up and cool down



2.2.8 LEDs

WARWICK

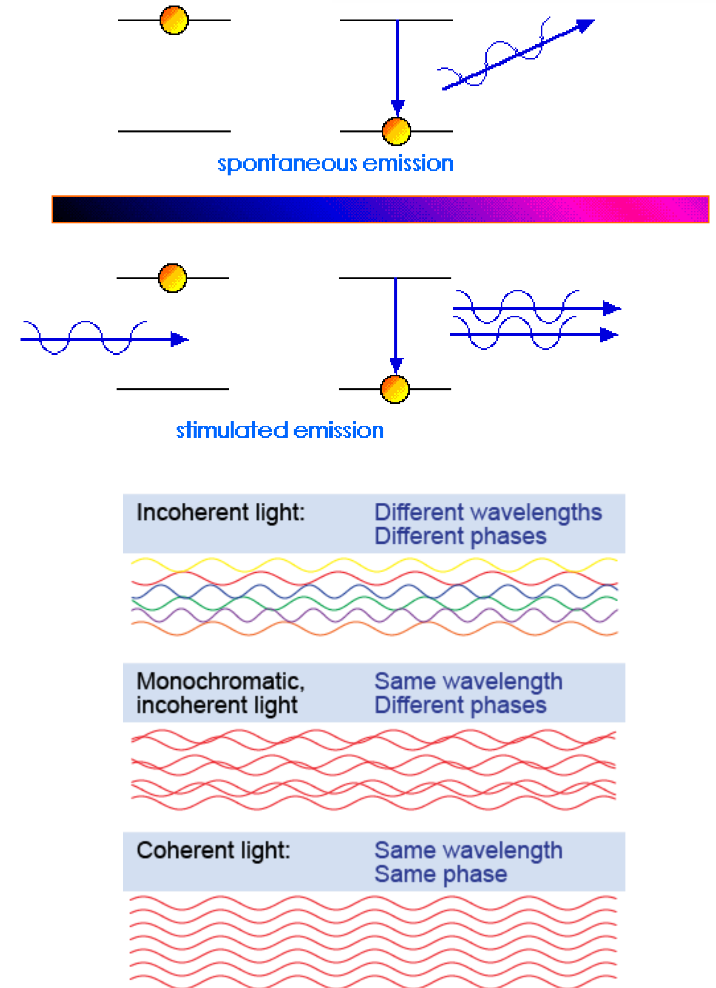
- Long lifetime
- Relatively narrow spectra
- Typical light engine might consist of several LEDs covering a range of wavelengths
- Quick switching on/off



2.2.9 Lasers

WARWICK

- Use stimulated emission to create a beam of coherent (in phase) photons
- Single wavelength emission for precise excitation without excitation filters
- Coherence means can focus down to a smaller point
- Higher power possible
- High cost and laser safety protocols required



education.pasco.com/epub/PhysicsNGSS/BookInd-809.html

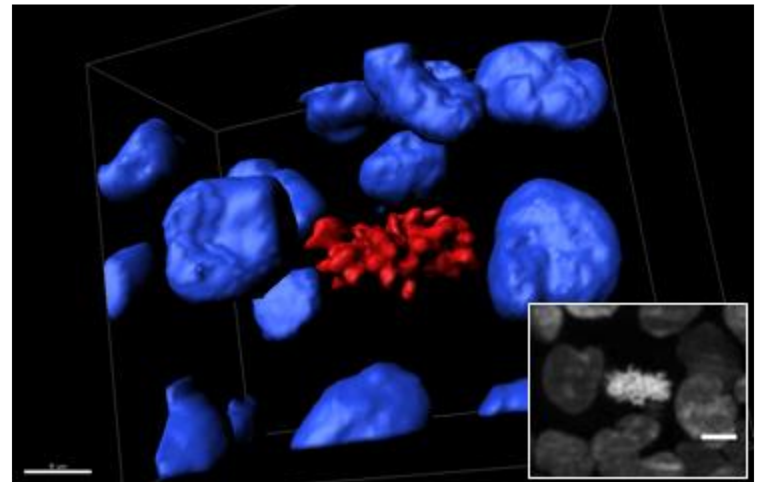
https://www.ux1.eiu.edu/~cfadd/1160/Ch29Atm/Laser.html

2.3 Volumetric microscopy

2.3 Volumetric microscopy

WARWICK

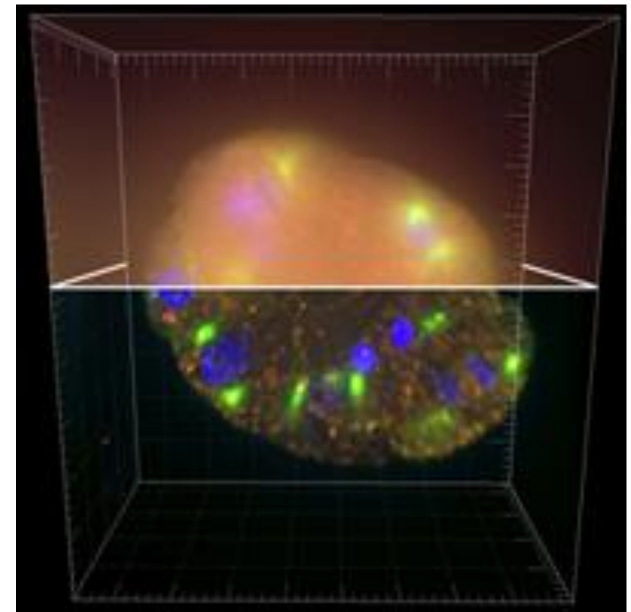
- Biology happens in 3D!
- Microscope objectives collect light from above and below the focal plane
- Better NA -> better sectioning
- Microtomes can be used for thick tissue
- We need optical methods to improve optical sectioning to build up volumetric information



www.bitplane.com

2.3.1 Deconvolution

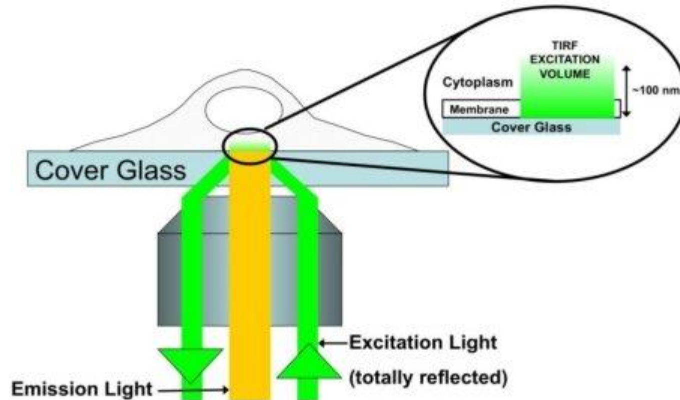
- Computationally restore light back to the correct plane
- Improves SNR and axial resolution
- Several methods, the more computationally intensive, generally the better
- Can cause artifacts



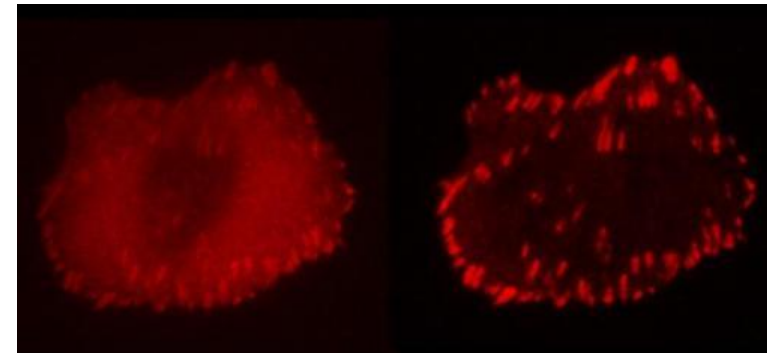
bigwww.epfl.ch

2.3.2 Total internal reflection fluorescence (TIRF)

WARWICK



Fogarty et al, Viruses 3(6): 770-93 (2011)

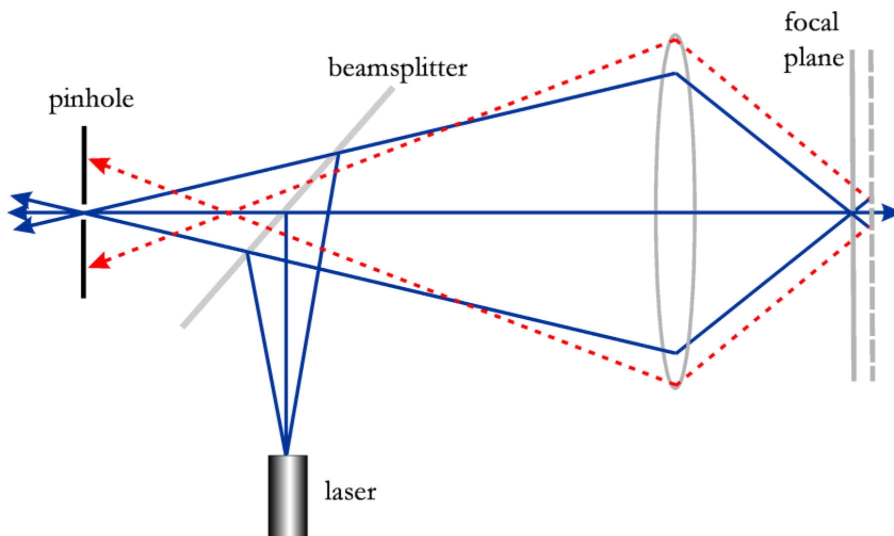


sharedresources.fredhutch.org

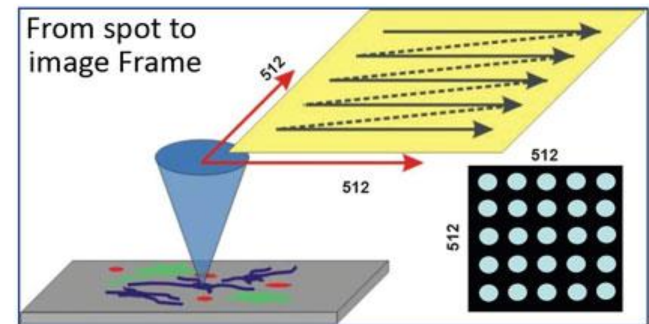
- Excites 100 nm closest to the coverslip
- V. High axial resolution
- Poor penetration depth

