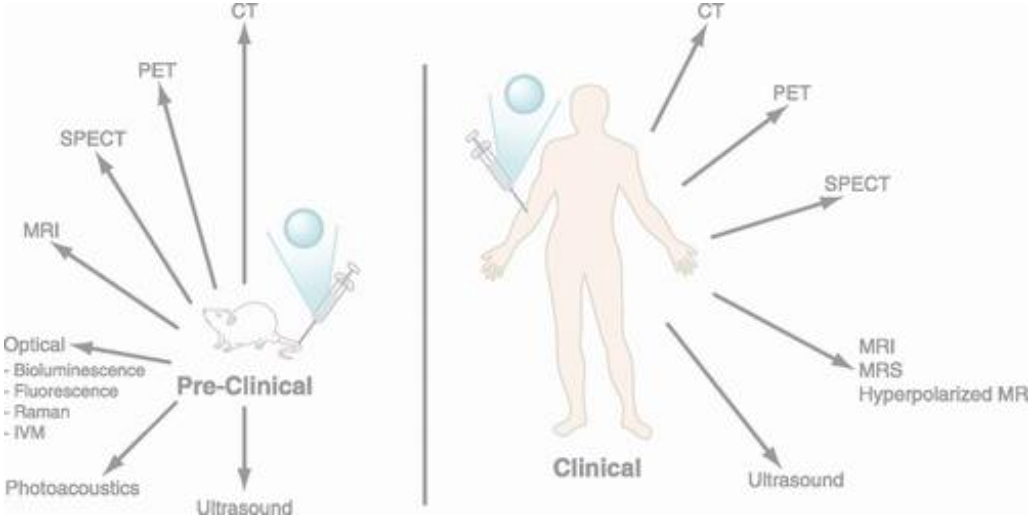
Memorial Sloan Kettering Cancer Center

Cancer Imaging



https://doi.org/10.1152/physrev.00049.2010

Ross Boltyanskiy
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Center for Molecular Imaging and Bioengineering

My objectives for this course

- Familiarize with the fundamentals of different imaging modalities used in cancer research
- Develop an engineering approach to the design and development of tools and methods in cancer imaging
- Get some hands-on practice with imaging tools, acquisition process, reconstruction, analysis
- Be able to better understand papers and talks in Cancer Imaging
- Build up a bit more background to make an informed decision about what research group to join.

A few important notes about my class

- My class is active and interactive: ask questions and don't by shy to answer!
- I'd like to cater as much as possible to what you want to get out of the class
- This is still just the beginning of the program so it's even more important for you to let me know what you found useful and interesting and what should be cut / added for next year.
- How is this different from an undergrad class? How is it different from a typical graduate level class?

Schedule + grading rubric

This week: Optics (1.5 days) + Acoustics
2nd week: Nuclear imaging, X-rays, Paper
3rd week: Magnetic resonance, hands-on activity

Assignment	% of grade
Problem Sets	65%
Class Participation	35%
Total	**100%**

Quick round of intros

- Your name
- Why did you choose to enroll in this new Cancer Engineering PhD program?
- What are some of the main things you'd like to get out of this class?

Take 1 min to think about it and we'll go around the room very quickly

Questions?

Common aspects of all imaging

1. Source of signal
   - Light, sound, radioactive decay, magnetic moment etc.

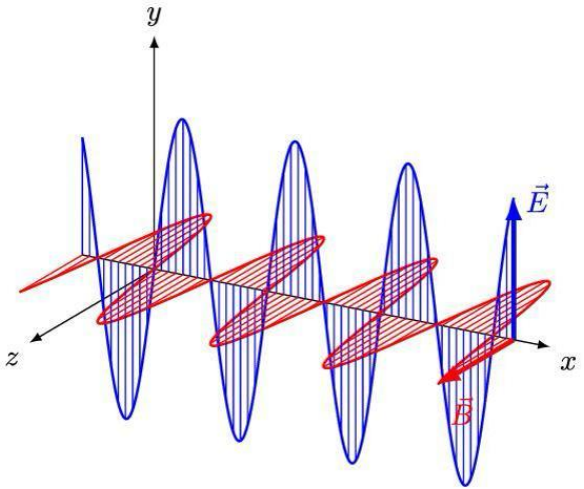
2. Method of detection
   - How does the signal get captured?
   - How is the signal processed/converted into interpretable data

3. Generation of images
   - What creates contrast?
   - Signal to noise ratio (SNR) considerations
   - Does contrast represent a qualitative or quantitative metric?
   - Is the contrast coming from a material property or a biological function?

In optics the source of signal is light

- Light is a particular form of energy
- Moving charges create an oscillating electric field, which creates an oscillating magnetic field (or other way around)

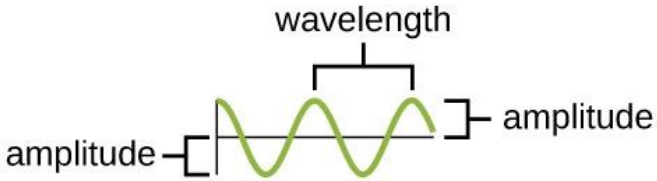
$$\nabla \cdot \mathbf{E} = \frac{\rho}{\varepsilon_0}$$
$$\nabla \cdot \mathbf{B} = 0$$
$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}$$
$$\nabla \times \mathbf{B} = \mu_0 \left(\mathbf{J} + \varepsilon_0 \frac{\partial \mathbf{E}}{\partial t} \right)$$

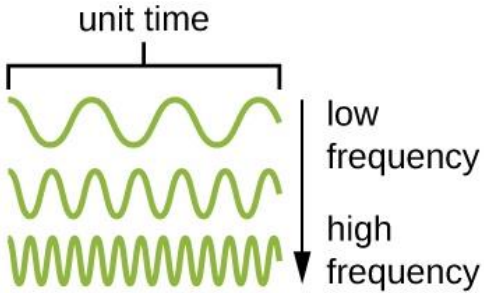


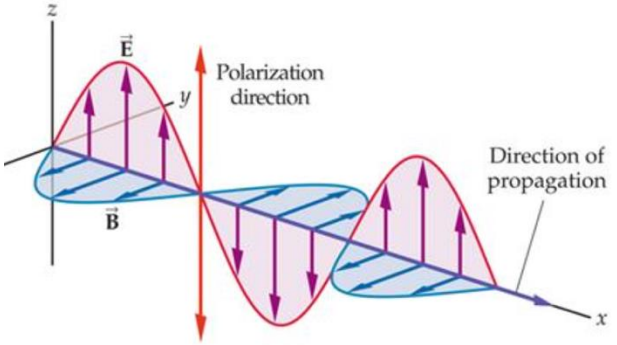
A few important parameters for us:

- Amplitude
- Wavelength / Frequency
- Direction of propagation
- Phase
- Polarization
- Speed

Light wave properties







What property of light do we perceive as brightness?

What property is the color?

What determines the speed of light?

$$\mathbf{S} = \frac{1}{\mu_0}(\mathbf{E} \times \mathbf{B}).$$

$$I(t) = \langle \mathbf{S}(t) \rangle = \frac{1}{2c\mu_0} E_0^2.$$

$$v = c/n \qquad v = \lambda f$$

- Light waves interfere creating amplification of intensity (constructive interference) or reduction of intensity (destructive interference)

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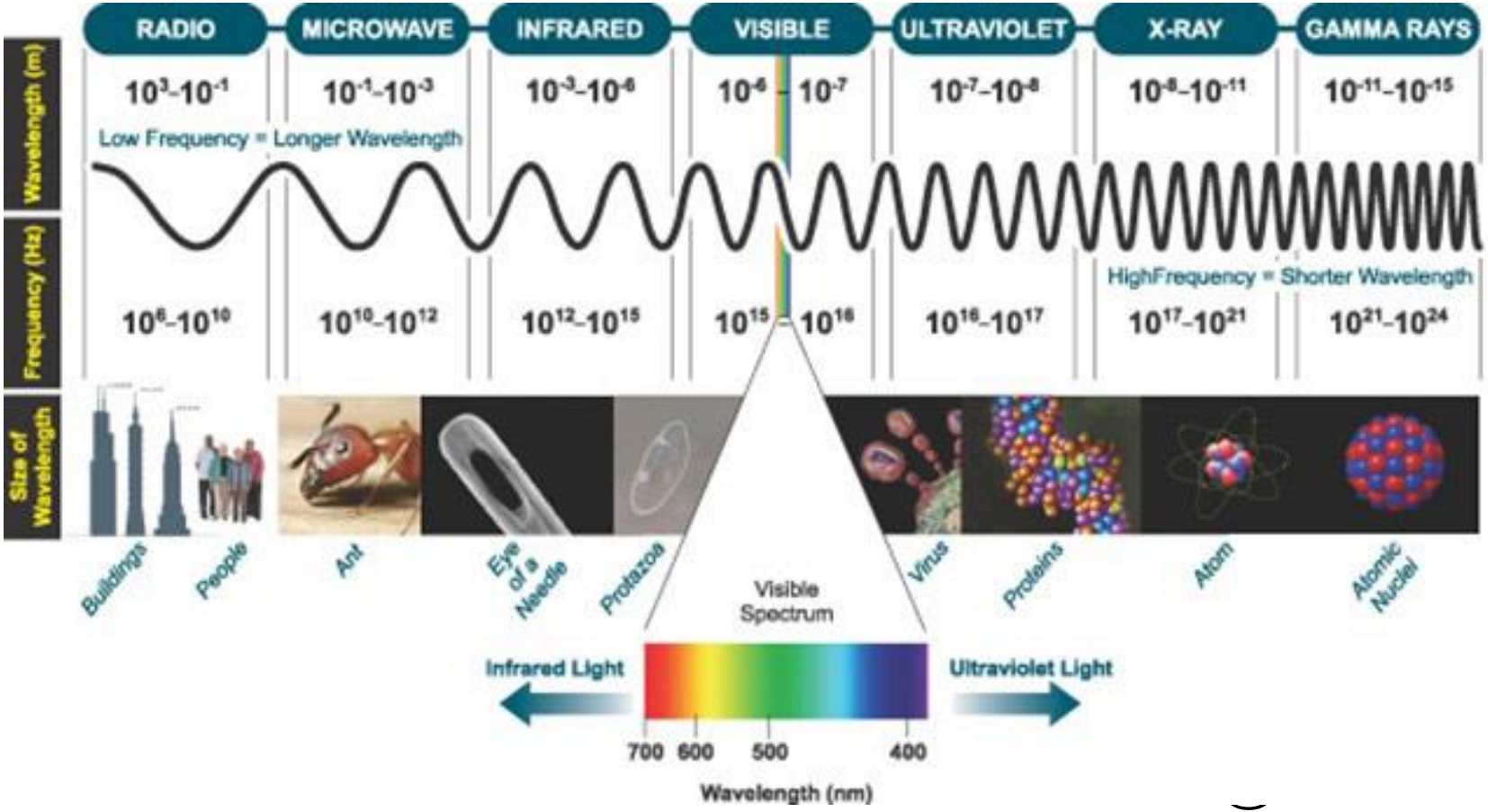
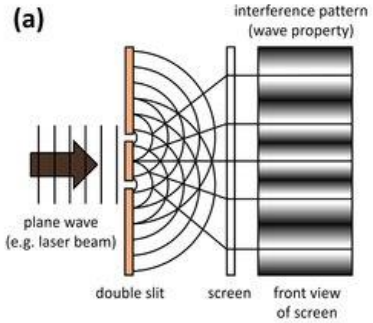
Electromagnetic spectrum



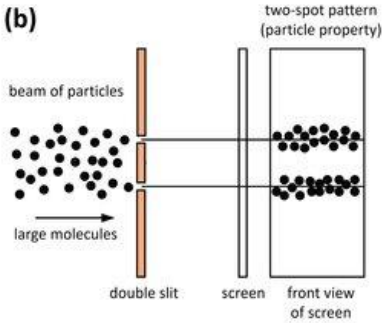
ring

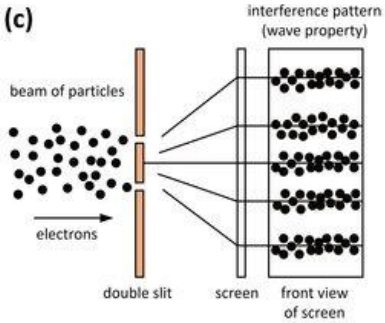
Light particle properties (glimpse at quantum picture)

https://www.bu.edu/articles/2016/single-photon-avalanche-diode-camera/



1801





1909 (photons) /
1961 (electrons)

Color is still related to the frequency of the light

The energy of a photon is proportional to its frequency: E = $\hbar$ f

Intensity is proportional to number of photons per area: $I = n \cdot \frac{hf}{A\Delta t}$.

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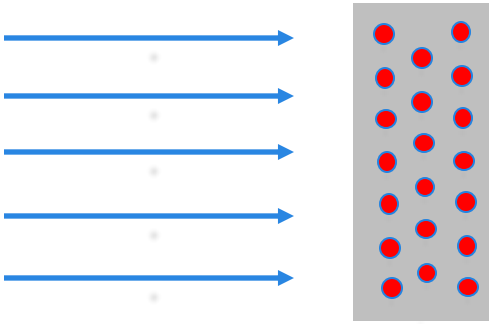
Interaction of light with matter

What kind of interactions exist between light and matter?

- Reflection
- Transmission
- Absorption
- Refraction
- Diffraction
- Scattering

- We learn about biological matter based on how it interferes with light
- We use these properties to design instruments to guide light and develop microscopy tools

Light ←→ Matter interaction one step deeper



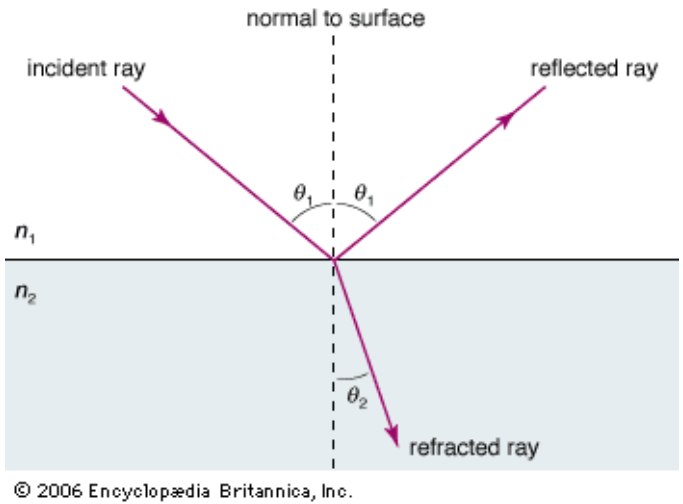
Transmission: Electrons absorb the radiation and perfectly re-emit it to the neighboring electrons until the light leaves the material.

Reflection: Electrons absorb radiation and re-emit phase-shifted by 180° Light therefore is seen traveling back.

Absorption: Electrons and atoms absorb the radiation and do not reflect it back. The energy is turned into vibration and heat (usually).

Refraction: Electrons absorb the radiation and re-emit it with a phase shift and a change in speed.

Light ←→ Matter interaction one step deeper



n is the **index of refraction** and is based on the dielectric properties of the material.

Snell's law: $n_1 \sin\theta_1 = n_2 \sin\theta_2$

n is **wavelength-dependent**! This is called **dispersion**

$$n(\lambda) = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^4} + \cdots$$ Cauchy's equation

The speed of light in the medium depends on the index of refraction. As does the wavelength, but not the frequency.

$$v(n) = \frac{c}{n} \qquad v(n) = \lambda(n) \cdot f$$

Light ←→ Matter interaction one step deeper

In reality *n* is complex:

→ Real part corresponds to refraction

→ The imaginary part corresponds to absorption/extinction

$$\underline{n} = n + i\kappa.$$

$$\begin{aligned}\mathbf{E}(x,t) &= \mathrm{Re}\left[\mathbf{E}_0 e^{i(\underline{k}x-\omega t)}\right] \\ &= \mathrm{Re}\left[\mathbf{E}_0 e^{i(2\pi(n+i\kappa)x/\lambda_0-\omega t)}\right] \\ &= e^{-2\pi\kappa x/\lambda_0}\ \mathrm{Re}\left[\mathbf{E}_0 e^{i(kx-\omega t)}\right]\end{aligned}$$

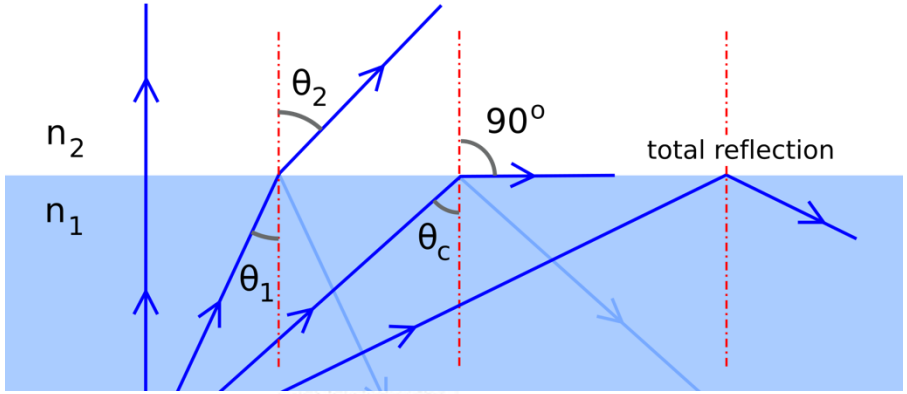
Wave number: $\underline{k} = 2\pi\underline{n}/\lambda_0$

$$I(x) = I_0 e^{-4\pi\kappa x/\lambda_0}$$

Absorption coefficient: $\alpha = 4\pi\kappa/\lambda_0$

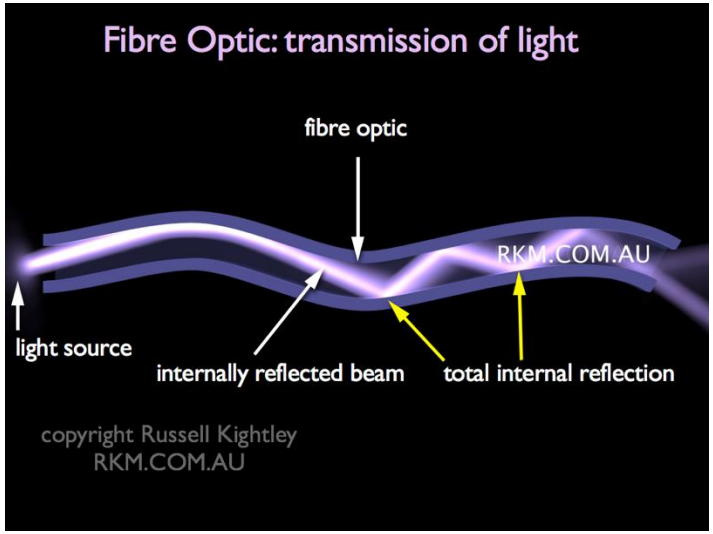
Penetration depth: $\delta_p = 1/\alpha = \lambda_0/4\pi\kappa$

Total Internal Reflection

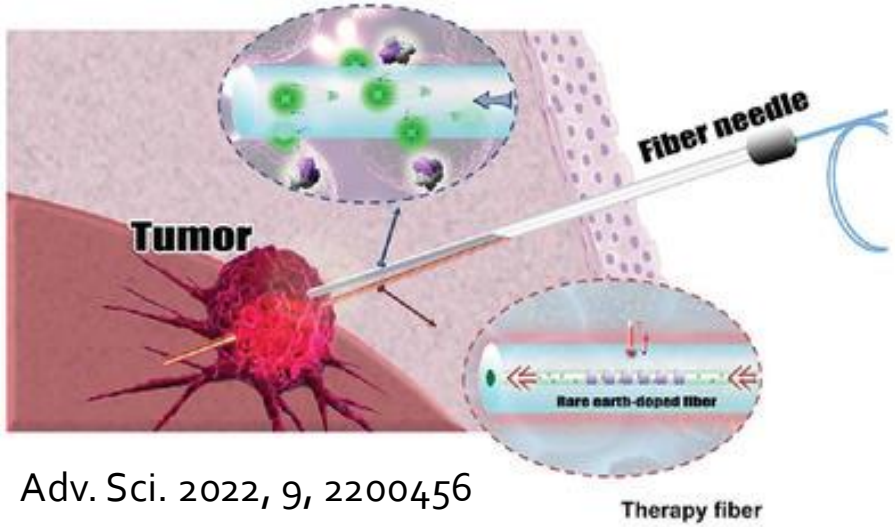


Snell's law: $n_1 \sin\theta_1 = n_2 \sin\theta_2$

$$\theta_c = \arcsin(\frac{n_2}{n_1})$$



Fiberoptic theranostics



Adv. Sci. 2022, 9, 2200456

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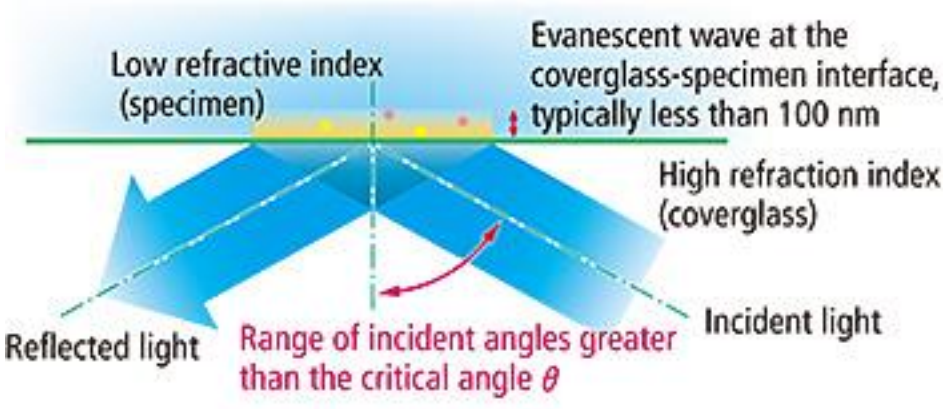
Evanescent wave and TIRF microscopy

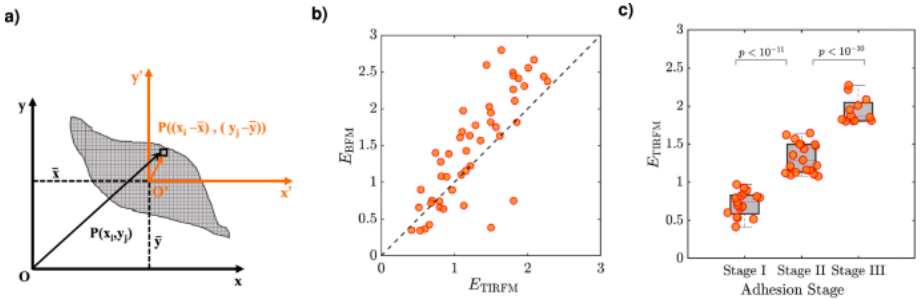
Evanescent wave: Even though no light propagates in the lower refractive index medium, some EM energy seeps in and decays exponentially away from the surface.



Quantifying F-actin patches in single melanoma cells using total-internal reflection fluorescence microscopy

Elham Sheykhi, Behnaz Shojaedin-Givi, Batool Sajad, Hossein Naderi-Manesh & Sharareh Tavaddod

Scientific Reports **12**, Article number: 19993 (2022) | Cite this article



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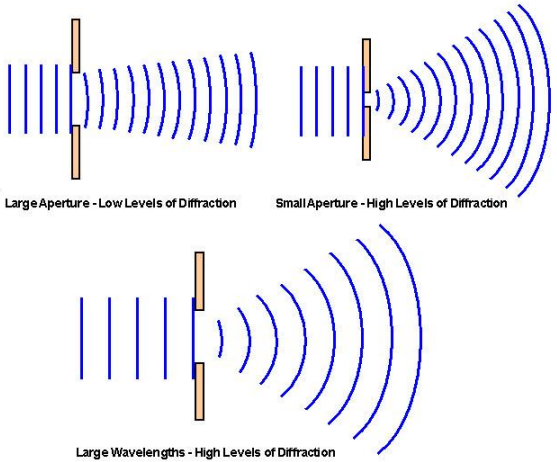
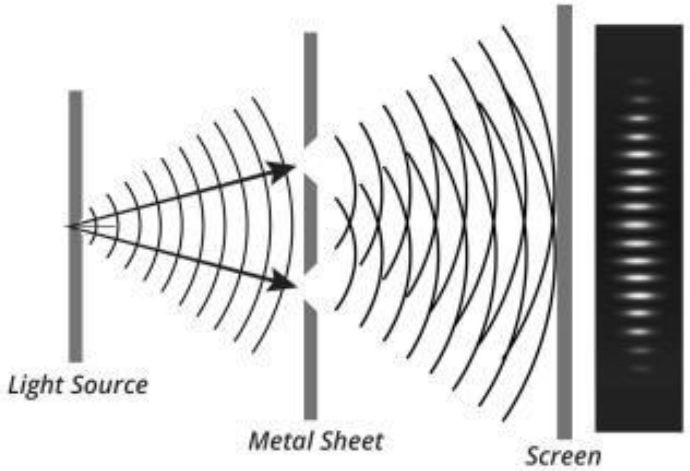
Interaction of light with matter

What kind of interactions exist between light and matter?

