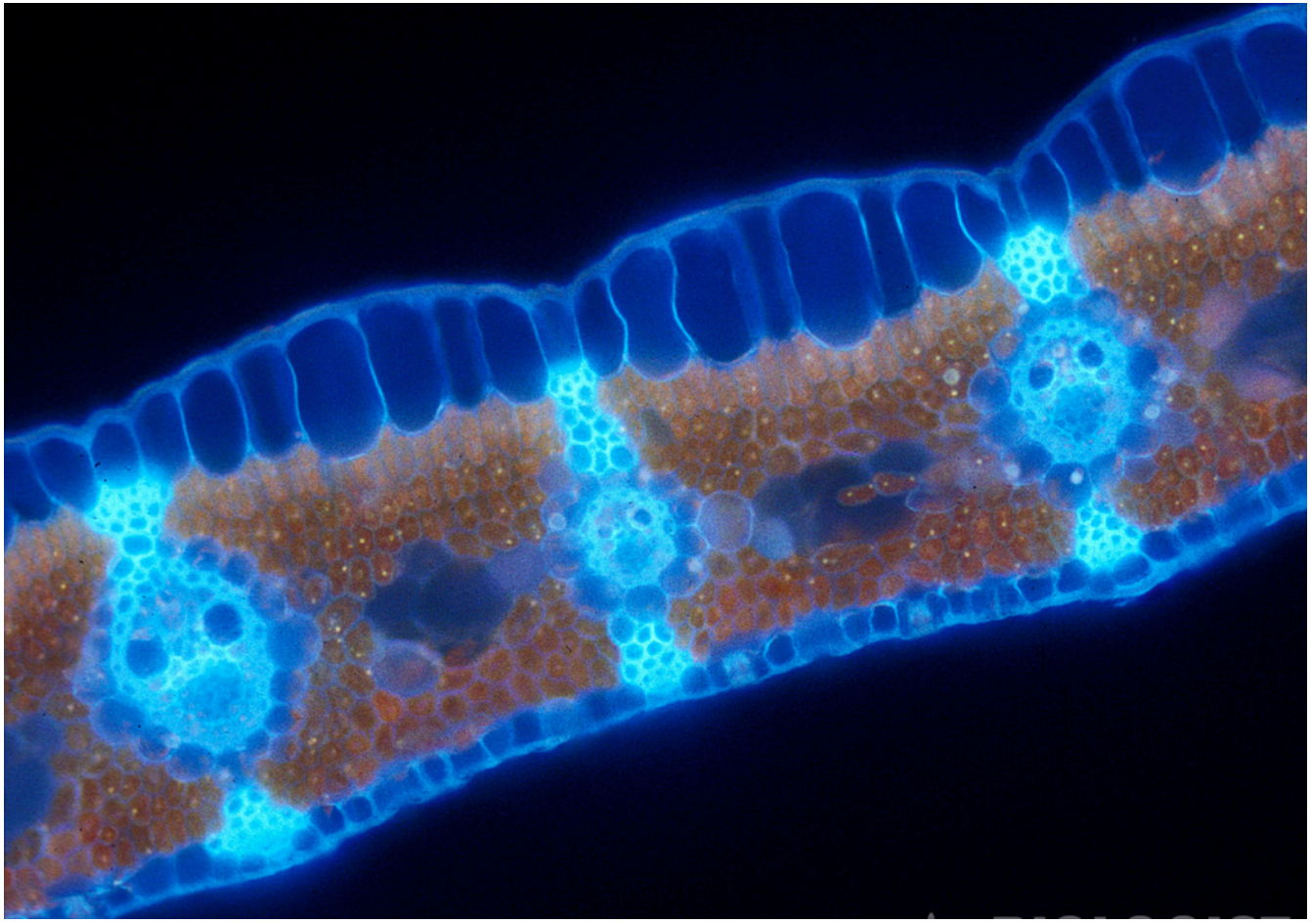
11 – Biophotonics

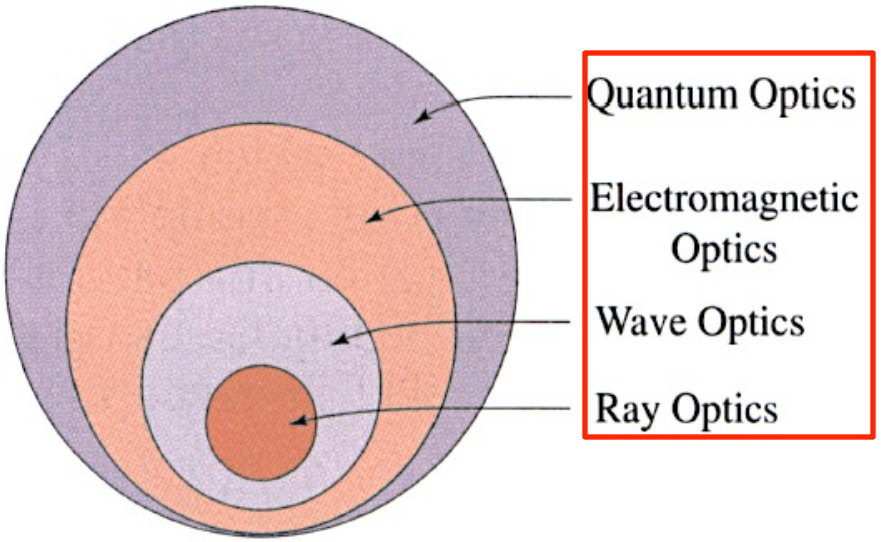


Cross section of umbrella plant with intense UV-induced blue-green and chlorophyll (red) fluorescence. The cells with intense red fluorescence are mesophyll cells, while the cells which are highly blue fluorescent are part of the leaf's vascular tissue (e.g. xylem and phloem).

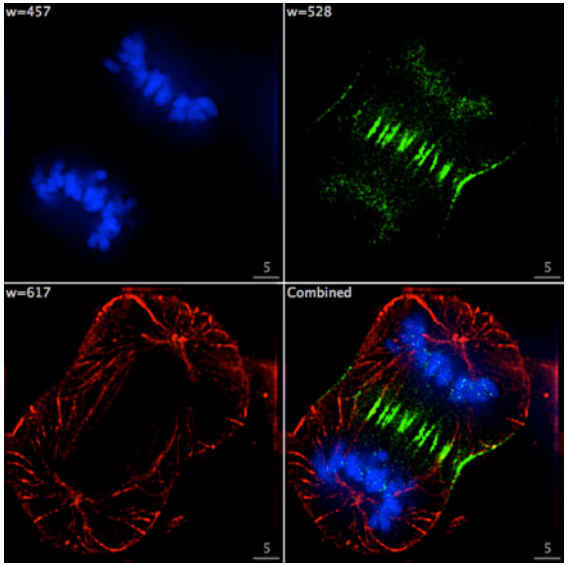
- Last regular lecture! Nice work this semester!

- We will cover several applications related to bio photonics today. Biophotonics has become the established general term for all techniques that deal with the interaction between biological items and photons.

1) Fluorescence Imaging
2) 96 Well Plate
3) Flow Cytometry
4) Laser Surgery
5) Pulse Oximetry
6) Capnography