2.3.3 Confocal microscopy

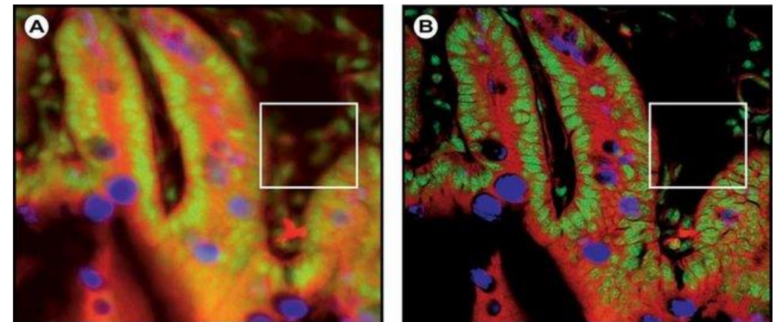
WARWICK



li155-94.members.linode.com/myscope/images/confocal/confoc1.png



microscopist.co.uk/wp-content/uploads/2017/04/confocal-working.jpg



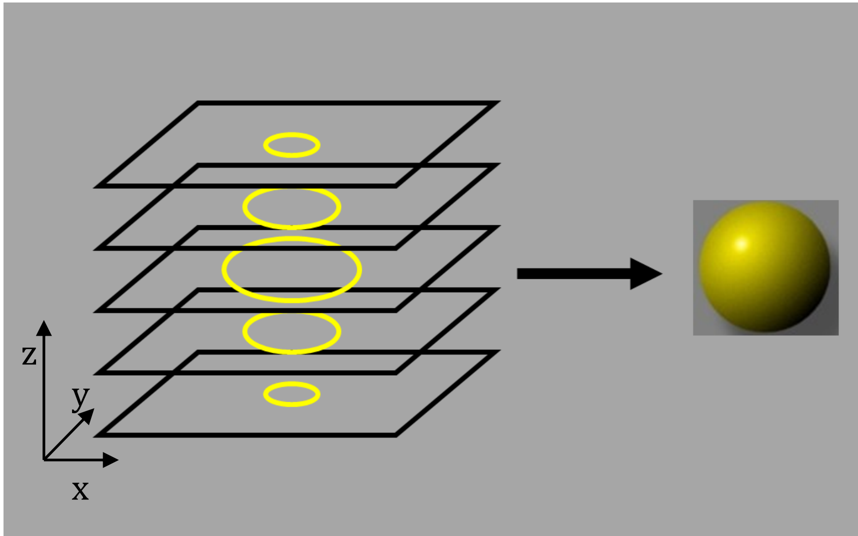
Ishikawa-Ankerhold et al, Molecules 17(4):4047-132 (2012)

Several point-scanning confocals available through the SLS Imaging Suite

2.3.4 Capturing 3D light microscopy data

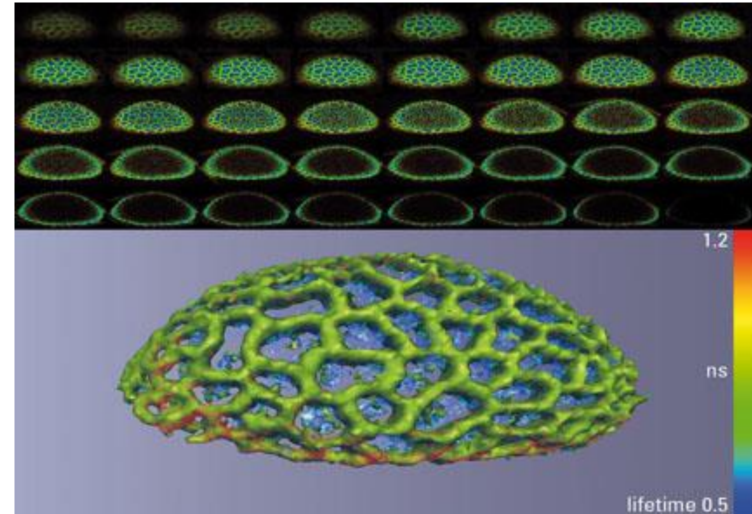
WARWICK

Take optical sections at evenly spaced axial intervals – a "z-stack"



cam.facilities.northwestern.edu/files/2014/07/Picture-17.png

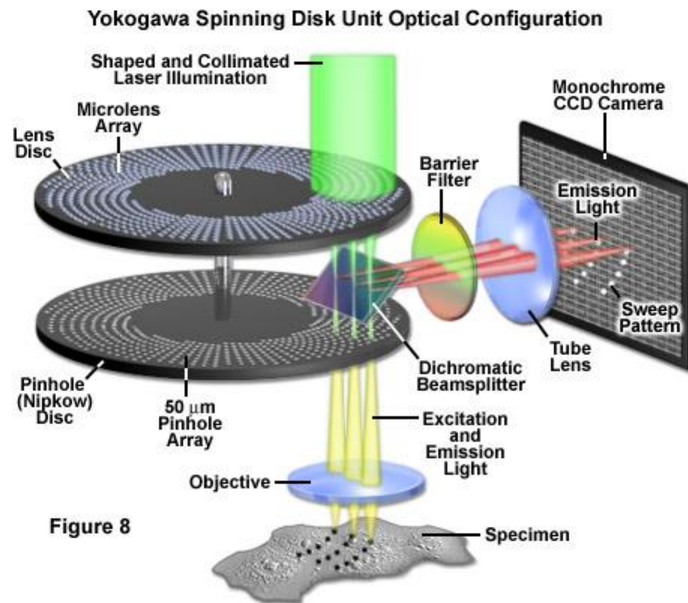
A z-stack and 3D reconstruction of lily pollen



imaging-git.com/sites/imaging-git.com/files/images/special/1177__original.jpg

2.3.5 Spinning disk confocal microscopy

WARWICK



zeiss-campus.magnet.fsu.edu/articles/spinningdisk/images/spinningdiskintrofigure8.jpg

- Large beam is split into beamlets using a microlens array/pinhole disk
- Nipkow disk ensures beamlets sweep across sample evenly
- Camera captures reflected emission beamlets
- Multiple beams make for faster image acquisition and reduced phototoxicity

2.3.6 Confocal wars

WARWICK

Point scanning confocal

- Variable pinhole size for optimal resolution (particularly in z)
- Greater flexibility in image collection
- Greater spectral flexibility
- Better background rejection
- Better penetration depth

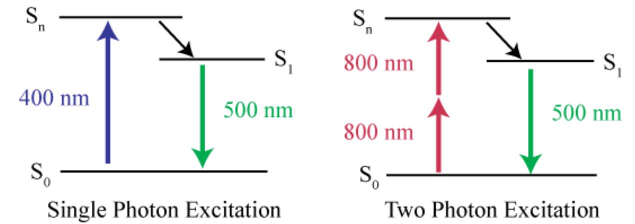
Spinning disk confocal

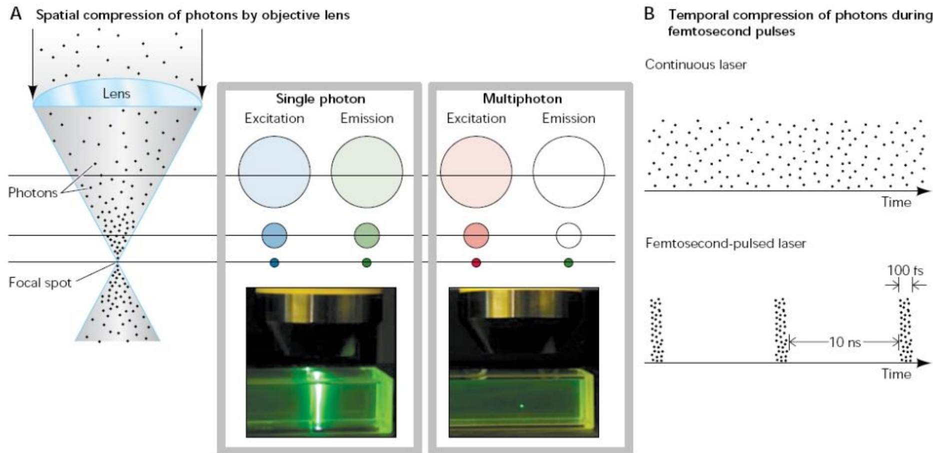
- **Faster**
- **Gentler**
- Simpler to use

2.3.7 Multiphoton microscopy

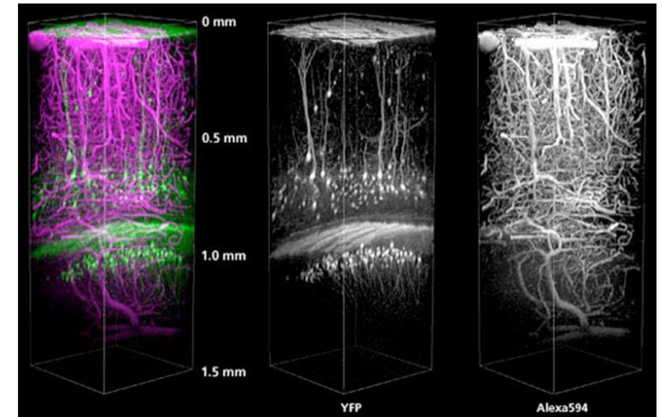
nobelprize.org

Maria Goeppert-Meyer first predicted two-photon absorption in 1931





parkerlab.bio.uci.edu



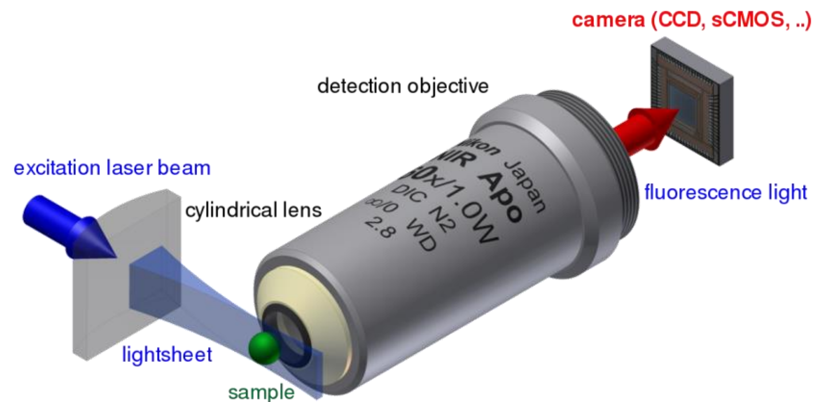
nikoninstruments.com

- Multiphoton excitation is confined to the focal plane
- Long wavelength excitation means very high penetration depth

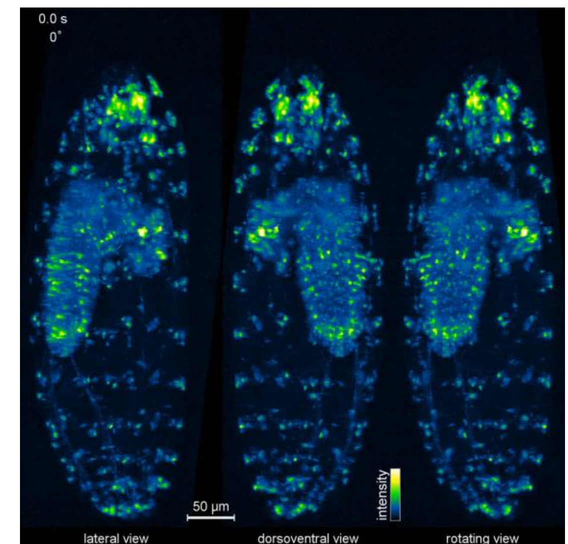
Multiphoton in SLS

2.3.8 Light sheet microscopy

WARWICK



https://upload.wikimedia.org/wikipedia/commons/thumb/5/53/Spim_prinziple_en.svg/1200px-Spim_prinziple_en.svg.png