- ✓ Reflection
- ✓ Transmission
- ✓ Absorption
- ✓ Refraction
- ▪ Diffraction
- ▪ Scattering

Diffraction



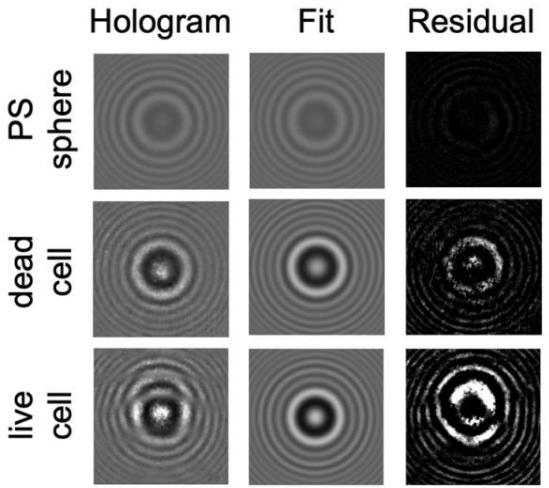


Interaction of light with matter

Scattering: In the context of optics, it's the collision of light and a particle (very broadly)

$$\alpha = \frac{\pi\, d}{\lambda}$$

$\alpha << 1$: Rayleigh scattering
$\alpha \sim 1$: Mie scattering
$\alpha >> 1$: Geometric scattering

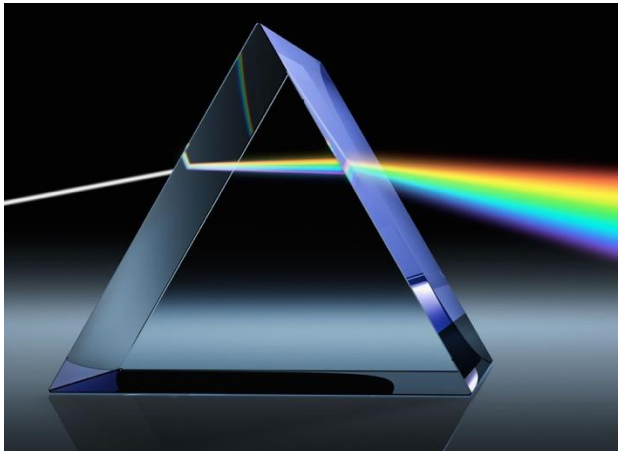


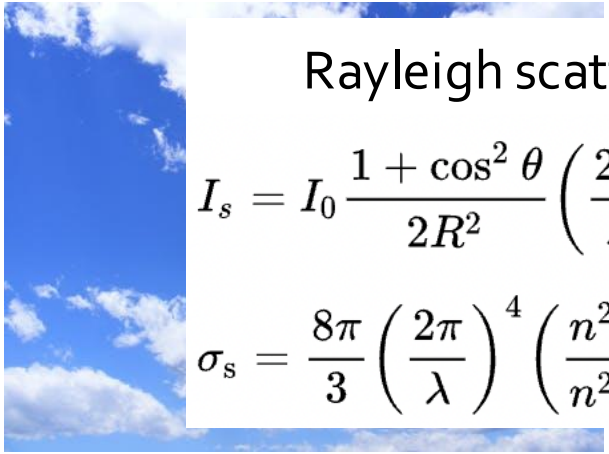
Boltyanskiy et al, Scientific Reports, 2022

Scattering can be:

- Indicative of the presence of cells/biological matter of interest
- We can quantitatively describe the scatter and learn about the underlying biological properties
- An obstacle to the desired imaging

Let's take a step back





Rayleigh scattering

$$I_s = I_0 \frac{1+\cos^2\theta}{2R^2}\left(\frac{2\pi}{\lambda}\right)^4\left(\frac{n^2-1}{n^2+2}\right)^2 r^6$$

$$\sigma_s = \frac{8\pi}{3}\left(\frac{2\pi}{\lambda}\right)^4\left(\frac{n^2-1}{n^2+2}\right)^2 r^6$$

ttering

Optical properties of biological tissues

IOP PUBLISHING PHYSICS IN MEDICINE AND BIOLOGY

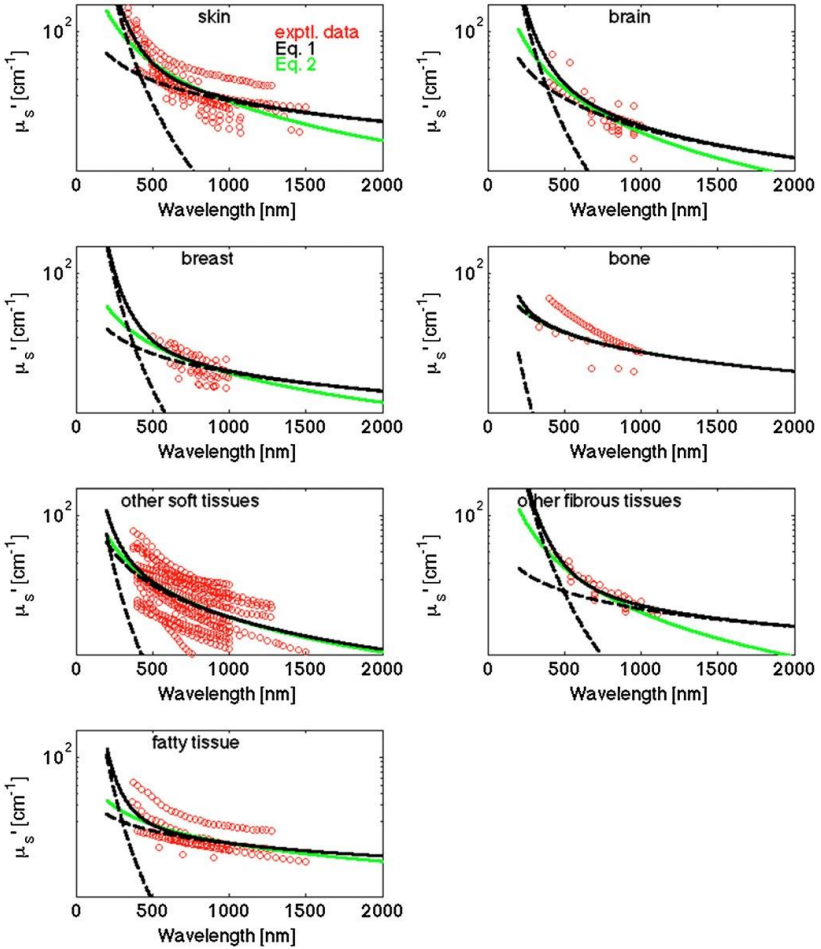
Phys. Med. Biol. **58** (2013) R37–R61 doi:10.1088/0031-9155/58/11/R37

TOPICAL REVIEW

Optical properties of biological tissues: a review

Steven L Jacques[1,2]

Optical properties of biological tissues



$$\mu_s' = a\left(\frac{\lambda}{500\ (\text{nm})}\right)^{-b}$$

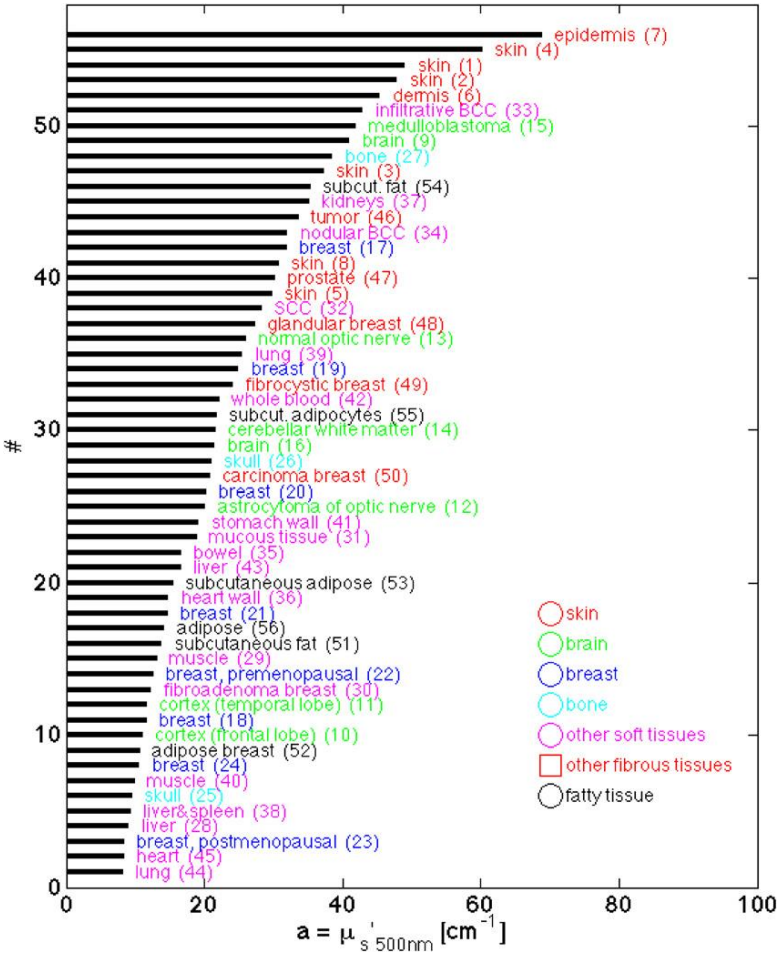
$$\mu_s'(\lambda) = a'\left(f_{\text{Ray}}\left(\frac{\lambda}{500\ (\text{nm})}\right)^{-4} + (1 - f_{\text{Ray}})\left(\frac{\lambda}{500\ (\text{nm})}\right)^{-b_{\text{Mie}}}\right)$$

μ_s' = reduced scattering coefficient

Solid line = Rayleigh scattering component
Dotted line = Mie scattering component

Main take away: Generally, much less scattering at higher wavelengths

Optical properties of biological tissues



A few take aways

- Skin generally scatters more. Need ways around it to image tissues
- Diversity of results for scattering of different tissues
- Soft tissues overall scatter less than fibrous tissues

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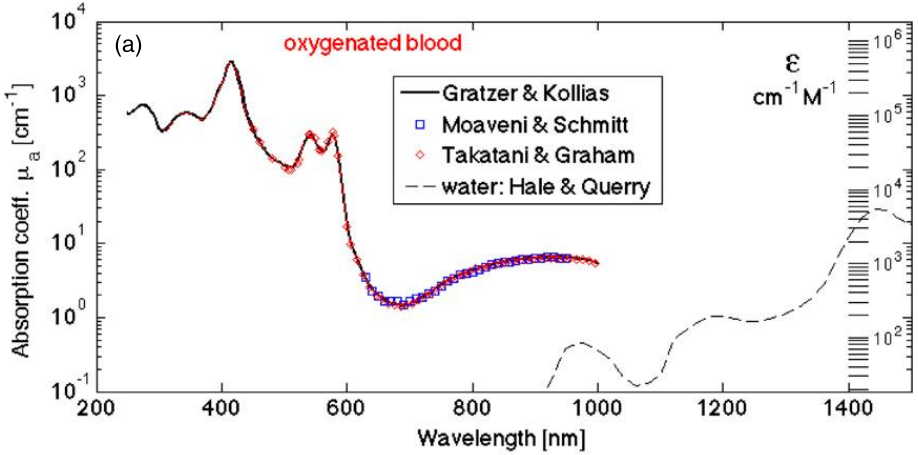
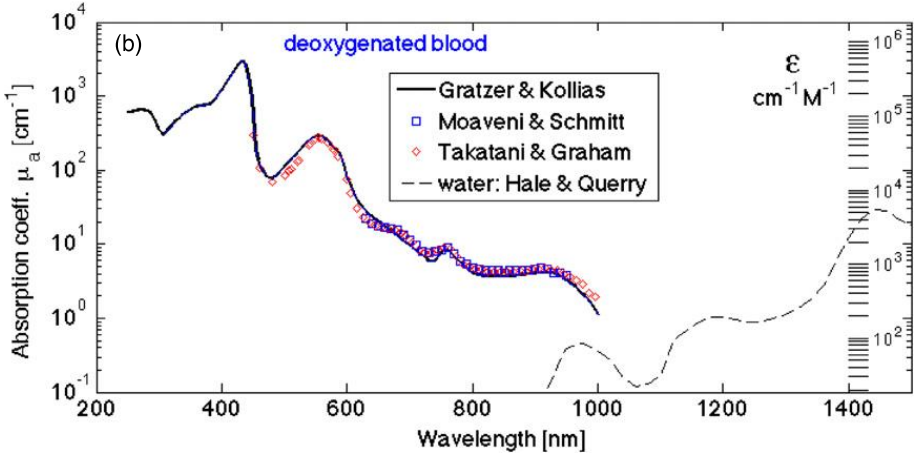
Optical properties of biological tissues





A few take aways

- Here is roughly what's behind the optical finger oxygen meters
- Overall less absorption at higher wavelengths

Quick demo: use flashlight

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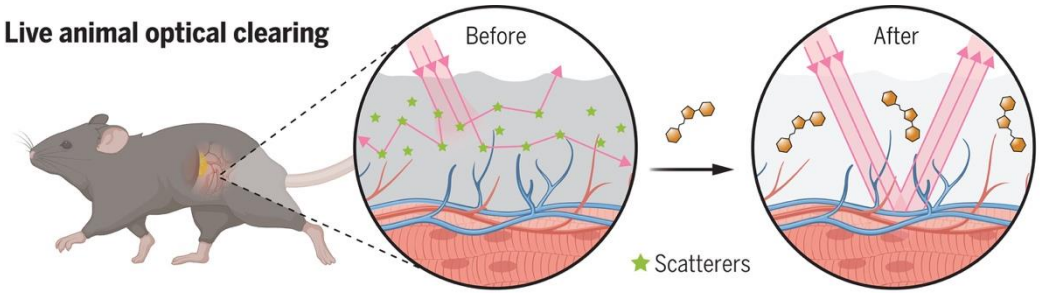
Examples research of optimizing tissue refractive index

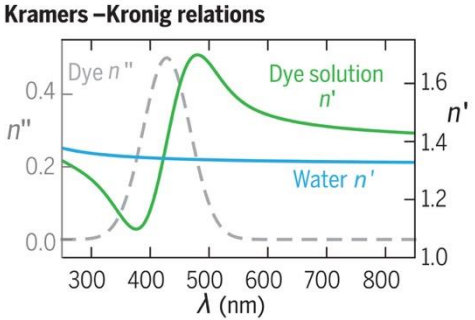
Achieving optical transparency in live animals with absorbing molecules

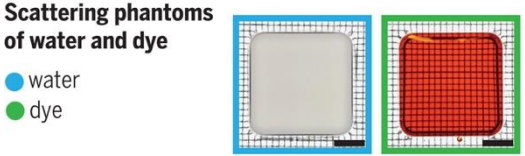
ZIHAO OU, YI-SHIOU DUH, NICHOLAS J. ROMMELFANGER, CARL H. C. KECK, SHAN JIANG, KENNETH BRINSON JR, SU ZHAO, ELIZABETH L. SCHMIDT, XIANG WU, [...], AND GUOSONG HONG +11 authors Authors Info & Affiliations

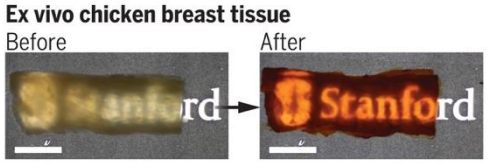
SCIENCE · 6 Sep 2024 · Vol 385, Issue 6713 · DOI: 10.1126/science.adm6869

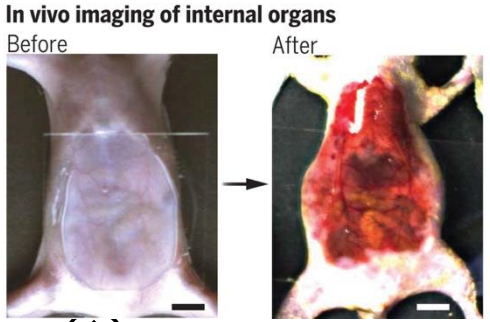
https://www.science.org/doi/10.1126/science.adm6869











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Microscopy tools

Light sources:

- White light: LEDs and incandescent bulbs
- One color + coherence: Lasers

Lenses, mirrors etc

Cameras

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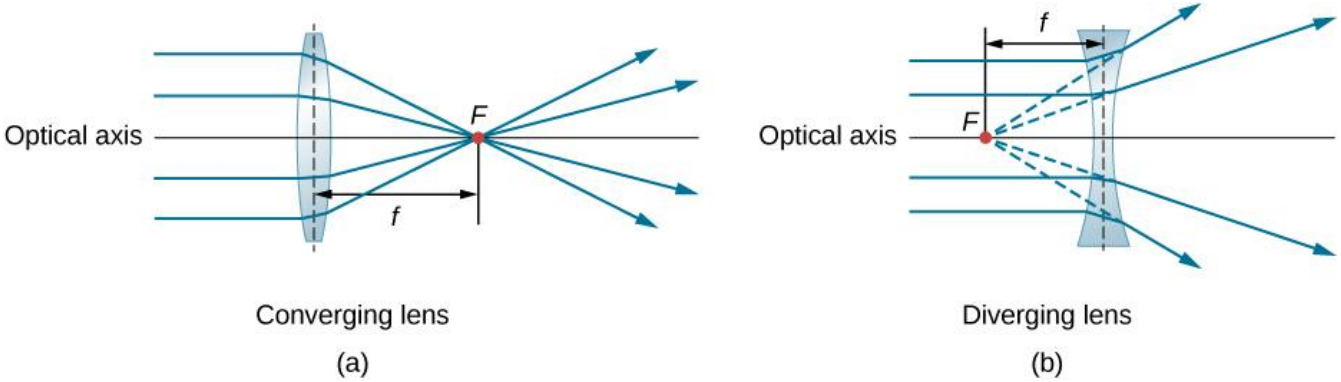
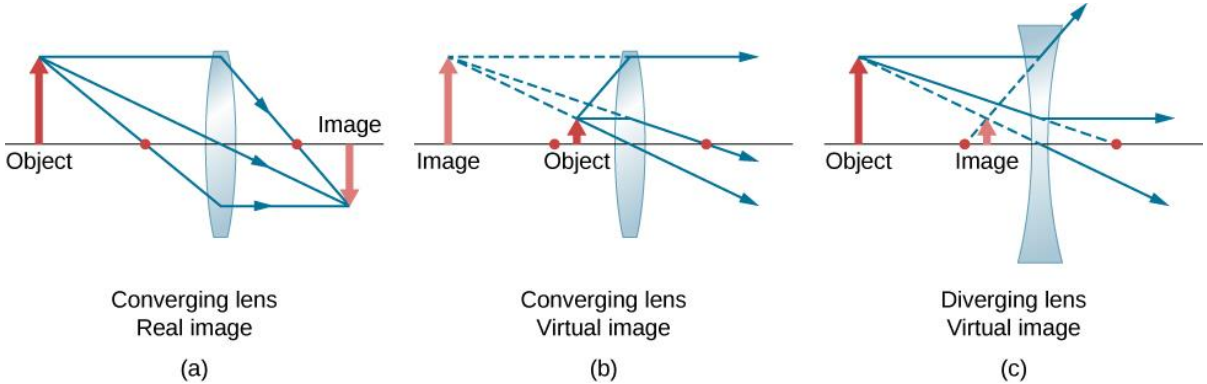
Microscopy tools

Lenses: Focus and collimate light based on Snell's law (and Len's Maker's formula)
Carry and transform imagine information (such as magnification)





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Microscopy tools

Lenses: Focus and collimate light based on Snell's law (and Len's Maker's formula)
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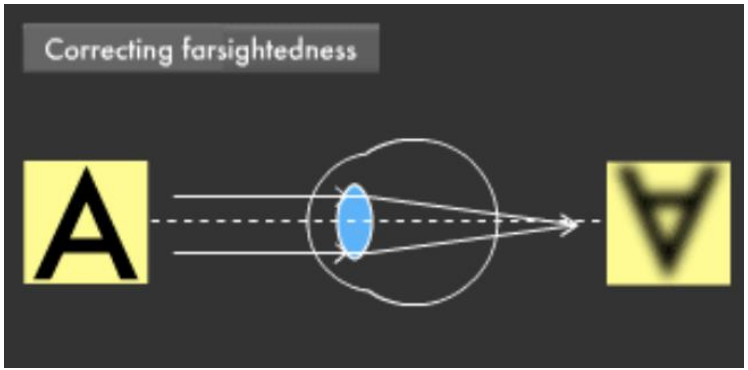
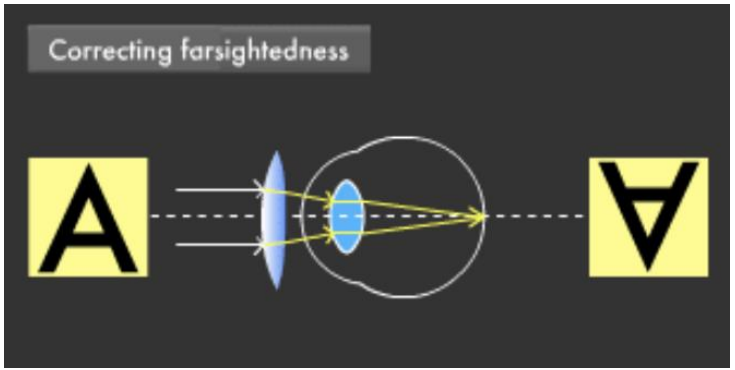
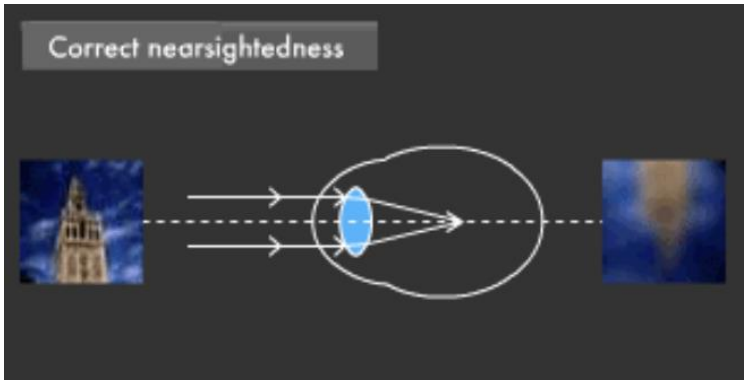
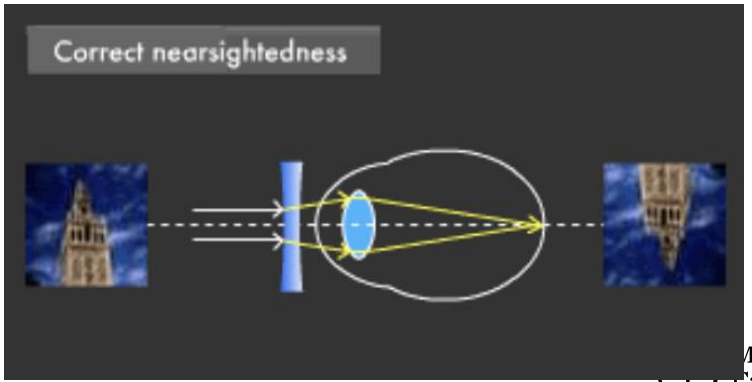




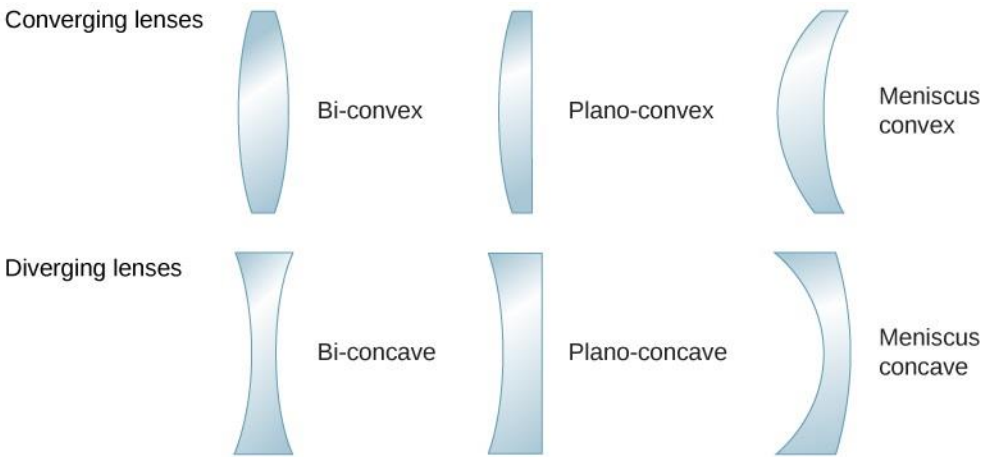


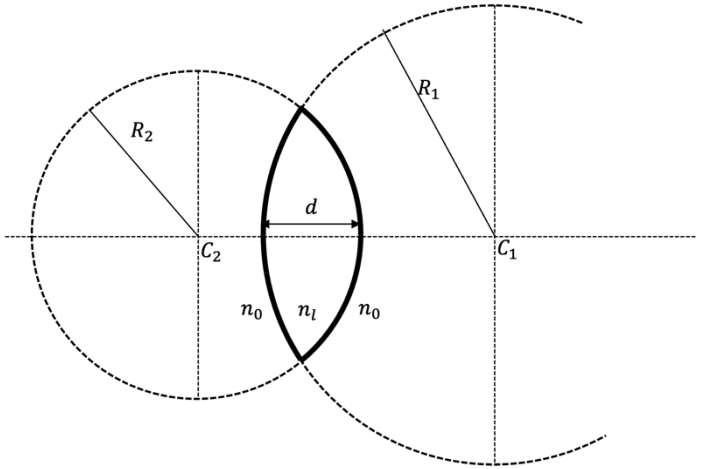


https://global.canon/en/technology/s_labo/light/003/02.html

Microscopy tools

Lenses: Focus and collimate light based on Snell's law (and Len's Maker's formula)
Carry and transform imagine information (such as magnification)