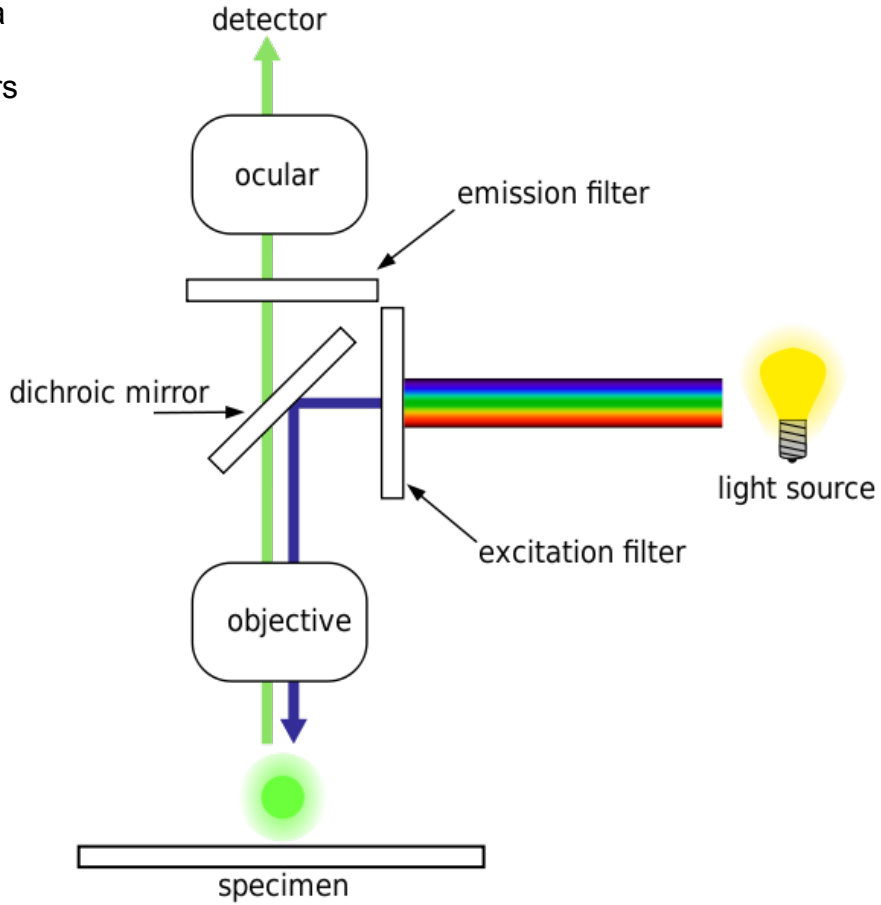


Credit: Fund. Photonics – Fig. 1.0-1

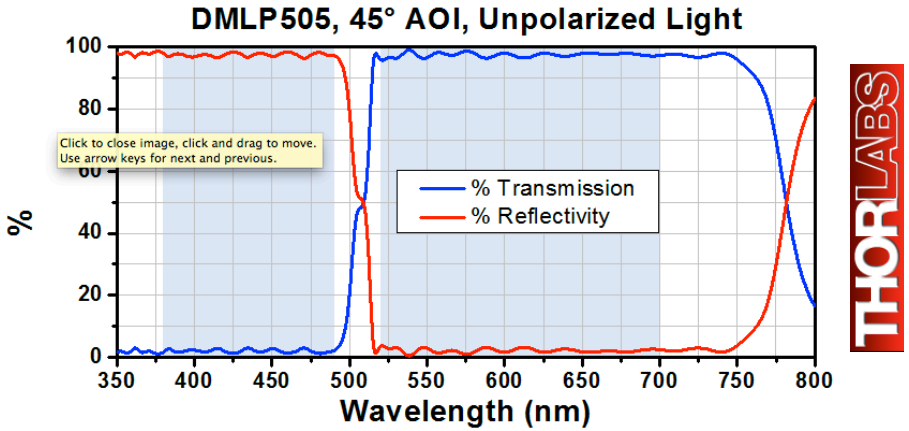


Epifluorescent imaging of a dividing human cancer cell. DNA is stained blue, a protein called INCENP is green, and the microtubules are red. Each is imaged separately using a different combination of excitation and emission filters and then overlaid.

Src: Wiki Commons



- How would you make a dichroic mirror?



- Choosing dyes for fluorescence imaging involves:

(1) Specificity… only bind to the protein, etc. of interest!

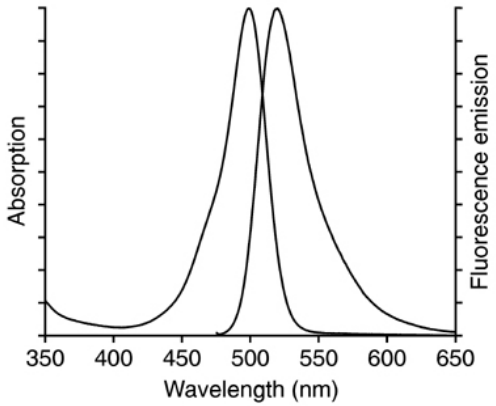
(2) Quantum yield (Φ): $$\Phi = \frac{photons\ emitted}{photons\ absorbed}$$

(3) Absorption cross-section (σ) at the excitation wavelength you use:

$$\sigma = \alpha N\ (kg/cm^4)$$
$$N = atomic\ dens.\ (kg/cm^3)$$
$$\alpha = 1/cm,\ I = I_0 e^{-\alpha x}$$

life technologies™

Catalog Number	D12371
Size	5 mg
List Price	(USD) 341.70
	My Price & Availability
Qty 1	Add To Cart



Staining G-Actin with Fluorescent DNase

1. Grow adherent cells on the surface of a coverslip.

2. Wash cells with PBS to remove excess medium.

3. Fix cells in 3.7% formaldehyde in PBS at room temperature for 10–15 minutes. Methanol disrupts the cytoskeletal structure or dye binding, which results in the absence of filament staining.

4. Wash three times with PBS.

5. Permeabilize cells in cold 100% acetone at –20°C for 5 minutes. Permeabilization can also be achieved by incubation with 0.1% Triton X-100 in PBS for 5 minutes.

6. Air-dry the samples or immediately rehydrate in PBS for 5–10 minutes.

7. Wash three times with PBS.

8. To the coverslip, add 200 µL of a 9 µg/mL (or 0.3 µM) solution of fluorescent DNase I in buffer. *Optional:* To simultaneously label F-actin, also add 1 unit (200 µL of a 0.165 µM solution) of a fluorescent phallotoxin. Stain for 15–20 minutes.

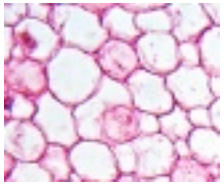
9. Wash three times with PBS.

10. Air-dry the samples. *Optional:* Add a drop of *SlowFade®* Gold or ProLong® Gold antifade reagent to reduce photobleaching. Mount and seal.

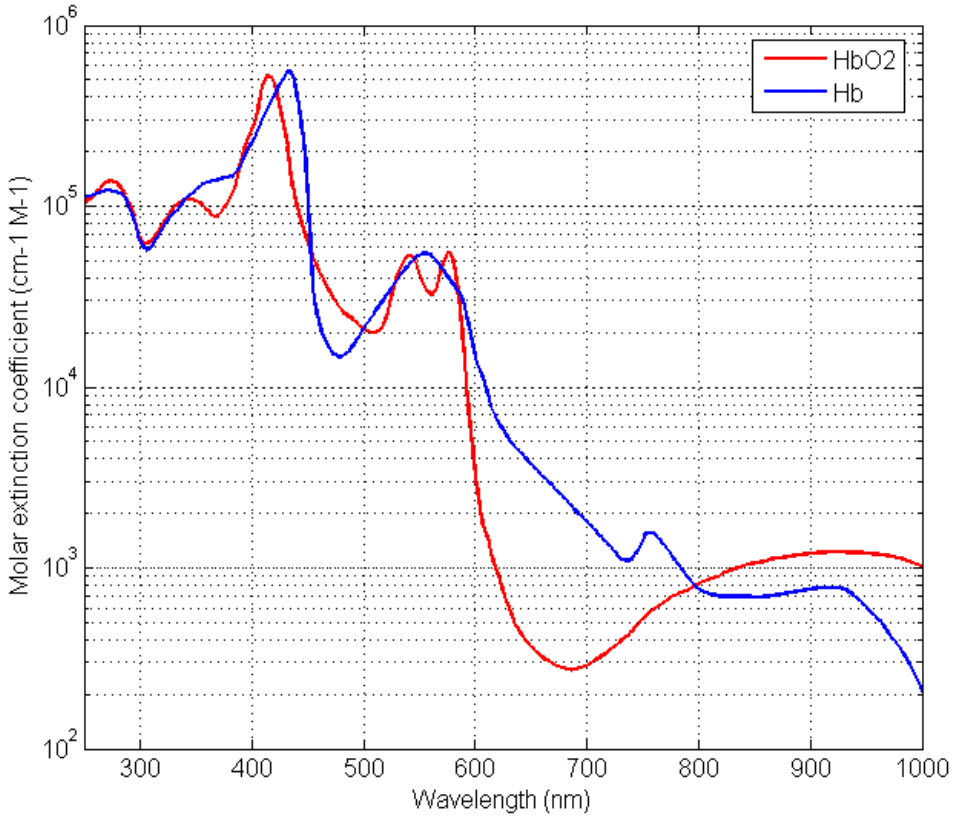
Actin is one of the most ubiquitous structural proteins of cells and is generally present in two forms: G-actin (globular) and F-actin (fibrous). The transformation between these two forms relates to the need of the cell for structural rigidity or motion. By using selective fluorescent probes with very high affinity for G- or F-actin, one can obtain information on the localization of G- and F-actin in fixed cells.