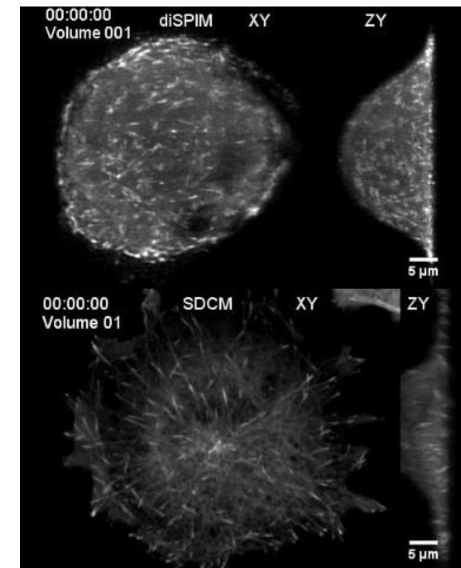
Chhetri et al, Nat. Methods 12:1171-1178 (2015)

- Orthogonal excitation sheet excites sample from side
- Detection objective projects illuminated section onto camera
- High speed, low phototoxicity
- Limiting geometry

WARWICK

diSPIM in the WMS

Marianas Lightsheet | University of Warwick System



diSPIM

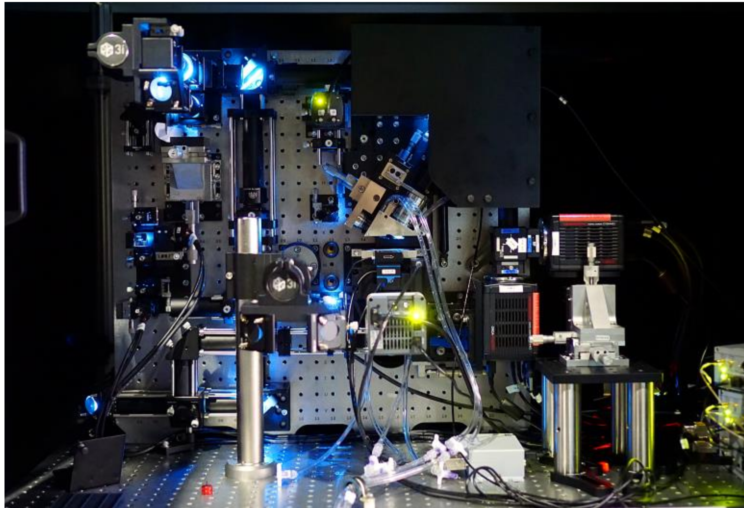
Spinning Disk Confocal

Comparison Between SDCM & D*i*SPIM on GFP-EB3 Microtubules in Live Human Umbilical Vein Endothelial Cells

Yicong Wu *et al.* 2013

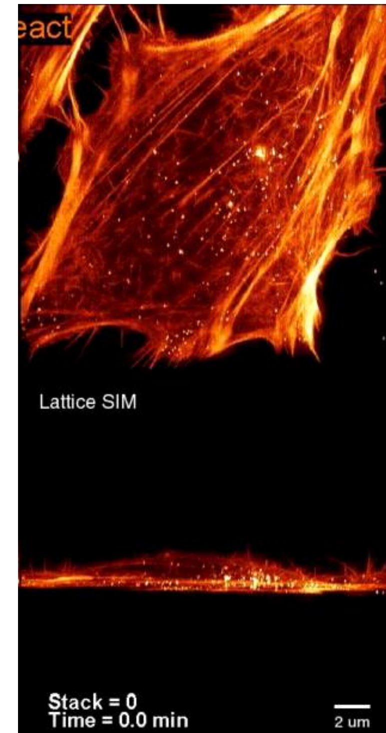
Lattice light sheet – the newest WMS acquisition!

WARWICK



rcc.uq.edu.au

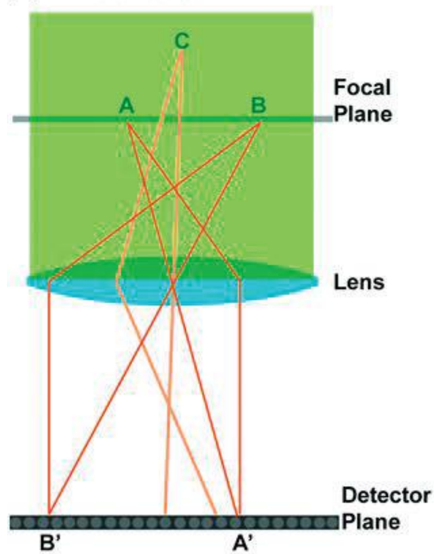
- Developed by Eric Betzig's lab in 2011.
- Uses advanced beam shaping to create a super-thin sheet
- High spatial and temporal resolution possible
- Optimal for thin samples e.g. cell monolayers

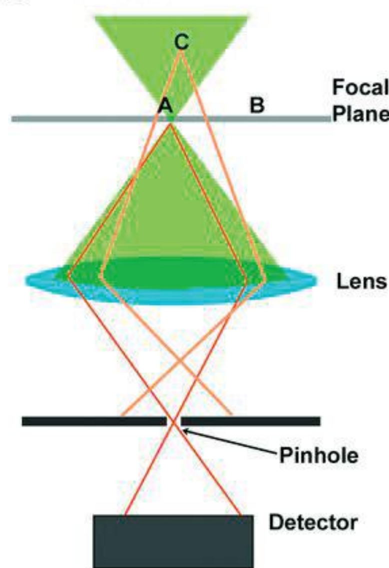


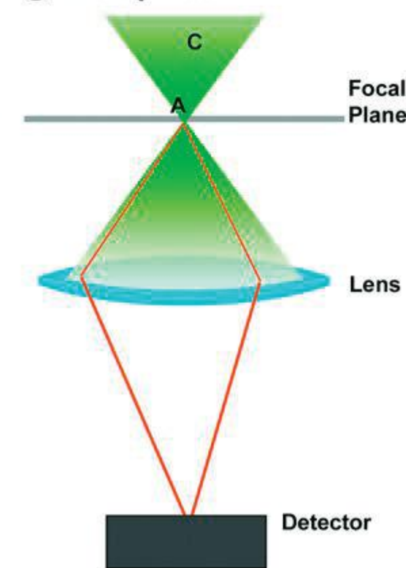
Chen et al, Science 346(6208):1257998 (2014)

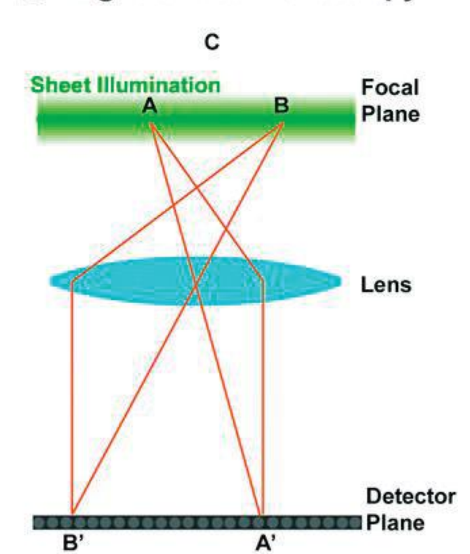
2.3.9 Summary of 3D microscopy data collection techniques

WARWICK









nap.edu/18433

2.4 Super-resolution microscopy

WARWICK

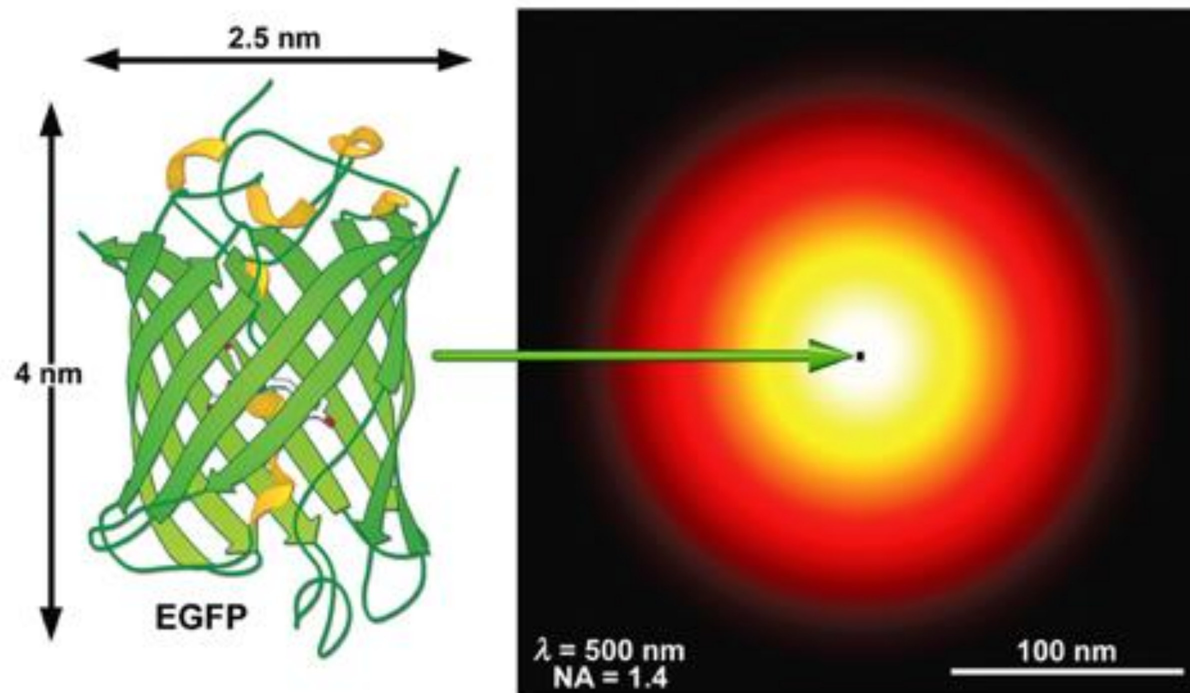


Figure 15.1

Enhanced green fluorescence protein (EGFP) and a comparison of its physical size to the size of its image in the fluorescence microscope.