Lens-maker's formula:

$$\frac{1}{f} = \left(\frac{n_l - n_0}{n_0}\right)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

Thin lenses:

$$\frac{1}{f} = \frac{1}{d_0} + \frac{1}{d_i}$$

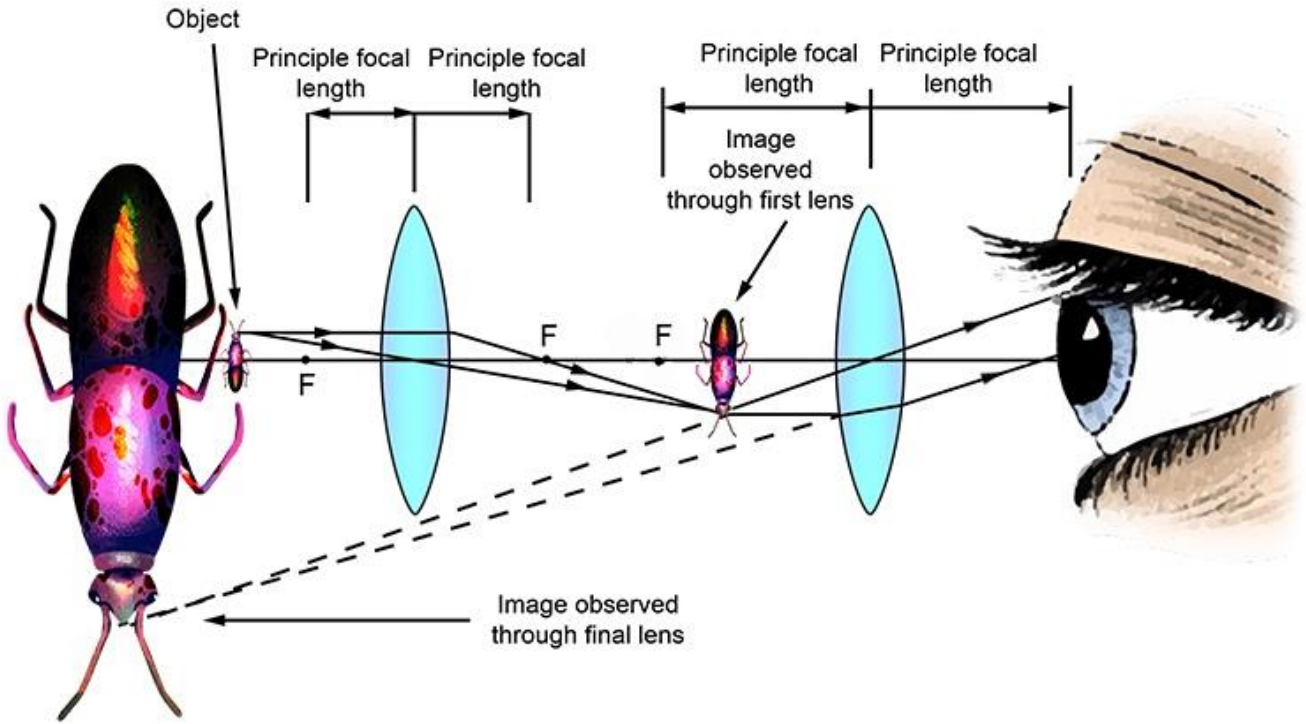
Magnification

$$M = \frac{d_i}{d_o}$$

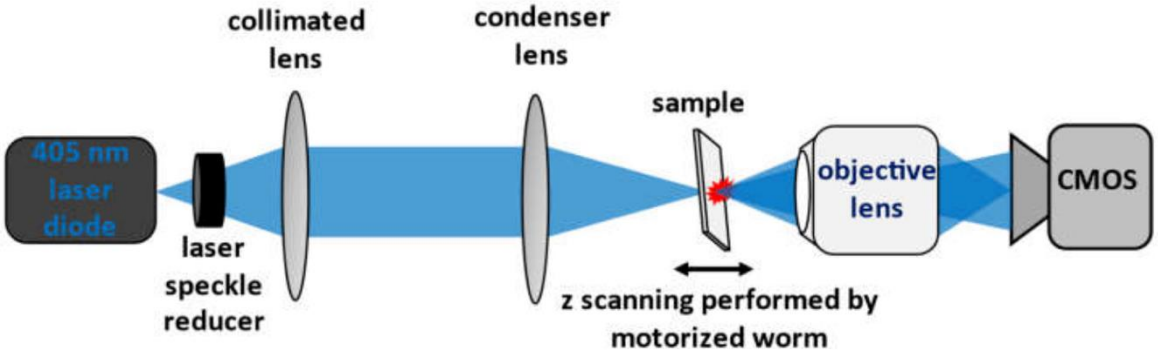
Microscopy tools: combination of lenses

Combinations of lenses can be used to relay and magnify and image

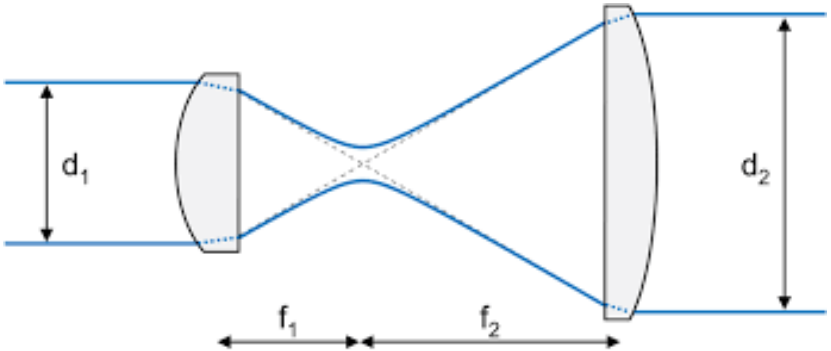
This is the fundamental principle behind compound microscopes



Bare bones of a microscope



Dion, Sylvere & Agre, Don & Akpa Marcel, Agnero & Zoueu, Jeremie. (2023). Multiplane Image Restoration Using Multivariate Curve Resolution: An Alternative Approach to Deconvolution in Conventional Brightfield Microscopy. Photonics. 10. 163. 10.3390/photonics10020163.



Soliman, Dominik. (2016). Augmented microscopy: Development and application of high-resolution optoacoustic and multimodal imaging techniques for label-free biological observation. 10.13140/RG.2.2.24410.03525.

- Lenses are placed at focal lengths from each other.
- Can resize a beam with 2 lenses at focal lengths apart (magnification = ratio of focal lengths)
- Due to dispersion, the exact workings of all optics will be wavelength dependent!

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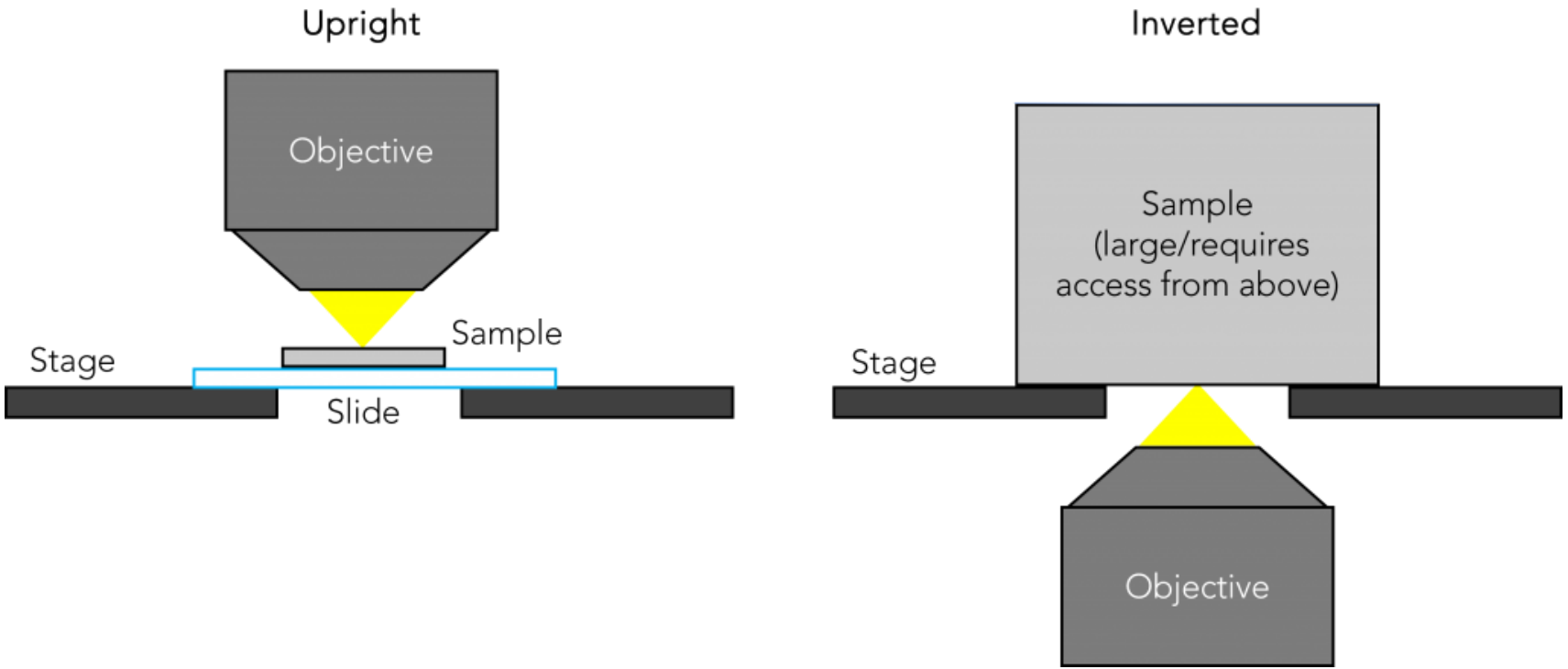
Upright vs. Inverted Microscopes



https://www.edinst.com/blog/illumination-techniques-in-optical-microscopy/

Little history break: first microscopes

Letters, however small and dim, are comparatively large and distinct when seen through a glass globe filled with water. Seneca (c. 4 BC – AD 65)

Zacharias Janssen (1585–c. 1632) and his father Hans are thought to have made one of the earliest (c. 1600) compound microscopes

Robert Hook Microscope, 1665

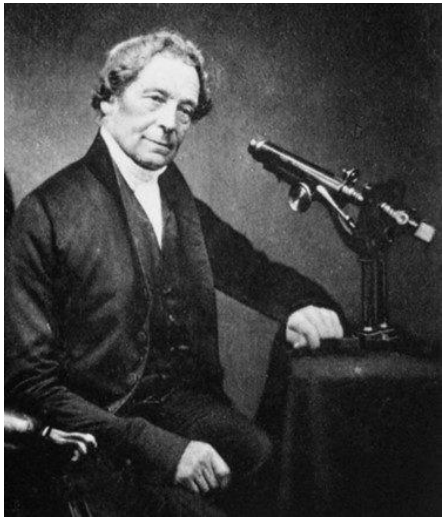
First picture illustrating use of a microscope ~1686

Leeuwenhoek (1632–1723) made simple one-lens microscopes using small, high-quality lenses that he ground himself. Leeuwenhoek observed animal and plant tissues, protozoa, human sperm and red blood cells etc

First microscopes

Joseph Lister (1786–1869) and his son: doctor and engineer.

First achromatic lenses (minimizing dispersion)

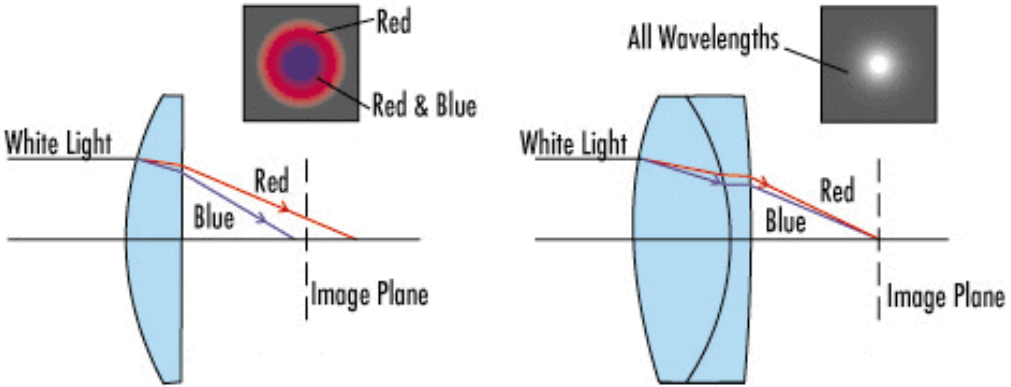


Nikon's first microscope ~ 1920's

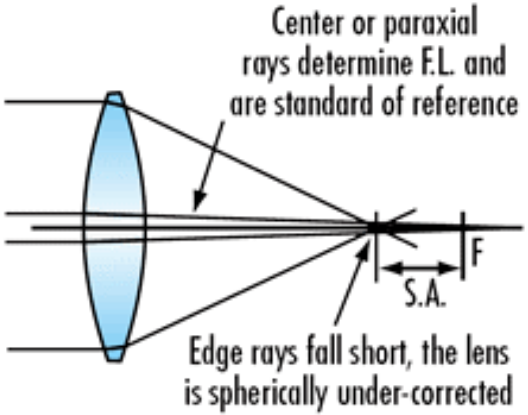
- **The invention of a microscope completely revolutionized cell biology.**
- **This is our dream in Cancer Engineering: to revolutionize cancer research and patient care through novel tools and technologies.**

How to fix dispersion and spherical aberrations?

Dispersion

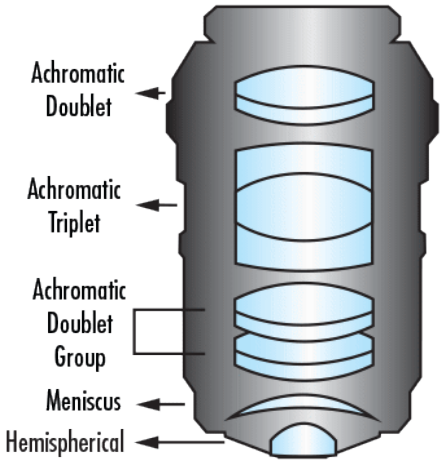


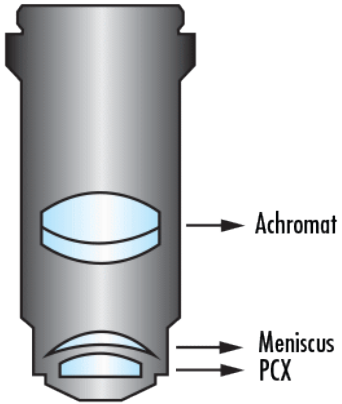
Spherical aberrations

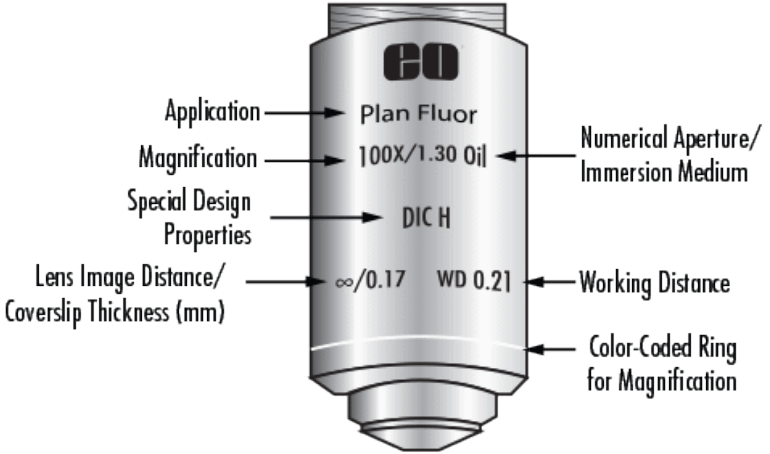


Modern objectives often have numerous combinations of lenses that try to compensate both of these effects

The anatomy of an objective







The objective is one of the most important choices when designing an imaging system

Important imaging / microscopy concepts

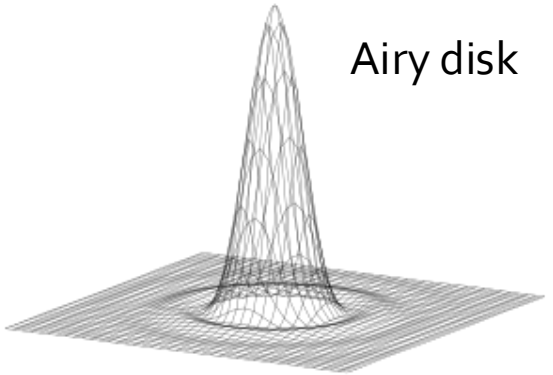
Resolution: the ability to distinguish 2 features close together

This blurred image of a feature is called the **point spread function (PSF)**

The PSF comes from all imperfections in the optics combined.

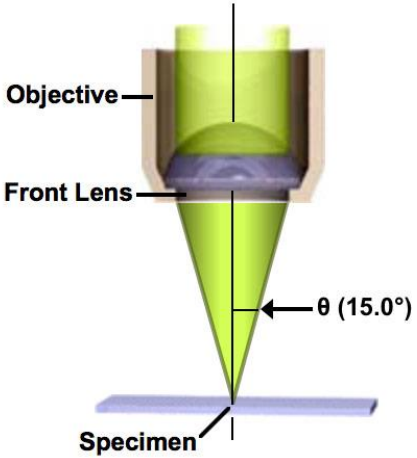
Cutting off part of the light from the finite size of objective is a bit part of it

$$\mathrm{PSF}(f,z) = I_r(0,z,f)\exp\left[-z\alpha(f) - \frac{2\rho^2}{0.36\frac{cka}{\mathrm{NA}f}\sqrt{1+\left(\frac{2\ln 2}{c\pi}\left(\frac{\mathrm{NA}}{0.56k}\right)^2 fz\right)^2}}\right]$$



Airy disk

Important imaging / microscopy concepts



The objective doesn't capture all of the light and the sample scatters light as well. Both of those contribute to the loss in resolution:

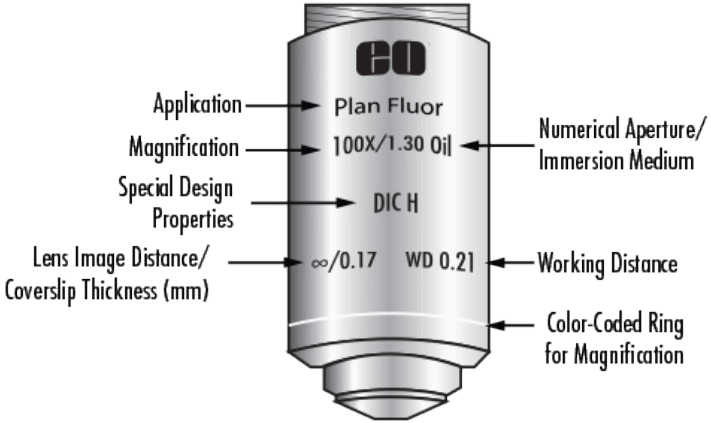
Numerical aperture (NA) = $n\ sin(\theta)$

Where n is the refractive index of the immersion medium
High NA = better resolution

Microscopy resolution: $\Delta X \approx \frac{\lambda}{2\ NA}$

- Exact numbers depends on how you define overlap of Airy disks
- Higher NA means better resolution
- Imaging with lower frequency / higher wavelength gives better resolution
- This is just an approximation. Other optics aberrations can degrade the resolution.

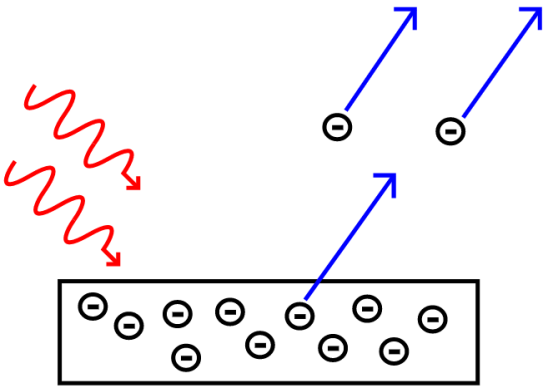
One more word on objectives



Working distance of an objective (**WD**) is the maximum distance between the surface of the objective and the closest surface of the glass slide

Manufacturer	Correction	Magnification	Numerical Aperture	Working Distance
Nikon	PlanApo	10x	0.45	4.0 mm
Nikon	PlanFluor	20x	0.75	0.35 mm
Nikon	PlanFluor (oil)	40x	1.30	0.20 mm
Nikon	PlanApo (oil)	60x	1.40	0.21 mm
Nikon	PlanApo (oil)	100x	1.40	0.13 mm

Basics of cameras



https://www.photometrics.com/learn/imaging-topics/what-happens-when-light-hits-a-pixel

The most fundamental working principle of all cameras is the **photoelectric effect**

- Light is incident on camera chip (usually silicon), electrons are released from the surface
- The number of electrons released is proportional to the intensity of the light
- The released electrons generate a current that is then converted into an intensity level displayed to the user

CMOS sensors: Complementary Metal Oxide Semiconductor
Each pixel has an amplifier
Usually more noise, but cost-effective and light

CCD sensors: Charged coupled devices
Each pixel is a photodiode and collects it's photoelectrons
Usually produce better resolution

Signal to noise ratio (SNR)

- Just like every measurement has an error bar and the measurement is only meaningful with an error bar, every image has noise.

- In optics: $\mathrm{SNR} = \frac{S}{\sqrt{\sigma_S^2+\sigma_D^2+\sigma_R^2}}$

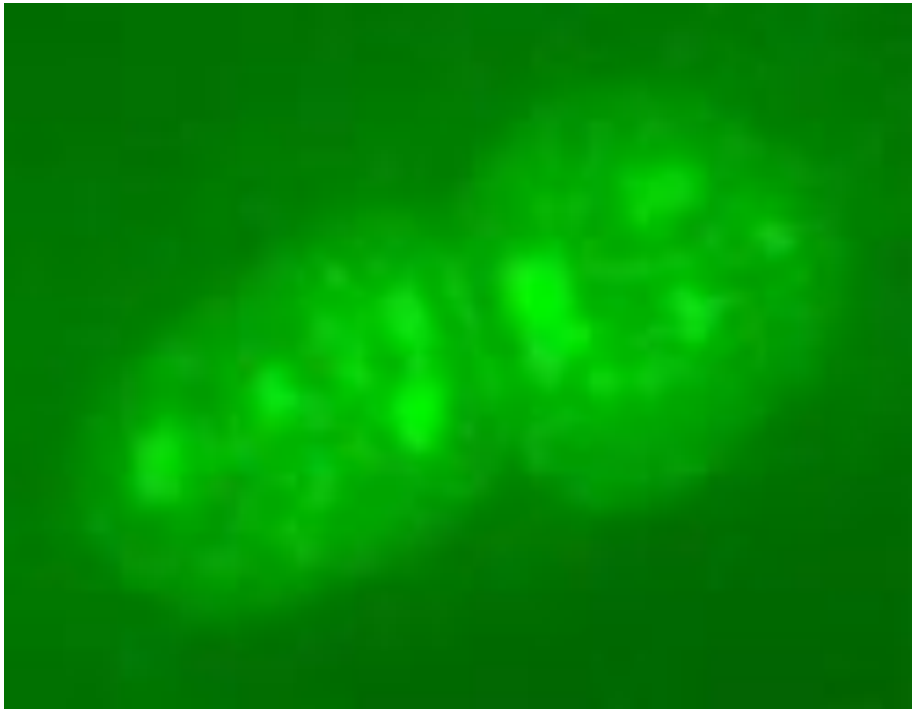
S = total signal (total number of photons)
σ_s = photon shot noise
σ_D = dark noise
σ_R = read noise

σ_s = photon shot noise: quantum nature of light ($1/\sqrt{N}$)
σ_D = dark noise: electronic noise from thermal fluctuations in a camera
σ_R = read noise: noise in converting charge to pixel value

- In practice, you also have extraneous signal from non-relevant sources contributing to the noise

Signal to noise ratio (SNR): example

- Think of 3 different ways to define SNR of the following image.
- Identify at least one pitfall of each



Reminder of where we are: common aspects of all imaging

1. Source of signal
   - Light, sound, radioactive decay, magnetic moment etc.

2. Method of detection
   - How does the signal get captured?
   - How is the signal processed/converted into interpretable data

3. Generation of images
   - What creates contrast?
   - SNR and not signal is what matters
   - Does contrast represent a qualitative or quantitative metric?
   - Is the contrast coming from a material property or a biological function?