- So why is fluorescent imaging not often used deep inside the body? Two reasons, one has to do with light loss, the other has to do with light refraction…

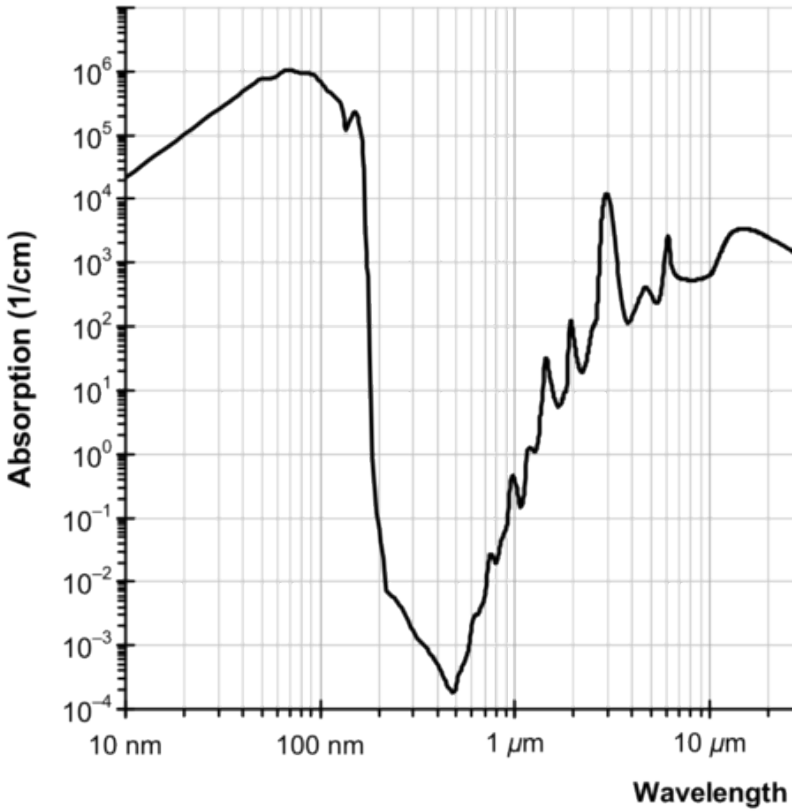
Scattering: each cell is like a mini-sphere that refracts and scatters light!



Absorption: oxygenated and de-oxygenated hemoglobin

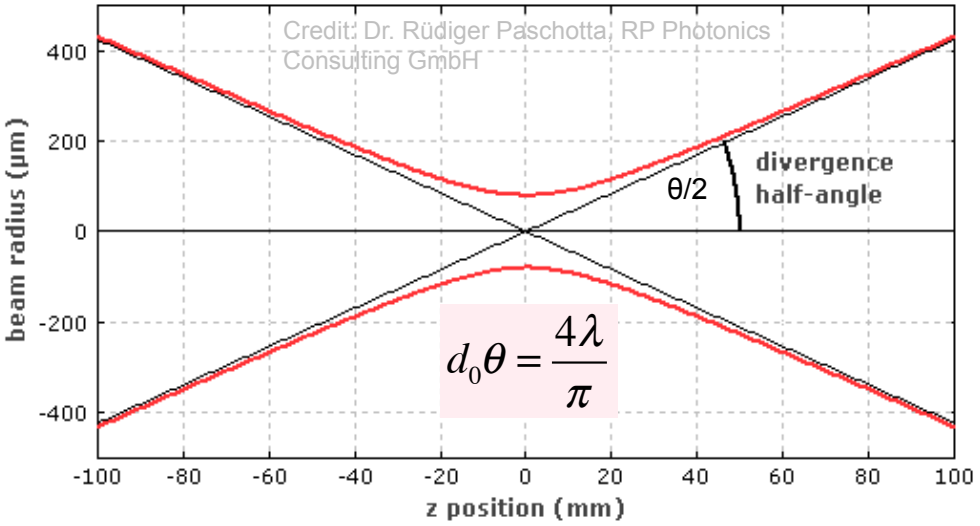


Absorption: water



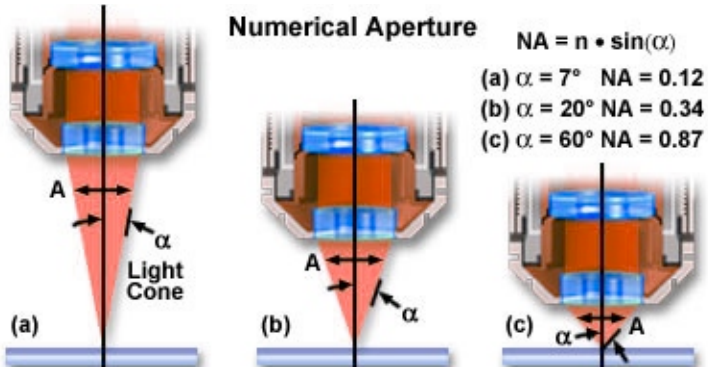
Src: Wiki Commons

- Remember! Diffraction limits smallest light spot! Same limit for seeing something very small (but light moves in reverse)



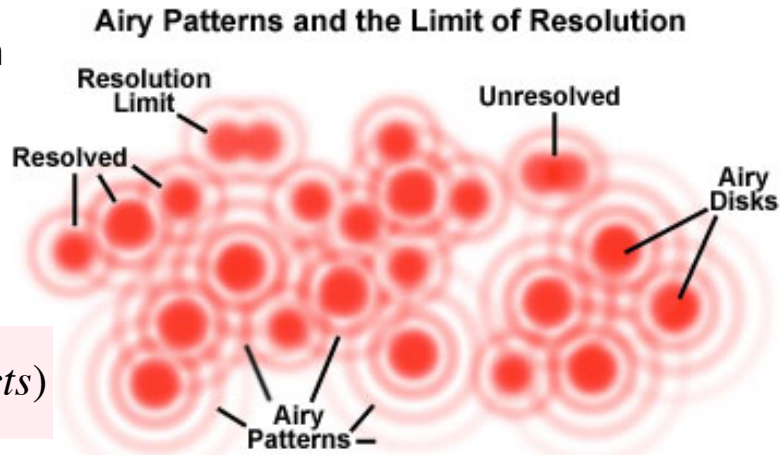
- Numerical aperture (NA) represents focusing power (largest θ) and light gathering power. Higher NA, lens smaller d_0.

$$NA = n \times \sin(\theta) \approx nD/2f \quad (D = lens\ diamter)$$



- Example: 50X Nikon objective with NA=0.8, and red light (650 nm) could barely resolve objects as close as ~406 nm). Typically a bit worse!

- What causes the Airy disks to look like they do? Only see them when get close to limit for r...