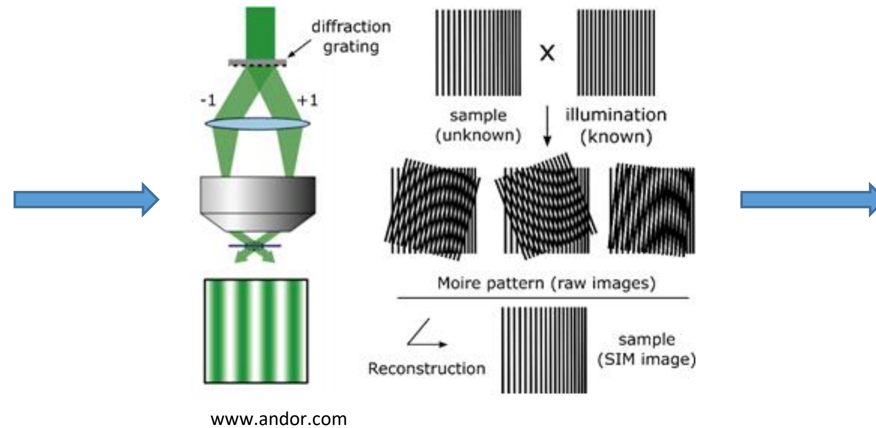
2.4.1 Structured illumination microscopy - SIM

WARWICK

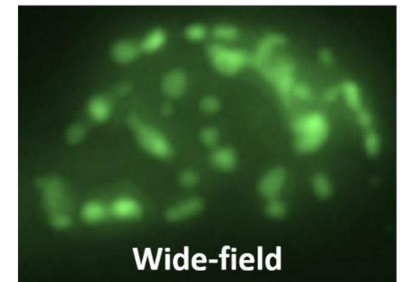
focusonmicroscopy.org

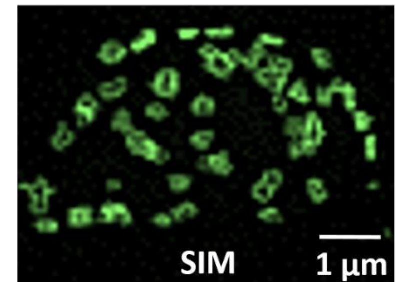
- Developed in 2000 by Mats Gustaffsson
- Surpasses Abbe limit by a factor of 2
- 9-15 images required to make one SIM image
- Can use standard fluorophores

wikimedia.org
Moire fringes



www.andor.com





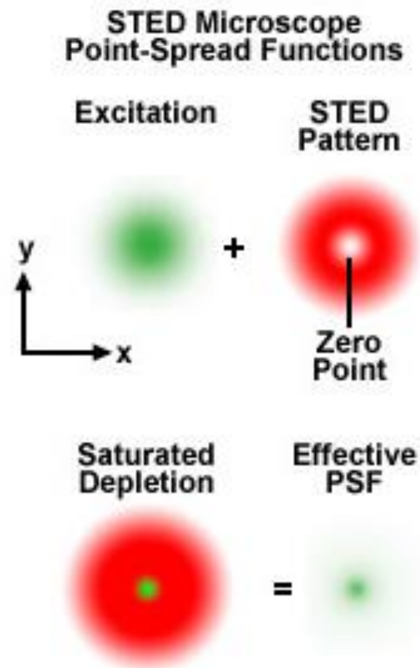
www.pages.drexel.edu

2.4.2 Stimulated emission depletion - STED

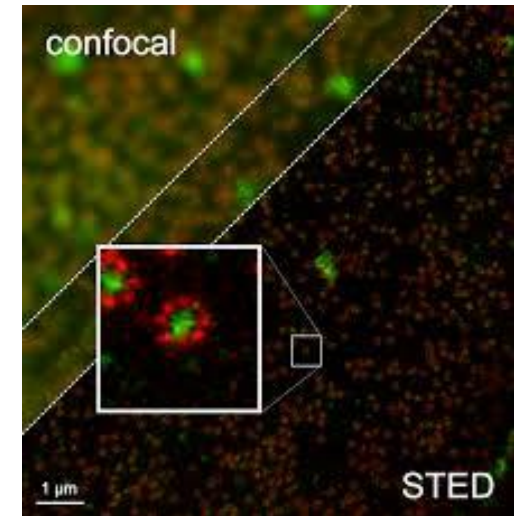
WARWICK

www.starmus.com

- Stefan Hell received the 2014 Nobel Prize in Chemistry for inventing STED microscopy
- Use a donut-shaped beam to induce stimulated emission in fluorophores
- Image is built up by point-scanning
- 10-20 nm resolution achievable
- Works best with specific fluorophores



zeiss-campus.magnet.fsu.edu

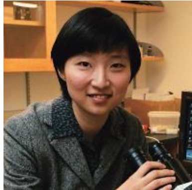


bitesizebio.com

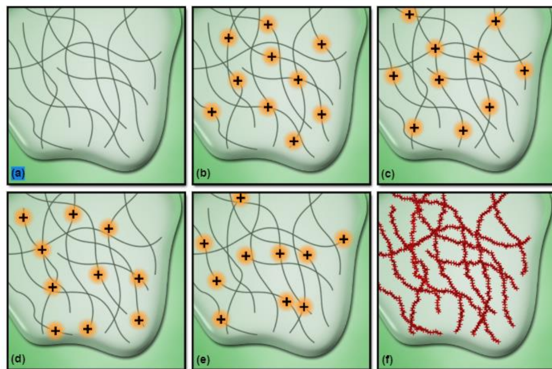
2.4.3 PALM/STORM – Single molecule localisation techniques

WARWICK

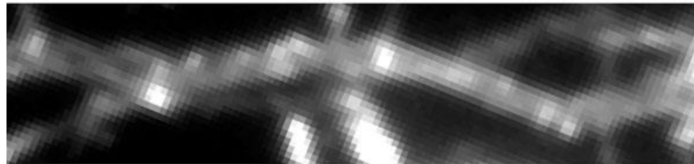
janelia.org

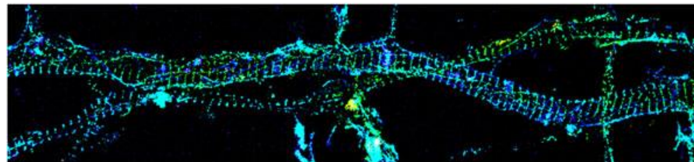

hhmi.org

- Developed in several labs concurrently in 2006
- Excite only a subsection of fluorophores in a sample
- Then determine the centroid of each resulting fluorophore
- 10-20 nm accuracy achievable
- 100s-1000s of images required
- Specialised photoswitchable fluorophores are needed
- Computationally expensive but simplest microscopy geometry



microscopyu.com

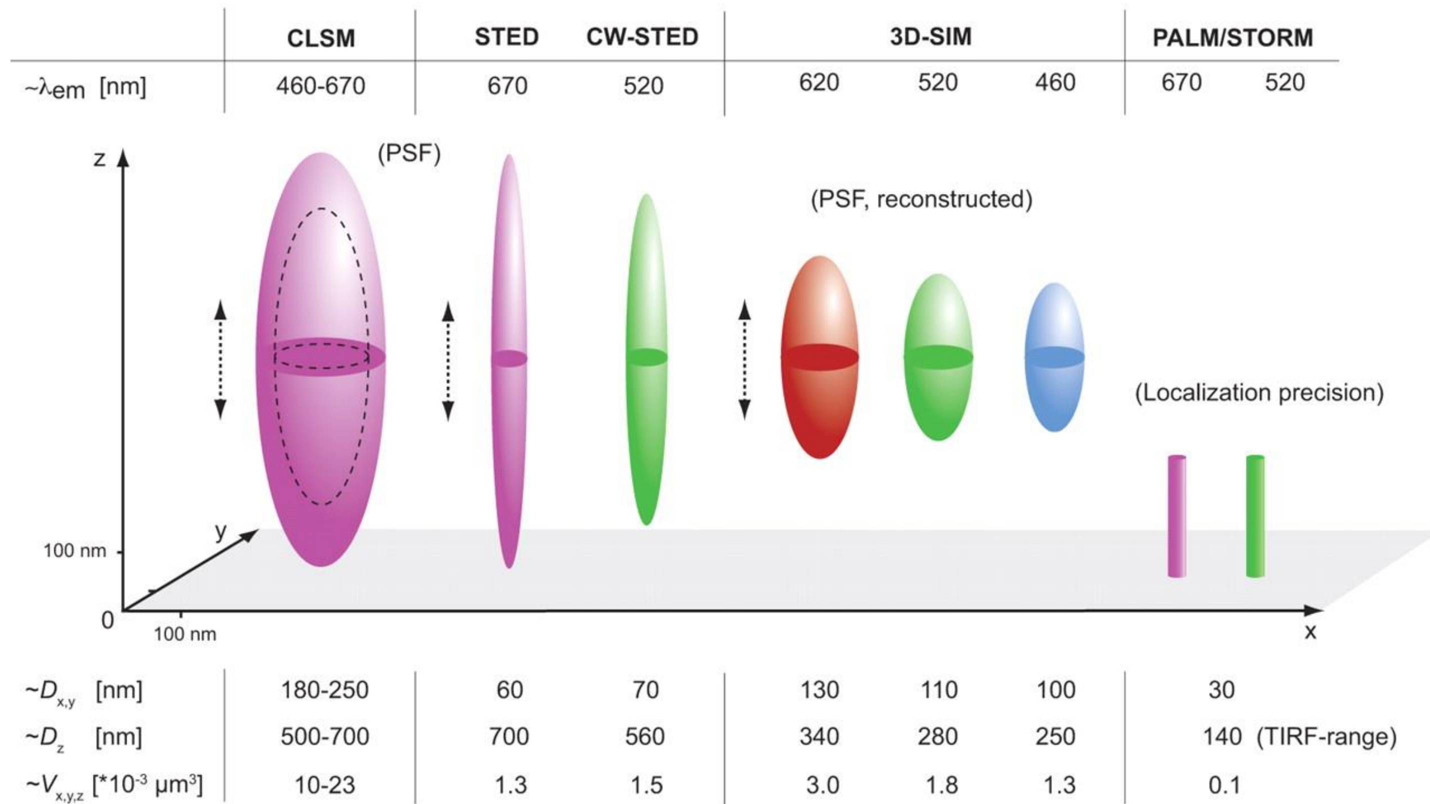

hhmi.org


Actin forms rings in axons – a new discovery using STORM!

@NateKrefman

2.4.4 Going beyond the diffraction limit

WARWICK



Schermelleh et al. JCB 190(2):165 (2010)

Recap of Section 2

- Brightfield microscopy
  - Phase contrast
  - Differential interference contrast
- Fluorescence microscopy
  - What is fluorescence microscopy?
  - Filtering fluorescence
  - Fluorescent excitation sources
- Volumetric microscopy
  - Deconvolution
  - TIRF
  - Confocal – point-scanning and spinning disk
  - Light sheet microscopy
- Super-resolution microscopy
  - SIM
  - STED
  - PALM/STORM