Widefield microscopy

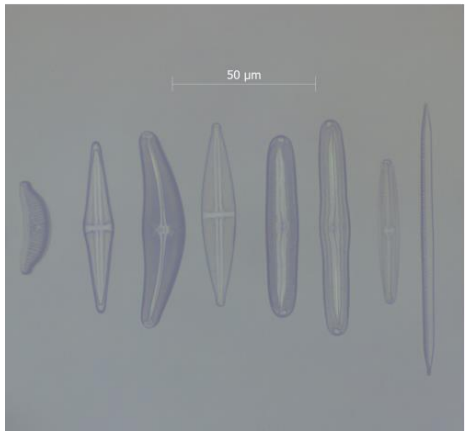
Transmission: The light detected in the camera (or eye piece) is what the sample transmitted. (bright = less scattered etc)

Reflection: The light detected is what's reflected from the sample

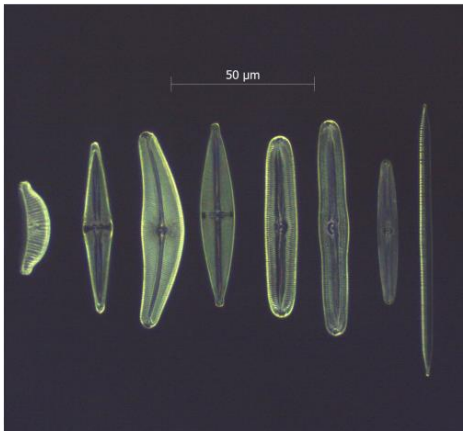
Brightfield

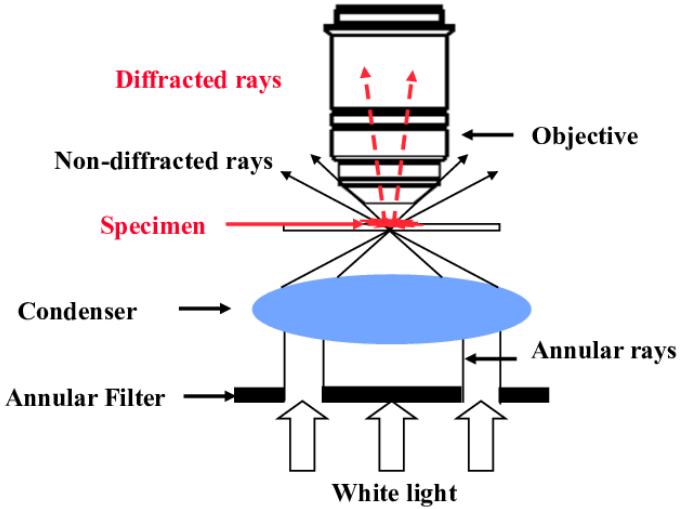
Darkfield: Block any light that's transmitted and only keep what has been scattered by the sample

Brightfield

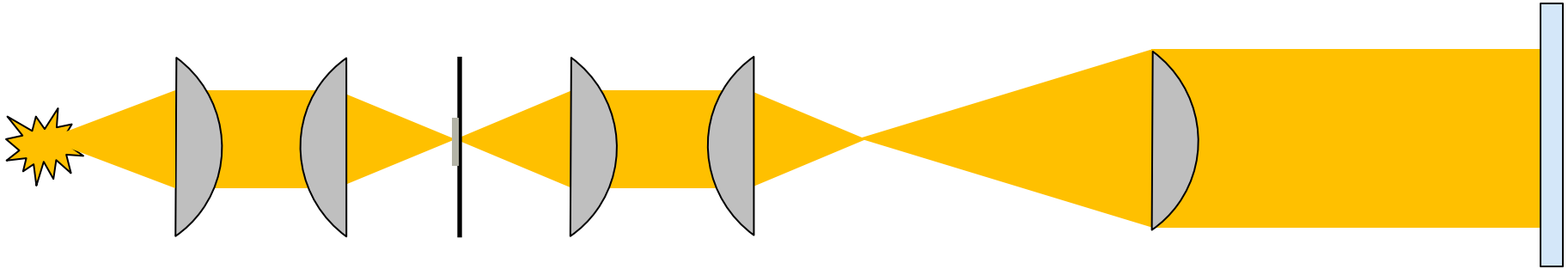


Darkfield

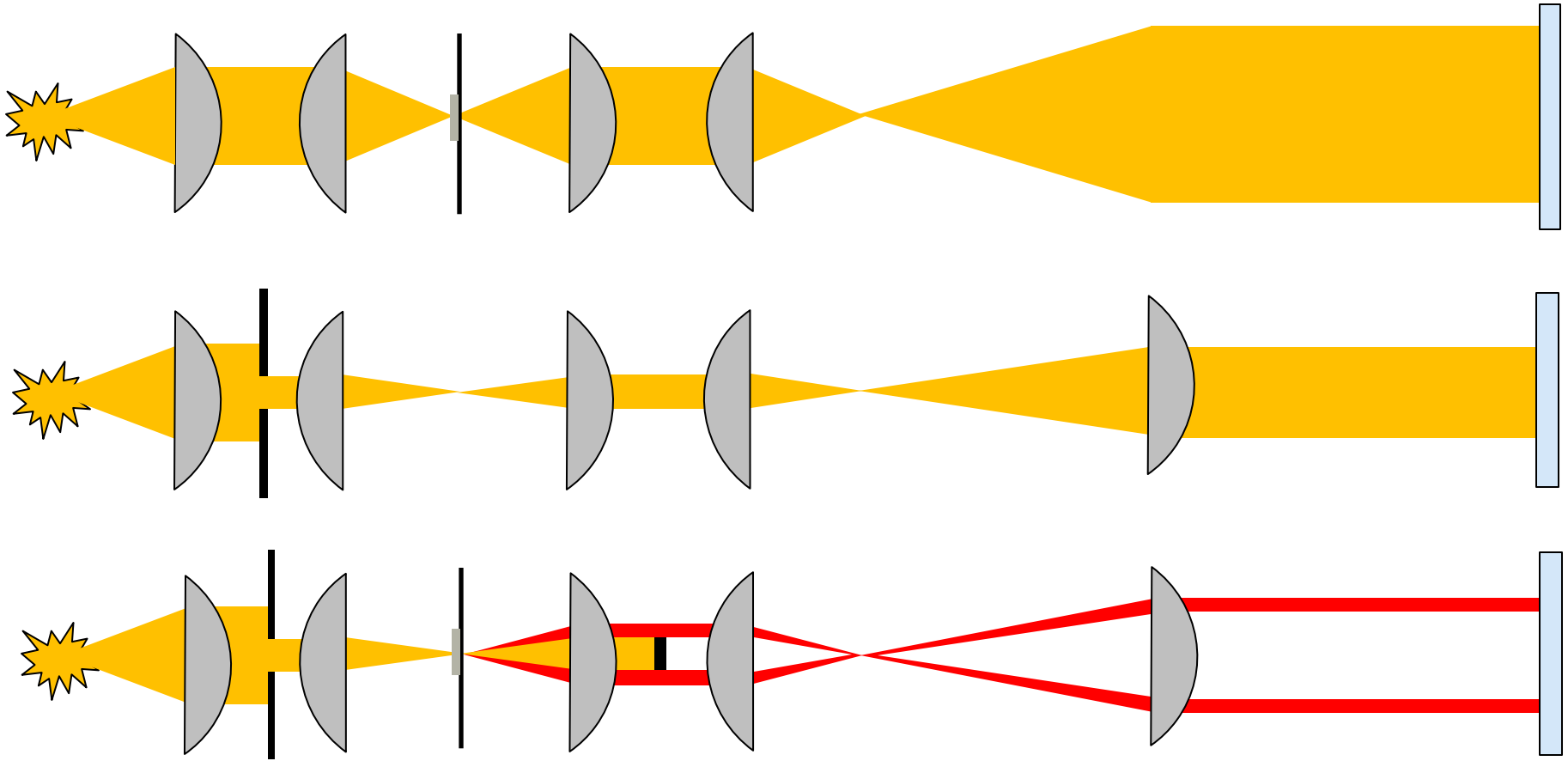




Transmission

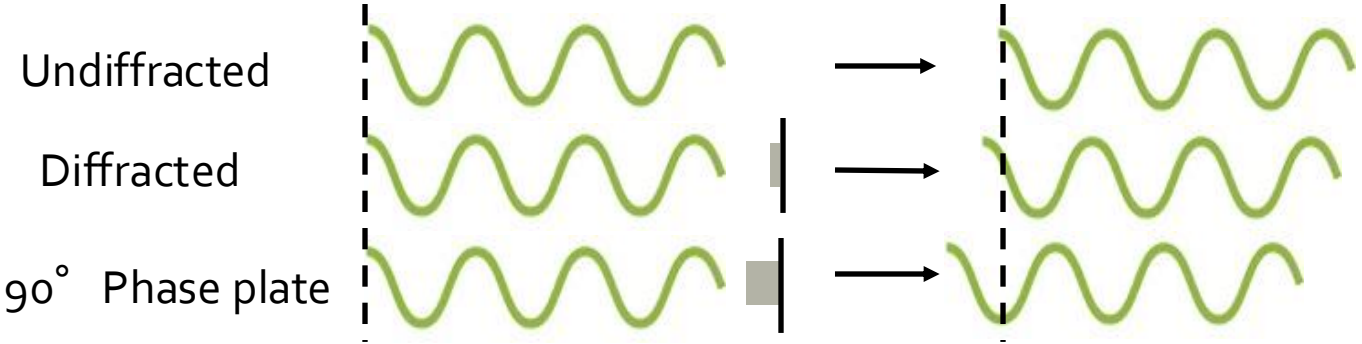


Darkfield



Widefield microscopy: phase imaging

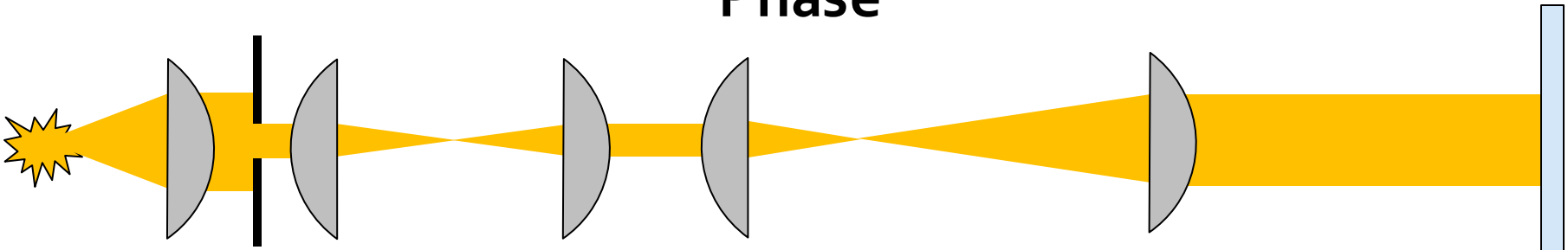
Phase: Use the fact that samples can change the phase of light to create contrast



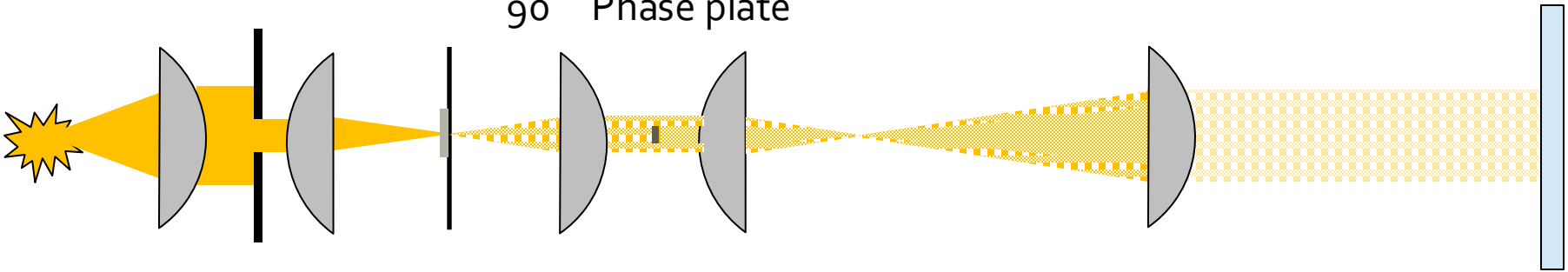
Idea of phase imaging: Make it such that light that did not interact with a sample interferes destructively and disappears. This occurs through a phase plate (or multiple).

Fritz Zernike got the Noble Prize for this in 1953

Phase



90° Phase plate



Widefield microscopy: phase imaging

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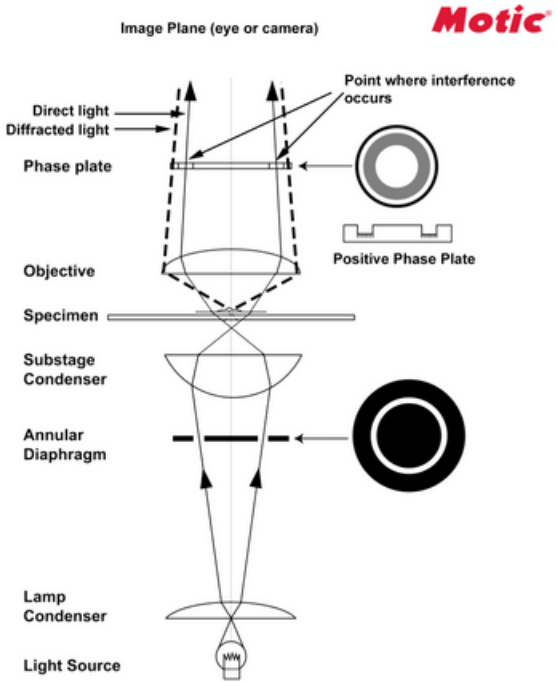
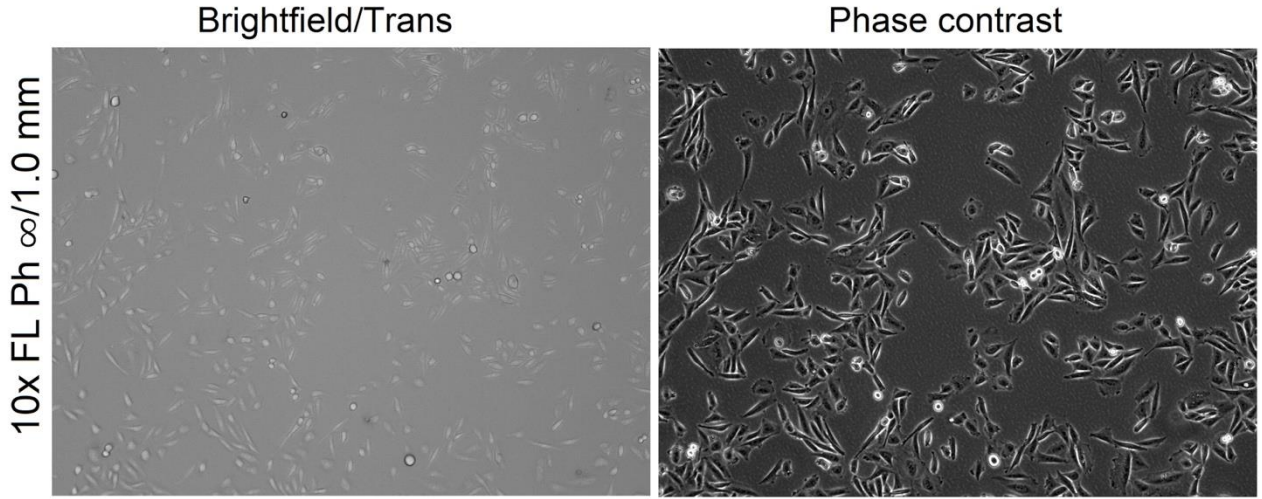


https://moticmicroscopes.com/blogs/articles/phase-contrast-by-motic



https://www.thermofisher.com/us/en/home/life-science/cell-analysis/cellular-imaging/cell-imaging-systems/evos-objectives/selection-guide-evos-objectives.html

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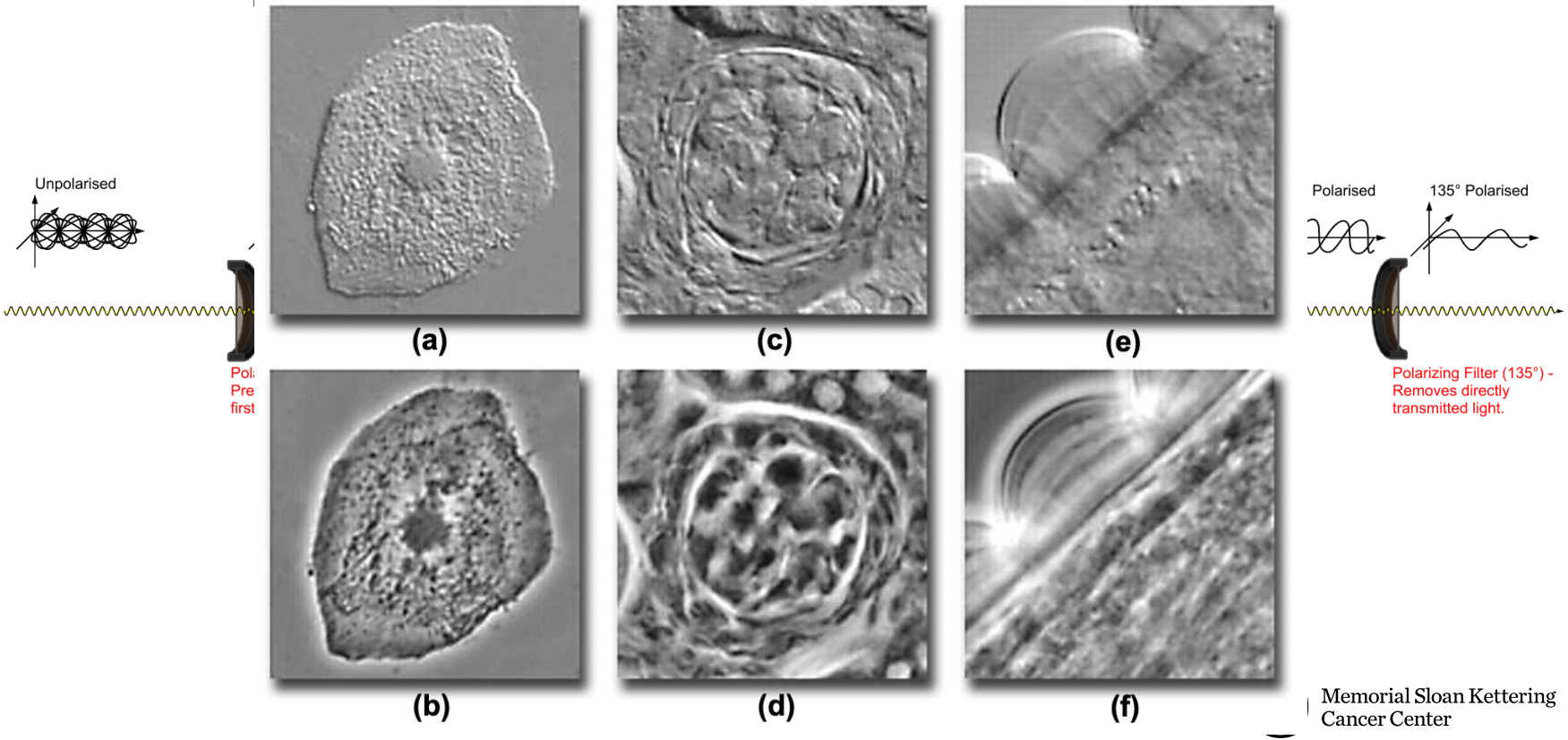
Widefield microscope: beyond transmission

DIC: Differential interference contrast. Use polarization to separate beams of light and utilize phase delays caused by the sample to create contrast



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Quick recap

Some aspects of light we have discussed

Amplitude

Frequency / wavelength

Direction of propagation

Phase

Speed

Polarization

Which aspect is most relevant in the following imaging techniques

Transmission

Phase

DIC

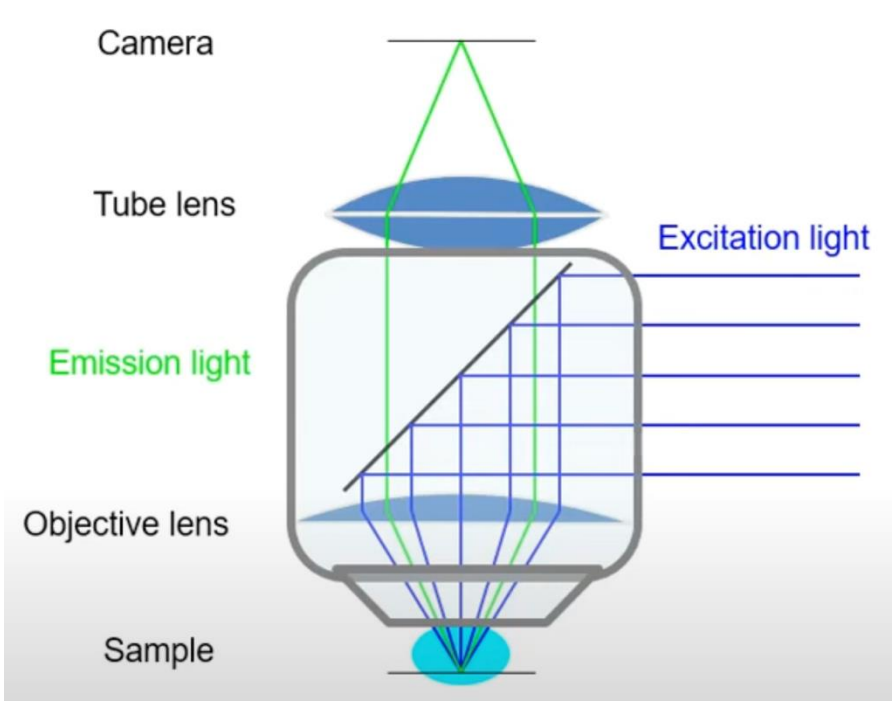
Reflection

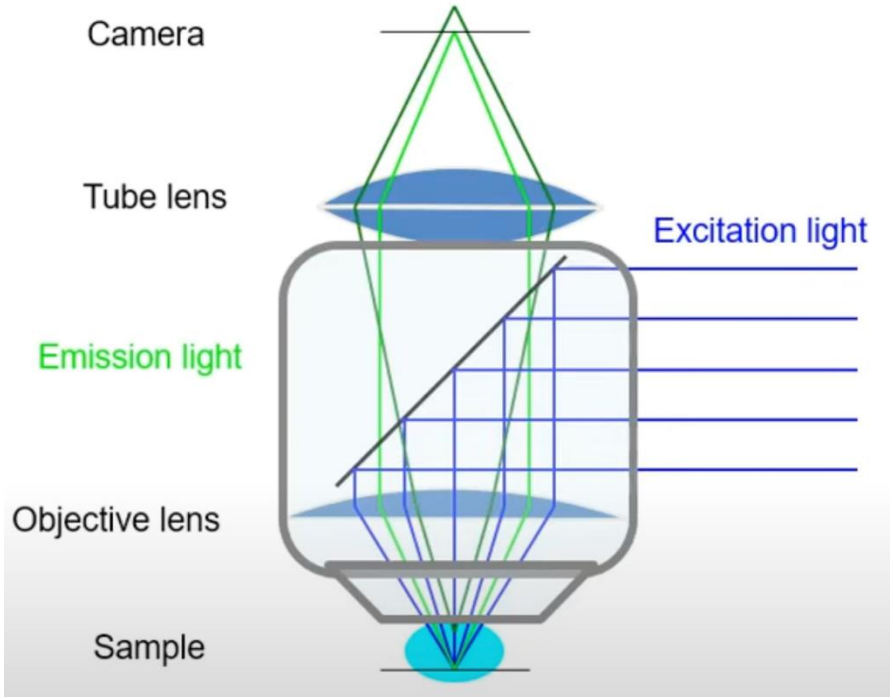
Darkfield

What is not a widefield scope? A confocal

Confocal: A selective way of imaging a thin slice of a sample.