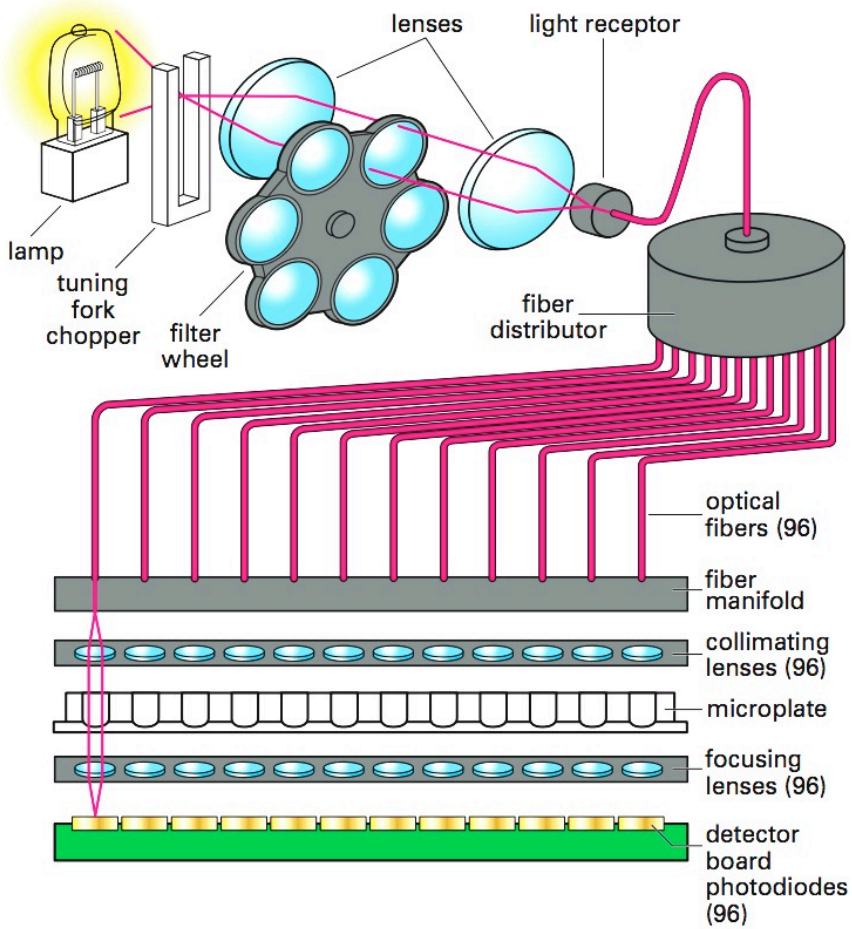


$$r = \frac{\lambda}{2NA} = resolution\ (smallest\ resolvable\ dist.\ between\ objects)$$

Src: Nikon

- A Microtitre plate (or microwell plate) is a flat plate with multiple "wells" used as small test tubes.

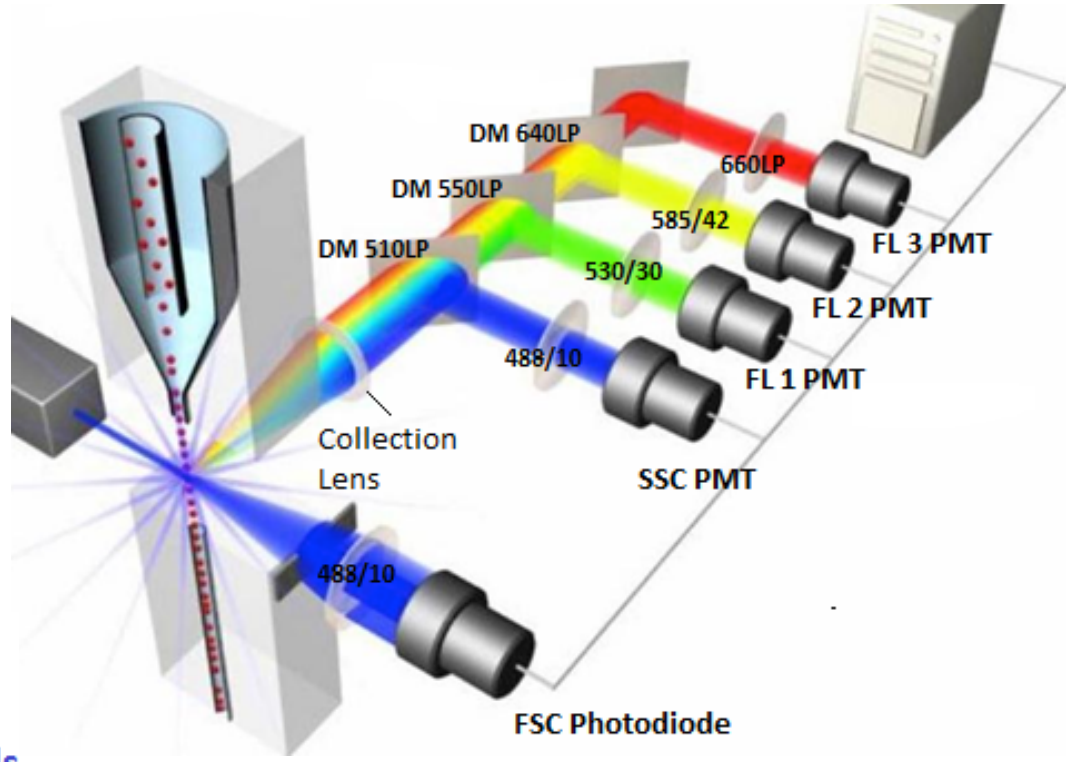
- It is the standard tool in analytical research and clinical diagnostic testing laboratories. A very common usage is in the enzyme-linked immunosorbent assay (ELISA), the basis of most modern medical diagnostic testing in humans and animals.



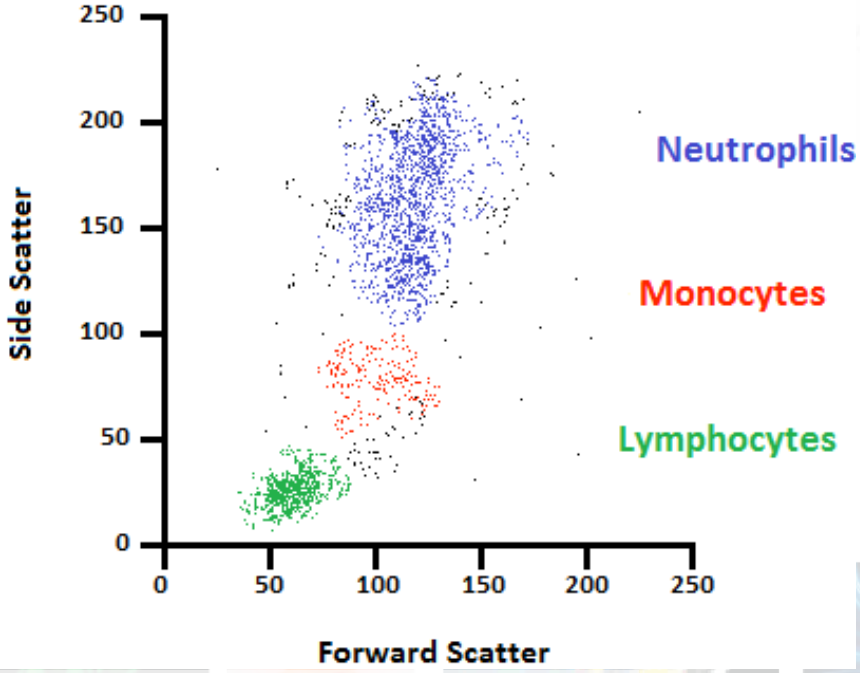
- Also, Dr. Ahn's company released this product in 2011! ("Siloam Optimax")
  http://www.youtube.com/watch?v=0eoHU9U1ZI0&feature=relmfu

- Flow cytometry is a technique for analyzing (counting) a large population of fluorescently labeled cells in a fluid stream (works for non-fluorescent, but less information).

- As particles pass through focused light the amount scattered (forward FSC and side SSC) and the emission (fluorescence label) can BOTH be measured. *Why use photodiodes in forward scatter and PMTs for the rest? What are 'DM's?*



Size and other aspects change scatter..



The 'flow cell' above has a liquid stream (sheath fluid), which carries and aligns the cells so that they line up single file, if Reynolds # (Re) is <2300 (non-turbulent)

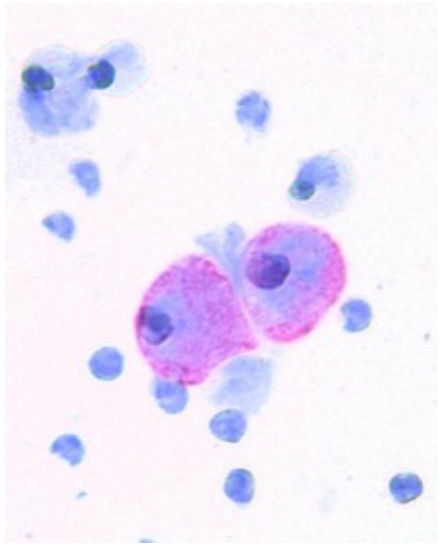
$$R_E = pVD/\mu = \frac{density \times velocity \times tube\ diameter}{viscosity}$$