WARWICK

3. Fluorescence techniques

3.1 Fluorescence theory

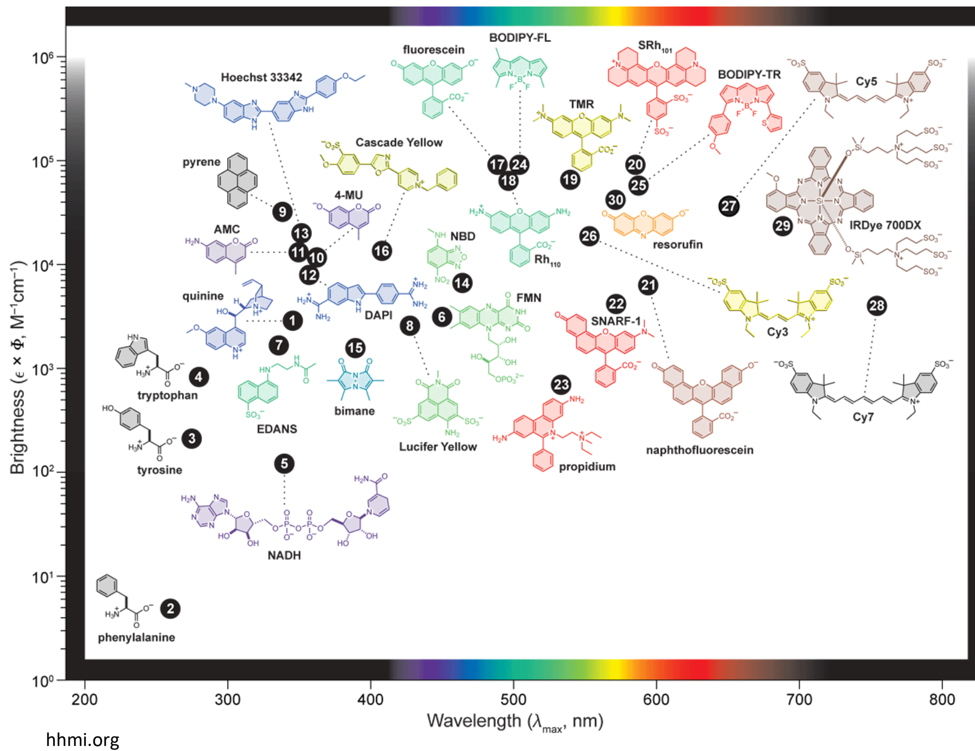
3.2 Photobleaching

3.3 Quantitative fluorescence techniques

3.1 Fluorescence under the hood

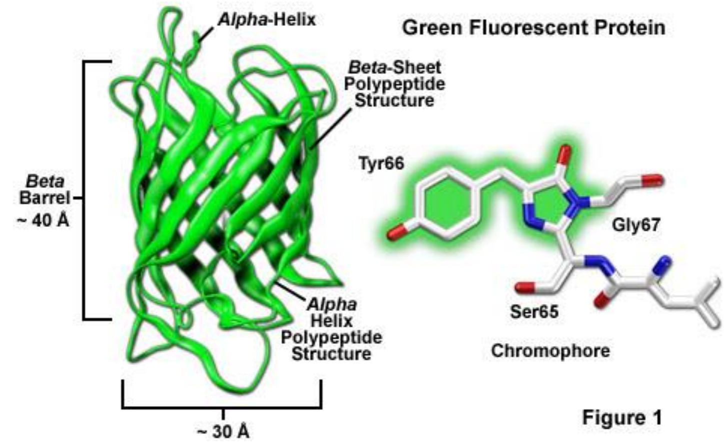
WARWICK

Synthetic fluorophores



hhmi.org

Fluorescent proteins



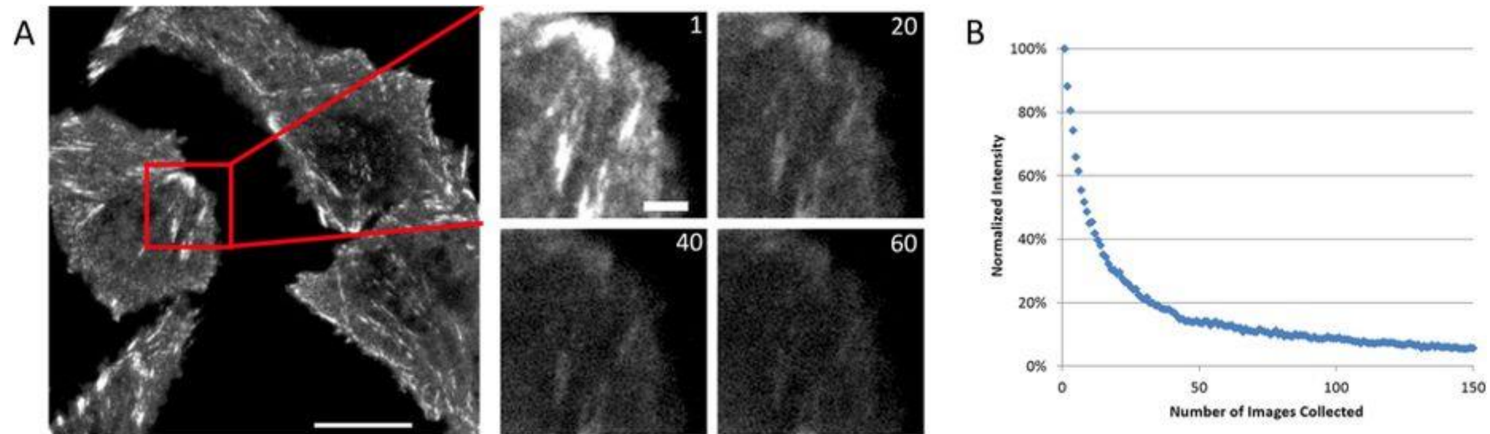
expertcytometry.com

It's all about delocalised electrons!

3.2 Photobleaching

WARWICK

After rounds of emission-excitation, fluorophores become unstable and unable to fluoresce



Boudreau et al. Sci. Rep. **6**, 30892 (2016)

Minimising photobleaching

- Lower illumination intensity
- Reduce exposure time
- Use a more photostable fluorophore
- Use anti-fade reagents
- "Pulsed illumination"

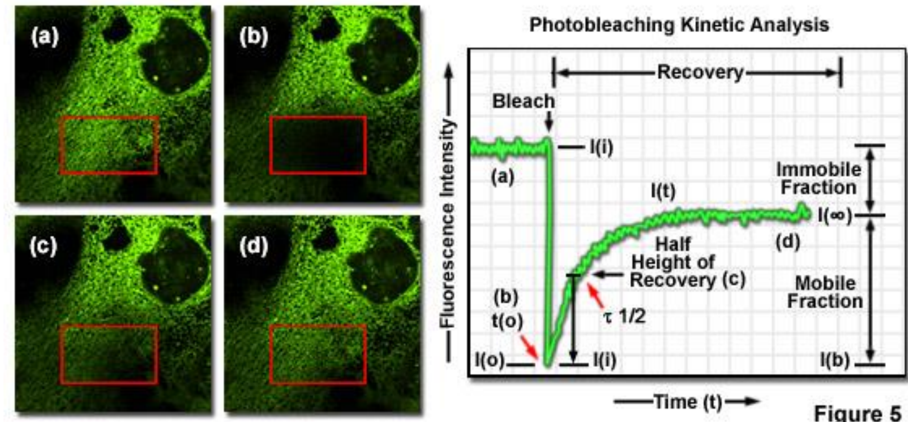
- When reduced but not eliminated – can correct post-processing

3.3.1 FRAP: Fluorescence recovery after photobleaching

WARWICK

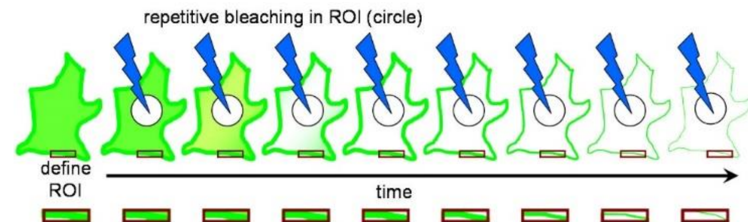
Using high laser power to purposefully bleach part of the sample

Dynamics of fluorescence recovery provides information about sample



olympus.magnet.fsu.edu

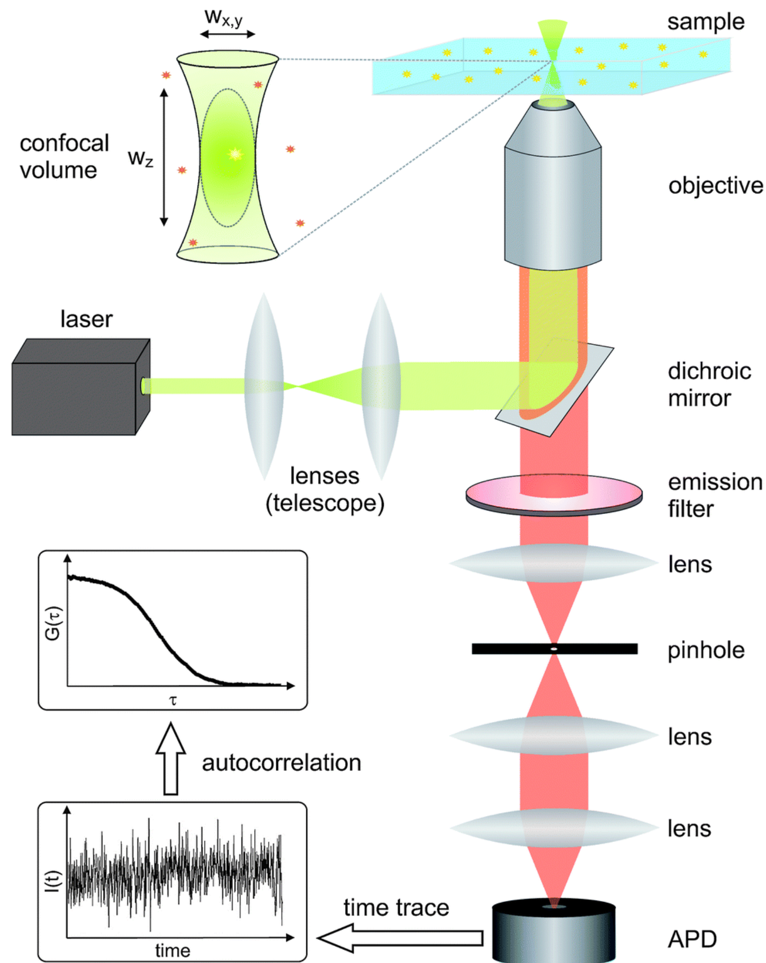
A sister technique: FLIP (fluorescence loss in photobleaching)



en.wikipedia.org

3.3.2 FCS: Fluorescence correlation spectroscopy

WARWICK



- Measuring particles diffusing in and out of a focal volume
- Can be used for local measurements of particle size and concentration with appropriate models.

cam.facilities.northwestern.edu/588-2/fluorescence-correlation-spectroscopy/

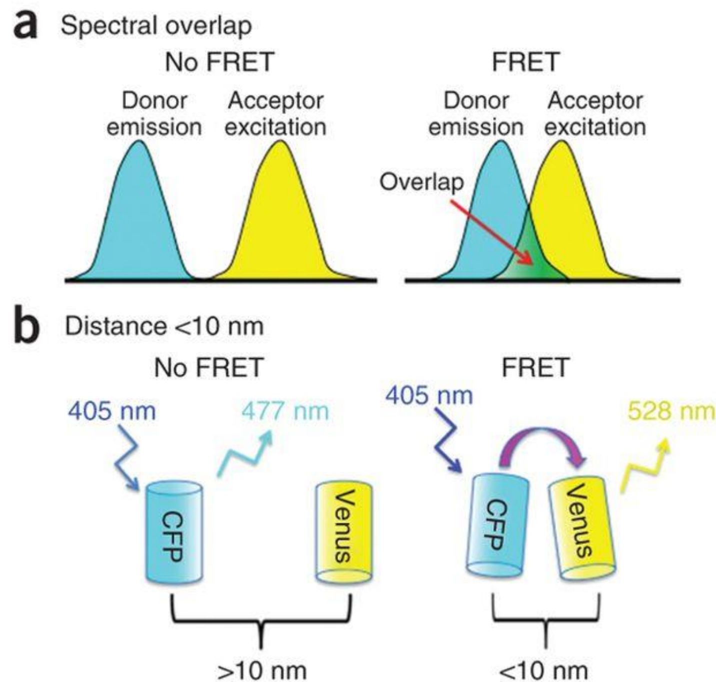
3.3.3 FRET: Forster resonance energy transfer