Widefield



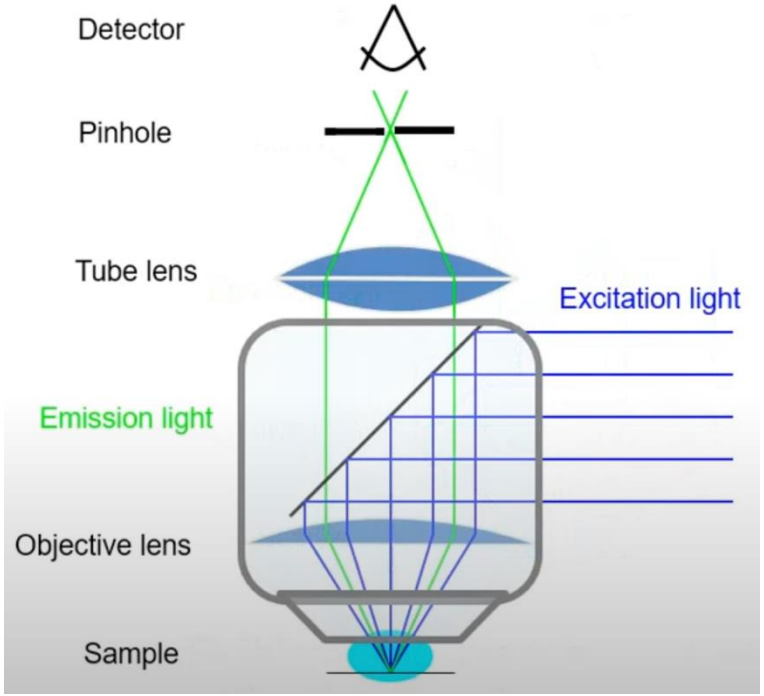


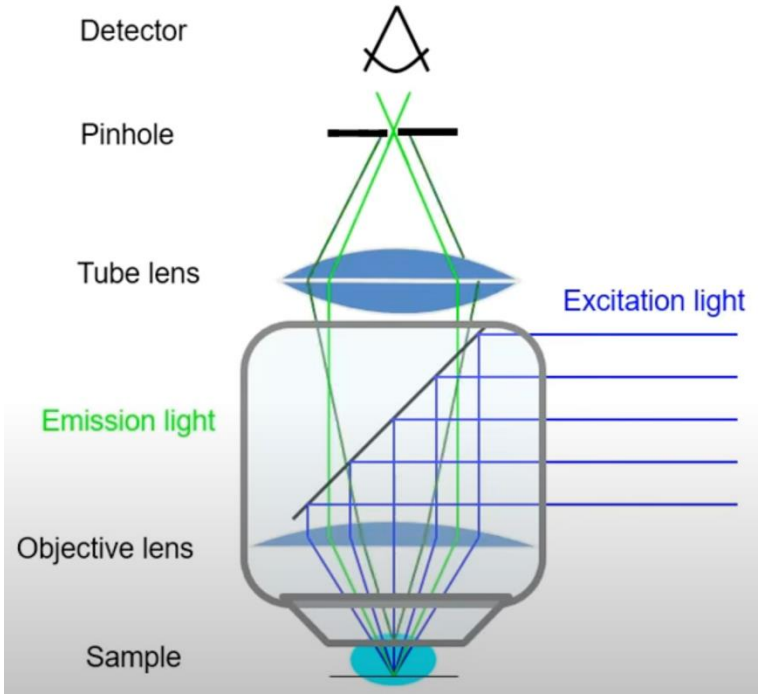
https://www.youtube.com/watch?v=FJbn_eXzA4o

What is not a widefield scope? A confocal

Confocal: A selective way of imaging a thin slice of a sample.

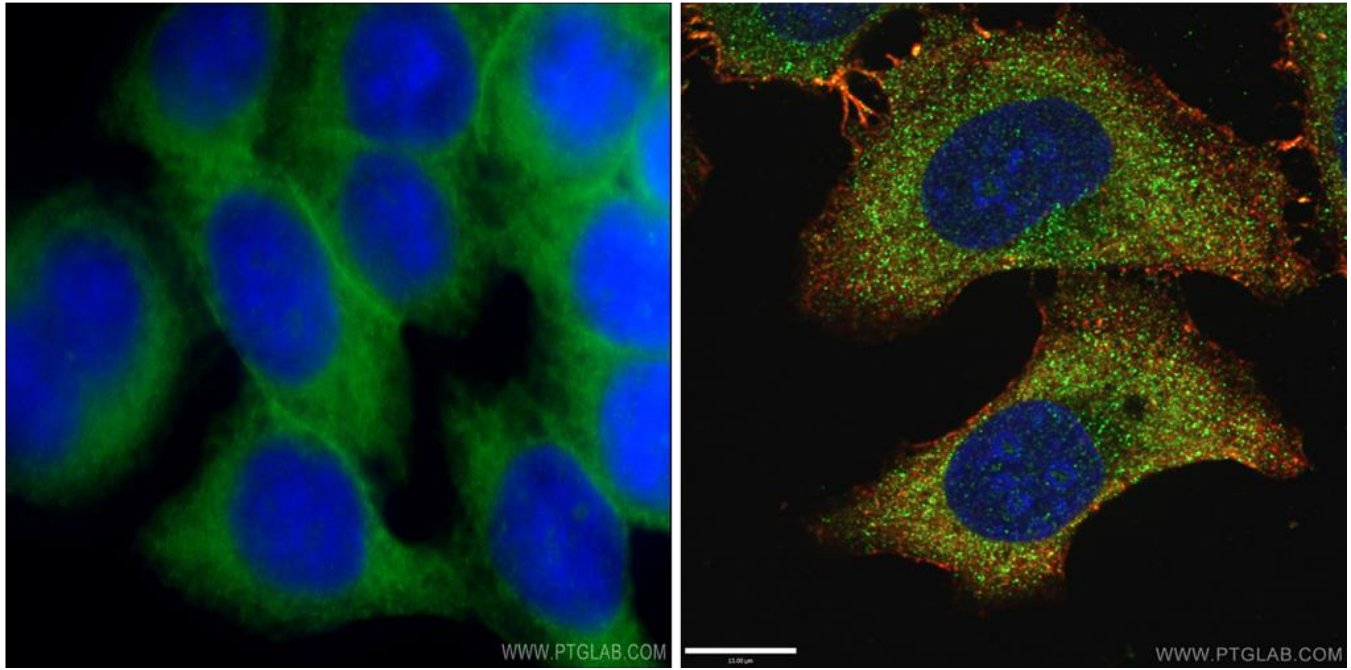
Confocal





https://www.youtube.com/watch?v=FJbn_eXzA4o

Confocal imaging

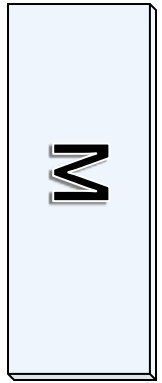


https://www.ptglab.com/news/blog/if-imaging-widefield-versus-confocal-microscopy/

Benefits and drawbacks?

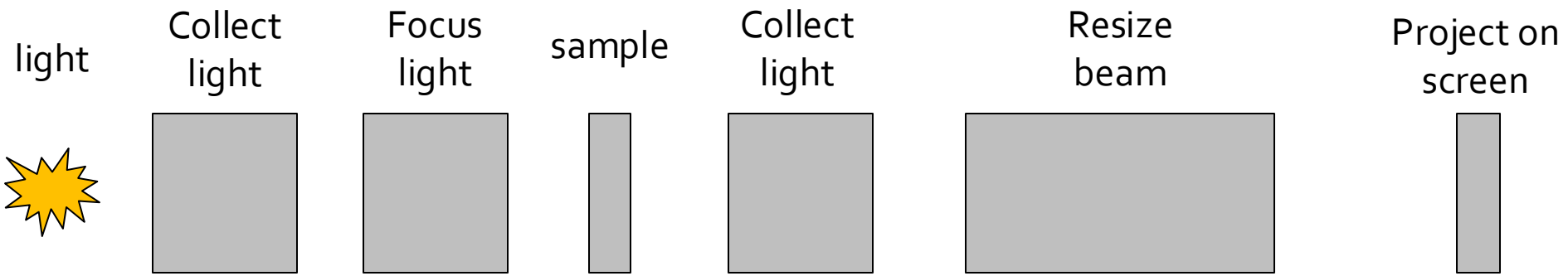
While the signal may be decreased, the contrast and resolution are usually better.

Exercise: to build the bare bones of a simple microscope



Exercise: to build the bare bones of a simple microscope

Transmission

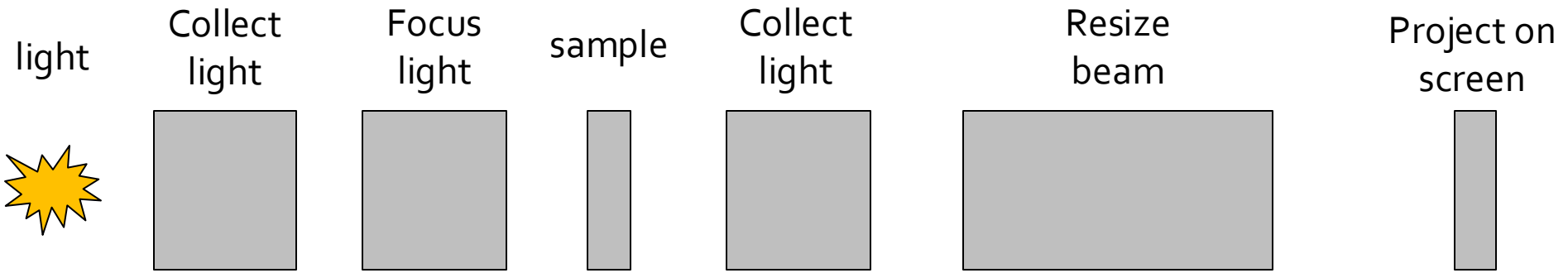


Part 1: Break up into groups of 2 or 3 and draw where you would put the lenses to project a transmission image (5 minutes)
Can use handout as a guide
You have four plano-covex 60mm lenses, one plano-covex 125mm lens

Exercise: to build the bare bones of a simple microscope

Darkfield

Part 2: In the same groups discuss what and where you would need to add to the system to turn it into darkfield imaging.



Exercise: to build the bare bones of a simple microscope

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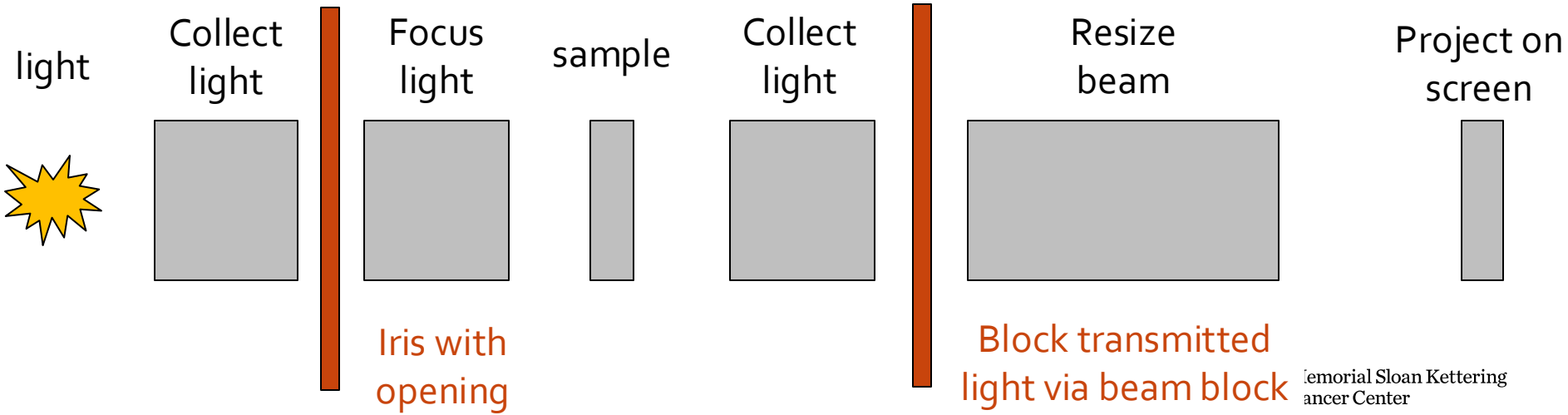


Exercise: to build the bare bones of a simple microscope

Objective #1: Build a simple transmission microscope and project the image of your sample (letter) on a far placed screen

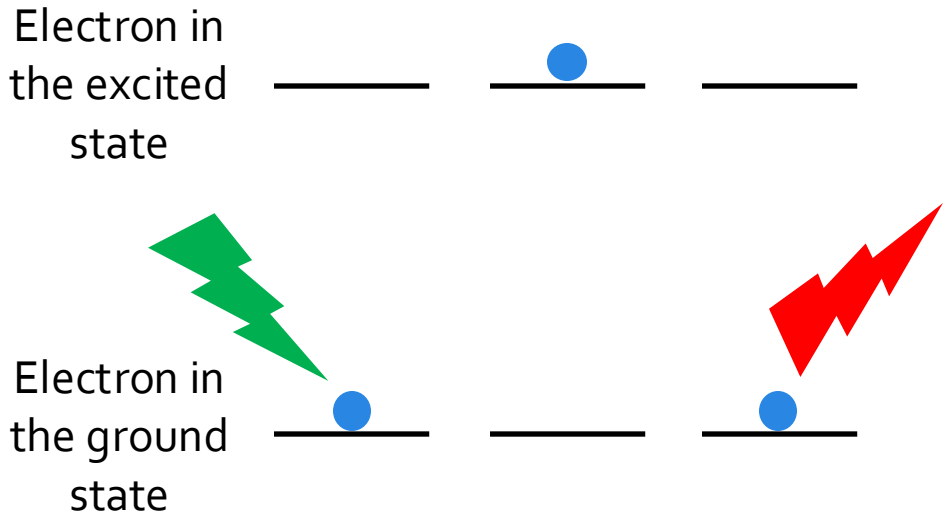
Objective #2: Convert your setup into a darkfield microscopy and demonstrate that you are imaging scattered light almost exclusively
(In addition to lenses, have an iris and a beamblock)

Tips:
→ Feel free to use the cheat sheet of suggestions
→ Make sure everyone has a part in the building

Fluorescence imaging

Fluorescence imaging

Fluorophore: Usually a small molecule or a protein that has "excitable" electrons which absorb one frequency of light and emit another.



The frequency of light that illuminates the fluorophore is called the **excitation frequency.**

The frequency of light that is emanated is called the **emission frequency.**

The length of time the electron is in the excited state is called its **lifetime.**

- Excitation frequency is always higher than the emission frequency
- Excitation wavelength is always lower than emission wavelength

$E = \hbar f$

$v = \lambda f$

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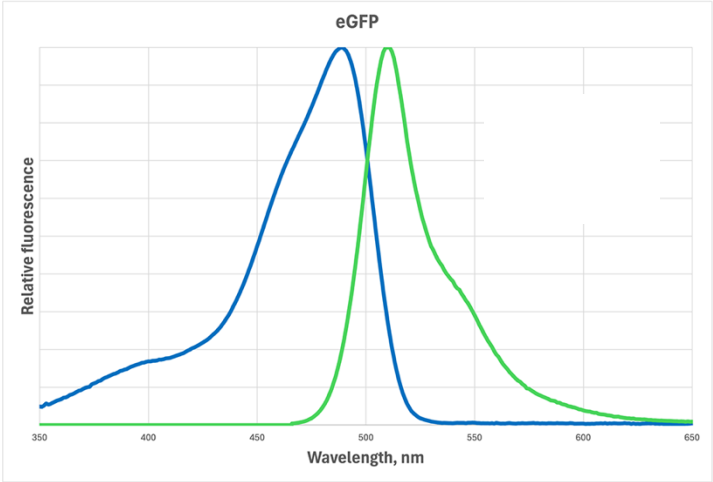
Fluorescence imaging

GFP: Green fluorescent protein

2008 Noble prize in chemistry

Different variants but most common one is illuminated in blue and fluoresces in green



At this points, probably hundreds of fluorescent dyes, proteins etc with the possibility to conjugate to different cell proteins / components.

Fluorophore	Fluorescence Colour	Maximal Absorbance, nm	Maximal Emissions, nm	Relative Brightness
Alexa Fluor®405		401	421	3
Pacific Blue		410	455	1
CoraLite®488		495	519	3
FITC		490	525	3
PE*		490; 565	578	5
CoraLite®594		590	617	4
APC		650	661	4
CoraLite®647		650	665	4
PerCP		490	675	2
Alexa Fluor®700		702	723	2

*PE is the same as R-phycoerythrin

Cy = cyanine. APC = allophycocyanin. FITC = fluorescein isothiocyanate. PE = phycoerythrin. PerCP = peridinin chlorophyll protein.

g

A few important fluorescence properties

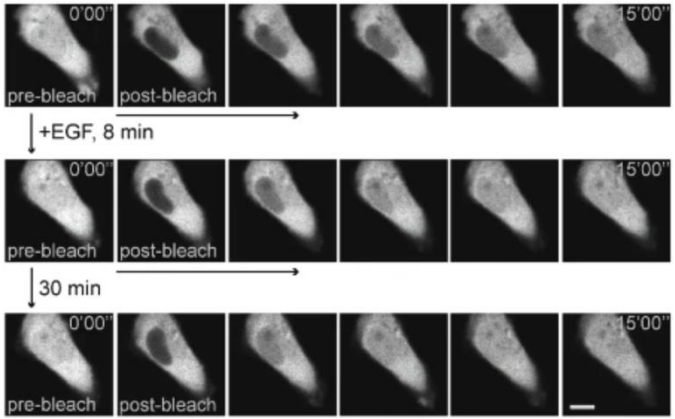
Photobleaching:

- Most applications want to avoid it, but in several cases it's a usual feature
  - Background fluorescence can sometimes be bleached for better SNR
  - **FRAP**: fluorescent recovery after photobleaching is an imaging technique

Repeated FRAP of the actin-binding protein CapG in the cell nucleus—a functional assay for EGF signaling in the single live breast cancer cell

M. K. Fernandez, M. Sinha, R. Kühnemuth & M. Renz

Scientific Reports **14**, Article number: 23159 (2024) | Cite this article

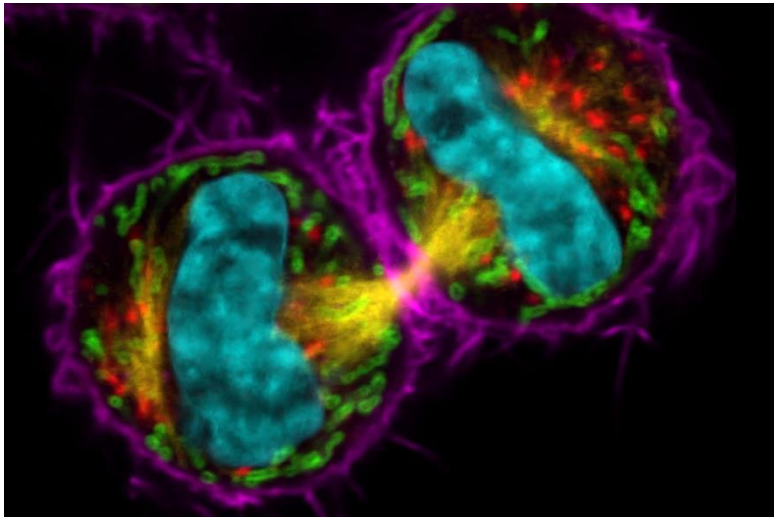


Autofluorescence: Fluorescence of the naturally abundant molecules in cells / tissues of interest

Fluorescence Imaging in Cancer Research
In vitro vs. In vivo

In vitro: "On a glass slide" which usually means with cultured cells, or thin tissue sections

- Could be live cells or stained
- Often a combination of fluorescently tagged cells components and a corresponding phase image of entire cell
- Static or dynamic



Example: COS7 mitotic cells.

- Chromatin (cyan, mCherry)
- mitotic spindle (yellow, EGFP),
- Golgi apparatus (red, Atto647N)
- mitochondria (green, AF532)
- actin filaments (magenta, SiR700)

https://www.leica-microsystems.com/science-lab/cancer-research-image-gallery/

Fluorescence in-vitro applications span wide areas of cancer

Labeling antibodies: By fluorescently tagging specific antibodies one can track the proteins of interest (both in vivo and in vitro).
→ Development and identification of cancer biomarkers

Tagging signaling pathways: Ras, PI3K and other common cancer-related proteins can be tracked when tagged with one of the fluorophores we discussed

Viability assays: Certain dyes only become permeable when cells dye allowing for viability assessment

Light up ECM: Extra-cellular matrix can be visualized either by fluorescently labeling ECM components (collagen etc) or by tracer particles

Labeling specific cells: Can genetically modify cells to express GFP for example and tag their growth / migration etc

Fluorescence in-vitro applications span wide areas of cancer

Tracking immune response: Can tag cancer target cells and thus monitor efficiency of immune clearing.
Can tag immune-specific molecules like PD-1, PD-L1 and track the initiation of an immune response.

Tagging stem cells: Can tag stem cell biomarkers (CD44 or CD133) and look at the progression from stem cells to tumors etc
FACS is a fluoresncence-based protocol commonly used to sort cancer stem cells

Many others of course!

Fluorescence in-vivo applications

Intravital Imaging: Basically in-vivo microscopy, often fluorescence based

How do you image inside of a person or animal?