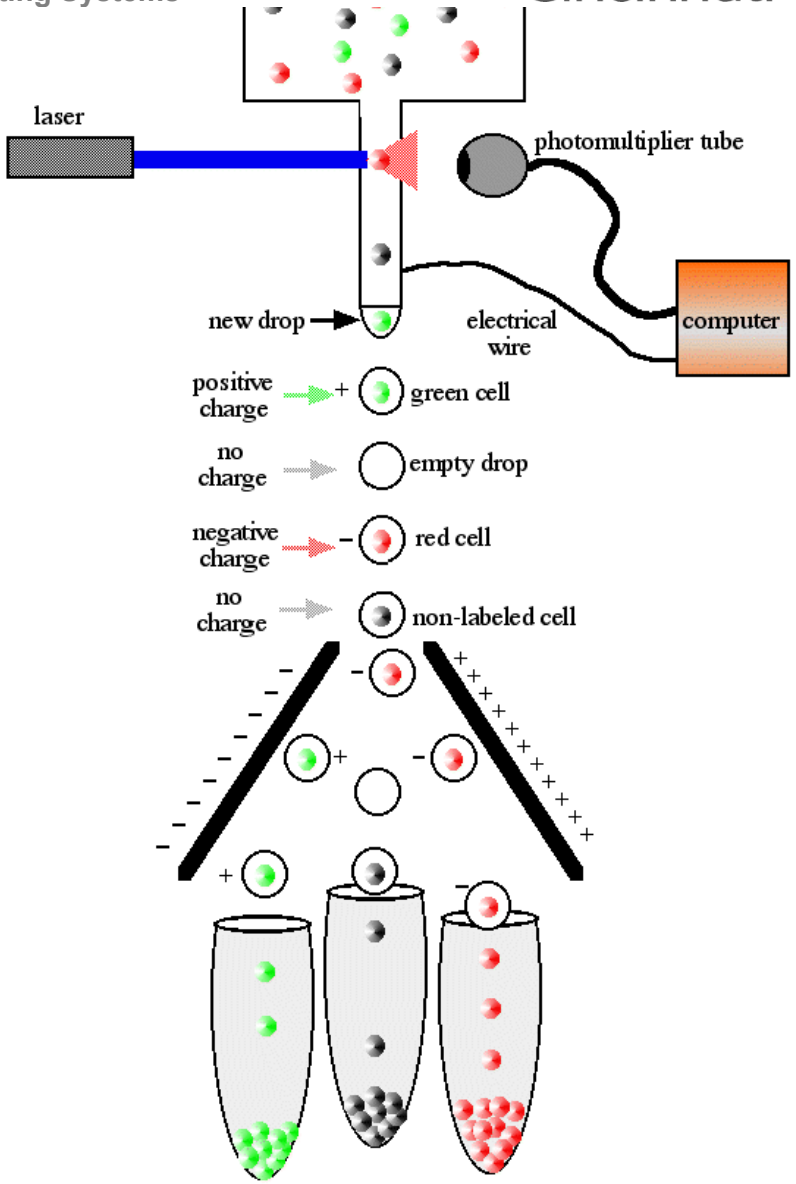
- Very fast throughput! Up to 100,000 counts per second is possible with commercial systems…

- Can also electrically sort! For example, sort circulating tumor cells (CTC's, cancer cells found in blood) to determine if treatment is working (hard if can't find them!).

Cell Number	Cell Type
10^6 µl	Erythrocytes
	Platelets
10^5 µl	Reticulocytes
10^4 µl	Neutrophils
10^3 µl	Lymphocytes
	Monocytes
10^2 µl	Eosinophils
	Basophils
10^1 µl	CD34+ Cells
10^3 ml	nRBC
	CECs
10^2 ml	CD34+CD38- Cells
10^1 ml	EPCs
10^3 L	CTCs
10^2 L	

10^6 / µL compared to 10^2/L
10,000,000,000 X !





- We can't do visible or infrared light imaging deep in the body because:

(a) Cells and tissue scatter light.
(b) Hemoglobin absorbs strongly in the visible spectrum.
(c) Water absorbs strongly in the infrared spectrum.
(d) All of the above.

- A 'dichroic' mirror is made from:

(a) A regular mirror and two color filters.
(b) Interference of thin films ('photonic crystal').
(c) Two mirrors.
(d) None of the above.

- Whew! Almost there!

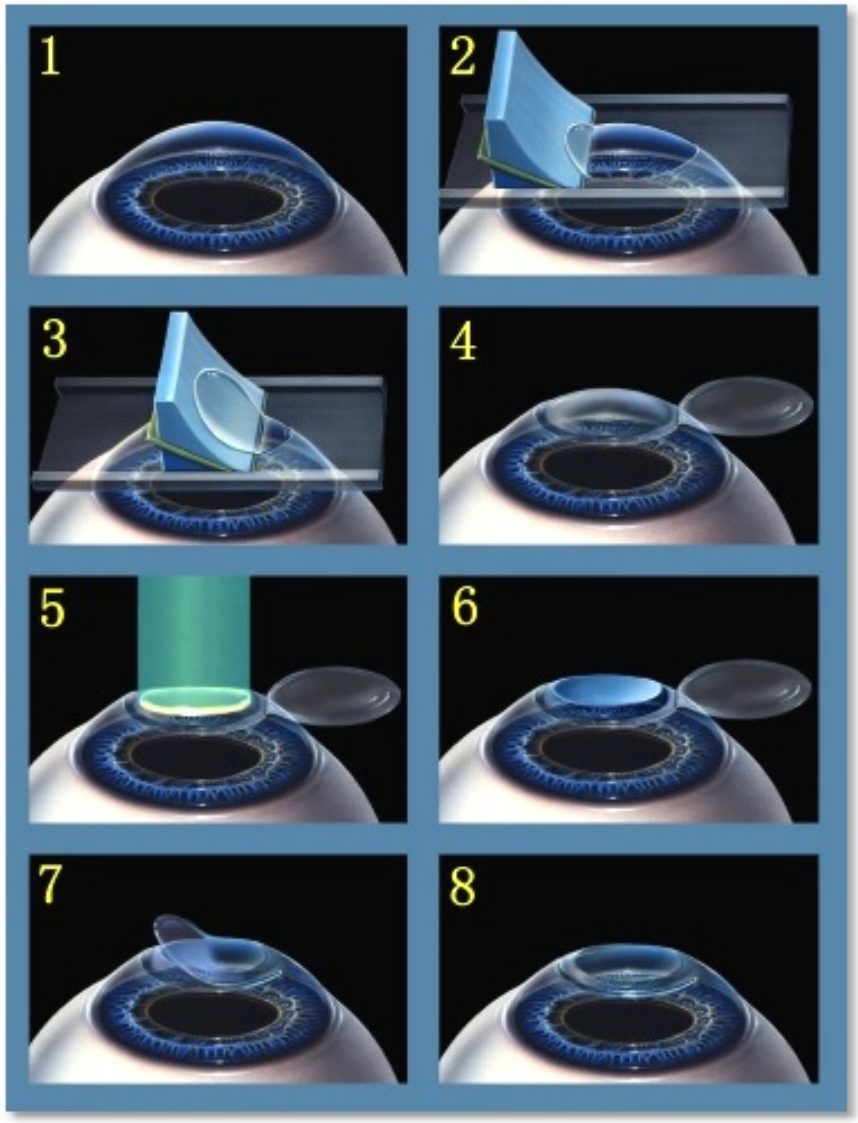
- **LASIK** is an acronym for **LAS**er **I**n-situ **K**eratomileusis, which simply means "to shape the cornea within using a laser."

- It corrects vision by reshaping the cornea (outer window of the eye) so that light rays focus more precisely on the retina, thereby reducing or eliminating refractive errors.

- Using an instrument known as a microkeratome, a thin protective flap of corneal tissue is folded back. The Excimer laser (UV, plasma generated) then removes a predetermined amount of tissue from the inner cornea to correct the individual's refractive error.

- The cornea is made flatter to treat nearsightedness , steeper to treat farsightedness (can't see near, need more curvature!), and/or more spherical to correct astigmatism.

- The corneal flap is placed back in its original position where it bonds without the need for stitches.