WARWICK

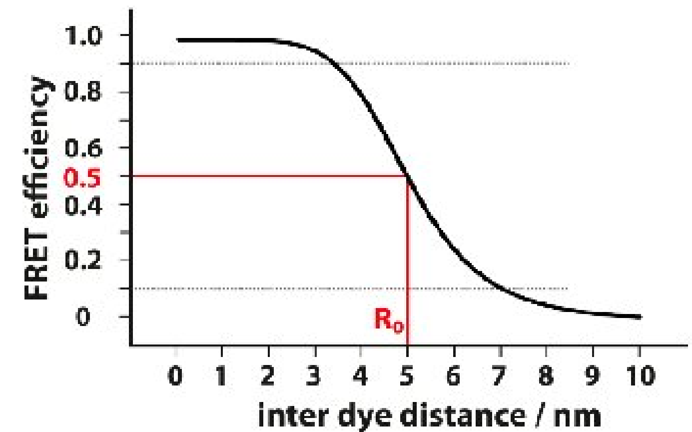
A proximity measurement

$$E = \frac{1}{1 + \left(\frac{r}{R_0}\right)^6}$$

E = coupling efficiency
r = intermolecular distance
R_0 = Forster radius



Broussard et al, Nat. Prot 8, 265-281 (2013)



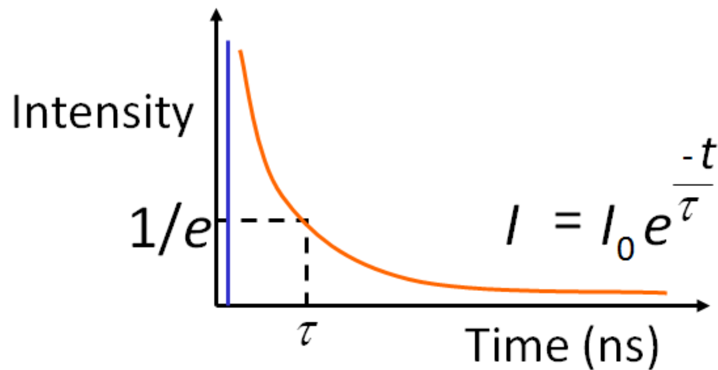
Gust et al, Molecules 19(10), 15824-15865 (2014)

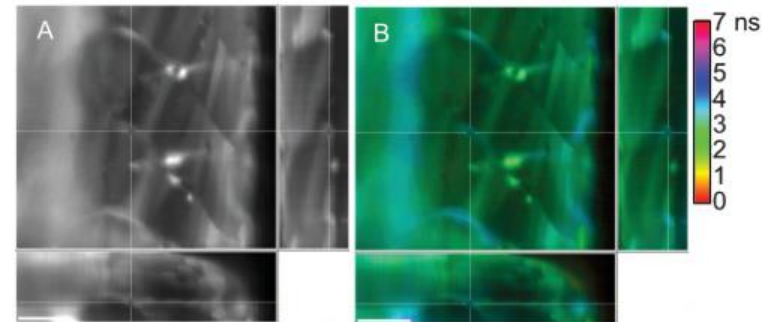
3.3.4 FLIM: Fluorescence lifetime imaging

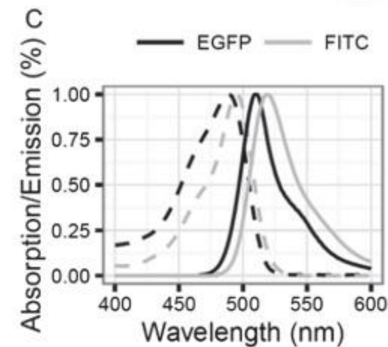
Each fluorophore has a characteristic lifetime (typically ns)

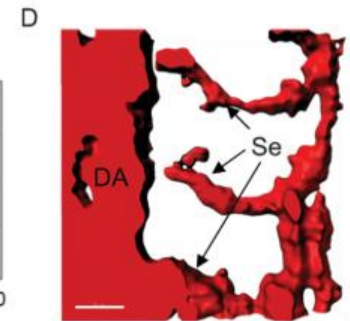
Excite fluorophores and measure the time delay of returning photons

Slow acquisition but the gold-standard for FRET measurements









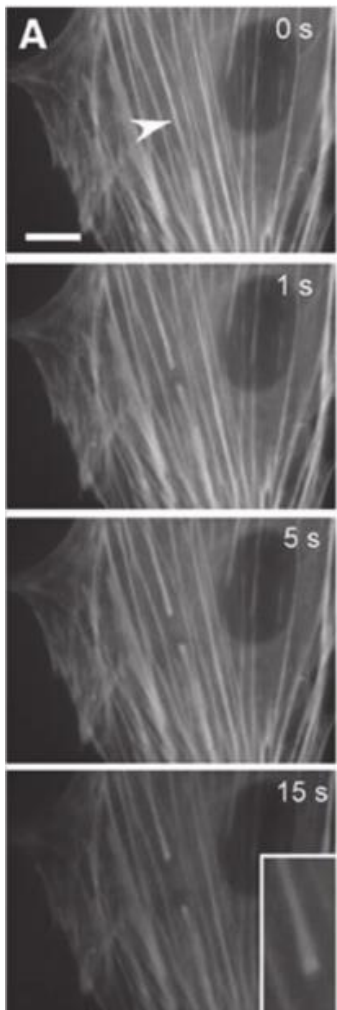
Mitchell et al. Opt. Letters **42**:7 1269-72 (2017)

Lambert Instruments

3.3.5 Other quantitative techniques

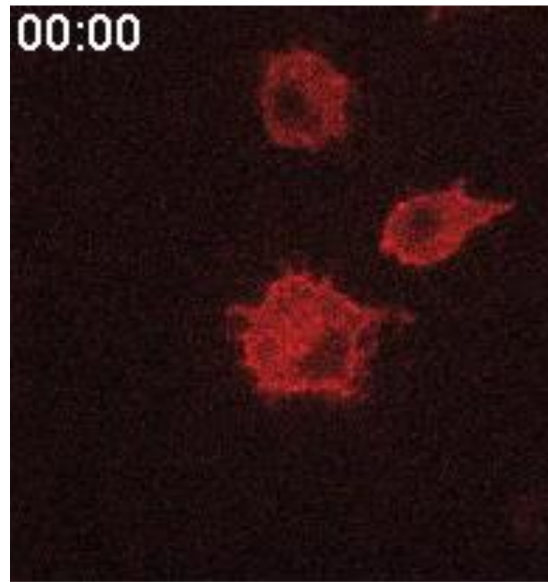
WARWICK

Laser ablation



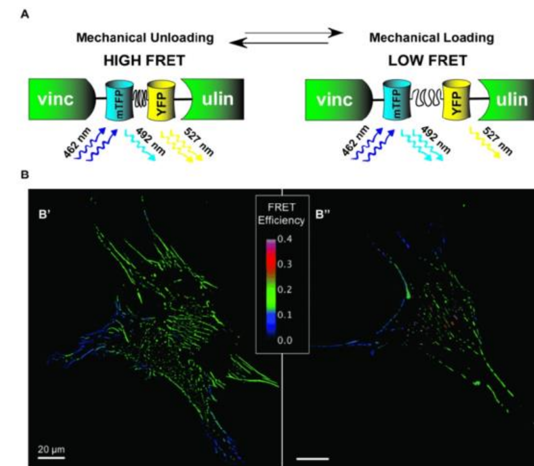
Wu et al. Int. Bio. 4(5):471-9 (2012)

Optogenetics for cell biology



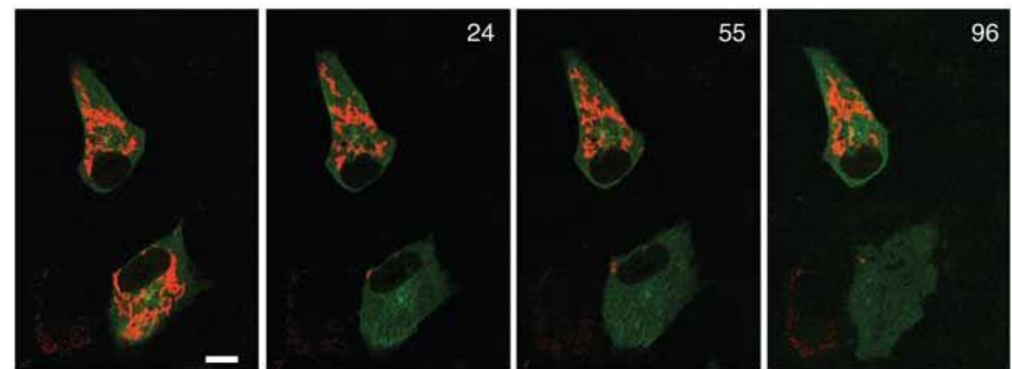
O'Neill et al, Mol Biol Cell 27(9):1442-50 (2016)

FRET-based tension sensors



Pomeroy et al, Theranostics 7(14): 3539-3558 (2017)

Chromophore-assisted light inactivation (CALI)



Bulin et al. Nat. Prot. 1: 947-953 (2006)

Recap of Section 3

WARWICK

- The origins of fluorescence
- Photobleaching
- Quantitative fluorescence techniques
  - FRAP
  - FCS
  - FRET
  - FLIM
  - Ablation, optogenetics, FRET biosensors and CALI

4. Microscopy for quantitative image analysis

WARWICK

4.1 Resolution and sampling

4.2 Noise

4.3 Optical aberrations

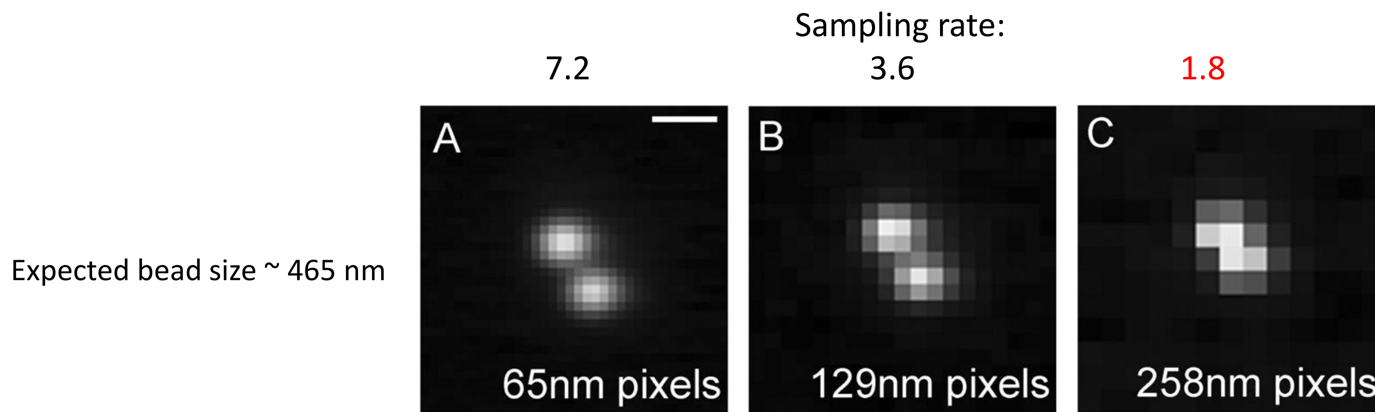
4.4 Crosstalk

4.1.1 Nyquist sampling

To achieve the maximum resolution:

Pixel size ≥ 2-2.3 x expected resolution

(known as Nyquist sampling)



Also relevant for z-stacks:

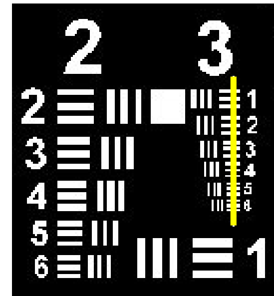
- Axial resolution $r_{ax} = \frac{2\lambda n}{NA^2}$
- Steps between z slices should be at least half this

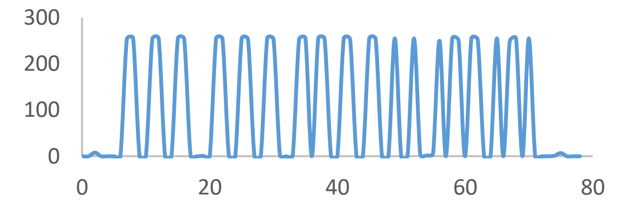
Waters JC, JCB (2009)

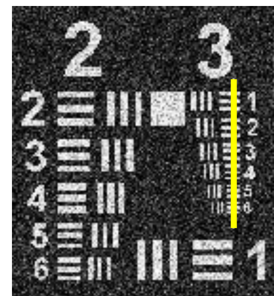
4.2.1 Noise in microscopy

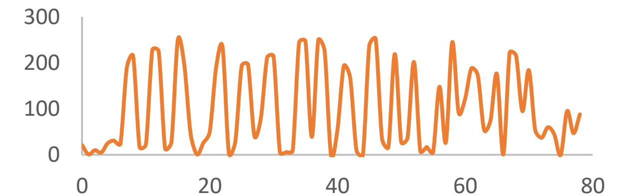
WARWICK

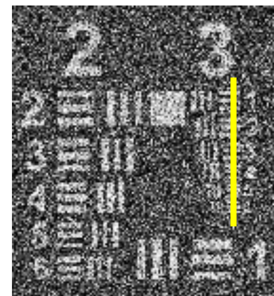
More noise means worse resolution

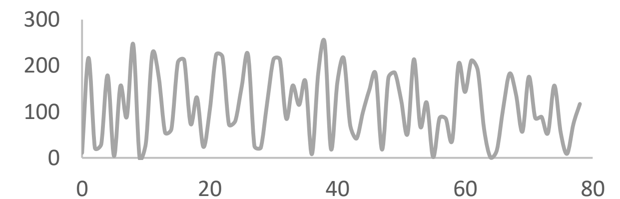






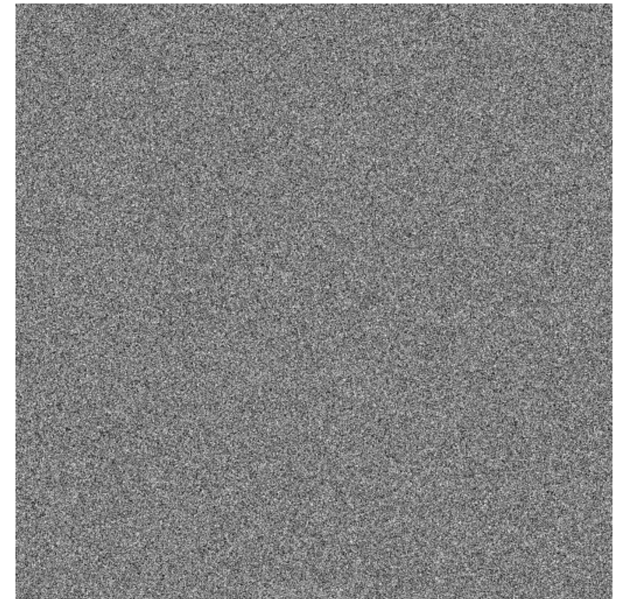






4.2.2 Read noise

- Electronic noise introduced when reading data from the camera chip.
- Intensity independent
- Gaussian distribution
- Only important at low intensities



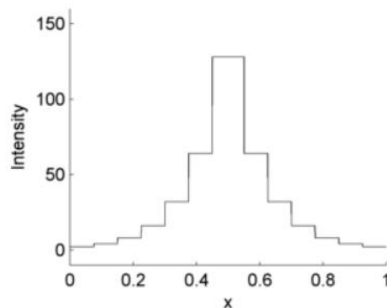
Simulated chip readout with zero illumination

4.4.3 Photon noise

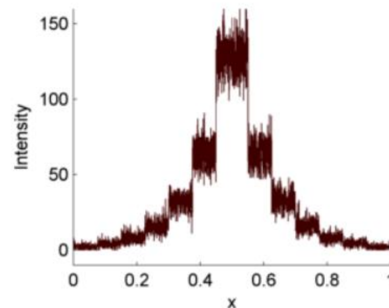
WARWICK

- Also called shot noise
- $\sigma_{noise} \propto \sqrt{intensity}$
- High intensity means more noise *but better signal-to-noise ratio (SNR)*

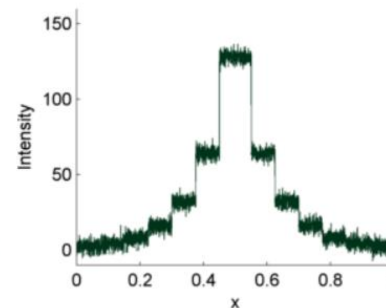
Intensity	σ_{noise}	SNR
100	10	10
10	3.2	3.2



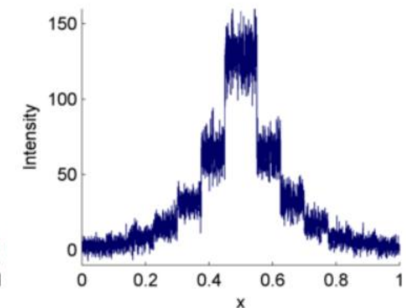
(a) A noise-free signal



(b) Photon noise



(c) Read noise



(d) Photon + Read noise

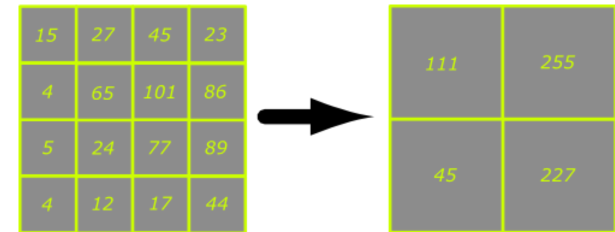
4.2.4 Noise reduction techniques

WARWICK

- Detect more photons
  - ✓ Increase exposure time
  - ✓ Increase laser power
  - × Increase photobleaching/toxicity

- Post-processing
  - ✓ Bin your data
  - ✓ Smooth your data
  - × Decrease your resolution

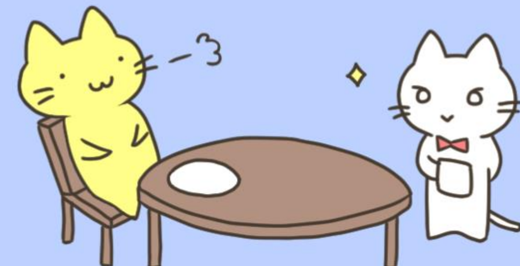
2x2 pixel binning



wiki.ucl.ac.uk/display/LMCBLMic/Glossary

Binning in ImageJ: Image -> Transform -> Bin

There's no such thing as a free lunch.



sirius-at-web.blogspot.com

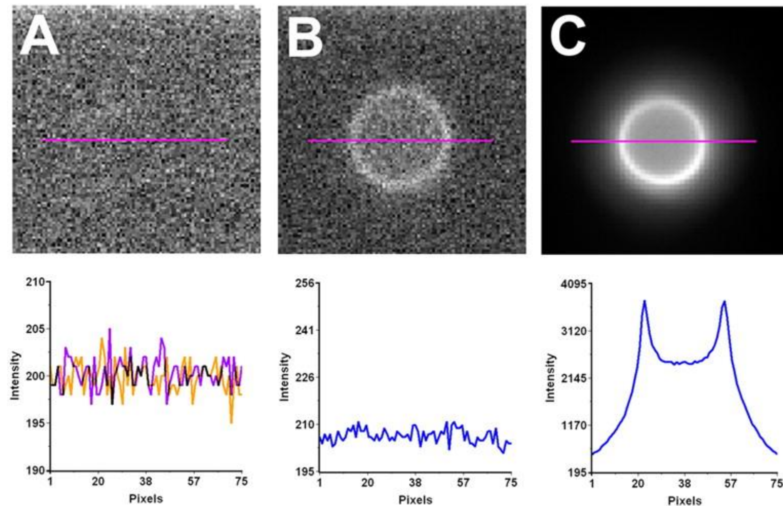
4.2.5 Bit-depth

Each detector will have a certain bit-depth which determines how many intensity levels your data is quantised into

Bit-depth (2^n)	No. int. levels
8	256
12	4096
16	65536

Higher bit-depth = more accurate representation of data

Higher bit-depth = bigger data



For quantitative microscopy:

- Fill your bins
- Don’t saturate

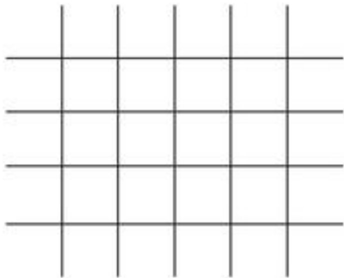
4.3 Optical aberrations

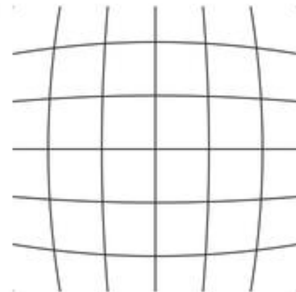
WARWICK

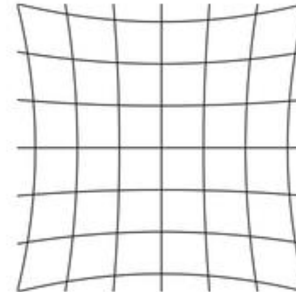
4.3.1 Distortion

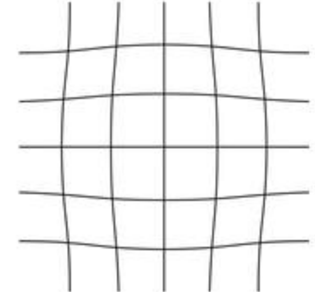
WARWICK

A change in the shape of the image


No Distortion


Barrel Distortion


Pincushion Distortion


Mustache Distortion

Drew Steven Photography

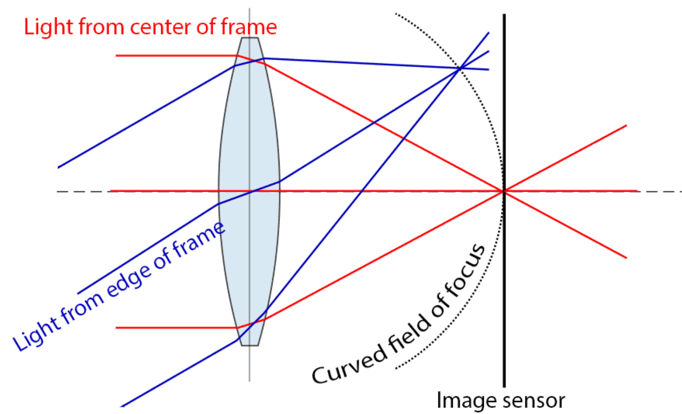
Usually negligible in research-grade microscopes