- Make sure the fluorescent signal (excitation and emission) penetrate through tissues
  - ➢ Use confocal or multi-photon imaging with "regular" fluorophores
  - ➢ Use fluorophores that penetrate deeper into tissues
    - Bioluminescence
    - NIRF / SWIRF

- Create a window to image through
  - ➢ Fix a window (usually in animals) for imaging
  - ➢ Surgically open the tissue to visualize tumor

nature reviews cancer

https://doi.org/10.1038/s41568-022-00527-5

Review article

Check for updates

Intravital imaging to study cancer progression and metastasis

David Entenberg[1,2,3], Maja H. Oktay[1,2,3,4] & John S. Condeelis[1,2,4,5]

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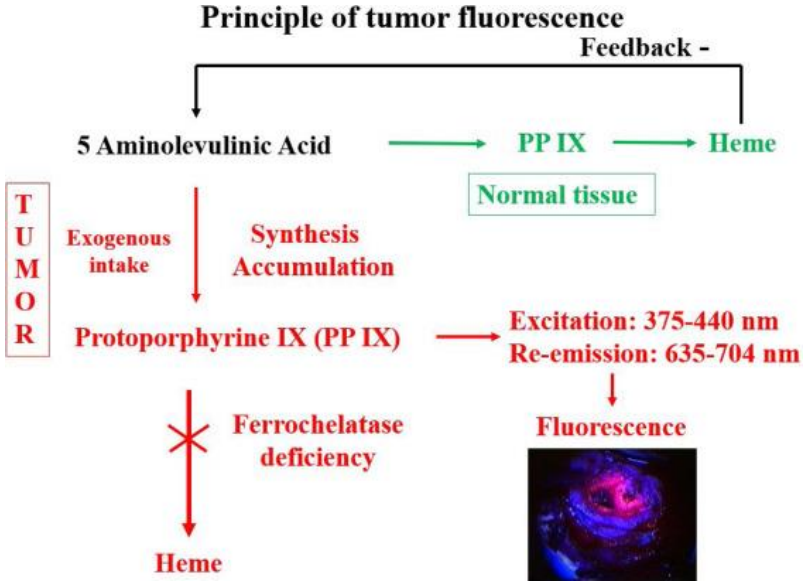
Fluorescence guided surgery



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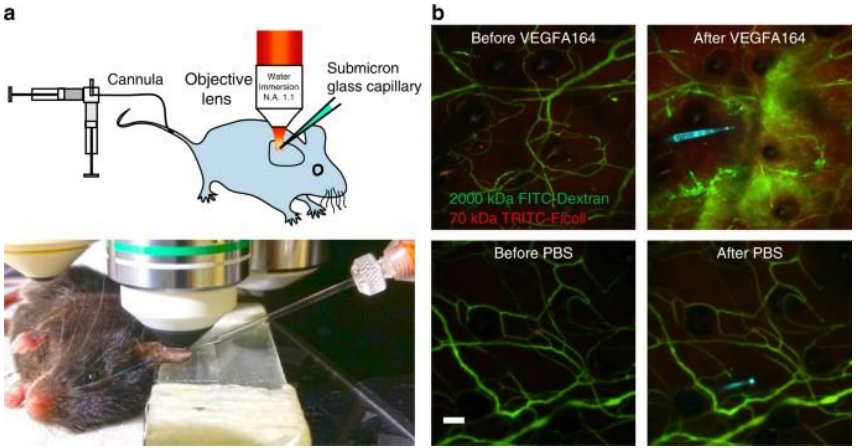
Window for intravital imaging



Intravital imaging-based analysis tools for vessel identification and assessment of concurrent dynamic vascular events

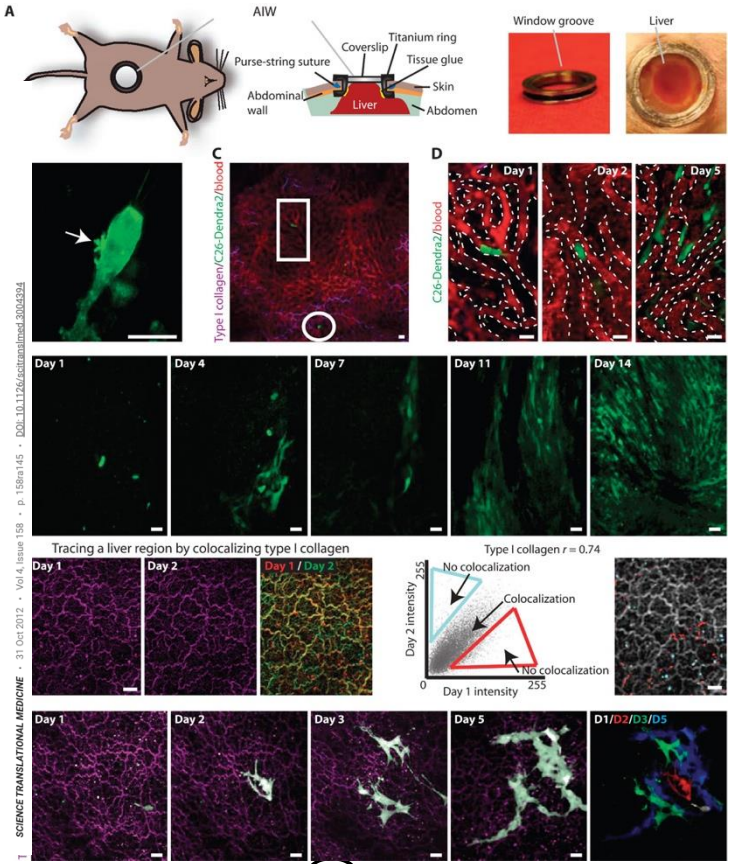
Naoki Honkura, Mark Richards, Bàrbara Laviña, Miguel Sáinz-Jaspeado, Christer Betsholtz & Lena Claesson-Welsh

Nature Communications **9**, Article number: 2746 (2018) | Cite this article

Intravital Microscopy Through an Abdominal Imaging Window Reveals a Pre-Micrometastasis Stage During Liver Metastasis

LAILA RITSMA, ERNST J. A. STELLER, EVELYNE BEERLING, CINDY J. M. LOOMANS, ANOEK ZOMER, CARMEN GERLACH, NIENKE VRISEKOOP, DANIËLLE SEINSTRA, LEON VAN GURP, […], AND JACCO VAN RHEENEN +7 authors Authors Info & Affiliations

SCIENCE TRANSLATIONAL MEDICINE • 31 Oct 2012 • Vol 4, Issue 158 • p. 158ra145 • DOI: 10.1126/scitranslmed.3004394



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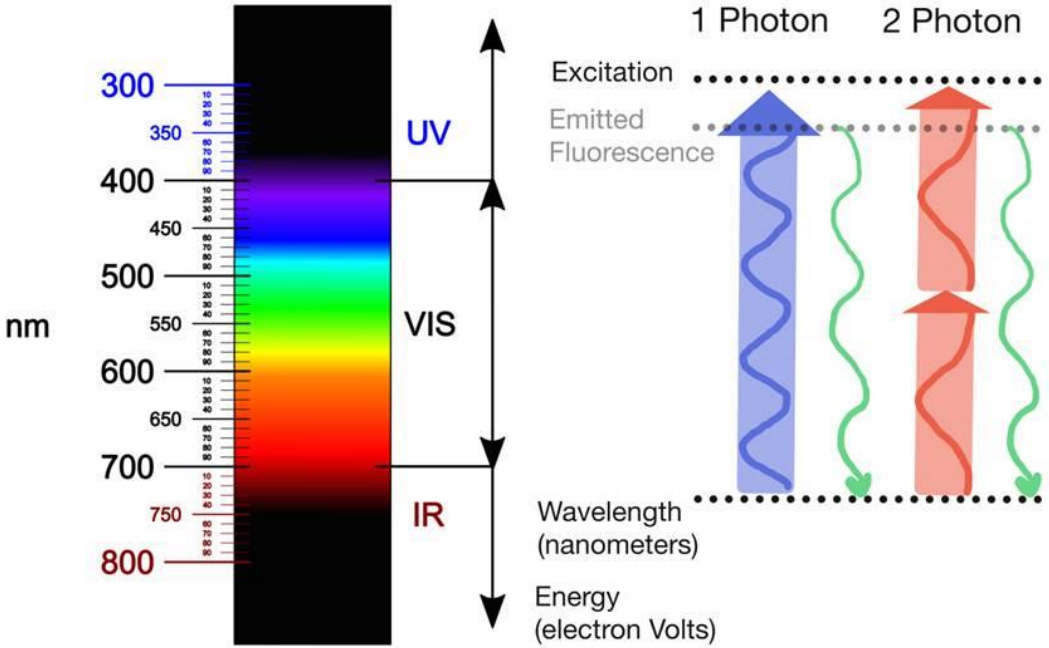
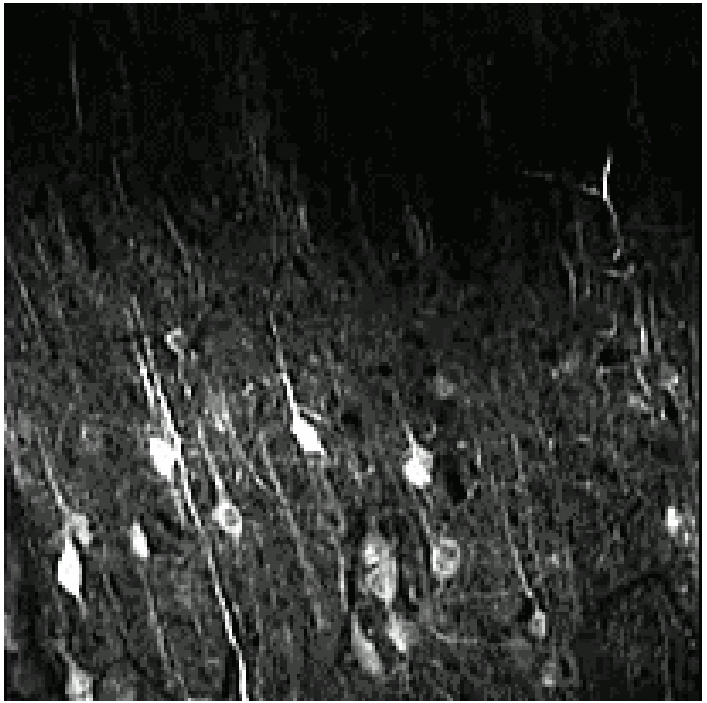
Two-photon / Multiphoton microscopy



https://www.neurotar.com/contract-research/two-photon-microscopy/

In-vivo calcium imaging



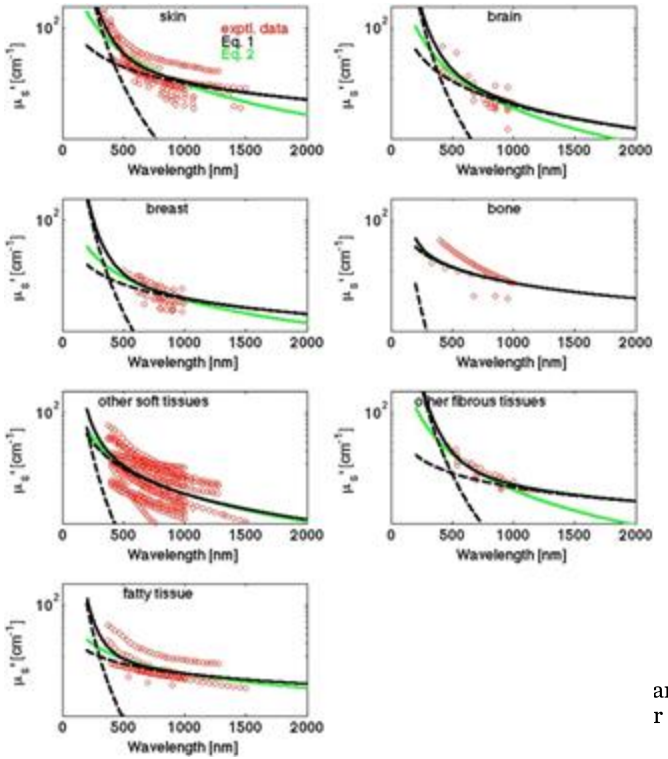
Advantages and costs of multiphoton microscopy

Multiphoton: Each photon is lower energy → Each photon is higher wavelength

$E = \hbar f$ $v = \lambda f$

- Deeper tissue penetration
- Lower toxicity

- Higher costs / complexity
- Longer imaging time
- Resolution



Cell

CellPress

Volume 187, Issue 17, 22 August 2024, Pages 4458-4487

Review

Multiphoton fluorescence microscopy for *in vivo* imaging

Chris Xu [1], Maiken Nedergaard [2 3], Deborah J. Fowell [4], Peter Friedl [5], Na Ji [6]

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r

NIRF / SWIRF

NIRF: Near infrared fluorescent imaging (~ 700 – 1400 nm)
SWIRF: Short wave infrared fluorescence imaging (~ 1400 – 3000 nm)

- Deeper penetration:
  - Less scattering
  - Less absorption

- Less tissue autofluorescence in this range, hence better SNR

International Journal of Nanomedicine

Dovepress
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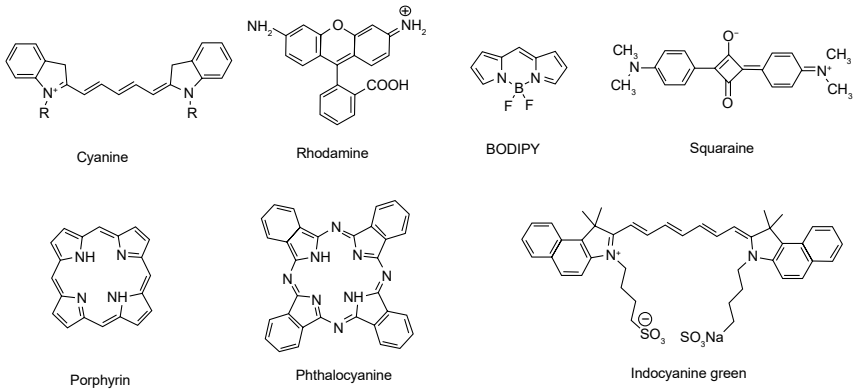
REVIEW

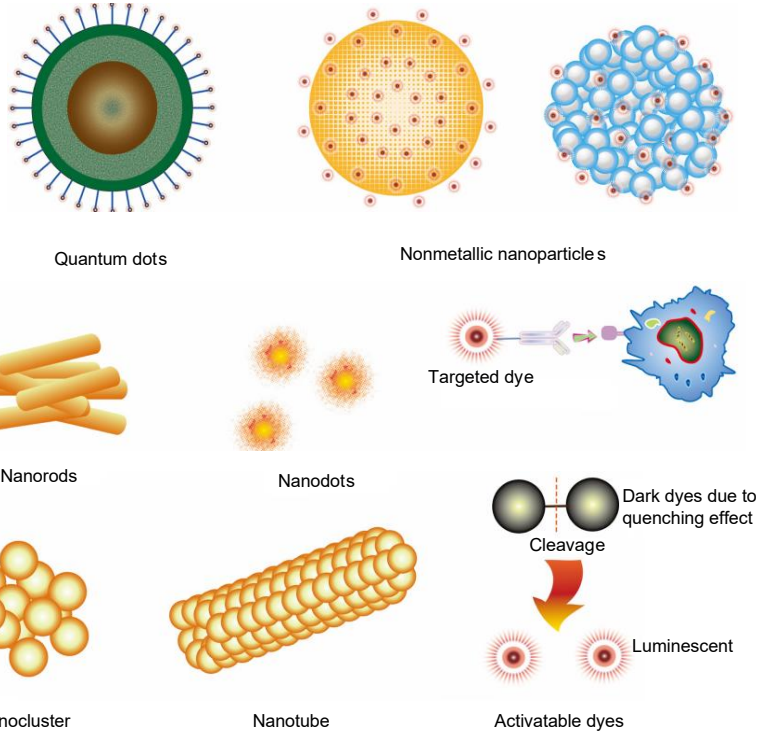
Near-infrared fluorescent probes in cancer imaging and therapy: an emerging field

NIRF / SWIRF probes

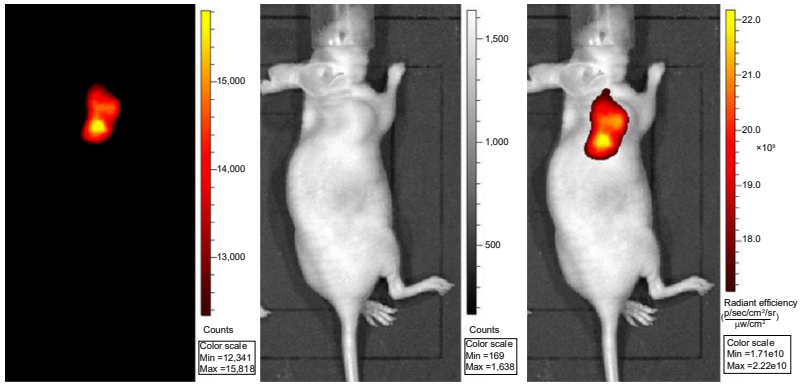
Several NIRF dyes



NIRF nanoparticles



Mouse with subcutaneous prostate tumor



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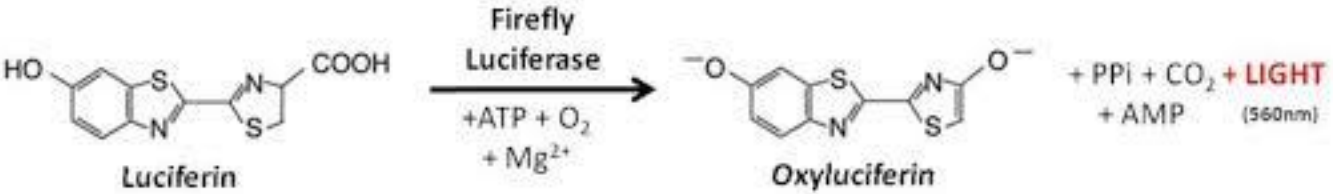
Bioluminescence

There is a way to make cells or tissues fluoresce without an excitation light.
Everyday example: firefly

A luciferase enzyme catalyzes luciferin (with other ingredients) to provide light (and other products)

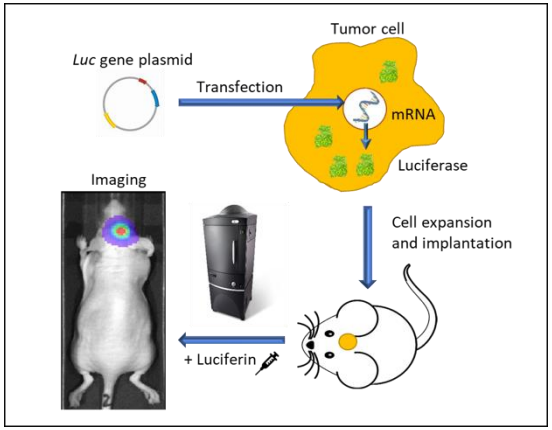


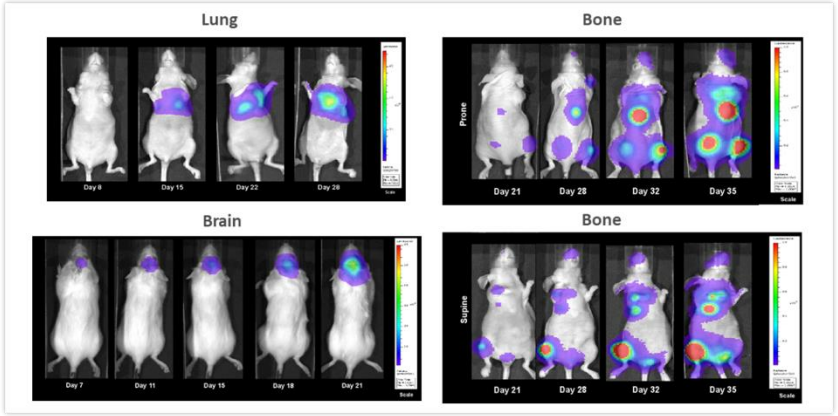
https://goldbio.com/articles/article/a-crash-course-on-luciferase-assays

- Can genetically encode cells to produce luciferase
- Provide luciferin as a substrate in the media
- The wavelength of light produced depends on the source / type of luciferase

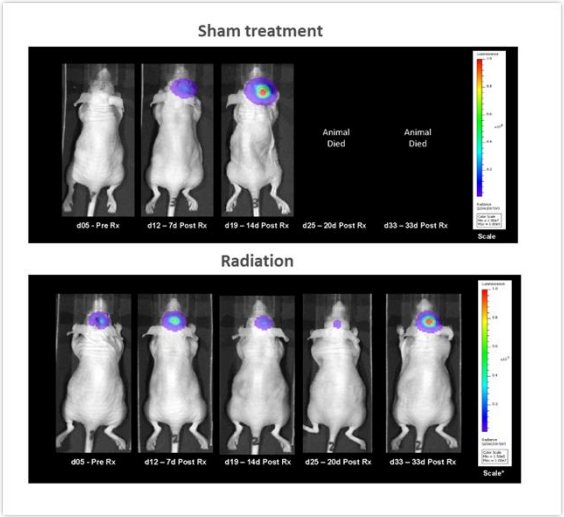
➢ Can essentially tag cells (such as cancer cells of interest) and observe their growth, migration etc

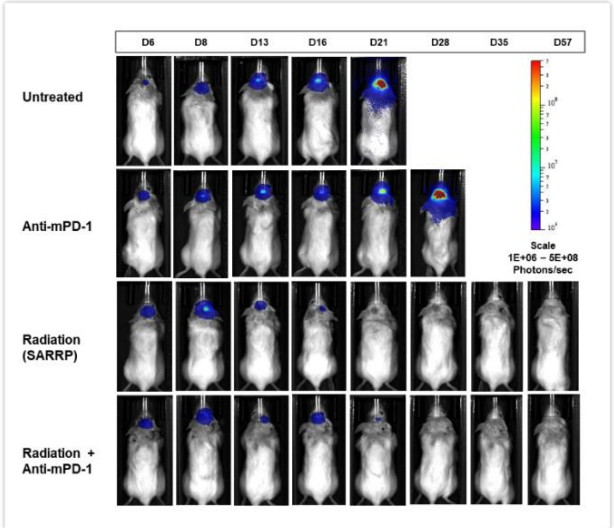
Bioluminescence example

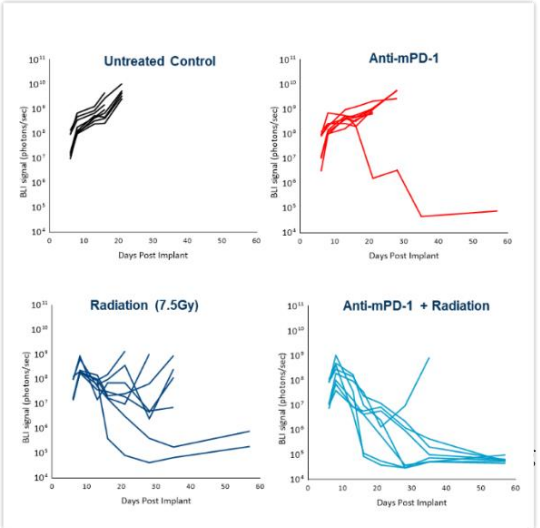




https://www.labcorp.com/bioluminescence-imaging-vivo-monitoring-tumor-development-disease-dissemination-and-treatment-o



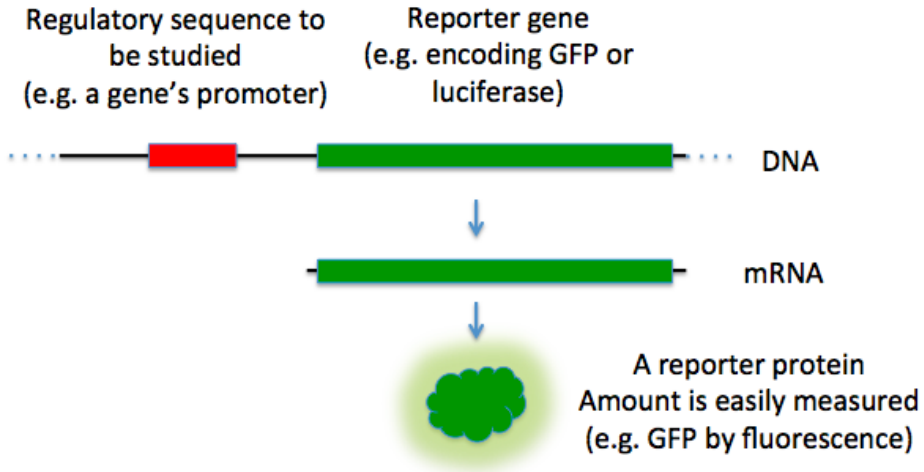




Genetic reporter systems

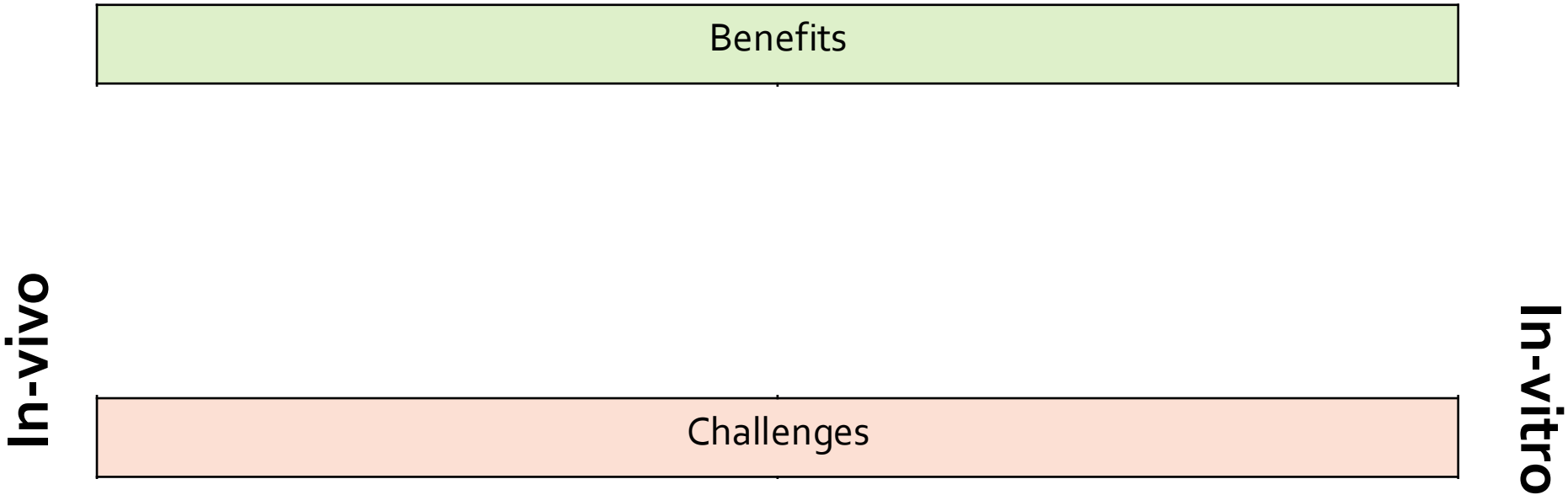
Reporter gene: Usually traditional fluorescence (like GFP) or bioluminescence that is genetically introduced and coupled to an experimental promoter gene

- Fluorescence or bioluminescence is then indicative of expression of the gene of interest



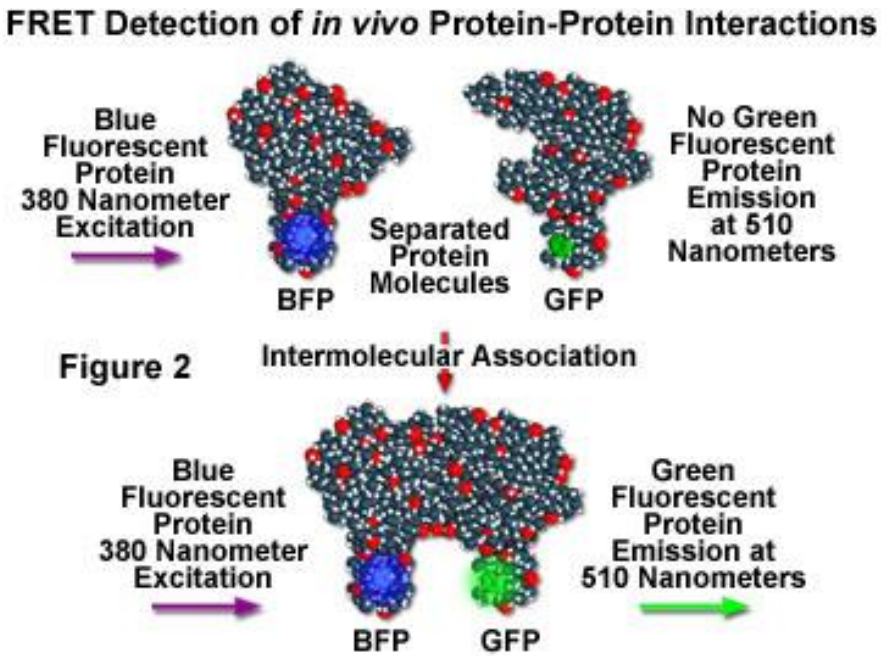
- In a sense, bioluminescence can be cast as a genetic reporter system as well
- The reporter, like luciferase, can be placed downstream of a particular signaling cascade