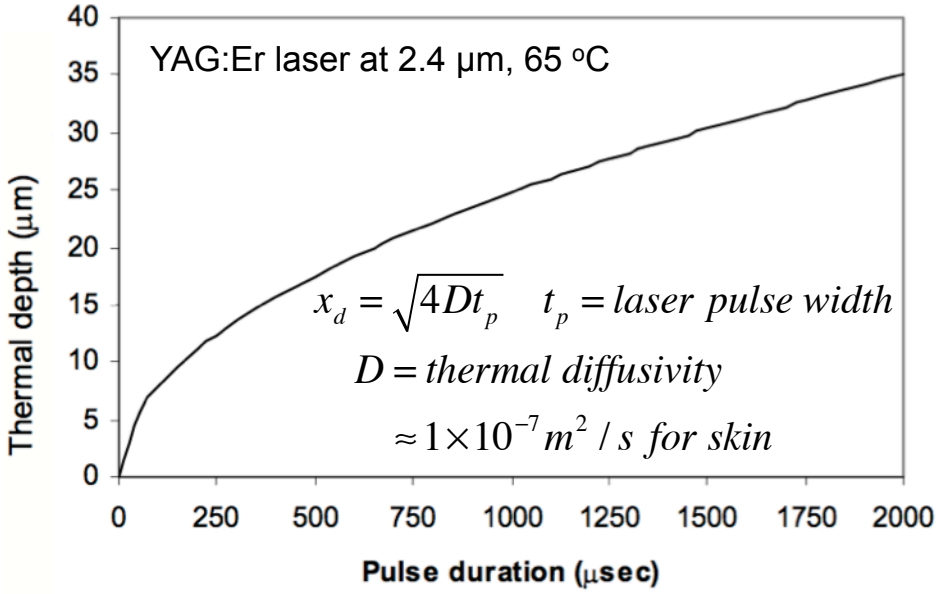
Src: www.eyeclinicpc.com

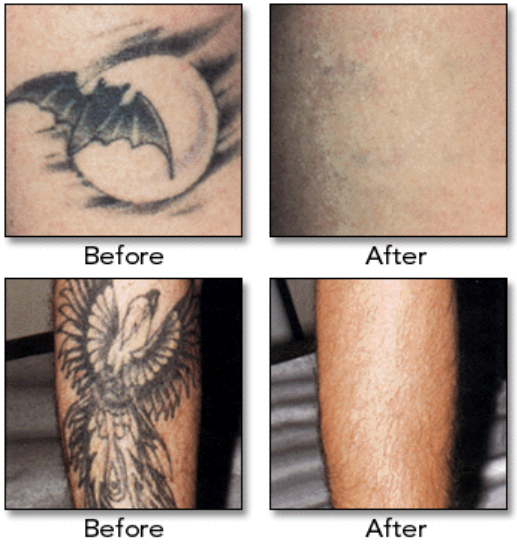
Journal of the Laser and Health Academy
Vol. 2010, No. 1; www.laserandhealth.com

- **Dermatological - *Skin Resurfacing by Ablation:*** 10.6 μm CO_2 laser for 30 μm depth. YAG:Er laser at 2.4 μm for 3 μm (10X less absorption coef. , see slide #5!).

- **Dermatological - *Hair Removal:*** 0.7 to 1.0 μm laser (strong absorption in hair melanin, not skin!).

- **Dermatological - *Tattoo Removal:*** 0.532 μm frequency doubled Nd:YAG (like green laser pointer). Tattoos are just pigments put in the skin that are too large for the body to remove, and the laser just breaks them into smaller particles! Carefully matching to laser wavelengths is better, why?





- **Surgery - *Tissue Bonding, 3 types:***

(1) Direct welding of collagen (uncoils and bonds)

(2) Proteineous solder (just add some albumen and heat it up!)

(3) Dye-enhanced Proteineous solder (dye only absorbs in selective tissue type, and localizes the heating).

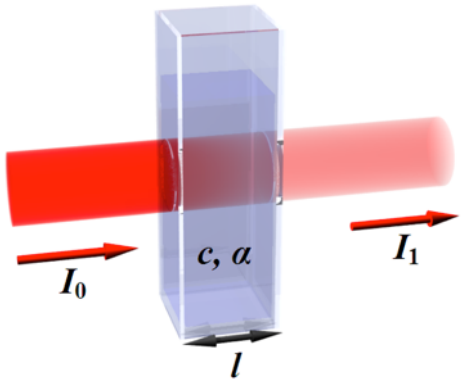
- Man… what a crazy weekend. You found a green laser and an IR laser, and are going to try to use one of them yourself to remove that new tattoo (yes… bad decisions on top of bad decisions). Well, at least because of this class you know that:

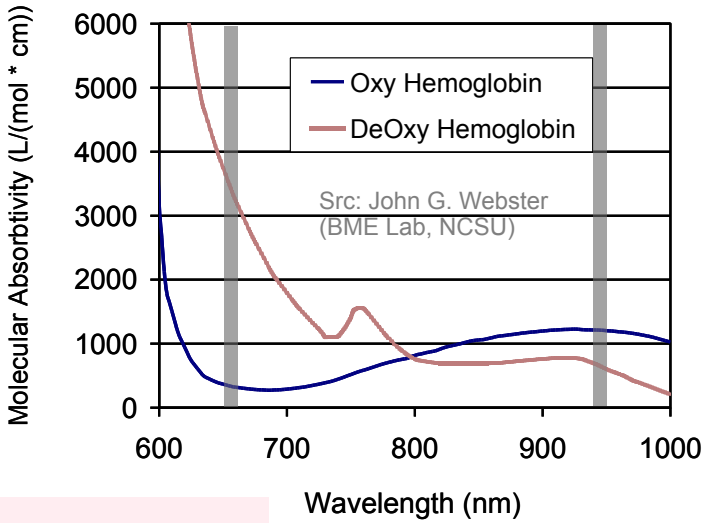
(a) The green laser is best.
(b) The IR (infrared) laser is best.
(c) Either is fine.
(d) Tattoos of ray traces through beam expanders are 'cool' and a great conversation starter…

- Whew! Almost there!

- In lab, grab a pulse ox from the storage bin and try it (read oxygen level, and heart rate).

- So, we can maybe measure absorption differences to get oxygen level in blood, but how do we account for all the variance in our tests? We don't know exact path length l! Ideas?





Warning – we are now going to analyze like chemists, engineers use ln & grams, chemists like log & moles…

$$A = \varepsilon cl = -\log(I / I_0) = absorbance\ (unit\ less)$$
$$\alpha = \varepsilon c = -\log(I / I_0) / l = absorb.\ or\ atten.\ coef.\ (1/cm)$$
$$c = molarity\ or\ concentration\ (moles / cm^3)$$
$$\varepsilon = molar\ absorbtivity\ (cm^2 / mole)$$
$$l = path\ length\ (cm) \qquad mole = 6.022 \times 10^{23} = \#\ atoms\ in\ 12g\ of\ carbon$$

- Key point, absorbance and absorption coef. are proportional to the concentration of absorbers!

- This makes sense! If I have one sheet with X amount of dye and it transmits 40% of light, then if I stacked 2 sheets I would transmit 16% of light (40% for 1st one, 40% of 40% = 16% after the second one).

The light sees the double the number of dye molecules. I could therefore also just have one sheet with 2X amount of dye!

$$I / I_0 = 0.4 \times 0.4 = 10^{\varepsilon cl} \times 10^{\varepsilon cl}$$
$$I / I_0 = 0.16 = 10^{\varepsilon 2cl}$$

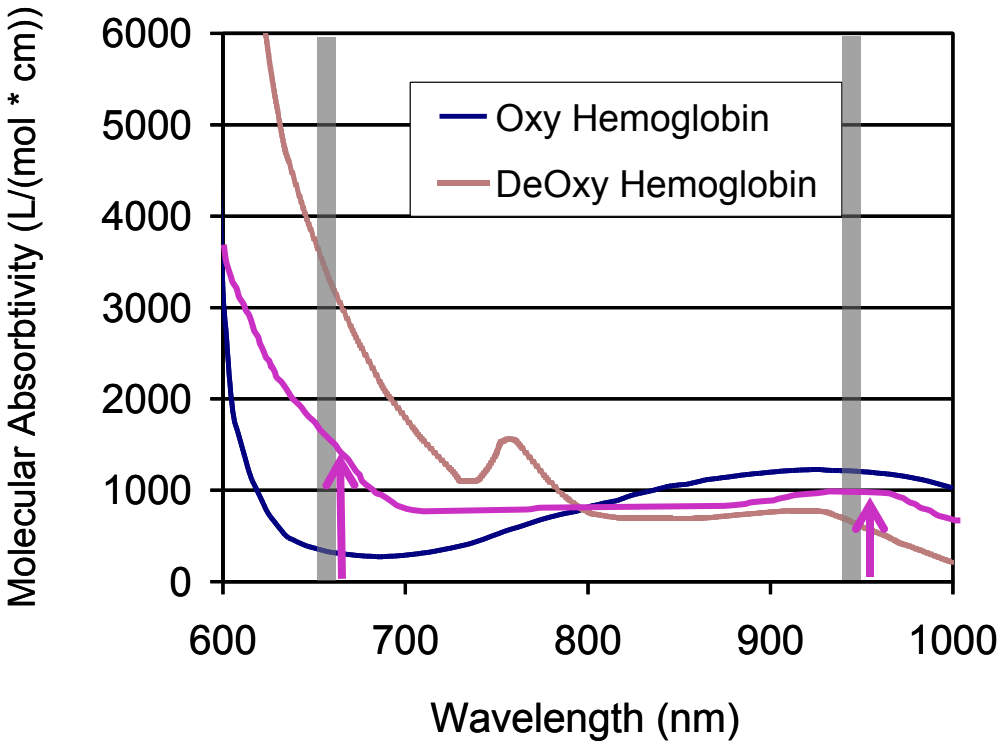
- The total absorbance in blood will be the sum of the absorbance by oxygenated and deoxygenated hemoglobin:

$$A = \varepsilon cl = (\varepsilon_{ox} c_{ox} + \varepsilon_{deox} c_{deox}) l$$