Can check for by imaging a test grid

4.3.2 Field curvature

WARWICK

A simple lens images an extended flat object to a spherical surface



discoverdigitalphotography.com

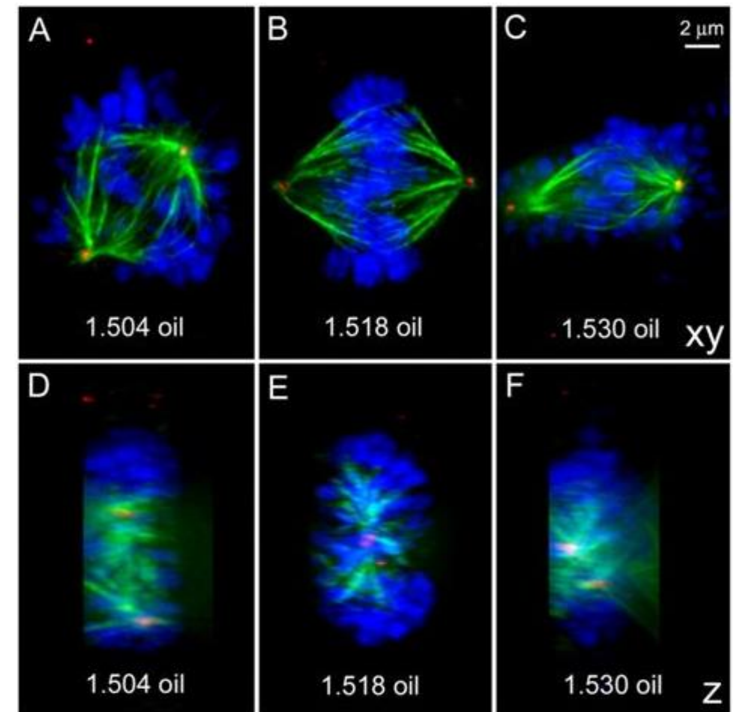
eckop.com

Objectives with Plan correction have many internal lenses designed to eliminate field curvature

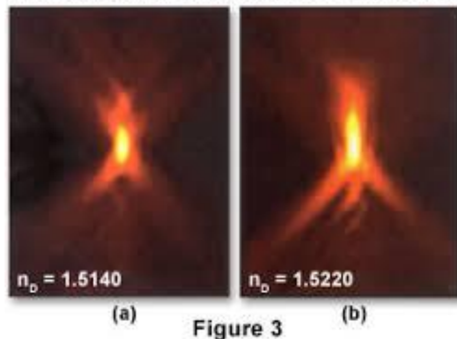
4.3.3 Spherical aberration

WARWICK

- Caused by refractive index mismatches
- Reduces the intensity of your image and introduces blur
- Observed by asymmetric PSF
- Improved using a correction collar or RI matching
- Increases with increasing depth in live samples when using an oil immersion objective



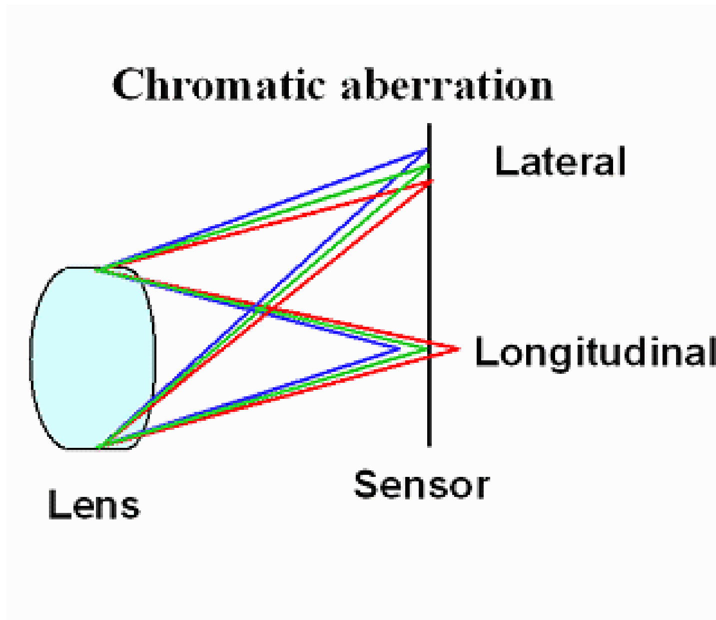
slideplayer.com/slide/8632392/26/images/53/



med.unc.edu

4.3.4 Chromatic shift

WARWICK

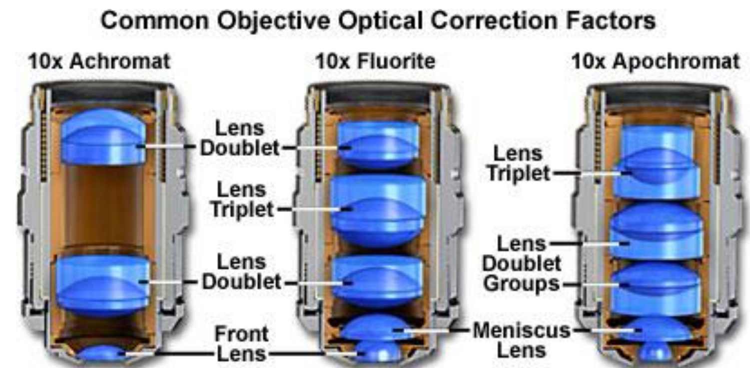


imatest.com/images/Chromatic_diag.gif

Refractive index varies with wavelength

Different excitation wavelengths will focus at slightly different points on the sample

Higher quality objectives minimise chromatic shift



olympus-lifescience.com

4.4 Crosstalk/spectral bleedthrough

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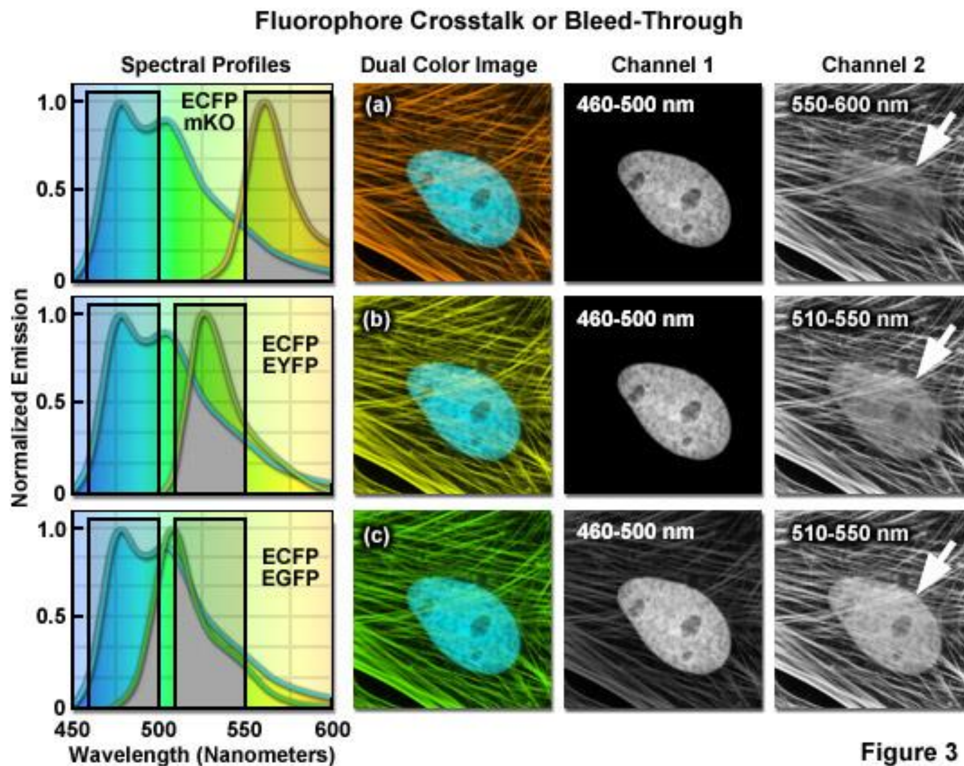
When more than one fluorophore is present we get crosstalk between channels



How to avoid/minimise:

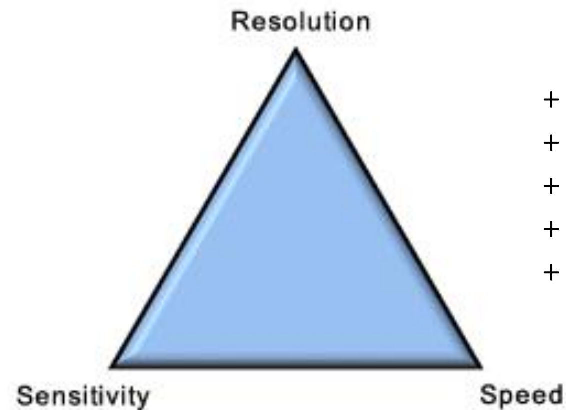
- Use spectrally separated fluorophores
- Check your fluorophore spectra before
- Match intensities as much as possible
- Image each channel separately
- Have single-colour controls
- Spectral unmixing

Nikon - MicroscopyU

Recap of Section 4

WARWICK

- Resolution and sampling
  - The Nyquist limit
- Noise
  - Read noise
  - Photon noise
  - Noise reduction
- Optical aberrations
  - Distortion
  - Spherical aberration
  - Chromatic shift
  - Crosstalk



+ Low photobleaching
+ Large FOV
+ Additional functionality
+ ....
+ ...

www.ammrf.org.au/myscope/confocal/confocal/image/

Summary

WARWICK

- Light and its properties
  - Wave-particle duality
  - Refraction
  - Diffraction
  - Interference
- A simple microscope
  - Lenses for magnification
  - Set-up of a simple microscope
  - The role of the objective
  - The resolution limit
  - Detectors for microscopy
- Light microscopy techniques
  - Label-free microscopy
  - Fluorescence microscopy
  - Volumetric fluorescence microscopy
- Fluorescence microscopy
  - Fluorophores
  - Photobleaching
  - Quantitative fluorescence techniques
- Acquiring quantitative microscopy data
  - Resolution and sampling
  - Sources of noise in light microscopy
  - Optical aberrations and how to minimise them

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ext. 51934