Summary: in-vivo vs. in-vitro

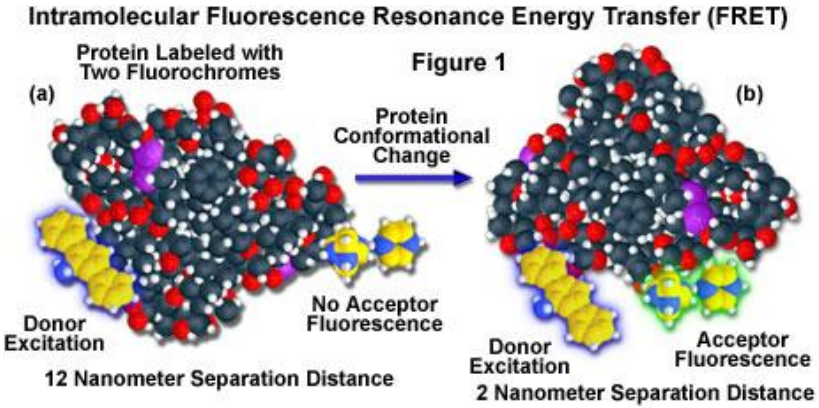


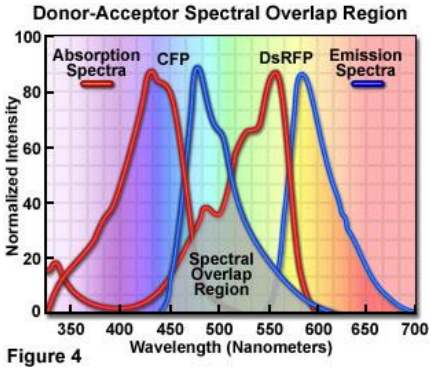
FRET

FRET: Fluorescence Resonance Energy Transfer: the emission of one fluorophore is the excitation of another



https://www.olympus-lifescience.com/en/microscope-resource/primer/techniques/fluorescence/fret/fretintro/





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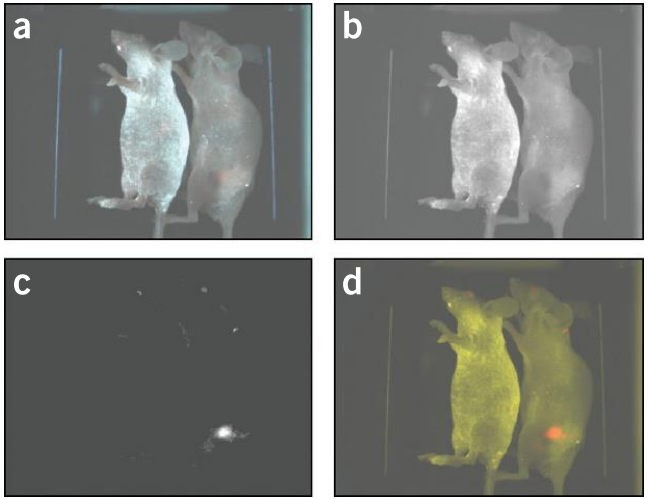
Beyond fluorophores

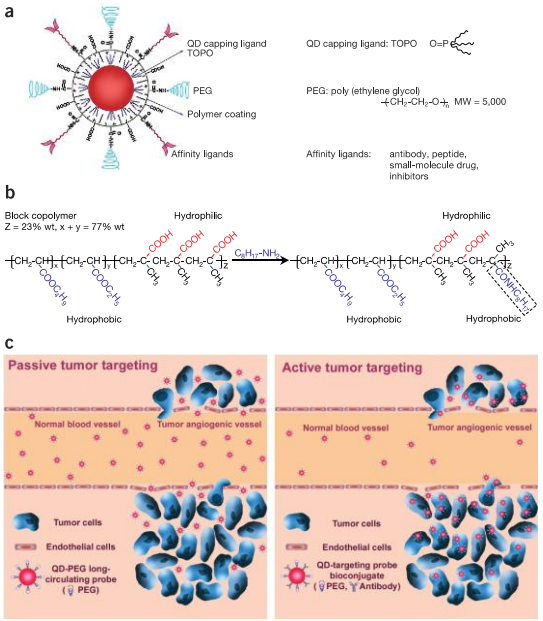
Quantum dots: Semiconductors with interesting quantum optics properties
Size-dependent emission (2-20 nm)
Much more resistant to photobleaching
Switch to non-radiative states cause quantum dots to blink

nature biotechnology

In vivo cancer targeting and imaging with semiconductor quantum dots

Xiaohu Gao,[1] Yuanyuan Cui,[2] Richard M Levenson,[3] Leland W K Chung[2] & Shuming Nie[1]





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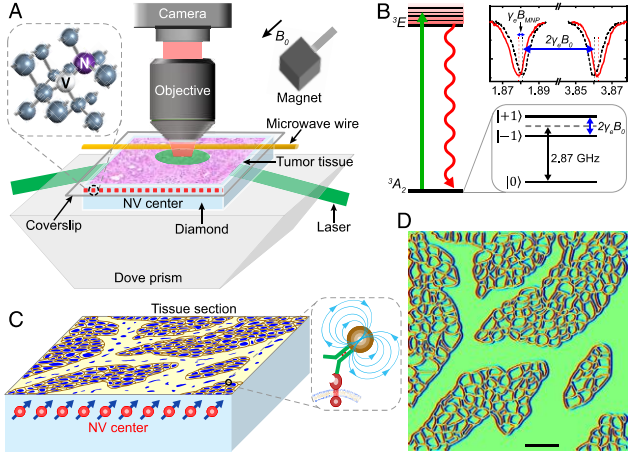
Beyond fluorophores

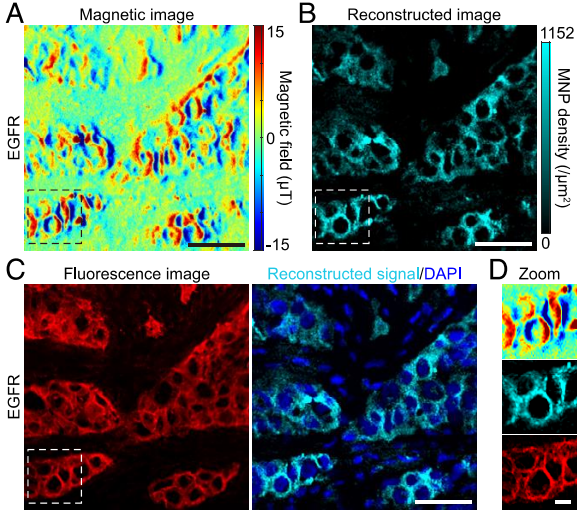
Nitrogen Vacancy: A fluorescent defect in a diamond
Defects in a slab or nanodiamonds
No bleaching or blinking
Optical output indicative of many biophysical parameters

- Magnetic fields (NMR)
- pH
- Temperature

Immunomagnetic microscopy of tumor tissues using quantum sensors in diamond

Sanyou Chen[a,b,c,d,e,f,1], Wanhe Li[a,b,c,d,1], Xiaohu Zheng[a,g,1], Pei Yu[a,b,c,d], Pengfei Wang[a,b,c,d], Ziting Sun[a,b,c,d], Yao Xu[a,b,c,d], Defeng Jiao[a,g], Xiangyu Ye[a,b,c,d], Mingcheng Cai[a,b,c,d], Mengze Shen[a,b,c,d], Mengqi Wang[a,b,c,d], Qi Zhang[a,b,c,d,e,f], Fei Kong[a,b,c,d], Ya Wang[a,b,c,d], Jie He[h], Haiming Wei[a,g], Fazhan Shi[a,b,c,d,e,f,2], and Jiangfeng Du[a,b,c,d,2]





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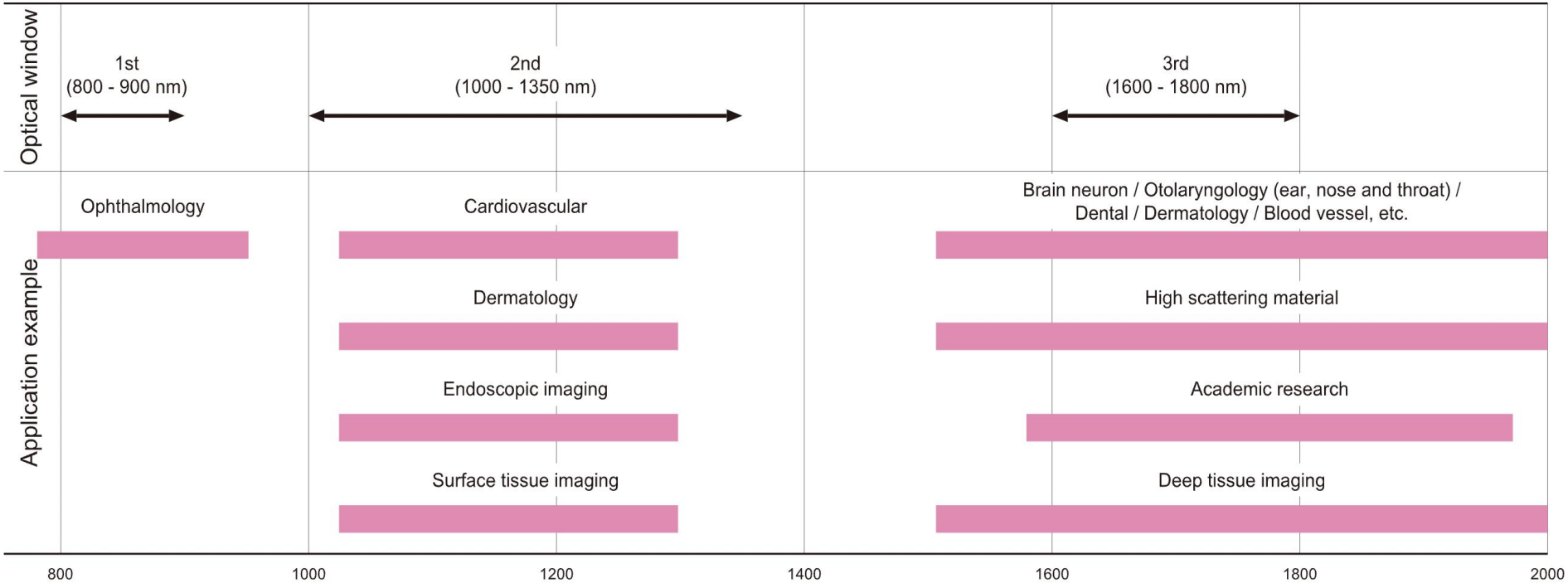
Optical Coherence Tomography

OCT: Imaging based on light interference at different wavelengths

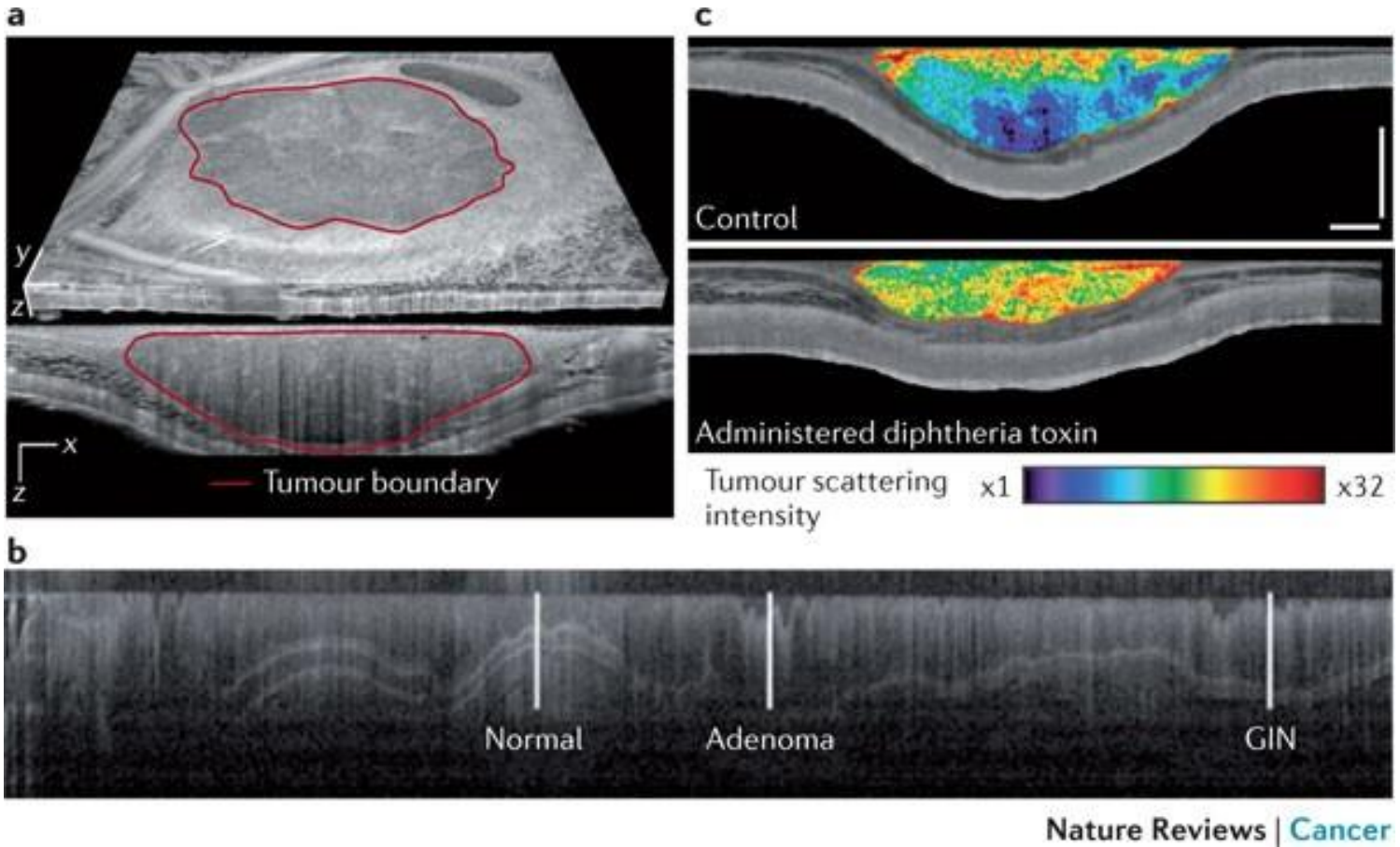
Sample applications by wavelength



Optical Coherence Tomography example

Cancer imaging by optical coherence tomography: preclinical progress and clinical potential

Benjamin J. Vakoc, Dai Fukumura, Rakesh K. Jain and Brett E. Bouma

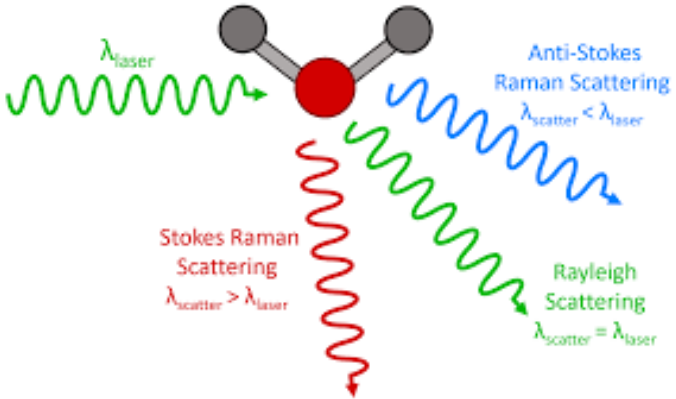


Nature Reviews | Cancer

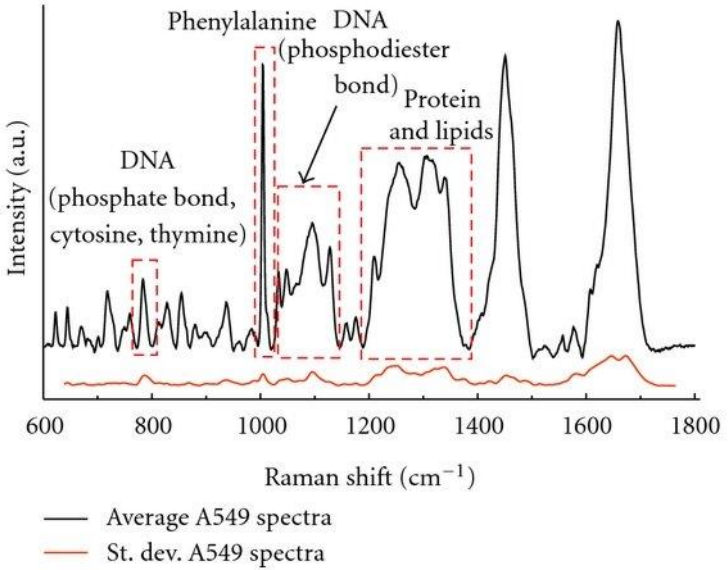
Raman spectroscopy

Raman scattering: Light scattering by molecules can come in two forms:

- Elastic = no energy change
- Inelastic = change in energy (due to molecular vibration)
  - This inelastic scattering is particular to each molecule and can be used as a molecular signature.



https://www.edinst.com/blog/what-is-raman-spectroscopy/



Raman Spectroscopy for Clinical Oncology

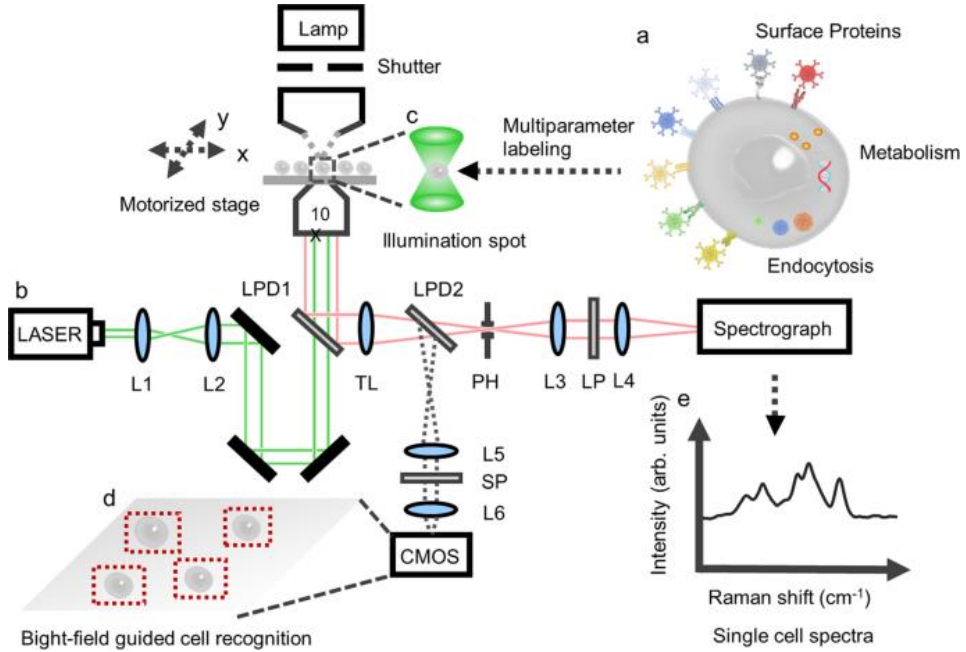
January 2011 · Advances in Optical Technologies · 2011

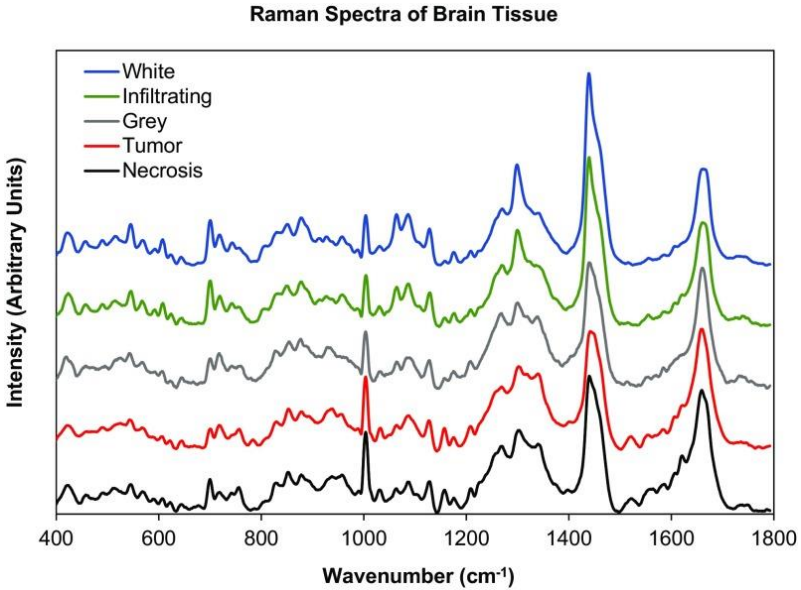
DOI: 10.1155/2011/213783

Michael B. Fenn · Petros Xanthopoulos · Georgios Pyrgiotakis · Show all 6 authors · Larry L. Hench

g

Raman spectroscopy examples





Multiplexed live-cell profiling with Raman probes

Chen Chen, Zhilun Zhao, Naixin Qian, Shixuan Wei, Fanghao Hu & Wei Min

Nature Communications **12**, Article number: 3405 (2021) | Cite this article

Cancer and Metastasis Reviews (2018) 37:691–717
https://doi.org/10.1007/s10555-018-9770-9

Applications of Raman spectroscopy in cancer diagnosis

Gregory W. Auner[1,2,3,4] · S. Kiran Koya[1,2,3] · Changhe Huang[1,2,3] · Brandy Broadbent[2,3] · Micaela Trexler[2,3] · Zachary Auner[3,5] · Angela Elias[2,3] · Katlyn Curtin Mehne[2,3] · Michelle A. Brusatori[1,2,3]

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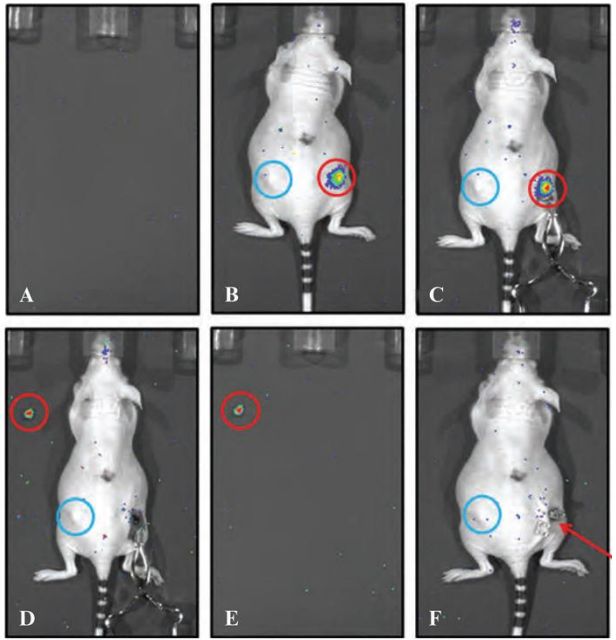
Cerenkov imaging in cancer

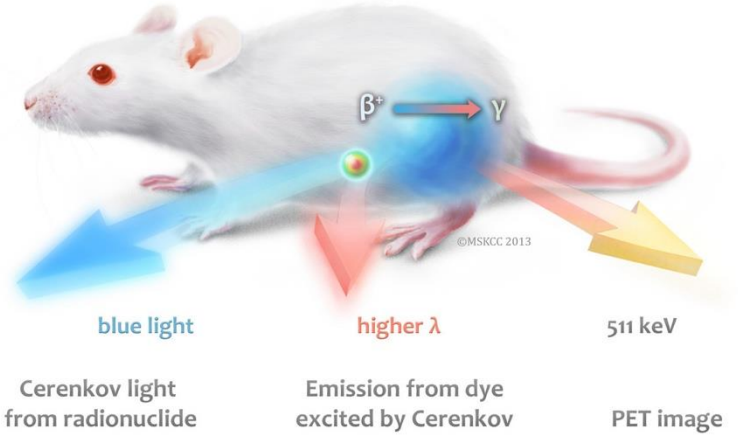
Cerenkov radiation: Charged particle can travel in a medium faster than the speed of light. As they do, they emit characteristic light that can serve as an imaging tool

Intraoperative Imaging of Positron Emission Tomographic Radiotracers Using Cerenkov Luminescence Emissions

Jason P. Holland, Guillaume Normand, Alessandro Ruggiero, Jason S. Lewis, and Jan Grimm



SCIFI = Secondary Cerenkov-induced Fluorescence Imaging



Shaffer, T., Pratt, E. & Grimm, J. Utilizing the power of Cerenkov light with nanotechnology. *Nature Nanotech* **12**, 106–117 (2017). https://doi.org/10.1038/nnano.2016.301

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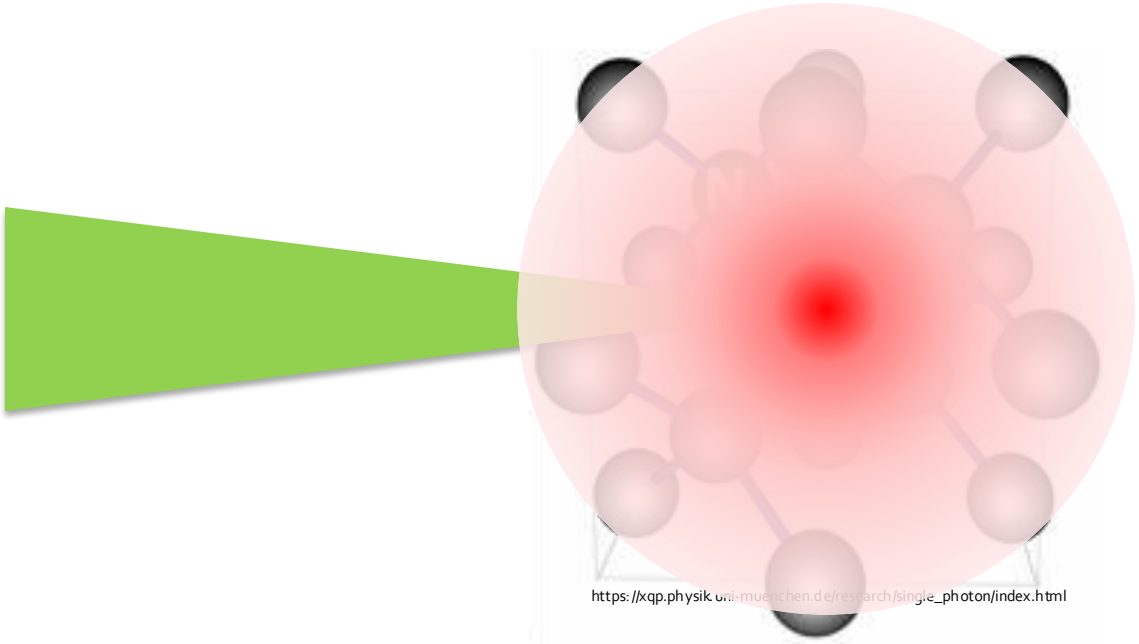
So much more to say!