- The Isosbestic point is the point where the 0% and 100% concentration plots overlap, all additional concentrations will cross through this point as well.

- At the Isobestic point, we know the molar absortivities (ε) are the same, so we can reduce the number of variables:

$$A = \varepsilon (c_{ox} + c_{deox}) l$$



- However, even a commercial pulse oximeter does not measure the exact absorption, because that will change based on skin pigmentation, size, how the finger is placed, etc…

- A commercial pulse oximeter uses a 660 nm LED and 940 nm LED, and determines the RATIO of the absorbance at each wavelength and compares this to a look-up table.

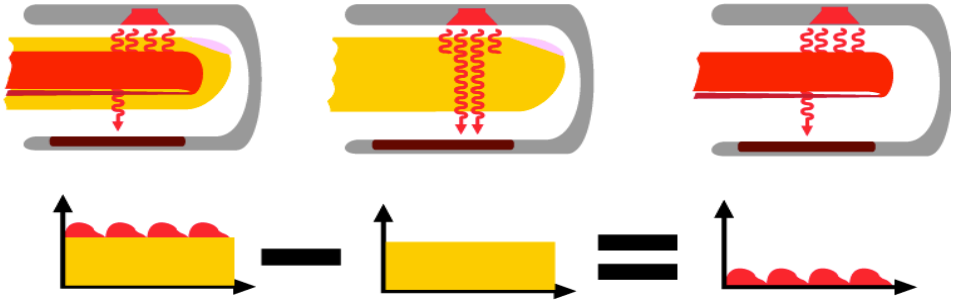
$$\varepsilon_{660nm} / \varepsilon_{960nm}$$

- Pulse oximeters also measure PULSE (heart rate) and they use this to further improve the oximetry data quality… how? Think AC/DC…

Blood (AC) + blood (DC) + tissue (DC).

Blood (DC) + tissue (DC).

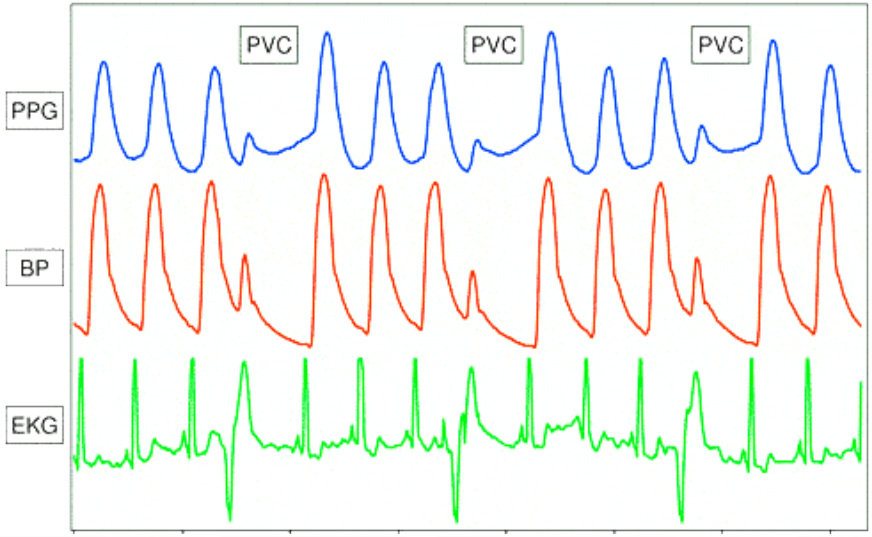
Blood (AC).



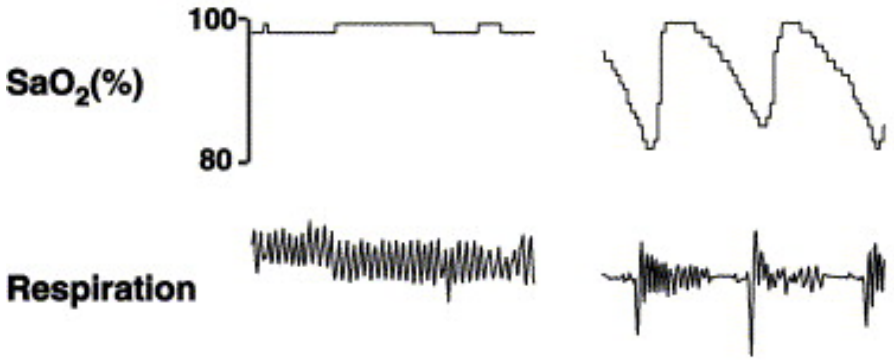
- AC signal from heart-rate is only about 2% signal (98% is DC),

- This optically measured AC blood signal is called a photoplethysmograph (PPG).

- Below is a plot of PPG along with blood pressure (BP), elektrokardiogramm (EK), and showing premature ventricular contraction (PVC).



- Compare a healthy vs. repetitive sleep apnea signal…



- Pulse oximeters use:

(a) Two measurement wavelengths for good accuracy.
(b) Pulse to remove the background light from tissue.
(c) Both of the above.
(d) Magic.

- Whew! Almost there!

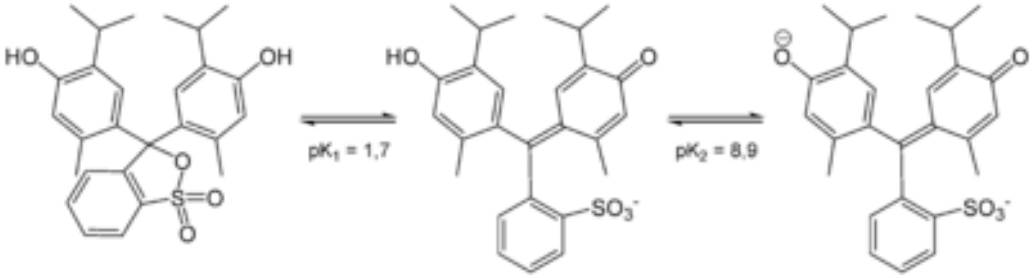
- It is also highly desirable to measure CO_2 in breath, is often used to monitor breathing rate during anaesthesia and intensive care.

- One way, is to just have a 4.25 μm IR source (broad, or laser) and detect changes in absorption across a breathing tube (CO_2 has a strong peak at 4.25 μm).

- Another, and lower cost way, is to remember that CO_2 breaks down in water to form carbonic acid… then you can use the same materials found in pH strips to determine CO_2 level!