Short feedback form

Perhaps a case in point about how we built one -- Air table, all of the optics, the energy diagram (like my version for CERIP)

We have developed a multi-purpose intracellular optical sensor based on a defect in a diamond lattice

Nitrogen vacancy (NV) color center:



- ✓ A magnetic field
  - ➢ NMR
- ✓ A pH change
  - ➢ Intracellular pH
- ✓ A temperature change
  - ➢ Nano-thermometry

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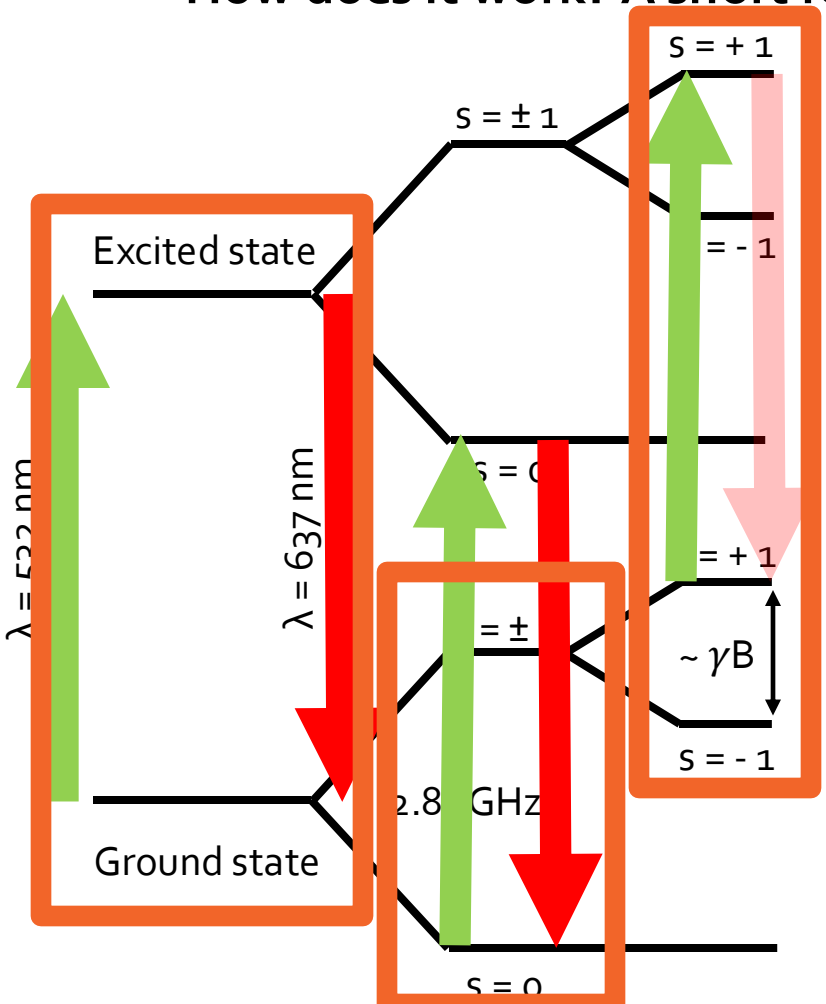
How does it work? A short foray into quantum mechanics



Optically **D**etected **M**agnetic **R**esonance

- Optical (fluorescent) readout of the spin state of the system
- Sensing of external magnetic fields (i.e. performing NMR) is done by properly pulsing between the magnetically split (+1 or -1) and 0 states
- Temperature changes the 0 → ± 1 transition
- pH changes the emission frequency

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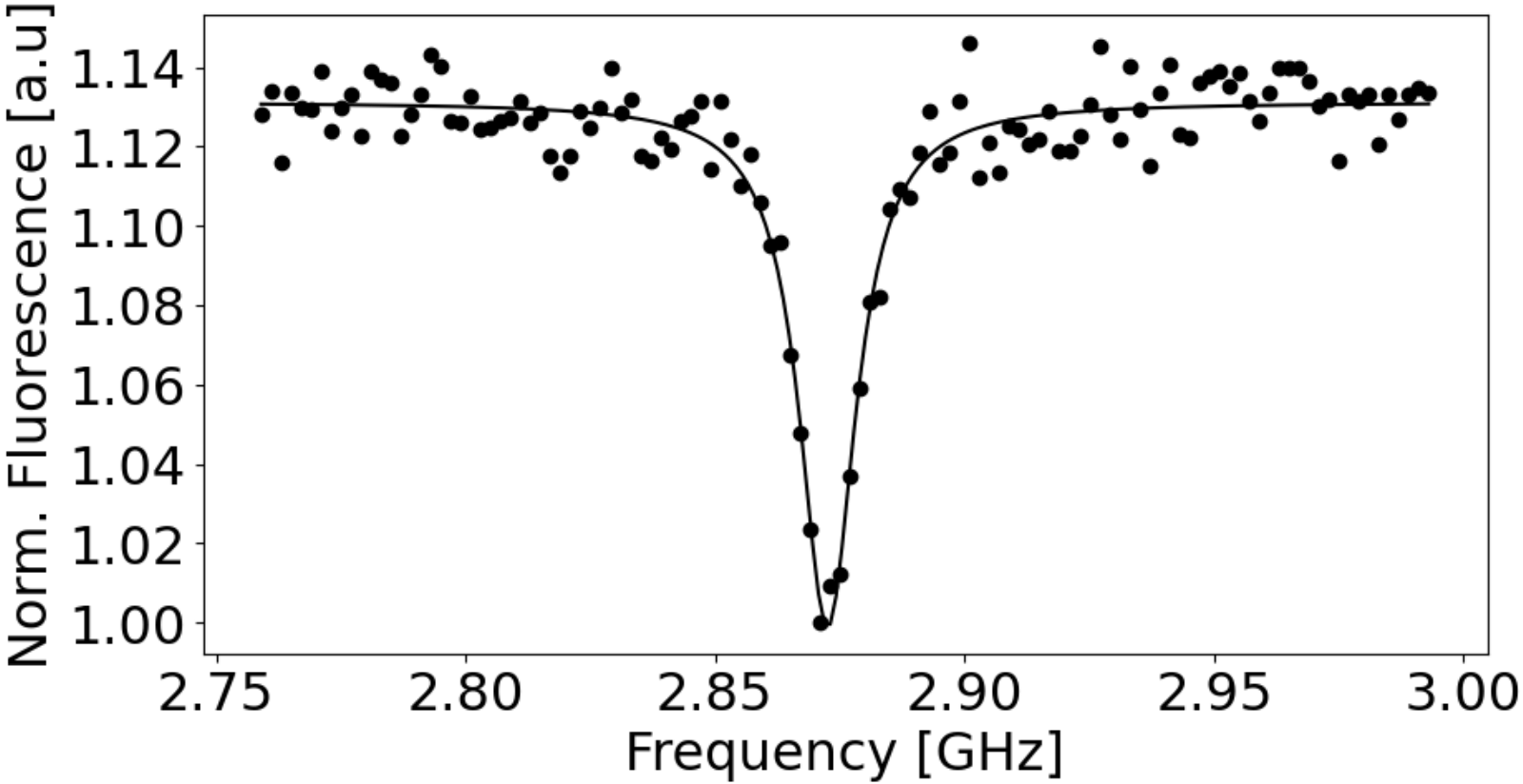
Shifts in ODMR represent temperature changes

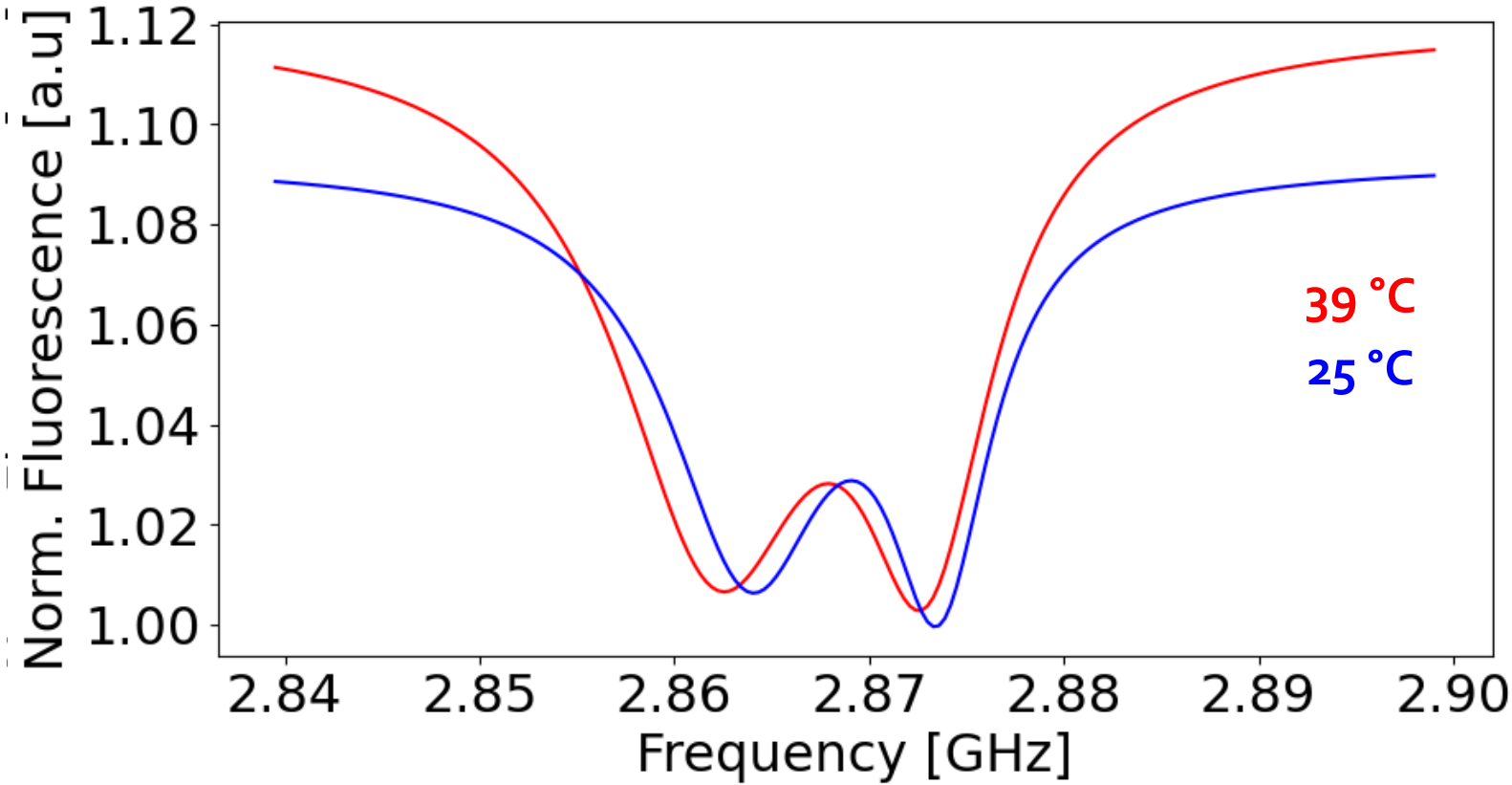
Example ODMR from a single NV



tering

Shifts in ODMR represent temperature changes

Example ODMR from a nanodiamond



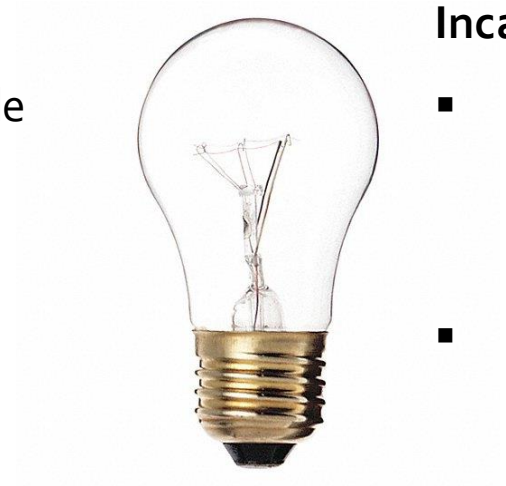
Microscopy tools

Light sources:

- White light: LEDs and incandescent bulbs
- One color: lasers

LED:

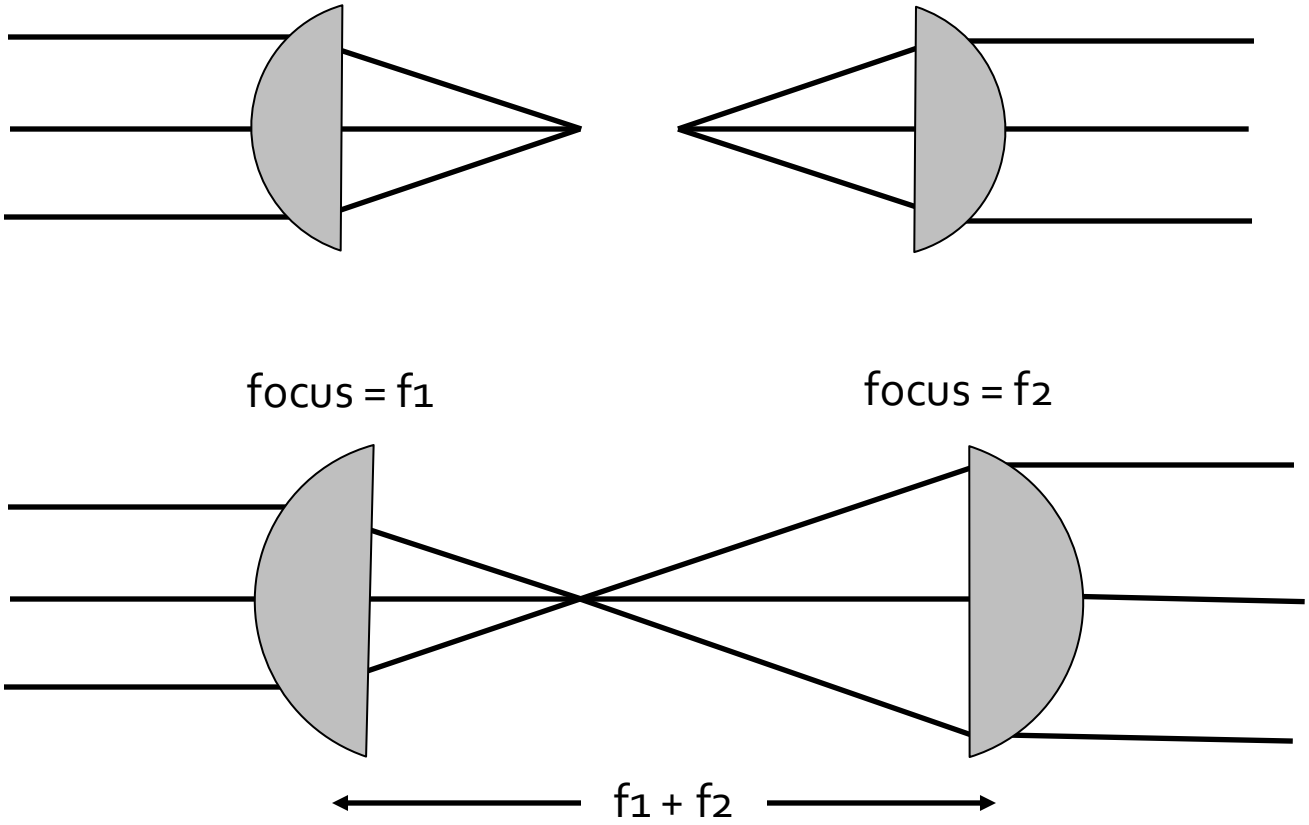
- Light emitting diode
- Semiconductors with specific photonic band gap properties



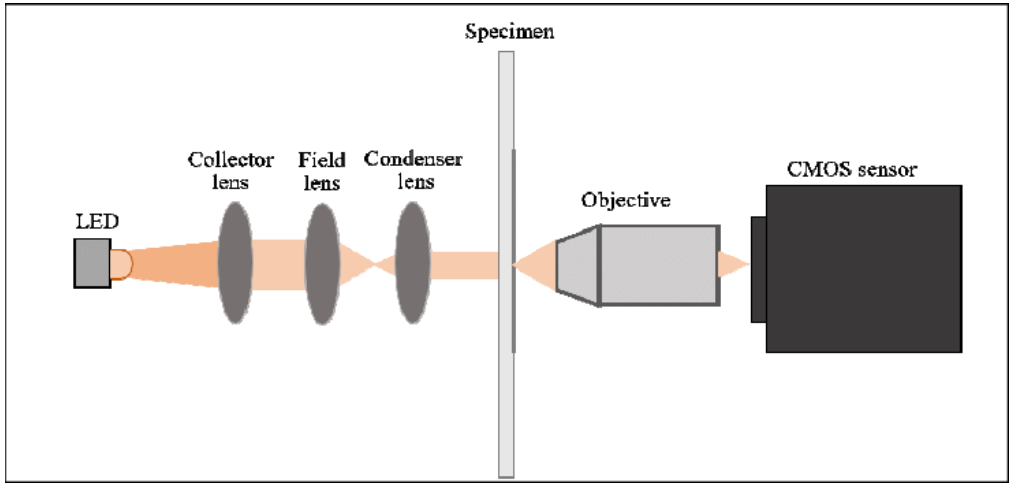
Incandescent bulb:

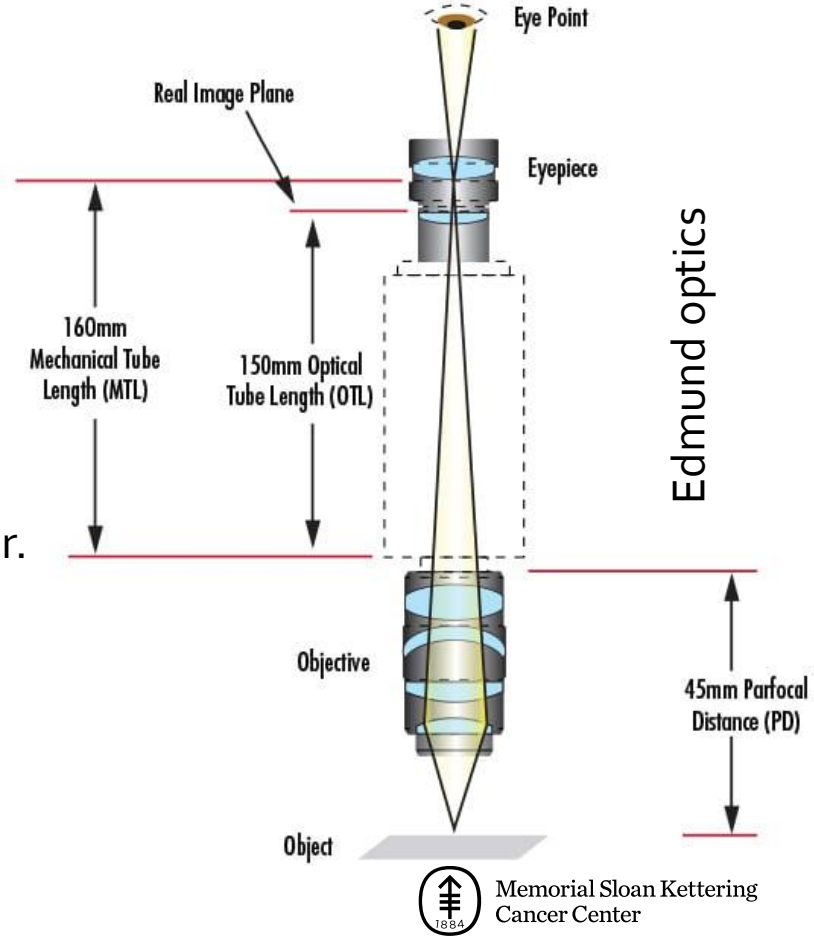
- Filament inside heats up and emits light
- Quite inefficient and shorter lifetime
- Simple, more time on the market

- Color depends on energy band gap
- When electrons meet holes, light is emitted
- Efficient, long-lasting



Bare bones of a microscope





- Lenses are placed at focal lengths from each other.
- Can resize a beam with 2 lenses at focal lengths apart (magnification = ratio of focal lengths)
- Due to dispersion, the exact workings of all optics will be wavelength dependent!

Memorial Sloan Kettering Cancer Center

Mode	Principle	Main Advantages	Main Disadvantages
Transmission (Brightfield)	Detects light transmitted through sample; contrast arises from absorption or staining.	• Simple and inexpensive • Works well with stained or pigmented samples • Color and structural information visible	• Poor contrast for transparent (unstained) samples • Not suitable for thick or opaque specimens
Reflection (Epi-illumination)	Detects light reflected or backscattered from sample surface.	• Ideal for opaque samples (e.g., metals, semiconductors, tissue surfaces) • Provides surface topography and reflectivity info • Compatible with fluorescence and confocal modes	• Limited depth information (surface-sensitive) • Can have glare/specular reflections • Not useful for fully transparent specimens
Phase Contrast	Converts optical phase shifts (from thickness/refractive index differences) into intensity differences using a phase ring and annulus.	• Excellent for viewing live, unstained cells • Simple setup and easy interpretation • Works with transparent aqueous samples	• Produces halos and shade-off artifacts • Not quantitative • Limited compatibility with color imaging and thick samp
Darkfield	Blocks direct light; only scattered light enters the objective, so features appear bright on dark background.	• High contrast for small or weakly scattering features (e.g., bacteria, nanoparticles) • No need for staining • Highlights edges and fine structures	• No internal structural info (only scatterers visible) • Very sensitive to dust and alignment • Not suitable for thick or highly scattering specimens
DIC (Differential Interference Contrast)	Uses polarized, sheared beams that interfere after passing through slightly offset regions; converts phase *gradients* to intensity.	• Very high-resolution, crisp images • Pseudo-3D “relief” contrast • Works well for live transparent samples • No halos, adjustable contrast direction	• Directional (contrast depends on shear direction) • Expensive, complex optics (one prism per objective) • Not quantitative • Doesn’t work well on birefringent materials