Indicator	Low pH color	Transition pH range	High pH color
Gentian violet (Methyl violet 10B)	yellow	0.0–2.0	blue-violet
Leucomalachite green (first transition)	yellow	0.0–2.0	green
Leucomalachite green (second transition)	green	11.6–14	colorless
Thymol blue (first transition)	red	1.2–2.8	yellow
Thymol blue (second transition)	yellow	8.0–9.6	blue
Methyl yellow	red	2.9–4.0	yellow
Bromophenol blue	yellow	3.0–4.6	purple
Congo red	blue-violet	3.0–5.0	red
Methyl orange	red	3.1–4.4	yellow
Screened methyl orange (first transition)	red	0.0–3.2	grey
Screened methyl orange (second transition)	grey	3.2–4.2	green
Bromocresol green	yellow	3.8–5.4	blue
Methyl red	red	4.4–6.2	yellow
Azolitmin	red	4.5–8.3	blue
Bromocresol purple	yellow	5.2–6.8	purple
Bromothymol blue	yellow	6.0–7.6	blue
Phenol red	yellow	6.4–8.0	red
Neutral red	red	6.8–8.0	yellow
Naphtholphthalein	colorless to reddish	7.3–8.7	greenish to blue
Cresol Red	yellow	7.2–8.8	reddish-purple
Phenolphthalein	colorless	8.3–10.0	fuchsia
Thymolphthalein	colorless	9.3–10.5	blue
Alizarine Yellow R	yellow	10.2–12.0	red

- Thymol blue (thymolsulphonephthalein) is a brownish-green or reddish-brown crystalline powder that is used as a pH indicator.



Acidic Neutral Basic

- Could the Easy Cap II monitor use Thymol blue? I'm not sure, but based on what we have above and is shown at left, what do you think?

Precise pH measurement

- Recognize this? Same technique!

[edit]

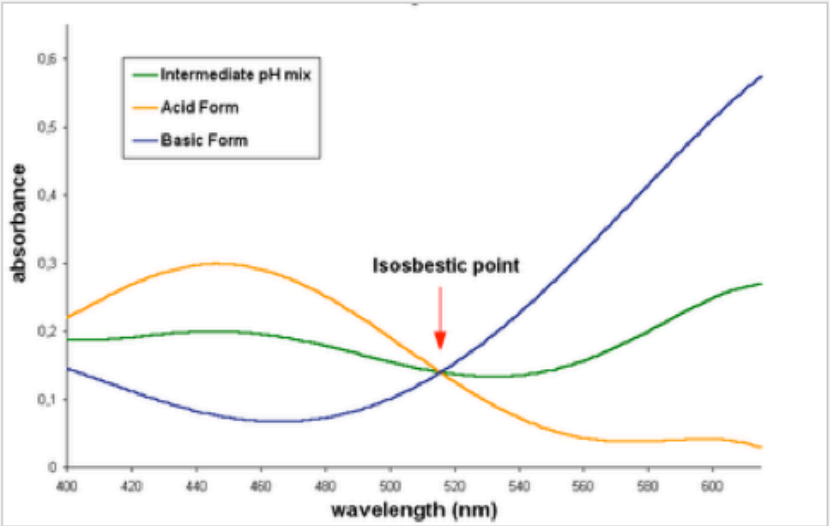
An indicator may be used to obtain quite precise measurements of pH by measuring absorbance quantitatively at two or more wavelengths. The principle can be illustrated by taking the indicator to be a simple acid, HA, which dissociates into H^+ and A^-.

$$HA \rightleftharpoons H^+ + A^-$$

The value of the acid dissociation constant, pK_a, must be known. The molar absorbances, ε_{HA} and ε_{A^-} of the two species HA and A^- at wavelengths λ_x and λ_y must also have been determined by previous experiment. Assuming that Beer's law is obeyed, the measured absorbances A_x and A_y at the two wavelengths are simply the sum of the absorbances due to each species.

$$A_x = [HA]\epsilon^x_{HA} + [A^-]\epsilon^x_{A^-}$$

$$A_y = [HA]\epsilon^y_{HA} + [A^-]\epsilon^y_{A^-}$$



Absorption spectra of bromocresol green at different stages of protonation.

These are two equation in the two concentrations [HA] and [A^-]. Once solved, the pH is obtained as

$$\mathrm{pH} = \mathrm{pK}_a + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]}$$

If measurements are made at more than two wavelengths the concentrations [HA] and [A^-] can be calculated by linear least squares[*disambiguation needed*]. In fact a whole spectrum may be used for this purpose. The process is illustrated for the indicator bromocresol green. The observed spectrum (green) is the sum of the spectra of HA (gold) and of A- (blue), weighted for the concentration of the two species.

When a single indicator is used this method is limited to measurements in the pH range $pK_a \pm 1$, but this range can be extended by using mixtures of two or more indicators. Because indicators have intense absorption spectra the indicator concentration is relatively low so that it can usually be assumed that the indicator itself has negligible effect on pH.

Lab on a Chip

View Online / Journal Homepage
Dynamic Article Links

Cite this: DOI: 10.1039/c2lc40741h

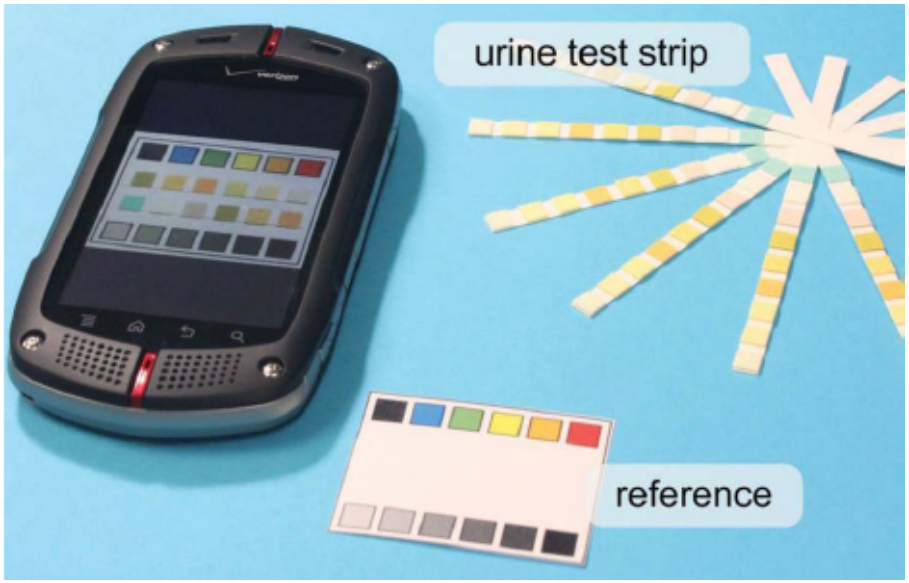
www.rsc.org/loc

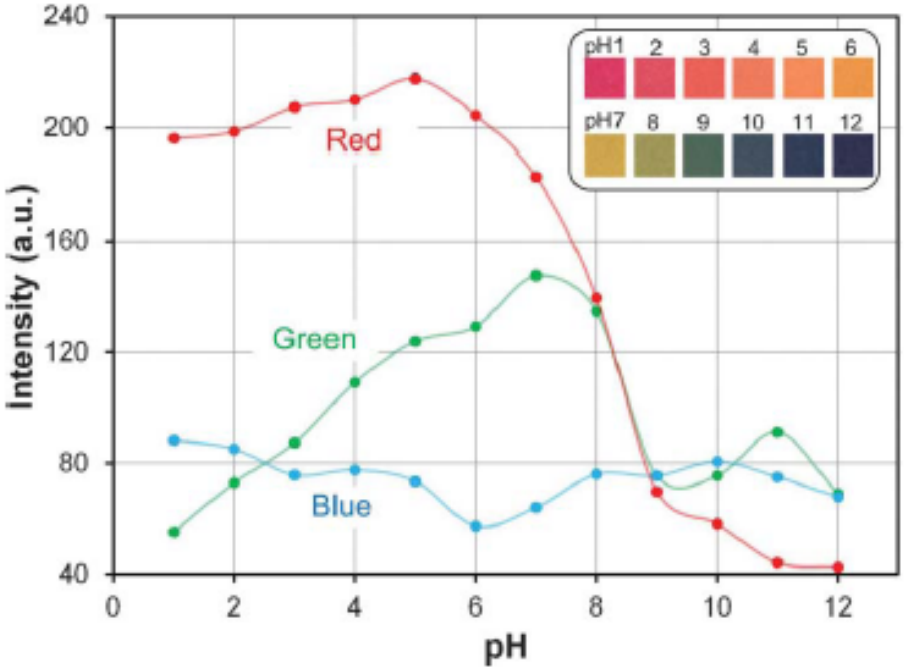
TECHNICAL INNOVATION

Point-of-care colorimetric detection with a smartphone†

Li Shen,[a] Joshua A. Hagen[b] and Ian Papautsky*[a]

Received 1st July 2012, Accepted 7th September 2012
DOI: 10.1039/c2lc40741h





- Nice job! Just a few more weeks away from a longer break…